FAQs
on Vaccines and Immunization Practices

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Second Edition

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IAP Advisory Committee on Vaccines and
Immunization Practices, 2013–14
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Foreword

Walter A Orenstein

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Foreword

Few measures in preventive medicine can compare with the impact of vaccines. Smallpox has been eradicated and polio is on the verge of eradication. Measles transmission has been terminated in large areas of the world. Most recommended vaccines are highly effective in preventing vaccine-preventable diseases in vaccine recipients. But nearly all vaccines have another special property, the induction of herd immunity or community protection. Most vaccine-preventable diseases are transmitted from person to person in a chain of transmission from infectious case to susceptible. When a transmitting case comes in contact with an immune person, transmission does not occur and the chain of transmission is broken. Since infectious cases do not have unlimited contacts, if the immunity levels are high enough in a community, the likelihood that such cases will meet a susceptible is very low. Thus, transmission can be terminated for many diseases in a given community before 100% vaccine coverage is achieved. Who are the people indirectly protected? They are children too young for vaccination, persons with compromised immunity who cannot make adequate immune responses to vaccines, persons with contraindications to vaccination, and persons who do not respond to vaccine for other reasons. All are protected indirectly by high levels of immunity in the population.

Prevention from vaccines is very attractive because generally with a few doses, long term, even lifelong, protection can be achieved with many vaccines. In contrast, the lifestyle changes needed to prevent many chronic diseases require a lifetime of continued implementation.

During the last few decades, there has been a revolution in vaccinology. Vaccines have been produced that prevent cancer. The human papilloma virus vaccine (HPV) prevents approximately 70% of cervical cancers globally. Similarly, hepatitis B vaccine can prevent one of the major causes of liver cancer. Antibodies against polysaccharides are key to the defense against several bacteria that cause pneumonia, meningitis, and other serious clinical syndromes. However, young infants make poor immune responses against plain polysaccharides. By conjugating polysaccharides to protein carriers, excellent immune responses can be achieved even in very young infants. Conjugation technology is the basis for vaccines against *Haemophilus influenzae* type b (Hib), pneumococci, and meningococci. We can now prevent one of the most common causes of severe diarrhea and dehydration, leading to death, rotavirus. Other new technologies, including the use of recombinant DNA, vaccine vectors, genetic reassortment and other techniques are being brought to bring us new and better vaccines.

This decade has been called the "Decade of Vaccines". Efforts are underway to develop new vaccines against more infectious diseases as well as enhancing more widespread and better use of existing vaccines.

The most favorable benefits of vaccines are only achieved when they are used widely in populations for whom they are recommended. Vaccines don't save lives, vaccinations save lives. This handbook provides a major resource to vaccine providers in using vaccines optimally. This book is devoted to answering practical questions on recommended vaccines and schedules, indications, contraindications, and precautions. It tries to answer questions regarding particular clinical situations, which may not be easily answered from reading existing recommendations. Use of the book, will help you minimize the risks of vaccine-preventable diseases in the populations you serve, while maximizing the safe use of those vaccines.

Walter A Orenstein MD

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Preface to the Second Edition

Vaccination continues to be the most cost-effective and reliable preventive measure. Its important has increased all the more in recent times when we are having difficulty in treating infections in the face of antibiotic resistance.

It is an extremely dynamic subject where new developments take place at an appreciable speed. No wonder, it attracts the maximum number of queries compared to any other field of medicine. The last edition of the book was conceived to answer the questions arising at that time. The response to the first edition has been quite gratifying.

Ever since then, many changes have occurred. There are new vaccines and so many new studies. These have led to new guidelines and new schedules. Hence, many new questions and the motivation impelled us to work on the second edition of this fascinating subject. Hope it delivers what it is aimed for.

Vipin M Vashishtha
Ajay Kalra
Naveen Thacker

Preface to the First Edition

"The Greeks had two gods of health: Aesculapius and Hygeia, therapy and prevention, respectively. Medicine in the twentieth century retains those two concepts, and vaccination is a powerful means of prevention. What follows is information on the vaccines that, together with sanitation, make modern society possible, and that if wisely used, will continue to bestow on humankind the gift of prevention, which according to proverb is worth far more than cure."

—*Stanley A Plotkin and Edward A Mortimer, Jr*
in the preface to the first edition of their book, "Vaccines"

Immunization is a lively subject where changes in the field happen more rapidly than one can anticipate. This makes it a very dynamic subject and underlines the need to remain updated more frequently. Though the general attitude of both physicians and patients finds cure more interesting than prevention, the truth is the latter is far more vital than the former, though less appealing. While dealing with infections, development of newer antibiotics may have some limitations but the possibilities of developing vaccines are immense. Despite the high cost involved in developing a new vaccine, the opening up of the huge markets of developing world has offered a new incentive to vaccine industry. With the advent of vaccine industry in the developing world, and ample availability of global funding opportunities through giant philanthropic organizations have further reinvigorated the vaccine manufacturers to readily invest in developing newer products for future needs by embracing newer technologies. Therefore, any discussion on the subject of vaccinology covers not only the several vaccines per se but also the changes in technology and the exciting possibilities of various combinations and permutations in the making of various vaccines, which further extend to the ingenuity of newer modes of vaccines' delivery.

In view of the above, it is no wonder that medical students, pediatricians and general practitioners are paying more attention to vaccines than ever before. As a proof of this trend, the sessions on vaccinology get a prime slot in all pediatric conferences and are usually jam-packed. Yet, in the short time frame that these conferences allow, not all questions can be answered and many delegates have often gone back home with doubts and queries unanswered.

Therefore, this book is an attempt to fill this void by providing contributions from experts who have a vast information and knowledge on the subject. They have devoted a lot of time and efforts to answer the questions on the basis of the latest information that is available today right from the Basics of Immunology to Vaccines in the Pipeline and Therapeutic Vaccines, besides talking about the changes in technologies and evolution of different adjuvants and combinations.

Of late, there is a paucity of a decent book on immunization in this format. Often, the FAQ books are criticized as being meant only to provide a superficial account of the subject; however, our effort is to provide a more in-depth account of the given subject. The hallmark of this book is its comprehensive nature—almost all the aspects related to pediatric vaccination today are covered in depth. Hopefully, the book will not only be handy for the busy practitioners but even postgraduate students will find it attractive to browse pages to find answers of their queries.

Vipin M Vashishtha
Ajay Kalra
Naveen Thacker

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Section 1

General Vaccination

Chapters

CHAPTER

1

Vaccine Immunology: Basics and Beyond

Yash Paul, Vipin M Vashishtha

1. Are vaccination and immunization same?

Broadly speaking both terms appear to be same and frequently used interchangeably. However, there is minor technical difference. 'Vaccination' is a process of inoculating the vaccine/antigen in to the body. The vaccinee may or may not seroconvert to vaccine whereas the process of inducing immune response, which can be 'humoral' or 'cell-mediated' in the vaccinee is called 'immunization'.

Vaccines can be administered through different routes e.g. nasal mucosa, gut mucosa or by injection which may be given intradermal, subcutaneous or intramuscular. This process is called 'vaccination' or 'active immunization'. In case immunoglobulins or antisera are administered it is called 'passive immunization'. Thus administration of immunoglobulins or antisera is not vaccination, although it provides immunity or protection for a short period.

2. What are 'humoral' and 'cell-mediated immunity'?

Vaccines confer protection against diseases by inducing both antibodies and T cells. The former is called 'humoral' response and the latter, 'cellular' response or 'cell-mediated immunity'. Antibodies are of several different types (IgG, IgM, IgA, IgD and IgE) and they differ in their structure, half life, site of action and mechanism of action. Humoral immunity is the principal defence mechanism against extracellular microbes and their toxins. B lymphocytes secrete antibodies that act by neutralization, complement activation or by promoting opsonophagocytosis. Cell mediated immunity (CMI) is the principal defence mechanism against intracellular microbes. The effectors of CMI, the T cells are of two types. The helper T cells secrete proteins called cytokines that stimulate the proliferation and differentiation of T cells as well as other cells including B lymphocytes, macrophages and NK cells. The cytotoxic T cells act by lysing infected cells.

3. What are innate and adaptive immunity?

Innate immunity comprises of the skin and mucosal barriers, phagocytes (neutrophils, monocytes and macrophages) and the natural killer (NK) cells. It comes into play immediately on entry of the pathogen and is non specific. Adaptive immunity is provided by the B lymphocytes (humoral/antibody-mediated immunity) and T lymphocytes (cellular/cell-mediated immunity). The innate immune system triggers the development of adaptive immunity by presenting antigens to the B lymphocytes and T lymphocytes. Adaptive immunity takes time to evolve and is pathogen specific (Figure 1.1 and Table 1.1).

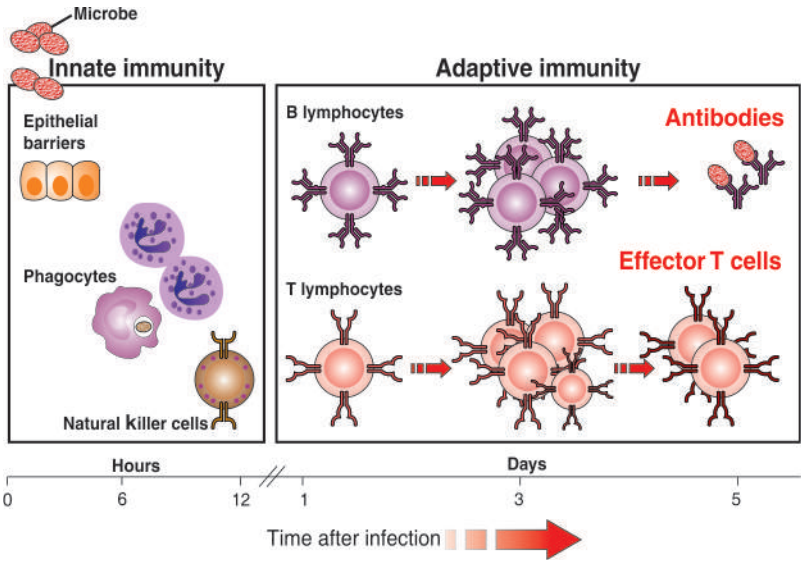

FIGURE 1.1 Innate and adaptive immunity

TABLE 1.1 Comparison of innate and adaptive immunity

Non-specific Immunity (innate)	*Specific Immunity (adaptive)*
Its response is antigen-independent	Its response is antigen-dependent
There is immediate response	There is a lag time between exposure and maximal response
It is not antigen-specific	It is antigen-specific
Exposure does not result in induction of memory cells	Exposure results in induction of memory cells
Some of its cellular components or their products may aid specific immunity	Some of its products may aid non-specific immunity

4. What are 'B' and 'T' cells? What role do they play in regard to immunology of vaccines?

Immune system is almost not existent at birth; maternal antibodies transferred transplacentally provide some protection during early childhood. After birth baby comes in contact with microbes which gradually activate immune system. B cells form the most important component of immune system in the body. These are produced in liver in fetal life and mature in bone marrow in humans. In other species these cells mature in an organ called "bursa of Fabricius", thus these lymphocytes are called B cells. On activation by an antigen contained in microorganisms and vaccines, the B cells proliferate and get converted to plasma cells, which in turn produce antibodies. For effective production of antibodies, B cells need help from T helper cells. T lymphocytes are the cells that originate in the thymus, mature in the periphery, become activated in the spleen/nodes if 1) their T cell receptor bind to an antigen presented by an MHC molecule and 2) they receive additional costimulation signals driving them to acquire killing (mainly CD8+ T cells) or supporting (mainly CD4+ T cells) functions.

B cells have immunoglobulin surface receptor, which binds with the appropriate antigen present on the infective pathogen. The processed antigen stimulates the B cell to mature into antibody secreting plasma cell and generate IgM. T helper2 (Th2) cell leads to switch in the production from IgM to IgG, IgA or IgD. The B cells can directly respond to the antigen and process the antigen, but the T cells do not react with the antigen directly unless processed and presented by special cells called antigen presenting cells (APCs).

5. What are antigen-presenting cells (APCs) and dendritic cells? What functions do they perform?

Antigen-presenting cells (APCs) are the cells that capture antigens by endo- or phagocytosis, process them into small peptides, display them at their surface through MHC molecules and provide co-stimulation signals that act synergistically to activate antigen-specific T cells. Antigen presenting cells include B cells, macrophages and dendritic cells, although only dendritic cells are capable of activating naïve T cells (Figure 1.2).

Dendritic cells are major APC in the body in addition to the B cells and the macrophages. The major role of these cells is to identify dangers, which is done by the special receptors on the APC named toll-like receptors (TLR).

Vaccine antigens are taken up by immature dendritic cells (DCs) activated by the local inflammation, which provides the signals required for their migration to draining lymph nodes. During this migration, DCs mature and their surface expression of molecules changes. DCs sense "danger signals" through their Toll-like receptors and respond by a modulation of their surface or secreted molecules. Simultaneously, antigens are processed into small fragments and displayed at the cell surface in the grooves of Major Histocompatibility Complex (MHC-HLA in humans) molecules. As a rule, MHC class I molecules present peptides from antigens that are produced within infected cells, whereas phagocytosed antigens are

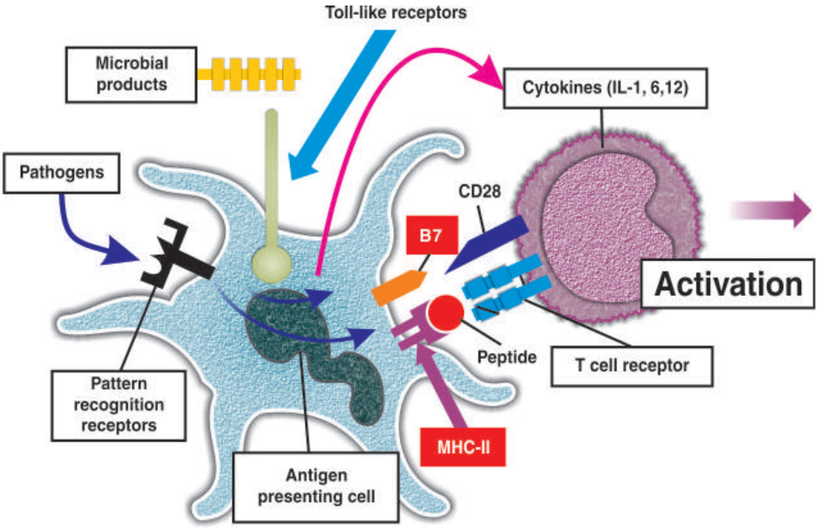

FIGURE 1.2 Schematic presentation of a dendritic cell and its activation by pathogens

displayed on MHC class II molecules. Thus, mature DCs reaching the T cell zone of lymph nodes display MHC-peptide complexes and high levels of costimulation molecules at their surface. CD4+ T cells recognize antigenic peptides displayed by class II MHC molecules, whereas CD8+ T cells bind to class I MHC peptide complexes.

Antigen-specific T cell receptors may only bind to specific MHC molecules (e.g. HLA A2), which differ among individuals and populations. Consequently, T cell responses are highly variable within a population.

6. What are adjuvants? How do they affect performance of a vaccine?

Adjuvants are agents which increase the stimulation of the immune system by enhancing antigen presentation (depot formulation, delivery systems) and/or by providing co-stimulation signals (immunomodulators). Aluminium salts are most often used in today's vaccines. Hence, the adjuvants improve the immunogenicity of vaccines. Many new generations of adjuvants are in fact analogues of TLRs, for example CpG-ODN used in new generation of Japanese encephalitis vaccines.

Most non-live vaccines require their formulation with specific adjuvants to include danger signals and trigger a sufficient activation of the innate system. These adjuvants may be divided into two categories: delivery systems that prolong the antigen deposit at site of injection, recruiting more dendritic cells (DCs) into the reaction, and immune modulators that provide additional differentiation and activation signals to monocytes and DCs. Although progress is being made, none of the adjuvants currently in use trigger the degree of innate immune activation that is elicited by live vaccines, whose immune potency far exceed that of non-live vaccines.

7. What are 'germinal centers' and 'marginal zone'?

Germinal centers (GCs) are dynamic structure that develop in spleen/nodes in response to an antigenic stimulation and dissolves after a few weeks. GCs contain a monoclonal population of antigen-specific B cells that proliferate and differentiate through the support provided by follicular dendritic cells and helper T cells. Immunoglobulin class switch recombination, affinity maturation, B cell selection and differentiation into plasma cells or memory B cells essentially occur in GCs.

'Marginal zone' is the area between the red pulp and the white pulp of the spleen. Its major role is to trap particulate antigens from the circulation and present it to lymphocytes.

8. What do the terms 'epitope' and 'paratope' mean?

An 'epitope', also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. The part of an antibody that recognizes the epitope is called a 'paratope'. Although epitopes are usually thought to be derived from non-self proteins, sequences derived from the host that can be recognized are also classified as epitopes.

T cell epitopes are presented on the surface of an antigen-presenting cell, where they are bound to MHC molecules. T cell epitopes presented by MHC class I molecules are typically peptides between 8 and 11 amino acids in length, whereas MHC class II molecules present longer peptides, and non-classical MHC molecules also present non-peptidic epitopes such as glycolipids.

Epitopes are sometimes cross-reactive. This property is exploited by the immune system in regulation by anti-idiotypic antibodies. If an antibody binds to an antigen's epitope, the paratope could become the epitope for another antibody that will then bind to it. If this second antibody is of IgM class, its binding can upregulate the immune response; if the second antibody is of IgG class, its binding can downregulate the immune response.

9. What is the difference between 'antibody affinity' and 'avidity'?

The antibody affinity refers to the tendency of an antibody to bind to a specific epitope at the surface of an antigen, i.e. to the strength of the interaction. The avidity is the sum of the epitope-specific affinities for a given antigen. It directly relates its function.

10. What do the terms 'CD4+ T cells' and CD8+ T cells' stand for? What are the functions of these lymphocytes?

T lymphocytes are the cells that originate in the thymus, mature in the periphery, become activated in the spleen/nodes if 1) their T cell receptor bind to an antigen presented by an MHC molecule and 2) they receive additional costimulation signals driving them to acquire killing (mainly CD8+ T cells) or supporting (mainly CD4+ T cells) functions.

CD4+ T cells are those T-lymphocytes that express the CD4 glycoprotein at their surface. CD4 (cluster of differentiation 4) is a glycoprotein expressed on the surface of T helper cells, regulatory T cells, monocytes, macrophages, and dendritic cells. It was discovered in the late 1970s and was originally known as leu-3 and T4 (after the OKT4 monoclonal antibody that reacted with it) before being named CD4 in 1984. In humans, the CD4 protein is encoded by the CD4 gene. CD4 is a co-receptor that assists the T cell receptor (TCR) to activate its T cell following an interaction with an antigen presenting cell. Using its portion that resides inside the T cell, CD4 amplifies the signal generated by the TCR by recruiting an enzyme, known as the tyrosine kinase lck, which is essential for activating many molecules involved in the signalling cascade of an activated T cell. CD4 interacts directly with MHC class II molecules on the surface of the antigen presenting cell using its extracellular domain.

T cells expressing CD4 molecules (and not CD8) on their surface, therefore, are specific for antigens presented by MHC II and not by MHC class I (they are MHC class II-restricted). The short cytoplasmic/intracellular tail (C) of CD4 contains a special sequence of amino acids that allow it to interact with the lck molecule described above.

CD8 cells are those T-lymphocytes that express the CD8 glycoprotein at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of nearly every cell of the body. Through IL-10, adenosine and other molecules secreted by regulatory T cells, the CD8+ cells can be inactivated to an anergic state, which prevent autoimmune diseases such as experimental autoimmune encephalomyelitis. CD8 cells are cytotoxic T cells (CTLs) and destroy virally infected cells and tumor cells, and are also implicated in transplant rejection (Figure 1.3).

CD8+ T cells do not prevent but reduce, control and clear intracellular pathogens by:

- Directly killing infected cells (release of perforin, granzyme, etc.)
- Indirectly killing infected cells through antimicrobial cytokine release

CD4+ T cells do not prevent but participate to the reduction, control and clearance of extra and intracellular pathogens by:

- Producing IFN-γ, TNF-α/-β, IL-2 and IL-3 and supporting activation and differentiation of B cells, CD8+T cells and macrophages (Th1 cells).
- Producing IL-4, IL-5, IL-13, IL-6 and IL-10 and supporting B cell activation and differentiation (Th2 cells).

11. What are TLRs and their role in vaccine immunogenicity?

Toll-like receptors are a family of 10 receptors (TLR1 to TLR10) present at the surface of many immune cells, which recognize pathogens through conserved microbial patterns and activate innate immunity when detecting danger.

Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system. They are single membrane-spanning non-catalytic receptors that recognize structurally conserved molecules derived from microbes. Once these microbes have breached physical barriers such as the skin or intestinal tract mucosa, they are recognized by TLRs which activates immune cell responses.

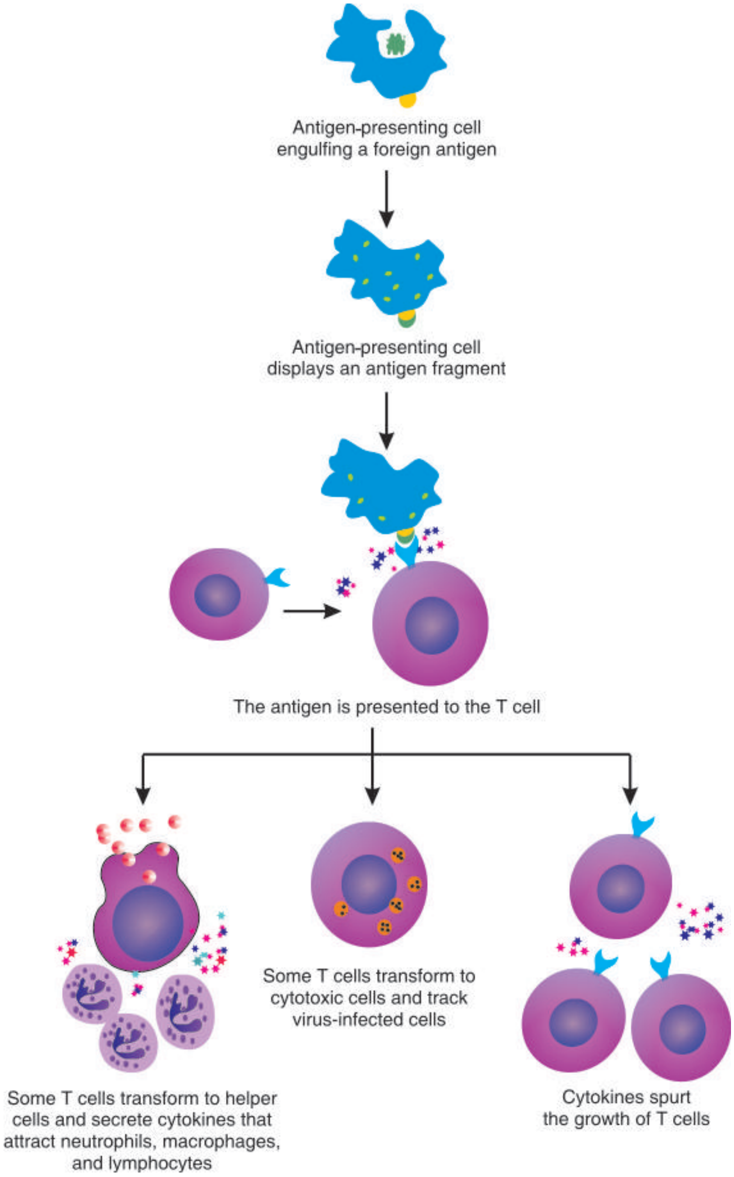
Antigen-presenting cell engulfing a foreign antigen
Antigen-presenting cell displays an antigen fragment
The antigen is presented to the T cell
Some T cells transform to helper cells and secrete cytokines that attract neutrophils, macrophages, and lymphocytes
Some T cells transform to cytotoxic cells and track virus-infected cells
Cytokines spurt the growth of T cells

FIGURE 1.3 T lymphocyte activation pathway

T Cell activation is triggered when a T cell encounters its cognate antigen, coupled to a Mhc molecule, on the surface of an infected cell or a phagocyte. T cells contribute to immune defences in two major ways: Some direct and regulate immune responses; others directly attack infected or cancerous cells

TLRs are a type of pattern recognition receptor (PRR) and recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as pathogen-associated molecular patterns (PAMPs). TLRs together with the interleukin-1 receptors form a receptor superfamily, known as the “interleukin-1 receptor/toll-like receptor superfamily”; all members of this family have in common a so-called TIR (Toll-IL-1 receptor) domain.

Three subgroups of TIR domains exist. Proteins with subgroup 1 TIR domains are receptors for interleukins that are produced by macrophages, monocytes and dendritic cells and all have extracellular immunoglobulin (Ig) domains. Proteins with subgroup 2 TIR domains are classical TLRs, and bind directly or indirectly to molecules of microbial origin. A third subgroup of proteins containing TIR domains consists of adaptor proteins that are exclusively cytosolic and mediate signaling from proteins of subgroups 1 and 2.

TLRs are present in vertebrates, as well as in invertebrates. Molecular building blocks of the TLRs are represented in bacteria and in plants, and in the latter kingdom, are well known to be required for host defence against infection. The TLRs thus appear to be one of the most ancient, conserved components of the immune system.

Toll-like receptors bind and become activated by different ligands, which, in turn are located on different types of organisms or structures. They also have different adapters to respond to activation and are located sometimes at the cell surface and sometimes to internal cell compartments. Furthermore, they are expressed by different types of leucocytes or other cell types.

Following activation by ligands of microbial origin, several reactions are possible. Immune cells can produce signaling factors called cytokines which trigger inflammation. In the case of a bacterial factor, the pathogen might be phagocytosed and digested, and its antigens presented to CD4+ T cells. In the case of a viral factor, the infected cell may shut off its protein synthesis and may undergo programmed cell death (apoptosis). Immune cells that have detected a virus may also release anti-viral factors such as interferons.

The discovery of the Toll-like receptors finally identified the innate immune receptors that were responsible for many of the innate immune functions that had been studied for many years. Interestingly, TLRs seem only to be involved in the cytokine production and cellular activation in response to microbes, and do not play a significant role in the adhesion and phagocytosis of microorganisms.

12. What are the differences between live attenuated and inactivated vaccines?

Live vaccines are attenuated (modified) live organisms, which have immunogenicity i.e. can generate antibodies, but have lost pathogenicity i.e. capability to cause disease. Live vaccines can be viral as well as bacterial. The live vaccine particles (viruses or bacteria) replicate or multiply in the body after administration and stimulate the immune system. The older concept that single dose of live vaccines induces life long immunity perhaps does not hold true, we need many doses of OPV, and booster doses are required for live vaccines like measles vaccine, varicella, rubella and live oral typhoid vaccines. Inactivated vaccines may consist of whole inactivated organisms like whole cell pertussis, typhoid, rabies; inactivated polio vaccine, modified exotoxins called ‘toxoids’ like diphtheria toxoid or tetanus toxoid; subunits like polysaccharide antigents of *Salmonella typhi, Haemophilus influenzae* type-B (Hib), and surface proteins of hepatitis B virus. Conjugation of the polysaccharide with a protein carrier significantly improves the immune response.

Live viral vaccines do efficiently trigger the activation of the innate immune system, presumably through pathogen-associated signals (such as viral RNA) allowing their recognition by pattern recognition receptors-Toll-like Receptos. Following injection, viral particles rapidly disseminate throughout the vascular network and reach their target tissues. This pattern is very similar to that occurring after a natural infection, including the initial mucosal replication stage for vaccines administered through the nasal/oral routes. Following the administration of a live viral vaccine and its dissemination, dendritic cells are activated at multiple sites, migrate towards the corresponding draining lymph nodes and launch multiple foci of T and B cell activation. This provides a first explanation to the generally higher immunogenicity of live versus non-live vaccines.

The strongest antibody responses are generally elicited by live vaccines that better activate innate reactions and thus better support the induction of adaptative immune effectors. Non-live vaccines frequently require formulation in adjuvants, of which aluminium salts are particularly potent enhancers of antibody responses, and thus included in a majority of currently available vaccines. This is likely to reflect their formation of a deposit from which antigen is slowly de-absorbed and released, extending the duration of B and T cell activation, as well as the preferential induction of IL-4 by aluminium-exposed macrophages.

Very few non-live vaccines induce high and sustained antibody responses after a single vaccine dose, even in healthy young adults. Primary immunization schedules therefore usually include at least two vaccine doses, optimally repeated at a minimal interval of 3-4 weeks to generate successive waves of B cell and GC responses. These priming doses may occasionally be combined into a single “double” dose, such as for hepatitis A or B immunization. In any case, however, vaccine antibodies elicited by primary immunization with non-live vaccines eventually wane.

13. What is the difference between T cell-dependent and T cell-independent immune response?

Certain antigens, primarily proteins, induce both B cell and T cell stimulation leading to what is called T cell-dependent immune response. Infants of 6 weeks of age onwards are capable of T cell-dependent response. This type of response usually results in higher titers of IgG type and long lasting. It also shows booster effects with repeated exposures.

On the other hand T cell-independent response being only B-cell mediated is not possible below 2 years of age. It is predominantly IgM type with low titers. The response is short lasting, repeated doses of vaccine does not lead to boosting effect. IgA is not produced and hence there is no local mucosal protection with this type of antigens, while in case of T cell-dependent response IgA antibodies are also produced which helps in providing mucosal protection and eradication of the carrier state. Few examples of T cell-independent vaccines include bacterial polysaccharide (PS) vaccines such as *S. pneumoniae, N. meningitidis, H. influenzae, S. typhi*.

14. What are conjugate vaccines?

As already mentioned in answer to above question, regarding the difference between T cell-dependent and T cell-independent immune response that T cell-independent response being B cell-mediated younger children do not respond to such vaccines. A T cell-independent antigen like polysaccharide can be made into T cell-dependent by the technique of conjugation. Such conjugated vaccines can be administered to children less than 2 years of age also. This technique is used to produce conjugated Vi typhoid, Hib, pneumococcal and meningococcal vaccines.

15. How do vaccines elicit their responses? Which are the main effectors of vaccine responses?

The nature of the vaccine exerts a direct influence on the type of immune effectors that are predominantly elicited and mediate protective efficacy (Table 1.2). Capsular polysaccharides (PS) elicit B cell responses in what is classically reported as a T-independent manner, although increasing evidence supports a role for CD4+ T cells in such responses. The conjugation of bacterial PS to a protein carrier (e.g. glycoconjugate vaccines) provides foreign peptide antigens that are presented to the immune system and thus recruits antigenspecific CD4+ Th cells in what is referred to as T-dependent antibody responses. A hallmark of T-dependent responses, which are also elicited by toxoid, protein, inactivated or live attenuated viral vaccines is to induce both higher-affinity antibodies and immune memory. In addition, live attenuated vaccines usually generate CD8+ cytotoxic T cells. The use of live vaccines/vectors or of specific novel delivery systems (e.g. DNA vaccines) appears necessary for the induction of strong CD8+ T cell responses. Most current vaccines mediate their protective efficacy through the induction of vaccine antibodies, whereas BCG-induced T cells produce cytokines that contribute to macrophage activation and control of *M. tuberculosis*. The induction of antigen-specific immune effectors (and/or of immune memory cells) by an immunization process does imply that these antibodies, cells or cytokines represent surrogates, or even correlates—of vaccine efficacy. This requires the formal demonstration that vaccine-mediated protection is dependent—in a vaccinated individual—upon the presence of a given marker such as an antibody titer or a number of antigen-specific cells above a given threshold. Antigen-specific antibodies have been formally demonstrated as conferring vaccine-induced protection against many diseases.

TABLE 1.2 Correlates of vaccine-induced immunity

Vaccines	*Vaccine type*	*Serum IgG*	*Mucosal IgG*	*Mucosal IgA*
EPI vaccines				
Diphtheria toxoid	Toxoid	++	(+)	
Pertussis, whole cell	Killed	++		
Pertussis, acellular	Protein	++		
Tetanus toxoid	Toxoid	++		
Measles	Live attenuated	++		
Polio sabin	Live attenuated	++	++	++
Polio salk	Killed	++	+	
Tuberculosis (BCG)	Live mycobacterial			
Non-EPI vaccines				
Hepatitis A	Killed	++	(+)	
Hepatitis B (HbsAg)	Protein	++		
Hib PS	PS	++	(+)	
Hib glycoconjugates	PS-protein	++	++	
Influenza	Killed, subunit	++	(+)	
Influenza intranasal	Live attenuated	++	+	+
Meningococcal PS	PS	++	(+)	
Meningococcal conjugate	PS-protein	++	++	
Mumps	Live attenuated	++		
Pneumococcal PS	PS	++	(+)	
Pneumoccoccal conjugates	PS-protein	++	++	
Rabies	Killed	++		
Rotavirus	VLPs	(+)	(+)	++
Rubella	Live attenuated	++		
Typhoid PS	PS	+	(+)	
Varicella	Live attenuated	++		
Yellow fever	Live attenuated	++		

Abbreviations: IgG, immunoglobulin G; IgA, immunogloblulin A; EPI, expanded program on immunization; BCG; Bacillus Calmette-Guèrin; Hib, *Haemophilus influenzae* type B; PS, polysaccharide; VLPs, Virus-like particles.

(*With permission from:* Siegrist CA. Vaccine Immunology. Vaccines. In: Plotkin SA, Orenstein W, Offit P (Eds) Saunders Elsevier, 5th Edition, 2008.p.17-36)

Passive protection may result from the physiological transfer of maternal antibodies (e.g. tetanus) or the passive administration of immunoglobulins or vaccine-induced hyperimmune serum (e.g. measles, hepatitis, varicella, etc.). Such antibodies may neutralize toxins in the periphery, at their site of production in an infected wound (tetanus) or throat (diphtheria). They may reduce binding or adhesion to susceptible cells/receptors and thus prevent viral replication (e.g. polio) or bacterial colonization (glycoconjugate vaccines against encapsulated bacteria) if present at sufficiently high titers on mucosal surfaces. The neutralization of pathogens at mucosal surfaces is mainly achieved by the transudation of vaccine-induced serum IgG antibodies. It requires serum IgG antibody concentrations to be of sufficient affinity and abundance to result into "protective" antibody titers in saliva or mucosal secretions. As a rule, such responses are not elicited by PS bacterial vaccines but achieved by glycoconjugate vaccines, which therefore prevent nasopharyngeal colonization in addition to invasive diseases.

Under most circumstances, immunization does not elicit sufficiently high and sustained antibody titers on mucosal surfaces to prevent local infection. It is only after having infected mucosal surfaces that pathogens encounter vaccine-induced IgG serum antibodies that neutralize viruses, opsonize bacteria, activate the complement cascade and limit their multiplication and spread, preventing tissue damage and thus clinical disease. That vaccines fail to induce sterilizing immunity is thus not an obstacle to successful disease control, although it represents a significant challenge for the development of specific vaccines such as against HIV-1. Current vaccines mostly mediate protection through the induction of highly specific IgG serum antibodies. Under certain circumstances, however, passive antibody-mediated immunity is inefficient (tuberculosis).

16. What are the clinical scenarios where evidences of T cells protection available?

BCG is the only currently used human vaccine for which there is conclusive evidence that T cells are the main effectors. However, there is indirect evidence that vaccine-induced T cells contribute to the protection conferred by other vaccines. CD4+ T cells seem to support the persistence of protection against clinical pertussis in children primed in infancy, after vaccine-induced antibodies have waned. Another example is that of measles immunization in 6-month-old infants. These infants fail to raise antibody responses because of immune immaturity and/or the residual presence of inhibitory maternal antibodies, but generate significant IFN-g producing CD4+ T cells. These children remain susceptible to measles infection, but are protected against severe disease and death, presumably because of the viral clearance capacity of their vaccine-induced T cell effectors. Thus, prevention of infection may only be achieved by vaccine-induced antibodies, whereas disease attenuation and protection against complications may be supported by T cells even in the absence of specific antibodies. The understanding of vaccine immunology thus requires appraising how B and T cell responses are elicited, supported, maintained and/or reactivated by vaccine antigens.

17. What happens once a vaccine is administered to a vaccinee?

Following injection, the vaccine antigens attract local and systemic dendritic cells, monocytes and neutrophils. These activated cells change their surface receptors and migrate along lymphatic vessels, to the draining lymph nodes where the activation of T and B lymphocytes takes place. In case of killed vaccines there is only local and unilateral lymph node activation. Conversely for live vaccines there is multifocal lymph node vaccination due to microbial replication and dissemination. Consequently the immunogenicity of killed vaccines is lower than the live vaccines; killed vaccines require adjuvants which improve the immune response by producing local inflammation and recruiting dendritic cells/monocytes to the injection site. Secondly, the site of administration of killed vaccines is of importance; the intramuscular route which is well vascularised and has a large number of patrolling dendritic cells is preferred over the subcutaneous route. The site of administration is usually of little significance for live vaccines. Finally due to focal lymph node activation, multiple killed vaccines may be administered at different sites with little immunologic interference. Immunologic interference may occur with multiple live vaccines unless they are given on the same day/at least 4 weeks apart or at different sites.

18. What are the immune responses of T cell-independent antigens (i.e. polysaccharide vaccines) at the cellular level?

On being released from the injection site these antigens usually non-protein, polysaccharides in nature, reach the marginal zone of the spleen/nodes and bind to the specific Ig surface receptors of B cells. In the absence of antigen-specific T cell help, B cells are activated, proliferate and differentiate in plasma cells without undergoing affinity maturation in germinal centers. The antibody response sets in 2–4 weeks following immunization, is predominantly IgM with low titers of low affinity IgG. The half life of the plasma cells is short and antibody titers decline rapidly. Additionally the PS antigens are unable to evoke an immune response in those aged less than 2 years due to immaturity of the marginal zones. As PS antigens do not induce germinal centres, bona fide memory B cells are not elicited. Consequently, subsequent re-exposure to the same PS results in a repeat primary response that follows the same kinetics in previously vaccinated as in naïve individuals.

19. What is hyporesponsiveness of repeated doses of a vaccine referring to?

Revaccination with certain bacterial polysaccharides (PS), of which group C meningococcus is a prototype—may even induce lower antibody responses than the first immunization, a phenomenon referred to as hyporesponsiveness whose molecular and cellular bases are not yet fully understood.

20. What are the immune responses of T cell-dependent antigens at the cellular level?

T cell-dependent antigens include protein antigens which may consist of either pure proteins (Hepatitis B, Hepatitis A, HPV, Toxoids) or conjugated protein carrier with PS antigens (Hib, meningo, pneumo). The initial response to these antigens is similar to PS antigens. However the antigen-specific helper T cells that have been activated by antigen-bearing dendritic cells trigger some antigen-specific B cells to migrate towards follicular dendritic cells (FDC's), initiating the germinal center (GC) reaction. In GC's, B cells receive additional signals from follicular T cells and undergo massive clonal proliferation, switch from IgM towards IgG/IgA, undergo affinity maturation and differentiate into plasma cells secreting large amounts of antigen-specific antibodies. Most of the plasma cells die at the end of germinal centre reaction and thus decline in antibody levels is noted 4-8 weeks after vaccination. However a few plasma cells exit nodes/spleen and migrate to survival niches mostly located in the bone marrow, where they survive through signals provided by supporting stromal cells and this results in prolonged persistence of antibodies in the serum.

21. What are 'memory B cells'?

Memory B cells are those B-lymphocytes that generate in response to T-dependent antigens, during the GC reaction, in parallel to plasma cells. They persist there as resting cells until re exposed to their specific antigens when they readily proliferate and differentiate into plasma cells secreting large amounts of high-affinity antibodies that may be detected in the serum within a few days after boosting.

22. What are the characteristics of immune response to live vaccines?

The live vaccines induce an immune response similar to that seen with protein vaccines. However, the take of live vaccines is not 100% with the first dose. Hence more than 1 dose is recommended with most live vaccines. Once the vaccine has been taken up, immunity is robust and lifelong or at least for several decades. This is because of continuous replication of the organism that is a constant source of the antigen. The second dose of the vaccine is therefore mostly for primary vaccine failures (no uptake of vaccine) and not for secondary vaccine failures (decline in antibodies over time).

23. What determines the intensity and duration of immune responses?

The nature of antigen is the primary determinant; broadly speaking live vaccines are superior (exception BCG, OPV) to protein antigens which in turn are superior to polysaccharide vaccines). Adjuvants improve immune responses to inactivated vaccines. Immune response is usually better with higher antigen dose (e.g. Hepatitis B). The immune response improves with increasing number of doses and increased spacing between doses. Technically, 0, 1 and 6 months is the best immunization schedule; The first two doses are for induction and the long gap between the 2nd and 3rd dose allows for affinity maturation of B cells and clonal selection of the fittest B cells for booster and memory response. Extremes of age and disease conditions lower immune response.

24. What are the limitations of young age immunization?

Young age limits antibody responses to most vaccine antigens since maternal antibodies inhibits antibodies responses but not T cell response, and due to limitation of B cell responses.

IgG antibodies are actively transferred through the placenta, via the FcRn receptor, from the maternal to the fetal circulation. Upon immunization, maternal antibodies bind to their specific epitopes at the antigen surface, competing with infant B cells and thus limiting B cell activation, proliferation and differentiation. The inhibitory influence of maternal antibodies on infant B cell responses affects all vaccine types, although its influence is more marked for live attenuated viral vaccines that may be neutralized by even minute amounts of passive antibodies. Hence, Antibody responses elicited in early life are short lasting. However, even during early life, induction of B memory cells is not limited.

Early life immune responses are characterized by age-dependent limitations of the magnitude of responses to all vaccines. Antibody responses to most PS antigens are not elicited during the first 2 years of life, which is likely to reflect numerous factors including: the slow maturation of the spleen marginal zone; limited expression of CD21 on B cells; and limited availability of the complement factors. Although this may be circumvented in part by the use of glycol-conjugate vaccines, even the most potent glycoconjugate vaccines elicit markedly lower primary IgG responses in young infants.

Although maternal antibodies interfere with the induction of infant antibody responses, they may allow a certain degree of priming, i.e. of induction of memory B cells. This likely reflects the fact that limited amounts of unmasked vaccine antigens may be sufficient for priming of memory B cells but not for full-blown GC activation, although direct evidence is lacking. Importantly, however, antibodies of maternal origin do not exert their inhibitory influence on infant T cell responses, which remain largely unaffected or even enhanced.

The extent and duration of the inhibitory influence of maternal antibodies increase with gestational age, e.g. with the amount of transferred immunoglobulins, and declines with post-natal age, as maternal antibodies wane.

25. Maternal antibodies interfere with neonatal immune responses, why Hepatitis B, BCG, and OPV are recommended at birth?

The first dose of Hepatitis B which is administered at birth acts as 'priming dose' while subsequent doses provide an immune response even in presence of maternal antibodies. As mentioned above, maternal antibodies do not interfere with induction of memory B cells, certain degree of priming is allowed. However, hepatitis B vaccine induces lower primary IFN-g responses and higher secondary Th2 responses in early life than adults. Similarly, antibodies of maternal origin do not exert their inhibitory influence on infant T cell responses. Since, BCG mainly works by inducing T cell immune response hence it can be given in the presence of maternal antibodies which may even enhance T cell responses. OPV is given at birth since there are no maternal IgA in the gut to neutralize the virus. However, IFN-g responses to oral polio vaccine are significantly lower in infants than in adults.

26. How maternal antibodies can sometimes enhance T cell responses of BCG vaccine administered at birth?

After administration of BCG, the maternal antibodies form immune complexes with the vaccine antigens. These immune complexes are taken up by more and more number of macrophages and dendritic cells, which in turn are dissociated into their acidic phagolysosome compartment and are processed into small peptides. These peptides are displayed at the surface of antigen-presenting cells, thus available for binding by more number of CD4+ and CD8+ T cells.

27. Considering the numerous limitations of young age immunization, why still vaccines are administered at much younger age in developing countries than in developed world?

This can be explained on the basis of disease epidemiology of vaccine-preventable diseases (VPDs). Since, majority of childhood infectious diseases cause early morbidity and mortality in poor, developing countries, hence the need to protect the children before wild organisms infect them. This is the reason why early, accelerated schedules are practiced in developing countries. According to W.H.O estimates, 2.5 to 3 million infants are born healthy but succumb to acute infections between the age of 1 and 12 months. These early deaths are caused by a limited number of pathogens, such that the availability of a few additional vaccines that would be immunogenic soon after birth would make a huge difference on this disease burden.

28. How limitations of young age immunization can be taken care of?

They can be countered by increasing the number vaccine doses for better induction, use of adjuvants to improve immunogenicity of vaccines, and by use of boosters at later age when immune system has shown more maturity than at the time of induction. Increasing the dose of vaccine antigen may also be sufficient to circumvent the inhibitory influence of maternal antibodies, as illustrated for hepatitis A or measles vaccines.

29. Which is the best vaccination schedule for non-live vaccines acting on the principal of 'prime-boost' mechanism?

Traditionally, 0-1-6 month schedule is considered as a most immunogenic schedule than 6-10-14 weeks or 2-3-5 months schedules for non-live T cell-dependent vaccines like Hepatitis-B vaccine. This is mainly due to proper spacing of the vaccine doses and adequate time interval between first few doses which act by inducing immune responses and last dose that works as boosters. Since, affinity maturation of B cells in GCs and formation of memory-B cells take at least 4-6 months, this schedule quite well fulfills these requirements. More than one dose is needed for better induction and recruitment of more number of GCs in young age considering young age limitations of immune system (Figure 1.4).

Immunization schedules commencing at 2 months and having 2 months spacing between the doses are technically superior to that at 6, 10 and 14 wks. However for operational reasons and for early completion of immunization and attainment of protection the 6, 10, 14 week's schedule is chosen in developing countries.

Accelerated infant vaccine schedules in which 3 vaccine doses are given at a 1 month interval (2, 3, 4 or 3, 4, 5 months) result into lower responses than

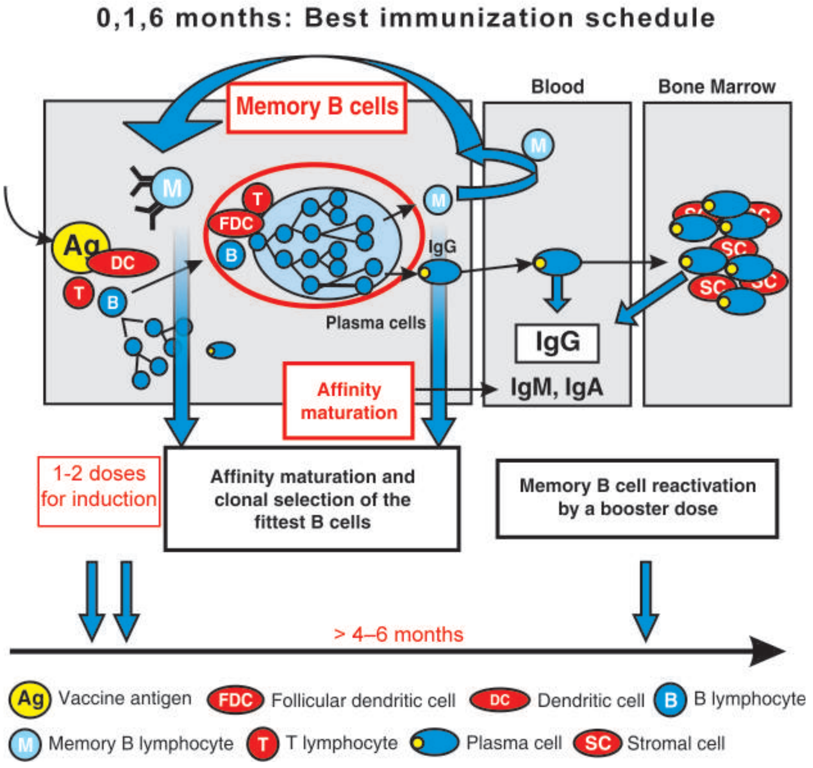

FIGURE 1.4 Schematic presentation of various components of 0, 1, 6 months immunization schedule at cellular level

schedules in which more time elapses between doses (2, 4, 6 months), or between the priming and boosting dose (3, 5, 12 months). However, the magnitude of infant antibody responses to multiple dose schedules reflects both the time interval between doses, with longer intervals eliciting stronger responses, and the age at which the last vaccine dose is administered.

30. What is a primary and secondary immune response?

When an antigen is introduced for the first time, the immune system responds primarily after a lag phase of up to 10 days. This is called the primary response. Subsequently, upon reintroduction of the same antigen, there is no lag phase and the immune system responds by producing antibodies immediately and this is called the secondary response. However, there are some differences in both these responses—primary response is short-lived, has a lag phase, predominantly IgM type, and antibodies titers are low, whereas secondary response is almost immediate without a lag phase, titers persist for a long time, predominantly of IgG type, and antibodies titers are very high. Figure 1.5 describes the background developments at the cellular

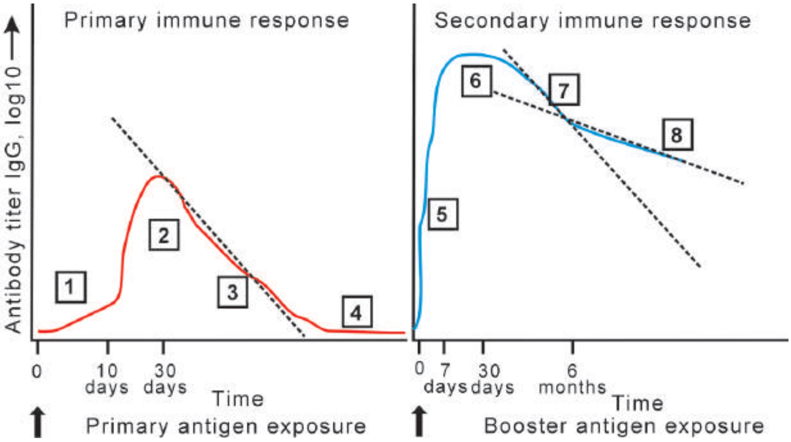

FIGURE 1.5 Correlation of antibody titers to various phases of the vaccine response

The initial antigen exposure elicits an extrafollicular response (1) that results in the rapid appearance of low IgG antibody titers. As B cells proliferate in germinal centers and differentiate into plasma cells, IgG antibody titers increase up to a peak value (2) usually reached 4 weeks after immunization. The short lifespan of these plasma cells results in a rapid decline of antibody titers (3) which eventually return to baseline levels (4) In secondary immune responses, booster exposure to antigen reactivates immune memory and results in a rapid (<7 days) increase (5) of IgG antibody titer. Short-lived plasma cells maintain antibody (6) during a few weeks—after which serum antibody titers decline initially with the same rapid kinetics as following primary immunization. Long-lived plasma cells that have reached survival niches in the bone marrow continue to produce antigen-specific antibodies, which then decline with slower kinetics

Note: This generic pattern may not apply to live vaccines triggering long-term igG antibodies for extended periods of time. (*With permission from* Siegrist ca. Vaccine immunology. In Vaccines. In: Plotkin sa, Orenstein w, Offit P (Eds). Saunders Elsevier, 5th edition, 2008.pp.17-36) level and interactions of B cells, memory B cells and T cells at the follicular level in a lymphnode. The secondary response is mainly due to booster response and is seen with vaccines that work on a 'prime-boost' mechanism inducing T cells such as conjugate vaccines. On the other hand, non-conjugate, polysaccharide vaccines mainly induces primary response and the repeat dose produces another wave of primary response and not acts as a booster since they do not induce T cells.

31. What are the hallmarks of 'memory B cell' responses?

These cells are only generated during T-dependent responses inducing germinal centers (GC) responses. These cells are resting cells that do not produce antibodies. Memory B cells undergo affinity maturation during several (4-6) months. A minimal interval of 4-6 months is required for optimal affinity maturation of memory B cells. Memory B cells rapidly (days) differentiate into antibody-secreting plasma cells upon re-exposure to antigen. Memory B cells differentiate into PCs that produce high(er) affinity antibodies than primary plasma cells. As plasma cells and memory responses are generated in parallel in GCs, higher post-primary Ab titres reflect stronger GC reactions and generally predict higher secondary responses. During induction, a lower antigen dose at priming results in inducing B cells differentiation away from PCs, towards memory B cells. This phenomenon can be exploited by using small amount of expensive conjugate vaccines such as PCV (pneumococcal conjugate vaccine) followed by use of less expensive PPV(pneumococcal polysaccharide vaccine) as booster. Exposure to exogenous antigens may reactivate or favour the persistence of memory B cells.

32. What are the implications of 'immune memory' for immunization programs?

Immune memory is seen with live vaccines/protein antigens due to generation of memory B cells which are activated on repeat vaccination/natural exposure. Immune memory allows one to complete an interrupted vaccine schedule without restarting the schedule. Hence, immunization schedule should never be started all over again regardless of duration of interruption. Regular boosters are not required to maintain immune memory during low risk periods (travellers). Certain immunization schedules may not need boosters if exposure provides regular natural boosters. Activation of immune memory and generation of protective antibodies usually takes 4-7 days. Diseases which have incubation periods shorter than this period such as Hib, tetanus, diphtheria and pertussis require regular boosters to maintain protective antibody levels. However diseases such as hepatitis A, hepatitis B do not need regular boosters as the long incubation period of the disease allows for activation of immune memory cells. This is to be noted that memory B cells do not produce antibodies unless re-exposed to antigen which drives their differentiation in to antibody producing plasma cells.

33. Why is number of doses for each vaccine different?

Live attenuated vaccines replicate (in case of viruses) or multiply (in case of bacteria) in the body thus the number of vaccine particles increases many folds which are capable of generating antibodies in large quantity to reach seroprotective levels. Due to some reasons not fully understood multiple doses of OPV are needed. On the other hand inactivated vaccines do not multiply in the body, and quantity of vaccine (antigens) required to provide full protection is large, fever and local reaction like swelling, tenderness and pain may be very severe if the required quantity of vaccine is administered at a time, so the quantity of vaccine is generally divided in two or more doses. DPT is divided in three doses while rabies vaccine is divided in four or five doses.

34. Why do we need booster (booster doses)?

The body starts antibody generation after administration of vaccines, which reach a peak after a period of time which is different for different vaccines. As already stated multiple doses of some vaccines have to be administered to attain optimal level of immunity. Over a period of time, which also varies for different vaccines, antibody level declines and revaccination or booster dose(s) is/are required to raise the antibody levels above the required protective levels.

In most cases sub clinical infection acts as a booster dose. As the percentage of vaccinated and immune population increases, circulation of the causative organisms declines in the community. This decline in circulation of organisms lessens the chances of non-immune individuals in coming in contact with organisms (which is a beneficial for non immune people), but those immune following vaccination may be deprived of the benefit of repeated sub clinical exposure leading to boosting effect. This is the reason that booster dose for varicella vaccine has been introduced in those countries where vaccine coverage is very high.

35. Why we need to give only one dose of a particular vaccine while multiple doses are needed for another vaccine?

In general live vaccines generate antibodies to protective levels after administration while antigens need multiple doses, because the quantity required to generate antibodies to protective levels in very large, so multiple doses are required.

It has been observed that natural infection with viral diseases provides very long or life long protection while infections by bacteria do not provide any long lasting protection. Typhoid disease, skin infections and other infections caused by bacteria can recur again and again while second attack of measles or chickenpox occurs rarely if at all. Similarly antibodies produced by antiviral vaccines persist for much longer period as compared to antibodies produced by antibacterial vaccines.

36. Why cannot we have an 'all-in-one vaccine'?

There are very strong scientific and logistic reasons against 'all-in-one vaccine'. Scientific reasons are: (i) different ideal ages for different vaccines e.g. OPV, BCG and hepatitis B vaccines can be administered soon after birth, other vaccines can not be administered at this age, (ii) different routes of administration eg. some are administered orally, others are administered parentally, some intradermally (BCG), some subcutaneously (measles, MMR, varicella vaccines) and other vaccines are administered intramuscularly. Logistic reasons are: (i) some vaccines need to be administered as a single dose (BCG and varicella vaccines) some need two doses (like measles, MMR, hepatitis A, rotavirus vaccine), some vaccines need three doses (hepatitis B, and human papilloma virus vaccines) while other vaccines have to be administered at different intervals, and (ii) quantity of such an 'all-in-one vaccine' would be too large. Certainly the idea appears to be very attractive, but does not appear feasible in foreseeable future.

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CHAPTER

2

Elementary Epidemiology in Vaccination

Vipin M Vashishtha, Yash Paul

1. What is epidemiology?

The study (observation, measurement, analysis, correlation, interpretation) of distribution (how many? in whom? (age group) where? when? Season and determinants (why there, why then?) of diseases (etiology and risk factors) is termed as epidemiology. Epidemiology is the study of the distribution and determinants of disease frequency in man. It is foundation science of public health. It provided insights for applying intervention. It informs if intervention is succeeding. It is the systematic study of the pathogen amplification and transmission systems. Epidemiology can often pin-point the weak links in the chains of the source and transmission pathways of the pathogen so that interventions can be directed at those points. Vaccination is one such intervention.

2. Why is it important to learn epidemiology?

From vaccinology perspective, there are three reasons to learn epidemiology. They are for the rational choice of vaccines for vaccination programmes; to design appropriate intervention programme including vaccinations; and to monitor and measure the progress and impact of any vaccination programme. Knowledge of epidemiology helps in choosing the appropriate vaccines for inclusion in public health programs after carefully assessing disease burden and economic factors. It also helps in designing disease specific control/elimination/eradication strategies after acquiring exact epidemiological data on prevalence, incidence, and transmission characteristics of target pathogens, and their transmission pathways. In the last, it also helps in monitoring intervention success/failure in order to improve performance/efficiency of the vaccination programs.

3. What are the basic measures of disease frequency? What do the terms 'incidence' and 'prevalence' refer to?

Basic measures of disease frequency is done by incidence and prevalence; Incidence relates to the number of new cases of the disease which occur during a particular period of time(e.g. new TB cases). Prevalence relates to total number of cases of a disease in a specified period of time usually during a survey. Often it is expressed as rate which is misnomer and it is actually proportion. In the long run, incidence should be more than the deaths and recoveries for prevalence to accumulate; Prevalence of various disease is a good indicator of the load on health services.

4. What is disease estimation? What are its merits and demerits?

Where measuring of exact incidence and/or prevalence of a disease is not practical, estimates are developed to have a rough idea about the burden of that particular disease in the community at a given geographic region. Hence, where incidence/prevalence is not 'measured' estimation is better than nothing. Estimates are for comparative purpose–intercountry and inter-disease. Comparison is for choice also for example, intervention vs none; or vaccination vs other. An estimate is by definition inaccurate, but maybe valid/invalid; reliable/unreliable. It is usually expressed in round figures, for example, 200,000 rabies; 2 million malaria; 3 million HIV infected; 40 million hepatitis B carriers, etc. No estimate is accurate for the actual burden- therefore arguments against hepatitis B vaccination program for the reason the estimate is inaccurate is an obvious misjudgment. For global leadership in specific diseases gross estimates are used. This is such an example for TB (Figure 2.1).

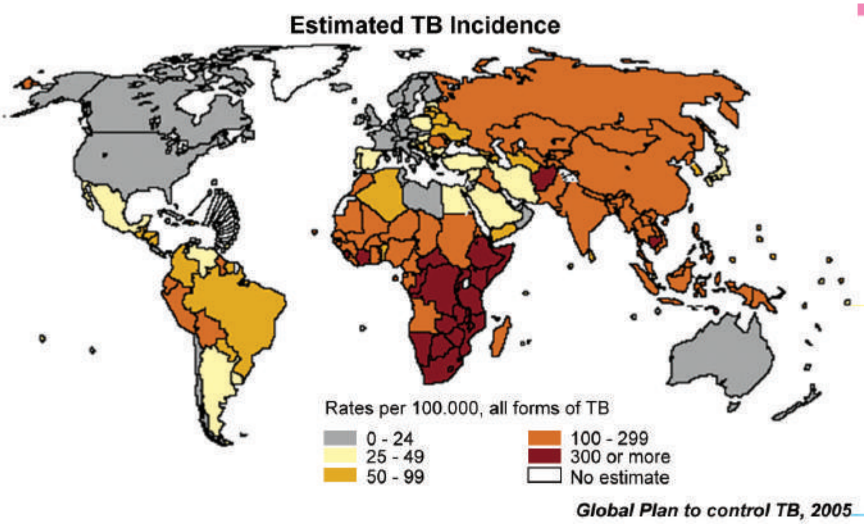

FIGURE 2.1 Global estimate of tuberculosis burden (estimate of TB incidence)
(*Source:* Manual of IAP Advancing Science of Vaccinology, 2009)

5. What are the differences between endemic, epidemic and pandemic patterns of diseases?

Endemic refers to normal occurrence of disease in defined population e.g. cholera, malaria, TB, etc. Epidemics/outbreaks is the occurrence of more cases of disease than expected in a given area or among a specific group of people over a particular period of time e.g. measles, influenza, meningococcal disease. Epidemic/outbreaks: Spreading rapidly and extensively by infection and affecting many individuals in an area or a population at the same time. The difference between epidemic and outbreak is arbitrary. The terms epidemic and outbreaks are often used similarly; however, former usually indicates less intensity, for example outbreak of salmonella in a neonatal unit. A community-based outbreak meningococcal disease is defined as the occurrence of >3 cases in <3 months in the same area who are not close contacts of each other with a primary disease attack rate of >10 primary cases/100,000 persons.

In terms of the flu, the difference between an epidemic an outbreak and is the percentage of overall deaths caused by the disease. Every week, if the number of flu caused deaths exceeds 7.7 percent of the total, then the USA officially has an epidemic on its hands (CDC). Currently, it is 7.2, so there's no epidemic yet. The 7.7 percent figure isn't static from year to year. During the flu epidemic of 1990, for example, the CDC's threshold was 6.7 percent of total deaths.

Pandemic is global epidemic. Disease originates in one country and then spreads to a number of countries e.g. AIDS, H1N1, etc.

6. What are the terms like vaccine immunogenicity, vaccine efficacy, and vaccine effectiveness refer to?

Vaccine immunogenicity is the ability of a vaccine to induce antibodies which may or may not be protective. These antibodies may be of no use in offering protection against the desired disease. The protective threshold for most vaccines is defined. However there is often controversy about the cutoffs (pneumococcus/Hib). Levels below the limits may be protective due to other reasons such as immune memory/T cell immunity.

Vaccine efficacy is the ability of the vaccine to protect an individual. It can be assessed through clinical trials, cohort studies or case control studies. It is calculated as:

$$\text{VE} = \frac{\text{Disease in unvaccinated} - \text{Disease in vaccinated}}{\text{Disease in unvaccinated}}$$

Vaccine effectiveness is the ability of the vaccine to protect the community and is a sum of the vaccine efficacy and herd effect. It is revealed after a vaccine is introduced in a program. Vaccine effectiveness is a combination of vaccine efficacy, coverage and herd effect. Hence, vaccine efficacy is the protection at the individual level while effectiveness is at the community level. Higher the force of transmission, younger the age at risk greater the need for vaccine effectiveness to reduce $R = 1$ to $R < 1$.

7. What do you mean by cost effectiveness of a vaccine or vaccination program?

Cost effectiveness is a method of economic evaluation which is carried out by mathematical modeling usually prior to introduction of a vaccine in a national program. It is expressed as cost per infections/deaths/hospitalizations prevented/life years gained.

8. What do the terms force of transmission and reproductive rate demote to? What is the significance of 'basic reproductive number'(R_o)?

The key determinant of incidence and prevalence of infection is depends on force of transmission which is determined by Reproductive rate. Reproductive rate is a simple concept in disease epidemiology. Incidence and prevalence of infection depends on reproductive rate. R_o measures the average number of secondary cases generated by one primary case in a susceptible population. Suppose all others were susceptible–then how many will be infected? That is basic reproductive number, R_o. Since population is a mix of susceptible and immune persons, one case must attempt to infect more than one person.

In the long term, pathogen can survive only if one case reproduces another case (effective reproductive rate, $R = 1$). If $R<1$ the disease is declining (e.g. herd effect). If $R>1$ an outbreak is occurring. For endemic diseases with periodic fluctuations, R may swing from <1 to >1 but in the long term the average may remain 1. Pathogen can survive if it reproduces. For all endemic infectious diseases (IDs), $R = 1$ for steady state or for long term endemicity. The community benefit of a vaccination program is to reduce R to <1 and sustain it for long periods. Such beneficial effect, measured as the degree of disease reduction due to a vaccination programme is sometimes called vaccine effectiveness to distinguish it from vaccine efficacy which refers to only the direct benefit of immunity in vaccinated individuals.

R_o is not a static entity and changes according at different time period even at a same geographic region.

9. How can one calculate the magnitude of 'basic reproductive number'(R_o)?

A series of simple relationships exist between key epidemiological, demographic and vaccination program related parameters. The magnitude of R_o and the average at infection prior to mass vaccination, A, plus life expectancy in the population are related as follows:

$$R \approx (L\ A)/(A\ M)$$

Here, 'M' is the average duration of maternal antibody protection (6 months) and 'L' is life expectancy.

10. How does vaccination impact natural epidemiology of an infectious disease?

Vaccination perturbs epidemiology by removing the vaccinated individual from susceptible pool: so, one less disease case, and by removing individuals from chain of transmission: so, lower incidence/prevalence of the disease. In other words, vaccination program interferes with natural epidemiology of the target disease.

11. What is epidemiological shift?

Epidemiologic shift refers to an upward shift in age of infection/disease in communities with partial immunization coverage. Owing to vaccination the natural circulation of the pathogen decreases and the age of acquisition of infection advances. This is especially important for diseases like rubella, varicella and hepatitis A wherein severity of disease worsens with advancing age.

12. What is herd immunity?

The term 'herd immunity' has been in use since 1920s i.e. for about 90 years. In fact it denotes resistance of a population to the spread of vaccine preventable diseases where causative organisms spread from human to human. Because immune individuals act as barrier in spread of infection and lessen the chances of an individual with the disease to come in contact of vulnerable person. Instead of the terms 'herd immunity' or 'herd effect' terms contact immunity and herd protection are being used now.

Herd immunity is the proportion immune in a herd. This can be deduced from the vaccination coverage. Herd effect is the protection offered to unvaccinated members when good proportion (usually more than 85%) of the herd is vaccinated. Herd effect is due to reduced carriage of the causative microorganism by the vaccinated cohort and thus is seen only with vaccines against those diseases where humans are the only source (there is no herd effect for tetanus). An effective vaccine is a prerequisite for good herd effect; OPV in India, BCG and unconjugated polysaccharide vaccines have no herd effect.

13. What is contact immunity?

Following administration live vaccine particles multiply in the body, induce immunity by generating antibodies and are excreted in body fluids like nasal secretions or passed out in feces. In case sufficient number of these live vaccine particles reach unvaccinated close contact may induce immunity in this person. As this person has developed immunity without taking the vaccine, benefit has occurred because of secondary spread so this is called contact immunity.

14. Which vaccines can cause contact immunity?

Any live attenuated vaccine may reach close contact through droplet infection, in case vaccine is administered as a nasal spray, ruptured pustule may result in spread of live vaccines as may happen in case of varicella vaccine, or the vaccine viruses shed in feces may reach close contact through feco-oral route. Thus, theoretically any live vaccine can induce contact immunity.

15. How commonly does this contact immunity occur?

Very rarely. There are only three documented cases where close contacts have developed immunity because of varicella vaccine.

16. How commonly does this contact immunity occur in case of OPV, because vaccine recipients continue to shed live polio vaccine viruses for up to a period of six weeks?

It is correct that polio vaccine viruses contained in OPV replicate in the gut are shed in feces and may infect the close contacts, and it was assumed that these vaccine viruses will provide protection. Now it is known that this secondary spread of polio vaccine viruses does not induce immunity because of two reasons: (i) attenuated polioviruses contained in OPV have markedly reduced infectivity, and (ii) low load of vaccine viruses spread through feces. There are about 1,000,000 type 1 polioviruses, about 100,000 type 2 polioviruses and about 600,000 type 3 polioviruses in each dose of two drops of OPV i.e. each dose of OPV contains about 1,700,000 polioviruses. On the other hand, one gram of fecal matter of vaccine recipient contains about 100 vaccine polioviruses. Thus, 17 kg of fecal matter may provide same quantity of vaccine polioviruses as are contained in one dose of OPV. How much antibodies would be generated by few thousand vaccine polioviruses spread through feces while many doses of OPV, each dose containing about 17 lakh vaccine polioviruses have failed to generate protective immunity in many children? This secondary spread of vaccine viruses may enhance the already existing antibodies.

17. What is herd protection?

Immunized people provide protection to the unimmunized individuals without inducing immunity, virtually by breaking the transmission of the infection or lessening the chances of susceptible individuals coming in contact with infective individual.

In clinical practice, contact immunity does not play significant role, while herd protection plays a major role, though to a limited extent, because unimmunized individuals do not develop immunity, but enjoy the protection because of break in spread of infection. Thus, the herd protection is the major beneficial component of immunization for unimmunized population for the infections which spread from person to person. It should be remembered that these unimmunized individuals enjoy protection till they are among immunized and resistant people. As they have not developed immunity, may develop disease if come in direct contact with infected person, in case they shift to the milieu where there is outbreak of the disease.

18. How effective is herd protection in real life?

It is not only a relevant question, but is very important to understand the phenomenon of herd protection. It is generally stated that when vaccine coverage is high, say 80% or more in the community, unvaccinated persons get benefit of herd protection. In certain situation such benefit may occur, in other situations this benefit may not be provided. If susceptible person does not come in direct contact of infected person because of presence of immune population, such person will escape infection, so higher the percentage of immune population, lesser are the chances of vulnerable person getting infection. In case a vulnerable individual comes in contact of infective person, such person may develop infection despite presence of very high percentage of immune population. Following example is cited for better understanding of the phenomenon.

One hundred persons go to a restaurant where all are served food which had been accidentally contaminated with *Salmonella typhi*. Ninety nine person in this group had received typhoid vaccine recently and have developed antibodies and thus resistance against typhoid disease, so, would not develop active disease despite being infected by *Salmonella typhi* from the infected food. But, one individual who had not received the typhoid vaccine may develop typhoid disease from this food. Thus, in this case even 99% vaccine coverage may fail to protect this single unvaccinated individual.

Can BCG vaccine administered to 90% persons of a large family from tubercular endemic area provide effective protection to the 10% unvaccinated persons, if all are exposed to some infective persons? Should we advise 90% vaccine coverage with diphtheria vaccine in a family and expect 10% unvaccinated will be protected against diphtheria disease? The answer for both situations is no. In other words, there is no 'free ride' even in case of the vaccines which prevent diseases where the causative organism spreads from person to person.

Thus, this so-called additional benefit to the community or presumptive public good does not provide any real benefit to an individual, except that it reduces the circulation of causative organisms in the community. As the proportion of vaccinated population increases, proportion of unvaccinated (susceptible) population decreases and their chances of coming in contact with infective individual reduce.

19. Do all vaccines provide some benefit to the community in form of contact immunity or herd protection?

The so called 'common good' may be provided by the vaccines which prevent infections where causative organism spreads from person to person, because some vaccines may provide contact immunity (by inducing immunity or by enhancing the existing immunity) as well as herd protection, while some vaccines may provide herd protection by lessening the chances of susceptible individual in coming in contact with infective persons. In case of vaccine preventable diseases where causative organism does not spread from person to person as happens in case of rabies and tetanus, immune people do not provide any benefit to un-immunized people in the community.

Depending upon whether the additional benefit(s) being provided to the community or not, the vaccines may be placed in three groups:

- Vaccines which can provide contact immunity and herd protection - oral polio vaccine (OPV) varicella vaccine, any live vaccine (theoretically possible).
- Vaccines which can provide herd protection only—inactivate polio vaccine (IPV), diphtheria, pertussis, measles, mumps, rubella, pneumococcal, H-influenzae (Hib), rotavirus, meningococcal, hepatitis A, typhoid and BCG.
- Vaccines which do not provide any additional benefit to the un-immunized persons—tetanus, rabies, Japanese encephalitis, hepatitis B, yellow fever.

20. How can the degree of herd immunity and the magnitude of R_o be assessed?

Various methods are employed to measure these parameters. Few include the following:

- Cross-sectional and longitudinal serological surveys
- Serum and saliva (viral infections)
- Activated T cells (bacteria and protozoa)
- Quantitative assays.

Scientific methods in the study of herd immunity include immunological and disease surveillance methods (provide the empirical base for analysis and interpretation), mathematical and statistical methods (play an important role in the analysis of infectious disease transmission and control). They help to define both what needs to be measured, and how best to measure define epidemiological quantities.

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CHAPTER

3

Vaccination Schedules

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1. What is the purpose of immunization?

Immunization alters the host's susceptibility to disease, while all other risk factors remain same, it protects the individual from disease in spite of exposure or even infection. This is for individual benefit. If many are immune, the community may have benefit above and beyond the total number of immune persons. If a large proportion is immune, that may affect the epidemiology of that particular disease. For control of disease burden or eradication of disease we need to immunize a large number in a population or all individual in a population.

2. What should be the ideal immunization schedule?

Ideal immunization schedule should be epidemiologicaly relevant, immunologicaly competent, technologicaly feasible, socially acceptable, affordable and sustainable. It wil vary from country to country and from time to time. In order to choose vaccines for vaccination program at government funding, not only incidence/prevalence/disease burden but their implication should be known. For government programs, usually it is cost first, efficacy next, and safety last. For an individual it is safety first, efficacy next, cost last. Though what is not in the best interests of the individual cannot be in the best interests of the community and what is in the best interests of the community is also in the best interests of the individual.

3. What are the determinants of optimal immunization schedules?

They can be summarized in three heads:

- **Immunological**
    - Minimum age at which vaccine elicit immune response
    - Number of doses required
    - Interval between doses, if multiple doses are required
- **Epidemiological**
    - Susceptibility for infection and disease
    - Disease severity and mortality

- **Programmatic**
  - Opportunity to deliver with other scheduled intervention
  - Increase coverage by limiting the required contacts

Balance between immunological and epidemiological determinants is mandatory.

One should aim for achieving protective immune response prior to the age when children are most vulnerable. There should be balance between inducing reasonable protection prior to vulnerable age versus inducing optimal immune response. For example, starting late, might induce a higher response, but miss the vulnerable age. Wider intervals between doses give a better response, but delays induction of immunity, leaving children vulnerable in a crucial period of life. Further, the disease epidemiology varies in different populations. A schedule that is used in one population may not be the best for another; need to individualize and tailor to suit local needs.

4. What are the determinants for requirement of doses of different vaccines?

Number of doses required vary by vaccine: Live vaccines induce immunity with a single dose; inactivated vaccines require multiple doses (initial doses to prime and later doses to boost).

- Some live vaccines induce immunity in small proportion of vaccinees, requiring multiple doses to induce good immunity, e.g. OPV.
- Number of doses required may also vary by age: More doses of conjugate vaccines required in young infants.
- In general, a larger interval between doses induces a higher level of antibody (though not provide better immunity).
- Duration of immunity and requirement for additional doses is needed either to boost or to reinduce immunity (for T-cell independent antigens). But one need to differentiate between decay in antibody level and immunity.
- Designing vaccination schedules is indeed a trade off which can be trade-off can be on efficacy, safety or cost.
- First aim of vaccination program is to prevent serious disease (in absolute numbers, severity or both). Second aim is to reduce spread of infection.

5. What are the vaccination schedules in different developing countries?

Vaccination schedules in developing countries:

- 6, 10, 14 weeks, e.g. India, Kenya, Madagascar, Mozambique, Philippines, Rwanda, South Africa
- 2, 4, 6 months, e.g. Egypt, Chile, Mexico, Thailand, Uruguay, Argentina, Brazil
- 2, 3, 4 months, e.g. Gambia, Indonesia, Turkey, Vietnam
- 2, 3, 5 months, e.g. Malaysia
- 3, 4, 5 months, e.g. China.

6. What is the National Immunization Schedule?

Present National Immunization Schedule as proposed by IAP is given in Tables 3.1 and 3.2.

TABLE 3.1 Vaccination schedule under UIP in India, 2013–14

Vaccine	*When to give*	*Dose*	*Route*	*Site*
For pregnant women				
TT-1	Early in pregnancy	0.5 mL	Intra-muscular	Upper arm
TT-2	4 weeks after TT-1*	0.5 mL	Intra-muscular	Upper arm
TT- booster	If received 2 TT doses in a pregnancy within the last 3 years	0.5 mL	Intra-muscular	Upper arm
For infants				
BCG	At birth or as early as possible till one year of age	0.1 mL (0.05 mL until 1 month of age)	Intra-dermal	Left Upper arm
Hepatitis B birth dose	At birth or as early as possible within 24 hours	0.5 mL	Intra-muscular	Antero-lateral side of mid-thigh
OPV Zero dose	At birth or as early as possible within the first 15 days	2 drops	Oral	Oral
OPV 1, 2, and 3	At 6, 10, and 14 weeks	2 drops	Oral	Oral
DPT 1, 2, and 3		0.5 mL	Intra-muscular	Anterolateral side of mid-thigh
Hepatitis B 1, 2, and 3		0.5 mL	Intra-muscular	Anterolateral side of mid-thigh
HiB 1, 2, and 3		0.5 mL	Intra-muscular	Anterolateral side of mid-thigh
Measles 1st dose	9 completed months–12 months. (give up to 5 years if not received at 9–12 months age)	0.5 mL	Sub-cutaneous	Right upper arm
JE 1st dose**	9 completed months	0.5 mL	Sub-cutaneous	Left upper arm
For children and adolescents				
DPT booster	16–24 months	0.5 mL	Intra-muscular	Anterolateral side of mid-thigh
OPV booster	16–24 months	2 drops	Oral	Oral
Measles 2nd dose	16–24 Months	0.5 mL	Sub-cutaneous	Right upper arm

Contd.

Contd.

Vaccine	*When to give*	*Dose*	*Route*	*Site*
Rubella***	16–24 months Adolescent girls	0.5 mL	Sub-cutaneous	Right upper arm
JE 2^{nd} dose	16–24 months with DPT/OPV booster	0.5 mL	Sub-cutaneous	Left upper arm
DPT booster 2	5–7 years	0.5 mL	Intra-muscular	Upper arm
TT	10 years and 16 years	0.5 mL	Intra-muscular	Upper arm
Vitamin A****				

*Give TT-2 or Booster doses before 36 weeks of pregnancy. However, give these even if more than 36 weeks have passed. Give TT to a woman in labor, if she has not previously received TT.

**JE vaccine (SA 14-14-2) is given in select endemic districts, after the campaign is over in that district.

***Rubella vaccine will be given as part of measles 2^{nd} dose

****The 2^{nd} to 9^{th} doses of vitamin A can be administered to children 1–5 years old during biannual rounds, in collaboration with ICDS.

TABLE 3.2 IAP Immunization Timetable 2014

I. IAP recommended vaccines for routine use

Age (completed weeks/months/years)	*Vaccines*	*Comments*
Birth	BCG OPV 0 Hep-B 1	Administer these vaccines to all newborns before hospital discharge
6 weeks	DTwP 1 IPV 1 Hep-B 2 Hib 1 Rotavirus 1 PCV 1	**DTP:** • DTaP vaccine/combinations should preferably be avoided for the primary series • DTaP vaccine/combinations should be preferred in certain specific circumstances/conditions only • No need of repeating/giving additional doses of whole-cell pertussis (wP) vaccine to a child who has earlier completed their primary schedule with acellular pertussis (aP) vaccine-containing products **Polio:** • All doses of IPV may be replaced with OPV if administration of the former is unfeasible • Additional doses of OPV on all supplementary immunization activities (SIAs) • Two doses of IPV instead of 3 for primary series if started at 8 weeks, and 8 weeks interval between the doses • No child should leave the facility without polio immunization (IPV or OPV), if indicated by the schedule **Rotavirus:** • 2 doses of RV1 and 3 doses of RV5 • RV1 should be employed in 10 and 14 week schedule, instead of 6 and 10 week • 10 and 14 week schedule of RV1 is found to be far more immunogenic than existing 6 and 10 week schedule

Contd.

Contd.

Age (completed weeks/months/years)	*Vaccines*	*Comments*
10 weeks	DTwP 2 IPV 2 Hib 2 *Rotavirus 2 PCV 2	**Rotavirus:** • If RV1 is chosen, the first dose should be given at 10 weeks
14 weeks	DTwP 3 IPV 3 Hib 3 *Rotavirus 3 PCV 3	**Rotavirus:** • Only 2 doses of RV1 are recommended at present • If RV1 is chosen, the 2nd dose should be given at 14 weeks
6 months	OPV 1 Hep-B 3	**Hepatitis-B:** The final (third or fourth) dose in the HepB vaccine series should be administered no earlier than age 24 weeks and at least 16 weeks after the first dose
9 months	OPV 2 MMR-1	**MMR:** • Measles-containing vaccine ideally should not be administered before completing 270 days or 9 months of life • The 2nd dose must follow in 2nd year of life • No need to give stand-alone measles vaccine
9–12 months	Typhoid conjugate vaccine	• Currently, two typhoid conjugate vaccines, Typbar-TCV® and PedaTyph® available in Indian market • PedaTyph® is not yet approved; the recommendation is applicable to Typbar-TCV® only • An interval of at least 4 weeks with the MMR vaccine should be maintained while administering this vaccine • Should follow a booster at 2 years of age
12 months	Hep-A 1	**Hepatitis A:** • Single dose for live attenuated H2-strain Hep-A vaccine • Two doses for all killed Hep-A vaccines are recommended now
15 months	MMR 2 Varicella 1 PCV booster	**MMR:** • The 2nd dose must follow in 2nd year of life • However, it can be given at anytime 4 to 8 weeks after the 1st dose **Varicella:** The risk of breakthrough varicella is lower if given 15 months onwards
16–18 months	DTwP B1/ DTaP B1 IPV B1 Hib B1	The first booster (4th dose) may be administered as early as age 12 months, provided at least 6 months have elapsed since the third dose **DTP:** • First and second boosters should preferably be of DTwP • Considering a higher reactogenicity of DTwP, DTaP can be considered for the boosters

Contd.

Contd.

Age (completed weeks/months/years)	*Vaccines*	*Comments*
18 months	Hep-A 2	**Hepatitis A:** 2nd dose for killed vaccines; only single dose for live attenuated H2-strain vaccine
2 years	Typhoid booster	• Either Typbar-TCV® or Vi-polysaccharide (Vi-PS) can be employed as booster • Typhoid revaccination every 3 years, if Vi-polysaccharide vaccine is used • Need of revaccination following a booster of Typbar-TCV® not yet determined
4–6 years	DTwP B2/ DTaP B2 OPV 3 Varicella 2 Typhoid booster	**Varicella:** the 2nd dose can be given at anytime 3 months after the 1st dose
10–12 years	Tdap/Td HPV	**Tdap:** is preferred to Td followed by Td every 10 years **HPV:** • Only 2 doses of either of the two HPV vaccines for adolescent/preadolescent girls aged 9–14 years; • For girls 15 years and older, and immunocompromized individuals 3 doses are recommended • For 2 dose schedule, the minimum interval between doses should be 6 months • For 3 dose schedule, the doses can be administered at 0, 1–2 (depending on brands) and 6 months

II. IAP recommended vaccines for High-risk* children (Vaccines under special circumstances):

1. Influenza vaccine
2. Meningococcal vaccine
3. Japanese encephalitis vaccine
4. Cholera vaccine
5. Rabies vaccine
6. Yellow fever vaccine
7. Pneumococcal polysaccharide vaccine (PPSV 23)

** High-risk category of children:*

- Congenital or acquired immunodeficiency (including HIV infection)
- Chronic cardiac, pulmonary (including asthma if treated with prolonged high-dose oral corticosteroids), hematologic, renal (including nephrotic syndrome), liver disease and diabetes mellitus
- Children on long term steroids, salicylates, immunosuppressive or radiation therapy
- Diabetes mellitus, cerebrospinal fluid leak, cochlear implant, malignancies
- Children with functional/anatomic asplenia/hyposplenia
- During disease outbreaks
- Laboratory personnel and healthcare workers
- Travelers
- Children having pets in home
- Children perceived with higher threat of being bitten by dogs such as hostellers, risk of stray dog menace while going outdoor.

7. Why is National schedule different from IAP schedule?

Perhaps it would be wrong to label it a National Immunization Schedule, because a National schedule is applicable for the whole country to be followed by all. At best, it can be called "Government Immunization Schedule" because vaccines recommended under this schedule are provided free. But, due to limited resources, government cannot provide all the vaccines which are required. On the other hand, IAP recommends vaccines depending upon availability of vaccines and disease burden. Depending upon perceptions regarding some diseases and need to prevent these diseases and also cost factors, parents have to decide which vaccines they would like to or can afford to provide to their children. Because of some wrong perceptions there are many parents who feel that chickenpox should not be prevented, we need to educate them, but should not coerce them to get their child vaccinated.

8. What are the considerations in deciding the age of administration of vaccines?

This may vary from place to place and country to country. Optimal response to a vaccine depends on a number of factors:

- Nature of the vaccine—killed, live, polysaccharide
- Age and immune status of the recipient, i.e. ability of persons of a certain age to respond to the vaccine and potential interference with the immune response by passively transferred maternal antibody or previously administered antibody containing blood products.
- Age-specific risks for disease, age specific risks for complications.

Vaccines usually are recommended for members of the youngest age group at risk for experiencing the disease for which efficacy and safety have been demonstrated.

9. Why does vaccination schedule vary from country to country?

Vaccination schedules for different countries may be different primarily due to prevalence of different communicable diseases in different countries. In India, Yellow fever disease is not prevalent, so there is no need to administer vaccine against, yellow fever disease. This vaccine is recommended to those persons who have to travel to those countries where this disease occurs, e.g. subsaharan countries in Africa and Saudi Arabia during Haj pilgrimage. Similarly, typhoid disease does not occur in many developed countries because of good sanitation and clean drinking water, so typhoid vaccine is not administered in routine, but people are advised to take typhoid vaccine prior to traveling to a place where typhoid disease occurs. Even BCG vaccine is not administered in routine at birth in most of the developed countries.

Thus, vaccines are recommended to prevent the vaccine preventable diseases according to the disease burden in a population, so some vaccines may be discontinued while new vaccines may be introduced from time to time as epidemiology of diseases changes. Some vaccines are administered universally, e.g. vaccines against polio, diphtheria, tetanus, pertussis, measles and hepatitis B diseases.

10. If a Western/NRI child comes and settles in India, which schedule to follow—native or Indian?

Such a child should be provided vaccines according to the Indian schedule, because this child would be exposed to those infections which are prevalent in India. For example, a six-year-old child who has migrated from UK, or USA, where BCG vaccine is not administered as a routine vaccine, should be administered BCG vaccine on a priority basis, after a Mantoux test.

11. With so many vaccines available what schedule should one follow?

Different countries have their own recommended schedule according to epidemiology of the diseases and other logistic considerations. In India we have mainly two schedules: National Immunization Schedule, and the one suggested by IAP (see above).

National Immunization Schedule comprises of those vaccines which are given free of cost to all children of country under EPI (Expanded Program of Immunization).

The IAP schedule is based on the recommendations of the IAP Advisory Committee on Vaccines and Immunization Practices (ACVIP). The committee submits its position on vaccines not included in the national schedule on a periodic basis as they are introduced in the private market. The process of issuing recommendations involves an exhaustive review of published literature including standard textbooks, vaccine trials, recommendations of various countries, World Health Organization (WHO) position papers (see annexure 1), literature from the vaccine industry, postmarketing surveillance reports, cost-effective analysis, epidemiology of disease in India and if available Indian studies on vaccine efficacy, immunogenicity and safety. These recommendations of ACVIP are primarily for pediatricians in office practice.

12. What are the different vaccines category formed by IAP and how are the vaccines categorized?

There are now only two vaccine categories:

- Category 1: IAP recommended vaccines for routine use
- Category 2: IAP recommended vaccines for high-risk children (Vaccines under special circumstances)

Routine vaccines mean those vaccines recommended for all the children all over the country, universally (*See* Table 3.2).

TABLE 3.3 Catch-up immunization schedule for an unimmunized child

Visit	*Suggested schedule*
First	Measles (MMR if more than 12 months) DTwP1/DTaP1 (Tdap if 7 years or more) OPV1/IPV1 (only if less than 5 years) Hib1(only if less than 5 years) Hep B1
Second visit (after 1 month of first visit)	BCG (Only in less than 5 years) DTwP2/DTaP2 (Td if 7 years or more) OPV2 (if OPV given earlier) Hep B2 Hib2 (if less than 15 months)
Third visit (after 1 month of second visit)	OPV3/IPV2 MMR (if more than 12 months) Typhoid (if more than 2 years)
Fourth visit (6 months after first visit)	DTwP3/DTaP3 (Td if 7 years or more) OPV4/IPVB1 Hep B3

Abbreviations: MMR, measles-mumps-rubella (vaccine); DTwP/DTaP, diptheria-tetanus-pertussis (vaccine); OPV, oral polio-vaccine; IPV, inactivated polio virus vaccine, BCG, bacillus-Calmette-Guèrin; Hib, *Haemophilus influenza* type b; Hep, Hepatitis; Td, tetanus-diptheria.

13. How do we plan for a child who has not received any vaccination?

Depending on the age of the child at the first contact, we can plan to catch up with the immunization. An accelerated schedule may be planned if required (Table 3.3).

14. How about adolescent immunization?

This is an important period for "top up" immunization. Additionally some of the vaccines might not have been available when these adolescents were in their early childhood. The catch up immunization may be done up to 18 years age (For details, please *see* Chapter 8).

SUGGESTED READING

1. Vashishtha VM, Kalra A, Bose A, et al. Indian Academy of Pediatrics (IAP) recommended immunization schedule for children aged 0 through 18 years—India, 2013 and updates on immunization: Position paper. Indian Pediatr. 2013;50:1-19.

CHAPTER

4

Practice of Vaccination

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1. What are the safe injection practices?

The hands should be washed with soap and water or cleaned with an alcohol based waterless hand rub before each patient contact to prevent contamination. Skin at the injection site should be prepared with 70% isopropyl alcohol or another disinfecting agent and allowed to dry before injection. Separate disposable syringes and needles should be used for each injection. One should not deviate from the recommended route of administration in the product label. Pre-filling of syringe should be avoided as most vaccines appear similar and administration error can occur.

2. How can we alleviate pain associated with vaccination?

Several methods are used for this purpose but they have not been tested widely. Superficial anesthesia can be induced by application of topical lidocaine-prilocaine emulsion cream or patch. However, drug interactions regarding development of methemoglobinemia should be kept in mind. Paracetamol or ibuprofen can be used as analgesic when required. Breastfeeding is a potent analgesic. Sweet fluids just before injection may also help. Distraction techniques, such as music can help children cope with discomfort.

3. What are the roles of preservatives, stabilizers, and antimicrobial agents in a vaccine?

Trace amounts of chemicals (e.g., mercurials, such as thimerosal) and certain antimicrobial agents (such as neomycin or streptomycin sulfate) commonly are included to prevent bacterial growth or to stabilize an antigen. Allergic reactions may occur if the recipient is sensitive to one or more of these additives.

4. What are the general instructions for vaccination?

Following instructions should be strictly followed while providing vaccination services at your practice:

- To prevent accidental needle-sticks or reuse, a needle should not be recapped after use
- Disposable needles and syringes should be discarded promptly in puncture-proof, labeled containers
- Changing needles between drawing vaccine into the syringe and injecting it into the child is not necessary
- Different vaccines should not be mixed in the same syringe unless specifically licensed and labeled for such use

Whenever possible, patients should be observed for an allergic reaction for 15–20 minutes after receiving immunization.

5. What are the instructions one should follow on orally administered vaccines?

The vaccine must be swallowed and retained. OPV should be repeated immediately if a child spits it out, fails to swallow, or regurgitates a dose within 10 minutes after administration. If the second dose is not retained, neither dose should be counted, and vaccine should be re-administered. Breastfeeding does not interfere with successful immunization with oral vaccines (e.g., OPV, Rotavirus).

Regarding rotavirus vaccines, instructions given by the manufacturer should be followed if the child spits out the vaccine.

6. What are the general instructions on administration of parenteral vaccines?

Injectable vaccines should be administered in a site as free as possible from risk of local neural, vascular, or tissue injury. Data do not warrant recommendation of a single preferred site for all injections.

Preferred sites for vaccines administered subcutaneously or intramuscularly include the anterolateral aspect of the upper thigh and the deltoid area of the upper arm.

Ordinarily, the upper, outer aspect of the buttocks should not be used for active immunization because:

- The gluteal region is covered by a significant layer of subcutaneous fat and
- The possibility of damaging the sciatic nerve
- Due to diminished immunogenicity, hepatitis B and rabies vaccines should not be given in the buttocks at any age.

People who were given hepatitis B vaccine in the buttocks should be tested for immunity and reimmunized if antibody concentrations are inadequate.

7. What are general instructions for administration of vaccines intramuscularly?

When the upper, outer quadrant of the buttocks is used for large-volume passive immunization, such as IM administration of large volumes of immune globulin (IG), care must be taken to avoid injury to the sciatic nerve.

- The site selected should be well into the upper, outer mass of the gluteus maximus, away from the central region of the buttocks, and the needle should be directed anteriorly, that is, if the patient is lying prone, the needle is directed perpendicular to the table's surface, not perpendicular to the skin plane.
- The ventrogluteal site may be less hazardous for IM injection, because it is free of major nerves and vessels. This site is the center of a triangle for which the boundaries are the anterior superior iliac spine, the tubercle of the iliac crest, and the upper border of the greater trochanter.

Vaccines containing adjuvants (e.g., aluminum-adsorbed DTaP, DTwP, DT, hepatitis B, and hepatitis A) must be injected deep into the muscle mass. These vaccines should not be administered subcutaneously or intracutaneously, because they can cause local irritation, inflammation, granuloma formation, and tissue necrosis.

8. Can two vaccines be administered in the same limb at a single visit? What should be the minimum distance between two injections?

Yes, they can be. When necessary, two vaccines can be given in the same limb at a single visit. The anterolateral aspect of the thigh is the preferred site for two simultaneous IM injections because of its greater muscle mass. The distance separating the 2 injections is arbitrary but should be at least 1 inch so that local reactions are unlikely to overlap.

9. Should one perform gentle aspiration by pulling back on the syringe while administering vaccine by IM route?

Although most experts recommend "aspiration" by gently pulling back on the syringe before the injection is given, there are no data to document the necessity for this procedure. If blood appears after negative pressure, the needle should be withdrawn and another site should be selected using a new needle.

10. What are the guidelines for site, route of administration and length and gauge of needles?

Table 4.1 shows details about route, site and needle length. If more than one vaccine are given at the same anatomic site they should be separated by a distance of at least one inch. Gluteal region should never be used as site of injection. In infants and younger children antero-lateral aspect of thigh is preferred where

TABLE 4.1 Injection site, type of needle and technique

Age	*Site*	*Type of Needle*	*Comments*
Intramuscular injections (needle should enter at 90 degree angle)			
Preterms and neonates	Anterolateral thigh (junction of middle and lower third)	22–25 gauge, 5/8 inch	Skin should be stretched between thumb and forefinger
Infants (1 to <12 months)	Anterolateral thigh	22–25 gauge, 1 inch	Bunch the skin, subcutaneous tissue and muscle to prevent striking the bone
Toddlers and older children (12 months-10 years)	Deltoid OR Anterolateral thigh	22–25 G, 5/8 inch 22–25 gauge, 1 inch	Skin should be stretched between thumb and forefinger Bunch the skin, subcutaneous tissue and muscle
Adolescents and adults (11 years onwards)	Deltoid or Anterolateral thigh	<60 kg, 1 inch >60 kg, 1.5 inch	
Subcutaneous injections (needle should enter at 45 degree to the skin)			
Infants >12 months	Thigh Outer triceps	22–25 G, 5/8 inch 22–25 G, 5/8 inch	
Intradermal injections			
All ages	Left deltoid	26/27 G, 0.5 inch	A 5 mm wheal should be raised

as deltoid is preferred site for older children and adolescents. If vaccine and an immunoglobulin preparation are administered simultaneously, two different anatomic sites should be used.

Needles used for IM injections

- For newborn infants, especially preterm infants, a 5/8-inch long needle usually is adequate
- A 7/8 to 1 inch long needle is recommended to ensure penetration of the thigh muscle of full-term infants 2 to 12 months of age
- For injection into the thigh or deltoid muscle in toddlers and older children, a 7/8 to 1 1/4 inch long needle is suggested, depending on the size of the muscle
- The deltoid is preferred for immunization of adolescents and young adults. The needle length should be 1 to 2 inches, depending on the vaccine recipient's weight
- A 22 to 25 gauge needle is appropriate for injection of most IM vaccines

11. What should be the ideal spacing between multiple doses of same vaccine?

Killed vaccines require multiple doses to boost the immune response and develop a protective antibody titre to last for the age till the individual is susceptible for the disease. Though live attenuated vaccines as BCG, rubella require only one dose as the attenuated organism multiplies in the body for a lasting immune response and formation of memory cells. Multiple doses of some live vaccines are recommended to stimulate an immune response to different types of the same virus, such as poliovirus types 1, 2, and 3, or to induce immunity in persons who failed to mount an immune response to an earlier dose of vaccine, such as measles. These multiple doses constitute a primary vaccination series and are not 'booster doses.'

Because of immunologic memory, intervals longer than routinely recommended between doses do not impair the immunologic response to live and inactivated vaccines that require more than one dose to achieve primary immunity. As result interruption of a recommended primary series or an extended interval between booster doses does not necessitate re-initiation of the entire vaccination series.

Minimum interval is usually 4 weeks. Interval of 8 weeks between multiple doses of DPT, IPV etc. have the best response in terms of antibody titres but the EPI schedule of 4 weeks between multiple doses of primary immunization is also effective.

Aministration of doses of a vaccine at intervals less than the minimum intervals or earlier than the minimum age may result in a reduced immune response with diminished vaccine efficacy and should be avoided.

12. What should be the spacing of different vaccines?

Two or more inactivated vaccines can be given simultaneously or at any interval between doses. Inactivated and live vaccines can be given at any interval between doses or simultaneously. But two or more live injectable or nasal vaccines should have an interval of 4 weeks if not administered simultaneously. There is a possibility that two doses of the same or different live virus vaccines administered within too short an interval may inhibit the immunologic response to the second dose is based on evidence from both animal and human studies. If parenterally or nasally administered live virus vaccines are separated by less than 4 weeks, re-administration of the live virus vaccine given second should be considered. As exception OPV, rotavirus and oral typhoid vaccines may be given at any time in relation to any live/inactivated vaccine. These guidelines are important for planning catch up immunization.

This interval between two doses of a vaccine is different for different vaccines, where multiple doses are to be administered. This time interval can be divided in three groups: ideal interval, minimum interval and maximum interval.

In case of rabies vaccine number of doses and gaps are different for pre-exposure and post-exposure schedules. In pre-exposure schedule three doses are given on days 0, 7 and 21 or 28. In post exposure schedule 5 doses are administered on days 0, 3, 7, 14, 28/30. In case of DPT vaccine, minimum interval between two doses should be 4 weeks, ideal interval is 8 weeks, and maximum interval between two doses can be one year. In case of hepatitis B vaccine variable intervals between 1st and 2nd, and 2nd and 3rd doses are recommended. In case of pre-exposure schedule recommendations for low risk individuals and high risk individuals are different. Ideal schedule for low risk population is 0, 1 and 6 months, for high risk population is 0, 1, 2 and 12 months.

13. How many different vaccines can be administered simultaneously?

Any number of vaccines can be given simultaneously on the same day but at different anatomic sites. All vaccines indicated as per schedule should be given together. Simultaneous administration of different vaccines is particularly important when return of the recipient for further vaccination is uncertain, when imminent exposure to several vaccine-preventable diseases is expected, or when preparing for international travel on short notice.

Vaccines licensed for injection in the same syringe can be given together. If more than one vaccine has to be administered a single limb of an infant or young child, the thigh usually is preferred because of its large muscle mass. The distance separating two injections in the same limb should be sufficient (e.g 1 to 2 inches) to minimize the chance of overlapping local reactions. Studies have shown that two injections on the same day does not increase stress or severity of reactions or immunological interference.

Scheduling immunizations

- Most vaccines are safe and effective when administered simultaneously
- This information is particularly important for scheduling immunizations for children with lapsed or missed immunizations and for people preparing for international travel
- Inactivated vaccines do not interfere with the immune response to other inactivated vaccines or to live vaccines
- Limited data indicate possible impaired immune responses and increased incidence of a break-through illness, when 2 or more live-virus vaccines are given non-simultaneously but within 28 days of each other. Therefore, parenterally administered live-virus vaccines not administered on the same day should be given at least 28 days (4 weeks) apart whenever possible, such as MMR and Varicella vaccines
- Combination vaccine products may be given whenever any component of the combination is indicated and its other components are not contraindicated
- The use of combination vaccines is preferred over separate injection of their component vaccines

14. Why IM route is preferred for certain vaccines and SC/ID for some other? and vice-versa?

The vaccines which contain stabilizer and preservative generally cause local reactions like pain and swelling. For this reason such vaccines should be administered intramuscular. BCG is administered intradermal. If BCG is administered subsctaneous or intramuscular, it could result in lower efficacy as well as some local abscess.

15. Do immunoglobulins acquired passively or administered IM/IV as available preparations interfere with immune response to live vaccines?

Passively acquired antibodies can interfere with the immune response leading to either absence of seroconversion or a blunting of the immune response with lower final antibody concentrations in the vaccinee. Passively acquired antibody does not affect the immune response to all vaccines. Maternal antibodies effect measles and rubella uptake not varicella , rotavirus and influenza.

Intramuscular or intravenous administration of immune globulin-containing preparations (e.g., serum immune globulin, hyperimmune globulins, intravenous immune globulin and blood) before or simultaneous with certain vaccines also can affect the immune response to live virus vaccines. Blunting of responses to measles and rubella have been demonstrated. Though low dose of anti-Rh(D) globulin administered to postpartum women has not been demonstrated to inhibit the immune response to RA27/3 strain rubella vaccine. Polio and yellow fever are not affected. Data are insufficient to determine the extent to which passively acquired antibodies interfere with the immune response varicella, mumps, and typhoid (Ty21a strain).

Minimum ages and minimum intervals between doses

Vaccine doses:

- Vaccines should not be administered at intervals less than the recommended minimum or at an earlier age than the recommended minimum (e.g., accelerated schedules)

Two exceptions to this may occur:

- The first is for measles vaccine during a measles outbreak, in which case the vaccine may be administered before 9 months of age
- However, if a measles-containing vaccine is administered before 9 months of age, the child should be reimmunized at 12 to 15 months of age with MMR vaccine
- The second consideration involves administering a dose a few days earlier than the minimum interval or age, which is unlikely to have a substantially negative effect on the immune response to that dose
- Vaccine doses administered 4 days or fewer before the minimum interval or age can be counted as valid
- This 4 day recommendation does not apply to rabies vaccine because of the unique schedule for this vaccine
- Doses administered 5 days or more before the minimum interval or age should not be counted as valid doses and should be repeated as age appropriate

16. Do immunoglobulins interfere with immune response to inactivated and component vaccines?

Response to inactivated and component vaccines is less affected with live vaccines and requires exposure to large doses of passively acquired antibodies. Moderate doses of parenterally administered immune globulins have not inhibited development of a protective immune response to DTP, tetanus toxoid, hepatitis B vaccines, and Hib conjugate vaccines. Administration of inactivated hepatitis A vaccine and immune globulin simultanesly does not affect seroconversion. Maternal antibody to hepatitis A virus do not affect seroconversion.

17. What is the recommendation regarding spacing with respect to administration of immunoglobulins or antibody containing products?

Antibody-containing products and inactivated antigen can be administered simultaneously at different sites or at any time interval between doses. Non simultaneous administration can also be done at any interval.

Antibody containing products and live antigen should not be administered simultaneously. If simultaneous administration of measles-containing vaccine or varicella vaccine is unavoidable, administer at different sites and revaccinate or test for seroconversion after the recommended interval. If live antigen is administered after antibody containing products interval is dose related. Antibody containing products can be administered after 2 weeks of live antigen without any interference with seroconversion. Measles and varicella containing vaccines should be administered 3-6 months after immunoglobulins or blood transfusion.

Vaccine dose

- Reduced or divided doses of DTP or any other vaccine, including those given to premature or low birth weight infants, should not be administered. The efficacy of this practice in decreasing the frequency of adverse events has not been demonstrated
- A previous immunization with a dose that was less than the standard dose or one administered by a nonstandard route should not be counted, and the person should be reimmunized as appropriate for age
- Exceeding recommended doses also may be hazardous
- Excessive local concentrations of injectable inactivated vaccines might result in enhanced tissue or systemic reactions, whereas administering an increased dose of a live vaccine constitutes a theoretic but unproven risk

18. Suppose expiry date of a vaccine is June 2010. Can it be used till June 30, 2010 or till May 31, 2010?

It can be used till June 30, 2010. To avoid any confusion, the manufacturers should state: ‘use till ....’ information.

19. Are vaccines of different manufacturers interchangeable?

Yes, these are interchangeable. Change of brand may be necessary in case of non availability of the same brand or if previous records are not clear about the brand used. Change of brand is acceptable. However as far as possible one should use the same brand in a given patient.

Simultaneous administration of multiple vaccines

- Most vaccines can be safely and effectively administered simultaneously
- No contraindications to the simultaneous administration of multiple vaccines routinely recommended for infants and children are known
- Immune responses to one vaccine generally do not interfere with those to other vaccines
  - An exception is a decrease in immunogenicity when cholera and yellow fever vaccines are given together or 1 to 3 weeks apart
  - When simultaneous vaccines are administered, separate syringes and sites should be used, and injections into the same extremity should be separated by at least 1 inch so that any local reactions can be differentiated
  - Individual vaccines should never be mixed in the syringe unless they are specifically licensed and labeled for administration in one syringe

20. Can 2 vaccines (to be given on the same day) be mixed in a single syringe?

Some vaccines are prepared as premixed vaccines like measles, mumps and rubella as MMR, diphtheria, tetanus and pertussis whole cell or acellular component are available as DPT or DaPT. Some manufacturers provide additional vaccines with DPT or DaPT like Hib or hepatitis B vaccines to be mixed together. In these vaccines some component is provided as fluid and other component as solid or powder, the final quantity of all these vaccines remains 0.5 mL. Only such preparations should be mixed in a syringe which are recommended by the manufacturers.

21. Should whole series of vaccination be repeated if one dose is missed/delayed?

The answer can not be simple no or yes. It needs to be elaborated. Two doses of rotavirus vaccines are recommended, ideally to be administered at 6 and 10 weeks. Second dose should be administered before 24 weeks of age, but if delayed beyond 24 weeks it should not be administered. In case of hepatitis B vaccine second dose should be administered after 4 weeks, but if the vaccinee comes in less than 12 months after first dose, second dose can be administered. If vaccinee comes for the second dose after more than 1 year of the first dose, then this second dose should be considered as first dose. In case of third dose if interval is more than two years then two doses at interval of six months should be advised. Same schedule can be applied for DPT/DaPT and polio vaccines.

22. What precaution is required in a child with bleeding tendency?

Unless contraindicated, subcutaneous route should be used. For those vaccines (aluminium adjuvanted) which need to be given only intramuscularly, vaccination should be planned after factor replacement. A sustained pressure for at least 5 minutes should be applied following injection.

23. Is it necessary to withhold vaccination in a sick child?

Vaccination may be with held only if child is seriously ill. However, in case of minor illnesses like URTI and mild diarrhea, vaccination need not be postponed.

24. What precaution should be undertaken in immunizing a child who is also advised Tuberculin skin test?

A tuberculin skin test can be applied at the same visit during which live viral vaccines, such as MMR, measles, varicella etc. are administered. Because measles vaccine temporarily can suppress tuberculin reactivity, if tuberculin testing is indicated and cannot be done at the same time as measles immunization, tuberculin testing should be postponed for 4 to 6 weeks.

The effect of other live-virus vaccines on tuberculin skin test reactivity is not known.

25. How should a serious anaphylaxis reaction to a vaccine be managed?

Following protocol should be followed immediately:

- Administer epinephrine (1:1000 solution) 0.01 mL/kg/dose (max 0.5 mL) intramuscular in anterolateral thigh.
- Set up IV access.
- Lay patient flat and elevate legs if tolerated. Give high flow oxygen and airway/ventilation if needed.
- If hypotensive also, set up additional wide bore access and give IV normal saline 20 mL/kg under pressure over 1–2 minutes.
- IM adrenaline may be repeated after 3–5 minutes if required.
- Oral antihistaminics may be given to ameliorate skin symptoms but IV antihistaminics are not recommended. Oral or injectable corticosteroids equivalent to prednisone 1–2 mg/kg may be given but benefit is yet unproven.

26. What precautions should be observed for proper storage and handling of vaccines?

Handling vaccines at the recommended temperature at all the time is very important to prevent loss of potency. Vaccines can be damaged by both excessive heat and excessive cold.

Vaccines differ in their sensitivity to heat. Loss of potency can not be restored by putting them back to correct temperature. With every exposure to improper

TABLE 4.2 Recommendation for storage

Vaccines which should not be frozen	*Vaccines which can be frozen*
DTwP, DTaP, TT, DT,Td, Hep B, combination vaccines, rotavirus, typhoid, Hib	OPV l, lyophilized measles, MMR, BCG, LAIV, certain brands of varicella and MMRV

Abbreviations: DTWP/DTaP, dipetheria tetanus-pertussis (vaccine); TT, tetanus toxoids; DT, diptheria tetanus (vaccine); Td, tetanus-diptheria; Hep, hepatitis; OPV, oral polio vaccine; MMR measles-mumps rubella (vaccine); BCG, bacillus Calmette-Guèrin; LAIV, live attenuated influenza vaccine; MMRV, measles-mumps-rubella and variable (vaccine); Hib, Haemophilus influenza type b.

temperature there is loss of potency and the damage is cumulative. Live vaccines are more sensitive to heat exposure and aluminum adjuvanted vaccines are more damaged by cold injury (Table 4.2).

Lyophilized and reconstituted BCG, measles, MMR, varicella, rotavirus, HPV and most DTaP containing vaccines are susceptible to exposure to light and need to be protected from strong light, sunlight, ultraviolet and fluorescent neon lights.

27. What storage equipment is required for office practice and how best one should use it?

A domestic refrigerator with combination of refrigerator and freezer is acceptable. Refrigerator and freezer should have separate external door. The recommended temperature for the main compartment is 2–8°C and for the freezer compartment is 5 to 15°C. A thermometer to monitor the temperature is must.

The appropriate places for different vaccines are given in Table 4.3.

TABLE 4.3 Storage at different levels in a refrigerator

Compartment	*Vaccines*
Freezer	BCG, OPV, Measles, MMR
Top shelf	OPV, Measles, MMR
Middle shelf	DTwP, DTaP, DT, TT, Tdap, Td, IPV, HPV, Typhoid, Hepatitis A, Hib, PCV 13, Influenza, Rotavirus
Lower shelf	Hepatitis B, Varicella
Crispator	Diluents
Baffle tray	No vaccines
Doors	No vaccines

Abbreviations: BCG, bacillus Calmette-Guèrin; OPV, oral polio vaccine; MMR, measles-mumps-rubella (vaccine); DTWP/DTaP/TDaP, diptheria-tetanus pertussis (vaccine); DT, diptheria-tetanus; TT, tetanus toxoids; Td, tetanus-diptheria; IPV, inactivated poliovirus vaccine; HPV, human papillomavirus vaccine; Hib, Haemophillus influenza type b; PCV, pneumococcal conjugate vaccine.

28. What are the do's and dont's for use of refrigerator?

Following instructions should be followed:
- Do not open the door unnecessarily
- Do not use for storage of any other items
- Keep ice packs and bottles filled with water in the freezer and door to maintain cold temperature in case of power failure
- The power plug should have a sticker saying "Do Not Unplug" or "Do Not Turn Off"
- In case of frequent power failure an alternative power source should be arranged
- Use principles of FEFO (first expired first out) and FIFO(first in first out).

29. How should one maintain record of immunization?

Complete and accurate records are mandatory. Failure to do so poses lot of difficulty and confusion as to how to complete the schedule. Ideally one should record date of vaccination, name of the vaccine, manufacturer, lot number, expiry date, site and route of administration and name, title and address of the health care provider.

30. What are the ethical issues of immunization?

With several new vaccines flooding the market, parents find it difficult to decide on which vaccines should be given to their child. The onus is on the pediatrician to give a balanced scientific advice to parents. One has to keep one self updated with knowledge regarding the vaccines. Special communication skills need to be developed to address many issues related to the decision making process. Some of the aspects which need to be clearly explained to parents are: risk of developing disease and need for vaccination, efficacy of vaccine, safety issues, cost of vaccine and affordability. The dynamic nature of recommendations should also be emphasized.

31. Should we encourage advertisements of various vaccines in lay press?

No advertisement of any sort should be permitted I would like to quote what I had stated earlier (Paul Y. Vaccines for whose benefit? Indian Journal of Medical Ethics 2010;7:30-31).

These days some of the new vaccines are advertised in the electronic media on the pretext of creating public awareness. Consumer products are advertised, but no new medicine is advertised in similar manner. Some of these vaccines are not recommended for universal immunisation but are recommended for specific conditions, but this is not mentioned in the advertisement. So when parents ask their doctors about such vaccines, doctors find themselves in a piquant situation. For example the influenza vaccine is recommended when a vaccinee is suffering from chronic pulmonary and cardiac disease, immunodeficiency, HIV infection etc. On the other hand the absence of such indications is no contraindication for this vaccine, and no harm is expected to occur. The doctor may choose to explain the true situation and spend a lot of time to convince the parents that the vaccine is not required for that child. Two questions may be asked. First, will any harm occur to the child if this vaccine is administered? The answer is “No”. Second, is there any possibility, although it is not likely, that this child could suffer from a severe form of influenza in future? The answer is “Yes”, as this possibility is always there. Thus, through advertisements a sort of fear is created to increase sales of this vaccine. Under these circumstances doctors cannot be blamed for administering such a vaccine. Such advertisements should not be permitted.

32. Should school administration be allowed to insist on vaccination of any particular vaccine even for hostellers?

In India no vaccine has been declared compulsory. Even for compulsory vaccines, the vaccine recipient or care takers can refuse vaccination with the undertaking to be responsible for any harm to self or ward. Some schools had insisted on varicella vaccine and hepatitis A vaccine administration to those children seeking admission specially as hostellers. It is for the doctors to suggest vaccines but ultimate decision to administer or not to administer any vaccine rests with the individuals or caretakers in case of children.

SUGGESTED READING

1. Manual of Advancing Science of Vaccinology. Indian Academy of Pediatrics; 2009.
2. Pickering LK, Baker CJ, Long SS, McMillan JA (Eds). Red Book: 2006 Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2006.

CHAPTER

5

Vaccination in Special Situations

Monjori Mitra

1. What are the special situations for vaccination?

They include the following:
- Immunocompromised
- Preterm/low birth weight infants
- Immunization in chronic diseases
- Immunization in bone marrow/organ transplant
- Immunization in asplenia/hyposplenia
- Immunization in corticosteroid/immunosuppressive therapy
- Transfusion of antibody containing products
- Immunization in the presence of H/o allergy
- Immunization in bleeding disorders/anticoagulant therapy
- Immunization during illness
- Lapsed/proponed/unknown immune status
- Immunization in pregnancy/lactation
- Immunization for travelers.

2. What are the issues faced by the clinicians in vaccinating an immunocompromised individual?

The need to protect the individual against serious infections is the primary goal. However, the public health point of view is also significant because it is important not to have an increasing number of individuals vulnerable to serious infectious agents (e.g. poliovirus). Both aspects require an analysis of risks and benefits for the individual patient. The dilemma is because of the following issues:
- Increased susceptibility to infection
- Increased severity of the disease
- Poor immune response to the vaccine
- Safety of vaccination would also be an issue
- Efficacy of vaccination id doubtful.

3. Why are vaccinations not optimally used in immunocompromised patients?

- Ignorance/minor interest for “old” preventive strategies.
- Fear of vaccine-associated risks.
- Perception of insufficient efficacy and hence a worry sets in regarding the stimulation of a compromised immune system.

4. How is immunosupression differentiated on the basis of CD4+ count?

Immune response in case of immune-deficient subjects is based on the CD4 counts and the response to any vaccination and the plan to vaccinate the child also depends on the same. If the CD4 count is >25% the immunity is conserved and hence vaccination with live vaccines based on risk benefit ratio can be given.

- Conserved—no immunosuppression:
  CD4+ T lymphocytes >25%
- Moderate immunosuppression:
  CD4+ T lymphocytes 15–24%
- Severe immuno-suppression (Impaired, absent):
  CD4+ T lymphocytes <15%

5. Name various types of immunocompromised conditions.

Various immunocompromised conditions which require special attention in planning vaccinations are:

- *Primary immunodeficiencies (congenital)*
  - B-lymphocyte defects
  - T-lymphocyte defects
  - Phagocytic function disorders
  - Complement deficiency
- *Acquired immunodeficiencies*
  - HIV infection
  - Chronic diseases and immunosuppression
  - Transplant/cancer patients
  - Immunosuppressive therapy—Steroids, CXT, RXT
  - Asplenic children
  - Autoimmune diseases patients.

6. What are the recommendations for vaccination of individuals having primary B lymphocyte defects?

- In B-lymphocyte defects there is abnormal humoral response to infections.
- All live bacterial (BCG and Oral Typhoid) and Live Viral (MMR, OPV, Measles and Varicella) vaccines—contraindicated
- Agammaglobulinemia: consider pertussis, influenza
- Selective IgG and IgA defects: all live vaccines other than OPV can be considered.

7. What are the recommendations for vaccination of individuals having primary T lymphocyte defects?

All live vaccines are contraindicated. No vaccine is useful.

8. What are the recommendations for vaccination of individuals having primary phagocytic function disorders?

- All live bacterial vaccines are contraindicated
- Live viral vaccines can be administered
- Consider Influenza vaccine to prevent secondary bacterial infections.

9. What are the recommendations for vaccination of individuals having primary complement deficiency?

- All vaccines can be safely administered
- More prone to pneumococcal and meningococcal infections
- C1, C4, C2, and C3 deficiency: all vaccines are effective; pneumo + meningo recommended
- C5-9, Properdin, Factor B deficiency: All vaccines are effective; meningo recommended.

10. What are the risks associated with vaccination of HIV infected patients?

With the decrease in the immunity because of HIV the chances of enhancement of certain diseases increases thus increasing the risk of the disease. Hence one needs to weigh the risk benefit ratio of the diseases and plan vaccination. Various studies have shown the increased number of chances of the following diseases:

- Risk of tuberculosis enhances by 10–300 times, S. pneumoniae by 5–50 times.
- The chronicity increases in case of hepatitis B exposure. Hepatitis A also has a severe duration of action.
- In case of measles infection the condition can be life-threatening. Similarly the risk enhances in case of influenza, varicella and zoster infection.

11. What are the various issues with HIV+ individual?

In case of progressive decline in CD4 T lymphocytes count, there is increased risk of complications from infections, impaired effectiveness of vaccines, there may be risk of serious adverse events from live vaccines. With significantly low CD4 count there may be loss of prior immunity because of lack of CMI.

12. What are the recommendations of WHO, AAP and ACIP for HIV patients on individual vaccines?

Table 5.1 summarizes recommendations of different authority on individual vaccine for HIV infected individuals.

TABLE 5.1 Recommendations on individual vaccines for HIV infected asymptomatic and symptomatic children

	IAP/WHO		*ACIP*		*AAP*	
Vaccine	As	S	As	S	As	S
BCG	Yes	No	No	No	No	No
DPT	Yes	Yes	Yes	Yes	Yes	Yes
OPV	Yes	No	No	No	No	No
IPV	Yes	Yes	Yes	Yes	Yes	Yes
MMR	MEASLES/ MMR	MEASLES/ MMR	Yes*	Yes*	Yes	Yes
Hib conj	Yes	Yes	Yes	Yes	Yes	Yes
Pneumo conj	Yes	Yes	Yes	Yes	Yes	Yes

*May be considered.

Abbreviations: As; Asymptomatic; S; Symptomatic; BCG, bacillus Calmette-Guèrin; DPT, diptheria pertussis-tetanus (vaccine); OPV, oral polio vaccine, IPV, inactivated poliovirus vaccine; MMR, measles-mumps-rubella (vaccine); Hib, Haemophilus influenza type b.

13. What are the risks associated graft rejection through immunization?

There is always a perception of increased risk in graft rejection following immunization but not a single study showing increased rates of graft rejection episodes following immunizations was seen. On the contrary it has been demonstrated that there is increased evidence of rates of rejection after viral infections such as influenza.

14. What points should considered during immunization of children with anatomical or functional asplenia?

Risk of mortality from septicemia increases by about 50 times in post splenectomy cases and is about 350 times in sickle cell disease and thalassemia. Risk is higher in younger (<5 years) than older children/adults and it has high risk for serious infections with encapsulated organism. All live and inactivated vaccines indicated and pneumococcal, Hib influenzae and meningococcal is a must.

15. Outline the various issues of vaccination of patients receiving steroids?

The immune status and the response to vaccine would depend on the dose and duration of steroid.

There will be absence of immunosuppression if the dose is <20 mg/day (prednisone) or <2 mg/kg in children and the duration of administration is <2 weeks. In these situations killed vaccines are safe but less efficacious but all vaccines are safe and efficacious during inhalation and topical therapy.

Outside of these conditions no live vaccines (until 1 month after discontinuation of corticoids) should be given and the individual vaccine responses should be assessed after vaccination.

16. What are the outcomes of vaccination of the patients having cancer or are under chemotherapy?

- Decreased immunogenicity
- Increased risk of complications.

Hence, all live vaccines should be avoided during and at least 3 months after chemotherapy and radiotherapy.

17. What are the outcomes of vaccination in the presence of chronic liver disease?

- *Prevention of hepatitis A:*
  - It has been seen before cirrhosis the seroconversion to 2 doses is >95% but the antibody titers are lower. But when cirrhosis sets in, the seroconversion ranges from 0–66% with low antibody titers, transient responses and need for boosters.
- *Prevention of hepatitis B:*
  - Poorer results are due to the lower immunogenicity of hepatitis B vaccine. In advanced liver disease (cirrhosis) better immunogenicity is achieved by double dose vaccines i.e. adult dose in children, and dialysis patient dose in adults. Also better immunogenicity may be achieved with additional doses as needed after standard (0, 1, 6 months) schedule.

18. What are the recommendations for individuals who have recently received antibody-containing products?

- Inactivated vaccines can be administered safely
- Live vaccines including MMR and Varicella should be avoided for 3 months
- Antibody containing products should be avoided for 2 weeks after these vaccinations
- Oral typhoid vaccine, LAIV, OPV and Yellow fever vaccine may be given at any time
- Rotavirus vaccine should be avoided for 6 weeks.

19. What are the general recommendations for immunizing preterm/LBW infant?

All vaccines may be administered as per schedule according to the chronological age irrespective of birth weight or period of gestation.

- BCG and birth dose of OPV can be safely and effectively given to low birth weight/preterm babies after stabilization and preferably at the time of discharge.

- The birth dose of hepatitis B vaccine can be administered at any time after birth in babies weighing ≥2 kg.
- In babies less than 2 kg, the birth dose of hepatitis B vaccine should be delayed for 1 month after birth as immunogenicity is lower if given earlier.

In babies less than 2 kg born to a hepatitis B positive mother, hepatitis B vaccine should be given along with HBIG within 12 hours of birth and 3 more doses at 1, 2 and 6 months are recommended.

Since preterm, low birth weight babies have increased susceptibility to infections, category 3 and 4 vaccines such as pneumococcal conjugate vaccines, rotavirus and influenza should be offered if resources permit.

20. What are the general recommendations for immunizing individuals with H/o allergy?

Vaccines contraindicated with H/o serious hypersensitivity/anaphylaxis

- In H/o egg allergy influenza and yellow fever vaccines contraindicated but measles, MMR may be given.
- H/o any hypersensitivity vaccination with JE should be cautious.
- Mild reactions not contraindications to vaccinations.
- Resuscitation equipment should be kept standby.

21. What are the general recommendations for immunizing individuals with bleeding disorders?

Preferred route is subcutaneous (unless contraindicated).

- Aluminum-adjuvanted vaccines should be avoided. Vaccination should be offered after factor replacement therapy.

22. What are the general recommendations for immunizing pregnant and lactating women?

All live vaccines in pregnancy contraindicated.

- Risk to fetus–Yes, and may be a risk of congenital anomalies
- Inactivated vaccines–Safe (but only if strongly indicated)
- All vaccines except yellow fever are safe in lactating women.

23. What are the general recommendations on interchangeability of vaccine brands?

There is sufficient data that brands of hepatitis, Hepatitis B and Hepatitis A may be safely interchanged with no compromise on immunogenicity and efficacy. However robust data for immunogenicity of vaccination with different brands of DTaP is lacking. Hence vaccination with DTaP should be completed with the same brand. However, If previous brand is not known or no longer available any brand may be used and vaccination should not be delayed/cancelled.

24. What are the general recommendations on 'Catch up immunization'?

For catch up immunization, doses should preferably be given at the minimum possible interval to entail early protection.

Any number of vaccines live/inactivated may be given on the same day either singly or as combination vaccines maintaining a gap of 5 cm between different vaccines.

- Except BCG and measles/ MMR should not be given on the same day
- Inactivated vaccines can be given at any time in relation to any other live/ inactivated vaccines
- If not given on the same day a gap of 4 weeks should be maintained between two live vaccines
- OPV, Rotavirus and oral typhoid vaccines may be given at any time in relation to any live/inactivated vaccine.

25. What are the general recommendations for immunization of the elderly people (>65 years)?

Annual influenza, and a single dose of PPV23 vaccine.

26. Outline the general principals involved in the vaccination of immunocompromised host?

- In severe immunodeficiency all live vaccines contraindicated. In mild/moderate it depends on the CD4+ count.
- All inactivated vaccines may be given but immunogenicity and efficacy low.
- Higher doses, more number of doses may be required in case of hepatitis B.
- Antibody titers should be checked post immunization. Regular boosters may be needed.
- Immunoglobulins (RIG, HIG) may be needed in some situations.
- Some Category 3 and 4 Vaccines including pneumococcal, varicella, hepatitis A, inactivated influenza should be given if resources permit.
- Insufficient data on safety and efficacy of rotavirus vaccine in immunocompromised subjects.
- Household contacts of immunocompromised should not receive transmissible vaccines such as OPV.
- All household contacts should be fully immunized including varicella and influenza to reduce risk of transmission to the immunocompromised subjects.

27. What are the recommendations of the different schedules of vaccines in special situations?

Immunizations of immunocompromised individuals are important from two points of view. Obviously, the need to protect the patient against serious infections is the primary goal. However, the public health point of view is also significant because it is important not to have an increasing number of individuals vulnerable to serious infectious agents (e.g., poliovirus). Both aspects require an analysis of risks and benefits for the individual patient.

Depicts the recommendation of the vaccines in different situations: Tables 5.2 to 5.5.

TABLE 5.2 Recommendations for routine immunization of HIV-infected children

HIV Status	*Administer*	*Consider*	*Do not administer*
Known asymptomatic HIV infection	HepB, DtaP, IPV, MMR, Hib, PCV, influenza	Varicella*	BCG
Symptomatic HIV infection	HepB, DtaP, IPV, MMR, Hib, PCV, influenza	Varicella*	BCG

* If the CD4+ count is >25% for more than 6 months then in case of asymptomatic or mild symptoms varicella vaccine can be given with 2 doses 3 months apart.

Abbreviations: HIV, human immunodeficiency virus; MMR, measles-mumps-rubella (vaccine); DTaP, diptheria-tetanus pertussis (vaccine); IPV, inactivated poliovirus vaccine; Hib, Haemophillus influenza type b; PCV, pneumococcal conjugate vaccine.

Data from American Academy of Pediatrics: Red Book: 2006 Report of the Committee on Infectious Diseases, 27th ed. Elk Grove Village, Ill, AAP, 2006.

TABLE 5.3 Immunization for cancer patients

Vaccine	*Indications and comments*
DTaP	Indicated for incompletely immunized children <7 yr, even during active chemotherapy
Td	Indicated 1 yr after completion of therapy in children 7 yr
Hib	Indicated for incompletely immunized children if <7 yr
HBV	Indicated for incompletely immunized children
23PS	Indicated for asplenic patients
PCV7	Indicated for incompletely immunized children <5 yr
Meningococcus	Consider in asplenic patients
IPV	Indicated for incompletely immunized children; also recommended for all household contacts requiring immunization to reduce the risk for vaccine-associated polio
MMR	Contraindicated until child is in remission and finished with all chemotherapy for 3–6 mo; may need to reimmunize after chemotherapy if titers have fallen below protective levels
Influenza	Defer in active chemotherapy; may give as early as 3–4 wk after remission and off chemotherapy if during influenza season; peripheral granulocyte and lymphocyte counts should be >1000/ìL; should also be given to household contacts of children with cancer
Varicella	Consider immunizing children who have remained in remission and have finished chemotherapy for >1 yr; with absolute lymphocyte count of >700/L and platelet count of >100,000/ìL within 24 hr of immunization; check titers of previously immunized children to verify protective levels of antibodies

Abbreviations: HIV, human immunodeficiency virus; MMR, measles-mumps-rubella (vaccine); DTaP, diptheria-tetanus pertussis (vaccine); IPV, inactivated poliovirus vaccine; Hib, Haemophillus influenza type b; PCV, pneumococcal conjugate vaccine.

Note: Immune reconstitution is slower for cancer patients who have received bone marrow transplants. Centers for Disease Control and Prevention: MMWR 2000;49 (No. RR-10):1–147 for vaccine schedule.

TABLE 5.4 Recommendations for pneumococcal immunization with PCV13 or 23PS vaccine for children at high risk of pneumococcal disease

Age (months)	*Previous doses*	*Recommendations*
≤ 23	None	PCV13 regular schedule
24–59	1–3 doses of PCV13	1 dose of PCV13 1st dose of 23PS vaccine at 24 months, at least 8 weeks after last dose of PCV13 2nd dose of 23PS vaccine 3–5 years after 1st dose of 23PS vaccine
24–59	4 doses of PCV13	1st dose of 23PS vaccine at 24 months, at least 8 weeks after last dose of PCV13 2nd dose of 23PS vaccine 3–5 years after 1st dose of 23PS vaccine
24–59	None	Two doses of PCV 13 at 8 weeks apart 1st dose of 23PS vaccine 6–8 weeks after last dose of PCV13 2nd dose of 23PS vaccine 3–5 years after 1st dose of 23PS vaccine
24–59	1 dose of 23PS	Two doses of PCV13, 8 weeks apart, beginning at last dose of 23PS vaccine One dose of 23PS vaccine 3–5 years after 1st dose of 23PS vaccine

Abbreviations: PCV, pneumococcal conjugate vaccine

Data from American Academy of Pediatrics: Red Book: 2006 Report of the Committee on Infectious Diseases, 27th ed. Elk Grove Village, Ill, AAP, 2006.

TABLE 5.5 Live-virus immunization for patients receiving corticosteroid therapy

Steroid dose	*Recommended guidelines*
Topical or inhaled therapy or local injection of steroids	Live-virus vaccines may be given unless there is clinical evidence of immunosuppression; if suppressed, wait 1 month after cessation of therapy to give live-virus vaccines
Physiologic maintenance doses of steroids	Live-virus vaccines may be given
Low-dose steroids (<2 mg/kg/day prednisone or equivalent, or <20 mg/day if >10 kg)	Live-virus vaccines may be given
High-dose steroids (≥2 mg/kg/day prednisone or equivalent, or 20 mg/day if >10 kg) • Duration of therapy <14 days • Duration of therapy ≥14 days	May give live-virus vaccines immediately after cessation of therapy. (Consider 2 weeks delay in administration) Do not give live-virus vaccines until therapy has been discontinued for 1 month
Children with immunosuppressive disorders receiving steroid therapy	Live-virus vaccines are contraindicated, except in special circumstances

From American Academy of Pediatrics: Red Book: 2006 Report of the Committee on Infectious Diseases, 27th edition Elk Grove Village, Ill, AAP, 2006.p77.

SUGGESTED READING

1. American Academy of Pediatrics: Red Book: 2006 Report of the Committee on Infectious Diseases, 27th ed. Elk Grove Village, Ill, AAP, 2006.
2. Centers for Disease Control and Prevention: MMWR 2000;49 (No. RR-10): 1–147 for vaccine schedule.
3. Singhal T, Amdekar YK, Agarwal RK. IAP Guidebook on Immunization. Indian Academy of Pediatrics; 2007-08.

CHAPTER

6

Adverse Events Following Immunization (AEFI), Vaccine Safety, and Misinformation Against Vaccination

Baldev S Prajapati, Vipin M Vashishtha

1. What is the definition of adverse events following immunization?

An adverse event following immunization (AEFI) or vaccine-associated adverse event (VAE) is one that is believed to be caused by immunization. It is defined as an untoward, temporally associated event following immunization that might or might not be caused by the vaccine or the immunization process.

Reported adverse event can be true adverse event or an event coincidental to the immunization. These events may be recognized during clinical trials or during post marketing surveillance.

2. What do you mean by AEFI surveillance?

AEFI surveillance includes:

- Detecting, monitoring and responding to adverse events following immunization (AEFI)
- Implementing appropriate and immediate action to correct any unsafe practices detected through the AEFI surveillance system, in order to reduce the negative impact on the health of individuals and the reputation of the immunization program.

3. What are different types of AEFI?

AEFIs are classified into five categories.

- *Vaccine reactions:* Event caused or precipitated by the vaccine when given correctly, caused by the inherent properties of the vaccine, e.g. VAPP due to OPV, anaphylaxis.

- *Program error*: Event caused by an error in vaccine preparation, handling or administration, e.g. toxic shock syndrome following measles vaccination.
- *Coincidental*: Event that happens after immunization but not caused by the vaccine. It is a chance association, e.g. Pneumonia few days after the vaccination.
- *Injection reaction:* Event from anxiety about, or pain from the injection itself rather than the vaccine, e.g. syncope, abscess at injection site, sciatic nerve damage.
- *Unknown:* The cause of event cannot be determined.

4. What are different types of vaccine reactions?

Vaccine reactions are classified as:

- Common minor reactions
- Rare, more serious reactions.

Most vaccine reactions are minor, and include mild side effects, such as local reactions (pain, swelling and/or redness), fever and systemic symptoms (e.g. vomiting, diarrhea, malaise), which can result as part of the normal immune response to the vaccine. Some of the nonantigenic vaccine components (e.g. adjuvants, stabilizers or preservatives) can cause reactions.

5. What are the serious vaccine reactions?

AEFI may also be classified as serious and nonserious. A serious adverse event (SAE) is defined as an event which is either (i) fatal or life threatening or (ii) results in persistent or significant disability, incapacity or (iii) results in or prolongs hospitalization or (iv) leads to congenital anomalies or birth defects. Important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient should also be considered as serious adverse events (Table 6.1).

6. What are common local reactions following vaccination?

Most parenteral vaccines induce some degree of local reactions such as pain, erythema and induration. Local reactions are more frequent with whole cell pertussis vaccines and vaccines containing aluminium as an adjuvant. The frequency of local reactions increase with subsequent doses and frequently administered doses.

7. What are common systemic reactions?

Fever is the most common systemic reaction and like other local reactions, fever is more common with pertussis vaccines and aluminium adjuvanted vaccines. However, unlike local reactions, the incidence of fever and other systemic reactions usually declines with increasing age and increasing number of doses. Vaccination induced fever does not usually last for more than 48 hours and any fever persisting beyond this time should be evaluated for other causes.

TABLE 6.1 Rare serious vaccine reactions, onset interval, and rates

Vaccine	*Reaction*
BCG	Suppurative adenitis BCG osteitis Disseminated BCG osteitis
Hib	None known
Hepatitis B	Anaphylaxis
Measles/MMR*	Febrile seizures Thrombocytopenia (low platelets) Anaphylaxis
Oral polio (OPV)	Vaccine associated paralytic poliomyelitis (VAPP)
Tetanus/DT/Td	Brachial neuritis Anaphylaxis Sterile abscess
DTP	Persistent (>3 hours) inconsolable screaming Seizures Hypotonic hyporesponsive episode (HHE) Anaphylasix/shock
Japanese encephalitis	Serious allergic reaction
Yellow fever	Allergic reaction/anaphylaxis

Abbreviations: HIV, human immunodeficiency virus; MMR, measles-mumps-rubella (vaccine); DTaP, diptheria-tetanus pertussis (vaccine); IPV, inactivated poliovirus vaccine; Hib, Haemophillus influenza type b; PCV, pneumococcal conjugate vaccine.

* Reactions (except anaphylaxis) do not occur if already immune (~90% of those receiving a second dose); children over six years are unlikely to have febrile seizures.

Source: Training Manual for mid-level managers. WHO/IVB/08.03.

8. What are the severe allergic reactions which are known to occur following vaccination?

Severe allergy or anaphylaxis or anaphylaxis like reactions are known to occur following vaccination, these include generalized urticaria, hives, wheezing, swelling of the mouth and throat, difficulty in breathing, hypotension and shock, but these reactions occur very rarely, with a frequency of 1 per 10,00,000 vaccines. These reactions are rarely due to vaccine antigen, they are usually due to other vaccine constituents like egg, gelatin like stabilizers, antimicrobials like neomycin, streptomycin or preservatives, like thiomersal.

9. What measures should be taken to prevent severe allergic reactions?

A complete detailed history of any past allergies must be obtained. Patients with history of severe allergic reaction to any constituent of the vaccine should not be given the vaccine, except for the children with egg allergy, they can be given measles and MMR vaccines. As occurrence of anaphylaxis cannot be predicted, the vaccinee should be observed for 15 minutes after vaccination.

The resuscitative equipments like oxygen delivery system, Ambu bag and mask, laryngoscope, endotracheal tubes, IV access devices, epinephrine, hydrocortisone, antihistaminics and ionotropes should be kept ready.

10. What is the emergency management of anaphylaxis?

- The patient must be made to lie down flat and legs must be elevated if possible
- Epinephrine (1:1000 solution) 0.01 mL/kg/dose (maximum 0.5 mL) by IM injection must be administered on anterolateral aspect of thigh. It can be repeated after 3–5 minutes if required
- Airway must be cleared, breathing and circulation must be established
- IV access must be set up
- Wide bore access and IV normal saline 20 mL/kg as a bolus must be given in hypotension
- Oral antihistaminics may be given. IV antihistaminics are not recommended
- Oral or IV corticosteroids equivalent to prednisolone 1–2 mg/kg may be given, but benefit is not proven.

11. What are common programmatic errors?

It is a misconception that vaccines are the most common cause of AEFI. On the contrary, incorrect immunization practices that can be prevented are more often the cause of AEFI (Table 6.2). Careful epidemiological investigation of an AEFI is needed to pinpoint the cause and to correct these malpractices.

TABLE 6.2 Examples of incorrect immunization practices and associated AEFI

Incorrect practices	*Possible severe reactions following immunization*
Nonsterile injection • Reuse of disposable syringe or needle • Improperly sterilized syringe or needle • Contaminated vaccine or diluent	• Infection, such as local abscess at injection site, sepsis, toxic shock syndrome or death • Blood-borne infection transmitted, such as hepatitis, HIV
Reconstitution error • Inadequate shaking of vaccine • Reconstitution with incorrect diluents • Drug substituted for vaccine or diluents • Reuse of reconstituted vaccine at subsequent session	• Local abscess • Vaccine ineffective • Negative effect of drug, e.g. insulin, oxytocine, muscle relaxants • Death
Injection at incorrect site • BCG given subcutaneously • DTP/DT/TT too superficial • Injection into buttocks	• Local reaction or abscess • Local reaction or abscess • Sciatic nerve damage
Vaccine transportation/storage incorrect • VVM changed color • Clumbing of absorbed vaccine	• Local reaction from frozen vaccine • Vaccine infective*
Contraindications ignored	Avoidable severe reaction

* Vaccine being ineffective is an 'effect', it is not strictly an adverse event.

12. What precautions should be taken while reconstituting a vaccine with diluents?

In the past, tragedies related to reconstitution of freeze-dried vaccines with insulin, muscle relaxant and other inappropriate solutions have occurred. Managers should ensure that such products are not stored in the vaccine refrigerators or cold boxes. To avoid this confusion, WHO now encourages vaccines and diluents to be distributed together. The diluent supplied with a vaccine is part of the licensed product and is specifically designed for the needs of that vaccine with respect to volume, pH level and chemical properties. Following precautions should be taken for safe use of diluents:

- Careful stock control and accurate records are vital to monitor that the correct diluent is always kept and distributed with each vaccine type and batch
- In order to avoid confusion during reconstitution, diluents should be supplied, transported and distributed together with the vaccine types to which they correspond
- Only use the diluents supplied and packaged by the manufacturer with the vaccine
- Vaccines and diluents must be clearly labeled and identified
- Health workers must always read the labels to be sure that they have the diluent provided by the manufacturer for that specific vaccine and vial. If the label is missing or cannot be read, the product should not be used
- Diluents must be cooled to between 2°C and 8°C before reconstitution
- Draw up all the diluent in the vial and then reconstitute the vaccine to make sure the correct number of doses per vial is obtained
- Diluents should be handled with the same care as vaccines. Health workers should be trained to know the proper way to reconstitute each of the vaccines they are using
- Discard reconstituted vaccines within six hours of reconstitution
- Diluents must not be frozen
- Diluents from other vaccines or from other manufacturers must NOT be used
- Sterile water for injection must NOT be used as a vaccine diluent
- Inside the refrigerator, proper grouping and marking of medical products should be done
- Do not leave the mixing needle in the vial ; this leaves the vial open to contamination
- Do not reconstitute vaccine until the person needing the vaccine injection is present.

13. What are the critical steps to reconstitute vaccines safely?

They include the following 10 critical steps:

- Read the label on the diluent to make sure that it is the correct diluents provided by the manufacturer for that specific vaccine and vial size.
- Check the expiry date to make sure that it has not passed
- Check the status of the vaccine vial monitor (VVM) to make sure that it is not at, or beyond the discard point

- Cool the diluent to between 2°C and 8°C, preferably a day prior to its use
- Draw the entire contents of the diluent into a new sterile mixing syringe and empty the entire contents of the diluent into the vaccine vial
- Discard the used mixing syringe and needle into a safety box without recaping
- Do not leave the mixing needle in the vaccine vial
- After reconstitution, insert the vial in the foam pad of a vaccine carrier. Never allow the vial to become immersed in water
- Discard all reconstituted vaccine at the end of the session, or within six hours, whichever comes first
- Use a new autodisable (AD) syringe and needle to withdraw each dose of the vaccine, and use the same needle and syringe for injecting the vaccine. After giving the injection, drop the used syringe and needle into the safety box without recaping.

14. What is the revised 'multidose vial policy' (MDVP)?

Multidose vials of OPV, DTP, TT, DT, Td, hepatitis B and liquid formulations of Hib vaccines from which one or more doses of vaccine have been removed during an immunization session, may be used in subsequent immunization sessions for up to a maximum of four weeks, provided that all the following conditions are met:

- The expiry date has not passed
- The vaccines are stored under appropriate cold-chain conditions (2°C to 8°C)
- The vaccine vial septum has not been submerged in water
- Aseptic technique has been used to withdraw all doses
- The VVM, if attached, has not reached the discard point.

Note: The revised policy does not change the recommended procedures for handling vaccines that must be reconstituted; for example BCG vaccine, measles, yellow fever and some formulations of Hib vaccines. Once a vial of any of these vaccines is reconstituted, it must be discarded at the end of each immunization session or at the end of six hours, whichever comes first.

15. What are coincidental AEFI?

Vaccines are normally scheduled early in life, when infections and other illnesses are common and underlying congenital or neurological conditions may be present. Consequently, many events including deaths are falsely attributed to vaccines (rather than a chance association).

Coincidental events are unrelated to the immunization but medical officers should be encouraged to ensure the proper diagnostic workup and management of the AEFI cases even when not related to the vaccination.

Parents or the community may blame the vaccine, especially if the child was previously healthy. These cases still require investigation to allay public fears and to maintain credibility. Responding to a community's concerns about immunization safety is important in maintaining confidence in the immunization program.

An event is more likely to be coincidental if a similar event affected others in the same age group around the same time, although they did not receive the suspect vaccine(s). There may also be evidence showing that the event is not related to immunization.

16. What do you mean by injection reactions?

Individuals and groups of individuals may react before and after an injection of any kind. This reaction, unrelated to the content of the vaccine, can include hyperventilation resulting in light-headedness, dizziness, tingling around the mouth and in the hands, vomiting, breath-holding, fainting (relatively common), and convulsions.

Some individuals may be needle-phobic, aggravating such reactions. In a group situation, mass hysteria is possible, especially if a patient is seen to faint or have some other reaction. Clear explanations about the immunization, and calm, confident delivery, will decrease the level of anxiety about the injections, and thus reduce the likelihood of an occurrence.

17. Why AEFI detection and reporting rates are low in our country?

Whenever AEFI are detected, they must be reported on a timely basis so that the cause can be identified.

Peripheral health workers may not report AEFI for one or more of the following reasons:

- Not considering the event as related to immunization
- Not knowing about the reporting system and process
- Fear that the report will lead to personal consequences
- Guilt about having caused harm and being responsible for the event
- Uncertainty about reporting an event when not confident about the diagnosis.

A manager can overcome these reporting barriers by:

- Increasing awareness of the importance of reporting
- Teaching staff how to report AEFI
- Encouraging staff to report, even in situations of uncertainty
- Emphasizing that investigations are about finding problems with the system, and not blaming individuals
- Giving positive feedback to health workers for reporting AEFI.

18. What are the measures to be taken to prevent vaccine reactions?

It is mandatory for the person administering the vaccine to have sufficient knowledge regarding vaccines and expected side effects and to inform the parents thoroughly regarding such adverse effects. One should be prepared to manage the untoward condition if it develops and to have the life saving drugs and equipment at the place of vaccination.

Advice on managing the common reactions should be given to parents and to return if there are more serious symptoms. It will help to reassure them.

WHO guidelines to avoid program errors are as follows:

- Vaccine must only be reconstituted with the diluent supplied by the manufacturer

- Reconstituted vaccines must be discarded at the end of each immunization session
- No other drugs or substances should be stored in the refrigerator of the immunization center
- Immunization workers must be adequately trained and closely supervised to ensure that proper procedures are being followed
- Careful epidemiological investigation of an AEFI is needed to pinpoint the cause and to correct immunization practices.

19. What are reportable AEFIs?

The reportable AEFI must include any death or serious event believed by the public or health worker to be caused by immunization. The reportable AEFIs are shown in Table 6.3. The minor common reactions such as local pain, induration, fever and self-limiting symptoms need not be reported. For more serious problems patient should be advised to return or to seek medical attention and to allow detection of AEFI. They should also be advised not to delay treatment of coincidental illness falsely attributed as vaccine reaction. Severe local reactions especially if occurring in clusters should be reported as they can be markers for program errors and for problems with specific vaccine lots.

TABLE 6.3 Reportable AEFIs

• Occurring within 24 hours of immunization	• Anaphylaxis • Persistent (>3 hours) inconsolable screaming • Hypotonic hyperresponsive episode (HHE) • Toxic shock syndrome (TSS)
• Occurring within 5 days of immunization	• Severe local reaction • Sepsis • Injection site abscess
• Occurring within 15 days of immunization • Occurring within 3 months of immunization	• Seizures • Encephalopathy • AFP (4–30 days for OPV recipient and 4–75 days for contact) • Brachial neuritis • Thrombocytopenia (15–35 days after measles/MMR)
• Occurring between 1 and 12 months after BCG immunization • No time limit	• Lymphadenitis • Disseminated BCG infections • Osteitis, osteomyelitis. • Any death, hospitalization or other severe or unusual events that are thought by health worker or public to be related to immunization

Abbreviations: HIV, human immunodeficiency virus; MMR, measles-mumps-rubella (vaccine); DTaP, diptheria-tetanus pertussis (vaccine); IPV, inactivated poliovirus vaccine; Hib, Haemophillus influenza type b; PCV, pneumococcal conjugate vaccine.

20. Regarding AEFIs, when to report? Who should report? To whom it should be reported?

A system for reporting vaccine associated adverse event is crucial in any immunization program so as to pick up previously unrecognized adverse effects and generate further data on vaccine safety. A robust system for reporting VAE exists in most developed countries like the USA. However, such system is not currently available in India.

Reporting should be done as quickly as possible so that an immediate decision on the need for action and investigations can be made. Private physicians and hospitals should also report the events that they come across. In the community, peripheral health worker or supervisor should report to the district office. The report should contain patients identifying details, vaccine given, timing of event in relation of immunization, description of the event, etc.

21. A one-day-old infant died within 15 minutes of administrating OPV by a health worker in a periurban slum health center. A scare was created in the community, refused vaccination, blocked roads and assaulted medical officer and staged 'dharana' at district DM office.

I. What should be the appropriate action?
II. Which category of AEFI are you dealing with?
III. Why it couldn't be a true vaccine induced?
IV. How to investigate such a case?
V. How to pacify parents and convince community?

- Pacify and reassure the grieved family. Try to take them into confidence for a fair trial of the incident
- Coincidental reaction
- The very short-time interval does not fit well the cause-effect assessment. The clinical course is incompatible with the process of scientific causality assessment
- Obtain all the details pertaining to neonate's and mother's health prior to the incident

  Whether the baby was healthy or not? Was she immunocompromised
- Pacify parents by reassuring them about the safety and quality of the vaccine. Inform or arrange interview of the parents whose kids have also administered the same vaccine on the same day. Try to cite success of the ongoing program.

22. In the above case, detailed history from attendants revealed that the newborn was delivered on 'Tonga' during the transport of pregnant mother to nearest health center. The newborn was asphyxiated, and passed meconium in utero, and aspirated. The death was due to meconium aspiration syndrome (MAS), not due to OPV per se. Now, would you like to revise your diagnosis?

Yes. Now the correct diagnosis would be "programmatic error" since a known contraindication was overlooked by the ANM/health worker.

23. An infant has died five days after receiving his first dose of DPT in a rural health center. The center serves a community with a total population of 50,000 people. Since the death of this infant, many parents have refused to have their children immunized. A full investigation revealed no error of administration or problem with the vaccine.

I. What category of AEFI are you dealing with here?
II. How will you investigate?
III. What strategies would you implement to convince the community that vaccination is safe and necessary, despite the recent infant death?

- Coincidental reaction
- Similar line as with the previous case.
- Pacify parents by reassuring them about the safety and quality of the vaccine. Inform or arrange interview of the parents whose kids have also administered the same vaccine on the same day. Try to highlight that the diseases prevented by the vaccines carry far greater risks to life than the vaccines themselves. cite success of the vaccines in preventing diseases. Be patient, honest, frank, and open. Be aware of context; psychological and social issues. Be clear on message- and keep returning to message -vaccines protect children from disease. Use: logic, evidence, emotion, imagery. Story telling can be powerful. Draw attention to evidence supporting vaccination. Educate parents, public, professionals, journalists, and politicians about causal assessment

24. What are the reasons for delaying or withholding vaccines (contra indications)?

Health workers should use every opportunity to immunize eligible infants and adults, unless the infant or adult has a health condition that does not permit vaccination. Sometimes there are reasons why a specific vaccine should NEVER be administered (also called an absolute contraindication) and sometimes the health worker should delay giving the vaccine for a short time (also called a temporary contraindication). These different types of reasons for delaying or withholding a vaccine. Health workers must know about the correct reasons for withholding immunization. The incorrect reasons for withholding a vaccine are called 'false contraindications'. Delaying immunization because of false reasons (false contraindications) results in a missed opportunity to fully immunize an infant or adult.

Reasons for never administering a specific vaccine (also called absolute contraindications).

If the infant or person has:

- Symptomatic (showing symptoms) or documented human immunodeficiency virus (HIV) infection-do NOT immunize with BCG
- Symptomatic (showing symptoms) HIV infection-do NOT immunize with yellow fever vaccines

- A history of a severe adverse event following a dose of a specific vaccine (anaphylactic reaction or severe shock)—do NOT give follow-up doses of that particular vaccine, but provide the infant or adult with other vaccines.

Reasons for delaying administering a specific vaccine (also called temporary contraindications).

The following vaccines should not be administered until the specific condition is no longer present.

- On theoretical grounds, measles and yellow fever vaccines are not recommended during pregnancy
- Do not give measles vaccine to persons with a history of an anaphylactic reaction to neomycin, gelatin or other components
- Yellow fever vaccine is contraindicated for persons with severe allergy to egg
- Measles and yellow fever vaccine are contraindicated in persons who are severely immunocompromised as a result of congenital disease, HIV infection, advanced leukemia or lymphoma, serious malignant disease, or treatment with high-dose steroids, alkylating agents or antimetabolites, or in persons who are receiving immunosuppressive therapeutic radiation.

The incorrect reasons for withholding a vaccine are called "false contra-indications". The list below of conditions comprises some examples of "false contraindications". If an infant or adult presents with any of these, they should be vaccinated.

- Minor illnesses such as upper respiratory infections, or diarrhoea with fever <38.5°C
- Allergy, asthma, or other atopic manifestations such as hay fever or 'snuffles'
- Prematurity; low-birth-weight infant
- Malnutrition
- Infant being breastfed
- Family history of convulsions
- Treatment with antibiotics, low-dose corticosteroids or locally acting (e.g. topical or inhaled) steroids
- Dermatoses, eczema or localized skin infection
- Chronic diseases of the heart, lung, kidney and liver
- Stable neurological conditions, such as cerebral palsy and Down syndrome
- History of jaundice after birth.

None of the above list is a true reason for withholding vaccination. If an infant or adult has any of these health issues they should be vaccinated.

25. What is the difference between 'misinformation' and 'missing information'?

- *'Misinformation':* Those vaccine safety concerns that persist despite the evidence against them. There is intentional dissemination of false information.
- *'Missing information':* Those vaccine safety concerns for which the necessary data to support or reject the hypothesis are not available.

26. Thiomersal is used as preservative in some vaccines. Its presence in the vaccines has been doubted for mercury toxicity and autism like neuropsychological disorders. What is the scientific fact regarding this matter?

Thiomersal is mercury based preservative used in some vaccines, is an organic compound containing 49.6% of ethylmercury by weight. It is used in very small amount in some vaccines to prevent bacterial and fungal contamination. This organic compound of mercury is in two forms, ethylmercury (Thiomersal and methylmercury. Thiomersal does not accumulate in the body because its half life is 7–10 days and it is rapidly converted in the body to inorganic mercury which is excreted in the stool. The methylmercury which is more potent is accumulated in the body because time taken by the body to eliminate it is about 50 days.

The safe level of mercury consumption lies somewhere between 0.7 mg/kg/week (Environmental Protection Agency, USA) to 3.3 mg/kg/week (WHO). The total intake of 175 mg of ethylmercury occurs in a child who is given all routine vaccines, is equivalent to 1.9 mg/kg/week. This level is much below the WHO limit for methylmercury which is converted to ethylmercury and rapidly excreted.

The Global Advisory Committee on Vaccine Safety (GAVSC) of WHO has concluded that "There is currently no evidence of mercury toxicity in infants, children or adults exposed to thiomersal containing vaccines" and there is no reason to change current immunization practices with Thiomersal containing vaccines on ground of safety. Various studies in Denmark, Sweden, United States and UK indicated that autism and neurodevelopmental disorders are not associated with Thiomersal in the vaccines.

27. The media plays an important role in public perception following any AEFIs. What points should be kept in mind while communicating with media on such occasions?

The media provide the interface between immunization programs and the public. We cannot assume that the media share our views and we must recognize their independence.

Ignoring any stray report carries the risk of providing an "information vacuum" that ultimately proves detrimental to the vaccine program and consequently to public health.

Some key messages should be prepared before media contact and following facts should be included in it:

- The benefits of immunization in preventing disease are well proven. The risks of diseases and complications are more serious than that of immunization
- No vaccine is 100% safe
- No vaccine is 100% effective
- All vaccines have possible side effects, mostly mild, rarely severe
- The risk of disease far outweighs the risk of vaccine
- Immunization safety is of utmost importance and any suspicion of a problem is investigated

- The AEFI is currently being investigated, but is likely to be coincidental or due to a local problem (depending on the type of event) and the immunization program must continue to keep the population safe from disease.

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CHAPTER

7

Cold Chain and Vaccine Storage

Digant D Shastri

INTRODUCTION

An effective logistics system and a well-maintained cold chain are essential for safe and effective immunization service delivery, and it is a shared responsibility from the time the vaccine is manufactured until it is administered. The cold chain has three main components: (i) transport and storage equipment, (ii) trained personnel, and (iii) efficient management procedures. All three elements must combine to ensure safe vaccine transport and storage.

An improperly functioning of cold chain can lead to wasted vaccines, missed opportunities to immunize due to lack of vaccines, and children receiving vaccines that do not protect them as intended or that actually make them sick. Cold chain breaches can occur even in well-designed and well-managed systems as a result of technical malfunctions but if there are good procedures in place, problems will be detected and effectively managed. Efficient vaccine storage management is an essential quality assurance measure for vaccine service providers. Majority of vaccine storage and handling mistakes are easily avoidable.

1. What is cold chain?

The "cold chain" is the system of transporting and storing vaccines within the temperature range of 2–8°C from the place of manufacture to the point of administration. Immunization service providers should maintain their vaccine refrigerators as close as possible to 5°C, as this gives a safety margin of ± 3°C (Table 7.1).

2. What is the importance of cold chain?

The vaccines which are exposed to the higher temperature causes degradation and consequently total or partial loss of potency, while vaccine which are exposed to freezing causes and increase the local reactivity with or without loss of potency. Commonly the degradation rate of a vaccine is determined by the

TABLE 7.1 WHO recommended vaccine storage conditions

	National	*Intermediate*		*Primary health center*	*Subcenter/ session site*
		Regional	*District*		
OPV	−15°C to −25°C				
BCG	WHO no longer recommends that freeze-dried vaccines be stored at −20°C. Storing them at −20°C is not harmful but is unnecessary. Instead, these vaccines should be kept in refrigeration and transported at +2°C to 8°C.				
Measles			+2°C to +8°C		
Hep B					
DT					
DPT					
TT					

Diluent vials must NEVER be frozen. If the manufacturer supplies a freeze-dried vaccine packed with its diluent, ALWAYS store the product at between + 2°C and + 8°C. If space permits, diluents supplied separately from vaccine may safely be stored in the cold chain between + 2°C and + 8°C.

Abbreviations: OPV, Oral polio vaccine; BCG, Bacillus of Calmette and Guérin; Hep B, hepatitis B; DT, diphtheria and tetanus; DPT, diphtheria, pertussis and tetanus; TT, tetanus toxoid.

storage temperature: the higher the temperature, the more rapid and extensive is the degradation. There are considerable differences between degradation rates for different vaccines. An effective logistics system and a well-maintained cold chain are essential for safe and effective immunization service delivery.

3. What are the drawbacks of improper cold chain?

An improperly function of cold chain can lead to increased chances of adverse events, wastage of vaccines, missed opportunities to immunize due to lack of vaccines, and inability to provide immunity to recipient of vaccines and that may ultimately lead to loss of confidence in parents.

4. What are the important components of cold chain?

The cold chain has three main components:

1. Transport and storage equipment
2. Trained personnel
3. Efficient management procedures.

All the three elements must combine to ensure safe vaccine transport and storage.

5. Which are the vaccine storage equipments used in the immunization Program?

There are equipments of different capacity for storage of vaccines at different levels. They are:

- Walk-in freezer
- Walk-in coolers
- Deep freezer
- Ice-lined refrigerator (ILR)
- Purpose-built refrigerator
- Domestic refrigerator
- Isothermic cold boxes
- Vaccine carrier.

6. Between freezer and refrigerator, which is to be preferred for vaccine storage?

All vaccines except varicella and live intranasal influenza vaccine can safely be kept in the refrigerator within a temperature range of 2–8°C. In addition to not getting too warm (>8°C), most vaccines (the inactivated ones, in particular) must never freeze. Measles-mumps-rubella (MMR), a live virus vaccine, can be stored either frozen or refrigerated. Varicella vaccine can be temporarily placed in a refrigerator for up to 72 hours before use (as long as it is not reconstituted), but if it is not used within 72 hours, it must be discarded. Once stored in the refrigerator, it must not be refrozen.

7. Which is the most ideal vaccine storage device in vaccination clinic? Why?

Purpose-built vaccine refrigerator is the most ideal refrigerator for vaccine storage in vaccination clinic. The purpose-built refrigerators have following advantages over the domestic refrigerator:

- They are programmed to maintain an internal temperature between 2°C and 8°C
- Good temperature recovery—when the fridge is open to access the vaccines
- Cabinet temperature is not affected by ambient temperature and is stable and uniform
- They have an external temperature reading display and a maximum/minimum temperature continuous display, and an alarm for deviations outside the programmed temperature range
- Defrost cycle allowing defrosting without rise in cabinet temperature
- They automatically defrost
- Do not required to be modified for vaccine storage
- Nearly all internal space can be used to store the vaccines, so the size of the purpose built refrigerator may be smaller than the previously used domestic refrigerator.

8. What are the drawbacks of domestic refrigerator in storage of vaccines?

Domestic refrigerators used for the vaccine storage are basically designed for the storage of food and drink and usually have several temperature zones to meet the requirements of different foods. Since they are not designed for the special temperature needs of vaccines and place the safety of vaccines at risk. For vaccine storage the domestic refrigerator suffer from following drawbacks:

- Cabinet temperature varies significantly every time when the door is opened
- Cabinet temperature rises during defrosting in cycle in cyclic defrost and frost free refrigerator
- Cabinet temperature is easily affected by ambient temperature
- Temperature setting using dial is crude and inaccurate
- Whole inner space cannot be used to store the vaccines.

9. What are the different types of domestic refrigerator?

- Dormitory/bar style refrigerator: Single door with common freezer and cabinet compartment
- Cyclic type refrigerator
- Frost-free refrigerator
- Chest type refrigerator: Front opening door refrigerator
- Top lid opening refrigerator.

10. Why bar refrigerators are not recommended to use to store vaccines?

There are high chances of temperature instability and freezing injury to stored vaccines as well, and it has risk and susceptibility to ambient temperature. For all these reasons *bar* refrigerators are not strongly recommended.

11. Why frost-free refrigerators are recommended to use to store vaccines?

Frost-free refrigerators do not have heating cycles but have low level warming cycles and hence there are less chances of fluctuation of cabinet temperature outside 2–8°C range. The cyclic refrigerators have regular internal heating and that can cause wide fluctuation in the internal temperature. Hence, “frost-free” rather than cyclic type domestic refrigerators are recommended for storage of vaccines. Frost-free refrigerators usually have several temperature zones.

12. What are the prerequisites in domestic refrigerators for safe vaccine storage?

- Should have a separate freezer compartment
- Should exclusively used to store the vaccines

- Should maintains temperatures without fluctuating into the danger zones (< 2°C, >8°C)
  - The refrigerator compartment should maintain temperatures between 2°C and 8°C
  - The freezer compartment should maintain temperatures at or below 5°F (–15°C).
- Should not have required repairing for last 2 years
- Should be free of any water or coolant leaks
- The door seals should be in good condition and should seal tightly
- The door should close properly automatically on leaving it free
- The refrigerator compressor should be quiet and does not make sound
- The refrigerator is of an adequate size for the individual storage needs.

13. What are the points to be considered in placement of refrigerator?

- Refrigerator should be placed out of direct sunlight and away from heat
- It should be at least 10 cm away from wall
- *Should be placed in* secure area *so that the accessibility to* the vaccine refrigerator is restricted to identified staff only. This can minimize the risk of unnecessary door opening, accidental switched off of power at the power point.

14. What precautions should be observed while storing vaccines in domestic refrigerator?

- Vaccines must never be stored in the door of the refrigerator
- Place *freeze-tolerant vaccines* [measles, mumps, rubella, oral poliomyelitis vaccine (OPV) and bacillus of Calmette and Guérin (BCG)] in the shelves identified as being the coldest and *freeze-sensitive vaccines* [DTP containing vaccines; Hib, pneumococcal, influenza, hepatitis, inactivated polio vaccine (IPV) and some varicella vaccines] on shelves identified as having more stable temperatures (e.g. no "cold spots").
- Store the vaccines in enclosed plastic containers/labeled baskets
  - This will allows easy identification of vaccines and minimizes time spent with the door opened searching for vaccines
  - Enclosed plastic containers will help to stabilize temperatures a little and provide some protection in borderline freezing episodes as well as against effects from the cooling plate and blasts of cold air from outlets.
- Do not crowd the vaccines by overfilling the shelves. Allow space between containers for air circulation
- Storage of vaccine against refrigerator walls increases the risk of freezing. Ensure a *gap of at least 4 cm from all walls of refrigerator and between two large packs of vaccines*
- Keep a distance of at least 4 cm above and below the vaccine container to allow free air circulation
- Rotate stock so vaccines with the shortest expiry date are used first.

15. Can we straight away start using the refrigerator/freezer unit that is new or has just repaired?

- If the refrigerator/freezer unit is new or just repaired, allow one week of recorded temperature checks within normal ranges before placing vaccines into the unit

16. What is the importance of placing ice packs/gel packs in the freezer and water bottles in lower drawer and door?

- This will assist in stabilizing the temperature in refrigerator compartment, as in most frost-free refrigerators, cold air is distributed from the freezer to the fresh food compartment.
- It reduces warming periods when the refrigerator is opened. This is particularly useful if there is a power cut or other cause of refrigerator failure.
- It will be available for vaccine transport also.

17. What are the protocols for maintenance of the vaccine refrigerator?

- Refrigerator breakdowns should be repaired immediately
- Regularly check the door seals to ensure that a good seal is maintained. Replace the seals if they are damaged or cold air is leaking from the refrigerator
- Refrigerators that are not "frost-free" should be defrosted regularly to prevent ice build-up. If the freezer is not frost-free, ice should not be allowed to build-up more than 1/4 inch. Ice build-up reduces the efficiency and performance of a refrigerator. During defrosting or cleaning of the refrigerator, move the vaccines to a second refrigerator
- If there are exposed coils on the back of the refrigerator keep them clean and dust free to improve operating efficiency
- A summary of preventive maintenance of the vaccine refrigerator is described in Table 7.2.

TABLE 7.2 Checklist for preventive maintenance

External	*Internal*	*Technical*
• The exterior is clean • It is firm on the floor • It is properly leveled • Its sides are at least 10 cm away from walls • It is away from direct sunlight • Room is well ventilated • It is opened only when necessary	• Doors seals properly without gap • The door seal is clean • Ice packs are in proper position • Vaccines are neatly placed with space for air circulation • DPT, TT, Hep B and DT are not touching the cooling surface • Thermometer has been kept amongst the vaccine • Temperature is recorded twice a day	• Temperature is within prescribed limit (if not, set the thermostat) • Voltage stabilizer is working properly and equipment are connected through it • Plug of the voltage stabilizer is fitted properly to the power line • There is no abnormal noise • Compressor mounting bolts are tight

Abbreviations: DPT, diphtheria, pertussis and tetanus; TT, tetanus toxoid; Hep B, hepatitis B; DT, diphtheria and tetanus.

18. Which are cold sensitive vaccines?

- Diphtheria-tetanus-pertussis containing vaccines
- *Haemophilus influenzae* type B (the exception being the lyophilized PRP-T vaccines)
- Hepatitis B-containing vaccines
- Hepatitis A vaccine
- Influenza vaccine
- Pneumococcal (polysaccharide and conjugate) vaccines
- Meningococcal C conjugate vaccines
- Japanese encephalitis vaccine.

19. Which vaccines are heat sensitive?

- Bacille Calmette-Guérin vaccine
- Measles-mumps-rubella (MMR) vaccine
- Oral poliomyelitis vaccine
- Varicella-zoster vaccine
- Yellow fever vaccine
- All reconstituted vaccines

20. Which are light-sensitive vaccines?

Following vaccines are sensitive to strong light, sunlight, ultraviolet, fluorescents (neon), and exposure to light should be avoided.

- BCG
- Measles
- Rubella
- MMR
- Varicella.

21. What precautions should be taken while storing vaccines in ILR?

The ILR has got two sections: the top and bottom. The bottom of the refrigerator is the coldest place. The DPT, DT, TT and Hepatitis B vaccines should not be kept directly on the floor of the refrigerator, as they can freeze and get damaged. The DPT, DT, TT and hepatitis B vaccine along with diluents are to be placed in basket. A thermometer should also be placed in the basket along with the vaccines, as this gives the correct temperature. If no basket is available, place two rows of empty/water filled with ice packs on the floor of the ILR.

22. How to pack vaccines in isothermic cold boxes?

Before the vaccines are placed in the cold boxes, fully frozen ice packs should be placed at the bottom and sides of the cold box. The vaccines should be placed in cartons or polythene bags and then placed in the cold box. The vaccines are to be covered with a layer of fully frozen ice packs and the cold box is then closed. The vials of DPT, DT, HepB and TT vaccines should not be placed in direct contact with frozen ice packs and should be surrounded by OPV vials. Hard frozen ice packs (frozen at –20°C) should be conditioned before laying out in the cold box. This will protect "T" series vaccine from getting frozen.

23. What precautions should be taken while storing vaccines in vaccine carriers?

Vaccine carriers are used by health workers for carrying vaccines (16–20 vials) to subcenters or to villages or for delivery of vaccines from the supplier's shop to vaccination clinic. They maintain the cold chain during transport and for one day's use in the field. The inside temperature of a vaccine carrier is maintained between 2 to 8°C with four frozen ice packs for one day (if not opened frequently).

- Only vaccine carriers with four ice packs should be used
- Do not leave vaccine carriers in the sunlight
- Do not open the lid unnecessarily, as this can allow heat and light into the carrier, which may spoil vaccines
- Do not drop or sit on the vaccine carrier: this can damage the carrier.

24. How to safeguard vaccines during immunization sessions?

- Keep refrigerator door/cooler openings to a minimum
- Reconstitute vaccines immediately prior to administering
- When the vaccines are outside the vaccine carrier, keep them out of direct sunlight and away from other sources of heat and ultraviolet light (e.g. fluorescent light)
- Avoid handling vaccines any more than absolutely necessary. Take vaccine (and diluents if needed) from the cooler only as required.

25. How to safeguard vaccines during power failure?

- During a power failure of 4 hours or less, the refrigerator door should be kept closed
- During power failures more than 4 hours: if the back-up generator facility is lacking, identify an available unit at another nearby site and shift the vaccines to the alternative storage site
- If a refrigerator with a backup generator has not been located or is not working, and for power failures more than 4 hours, store vaccines in a cooler with conditioned ice packs/gel packs.
- Continue to monitor the temperature of the vaccines by placing the thermometer probe inside a vaccine box inside the cooler.

26. What should be line of action when cold chain breach is found?

- Immediately isolate the vaccines until you have been in touch with the relevant authority
- Keep vaccines refrigerated between 2°C and 8°C and label "Do not use".

- Do not discard any vaccine until advice has been sought. Depending on manufacturer specifications, the vaccine may still be viable.
- For privately purchased vaccines contact the manufacturer for advice.

27. What are the different temperature monitoring devices?

To measure the temperature during storage of vaccines, different types of thermometers are used. The different types of thermometers commonly used are:

- Standard fluid-filled: very easy to see and read temperature
- Dial: most common, but not the most accurate
- Minimum/maximum: tells the highest and lowest temperatures reached, but can be difficult to read
- Digital: very easy to read and some come with an alarm, but the temperature probe must be placed in the proper location inside the unit in order to get an accurate reading
- Continuous reading: It will record the temperature inside the unit at all times, 24 hours a day, on a sheet of paper, but the paper must be changed when it is running low. Using this thermometer is the most effective method of tracking the refrigerator/freezer temperature over time.

28. How to use them?

- Minimum/maximum: It should be placed on a middle shelf. Minimum/maximum thermometer must be reset regularly, the thermometer battery must be checked and replaced time to time and one should choose a thermometer which records temperature in Celsius
- Digital: Place the probe directly in contact with vaccine vial or package
- Continuous reading: It will record the temperature inside the unit at all times, 24 hours a day, on a sheet of paper, but the paper must be changed when it is running low. Using this thermometer is the most effective method of tracking the refrigerator/freezer temperature over time.

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CHAPTER

8

Adolescent Immunization

CP Bansal, Ashok K Banga

Why so much buzz around "adolescent immunization"?

Twenty-two percent of the country's population is of adolescents. In absolute numbers, it becomes 250,000,000, bigger than the whole population of many countries. Adolescents are the biggest and strongest resource of any country and their health represents the country's true growth potential. Unfortunately, the vaccination coverage in adolescents is dismal in relation to early childhood immunization. *Immunization is one of the most important preventive health services that can be provided to an adolescent.*

Adolescents are more vulnerable to a number of diseases and their complications because of their risk taking behavior. Their falling sick jeopardize the growth of the entire family and the nation. Hence, it becomes imperative that they receive the recommended vaccines to prevent them from morbidity and mortality.

With a better coverage of expanded program on immunization (EPI), the epidemiological trend of childhood diseases has seen a rightward shift leading to a surge of vaccine preventable diseases, such as diphtheria, pertussis, measles, mumps, etc. in adolescence and early adulthood. The susceptibility of the older population is also because of waning antibody levels induced by childhood immunization and lack of booster effect induced by subclinical natural infection. These are all the reasons to focus on adolescent immunization.

1. Which vaccines need to be recommended to adolescents?

Tetanus and diphtheria toxoids and acellular pertussis (Tdap), human papillomavirus (HPV), meningococcal vaccine, influenza, pneumococcal vaccine, hepatitis A, hepatitis B, measles, mumps and rubella (MMR), varicella and typhoid.

2. What is catch-up immunization?

Administration of vaccines at a later age, if due to any reason the vaccines have not been given at recommended age.

3. What are the recommendations about these vaccines?

Following are the recommendations for individual vaccines:

Tetanus and Diphtheria Toxoids and Acellular Pertussis (Tdap) Vaccine

Routine Vaccination

- *Minimum age:* 7 years [Adacel® is approved for 11–64-year olds by the Advisory Committee on Immunization Practices (ACIP) and 4–64 years old by the Food and Drug Administration USA (FDA), while Boostrix® for 10 years and older by ACIP and 4 years of age and older by FDA in USA]
- Administer 1 dose of Tdap vaccine to all adolescents aged 11 through 12 years
- *Tdap during pregnancy:* One dose of Tdap vaccine to pregnant mothers/ adolescents during each pregnancy (preferred during 27 through 36 weeks gestation) regardless of number of years from prior tetanus and diphtheria toxoids vaccine (Td) or Tdap vaccination.

Catch-up Vaccination

- *Catch up above 7 years:* Tdap, Td, Td at 0, 1 and 6 months
- Persons aged 7 through 10 years who are not fully immunized with the childhood diphtheria and tetanus toxoids and whole-cell pertussis (DTwP)/ diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccine series, should receive Tdap vaccine as the first dose in the catch-up series; if additional doses are needed, use Td vaccine. *For these children, an adolescent Tdap vaccine should not be given.*
- Persons aged 11 through 18 years who have not received Tdap vaccine should receive a dose followed by tetanus and diphtheria toxoids (Td) booster doses every 10 years thereafter.

Tdap vaccine can be administered regardless of the interval since the last tetanus and diphtheria toxoid-containing vaccine.

Human Papillomavirus (HPV) Vaccine

Routine Vaccination

- *Minimum age:* 9 years
- HPV4 [Gardasil®] and HPV2 [Cervarix™] are licensed and available
- Either HPV4 (0, 2, 6 months) or HPV2 (0, 1, 6 months) is recommended in a 2-dose series for females 9 to 13 years and 3-dose series from 14 to 45 years
- HPV4 can also be given in a 3-dose series for males aged 11 or 12 years, but not yet licensed for use in males in India
- The vaccine series can be started beginning at age 9 years
- Administer the second dose 1–2 months after the *first* dose and the third dose 6 months after the *first* dose (at least 24 weeks after the first dose).

Catch-up Vaccination

- Administer the vaccine series to females (either HPV2 or HPV4) at age 13 through 45 years, if not previously vaccinated.
- Use recommended routine dosing intervals (see above) for vaccine series catch-up.

Meningococcal Vaccine

- Recommended only for certain high-risk group of children, during outbreaks, and international travelers, including students going for studying abroad and travelers to Hajj and sub-Sahara Africa
- Both meningococcal conjugate vaccines (Quadrivalent MenACWY-D, Menactra® by Sanofi Pasteur and monovalent group A, PsA-TT, MenAfriVac® by Serum Institute of India) and polysaccharide vaccines (bi- and quadrivalent) are licensed in India. PsA-TT is not freely available in market
- Conjugate vaccines are preferred over polysaccharide vaccines due to their potential for herd protection and their increased immunogenicity, particularly in children younger than 2 years of age
- As of today, quadrivalent conjugate and polysaccharide vaccines are recommended only in children 2 years and above. Monovalent group A conjugate vaccine, PsA-TT can be used in children 1 year and above
- Meningococcal conjugate vaccine (MCV4):
  - Administer at age 11–12 years, or at age 13 through 18 years if not previously vaccinated
  - Administer to previously unvaccinated students living in a dormitory (crowded living)
  - Administer MCV4 to children aged 2 through 10 years with persistent complement component deficiency, anatomic or functional asplenia, or certain other conditions placing them at high risk
  - Administer to children previously vaccinated with MCV4 or meningococcal polysaccharide vaccine (MPSV4) who remain at increased risk after 3 years (if first dose administered at age 2 through 6 years) or after 5 years (if first dose administered at age 7 years or older)
- Persons whose only risk factor is living in on campus housing are not recommended to receive an additional dose.

Influenza Vaccine

Routine Vaccination

It is recommended only for the vaccination of persons with certain high-risk conditions.

- *First time vaccination:* 6 months to below 9 years: two doses 1 month apart; 9 years and above: single dose
- Annual revaccination with single dose
- *Dosage (TIV):* aged 6–35 months: 0.25 mL; 3 years and above: 0.5 mL
- For children aged 6 months through 13–14 or 14–15, administer 2 doses (separated by at least 4 weeks) to children who are receiving influenza vaccine for the first time
- All the currently available trivalent inactivated vaccines (TIVs) in the country contain the "Swine flu" or "A (H1N1)" antigen; no need to vaccinate separately
- Best time to vaccinate:
  - As soon as the new vaccine is released and available in the market
  - Just before the onset of rainy season.
- Administer annually to children aged 6 months through 18 years.

Pneumococcal Vaccines (PCVs)

Pneumococcal Polysaccharide Vaccine (PPSV), (PSV13) and Pneumococcal Polysaccharide Vaccine (PPSV23)

Routine Vaccination

- *Minimum age:* 6 weeks
- Both PCV10 and PCV13 are licensed for children from 6 weeks to 5 years of age (although the exact labeling details may differ by country). Additionally, PCV13 is licensed for the prevention of pneumococcal diseases *in adults older than 50 years of age*
- *Primary schedule (for both PCV10 and PCV13):* 3 primary doses at 6, 10 and 14 weeks with a booster at age 12 through 15 months.

Vaccination of Persons with High-risk Conditions

- PCV and pneumococcal polysaccharide vaccine (PPSV) both are used in certain high-risk group of children
- For children aged 24 through 71 months with certain underlying medical conditions, administer 1 dose of PCV13 if 3 doses of PCV were received previously, or administer 2 doses of PCV13 at least 8 weeks apart if fewer than 3 doses of PCV were received previously
- A single dose of PCV13 may be administered to previously unvaccinated children aged 6 through 18 years who have anatomic or functional asplenia (including sickle cell disease), HIV infection or an immunocompromising condition, cochlear implant or cerebrospinal fluid leak.

Pneumococcal Polysaccharide Vaccine (PPSV23)

- *Minimum age:* 2 years
- Not recommended for routine use in healthy individuals. Recommended only for the vaccination of persons with certain high-risk conditions as mentioned above
- Administer PPSV at least 8 weeks after the last dose of PCV to children aged 2 years or older with certain underlying medical conditions like anatomic or functional asplenia (including sickle cell disease), HIV infection, cochlear implant or cerebrospinal fluid leak
- An additional dose of PPSV should be administered after 5 years to children with anatomic/functional asplenia or an immunocompromising condition.
- PPSV should never be used alone for prevention of pneumococcal diseases amongst high–risk individuals.

Hepatitis A Vaccine (Hep A)

Administer 2 doses of killed vaccine at least 6 months apart or single dose of live vaccine. It can be given to children aged 7 through 18 years if not already received.

Hepatitis B Vaccine (Hep B)

Administer a 3-dose series to those not previously vaccinated.

A 2-dose series (separated by at least 4 months) of adult formulation for children aged 11 through 15 years.

Japanese Encephalitis (JE) Vaccine

Routine Vaccination

- Recommended only for individuals living in endemic areas
- Three types of new generation JE vaccines are licensed in India: one, live attenuated, cell culture derived SA 14-14-2, and two inactivated JE vaccines, namely "vero cell culture-derived SA-14-14-2 JE vaccine" (JEEV® by BE India) and "vero cell culture-derived, 821564XY, JE vaccine" (JENVAC® by Bharat Biotech).
- Live attenuated, cell culture derived SA-14-14-2:
  - *Minimum age:* 8 months
  - *Two-dose schedule:* first dose at 9 months along with measles vaccine and second at 16 to 18 months along with DTP booster
  - Not available in private market for office use.
- Inactivated cell culture derived SA-14-14-2 (JEEV® by BE India):
  - *Minimum age:* 1 year (US-FDA: 2 months)
  - *Primary immunization schedule:* 2 doses of 0.25 mL each administered intramuscularly on days 0 and 28 for children aged ≥1 to ≤3 years
  - Two doses of 0.5 mL for children >3 years and adults aged ≥18 years
  - Need of boosters still undetermined.
- Inactivated vero cell culture-derived Kolar strain, 821564XY, JE vaccine (JENVAC® by Bharat Biotech)
  - *Minimum age:* 1 year
  - *Primary immunization schedule:* 2 doses of 0.5 mL each administered intramuscularly at 4 weeks interval
  - Need of boosters still undetermined.

Catch-up Vaccination

- All susceptible children up to 15 years should be administered during disease outbreak or ahead of anticipated outbreak in campaigns.

Typhoid Vaccines

Routine Vaccination

- Both Vi-PS (polysaccharide) and Vi-PS conjugate vaccines are available
- Minimum ages:
  - Vi-PS (polysaccharide) vaccines: 2 years
  - Vi-PS (Typbar-TCV®): 6 months
- Vaccination schedule:
  - *Vi-PS (polysaccharide) vaccines:* single dose at 2 years; revaccination every 3 years; (no evidence of hyporesponsiveness on repeated revaccination so far)
  - *Vi-PS conjugate (Typbar-TCV®):* Single dose at 9–12 months and a booster during second year of life

- Vi-PS conjugate vaccine (PedaTyph®): data not sufficient to recommend for routine use
- Greater experience and more robust data with Vi-PS polysaccharide vaccines; whereas there is limited experience with Vi-PS conjugate vaccines.

Catch-up Vaccination

- Recommended throughout the adolescent period, i.e. 18 years.

Measles, Mumps, and Rubella (MMR) Vaccine

Routine Vaccination

- *Minimum age:* 12 months
- Administer the first dose of MMR vaccine at age 12 through 18 months, and the second dose at age 4 through 6 years
- The second dose may be administered before age 4 years, provided at least 4 weeks have elapsed since the first dose.

Catch-up Vaccination

- Ensure that all school-aged children and adolescents have had two doses of MMR vaccine; the minimum interval between the two doses is 4 weeks
- One dose, if previously vaccinated with one dose.

Varicella Vaccine

For children aged 7 through 18 years without evidence of immunity, administer two doses if not previously vaccinated or the second dose if only one dose has been administered.

For children aged 7 through 12 years, the minimum interval between doses is 3 months. However, if the second dose was administered at least 28 days after the first dose, it can be accepted as valid.

For children aged 13 years and older, the minimum interval between the doses is 28 days.

4. Can MMR and chickenpox vaccine be given at the same time?

Yes, but at different sites or as a combination vaccine if available.

5. Do you recommend MMR or rubella vaccine to adolescent girls?

MMR is the ideal recommendation for both boys and girls.

6. Rubella vaccination certificate is insisted by temple authorities at Thiruannamalai, Tamil Nadu, India for performance of marriage in Arunachala Eswara temple. Why not this is practiced everywhere?

Yes, it would be ideal if temple and school authorities everywhere insist on such a certificate for MMR (rather than rubella alone).

7. What are the recommendations for adolescents travelers?

All age appropriate vaccines should be completed before travel in addition to those listed in Table 8.1.

8. Why do American universities require MMR vaccination certificate from students coming from India?

Because measles still continues to be a problem in adolescents in some parts of USA. Mumps and rubella infection, although under control in USA, if emerges in a foreign student, can cause epidemiological concern.

9. What can be the adolescent immunization schedule card?

Figure 8.1 displays an IAP ACVIP immunization schedule for persons aged 7 through 18 years.

Figure 8.2 shows the IAP recommended immunization schedules for children aged 0–18 years, 2014.

- This schedule includes recommendations in effect as of 2014
- These recommendations must be read with the footnotes that follow. For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity as indicated by the green bars in Figure 8.2.

TABLE 8.1 Vaccines for adolescent travelers

Vaccine	*Place of travel**	*Dose recommended*
Meningococcal vaccine	USA/UK/endemic areas Saudi Arabia and Africa[#]	2 doses 4–8 weeks apart
Yellow fever^	Yellow fever endemic zones**	10 days before travel
Oral cholera vaccine	Endemic area or area with an outbreak	2 doses 1 week apart
Japanese B encephalitis	Endemic areas for Japanese encephalitis. In India: North Arcot district of Tamil Nadu and neighboring districts of Andhra Pradesh; also in most parts of South, Central, Northern, and North Eastern states; South-East Asian and East Asian countries	Single dose (upto 15 years)
Rabies vaccine (Pre-exposure prophylaxis)	For adolescents going on trekking, for children where pet dogs are present	0, 7, 28

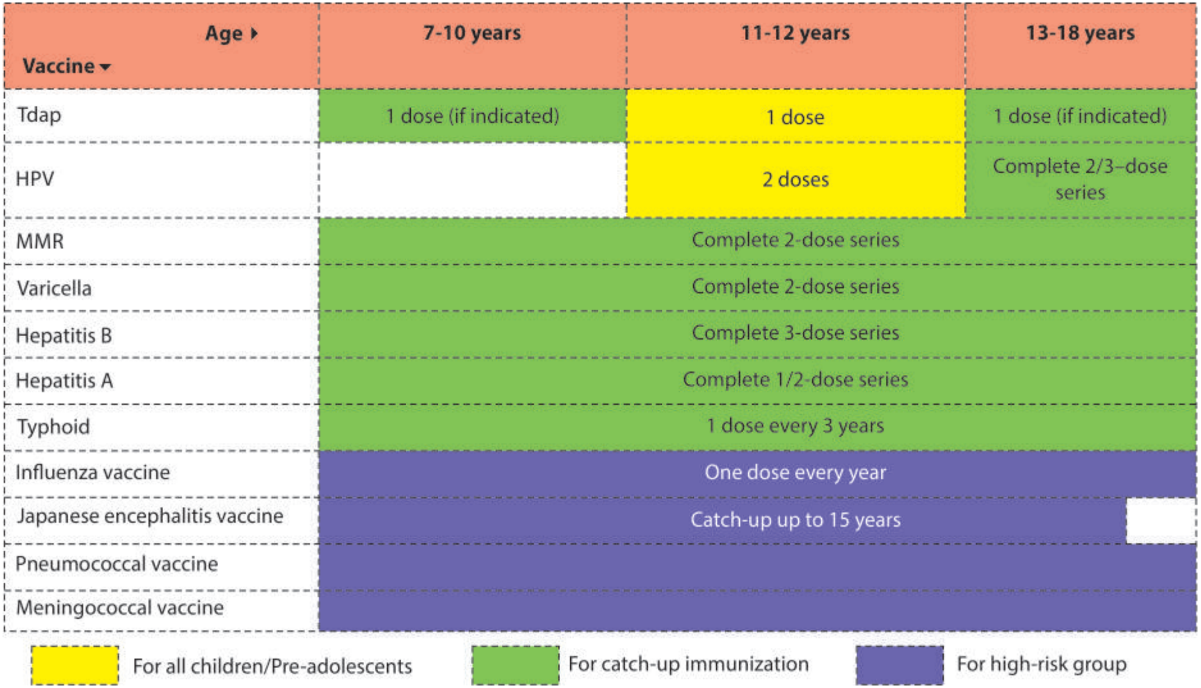
*All the persons, including adults going to Saudi Arabia for Hajj pilgrimage are required to take one dose of oral polio vaccine (OPV) in addition to quadrivalent meningococcal vaccine.

[#] Quadrivalent vaccine for those traveling to the US and bivalent (A+C) or quadrivalent for those traveling to the UK

^Mandatory for all travelers to yellow fever endemic zones as per International Health Regulations.

**The list of endemic countries can be obtained at http://wwwn.cdc.gov/travel/yellowBookCh4-YellowFever.aspx currently available only at select government controlled centers in India.

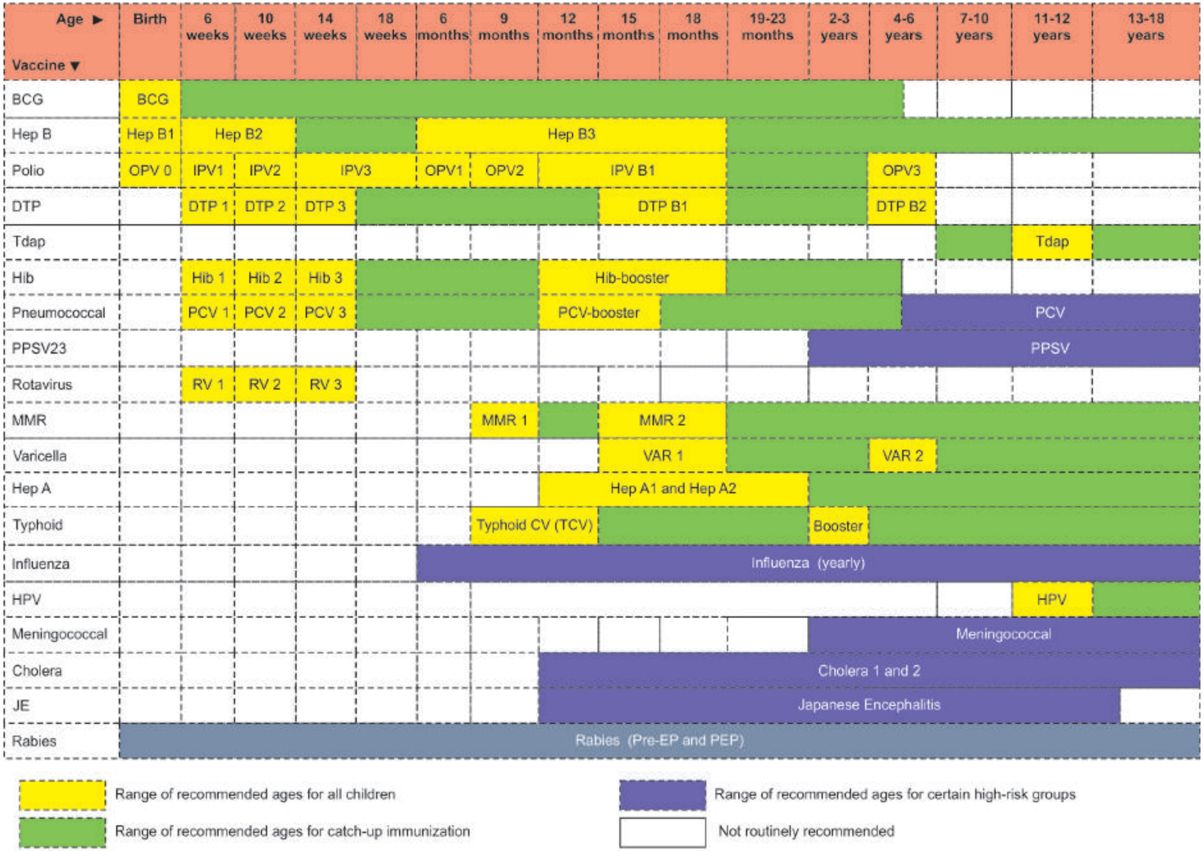
For more information on travelers vaccination, visit http://wwwnc.cdc.gov/travel/default.aspx



Vaccine ▾ / Age ▸	7-10 years	11-12 years	13-18 years
Tdap	🟩 1 dose (if indicated)	🟨 1 dose	🟩 1 dose (if indicated)
HPV		🟨 2 doses	🟩 Complete 2/3–dose series
MMR	🟩 Complete 2-dose series		
Varicella	🟩 Complete 2-dose series		
Hepatitis B	🟩 Complete 3-dose series		
Hepatitis A	🟩 Complete 1/2-dose series		
Typhoid	🟩 1 dose every 3 years		
Influenza vaccine	🟪 One dose every year		
Japanese encephalitis vaccine	🟪 Catch-up up to 15 years		
Pneumococcal vaccine	🟪		
Meningococcal vaccine	🟪		

🟨 For all children/Pre-adolescents 🟩 For catch-up immunization 🟪 For high-risk group

FIGURE 8.1 IAP ACVIP immunization schedule for persons aged 7 through 18 years, 2014 (with range)
For more details visit the URL: http://www.indianpediatrics.net/oct2014/oct-785-803.htm



Age ► / Vaccine ▼	Birth	6 weeks	10 weeks	14 weeks	18 weeks	6 months	9 months	12 months	15 months	18 months	19-23 months	2-3 years	4-6 years	7-10 years	11-12 years	13-18 years
BCG	BCG	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩			
Hep B	Hep B1	Hep B2		🟩	🟩	Hep B3					🟩	🟩	🟩	🟩	🟩	🟩
Polio	OPV 0	IPV1	IPV2	IPV3		OPV1	OPV2	IPV B1			🟩	🟩	OPV3			
DTP		DTP 1	DTP 2	DTP 3	🟩	🟩	🟩	🟩	DTP B1		🟩	🟩	DTP B2			
Tdap														🟩	Tdap	🟩
Hib		Hib 1	Hib 2	Hib 3	🟩	🟩	🟩	Hib-booster			🟩	🟩				
Pneumococcal		PCV 1	PCV 2	PCV 3	🟩	🟩	🟩	PCV-booster		🟩	🟩	🟩	PCV	🟪	🟪	🟪
PPSV23												PPSV	🟪	🟪	🟪	🟪
Rotavirus		RV 1	RV 2	RV 3												
MMR							MMR 1	🟩	MMR 2		🟩	🟩	🟩	🟩	🟩	🟩
Varicella									VAR 1		🟩	🟩	VAR 2	🟩	🟩	🟩
Hep A								Hep A1 and Hep A2				🟩	🟩	🟩	🟩	🟩
Typhoid							Typhoid CV (TCV)		🟩	🟩	🟩	Booster	🟩	🟩	🟩	🟩
Influenza						Influenza (yearly)	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪
HPV															HPV	🟩
Meningococcal												Meningococcal	🟪	🟪	🟪	🟪
Cholera								Cholera 1 and 2	🟪	🟪	🟪	🟪	🟪	🟪	🟪	🟪
JE								Japanese Encephalitis	🟪	🟪	🟪	🟪	🟪	🟪	🟪	
Rabies	Rabies (Pre-EP and PEP)	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦

🟨 Range of recommended ages for all children

🟩 Range of recommended ages for catch-up immunization

🟪 Range of recommended ages for certain high-risk groups

⬜ Not routinely recommended

- This schedule includes recommendations in effect as of September 2014
- For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity.

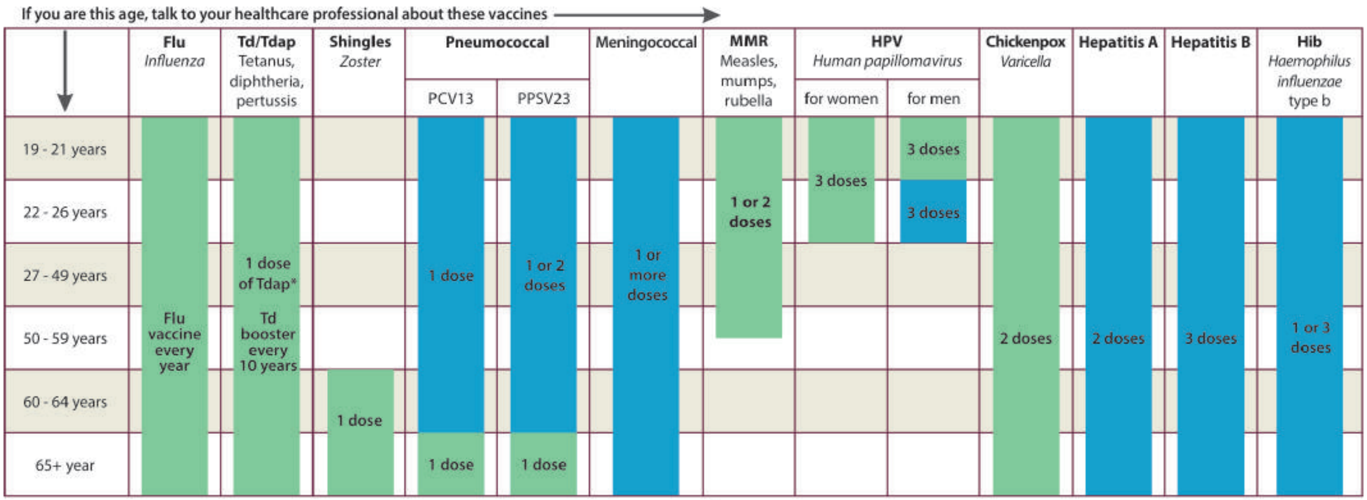
FIGURE 8.2 IAP recommended immunization schedule for children aged 0–18 years (with range), 2014
For more details visit the URL: http://www.indianpediatrics.net/oct2014/oct-785-803.htm

General instructions for immunizations:

- Vaccination at birth means as early as possible within 24 to 72 hours after birth or at least not later than one week after birth
- Whenever multiple vaccinations are to be given simultaneously, they should be given within 24 hours if simultaneous administration is not feasible due to some reasons
- The recommended age in weeks/months/years mean completed weeks/months/years
- Any dose not administered at the recommended age should be administered at a subsequent visit, when indicated and feasible
- The use of a combination vaccine generally is preferred over separate injections of its equivalent component vaccines
- When two or more live parenteral/intranasal vaccines are not administered on the same day, they should be given at least 28 days (4 weeks) apart; this rule does not apply to live oral vaccines
- If given <4 weeks apart, the vaccine given 2nd should be repeated
- The minimum interval between 2 doses of inactivated vaccines is usually 4 weeks (exception rabies)
- Vaccine doses administered up to 4 days before the minimum interval or age can be counted as valid (exception rabies). If the vaccine is administered >5 days before minimum period it is counted as invalid dose. Any number of antigens can be given on the same day
- Changing needles between drawing vaccine into the syringe and injecting it into the child is not necessary
- Different vaccines should not be mixed in the same syringe unless specifically licensed and labeled for such use
- Patients should be observed for an allergic reaction for 15 to 20 minutes after receiving immunization(s)
- When necessary, 2 vaccines can be given in the same limb at a single visit
- The anterolateral aspect of the thigh is the preferred site for 2 simultaneous IM injections because of its greater muscle mass
- The distance separating the 2 injections is arbitrary but should be at least 1 inch so that local reactions are unlikely to overlap
- Although most experts recommend "aspiration" by gently pulling back on the syringe before the injection is given, there are no data to document the necessity for this procedure. If blood appears after negative pressure, the needle should be withdrawn and another site should be selected using a new needle
- A previous immunization with a dose that was less than the standard dose or one administered by a nonstandard route should not be counted, and the person should be reimmunized as appropriate for age.

10. Do American universities require any other immunization certificate from adolescents of India?

Yes, some universities insist on complete immunization certificate since birth, but some insist for varicella, hepatitis A and typhoid.



If you are this age, talk to your healthcare professional about these vaccines →

↓	**Flu** *Influenza*	**Td/Tdap** Tetanus, diphtheria, pertussis	**Shingles** *Zoster*	**Pneumococcal** PCV13	**Pneumococcal** PPSV23	Meningococcal	**MMR** Measles, mumps, rubella	**HPV** *Human papillomavirus* for women	**HPV** *Human papillomavirus* for men	**Chickenpox** *Varicella*	**Hepatitis A**	**Hepatitis B**	**Hib** *Haemophilus influenzae* type b
19 - 21 years	🟩 Flu vaccine every year	🟩 1 dose of Tdap* Td booster every 10 years		🟦 1 dose	🟦 1 or 2 doses	🟦 1 or more doses	🟩 1 or 2 doses	🟩 3 doses	🟩 3 doses	🟩 2 doses	🟦 2 doses	🟦 3 doses	🟦 1 or 3 doses
22 - 26 years	🟩	🟩		🟦	🟦	🟦	🟩	🟩	🟦 3 doses	🟩	🟦	🟦	🟦
27 - 49 years	🟩	🟩		🟦	🟦	🟦	🟩			🟩	🟦	🟦	🟦
50 - 59 years	🟩	🟩		🟦	🟦	🟦	🟩			🟩	🟦	🟦	🟦
60 - 64 years	🟩	🟩	🟩 1 dose	🟦	🟦	🟦				🟩	🟦	🟦	🟦
65+ year	🟩	🟩	🟩	🟩 1 dose	🟩 1 dose	🟦				🟩	🟦	🟦	🟦
More information:	There are several flu vaccines available. Talk to your healthcare professional about which flu vaccines is right for you	*If you are pregnant, you should get a Tdap vaccine during the **3rd trimester of every pregnancy** to help protect your babies from pertussis (whooping cough)	You should get zoster vaccine even if you've had shingles before	There are two different types of pneumococcal vaccine: PCV13 (conjugate) and PPSV23 (polysaccharide). Talk with your healthcare professional to find out if one or both pneumococcal vaccines are recommended for you		Your healthcare professional will let you know how many doses you need	**Recommended for you if you did not get it when you were a child** If you were born in 1957 or after, and don't have a record of being vaccinated or having had measles, mumps and rubella, talk to your health care professional about how many doses you may need	There are two HPV vaccines but only one HPV vaccine (Gardasil®) should be given to men If you are a male 22 through 26 years old and have sex with men you should complete the HPV vaccine series if you have not already done so					Your healthcare professional will let you know how many doses you need

🟩 **Recommended For You:** This vaccine is recommended for you ***unless*** your healthcare professional tells you that you cannot safely receive it or that you do not need it

🟦 **May Be Recommended For You:** This vaccine is recommended for you if you have certain risk factors due to your health, job, or lifestyle that are not listed here. Talk to your healthcare professional to see if you need this vaccine

FIGURE 8.3 Recommended adult immunization schedule by vaccine and age group—United States 2014

11. What is the role of Td/Tdap in routine immunization?

The World Health Organization (WHO) now recommends Td instead of TT in prophylaxis of wound management and against neonatal and maternal tetanus. Td is to be repeated every 10 years. Tdap is given as a single booster at 10 years to those who can afford it.

12. Why might some adults need vaccines?

Some adults incorrectly assume that the vaccines they received as children will protect them for the rest of their lives. Generally this is true, except that:

- Some adults were never vaccinated as children
- Newer vaccines were not available when some of them were children
- Immunity can begin to fade over time
- As we age, we become more susceptible to serious disease caused by common infections (e.g. flu, Pneumococcus).

The Advisory Committee on Immunization Practices (ACIP) annually reviews the recommended Adult Immunization Schedule to ensure that the schedule reflects current recommendations for the licensed vaccines. Figure 8.3 displays the current recommended adult immunization schedule of the United States.

SUGGESTED READING

1. IAP Immunisation time-table 2013.
2. Recommended Adult Immunization Schedule – United States 2010. MMWR. [online] Available from: http://www.cdc.gov/mmwr/PDF/wk/mm5901-Immunization.pdf [Accessed on December 13, 2013].
3. Recommended Immunization Schedule for Persons Aged 7 through 18 Years—United States 2010. Available from: http://www.cdc.gov/vaccines/recs/ schedules/downloads/child/2010/10_7-18yrs-schedule-pr.pdf Accessed on December 5, 2010.
4. Singhal T, Amdekar YK, Agarval RK (Eds). IAP Guide Book on Immunization, 4th edition. IAP Committee on Immunization. New Delhi,India: Jaypee Brothers Medical Publishers (P) Ltd; 2009.
5. Updated Recommendation from the Advisory Committee on Immunization Practices (ACIP) for Revaccination of Persons at Prolonged Increased Risk for Meningococcal Disease. Morbidity & Mortality Weekly Review (MMWR) 2009;58:1042-3. [online] Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5837a4.htm [Accessed on December 13, 2013].

Section 2

EPI Vaccines

Chapters

CHAPTER

9

Questions Pertaining to Bacillus Calmette-Guérin Vaccine

A Parthasarthy, Hitt Sharma

1. When can we give bacillus Calmette-Guérin (BCG) vaccine to a newborn who did not receive the same at birth?

Bacillus Calmette-Guérin (BCG) vaccine is given anytime from birth until 2 weeks of age, so that there is a gap of 4 weeks until the next immunization at 6 weeks. If the opportunity to give BCG is not available in the neonatal period, it may be given at 6 weeks, simultaneously with diphtheria, pertussis, and tetanus (DPT) vaccine and oral polio vaccine (OPV). It is to be given to all children as a part of Expanded Program on Immunization (EPI) schedule.

Bacillus Calmette-Guérin vaccine is preferably given at birth to provide protection in the early years when infection can often lead to devastating widespread disease, such as miliary tuberculosis or tuberculous meningitis. This is particularly important in high prevalence countries where the chance of being infected in very early life is high.

Catch up vaccination with BCG is recommended till the age of 5 years. Routine tuberculin testing prior to catch up vaccination is not necessary.

2. Can we give BCG vaccine to a preterm/low birth weight (LBW) newborn or should we wait for 2 months or till the baby gains 2 kg weight?

In general, any LBW baby should be given BCG vaccine as soon as the baby is ready to be discharged. Some neonatologists might consider sending a stable baby home at weight as low as 1,500 g but for such babies the priority is not to give early BCG, but to wait until the weight has caught up to about 1,800 gm. Therefore, one should wait until the baby's immune system is robust enough, for which purpose, it is better to delay vaccination.

In terms of minimum gestational age, about 38 weeks, may be satisfactory provided the baby has reasonable weight (such as about 1,800 g or more) and the baby is ready for discharge.

There are studies which have shown that when BCG is given at birth to LBW babies, they show a poor Mx conversion and defective lymphocyte migration inhibition (LMI) response as compared to appropriate for gestational age (AGA) babies.

3. If the baby does not develop a scar after BCG vaccination what to do?

As per IAPCoI (now ACVIP) recommendations (2009-2011), BCG may be repeated once in children less than 5 years of age in the absence of a reaction/ scar presuming that BCG has not been taken up (even though most patients with absent reactions/ scars have shown in vitro evidence of cell mediated immunity against tuberculosis). Routine tuberculin testing prior to administration of the second dose of BCG is not necessary.

4. Can we give BCG vaccine to human immunodeficiency virus (HIV) positive/acquired immunodeficiency syndrome (AIDS) inflicted children or adolescents?

Since severe adverse effects of BCG vaccination are extremely rare in asymptomatic HIV positive infants, all healthy neonates should be BCG-vaccinated in areas endemic for tuberculosis. However, where resources permit, long-term follow-up of BCG-vaccinated infants of known HIV positive mothers is desirable for early treatment, should disseminated BCG disease occur in children with rapid development of immunodeficiency.

Infants and children with symptomatic HIV or those known to have other immunodeficiency states should not be BCG vaccinated. Very rarely indeed, disseminated BCG disease may result in the immunocompromized infant, which is usually fatal. For this reason, BCG should not be given to symptomatic HIV positive individuals.

5. Do you recommend BCG after infancy? If so, what precautions one should take?

As already stated, BCG is usually given anytime from birth to 15 days of in the neonatal period along with the zero dose of oral polio vaccine. If it is given beyond 6 months it is preferable to do a prior TST/Mx to ascertain if the patient is already sensitized to tuberculosis. If patient is already sensitized as shown by positive TST/ Mx, BCG is not necessary.

The following are certain other conditions wherein BCG vaccination may be recommended:

- Individuals, including healthcare workers and laboratory workers, repeatedly exposed to persons with untreated, inadequately treated or drug-resistant active tubercle bacilli (TB) may benefit from BCG vaccine. Consultation with a TB and/or infectious disease expert is recommended
- BCG vaccine may be considered for travelers in areas of high TB incidence, particularly when a program of serial TST and appropriate chemotherapy is

not possible or where the prevalence of drug resistance, particularly multidrug-resistant (MDR) TB, is high. This decision should be made in consultation with an infectious disease or travel medicine specialist

- Factors that favor the BCG option might include poor access to repeat skin testing, personal preference against taking isonicotinyl hydrazine (INH), contraindications to taking INH, such as liver disease or previous intolerance to INH, and the limited number of treatment options if infected with an MDR strain
- Travelers with medical conditions, particularly HIV infection, which may be associated with an increased risk of progression of latent TB infection to active disease, should carefully weigh, with their physician, the risk of travel to a high-incidence area in determining the most appropriate means of prevention.

6. What are the precautions to be taken before administering BCG vaccine?

The precautions include:

- Administering BCG vaccine intradermally and not to inject subcutaneously, intramuscularly, or intravenously
- Using a separate sterile needle and syringe, or a sterile disposable unit, for each individual patient to prevent disease transmission. All equipment, supplies and receptacles in contact with BCG vaccine should be handled and disposed of as biohazardous waste
- The vaccine should not be administered to individuals receiving drugs with antituberculous activity, since these agents may be active against the vaccine strain
- BCG immunization is contraindicated in persons with immune deficiency diseases, altered immune status due to malignant disease, and impaired immune function secondary to treatment with corticosteroids, chemotherapeutic agents or radiation
- Extensive skin disease or burns are also contraindications. BCG is contraindicated for individuals with a positive TST, although immunization of tuberculin reactors has frequently occurred without incident
- A review of each patient's immunization records to include history on reactions to immunizations should be completed prior to vaccination. All precautions should be taken for the prevention of allergic or any other side reactions. Epinephrine injection (1:1,000) for the control of immediate allergic reactions must be available should an acute anaphylactic reaction occur.

7. Almost all live vaccines now need a second dose following waning of immunity. Does BCG vaccine also need a second dose on that count? If so, at what age?

Subsequent booster inoculations of BCG are practiced in some countries, but this strategy is of no documented value. This also applies to revaccination of BCG-vaccinated individuals who remain negative by subsequent tuberculin testing. Neither Government of India (GoI) nor Indian Academy of Pediatrics recommends a second dose of BCG vaccine in India.

8. Some countries routinely recommend adolescent BCG vaccination in their National Immunization Program. What is the World Health Organization (WHO) recommendation?

BCG vaccination of adolescents and adults has shown variation in protective efficacy with geographical region, possibly as a consequence of differences in previous exposure to environmental mycobacteria. However, given the serious consequences of contracting MDR disease and the low reactogenicity of the vaccine, BCG vaccination should be offered to all unvaccinated, tuberculin-negative persons who are exposed to MDR tuberculous infection.

9. What are the reasons for variable efficacy of BCG vaccine? Why WHO still recommends BCG vaccination despite variable efficacy?

Though the reasons for the variable efficacy of BCG in different countries are difficult to understand, the following may stand true for the lack of efficacy in both low TB burden countries (US) and high TB burden countries (India):

- Background frequency of exposure to tuberculosis: It has been hypothesized that in areas with high levels of background exposure to tuberculosis, every susceptible individual is already exposed prior to BCG, and that the natural immunizing effect of background tuberculosis duplicates any benefit of BCG.
- Genetic variation in BCG strains: There is genetic variation in the BCG strains used and this may explain the variable efficacy reported in different trials.
- Genetic variation in populations: Difference in genetic make-up of different populations may explain the difference in efficacy.
- Interference by non-tuberculous mycobacteria: Exposure to environmental mycobacteria (especially *Mycobacterium avium*, *Mycobacterium marinum* and *Mycobacterium intracellulare*) results in a nonspecific immune response against mycobacteria. Administering BCG to someone who already has a non-specific immune response against mycobacteria does not augment the response that is already there. BCG will therefore appear not to be efficacious, because that person already has a level of immunity and BCG is not adding to that immunity.
- Interference by concurrent parasitic infection: Another hypothesis is that simultaneous infection with parasites changes the immune response to BCG, making it less effective. A T-helper type 1 (Th1) cell response is required for an effective immune response to tuberculous infection; one hypothesis is that concurrent infection with various parasites produces a simultaneous Th2-response which blunts the effect of BCG.

Despite the variable efficacy, WHO still recommends BCG vaccination as BCG is known to prevent life-threatening forms of tuberculosis, such as meningitis and disseminated disease in infants and young children. Vaccination with BCG remains the standard for tuberculosis prevention in most countries because it is available, is inexpensive and requires only one encounter with the patient; in addition, it rarely causes serious complications, and systems for early diagnosis and effective treatment of tuberculosis are lacking in many areas of the world.

10. Is BCG vaccine given routinely in developed countries as per WHO recommendation? If so, what are the countries which have included BCG vaccine in their national schedule?

The WHO recommends that BCG be given to all children born in countries highly endemic for TB because it protects against miliary TB and TB meningitis. However, developed countries like the US and the UK have not introduced the vaccine in their National Immunization Program. They rely more on the detection and treatment rather than universal immunization of all children. The age of the patient and the frequency with which BCG is given has always varied from country to country.

- *US:* The US has never used mass immunization of BCG, relying instead on the detection and treatment of latent tuberculosis.
- *UK:* The UK introduced universal BCG immunization in 1953 and until 2005; the UK policy was to immunize all school children at the age of 13, and all neonates born into high-risk groups. The injection was only given once during an individual's lifetime (as there is no evidence of additional protection from more than one vaccination). BCG was also given to protect people who had been exposed to tuberculosis. The peak of tuberculosis incidence is in adolescence and early adulthood, and the evidence from the Medical Research Council (MRC) trial was that efficacy lasted only 15 years at most. Styblo and Meijer argued that neonatal immunization protected against miliary TB and other noncontagious forms of TB and not pulmonary TB which was a disease of adults, and that mass immunization campaigns with BCG would therefore not be expected to have a significant public health impact. For these and other reasons, BCG was therefore given to time with the peak incidence of pulmonary disease. Routine immunization with BCG was withdrawn in 2005 because of falling cost-effectiveness; whereas in 1953, 94 children would have to be immunized to prevent one case of TB; by 1988, the annual incidence of TB in the UK had fallen so much that 12,000 children would have to be immunized to prevent one case of TB.
- *France:* The BCG was mandatory for school children between 1950 and 2007 and for healthcare professionals between 1947 and 2010. Vaccination is still available for French healthcare professionals and social workers but is now decided on a case by case basis.
- *India:* India introduced BCG mass immunization in 1948, the first non-European country to do so.
- *Brazil:* Brazil introduced universal BCG immunization in 1967–1968, and the practice continues until the present day. According to Brazilian law, BCG is given again to professionals of the health sector and to people close to patients with tuberculosis or leprosy.

- *Norway:* In Norway, the BCG vaccine was mandatory from 1947 to 1995. It is still available and recommended for high-risk groups.
- *South Africa:* In South Africa, the BCG vaccine is given routinely at birth, to all newborns, except those with clinically symptomatic AIDS.
- *Other countries:* In the UK, BCG was only ever given once (as there is no evidence of additional protection from more than one vaccination), but in some countries, such as the former USSR, BCG was given regularly throughout life. In South Korea, Singapore, Taiwan and Malaysia, BCG was given at birth and again at the age of 12. But in Malaysia and Singapore, from 2001, this policy was changed to once only at birth and it was discontinued in South Korea.

11. What are the results of recent meta-analysis on BCG vaccination? Despite repeated passages over many years, do the BCG strains retain their immunogenicity?

Meta-analysis of 10 randomized and controlled studies showed that the average protection against TB meningitis and disseminated disease was 86%; the corresponding result of case-control studies was 75%. In another analysis, that included 15 prospective and 12 case-control studies, the BCG-induced protection against TB disease was 51% and 50%, respectively. The protection against TB related death was 65%, against TB meningitis was 64% and against disseminated TB was 78%. Few reports show high protective efficacy following BCG vaccination of adults. However, in the late 1920s, BCG vaccination of tuberculin-negative Norwegian nursing students before entering TB wards reduced the development of tuberculous disease by greater than 80%, during a 3-year observation period.

The current vaccine strains are all descendants of the original *Mycobacterium bovis* isolate that Calmette and Guérin passaged through numerous cycles during the 13-year period 1909–1921. Subsequent passages under different laboratory conditions resulted in a variety of new BCG strains showing phenotypic as well as genotypic differences. Though, a number of BCG vaccine strains are available, in terms of efficacy, no BCG strain is demonstrably better than another, and there is no global consensus as to which strain of BCG is optimal for general use.

For instance, in India BCG vaccine manufactured by Serum Institute of India Ltd., Pune, India was developed using Moscow BCG-I (Russian) strain. This vaccine was licensed in India in October, 2001 and subsequently prequalified by WHO in June, 2003 for purchase by UN agencies for developing countries. Since then, millions of doses have been administered worldwide clearly indicating that this vaccine is safe and can be used effectively for prevention of miliary tuberculosis and tubercular meningitis. Apart from the BCG vaccines manufactured by BCG Vaccine Laboratory, Guindy, Chennai, India and Green Signal Bio Pharma Pvt. Ltd., Chennai, India the only WHO prequalified BCG vaccine being used by GoI in its National Immunization Program, is by Serum Institute of India Ltd., Pune, India.

12. What are the adverse effects following BCG vaccination and how to manage them?

The usual response to (intradermal) administration of BCG vaccine is the development of erythema, induration and either a papule or ulceration, followed by a scar at the immunization site. No treatment is required for this condition. Secondary infection at the vaccination site may require antimicrobials.

Rates of adverse reactions appear to vary with the strain of vaccine, dose and method of immunization and the age of the recipient. Adverse reactions are more common in young vaccinees (infants versus older children) and are frequently related to improper technique in administration (mainly improper dilution).

Intradermal administration of BCG vaccine usually results in the development of erythema and either a papule or ulceration (in about 50%), followed by a scar at the immunization site. Keloid formation occurs in 2% to 4% of vaccine recipients. Non-suppurative regional lymphadenopathy occurs in 1% to 10%. Most reactions are generally mild and do not require treatment. Antitubercular therapy is of no benefit in such situations and should not be administered. The nodes regress spontaneously after a few months. It should also be noted that if fine needle aspiration cytology of the nodes is carried out, stain for acid-fast bacilli may be positive. These are bovine vaccine bacilli and should not be misconstrued as being suggestive of tuberculous disease. In some children, the nodes may even liquefy and result in an abscess. Surgical removal of the nodes or repeated needle aspiration is the treatment of choice again, antitubercular therapy is not recommended in this situation also.

Since neonates have a higher risk of vaccine-induced suppurative lymphadenitis than older children, infants aged less than 30 days should receive a reduced dose of the vaccine.

A review of published and unpublished data, including a survey sponsored by the International Union Against Tuberculosis and Lung Disease, recorded 10,371 complications following almost 1.5 billion BCG vaccinations in adults and children. The most serious complication of BCG vaccination was disseminated BCG infection, which occurred in 3 per 1 million recipients. In that review, dissemination was fatal in 0.02 per 1 million vaccine recipients and occurred in children who had primary immunodeficiencies.

13. In view of the closure of public sector undertakings (PSUs) in India, only BCG vaccine manufactured by private sector is available. Are they WHO prequalified laboratories? Are there any efficacy studies available on the indigenous BCG vaccine formulations? Can we use them freely?

Bacillus Calmette-Guérin vaccine in India is manufactured by Serum Institute of India Ltd., Pune, India; BCG Vaccine Laboratory, Guindy, Chennai, India; and Green Signal Bio Pharma Pvt. Ltd., Chennai, India. BCG Vaccine Laboratory, Guindy, Chennai, India has since resumed production after the PSU revival.

The only private sector, WHO prequalified vaccine used in India, is by Serum Institute of India Ltd., Pune, India. The vaccine meets all the quality requirements and has been prequalified by WHO for purchase by UN agencies. Millions of doses of this vaccine have been administered worldwide. Numerous clinical studies conducted with the vaccine and number of doses administered over the years clearly indicates that this vaccine is safe and can be used effectively for prevention of serious tuberculosis infection and tubercular meningitis.

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CHAPTER

10

Polio

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1. What is the clinical spectrum of poliomyelitis?

Table 10.1 summarizes various types of clinical features and syndrome seen with a wild poliovirus infection.

2. What are the different vaccines available for polio?

There are two types of polio vaccines, first the inactivated polio vaccine (IPV) by Jonas Salk and other the live oral polio vaccine (OPV) by Albert Sabin. Both these vaccines contain all the three types of polioviruses.

TABLE 10.1 Clinical spectrum of poliomyelitis

S. No.	*Presentation*	*Frequency*	*Clinical features*
1	Asymptomatic	Up to 95%	Inapparent infection without symptoms
2	Minor illness ("Abortive")	4–8%	No CNS involvement Three syndromes: upper respiratory tract infection, gastrointestinal disturbances, and influenza-like illness
3	Major illness	3%	With CNS involvement
3.1	Nonparalytic	1–2%	Aseptic meningitis
3.2	Paralytic	<1%	
3.2.1	Spinal	79% of paralytic cases	Asymmetric paralysis that most often involves cases of the legs
3.2.2	Bulbar	2% of paralytic cases	Weakness of muscles innervated by bulbar cranial nerves
3.2.3	Bulbospinal	19% of paralytic cases	A combination of bulbar and spinal paralysis

The ratio of paralytic cases to infections was estimated per 100 infections at approximately 0.5 for serotype 1, 0.05 for serotype 2, and 0.08 for serotype 3.
Abbreviation: CNS, central nervous system.

3. What are the different types of OPV?

Different types of OPV are:

- *Trivalent OPV (tOPV):* tOPV used in national RI program for polio vaccination and in selective supplementary immunization activities (SIA) campaigns in pulse polio program, National Immunization Days (NIDs)
- *Monovalent OPV (mOPV):* mOPV (mOPV1 for type 1 and mOPV3 for type 3), licensed in 2005 to enhance the impact of SIAs in the key remaining reservoirs of wild polio virus (WPV)
- *Bivalent OPV (bOPV):* bOPV (for type 1 and type 3), licensed in 2010 and used effectively in SIAs to break the chain of transmission of both type1 and type 3 virus together in certain areas.

4. What are the features of tOPV?

Trivalent OPV is a trivalent vaccine consisting of a suspension of live attenuated poliovirus types 1, 2 and 3 grown in monkey kidney cell cultures and stabilized with magnesium chloride. It is presented in a buffered salt solution, with light pink color indicating the right pH. OPV is available as vial containing multidose. The dose is two drops per dose. It is a very safe vaccine.

5. What is the shelf life of OPV?

Oral polio vaccine is a very heat sensitive vaccine having a shelf life of 2 years at a temperature of –20°C, 6 months at 2–8°C and 1–3 days at room temperature (depending upon the season and room temperature). OPV should be stored at –20°C at the state and district level and in the freezer at the clinic level. The vaccine must reach the outreach facility at 2–8°C in vaccine carriers with ice packs. The dose of OPV is two drops orally.

6. What is the impact of thawing on OPV?

Multiple freeze thaw cycles should be avoided as the virus loses its potency. After thawing, it should be kept at temperatures between 2°C and 8°C for a maximum of 6 months.

7. Is there any difference in the vaccine if it is of a different color?

Usually, the color of an OPV is pink. However, sometimes the color may also be yellow or white. All the vaccines are the same and this color difference in no way affects the quality or type of vaccine.

8. If a child is given more than two drops at the time of immunization then what will be the harm to the child?

If more than two drops are given to the child, due to any reason, then there will be no harm to the child as OPV is among very safe vaccines.

9. If a child is vaccinated with an OPV vial having third stage of vaccine vial monitor (VVM), what will be the harm to the child?

Third stage of VVM on OPV vial indicates that vaccine in the vial has lost its potency due to breech in cold chain. If any child is immunized with such vial, then it will not produce the immunity in the child and such child will remain unprotected from disease, but there will be no harm to the child.

10. What are the contraindications of OPV?

Oral polio vaccine is contraindicated in patients with low immunity especially if inactivated polio vaccine (IPV) is available. OPV can be given to a patient with diarrhea, but that dose should not be counted and should be followed by an extra-dose.

11. What are the side effects of OPV?

Oral polio vaccine is very safe vaccine and has minimum side effects. It can lead to gastrointestinal (GI) upset like diarrhea and vomiting. It does not lead to fever. The most important but rare side effect with OPV is vaccine-associated paralytic poliomyelitis (VAPP). Massive benefits of OPV far outweigh the rare risk of paralysis.

12. What is VAPP?

Vaccine-associated paralytic poliomyelitis (VAPP) is serious adverse effect associated with OPV. It is defined as those cases of acute flaccid paralysis (AFP), which have residual weakness 60 days after the onset of paralysis and from whose stool samples, vaccine-related poliovirus but no WPV is isolated. VAPP may occur in the vaccine recipient (recipient VAPP, occurring within 4–40 days of receiving OPV) or contact of the vaccine recipient (contact of VAPP). VAPP occurs due to loss of attenuating mutations and reversion to neurovirulence during replication of the vaccine virus in the gut.

13. What is the incidence rate of VAPP?

The incidence of VAPP has been estimated at four cases per million (1/1,000,000) birth cohort per year in countries using OPV. The incidence of VAPP in developed countries, such as USA, has been reported to be 1 per 2.4 million doses distributed and 1 per 750,000 with first dose. The risk of VAPP in India has been estimated to be 1 per 4.1–4.6 million doses distributed and 1 per 2.8 million first dose recipient risks.

14. Is it true that Indian kids are immune against VAPP? If yes, why is it so?

The lower risk of VAPP in India might be attributed to high prevalence and titer of maternal antibodies, birth dose of OPV and early immunization with OPV in the RI schedule.

15. What is vaccine-derived poliovirus ? What is its importance?

Vaccine-derived polioviruses (VDPV) are rare but well-documented strains of poliovirus which emerge after prolonged multiplication of attenuated strains of the virus contained in the OPV in the guts of children with immunodeficiency or in populations with very low immunity. After prolonged multiplication, these vaccine viruses derived strains change and revert to a form that can cause paralysis in humans. Some VDPVs have shown a capacity for sustained circulation in communities.

16. How VDPV differs from original Sabin strain?

Vaccine-derived poliovirus arises due to mutation and recombination in the human gut and is 1–15% divergent of VP1 nucleotide from the parent vaccine virus. They are capable of both neurovirulence and transmissibility, VDPVs have caused outbreaks, including in the neighboring countries of China, Myanmar and Indonesia and huge outbreak in Nigeria. Since 2009 globally, the Sabin type 2 in the tOPV has been responsible for greater than 90% of all circulating vaccine-derived poliovirus (cVDPV) cases and 40% VAPP cases due to low immunity against type 2 hence for VDPV type 2, cases with greater than or equal to 6th difference from the Sabin in VP1; and for VDPV types 1 and 3, with greater than or equal to 10th difference from Sabin in VP1 are considered as VDPV.

17. What are the types of VDPV?

They have been classified into three groups:

1. *Circulating VDPV:* VDPV with evidence of virus circulation in the population causing two or more paralytic cases.
2. *Immunodeficiency-related VDPV (iVDPV):* VDPV in the immunodeficient person and
3. *Ambiguous VDPV (aVDPV):* VDPV isolated from environmental sources or evidence of circulation not established. During 2000–2013, 699 cases of cVDPVs were reported. Since 2006, majority of cVDPV cases are due to type 2.

18. How can a VDPV circulation be stopped?

The management of VDPVs is a necessary part of the global polio eradication effort, and is similar to management of wild poliovirus outbreaks, i.e. by rapid implementation of large-scale, high-quality SIAs. Global experience with VDPVs shows that they are less virulent than wild poliovirus strains, and can be rapidly stopped, with 2–3 rounds of high-quality, large-scale SIAs.

19. Is there a difference in a disease caused by a VDPV and one caused by wild poliovirus?

No, there is no clinical difference between paralytic polio caused by wild poliovirus or a VDPV.

20. What are the salient features of IPV?

Inactivated polio vaccine is formaldehyde killed poliovirus, grown in monkey kidney cell or human diploid cells. Old IPV contained 20, 8 and 32 D antigen units of types 1, 2 and 3 polioviruses respectively. All currently used IPV vaccines are enhanced potency IPV (eIPV) which contain 40, 8 and 32 D antigen units of type 1, 2 and 3 respectively. Currently the term IPV means eIPV. The vaccine should be stored at 2–8°C. It is highly immunogenic. Its immunogenicity is dampened by the presence of maternal antibody in the very young infant, especially up to the age of 8 weeks. IPV based on the attenuated Sabin virus strain (sIPV) was recently developed and licensed in Japan.

21. How IPV is to be given?

Inactivated polio vaccine should be given intramuscularly (preferably) or subcutaneously and may be offered as a component of fixed combinations of vaccines. The dose is 0.5 mL. IPV is available as single dose vial containing 0.5 mL of vaccine. It is also available in combination with other vaccines.

22. How efficacious is IPV?

Seroconversion rates of IPV are 90–100% after two doses given after the age of 2 months and at 2 months interval or in the Expanded Program on Immunization (EPI) schedule of three doses at 6,10 and 14 weeks. A third dose, given after a suitable interval boosts the antibody levels and ensures the perpetuation of immunity for decades and more.

23. Can IPV be given with other vaccines?

Inactivated polio vaccine can be administered along with all other childhood vaccines and can be used in combination with diphtheria, tetanus and pertussis (DTwP/DTaP), *Haemophilus influenzae* type B (Hib) and hepatitis B vaccines without compromising seroconversion or increasing side effects. The vaccine is very safe.

24. What are the demerits of IPV?

High cost, scarce availability—only 2 major manufacturers, injectable route—extra injections needed (monovalent use).

25. Can polio vaccine be given to a child who has received immunoglobulin?

The administration of polio vaccine should be delayed by at least 6 weeks after administration of immunoglobulin.

26. Can polio vaccines be administered to immunocompromized subjects?

Inactivated polio vaccine is the vaccine recommended for vaccination of immunodeficient persons and their household contacts. Many immunodeficient persons are immune to polioviruses as a result of previous vaccination or exposure to wild virus when they were immunocompetent. While OPV is contraindicated in such subjects, IPV is the vaccine of choice. Although a protective immune response in these persons cannot be ensured, IPV might confer a significant protection.

27. What should be schedule for polio immunization in children with human immunodeficiency virus (HIV)?

In HIV children, IPV to be given at 6, 10, 14 weeks; 15–18 months, and 5 years. If indicated, IPV to be given to household contact. If IPV is not affordable or available, OPV should be given as OPV has been found to be generally safe in HIV infected especially in early stages.

28. What is the risk of serious reactions following IPV?

In very rare circumstances, IPV can cause soreness, local reaction in 10–20% of vaccines due to poor aseptic precautions and rarely serious problems, such as a severe allergic reaction. It can lead to fever which is mild and lasts for 24–48 hours.

As IPV contains trace amounts of streptomycin, neomycin and polymyxin B, allergic reactions may be seen in individuals with hypersensitivity to these antimicrobials. IPV should not be administered to persons who have experienced a severe allergic reaction after a previous dose of IPV or to streptomycin, polymyxin B, and neomycin.

29. What is the minimum age for IPV vaccination?

Minimum age for primary vaccination of IPV is 6 weeks.

30. What is the National Immunization Schedule for polio vaccination?

As per the National Immunization Schedule, five doses of tOPV are to be given; 0 dose at birth, three primary doses at 6, 10 and 14 weeks and one booster dose at 16–24 months of age. Another dose is given at the age of 5 years. Apart from this, OPV is to be given to every child till the age of 5 years in every SIA being conducted in the area.

31. What is the primary schedule for polio vaccination as per recent Indian Academy of Pediatrics Committee on Immunization (IAPCOI) recommendation?

As per the recommendation of IAPCOI timetable 2012, the polio vaccination schedule is depicted in Table 10.2.

TABLE 10.2 Indian Academy of Pediatrics Committee on Immunization (IAPCOI) timetable 2012, the polio vaccination schedule

Birth	OPV0
6 weeks	IPV1
10 weeks	IPV2
14 weeks	IPV3
6 months	OPV1
9 months	OPV2
16–18 months	IPV B1
4½ to 5 years	OPV3

Abbreviations: OPV, oral polio vaccine; IPV, inactivated polio vaccine.

In addition to this, all OPV doses (mono-, bi- or trivalent) offered through SIAs, should also be provided.

32. What are the major changes for polio immunization recommendation in Indian Academy of Pediatrics (IAP) timetable 2012?

In the light of remarkable achievement in the field of polio eradication in India over the last few years, as per the IAPCOI timetable 2012, sequential IPV-OPV schedule is recommended for primary polio immunization in place of combined OPV and IPV schedule.

33. What are the reasons for these changes in recommendation?

This policy is in accordance with the recent decision taken by Global Polio Eradication Initiative (GPEI) where phased removal of Sabin viruses, beginning with highest risk (type 2) would be undertaken. This will pave the way to ultimate adoption of all-IPV schedule in future considering the inevitable cessation of OPV from immunization schedules owing to its safety issues (VAPP and cVDPVs). This will result in elimination of VDPV type 2 in “parallel” with eradication of last wild polioviruses by switching from tOPV to bOPV for routine EPI and campaigns.

34. What are the benefits of sequential schedule?

There are moderate level of scientific evidence that sequential immunization schedules starting with two or more doses of IPV and followed by two or more doses of OPV (at an interval of 4–8 weeks) induce protective immunological responses to all three polio virus serotypes in greater than or equal to 90% of recipients. Sequential schedules that provide IPV first, followed by OPV, can prevent VAPP while maintaining the critical benefits conferred by OPV (i.e. high levels of gut immunity). Data from several studies show that sequential schedules considerably decrease the risk of VAPP.

35. Is there any alternative schedule in IAPCOI timetable for primary vaccination of polio with two doses of IPV instead of three doses?

Yes, alternatively two doses of IPV can be used for primary series at 8 and 16 weeks. Though this schedule is immunologically superior to EPI schedule and the number of IPV doses is reduced, but will be more cumbersome due to extra-visits and incompatibility with combination formulations. Further, the child would be susceptible to WPV infection for the first 2 months of life considering the epidemiology of WPV in India till quite recently.

36. In the schedule recommended by IAPCOI, can OPV be used in place of IPV?

All doses of IPV may be replaced with OPV if IPV is unaffordable or unavailable. The primary schedule must be completed with three doses of OPV given at 6, 10, and 14 weeks. No child should be left without adequate protection against WPV (i.e. three doses of either vaccine).

37. If IPV first, followed by OPV can prevent VAPP then why IAPCOI has retained birth dose of OPV before IPV in immunization timetable 2012?

Providing the first OPV dose at a time when the infant is still protected by maternally derived antibodies may, at least theoretically, also prevent VAPP. A birth dose of OPV is considered necessary to induce gut immunity in countries where the risk of poliovirus transmission is high.

38. Why booster of IPV is required?

Since IPV administered to infants in EPI schedule (i.e. 6, 10 and 14 weeks) results in suboptimal seroconversion, hence, a supplementary dose of IPV is recommended at 15–18 months.

39. What is catch-up schedule of polio vaccination as per IAPCOI?

Inactivated polio vaccine may be offered as “catch-up vaccination” for children less than 5 years of age who have completed primary immunization with OPV. IPV can be given as three doses; two doses at 2 months interval followed by a third dose after 6 months. This schedule will ensure a long lasting protection against poliovirus disease.

40. What are the recommendations for polio vaccination for travelers?

The IAPCOI has now issued the following recommendations for travellers to polio-endemic countries:

- For those who have previously received at least three doses of OPV or IPV should be offered another dose of polio vaccine as a once-only dose before departure

- Nonimmunized individuals should complete a primary schedule of polio vaccine, using either IPV or OPV. Primary series includes at least three doses of either vaccine
- For people who travel frequently to polio-endemic areas but who stay only for brief periods, a one-time only additional dose of a polio vaccine after the primary series should be sufficient to prevent disease.

41. What is the schedule and dose in preterm and low birth weight (LBW) babies?

Dose and schedule are the same in preterm or LBW babies as after birth dose of OPV, vaccination starts at the age of 6 weeks. Birth dose of OPV can be given safely in these children.

42. What should be the schedule for polio immunization if a child comes late?

Polio immunization is given to any child presents till 5 years of age. Such a child should receive three primary doses followed by first booster 1 year after third primary dose and a second booster 3–4 years after the first booster dose provided the child is less than 5 years old. As per WHO recommendation, infants starting the routine immunization schedule late (age >3 months), the IPV dose should be administered at the first immunization contact.

43. What are the merits/demerits of OPV and IPV?

The difference of both vaccines is as given in Table 10.3.

TABLE 10.3 Merits and demerits of OPV and IPV

OPV	*IPV*
Oral, ease of administration, suitable for mass campaigns	Necessity of skilled personnel for injection
High systemic immunity except in developing countries	High systemic immunity
High gut immunity, pharyngeal immunity	Lower gut immunity but good oropharyngeal immunity
High efficacy (but suboptimal efficacy in tropical countries)	High efficacy
Risk of VAPP	No risk of VAPP
Risk of VDPVs	No risk of VDPVs
Contraindicated in immunodeficient individuals	The only polio vaccine recommended for immunodeficient individuals

Abbreviations: OPV, oral polio vaccine; IPV, inactivated polio vaccine; VAPP, vaccine-associated paralytic poliomyelitis; VDPV, vaccine-derived poliovirus.

44. What is immunogenicity and vaccine efficacy of tOPV in developing country like India?

In developed countries like USA, greater than 95% seroconverted after three doses of tOPV (to all three types). Excellent mucosal immunity induced by OPV and invisible immunization of others due to vaccine virus spread were believed to be two factors contributing to such striking herd effect. A greater degree of vaccine virus spread and herd effect was anticipated in developing countries with poor hygiene and sanitation conditions.

When OPV was introduced in developing countries including India, suboptimal immunogenic efficacy of type 1 and 3 component was observed in several places. Studies conducted by Dr T Jacob John and his colleagues from Vellore, India, have demonstrated low immunogenicity of tOPV in India as early as in 1970s and 1980s. This was further confirmed by a comprehensive review of 32 studies from developing countries which shows low immunogenicity of tOPV for type 1 and 3 in developing countries.

Within India when it comes to Uttar Pradesh, per dose efficacy of tOPV is calculated to be just 9% per dose. Exact cause for poor efficacy of OPV is not known but warm climate, interference by Enteroviruses, high incidence of diarrhea and prevalence of malnutrition are some of the factors attributed for lower efficacy of tOPV.

45. How can IPV be used as a complementary tool to OPV?

The advantages of using IPV along with OPV can be as follows:

- Excellent immunogenicity, efficacy and safety of IPV which guarantees individual protection
- The risk of VAPP with this combined OPV and IPV schedule is extremely low as the child receives OPV at the time when he/she is protected against VAPP by maternal antibodies. Subsequently, he/she is protected from VAPP by IPV
- Mucosal immunity as measured by stool excretion of virus after mOPV1 challenge is superior with combination of OPV and IPV as compared to IPV alone. IPV boost mucosal immunity very effectively in previously OPV vaccinated individuals
- Switch to IPV is part of end game strategy in post-polio eradication era in the form of at least one dose of IPV to be included in the routine EPI.

46. How many doses of OPV and IPV are needed to protect a child from wild polio?

There is no firm conclusion on the number of doses of tOPV, mOPV or bOPV required to ensure 100% protection, but administration of enhanced-potency vaccine in most studies has resulted in seroconversion to the three poliovirus types in 94–100% of vaccinees after two doses and high titers of serum neutralizing antibody in 99–100% of recipients after three doses. Two doses of IPV at 2 months interval produces 70–100% and three doses at 6, 10 and 14 weeks produces 63–91% seroconversion.

Various studies conducted in developed and developing countries, including India have shown consistent immunogenicity with IPV across different geographic and environmental variabilities. IPV also protects against VAPP, e.g. Hungary has not reported any case of VAPP after introduction of one IPV dose in sequential schedule in 1992.

47. What is the duration of protection of OPV and IPV?

Neutralizing antibody produced by OPV (or IPV) is long lasting. Long-lasting memory responses have been demonstrated by booster doses. Low levels of neutralizing antibody can be boosted by secondary exposure to disease, or by booster vaccination. Memory cells are prompted by a booster dose or secondary exposure to differentiate into plasma cells which secrete antibody.

48. If a child has received greater than 40 doses, does he still require further immunization during polio campaign?

Every child below 5 years of age should be given OPV in every polio campaign regardless of previous status of immunization (in routine as well as polio campaign), as gut immunity is of short duration and repeated frequent immunization with OPV is necessary to sustain gut immunity.

49. When there is no case of polio in India since Jan 2011, should still be OPV recommended to be given through SIAs?

Yes, because in 2013, polio remains endemic in three countries: Afghanistan, Nigeria and Pakistan. Until poliovirus transmission is interrupted in these countries, all countries remain at risk of importation of polio; hence, OPV to be administered to every child till the age of 5 years during all SIAs being conducted in that area as it is necessary to sustain high level of immunity against WPV till global success in polio eradication.

50. Why OPV should be given despite availability of IPV?

OPV use should be continued due to following reasons:

- In concordance with the government policy of using OPV for polio eradication
- Mucosal immunity as measured by stool excretion of virus after mOPV1 challenge is superior with combination of OPV and IPV as compared to IPV alone
- By not giving OPV we might create confusion in the minds of the parents whose children receive only IPV about the efficacy and safety of OPV and interfere with OPV uptake on the NIDs and Sub-National Immunization Days (SNIDs).

51. Since, Government of India (GOI) have not recommended IPV use, hence IAPCOI recommendation of using IPV contradicts national schedule. Giving both OPV and IPV in sequential dose is creating a lot of confusion and dissent amongst pediatricians. What's wrong if I use only IPV in my office practice?

As mentioned earlier, there is less than satisfactory response with IPV when used in 6, 10, 14 week schedule, particularly against types 1 and 2 serotypes. According to a study conducted by WHO in Gambia, Oman and Thailand, a three-dose schedule which combines OPV-IPV, demonstrated the highest seroconversion rates. This is the most quoted study put forward by groups advocating combined use of OPV and IPV. GOI has not recommended use of IPV in RI yet, but in coming years as a part of end game strategy, single dose of IPV will be part of RI.

52. What are the issues related to IPV as far as private practitioners are concerned?

There are many issues such as: Is there a need to use IPV in private practice? Whom should we offer this vaccine? How should we use it? Which schedule to follow? Do we need to offer OPV as well?

53. What are the dilemmas faced by the practitioners?

- How and where to incorporate current "stand-alone" IPV in existing schedule?
- How to start "switching to IPV" without harming the national program?
- How to convince parents about need for an extra injection?
- Why should we give both OPV and IPV?
- Those who can afford the vaccine already well protected and may not need it!

54. What are the recommendations for adult polio vaccination?

Routine adult polio vaccination or boosters are not recommended considering the epidemiology of the disease in India. Vaccination with IPV is recommended for certain adults who are at greater risk of exposure to poliovirus like laboratory workers who handle specimens that might contain polioviruses, etc.

For unvaccinated adults, two successive injections of 0.5 mL can be given at intervals of preferably 2 months. A third dose (booster) may be administered 8–12 months after the second injection.

55. What is mucosal immunity?

Mucosal immunity in polio refers to the resistance to mucosal infection by wild polioviruses due to prior infection with WPV or immunizing experience with polio vaccines. Mucosal immunity decreases the replication and excretion (shedding) of the virus and, thus, provides a potential barrier to its transmission.

56. Which vaccine OPV or IPV induces better mucosal immunity? If OPV induces good mucosal immunity then why highly vaccinated children with OPV participate in wild polio transmission?

Mucosal immunity refers to the resistance to mucosal infection by wild polioviruses due to prior infection or immunizing experience. OPV induces a high nasopharyngeal and duodenal IgA response. Some more facts about mucosal immunity are:

- Immunoglobulin A (IgA) response is induced more readily by infection than by stimulation by nonreplicating antigen. Thus IgA is a marker of gut infection not necessarily mucosal immunity. Moreover, IgA is not the only medium of local mucosal immunity
- It is not necessary that an OPV dose will cause infection every time if there is no uptake. This finding offers an explanation why repeated doses of OPV have to be given to the same children, with virtually 100% coverage, before wild virus transmission could be stopped in developing countries
- IPV induces only low levels of IgA antibody, thus, suggesting that there are other mechanisms of local immunity. So there is more to mucosal immunity than the IgA antibody production and transport. IPV induces a very strong humoral immunity
- There is evidence that resistance to reinfection wanes with time and high force of transmission of WPV in the area allows fully immunized children to participate in polio virus transmission.

57. What do you mean by "endgame" strategy?

Polio end game refers to management of the "post-eradication" risks due to OPV. The Polio Eradication and Endgame Strategic Plan 2013–2018 (the Plan) was developed by GPEI and spearheaded by the World Health Organization, Rotary International, the US Centers for Disease Control and Prevention and UNICEF, with support from the Bill and Melinda Gates Foundation in 2013 to capitalize on this new opportunity to end all polio disease. It accounts for the parallel pursuit of WPV eradication and cVDPV elimination.

58. What are the objectives of end game strategy?

The strategic plan involves four objectives:

1. Detect and interrupt poliovirus, interrupt all WPV transmission by the end of 2014.
2. Strengthen RI and withdrawal of OPV, to eliminate the risk of VDPV, the plan introduces a strategy to replace OPV with IPV. Strong RI programs will be critical for the rapid and successful introduction of these new vaccines.

3. Contain and certify, before certifying eradication, laboratories and vaccine production facilities worldwide must properly contain all virus samples to protect against future outbreaks.
4. Plan polio's legacy, the polio program provides a blueprint for accessing the most marginalized and hard-to-reach communities in the world. Sharing this expertise can benefit other health and development initiatives.

59. Should polio vaccination be continued after eradication of wild polio?

Once eradication of wild poliovirus has been confirmed, the public health benefits of RI with OPV may no longer outweigh the burden of disease due to VAPP and cVDPVs. Each OPV-using country must decide whether to stop all RI against polio after OPV cessation (after OPV cessation, IPV will be the only option for those countries which decide to continue RI).

60. Is there any plan to replace the tOPV with bOPV in RI?

Sabin type 2 in the tOPV has been responsible for greater than 90% of all cVDPV cases and about 40% cases of VAPP globally during the last few years. Strategic Advisory Group of Experts (SAGE), in 2012 recommended the withdrawal of the type 2 component of OPV as soon as possible from RI programs in all countries. It is because after the eradication of WPV, there is risk of cVDPV and all the countries will continue to face the risk of type 2 cVDPV as long as tOPV is being used in the program. In order to mitigate this risk, it is important to discontinue tOPV and switch to bOPV in both RI and SIAs as a part of the global "polio end game" strategy.

61. What are the risks associated with shift of bOPV use in RI?

There is risk of emergence of type 2 cVDPV after sudden withdrawal of type 2 component from Sabin vaccine. It will be critical to boost the population immunity against type 2 poliovirus prior to the tOPV to bOPV switch.

62. Is there any plan to introduce IPV in RI?

As mentioned earlier, there will be risk of cVDPV and VAPP in post-eradication era due to Sabin polio vaccine (mainly type 2). To address this risk, there is need of switch of vaccine in RI in phased manner. For Sabin type 2, cessation means that tOPV must be replaced with bOPV in a synchronized manner globally. For risk mitigation, SAGE has recommended at least one dose of IPV included in the routine EPI (starting >6 months before switch from tOPV to bOPV). Moradabad (UP, India) study has also revealed that single IPV dose to infants and older children with history of multiple OPV doses dramatically boosts intestinal mucosal immunity and effect is larger than with a bOPV dose. In previously

OPV immunized children, an IPV booster dose has greater impact than an OPV booster in closing immunity gap to all three serotypes.

63. What are benefits of 1 IPV dose prior to OPV2 cessation?

The benefits of 1 IPV dose prior to OPV2 cessation are to:

- Prevent polio if exposed to a VDPV2 or WPV2
- Improve response to mOPV2 in an outbreak
- Reduce transmission of a reintroduced type 2
- Boost immunity to WPV1 and WPV3.

64. What are the recommended schedules to add single dose of IPV in RI?

The schedules recommended by SAGE working group are as below:

- 6, 10, 14 weeks or 2, 3, 4 months schedule: Add IPV dose at the DPT3 contact
- 2, 4, 6 months schedule: Add IPV dose at the DPT3 contact, though DPT2 can be considered
- Countries with documented VAPP risk prior to 6 months of age may decide to consider alternative schedules

For current OPV-only countries, the working group has not recommended to change existing schedules.

In India, the most feasible option is one dose IPV at DTP3 contact (plus a buffer stock of IPV for outbreaks).

65. Is there any plan for cessation of OPV use after eradication of polio?

Yes, because as per the definition of polio eradication, it accounts for parallel pursuit of WPV eradication and cVDPV elimination. Once WPV transmission is eliminated, any polio thereafter would be exclusively vaccine-caused. To overcome the risk of cVDPV due to OPV in post eradication era, GPEI has recommended for cessation of OPV use globally in post-eradication era.

66. What are the risks associated with OPV cessation?

There are two main risks associated with OPV cessation: immediate risk of cVDPV emergence and; medium and long-term risk of polio virus reintroduction from a vaccine manufacturing site, research facility or diagnostic laboratory.

67. Is there any time line for switch of tOPV to bOPV and OPV cessation?

As per the projected timeline by GPEI, it is expected to interrupt the transmission of WPV globally by the end of 2014. In 2016, there will be introduction of IPV and withdrawal of OPV2 from RI. By the end of 2018, there will be certification of global polio eradication and in 2019, OPV use will be stopped.

In India, the most feasible option is one dose IPV at DTP3 contact (plus a buffer stock of IPV for outbreaks). Because India is a tier one country, IPV introduction should be accelerated (i.e. January 1, 2015). After cessation of all OPV, affordable hexavalent vaccine will likely be available.

68. What are the priorities for national policy makers in OPV using countries during preparatory phase of OPV cessation?

In the countries where OPV is being used, before its cessation certain preparations need to be ensured. These are:

- Strengthen polio (AFP) surveillance to guide interruption of WPV transmission, certify eradication, and detect potential importations and cVDPVs
- Fully implement and verify appropriate containment of all wild and VDPVs and prepare for Sabin virus containment
- Raise RI coverage (> 90%) to minimize the risk of spread of an imported WPV and risk of cVDPVs emergence
- Based on analysis of risks, benefits and opportunity costs, decide whether to stop all RI against polio after OPV cessation or continue with IPV which will be the only option
- Conduct an iVDPV risk assessment and establish a case management plan, if needed
- Establish national plans and mechanisms for the eventual cessation of all OPV use in RI programs and safe destruction of remaining tOPV stocks.

69. Once WPV is eliminated, what is the need of having a future vaccine policy?

When smallpox was declared eradicated, countries could decide independently whether or not to stop vaccination against the disease. For polio, this approach could prove disastrous. This is because of the safety problems associated with OPV use. Inadvertent escape of virulent virus from laboratories and malicious introduction of virulent polioviruses, an event that has already occurred in India are the few other risks involved with post-eradication era. Indeed, these are the main reasons why vaccination against polio cannot be stopped even after cessation of wild WPV transmission, and achieving eradication.

70. What is the WHO stand on post-eradication vaccination policy?

According to WHO, after interruption of wild poliovirus, continued use of OPV would compromise the goal of a polio-free world. The stated strategy of WHO is to stop using OPV in a single day, once eradication has been presumed to occur. This strategy involves simply observing for poliovirus circulation and for cases poliovirus-induced paralysis in the absence of vaccination.

71. What should be the ideal post-eradication strategy for India?

World Health Organization has suggested that developed countries continue to use IPV in RI to prevent possibilities of import of cVDPV or WPV through bioterrorism. Stopping all polio immunization and continuing AFP surveillance alone although would be cheaper, but this strategy is fraught with danger.

72. Do you think a strategy shift from OPV to IPV can avoid inevitable problems associated with OPV, such as vaccine associated polio, risk of polio in immunocompromized children and risk of vaccine derived polio outbreaks that have occurred in few countries even after successful eradication of wild polio?

Yes, as far as vaccine VAPP and risk of polio in immunocompromized children are concerned, IPV is definite answer. However, to thwart the possibility of future emergence of vaccine derived polio outbreaks, IPV still can work though not many precedents are available and experience is limited.

73. What is the current supply cost status of IPV?

Currently, IPV is only available in the private market in India. Estimated per dose cost of 10 dose vial in October 2012, for existing IPV product [whole dose, intramuscular (IM) Salk IPV] varied from US$1.25 to 2.75. Volume purchasing and guaranteed procurement can substantially reduce the price of IPV products below the current "UNICEF price" (US$2.75/dose). To achieve target price substantially below US$1.00, options are: intradermal (ID) fractional dose IPV (1/5 dose); and IM adjuvanted IPV.

Estimated per dose cost of 10-dose vial in October 2012, for pipeline products (2015–2017) is:

- Adjuvanted (1/5th) dose IM Salk IPV: US$0.50–1.00
- Fractional (1/5th) ID IPV dose: US$0.40–0.50

74. How can current IPV be made more efficacious?

To make the current IPV more efficacious and effective, following are the things that are being tried:

- Novel approaches to formulate and administer IPV that do not require trained medical personnel or hypodermic needles for administration, provide substantial dose sparing, and lower the overall cost without compromising efficacy
- Novel ways to substantially increase the level of intestinal mucosal immunity induced by IPV in order to prevent viral shedding
- Novel methods to produce an equally or more efficacious inactivated or subunit poliovirus vaccine that do not require wild virus for production and can be manufactured at scale in a cost-effective manner.

75. Why India or other developing nations are not allowed to indigenously produce IPV?

There are limitations on developing nations to produce IPV because the production requires highest level of biosafety (BSL 3+) and biocontainment which has only been certified for a few laboratories globally, which supply the bulk/finished formulation for the global requirements.

76. What about OPV contact immunization and herd effect?

Based on the experience from developed countries, it was presumed that advantage of the invisible vaccine virus spread will be more pronounced in developing countries with poor hygiene and sanitation where fecal-oral transmission would occur more frequently and polio virus transmission will be interrupted once 80% coverage will be reached with three doses of OPV. But this has never happened.

Herd Effect

Herd effect is reduction of incidence of disease in unvaccinated segment due to vaccinating a section of population. The prerequisites for herd effect of a vaccine include two elements. First, immunization should reduce the transmission potential of the individual, and second, sufficient proportion of individuals in a group should be immunized in order to have an effect on the transmission of the virus. The higher the vaccine coverage, the greater the potential of herd effect. When children in developing countries are given sufficient number of OPV doses and they seroconvert, they will be protected from the disease, most probably for life. On the other hand, gut immunity present in them soon after immunization, is likely to decline as time progresses, until its level is insufficient to prevent an outbreak. Such waning gut immunity particularly in the face of high force of virus transmission (dictated by inoculums size and/or repetitiveness of exposure) and the persistence or reintroduction of wild virus, would set the stage for large scale poliomyelitis epidemics in well-immunized populations after a period of control of disease or even interruption of transmission. Now fully vaccinated children would also participate in virus transmission.

Herd effect of tOPV is not visible in India on ground which is evident by the facts that repeated doses of OPV have to be given to the same group of children, with virtually 100% coverage, before wild virus transmission could be stopped, and the median age of polio in India has not shifted to the right and remained stationary at 12–18 months from prior to introducing immunization till now.

77. What is pulse polio immunization?

It is strategy for eradication of poliomyelitis in which mass immunization is done. Extra dose of OPV (in the form of pulse) is given to all children less than 5 years of age in an area regardless of previous status of immunization at a time on a given day. The aim is to achieve 100% coverage.

78. Do you need to give regular polio immunization when pulse OPV is taken?

Yes, OPV given in SIA (pulse immunization program) is an extra dose and RI for polio should be continued as per schedule.

79. Will the polio (AFP) surveillance continue in India even after certification of polio eradication which is likely in February, 2014?

Yes, even after certification, the polio (AFP) surveillance will continue and need to be sensitive enough to pick any importation of WPV or emergence of VDPV.

80. Can a child with isolated facial paralysis just like the Bell's palsy should also be reported as a case of AFP?

Rarely, poliomyelitis can manifest as isolated asymmetrical paralysis of face, a presentation quite indistinguishable from idiopathic "Bell's palsy". During a 16-months study period in South India, three out of five children presenting as "Bell's palsy" were in fact found suffering from poliomyelitis. This is also supported by AFP surveillance data of India. Hence, in polio-endemic countries, poliomyelitis should be considered when children present as "Bell's palsy".

81. What is polio like syndrome?

In the past decade, newly identified strains of enterovirus have been linked to polio-like paralytic cases among children in Asia and Australia,Enterovirus infections, especially coxsackieviruses A9 and A23 (echovirus 9) and group B coxsackieviruses, frequently caused meningoencephalitis often associated with transient paralysis. Coxsackievirus A7 infection occasionally resulted in permanent paralysis.

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CHAPTER

11

Diphtheria, Tetanus, and Pertussis

Sudhir Kumar Choudhary, Vipin M Vashishtha

1. I am confused about the various vaccines that contain diphtheria, tetanus, and pertussis. Can you explain?

There are three basic products that can be used in children younger than age 7 years [diphtheria, tetanus and whole cell pertussis (DTwP), diphtheria, tetanus and acellular pertussis (DTaP) and diphtheria and tetanus (DT)] and two that can be used in older children and adults [tetanus-diphtheria (Td) and tetanus-diphtheria-pertussis (Tdap)]. Some people get confused between DTaP and Tdap and others get confused between DT and Td. Here is a hint to help you remember. The pediatric formulations usually have three to five times as much of the diphtheria component than what is in the adult formulation. This is indicated by an upper-case "D" for the pediatric formulation (i.e., DTaP, DT) and a lower case "d" for the adult formulation (Tdap, Td). The amount of tetanus toxoid in each of the products is equivalent, so it remains an upper-case "T."

2. What do the acronyms DTwP, DTaP, DT, Tdap, etc. denote?

- wP: whole cell killed vaccine
- aP:
  - no cells, but its constituent components
  - pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin, agglutinogens 2 and 3
  - ap–contains lower dose of 3 acellular pertussis components (PT, FHA, Pertactin)
- D: diphtheria toxoid 25 Lf
- d: lower dose with 2 Lf
- T: tetanus toxoid 5 Lf.

3. What is the maximum age of a child to receive DTwP Vaccine?

Diphtheria, tetanus and whole cell pertussis DTaP, and DT are not given beyond 7 years of age because of high reactogenicity.

4. A child is brought for vaccination at 45 days with a background of prematurity with a birth weight of 1.8 kg and hypocalcemic seizures in the nursery. The mother is concerned about safety of diphtheria, tetanus and pertussis (DTP) vaccines. How will you counsel?

Diphtheria, tetanus, and pertussis vaccination should be given since prematurity, low birth weight (LBW) and an identifiable non-progressive neurologic disease (hypocalcemic seizures) are all not contraindications for DTP vaccination.

5. Can we interchange the different brands of DTaP vaccines?

The components of pertussis bacilli used for preparation of the acellular vaccines include pertussis toxin (PT) as the essential component with or without filamentous hemagglutinin (FHA), pertactin (PRN) and fimbrial hemagglutinins 1, 2, and 3 (FIM). Commercially available vaccines vary in number of components, quantity of components and method of inactivation of the components. Currently available aP vaccines in India include five component vaccines (Tripacel ™- PT, PRN, FHA, FIM 2, and 3), three component vaccines (Infanrix ™ - PT, FHA, PRN) and a two component combination vaccine [PentaximTM - PT and FHA, IPV, *Haemophilus influenzae* type b (Hib)].

Advisory Committee on Immunization Practices has recommended that, whenever feasible, healthcare providers should use the same brand of DTaP vaccine for all doses in the vaccination series. However, if we do not know the previously used brand, use whatever DTaP/DTwP vaccine is available for all subsequent doses.

6. Which is the most efficacious DTaP vaccine?

According to a systematic review involving 49 randomized clinical trials concluded that one- to two-component acellular pertussis (aP) vaccine had lower absolute efficacy (67–70%) than vaccine with three or more components (80–84%). A recent concurrent review summarized six aP vaccine efficacy trials, including a total of 46,283 participants and 52 safety trials. The review concluded that the efficacy of multicomponent (three or more) aP vaccine varied from 84 to 85% in preventing typical whooping cough and from 71 to 78% in preventing mild pertussis disease. In contrast, the efficacy of one- and two- component vaccine varied from 59 to 75% against typical whooping cough and from 13 to 54% against mild pertussis disease. Therefore, aP vaccine having three or more components is more efficacious than the one having lesser number of components.

7. We are routinely scheduling the fourth dose of DTaP in children at 15–18 months, but occasionally would like to give it earlier. Is that okay?

The fourth dose of DTaP may be given as early as age 12 months if at least 6 months have passed since the third dose, and if, in the provider's opinion, the child might not return for another visit at 15–18 months of age.

8. Should further doses of pertussis vaccine be given to an infant or child who has had culture-proven pertussis?

Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (ACIP) states that a child who has had culture-proven pertussis does not need additional doses of pertussis vaccine. The series may be completed with pediatric DT. However, if there is any doubt about the diagnosis (i.e., if the diagnosis was made without a culture), the pertussis vaccine series should be completed on schedule.

9. Is there any maternal protection against pertussis to a newborn?

There is no maternal protection against pertussis to the newborn.

10. Can a child or an adult who has had pertussis get the disease again?

Reinfection appears to be uncommon but does occur. Reinfection may present as a persistent cough rather than typical pertussis. Hence, it is essential to immunize even those children recovering from pertussis as natural disease may not offer complete protection.

11. As a pediatrician, I am concerned about protecting my newborn patients from pertussis, especially given the recent outbreaks in my community where infants have died. How many doses of pediatric DTaP vaccine do an infant need before she or he is protected from pertussis?

Vaccine efficacy is 80–85% following three doses of DTaP vaccine. Efficacy data following just one or two doses are lacking but are likely lower. Therefore, it is especially important that you advice parents of infants and all people who live with the infant or who provide care to him or her be protected against pertussis. Recommend that the entire infant's family members and visitors ages 10 through 64 years receive a one-time dose of adolescent/adult tetanus-diphtheria-acellular (Tdap) vaccine if they have not already done so.

12. When a child comes in for his vaccinations at age 4–6 years and presents with an incomplete history of 0–2 doses of DTwP/DtaP vaccine, how do we determine how many more doses are needed?

You should try to achieve at least four total doses. Give additional doses of DTwP/DtaP with 4 week intervals until you achieve three total doses. Then, give the last (third or fourth) dose at least 6 months after the previous dose.

13. If a child did not have the recommended 6-month interval between DTwP/DtaP doses number 3 and number 4, should it be repeated?

If DTwP/DtaP number 4 is given with at least a 4-month interval after DTwP/DtaP number 3, it does not need to be repeated. Decreasing the interval to less than 6 months, however, is not recommended.

14. What is the minimum interval between DTwP/DtaP number 4 and DTwP/DtaP number 5?

The minimum interval between DTwP/DtaP number 4 and DTwP/DtaP number 5 is 6 months. Remember that the minimum age for DTwP/DtaP number 5 is 4 years.

15. If a child has already received 5 doses of DTwP/DtaP by their fourth birthday (with the appropriate 6-month intervals between number 3 and number 4 and also between number 4 and number 5), is a booster dose after the fourth birthday necessary?

In general, a child should receive no more than four doses of DTwP/DtaP before 4 years of age (preferably by 2 years of age). The Advisory Committee on Immunization Practices (ACIP) recommends that a dose of DTwP/DtaP be given at 4–6 years of age. Many states have school immunization laws, which also require at least one dose of DTP/DTwP/DTaP on or after the fourth birthday. This dose is important to boost immunity to pertussis.

16. Is there a recommendation about how many doses of DTwP/DtaP a child can receive by a certain age? Does this include half doses?

Advisory Committee on Immunization Practices and AAP both recommend that children receive no more than 6 doses of diphtheria and tetanus toxoids (TT) (e.g. DT, DTwP/DtaP, DTP) before the seventh birthday because of concern about adverse reactions, primarily local reactions. Half doses of DTwP/DtaP are also not recommended under any circumstances, and should not be counted as part of the vaccination series. Only documented doses (those recorded on a written record) count toward the maximum of six doses.

17. The IAP has now revised its recommendations and advised use of whole-cell pertussis (wP) vaccine instead of aP in primary immunization. Why?

The recommendation on the exclusive use of wP vaccine in primary immunization series is based on the following reasons:

- There was no data on the efficacy/effectiveness of aP vaccines in India and almost all the recommendations were based on the performance of these vaccines in industrialized countries. However, all these countries have now reported upsurge and frequent outbreaks of the disease despite using highest quality aP vaccines with a very high coverage (close to 100%) since mid-1990s (Figure 11.1). Hence, there is no evidence of effectiveness of aP vaccines from the industrialized countries also

- The aP containing combinations were licensed in India on the basis of immunogenicity studies only. However, in the absence of any known correlate of protection for aP vaccines, mere presence of antibodies cannot be relied as a surrogate for efficacy or protection
- The studies from USA, Australia, and other industrialized countries post-2009 outbreaks have demonstrated superior priming with wP vaccines and more durability of immunity following wP vaccination than aP vaccines
- There is strong evidence of effectiveness, real-life performance of wP vaccines from India where the widespread use of them have markedly reduced the incidence of pertussis after the launch of Universal Immunization Program (UIP). We have achieved a good control of pertussis (high effectiveness, not merely the efficacy) with whatever type of wP was available in the country despite with a modest coverage of around 60–70%
- Worldover, the widespread use of wP vaccines had almost eliminated pertussis from almost all the countries that had employed them
- India is still a wP vaccine using country and more than 95% of children are still vaccinated with wP vaccines. There is no data on the efficacy or effectiveness of aP vaccines in India and almost all the recommendations are based on the

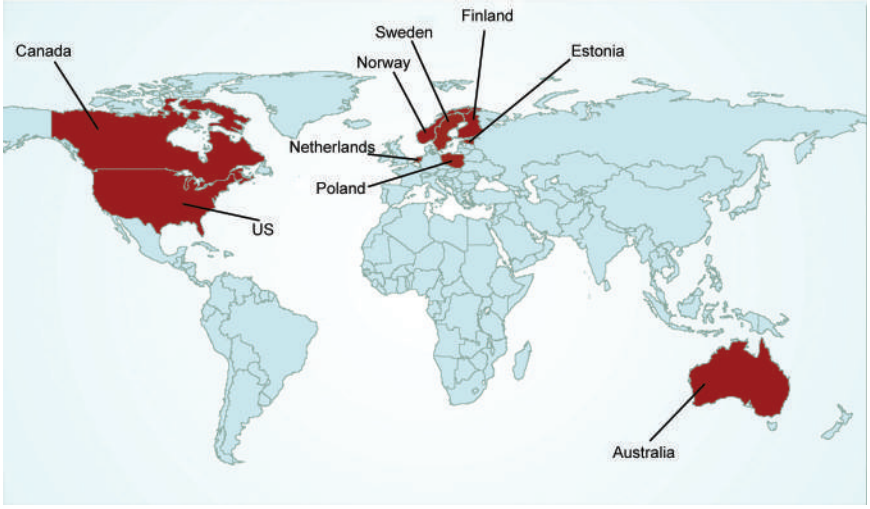

FIGURE 11.1 Global pertussis outbreaks in vaccinated populations: Pertussis re-emerged in countries highly vaccinated with DTaP vaccines

(*Source:* 1. Vaccine driven evolution of *B. Pertussis*: Changes in population structure and strain fitness. Igitur archive.library.uu.nl/dissertations/2003-0114-153343/full.pdf. 2. Gabutti G. Rota M. *C. Pertussis*: A Review of Disease Epidemiology Worldwide and in Italy. Int. J. Environ. Res. Public Health 2012, 9, 4626-4638).

performance of these vaccines in industrialized countries, mainly USA. On the contrary, we have strong evidence of effectiveness, real-life performance of wP vaccines from India where the widespread use of them have markedly reduced the incidence of pertussis. The incidence of pertussis declined sharply after launch of UIP. We have achieved a good control of pertussis (high effectiveness, not merely the efficacy) with whatever type of wP was available in the country despite with a modest coverage of around 60–70%. On the other hand, the epidemiology of pertussis and performance of wP and aP vaccines in US clearly shows that early use of wP vaccines had almost eliminated pertussis, which has now resurged after use of even the highest quality aP vaccines with a very high coverage (close to 100%) since mid-1990s.

18. Inspite of the poor efficacy and no effectiveness of aP vaccines, why Western countries have not reverted to wP vaccines?

Post-2012 outbreaks of pertussis in US, UK, and Australia have shifted the focus back on effectiveness of the pertussis vaccines from the safety. The ACIP and many US experts on pertussis have also discussed the option of going back to wP vaccines. But the problem with them and with the entire Western world is that they cannot now revert to wP vaccines owing to "poor public acceptance" of these products. Fortunately, this is not a big issue as yet in India. There is no report of poor acceptance or widespread rejection of wP vaccines both from the public or private sector.

19. When is DTaP vaccine preferred instead of DTwP?

DTaP vaccine may be preferred to DTwP vaccine in those children who have H/o severe adverse effects with DTwP vaccine or children with neurological disorders.

20. Can we give assurance to parents about full safety of the DTaP vaccine?

Serious adverse events following previous pertussis vaccination though less likely as compared to DTwP, may still occur with DTaP. So use precautions while using the vaccine. Don't assure there will be no side effects as incidence of minor and major adverse effects are reduced by two third only.

21. Can DT be used as second booster?

No it should not be used as second childhood booster. Pertussis vaccine is still required to protect against childhood pertussis.

22. When can one use Td?

Used in children above 7 years of age as replacement to TT.

23. What is the role of Td in Indian context? Can Td be used for subsequent boosters at 10 and 16 years? Can it replace TT in pregnancy?

Before replying to this query, let us consider two scenarios:

Scenario 1

- High DTP coverage! circulation of *Corynebacterium diphtheriae*
- Vaccine immunity to diphtheria gradually wanes off
- Adolescents and adults become susceptible
- Resurgence of diphtheria in adolescents and adults

Td is advisable 10 years onward and preferably every 10 years.

Scenario 2

With poor coverage of DTP this argument does not hold good and most parts of India still belong to this category.

Recommendations for Use

The DTwP, DTaP, and DT vaccines cannot be used in children aged 7 years and above due to increased reactogenicity due to the higher diphtheria toxoid and pertussis components.

This vaccine is indicated as replacement for DTwP/DTaP/DT for catch up vaccination in those aged above 7 years (along with Tdap) and as replacement for TT in all situations where TT is given.

Td in Pregnancy

Td is as safe as TT in pregnancy and hence should be preferably substituted. Being practiced in many developed countries like USA, UK, Australia, etc. The cost factor and logistics are almost the same.

On the other hand, few would argue in favor of TT. As we give DPT at 6 weeks and increasing maternal antibody through 'd' in pregnancy may be counterproductive. Any way diphtheria is not a problem in the neonate unlike tetanus. So, all in all, need and caution together should weigh more on TT only.

24. Is there any problem with repeated TT vaccinations?

Administration of booster doses more frequently leads to increased frequency and severity of local and systemic reaction as the preformed antitoxins binds with the toxoid and leads to immune complex mediated reaction (e.g. Arthus reaction).

25. When should a person receive TT alone?

Single antigen TT should only be used in rare instances, for example, when a person has had a documented severe allergic response to diptheria toxoid.

26. Which is the best vaccine at 10 years to protect adolescents from diphtheria, tetanus, and pertussis?

Tdap vaccine to check adolescent pertussis better then Td. Indian Academy of Pediatrics Committee of Immunization (IAPCOI) recommends to use Tdap vaccine instead of Td/TT vaccine in all catch up vaccination up to 18 years.

27. What is the rationale of introducing Tdap at 10 years of age?

Diphtheria and pertussis showed a dramatic decline after the introduction of vaccination against them in 1940s. However, recent years have witnessed a general resurgence of pertussis especially among the adolescent and adult population even in countries with high vaccine coverage. This indicated there is waning of immunity in due course of time. It has been found that such a waning occurs 5–10 years after the primary vaccination. Further, there is lack of boosting by natural immunity due to cessation of wild organism circulation in high vaccine coverage areas. Another important implication is that these adolescents and adults may not only suffer from pertussis but can be important carriers of the bacteria leading to widespread epidemic in the vulnerable infants and children. In India, the low coverage of routine immunization further compounds the problem.

A similar resurgence has been seen in cases of diphtheria in adolescents and adults, besides the increase in incidence in young children due to poor routine immunization. Further, tetanus toxoid administration at 10 years in national immunization schedule is virtually nonexistent. Immunity to all three components wanes over the next 6–12 years. Therefore, regular boosting is needed in as much intervals.

In view of the above, it is pertinent to give some vaccine against diphtheria, tetanus, and pertussis at the preadolescent stage or to boost the existing but waning immunity.

Since 10 years is the age at which tetanus toxoid is already recommended to be given under the NIS, it is worthwhile to replace it with a triple vaccine against all the three components. However, the conventional DPT vaccines being used in younger children can not be used beyond 7 years of age due to their high reactogenicity and possibility of damage to the neurological system by the wild type of pertussis virus vaccines. Therefore, Tdap which is the reduced antigen DPT vaccine is the right alternative. This vaccine has been found to be effective and safe in adolescents.

28. What is the time gap to give Tdap in those who have received TT/Td earlier?

A gap between previous TT/Td and Tdap should be 5 years.

A gap of two years between TT/Td and Tdap in those children with:

- During outbreak
- High chances of pertussis complication such as those with neurological or pulmonary disease
- Who are in contact with infant less than 12 months of age.

29. How will you immune a child who has not received complete primary immunization against diptheria/tetanus and pertussis and is more than 7 years?

One dose of Tdap + two doses Td at 0, 1, 6 months followed by Td every 10 years.

30. Should a teenage be given Tdap dose even if the child has received a dose of Td at age 11½ years?

Yes, all adolescents should receive dose of Tdap vaccine to protect them from pertussis, even if they have already received Td. t is important to do this right away if they are in contact with an infant younger than age 12 months, working in a healthcare center or live in a community where cases of pertussis are occurring.

31. What is the difference between the two Tdap products—Boostrix and Adacel?

Both of these vaccines provide protection against diphtheria, tetanus, and pertussis. Boostrix (GlaxoSmithKline) is licensed for people ages 10–64 years, and Adacel (sanofi pasteur) is licensed for people ages 11–64 years. Both are approved for one dose only, not multiple doses in a series. The two vaccines also contain a different number of pertussis antigens and different concentrations of pertussis antigen and diphtheria toxoid.

32. Can Tdap be given at the same visit as other vaccines?

Yes. Tdap can be administered with all other vaccines that are indicated (e.g. meningococcal conjugate vaccine, hepatitis B vaccine, MMR). Each vaccine should be administered at a different anatomic site using a separate syringe.

33. How many doses of Tdap can we give to a patient?

The vaccine is licensed for just one dose for people ages 10 through 64 years, depending on the age range of the product used. Subsequent doses, as well as vaccine given to children 7–9 years and adults ages 65 years and older, should be Td.

34. If an adolescent or adult who has never received their one-time dose of Tdap is either infected with or exposed to pertussis, is vaccination with Tdap still necessary, and if so when?

Yes. Adolescents or adults who have a history of pertussis disease generally should receive Tdap according to the routine recommendation. In the US, two Tdap products are licensed for use. Adacel (sanofi pasteur) is licensed for use in people age 11–64 years, and Boostrix (GlaxoSmithKline), is licensed for people age 10–64 years. This practice is recommended because the duration of protection induced by pertussis disease is unknown (waning might begin as early as 7 years after infection) and because diagnosis of pertussis can be difficult to confirm, particularly with tests other than culture for *B. pertussis*. Administering pertussis vaccine to people with a history of pertussis presents no theoretical risk. However, if the illness was recent (< 5 years) and the diagnosis was certain (i.e. culture confirmed), it is reasonable to wait 3–5 years before administration of Tdap, unless tetanus and diphtheria toxoids are needed.

35. Someone gave Tdap to an infant instead of DTaP. Now what should be done?

If Tdap was inadvertently administered to a child, it should not be counted as either the first, second, or third dose of DTaP. The dose should be repeated with DTaP. Continue vaccinating on schedule. If the dose of Tdap was administered for the fourth or fifth DTaP dose, the Tdap dose can be counted as valid.

36. Should I make an effort to give teenagers a Tdap dose, even if they have had a dose of Td at age 11–12 years?

Yes. All adolescents should receive one dose of Tdap vaccine to protect them from pertussis, even if they have already received Td. It is important to do this right away if they are in contact with an infant younger than age 12 months, work in a healthcare setting where they have direct contact with patients, or live in a community where pertussis is occurring.

37. I want to protect pregnant women and their unborn children from pertussis. Can I give Tdap to pregnant women?

Tdap is not contraindicated during pregnancy. It should be administered to a pregnant woman who is in contact with an infant younger than age 12 months, is in an outbreak setting, or is a healthcare provider who sees children. If there is no risk to the pregnant woman of acquiring or transmitting pertussis, ACIP recommends that Tdap vaccination be deferred until the immediate postpartum period. AAP has endorsed preferential Tdap vaccination of pregnant adolescents who were not vaccinated with Tdap at age 11–12 years (Pediatrics 2006; 117:965-78). Providers can follow either the AAP or ACIP recommendations.

However, IAPCOI states that there are not sufficient studies in this regard hence; Tdap should be deferred till immediate postpartum period. But Td can be given to these groups.

38. If a pregnant woman got a dose of Td during pregnancy, how soon after birth can she get her postpartum dose of Tdap?

The mother is taking home an infant who is susceptible to pertussis, so she should receive Tdap during the immediate postpartum period (e.g. before hospital discharge). There is no minimum interval between Td and Tdap.

39. Can the parents of a young infant be given a dose of Tdap right after birth to protect themselves and, indirectly, their newborn from pertussis, even though they had a dose of Td vaccine less than two years ago?

Yes. Parents should receive a single dose of Tdap as soon as possible to protect their baby from pertussis. If a dose of Td was given within the previous 2 years, parents should still be vaccinated with Tdap as soon as possible regardless of the time interval since the last dose of Td. Other household contacts that are not up to date with their pertussis-containing vaccinations should also be appropriately vaccinated.

40. How should Tdap be used in childhood immunization? What will be the role of Td during that time?

In those children who have received all three primary and the two booster doses of DTwP/DTaP, Tdap should be administered as a single dose at the age of 10–12 years. Catch up vaccination is recommended till the age of 18 years.

A single dose of Tdap may also be used as replacement for Td/TT booster in adults of any age if they have not received Tdap in the past.

A gap of 5 years should be maintained between Tdap and previous TT/Td vaccine.

A gap of 2 years between Tdap and TT/Td is acceptable in those children/adolescents:

- Who are at high risk for contracting pertussis, such as during an outbreak
- Who are at high risk for pertussis complications, such as those with neurological or pulmonary diseases
- Who are in contact with infants less than 12 months of age as infants are at the highest risk for pertussis complications.

It is also acceptable to use Tdap as a replacement for TT/Td in wound management of children aged 10 and above if they have not received Tdap in the past, and at least 5 years have elapsed since receipt of Td/ TT vaccine.

In children who have missed the 2nd booster of DTwP/DTaP and who are 7 years of age or more, Tdap single dose is recommended at the time of presentation.

In children who have not completed primary immunization with DTwP/ DTaP and are more than 7 years of age, one dose of Tdap and two doses of Td at 0, 1, and 6 months are recommended.

The single booster dose of Tdap may be followed by Td boosters every 10 years. There is no data at present to support repeat doses of Tdap (Austria is an exception where Tdap is recommended every 10 years).

No tetanus prophylaxis is required for minor wounds if less than 10 years have elapsed since receipt of Tdap. No tetanus prophylaxis is required for major wounds if less than 5 years have elapsed since receipt of Tdap; if more than 5 years (but less than 10 years) have elapsed a single dose of TT may be given.

In the absence of sufficient data on the efficacy, immunogenicity and duration of protection against pertussis with Tdap used as second childhood booster, the IAPCOI does not recommend the use of Tdap vaccine as an alternative to DTaP/ DTwP for the second childhood booster in children below the age of 7 years at present.

41. Is there any indication of DT now?

Absolute contraindications for DPT (very small group as discussed earlier) are still candidates for DT vaccine for primary and booster vaccination. Ideally DTaP is the vaccine of choice for 2nd booster at age 5, but the cost is prohibitive. IAP recommends DTP at 5 years while government of India (GOI) recommends DT because of the DPT reactogenicity. GOI has also recommended DPT at 5 years (second booster now).

42. Some children in my practice are not up to date on their immunizations, and pertussis is circulating in our community. Since neither pediatric diphtheria-tetanus-acellular pertussis (DTaP) vaccine nor adolescent/adult tetanus-diphtheria-acellular pertussis (Tdap) is licensed for use in children ages 7–9 years, I realize using either of these products in this age range is off-label. Can you guide me in determining how to make the decision about which vaccine to choose?

Providers may choose to use vaccines that are not licensed for a particular age range if they believe the benefit outweighs the risk. Because Td and Tdap contain less diphtheria toxoid than do pediatric DTaP and DT, giving Td and Tdap results in fewer local reactions in older children and adults. Therefore, in this scenario, if you decide to vaccinate, use Tdap.

43. If a dose of DTaP or Tdap is inadvertently given to a patient for whom the product is not indicated (e.g. wrong age group), how do we rectify the situation?

The first step is to inform the parent/patient that you administered the wrong vaccine. Next, follow these guidelines:

- Tdap given to a child younger than age 7 years as either dose one, two or three is not valid. Repeat with DTaP as soon as feasible
- Tdap given to a child younger than age 7 years as either dose 4 or 5 can be counted as valid for DTaP dose four or five
- Tdap given to a child age 7 through 9 years can be counted as valid for the one-time Tdap dose
- DTaP given to patients age 7 or older can be counted as valid for the one-time Tdap dose.

44. What if we mistakenly gave Tdap to a child ages 7–9 years?

Use of Tdap in children ages 7–9 years is considered off-label and is not recommended; however, the dose can be counted and does not need to be repeated with Td.

45. When a patient seen in ER needs tetanus protection, which vaccine should be given TT/ Td or Tdap?

Adolescent and adults ages 10–64 years who require a tetanus toxoid containing vaccine as part of wound management should receive a single dose of age appropriate Tdap instead of Td, if they have not previously received Tdap if Tdap is not available or was previously administered these people should receive Td.

46. We have a 16-years-old patient who received tetanus-diphtheria (Td) vaccine in the emergency room after a nail puncture a year ago. Can we give him a tetanus-diphtheria-acellular pertussis (Tdap) vaccine now?

No. Minimum interval is required between giving doses of Td and Tdap to an adolescent who is or might be in contact with an infant. This includes adolescents who are older siblings of infants, babysitters, or hospital employees or volunteers, etc. In circumstances like this, give Tdap without delay. For adolescents who will not be in contact with infants, CDC/ACIP recommends a routine wait of 5 years between Td and Tdap administration unless a school vaccination mandate requires giving Tdap.

47. I have a patient who received single-antigen tetanus (TT) in the emergency room for wound management rather than Td or Tdap. Should he be revaccinated?

Advisory Committee on Immunization Practices recommends that patients always be given Td or, if appropriate, Tdap rather than TT, as long as there is no contraindication to the other vaccine components. However, since it is already been given, you can wait until the next scheduled booster dose is due and administer Td (or Tdap) at that time. There are exceptions (e.g. the patient plans to travel internationally, is a contact of an infant younger than age 12 months) in which case you should administer Td (or Tdap).

48. When should tetanus immune globulin (TIG) be administered as part of wound management?

Tetanus immune globulin is recommended for any wound other than a clean minor wound if the person's vaccination history is either unknown, or s/he has had less than a full series of three doses of Td vaccine. TIG should be given as soon as possible after the injury.

49. How long after a wound occurs is tetanus immune globulin no longer recommended?

In the opinion of the tetanus experts at the CDC, for a person who has been vaccinated but is not up to date, there is probably little benefit in giving TIG more than a week or so after the injury. For a person believed to be completely unvaccinated, we would suggest increasing this interval to 3 weeks (i.e. up to day 21 post injury). Td or Tdap should be given concurrently.

50. There is lot of confusion about contraindications and precautions for administering triple antigen. Can you clarify?

Absolute contraindications to any pertussis vaccination (DTwP/DTaP/Tdap):

- Severe allergic reaction to vaccine component or following a prior dose;
- Encephalopathy within 7 days following previous DTwP vaccination (not due to another identifiable cause)

In case of anaphylaxis further immunization with any diphtheria/tetanus/pertussis vaccine is contraindicated as it is uncertain which component caused the event.

For patients with history of encephalopathy following vaccination any pertussis vaccine is contraindicated and only diphtheria and tetanus vaccines may be used.

Events such as persistent inconsolable crying of more than 3 hours duration/hyperpyrexia (fever >40.50°C)/HHE within 48 hours of DTwP administration and seizures with or without fever within 72 hours of administration of DTwP are considered as precautions but not contraindications to future doses of DTwP because these events generally do not recur with the next dose and they have not been proven to cause permanent sequelae. Progressive/evolving neurological illnesses, is a relative contraindication to first dose of DTwP immunization. However DTwP can be safely given to children with stable neurologic disorders.

Precautions – DTaP/DTwP:

- Moderate or severe acute illness
- Unexplained fever greater than 105°F (40.5°C) within 48 hours
- Hypotonic hyporesponsive episode within 48 hours
- Persistent inconsolable cry greater than 3 hours within 48 hours
- Convulsions ± fever within 3 days.

Tdap precautions:

- Guillain-Barré syndrome (GBS) within 6 weeks after a previous dose of TT containing vaccine
- Progressive neurologic disorder until the condition is stabilized
- Severe local reaction (Arthus reaction) following a prior dose of tetanus/diphtheria toxoid containing vaccine
- Moderate or severe acute illness.

51. If a baby had inconsolable crying for greater than three hours after the first DTwP vaccine, should we give second dose of DTaP or just DT?

Inconsolable crying for more than 3 hours is a contraindication to use it further. Here DTaP can be used with a precaution or warning and after discussing with the parents.

52. If a child shows reactions like high fever, convulsion, and "inconsolable crying can we give DTwP in subsequent doses? Is it preferable to switch to DTaP as the next doses?

Events such as persistent inconsolable crying of more than 3 hours duration/hyperpyrexia (fever >40.50°C)/HHE within 48 hours of DTwP administration and seizures with or without fever within 72 hours of administration of DTwP are considered as precautions but not contraindications to future doses of DTwP because these events generally do not recur with the next dose and they have not been proven to cause permanent sequelae. Wise option, but parents must be explained that such side effects can also occur with DTaP, though much less frequently.

Severe local/systemic reactions to DTP are two-third less with DTaP. Apprehensive parents should be explained on 1–1 basis including the expenses involved.

53. Do the same precautions that apply to DTaP also apply to Tdap?

No. Many of the precautions to DTaP (e.g. temperature of 105°F or higher, collapse or shock-like state, persistent crying lasting 3 hours or longer, seizure with or without fever) do not apply to Tdap.

54. Can an adult receive Tdap if they had a contraindication or precaution to DTP as a child?

Tdap has two contraindications and four precautions. The contraindications are:

1. Anaphylactic reaction to a prior dose of the vaccine or any of its components and
2. Encephalopathy within 7 days of a previous dose of DTaP or DTP; in this case, give Td instead of Tdap.

The precautions are:

1. Moderate or severe acute illness.
2. History of an Arthus reaction following a previous dose of a tetanus-containing and/or diphtheria toxoid-containing vaccine, including meningococcal conjugate vaccine.
3. (GBS) 6 weeks or sooner after a previous dose of tetanus-toxoid containing vaccine.
4. Progressive or unstable neurological disorder, uncontrolled seizures or progressive encephalopathy until a treatment regimen has been established and the condition has stabilized.

55. Can we give further doses of DTaP to an infant who had an afebrile seizure within 3 hours of a previous dose?

An infant who experiences an afebrile seizure following a dose of DTaP requires further evaluation. An infant with a recent seizure or an evolving neurologic condition should not receive further doses of DTaP, or DT until the condition has been evaluated and stabilized. Other indicated vaccines may be administered on schedule. To assure that the child is at least protected against tetanus and diphtheria, the decision to give either DTaP or DT should be made no later than the first birthday.

56. We have a 13-year-old patient who was given DT (pediatric) as a preschooler after she had experienced excessive crying following a dose of DTP. Now, we are wondering if we can give her Tdap since we know she may not be protected against pertussis.

Yes, You can. Many of the precautions to DTaP (e.g. temperature of 105°F or higher, collapse or shock-like state, persistent crying lasting 3 hours or longer, seizure with or without fever) do not apply to Tdap.

57. Instead of giving tetanus/diphtheria toxoid and acellular pertussis (Tdap) vaccine to a father-to-be who needed protection against pertussis, we mistakenly gave him tetanus/diphtheria (Td) toxoid. How soon after the Td dose can we give him the dose of Tdap he needs?

As long as they are younger than age 65 years and at least age 10 years, parents, grandparents, healthcare workers, and all others who have not already received Tdap, and who are close contacts of infants younger than age 12 months, should receive a single dose of this vaccine as soon as possible to protect infants from pertussis. When giving Tdap to protect infants, one does not need to observe a "minimum interval" between giving Td and Tdap. For example, if you had immediately realized that you had mistakenly given the father-to-be Td instead of Tdap, you could have given him the needed Tdap dose at the same visit at which you gave him the erroneous Td dose.

58. Can a booster dose of Tdap be given to people age 65 years and older?

No brand of Tdap is approved for people age 65 years or older. ACIP does not recommend off-label use of Tdap for this age group. However, a clinician may choose to administer Tdap to a person age 65 years or older if both patient and clinician agree that the benefit of Tdap outweighs the risk of a local adverse event.

59. I am a 66-year-old pediatrician. To protect our patients, my aging colleagues (65 years and older) and I received Tdap vaccine, even though it is not licensed for our age group. Regardless, I do not understand why this vaccine was not recommended or at least suggested for healthcare workers age 65 years and older who are in contact with young children.

There are no safety and efficacy data for this age group; FDA did not approve the vaccine for anyone older than age 64 years, and ACIP has not recommended off-label use of Tdap for this age group. However, there is no reason to believe that Tdap is any less safe for people age 65 years and older than it is for younger adults. Clinicians may decide that the benefit of Tdap exceeds the hypothetical risk in these situations.

60. Tetanus-diphtheria-acellular pertussis vaccine is licensed for use in adults through age 64 years. Are there exceptions for healthcare professionals or grandparents older than age 64 who are in contact with infants?

Advisory Committee on Immunization Practices has not recommended off-label use of Tdap for adults age 65 years and older. However, there is no reason to believe that Tdap is any less safe for people age 65 years and older than it is for younger adults. Clinicians are always free to use their clinical judgment; they may decide that in this situation the benefit of administering Tdap off-label exceeds any hypothetical risk of giving the vaccine.

61. We recently saw a 30-year-old man who remembers that he received a "tetanus booster" in another state within the past 2 years. The problem is he can not remember if he received Tdap or Td, and we cannot obtain an immunization record. His wife is pregnant, and we would like to immunize him against pertussis as a way to protect their soon-to-be-born child. Should we give him Tdap in this situation?

Yes. Whenever you lack vaccination documentation and vaccination is indicated, give the patient Tdap unless they are older than age 64.

62. Someone in our clinic gave DTaP to a 50-years-old instead of Tdap. How should this be handled?

The DTaP recipient received the appropriate amount of tetanus toxoid and more diphtheria toxoid and pertussis antigen than is recommended. Count the dose, but take measures to prevent this error in the future.

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CHAPTER

12

Measles

Baldev S Prajapati

1. What is modified (incomplete) measles?

Modified measles is an attenuated form of infection that may occur in individuals who have received immunoglobulin as prophylaxis after exposure to measles. It may occur in individuals with passively acquired antibodies, such as recipients of blood products or intravenous immunoglobulins few days before the exposure. The clinical manifestations of modified or incomplete measles are milder than those of typical infection and the incubation period is prolonged, 15–20 days. The rash may be indistinct, brief or rarely entirely absent. Complications are rarely observed following modified measles.

2. What is atypical measles?

Atypical measles occurs in individuals infected with natural virus who had previously received killed measles vaccine between 1963 and 1968. These persons have a sudden onset of fever accompanied by abdominal pain, cough, vomiting, and pleuritic chest pain. Koplik spots are rarely present and rash begins distally and progresses in a cephalad direction, with less involvement of face and upper part of trunk. The rash is not generalized and confluent as in typical measles. Although rash is erythematous and maculopapular, it often has a vesicular component. Cough and conjunctivitis are not prominent features of atypical measles. Pulmonary symptoms accompanied by radiographic evidence of pneumonia, hilar adenopathy and pleural effusions are common. Recovery from atypical measles may take two weeks or longer.

3. Which strains of measles virus are used for preparing measles vaccines at present?

All currently used measles vaccines are live attenuated vaccines and the strains originate from the original Edmonton strain. They are Schwarz, Edmonton Zagreb, Moraten and Edmonton–B strains. Indian vaccines are usually formulated from the Edmonton Zagreb strain grown on human diploid cells or purified chick embryo cells.

4. Being live viral vaccine, measles vaccine is more labile. What precautions should be taken to main its potency?

Measles vaccine is supplied as freeze-dried in single dose or multidose vials with distilled water as a diluent. The vaccine may be stored frozen or at 2–8°C (shelf-life 2 years). Reconstituted vaccine is destroyed by light and is very heat labile. It loses 50% potency at 20°C and 100% at 37°C after one hour. Therefore, it should be protected from light, kept at 2–8°C and used within 4–6 hours of reconstitution. This is particularly applicable to multidose vials.

5. What are the contraindications of measles vaccination?

- Severely immunocompromised
- H/o severe allergic reaction to constituents
- Pregnancy.

HIV infected individuals and history of egg allergy are not contraindications for measles vaccination.

6. What is the schedule of measles vaccination in an outbreak?

The immunogenicity and efficacy of the measles vaccine depends on the age of administration due to interference by preexisting maternal antibodies. Seroconversion rates are around 60% at the age of 6 months, 80–85% at the age of 9 months and more than 95% at the age of 12–15 months. Therefore, the best time for the measles vaccination is 12–15 months of age. However, in India a significant proportion of measles cases occur below the age of 12 months. Hence, in order to achieve the best balance between these competing demands of early protection and high seroconversion, completed 9 months of age has been recommended as the appropriate age for measles vaccination in India. In case of an outbreak, however, the vaccine can be given to infants as young as 6 months. (Scientifically feasible and epidemiologically relevant). An additional dose of measles vaccine preferably as MMR vaccine at the age of 15 months is required for durable and possibly life long protection against measles.

7. What are adverse reactions of measles vaccination?

Measles vaccine is remarkably safe vaccine, side-effects are few and generally mild. Measles like illness may develop 7–10 days after measles vaccination in 2–5% of the vaccinees. Thrombocytopenic purpura may occur in some vaccinees. The depression of cell mediated immunity may occur, it is harmless and recovers within 4–6 weeks. It does not create any problem with early HIV or un unrecognized tuberculosis. Subacute sclerosing panencephalomyelitis (SSPE) is rare complication following measles vaccination. It may occur 7–8 years after the vaccination in one out of 1 million vaccinees.

8. What is toxic shock syndrome following measles vaccination? How can it be prevented?

Toxic shock syndrome following measle vaccination is due to contamination of measles vaccine with *Staphylococcus aureus* bacteria due to use of unsterile syringes, needles, and using a vaccine vial beyond 4 hours after reconstitution. Reconstituted measles vaccine is susceptible to contamination as it does not have any preservative or antibiotics. It can occur after 30 minutes to few hours after vaccination presenting with fever, vomiting, diarrhea, and shock.

Use of sterile syringes, needles, aseptic precautions and prevention of contamination of reconstituted measles vaccine can prevent toxic shock syndrome. Hence, once reconstituted the measles vaccine should be used within 4 hours.

9. Is it true that measles vaccine may be given along with all childhood vaccines except BCG vaccine?

Earlier it was postulated that depression of cell mediated immunity following measles vaccination may cause disseminated tuberculosis due to vaccine strain of BCG vaccine if both the vaccines are given on the same day. The recommended minimum interval between two vaccines was four weeks. Now, it is known that depression of CMI takes at least 2 weeks. Therefore, even BCG vaccine can be given along with the Measles vaccine on the same day. In fact, in many African countries, simultaneous campaign of both BCG and measles vaccines are undertaken by WHO.

10. Can measles vaccine be given in a HIV-infected child? At what age? How many doses?

Children infected by HIV are vulnerable to develop severe measles disease with high mortality rate. In early life, most vaccines in this group are safe and efficacious including measles vaccine as the immune system is relatively well preserved. It is noted that in HIV infected infants following measles vaccination seroconversion rates are superior at 6 months of age compared to 9 months due to progressive immunodeficiency with the age. Hence, IAP COI recommends two doses of measles vaccine in HIV infected infants, at 6 and 9 months of age. The measles vaccine should be administered to those with HIV infection irrespective of degree of immunocompromise as here the benefits outweigh the risks.

11. If the mother gives the history of fever and measles like rashes to the child in the past, what decision should be taken to administer measles vaccine?

The measles vaccine should be given irrespective of prior history of measles as any exanthematous illness is often considered as measles.

12. What is the risk of epidemiological shift of measles cases?

Infants are protected from measles up to 6–9 months of age by maternal antibodies. Thereafter, measles and MMR vaccines protect them. If measles vaccination coverage is persistently more than 85%, children will be well protected from the disease. But the older children and adults who are not protected will be more susceptible to measles disease. Thus, the measles epidemiology may shift to older group and the disease severity is likely more in this group.

13. The Government of India has launched a Mass Measles Vaccination Program. Is this scientifically strong? Why should a child vaccinated with Measles and then MMR suffer unnecessary pricks?

The country needs 95% vaccination coverage to control measles. The First time the measles vaccine is given at 9 months. We know that giving measles at 9 months is an epidemiological compulsion; there is a 20% primary vaccination failure due to maternal antibodies. Therefore, at least 2–3 measles containing vaccines are required for protection. Even then, 5–8% remains susceptible. Therefore, giving a measles or measles containing vaccine for more than two times is scientifically sound. So, even if the child has already received a measles containing vaccine, the extra dose offered under the Government of India (GOI) Measles Immunization Program does not do any harm but is also scientifically valid. The measles mass vaccination program is like the supplementary immunization activity (SIA) that was carried out for polio eradication.

14. Is there any role of measles vaccine as postexposure prophylaxis?

Available data suggest that live measles virus vaccine if given within 72 hours of measles exposure, will prevent or modify the disease. Exposure to measles is not a contraindication to measles immunization.

15. Before launching the MR vaccination program, the Government of India had earlier launched a mass Measles Vaccination Program. Was this scientifically strong? Why should a child already vaccinated with measles and then MMR suffer unnecessary pricks?

The country needs 95% vaccination coverage to control measles. The first time that the measles vaccine is given at 9 months. We know that giving measles at 9 months is an epidemiological compulsion; there is a 15–20% primary vaccination failure due to maternal antibodies. Therefore, at least 2–3 doses of measles containing vaccines are required for protection. Even then, 5–8% remains susceptible. Therefore, giving a measles vaccine/measles containing vaccine for more than two times is scientifically sound. So, even if the child has already received a measles containing vaccine, the extra dose offered under the Government of India (GOI) Measles/ Measles Rubella immunization program does not do any harm but is also scientifically valid. The measles mass vaccine program is like the supplementary immunization activity that was carried out for polio eradication.

16. What are the new recommendations of NTAGI/GOI on measles-rubella immunization?

Recently, following the NTAGI Standing Technical Sub-Committee recommendation of two doses of MR vaccines in UIP at 9 months and 16–24 months at the time of Ist booster of DTP vaccine, the Government of India (GOI) has decided to implement this policy. Further, India has now joined the global initiative of elimination of Measles and rubella and control of CRS.

17. What is the IAP ACVIP's stand on the Government new policy of rescheduling the MR vaccine at 9 months and 16–24 months?

The academy has argued very strongly in favor of MMR instead of MR vaccine in UIP schedule because of various reasons. Mumps carries as much significance as rubella in terms of morbidity and complications. Moreover, the MMR vaccine has been invoked since decades and therefore, its acceptance by doctors and parents would be much easier than of MR vaccine with which no one has been so far acquainted. Also, it is more sensible in terms of logistics as it will require the same effort, money and manpower to eliminate three illnesses instead of two.

18. Does it mean stand-alone measles vaccine will become obsolete?

Yes, it is highly unethical to use stand-alone measles vaccine when effective MR and MMR vaccines are available in the country.

19. Why the Government of India is not willing to include MMR in place of MR in its UIP?

Probably, they are still not convinced about the burden of mumps in the country. Lack of large-scale studies has also hampered the moves to include mumps in national immunization program/UIP.

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CHAPTER

13

Hepatitis B Vaccine

Ajay Kalra, Sanjay Verma

1. What is the magnitude of problem of hepatitis B in the world?

Hepatitis B is one of the most common viral infections in the world. It is hyperendemic in many countries of Asia, Africa, and Oceania (tropical areas around Pacific Ocean), with the prevalence ranging from 30 to 100%. The WHO estimates that worldwide about 2 billion people are infected with hepatitis B. Of these, over 370 million are chronically infected. More than 10% of the population in some areas suffers from chronic liver disease due to hepatitis B. The WHO estimates that more than 600,000 persons died worldwide in 2002 of hepatitis B associated acute and chronic liver diseases. It is the cause of up to 80% hepatocellular carcinomas and is second only to tobacco among known human carcinogens.

2. What is its prevalence in different countries of the world?

Australia, North America and countries in Northern Europe have low prevalence rates. Countries of West and South Asia, South America, Eastern Europe, and those in the Mediterranean belt have intermediate prevalence. China, countries of tropical Africa and Pacific region and the Southeast Asian countries have carrier rates ranging from 7 to 20% and have, therefore, high prevalence rates.

3. Which zone does India belongs to?

With the average carrier rate being estimated as 3.8–4.2%, India belongs to the group of countries with intermediate prevalence rates.

4. What are the features of hepatitis B virus (HBV)?

Hepatitis B virus is a deoxyribonucleic acid (DNA) virus belonging to the family Hepadnaviridae. The virion of hepatitis B consists of surface and core components. The core contains DNA polymerase, core antigen and e-antigen.

The DNA structure of the virus is double stranded and circular. Hepatitis B surface antigen (HBsAg) (Australia antigen) is an antigen determinant found on the surface of virus. HBsAg is a marker of infectivity and its presence indicates either acute or chronic HBV infection. Anti-HBs antibody to HBsAg is a marker of immunity. Its presence indicates immune response to HBV infection or vaccination, or presence of passively acquired antibody. Hepatitis B e-antigen (HBeAg) is a marker of high degree of HBV infectivity, and it correlates with high level of HBV replication.

5. What are the modes of transmission of HBV infection?

The following are known modes of transmission:

- Vertical transmission: From pregnant mother to her fetus and baby during the perinatal period. Perinatal transmission is mostly acquired during birth. In utero transmission is rare
- Parenteral transmission: Transmission through blood and blood products is the major contributor for parenteral transmission. Needlestick injuries, unsterile needles, tattooing, ear piercing and acupuncture can also transmit infection
- Sexual transmission: It is a major mode of transmission in developed countries. It is mainly seen among male homosexual individuals and those with promiscuous heterosexual behavior. Transmission can also occur during artificial insemination
- Horizontal transmission: Virus transmission is known to occur unrelated to sexual, vertical and parenteral modes of transmission. This transmission is probably due to close contact. In 50% of cases, no definite history of contact can be elicited.

Fifty percent of the carrier pool is contributed by vertical transmission; while other modes of transmission account for the rest. Risk of developing HBV infection varies inversely with age.

6. Which categories of individuals are most susceptible of HBV infection?

The following individuals are at high risk to suffer from HBV infection:

- Infants born to HBsAg-positive mothers
- Homosexual men, heterosexuals individuals with multiple sex partners or with sexually transmitted diseases (STDs)
- Parenteral drug users who share needles
- Patients on hemodialysis therapy, health care professionals coming in contact with blood and secretions
- Patients receiving blood or blood products (especially on multiple occasions)
- Household contacts of HBsAg-positive individuals
- Sexual partners of persons with acute HBV infection or carriers
- Inmates of institution for developmentally disabled children
- Prison inmates and staff.

7. When does vertical transmission of HBV occur?

Vertical transmission is because of contact between secretions of mother and the baby. This most commonly occurs in late perinatal period or at the time of delivery. It is known to occur via breastfeeding and handling by mother in the postnatal period. Hence, there is the importance of timely vaccination in the neonatal period.

8. What is the efficacy of vertical transmission and what does it depend on?

The overall efficacy of vertical transmission is 30–40%. This increases to 70–90% if mother is also HBeAg-positive, but drops to less than 30% if she has anti-HBe antibodies. In India, about 10% of carrier mothers are also HBeAg-positive. As many as 90% of these newborns infected at birth have potential to become chronic carriers.

9. What happens to newborns infected through vertical transmission?

Newborns infected through vertical transmission usually do not develop acute symptoms, but have a high risk of developing chronic carrier state. Approximately 90% of newborns infected at birth become chronic carriers, and over 25% of these will die of cirrhosis or hepatocellular carcinoma in later life. Thus, perinatally acquired infection has serious consequences for the infant.

10. Is horizontal transmission known to occur with HBV?

Yes, familial clustering of hepatitis B is known. Children attending daycare centers can also possibly transmit HBV infection to each other. Whenever close and long-term contact exists, HBV may become widely disseminated through horizontal transmission. The 25% of HBV infections are known to follow familial clustering. Almost 50% of carrier pool is estimated to be due to horizontal transmission.

11. Can HBV be transmitted in daycare centers via saliva, e.g. drooling infants?

Though HBV has been found in saliva, there are no data to suggest that saliva alone transmits HBV infection. There have been reports of HBV transmission when an HBV-infected person bites another person. In these reports, bloody saliva was usually present in the infected person's mouth and the blood was more likely the vehicle of transmission. HBV is not spread by kissing, hugging, sneezing, coughing, food or water, sharing eating utensils or drinking glasses or casual contact.

12. Can HBV be transmitted by sharing cups or straws?

There are no data to suggest that sharing drinking cups, straws or other eating utensils have been associated with HBV transmission.

13. More of my patients are getting tattoos and body piercing. Should they be concerned about contracting a blood-born infection like HBV?

Yes, tattooing and body piercing have the potential to transmit blood-born infections, including HBV, hepatitis C virus (HCV) and human immunodeficiency virus (HIV), if the person doing the tattoos or body piercing does not use good infection control practices.

14. What is the risk for transmitting HBV by oral sex?

There are no specific data on transmission of blood-borne viruses through oral-genital sex. Saliva has not been associated with HBV transmission unless biting has taken place. HBV is not spread by kissing, hugging, sneezing, coughing, food or water, sharing eating utensils or drinking glasses, or casual contact.

15. Can kissing transmit HBV?

Although HBV has been found in saliva, there is no data to suggest that kissing transmits HBV; however, there have not been studies to specifically look at kissing.

16. I tested positive for chronic HBV infection about 5 months ago. I know there is a vaccine to prevent transmission; however, I would like to know how long my sex partner (I don't have one now) should wait after taking this vaccine, before having sex with me without any risk of transmission?

You should use condoms (the efficacy of latex condoms in preventing infection with HBV is unknown, but their proper use might reduce transmission) until a postvaccination blood test (anti-HBs) shows that your sex partner is protected from HBV infection. For example, your sexual partner should have the three-dose series of hepatitis B vaccine and postvaccination testing 1–2 months after the last dose of vaccine. If your sexual partner's test shows adequate anti-HBs (at least 10 mIU/mL), then he/she should be protected against HBV infection.

17. What are the signs and symptoms of hepatitis B?

About 7 out of 10 adults with acute hepatitis B have signs or symptoms when infected with HBV. Children under age of 5 years, who become infected rarely, show any symptoms. Signs and symptoms of hepatitis B might include nausea, lack of appetite, vomiting, malaise, pain in joints and muscles, headache, photophobia, jaundice, dark urine, clay colored or light stools and abdominal pain. People who have such signs or symptoms generally feel quite ill and might need to be hospitalized. The case fatality rate among persons with reported cases of acute hepatitis B is approximately 1.5%, with the highest rates occurring in adults who are over 60 years of age.

18. How long does it take to show signs of illness after a person becomes infected with HBV?

The incubation period ranges from 45 to 160 days (average 120 days).

19. If a patient is diagnosed with acute hepatitis B and then resolves the infection, can the patient ever get hepatitis B again?

Generally speaking, no. However, it is possible for a person to have two different HBV infections, the second due to an HBV variant or a different HBV subtype.

20. How stable is HBV in the environment and what types of equipment cleaners are virucidal against HBV?

Any high level disinfectant that is tuberculocidal will kill HBV. It is important to note that HBV is quite stable in the environment and remains viable for 7 or more days on environmental surfaces at room temperature. So, the virus is capable of transmitting HBV despite the absence of visible blood.

21. What are HBV mutants?

Table 13.1 describes HBV mutants that are known to occur.

TABLE 13.1 Hepatitis B virus mutants and their biological significance

Mutant form	*Mutation*	*Biological significance*
Precore	Single or multiple mutations in the precore region, at codon 28 or 29; preventing synthesis of HBeAg	Commonest mutation, association with severe disease and fulminant hepatitis
Core	Clustering mutations in core gene, often associated with precore mutations	Progressive liver disease
Envelope vaccine escape mutant	Does not synthesize HBsAg. HBV DNA positive	Responsible for vaccine failure sometimes associated with chronic liver disease
X-gene	Mutations in X-gene	Not known
Pre-S gene	Mutation in Pre-S region	Not known

Incidence of mutants in India varies from 5 to 14% of total HBV infections.

Abbreviations: HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; DNA, deoxyribonucleic acid.

22. How do mutants matter?

The significance of mutants is that:

- Some mutants can escape recognition by routine HBV serology
- HBV serology being negative, these persons may be accepted as blood donors and hence transmission of infection can occur
- Vaccination may not be able to offer protection against some of the mutants
- Some mutants do not have HBeAg, and hence do not induce formation of anti-HBe antibodies. The virus replication continues in spite of HBeAg being negative leading to a false sense of security.

23. I understand that if a person is HBeAg negative and HBsAg positive, he/she is not infectious. Am I correct?

No, HBsAg-positive people are infectious independent of their HBeAg status. HbeAg-positivity indicates higher levels of HBV in the blood compared to an HBeAg-negative person. A person who is HBsAg positive and HBeAg negative is still infectious, but has lower levels of HBV in their blood.

24. Can HBV infection be in a remission? Would you please comment on the appropriateness of this terminology?

"Remission" is not a good term to be used for a persistent infection, such as HBV. HBV infection should be described in terms of virologic markers, infectivity and evidence of liver disease. Some persons might resolve their infection (i.e. become HBsAg negative, and hence are not infectious) spontaneously or from antiviral therapy. Other persons might remain HBsAg positive and hence infectious, but have no evidence of chronic liver disease (i.e. the often used term "healthy carriers"). We assume that the use of "remission" in the question might refer to either of these scenarios.

25. What are possible risk factors for developing liver disease among persons with chronic HBV infection?

Risk factors include older age, male gender, presence of HBeAg, mutations in the precore and core promoter regions of the viral genome, and coinfection with hepatitis D (delta) virus. An association between alcohol use and progression to hepatocellular carcinoma in persons with chronic hepatitis B has been reported in some studies, but not in others; these discrepancies might be related to accuracy of the alcohol history.

26. What are the various serologic tests for hepatitis B?

See Table 13.2.

27. How do I interpret some of the common hepatitis B panel results?

See Table 13.3.

TABLE 13.2 Hepatitis B laboratory nomenclature

HBsAg	HBsAg is a marker of infectivity. Its presence indicates either acute or chronic HBV infection. Anti-HBs antibody to HBsAg is a marker of immunity. Its presence indicates an immune response to HBV infection, an immune response to vaccination, or the presence of passively acquired antibody (It is also known as HBsAb, but this abbreviation is best avoided since it is often confused with abbreviations such as HbsAg)
Anti-HBc (total)	Antibody to HBcAg is a nonspecific marker of acute, chronic or resolved HBV infection. It is not a marker of vaccine-induced immunity. It may be used in prevaccination testing to determine previous exposure to HBV infection (It is also known as HbcAb, but this abbreviation is best avoided since it is often confused with other abbreviations)
Anti-HBc IgM	IgM antibody subclass of anti-HBc. Positivity indicates recent infection with HBV (within the past 6 months)
HBeAg	HBeAg is a marker of a high degree of HBV infectivity, and it correlates with a high level of HBV replication. It is primarily used to assess and monitor the treatment of patients with chronic HBV infection
Anti-HBe	Antibody to HBeAg may be present in an infected or immune person. In persons with chronic HBV infection. Its presence suggests a low viral titer and a low degree of infectivity. HBV-DNA is a marker of viral replication and it correlates well with infectivity. It is used to assess and monitor the treatment of patients with chronic HBV infection

Abbreviations: HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBsAb, hepatitis B surface antibody; HBcAg, hepatitis B core antigen; HbcAb, hepatitis B core antibody; IgM, immunoglobulin M; HBeAg, hepatitis B e-antigen; DNA, deoxyribonucleic acid; HBc, hepatitis B core; HBs, hepatitis B surface.

TABLE 13.3 Interpretation of some common hepatitis B tests, results

Tests	*Results*	*Interpretation*	*Vaccinate?*
HBsAg Anti-HBc Anti-HBs	Negative Negative Negative	Susceptible	Vaccinate if indicated
HBsAg Anti-HBc Anti-HBs	Negative Negative Positive with ≥ 10 mIU/mL*	Immune due to necessary vaccination	No vaccination necessary
HBsAg Anti-HBc Anti-HBs	Negative Negative Positive	Immune due to natural infection	No vaccination necessary
HbsAg Anti-HBc IgM anti-HBc Anti-HBs	Positive Positive Negative Negative	Actually infected	No vaccination necessary

Contd....

Contd....

Tests	*Results*	*Interpretation*	*Vaccinate?*
HbsAg Anti-HBc IgM anti-HBc Anti-HBs	Positive Positive Negative Negative	Chronically infected	No vaccination necessary (may need treatment)
HbsAg Anti-HBc Anti-HBs Positive	Negative Positive Negative	Four interpretations possible**	Use clinical judgment

*Postvaccination testing, when it is recommended, should be performed 1–2 months after the last dose of vaccine. Infants born to HbsAg-positive mothers should be tested for HbsAg and anti-HBs after completion of at least three doses of a licensed hepatitis B vaccination series, at age 9–18 months (generally at the next well child visit)

**(1. May be recovering from acute HBV infection; 2. May be distantly immune, but the test may not be sensitive enough to detect a very low level of anti-HBs in serum; 3. May be susceptible with a false-positive anti-HBc; 4. May be chronically infected and have an undetectable level of HBsAg present in the serum)

Abbreviations: HBsAg, hepatitis B surface antigen; HBc, hepatitis B core; HBs, hepatitis B surface.

28. Is it safe for an HBsAg-positive mother to breastfeed her infant?

Yes. An HBsAg-positive mother, who wishes to breastfeed should be encouraged to do so, including immediately following delivery. However, the infant should receive hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine within 12 hours of birth. Although HBsAg can be detected in breast milk, studies done before hepatitis B vaccine was available; showed that breastfed infants born to HBsAg-positive mothers did not demonstrate an increased rate of perinatal or early childhood HBV infection. More recent studies have shown that, among infants receiving post-exposure prophylaxis to prevent perinatal HBV infection, there is no increased risk of infection among breastfed infants.

29. What is possibility of maternal HBV transmission when breastfeeding an infant if the mother is HBsAg positive and has cracked or bleeding nipples?

As stated before, although HBsAg can be detected in breast milk, there is no evidence that HBV is transmitted by breastfeeding. Babies born to HBsAg-positive mothers should be immunized with hepatitis B vaccine and HBIG, which will substantially reduce the risk of perinatal transmission and protect the infant from modes of postnatal HBV transmission, including the theoretical exposure to HBV from cracked or bleeding nipples during breastfeeding. To prevent cracked and bleeding nipples, all mothers who breastfeed should be instructed on proper nipple care.

30. What types of hepatitis B vaccines are available?

Earlier, two types of vaccines against hepatitis B were available:
- Plasma-derived vaccine, and
- DNA recombinant vaccine

The plasma-derived vaccine consisted of purified inactivated HBsAg particles obtained from the plasma of chronic carriers.

Now only the DNA recombinant vaccine is available. In the DNA recombinant vaccine the antigen particles are obtained from the yeast Saccharomyces cerevisiae through recombinant DNA technology. These are adjuvenated with aluminum salts and lipid A. Both thimerosal-preserved and thimerosal-free vaccines are available. The vaccines are available as single and multidose vials. They need to be stored at 2–8°C. Single pediatric dose vial of vaccine contains 10 µg of antigen and that for adult use contains 20 µg. Multidose vials are available containing 20 µg/mL.

31. What is the dose and schedule of routine vaccination?

The dose of vaccine is age dependent. It is 10 µg for children less than 10 years and 20 µg for children above 10 years or adults. There are a number of schedules. In all schedules, at least three doses are recommended. The classic schedule is 0, 1 and 6 months. It is preferable to start vaccination as early as possible. This may be preferably done within 12 hours of birth especially as routine antenatal screening for HBsAg is not practiced in our country. Importance of birth dose should be emphasized in preventing vertical transmission, which is important for our country. When used to conform to schedules for other childhood vaccines, the third dose should have minimum gap of 8 weeks after the second dose.

The route of vaccination is intramuscular (IM). The site of vaccination is anterolateral thigh in neonates and infants; deltoid in children and adults. Vaccination given by any other route than IM or any other site than anterolateral part of thigh or deltoid should not be considered as valid.

32. What if the child comes late for subsequent doses?

If there is a gap of up to 6 months between the first and second dose or a gap of up to 1 year between second and third dose, there is no need to restart vaccination. Complete the remaining doses as per the original schedule. However, such delays are not desirable, as the person remains unprotected till the schedule is completed. The Centers for Disease Control and Prevention (CDC) recommendations state:
- When the hepatitis B vaccine schedule is interrupted, the vaccine series does not need to be restarted
- If the series is interrupted after the first dose, the second dose should be separated by an interval of at least 8 weeks
- If only the third dose is delayed, it should be administered as soon as possible, after age of 24 weeks (168 days)
- It is not necessary to restart the vaccine series for infants switched from one vaccine brand to another, including combination vaccines.

33. What is the efficacy of hepatitis B vaccine?

Protective serum titers of anti-HBs (more than 10 mIU/mL) develop in 95–98% of healthy infants and children who receive a series of three IM doses. In carefully conducted field trials, efficacy has been estimated to be up to 95%. Vaccine has been shown to induce long-term protection for 20 or more years due to immunologic memory. Therefore, booster doses are not routinely recommended.

34. What are the reasons for vaccine failure?

Improper storage of vaccine and failure to maintain cold chain are the most important causes of vaccine failure. Immunocompromized individuals (those on chemotherapy, etc.) may also fail to respond. Surface mutants of HBV may be able to cause infection even in vaccinated children. In addition, there are individuals who do not respond to the vaccine for no apparent reason. However, half of these people who do not develop anti-HBs antibodies after a three-dose series will do so after additional dose(s).

35. What are other factors that can affect the extent of immunogenicity?

The recommended series of three IM doses of hepatitis B vaccine induces a protective antibody response in more than 90% of healthy adults younger than 40 years of age. After the age of 40 years, the cumulative age-specific decline in immunogenicity drops to less than 90%, and by age of 60 years, only 65–70% vaccines develop protective antibody level. Therefore, the earlier the vaccination starts, the better it is. Besides age, other factors, which decrease immunogenicity to hepatitis B vaccination include obesity, smoking, presence of chronic disease and HIV infection, malignancy like leukemia in whom the response has been seen to be as poor as 20–40% with the vaccines and these cases need to be protected by HBIG prophylaxis. Genetic nonresponders and injection given in the gluteal region also elicit decreased immune response. Whenever doubtful about uptake of vaccine, anti-HBs antibody titer should be done 2–3 weeks after last dose of vaccine to ensure vaccine uptake.

36. For how long is hepatitis B vaccine protective?

Hepatitis B vaccine has been shown to induce long-term protection for 20 or more years. Although antibody level declines after 15–20 years of age, anamnestic reaction develops after a challenge with hepatitis B virus and the person develops enough antibodies for protection. Studies show that protection against clinical illness and chronic HBV infection persists, even though anti-HBs levels might become low or decline below detectable levels. Therefore, booster doses are not routinely recommended.

37. What are the adverse effects of hepatitis B vaccination?

Adverse effects of hepatitis B vaccine are very few. They consist primarily of local reactions or low-grade fever. Serious reactions like anaphylaxis are very rare, but can occur as with any other vaccine.

38. Who should not receive the vaccine?

Persons with a history of serious adverse events, including anaphylaxis, after receipt of hepatitis B vaccine should not receive additional doses. Persons allergic to yeast should not be vaccinated with vaccines containing yeast. As with other vaccines, vaccination of persons with moderate or severe acute illness, with or without fever, should be deferred until the illness improves. Vaccination is not contraindicated in persons with a history of multiple sclerosis, Guillain-Barré syndrome, or autoimmune diseases such as systemic lupus erythematosus or rheumatoid arthritis.

39. What schedule is used for immunocompromized children or children on hemodialysis?

These include primarily those with advanced HIV infection, chronic renal failure, chronic liver disease and diabetes. It is estimated that 10% of the 40 million people infected with HIV infection worldwide are coinfected with HBV; the presence of HIV markedly increases the risk of developing HBV associated liver cirrhosis and hepatocellular carcinoma.

In immunocompromized hosts, one should use double the recommended dose for that age. They may also require additional doses in case they do not show seroconversion following three doses. In situations where early seroconversion is required, one may use accelerated schedule of doses at 0, 1, 2 and 12 months. Despite this, it has been shown that not more than 30% of children with leukemia undergoing chemotherapy show seroconversion. In such cases, it may be better to use regular passive prophylaxis with HBIG. For hemodialysis patients the need for booster doses should be assessed by annual testing for antibody levels and booster doses should be provided when antibody levels decline below 10 mIU/mL.

40. Can we give the vaccine by intradermal route?

Intradermal administration of hepatitis B vaccine reduces the cost tremendously as the dose consists of 0.1 mL. But it has not been found to result in protective antibody titers in all recipients. Hence, testing for antibody response becomes mandatory in these persons. Also, antibody titers may not persist for long in these patients. Thus, this route has not been routinely advocated.

As a matter of fact, hepatitis B vaccine administered by any route or site other than intramuscularly in the anterolateral thigh or deltoid muscle should not be counted as valid.

41. If you want to test and vaccinate your patient for hepatitis B on the same day, does it matter if you test or vaccinate first?

Yes, you should draw the blood first and then administer the first dose of vaccine, as transient HBsAg positivity has been found to occur after a dose of hepatitis B vaccine.

42. How long should a person wait to donate blood after a dose of hepatitis B vaccine?

It is advisable to wait for 1 month. Studies published in the last several years have found that transient HBsAg positivity (lasting less than 21 days) can be detected in certain persons after vaccination.

43. Which schedule is recommended for immunizing a neonate born to an HBsAg positive mother?

In infants born to HbsAg-positive mothers, the first dose of HBV should be given at birth (within 12 hours). This should be given along with HBIG in the dose of 0.5 mL. Both vaccine and HBIG can be given at the same time but they should be administered intramuscularly at separate sites, the second dose of vaccine should be given at the age of 1 month, and the third dose at 6 months of age. One can also use accelerated schedule of four doses given at 0, 1, 2, and 12 months.

44. What about vaccination in preterm and low birth weight infants?

Preterm infants born to HBsAg-positive women and women with unknown status must receive immunoprophylaxis with hepatitis B vaccine and HBIG beginning at shortly after birth. Preterm infants having low birth weight have a decreased response to hepatitis B vaccine administered before 1 month of age; however, by chronological age of 1 month. Preterm infants, regardless of initial birth weight or gestational age, are likely to respond as adequately as full-term infants. Since the HBsAg status of most mothers is usually not known in our country, it is advisable to give the vaccine as recommended above within 12 hours. The next dose given at 1 month age will take care of the immunological incompetence if any of the preterm baby.

For preterm infants weighing less than 2 kg at birth:

- If maternal HBsAg status is positive: Give HBIG plus hepatitis B vaccine within 12 hours of birth. Give three additional doses (with single-antigen vaccine at ages 1, 2, 3 and 6 months, or 2, 4 and 12–15 months. Test for HBsAg and antibody to HBsAg 1–2 months after completion of at least three doses of a licensed hepatitis B vaccine series (i.e. at age of 9–18 months, generally at the next well-child visit). Testing should not be performed before age of 9 months nor within 4 weeks of the most recent vaccine dose

- If maternal HBsAg status is unknown: Give HBIG plus hepatitis B vaccine within 12 hours of birth. Be sure to test the mother's blood for HBsAg. Give 3 additional hepatitis B vaccine doses (with single-antigen vaccine at ages 1, 2, 3 and 6 months, or hepatitis B containing combination vaccine at ages 2, 4 and 6 months or 2, 4, and 12–15 months)
- If the maternal HBsAg status is negative: If you are certain that appropriate maternal testing was done and a copy of the mother's original laboratory report indicating that she was HBsAg negative during this pregnancy is placed on the infant's chart, delay the first dose of hepatitis B vaccine until age 1 month or hospital discharge, whichever comes first. Administer vaccine as per the recommended schedule.

For preterm infants weighing 2 kg or more at birth, follow the recommendations for full-term infants, including the birth dose for all, keeping in mind the special needs of newborns whose mother's HBsAg status is positive or unknown.

45. An infant was given monovalent hepatitis B vaccine at birth. Later, we gave her monovalent vaccine at age of 1 month and age of 4 months. Did we give her the third dose too early?

Yes. Poorer immune response rates are seen in infants who complete the vaccination schedule prior to age of 6 months. Do not count dose number 3, which you gave at age 4 months. Repeat dose number 3 when the infant is at least 6 months old (not earlier than age 24 weeks).

46. What is the recommended time to do hepatitis B testing for evidence of success or failure of immunoprophylaxis given at birth to an infant born to an HBsAg-positive mother?

For infants born to HBsAg-positive mothers, postvaccination testing is recommended 1–2 months after completion of at least three doses of a licensed hepatitis B vaccine series (i.e. at age of 9–18 months, generally at the next well-child visit). Testing should not be performed before age 9 months, as HBIG might still be present for 6–8 months nor should testing be performed within 4 weeks of the most recent vaccine dose, as a false positive HBsAg might occur. Anti-HBc testing of infants or children is not recommended because passively acquired maternal anti-HBc might be detected up to age 24 months in children of HBV-infected mothers.

Hepatitis B surface antigen negative infants with anti-HBs levels of at least 10 mIU/mL are protected and need no further medical management. HbsAg-negative infants with anti-HBs levels less than 10 mIU/mL should be revaccinated with a second three-dose series and retested 1–2 months after the final dose of vaccine. Children who are HBsAg positive should receive medical evaluation and ongoing follow-up.

47. An infant of an HBsAg-positive mother received appropriate post-exposure prophylaxis and tested negative for anti-HBs and HBsAg at 12 months of age. How many more doses of hepatitis B vaccine do I need to give before I retest?

The recommended approach is to complete a second three-dose schedule of vaccine and re-test for both HBsAg and anti-HBs 1–2 months after the third dose of vaccine. If anti-HBs and HBsAg are still negative after revaccination, the child is considered a nonresponder to hepatitis B vaccine.

48. When screening an adopted infant for hepatitis B, at what age would you expect the infant to not show anti-HBs or anti-HBc if it were passively transferred antibody from the mother?

Passively acquired maternal anti-HBs might be detected until age 6–8 months and passively acquired maternal anti-HBc might be detected until age 24 months.

All foreign-born persons (including immigrants, refugees, asylum seekers and internationally adopted children) born in Asia, the Pacific Islands, Africa, and other regions with high endemicity of HBV infection should be tested for HBsAg regardless of vaccination status. Persons testing HBsAg positive should be referred for medical evaluation and ongoing follow-up.

49. Can adolescents be immunized on a 0-, 2-, 4-month schedule for hepatitis B?

Yes. There are data that show adequate seroprotection using this schedule in young adults. If this schedule is used, you should be aware that the studies were in young adults and might not translate to older adults (equal or greater than 40 years). There are other schedules that offer flexibility in vaccination, as well.

50. Three years ago at a middle school, my patient received the first dose of the hepatitis B vaccine series. Should I give her the second dose now, or do I need to start over again with the first dose?

There is no need to restart the series. Give the second dose now and be sure there are at least 8 weeks between that dose and the third dose. No apparent effect on immunogenicity has been documented when minimum spacing of doses is not achieved precisely. Increasing the interval between the first two doses has little effect on immunogenicity or final antibody concentration.

The third dose confers the maximum level of seroprotection, but acts primarily as a booster and appears to provide optimal ling-term protection. Longer intervals between the last two doses result in higher final antibody levels, but might increase the risk for acquisition of HBV infection among persons who have a delayed response to vaccination. No differences in immunogenicity have been observed when one or two doses of hepatitis B vaccine produced by one manufacturer are followed by doses from a different manufacturer.

51. At what anatomic site should hepatitis B vaccine be administered to adults? What needle size should be used?

The deltoid muscle is recommended for routine IM vaccination among adults. The gluteus muscle should not be used as a site for administering hepatitis B vaccine. The suggested needle size is 1–2 inch depending on the recipient's gender and weight (1 inch for females weighing less than 70 kg; 1.5 inch for females weighing 70–100 kg; 1–1.5 inch for males weighing less than 120 kg; and 2: for males weighing 120 kg or more and females more than 100 kg). A 22–25 gauge needle should be used. For optimal protection, it is crucial that the vaccine be administered IM, and not subcutaneously.

52. I would like more information about Twinrix®, the combination of hepatitis A and B vaccine.

Twinrix® [GlaxoSmithKline (GSK)] is an inactivated combination vaccine containing both hepatitis A virus (HAV) and HBV antigens. The vaccine contains 720 Enzyme linked immuno-garbent assay units (ELU) of hepatitis A antigen (half of the Havrix® adult dose) and 20 µg of hepatitis B antigen (the full Engerix-B adult dose). In the US, Twinrix® is licensed for use in people who are age 18 years or older. It can be administered to persons who are at risk for both hepatitis A and hepatitis B, such as certain international travelers, men who have sex with men, illegal drug users or to persons who simply want to be immune to both diseases. Primary immunization consists of three doses given intramuscularly on a 0-, 1- and 6-month schedule. For those who need rapid protection like travelers, earlier three dose schedule of Twinrix® was advised (0, 7 and 21 days). In March 2007, the US Food and Drug Administration (FDA) also approved a four-dose schedule. Now, it consists of three doses given within 3 weeks, followed by a booster dose at 12 months (0, 7, 21–30 days and 12 months). The four-dose schedule could benefit individuals needing rapid protection from hepatitis A and hepatitis B, such as persons traveling to high-prevalence areas imminently and emergency responders, especially those being deployed to disaster areas overseas. Twinrix® cannot be used for post-exposure prophylaxis.

53. I have seen adults who had one or two doses of Twinrix®, but we only carry single-antigen vaccine in our practice. How should we complete their vaccination series with single-antigen vaccines?

Twinrix® is licensed as a three-dose series for persons of age 18 years and older. If Twinrix® is not available or if you choose not to use Twinrix® to complete the Twinrix® series, you should do the following: If one dose of Twinrix® was given, complete the series with two adult doses of hepatitis B vaccine and two adult doses of hepatitis A vaccine. If two doses of Twinrix® were given, complete the schedule with one adult dose of hepatitis A vaccine and one adult dose of hepatitis B vaccine. Another way to consider this is as follows:

- Any combination of three doses of adult hepatitis B or 3 doses of Twinrix® is equal to a complete series of hepatitis B vaccine
- One dose of Twinrix® with two doses of adult hepatitis A is equal to a complete series of hepatitis A vaccine
- Two doses of Twinrix® plus one dose of adult hepatitis A is equal to a complete series of hepatitis A vaccine.

54. We're thinking of using Twinrix® and we're wondering whether we can use it for doses number 1 and number 3 only and use single antigen hepatitis B vaccine for dose number 2?

No. Twinrix® contains 50% less hepatitis A antigen component than Havrix®, GSK's monovalent hepatitis A vaccine (720 versus, 1,440 EIU), so the patient would not receive the recommended dose of hepatitis A vaccine antigen. For this reason, three doses of Twinrix® must comprise the series.

55. What blood test should be used to screen a pregnant woman to prevent perinatal HBV infection?

Screening should be done with the HBsAg test only. This blood test will tell whether a woman has current HBV infection that can be transmitted to her infant. Ordering other blood tests such as total antibody to hepatitis B core antigen (total anti-HBc) and/or antibody to HBsAg (anti-HBs) are not useful when screening to prevent perinatal HBV infections and should not be included in screening pregnant women for perinatal HBV infection. Total anti-HBc will be positive in all HBsAg-positive persons, and anti-HBs are rarely positive in an HBsAg-positive person. Women who are found to be HBsAg positive should then be referred for counseling and medical evaluation that will include further testing. If there is a reason to suspect recently acquired HBV infection in a pregnant woman, IgM class anti-HBc (IgM anti-HBc) could be tested to differentiate recently acquired HBV infection from chronic HBV infection. IgM anti-HBc is the blood test that is positive in recently acquired HBV infection.

56. A female patient was immunized against hepatitis B about 4 years ago. She was recently found to be "hepatitis B positive" by her gynecologist. Is this possible? Could it be a false-positive?

It is possible, but unlikely. The HBsAg test has high sensitivity and specificity, and is quite trustworthy. She might have already been HBsAg positive when she was vaccinated; therefore, the vaccine would not have been effective. You should make sure that the positive test was actually HBsAg and not another hepatitis B test, such as anti-HBs (sometimes confusingly referred to as HBsAb) or anti-HBc. A positive anti-HBs test is expected after vaccination with hepatitis B vaccine, but not a positive anti-HBc or HBsAg. If you are certain after careful checking that the test and reported result are correct, you should then make sure the laboratory that did the test repeated the test in duplicate and then did neutralization. If she is ultimately determined to be truly HBsAg positive, she should be referred to a liver disease specialist for counseling and medical evaluation.

57. Do women who have been vaccinated previously against HBV infection still need to be screened during pregnancy?

Yes. Women who have received hepatitis B vaccine should still be screened for HBsAg early in each pregnancy. Just because a woman has been vaccinated does not mean she is HBsAg negative. Since postvaccination testing is not performed for most vaccinated persons, she could have been vaccinated even though she was already HBsAg positive.

58. Is it safe to give hepatitis B vaccine to a pregnant woman?

Yes, limited data indicate no apparent risk for adverse events to developing fetus. Current vaccines contain noninfectious HBsAg and should cause no risk to the fetus. If the mother is being vaccinated because she is at risk for HBV infection [e.g. a healthcare worker (HCW), a person with an STD, an injection drug user (IDU), multiple sex partners], vaccination should be initiated as soon as her risk factor is identified during the pregnancy. In contrast, HBV infection affecting a pregnant woman might result in severe disease for the mother and chronic infection for the newborn.

59. Should a pregnant lady who is HBsAg positive, for liver disease should be evaluated during her pregnancy only, or should the evaluation wait until the postpartum period? What should I recommend for her husband and her children? How urgent is the time frame?

The earlier the evaluation is done, the better. Consultation with a liver disease specialist (i.e. hepatologist, gastroenterologist, infectious disease specialist) should be done. The consulting/referral physician should be completely aware of the patient's obstetrical status. In addition, the patient's sex partner and children or other household contacts should be tested for HBV infection (total anti-HBc and HBsAg) as soon as possible. If any of them are susceptible to HBV infection (anti-HBc and HBsAg negative), they should be vaccinated; if any are HBsAg positive, they should be referred to or have consultation with a liver disease specialist.

60. What advances have occurred with regards the hepatitis B vaccine?

Over the years, the following advances have occurred:

- Improvement in antigenicity by including the pre-S component

- Combining hepatitis B antigen with other antigens like diphtheria, pertussis and tetanus (DPT) or diphtheria, tetanus and pertussis (DTaP), killed polio vaccines, hepatitis A vaccine, *Haemophilus influenza* type b (Hib) vaccine, etc. For example:
  - Hepatitis A + hepatitis B (Twinrix®)
  - DTaP + killed polio vaccine + hepatitis B (pentavalent vaccine)
  - DTaP+ killed polio vaccine + hepatitis A + hepatitis B + Hib (septavalent vaccine).

61. What is the role of HBV vaccine containing pre-S component?

This vaccine has better immunogenicity especially protection against HBV surface mutant as well.

62. Till what age can the HBV vaccination be given?

Vaccination against HBV can be started at any age. The earlier one starts, the better it is.

After three IM doses of hepatitis B vaccine, more than 90% of healthy adults and more than 95% of infants, children and adolescents (from birth to 19 years of age) develop adequate antibody response. However, there is an age-specific decline in immunogenicity. After age 40 years, approximately 90% of recipients respond to three-dose series, and by 60 years, only 75% of vaccines develop protective antibody titers.

63. What is the role of HBIG?

Post-exposure prophylaxis with HBIG is recommended for newborn babies born to HBsAg-positive mothers. Post-exposure prophylaxis with HBIG is also recommended for health personnel who suffer from accidental needlestick injury and for patients who receive HBsAg-positive blood inadvertently. Preexposure passive prophylaxis with HBIG is needed for individuals failing to respond to vaccine (e.g. immunocompromized children), or in children with disorders that preclude a response (e.g. Agammaglobulinemia), when they are likely to be exposed to the risk of acquiring HBV infection.

64. What are the CDC recommendations for post-exposure prophylaxis after exposure to hepatitis B virus in occupational setting?

Table 13.4 summarize course of actions in this situation as recommended by CDC.

Pool may take decades to manifest. However, effects on carrier pool are already evident in countries that took to universal immunization earlier. Now the cost of vaccine has also come down drastically. Indian Academy of Pediatrics recommends that universal immunization of all children should be followed.

TABLE 13.4 Recommendations for post-exposure prophylaxis after percutaneous or mucosal exposure to hepatitis B virus (HBV) in an occupational setting

Vaccination and antibody response status of exposed persons	*Treatment*			
	Source is HBsAg positive	*Source is HBsAg negative*	*Source is unknown or not tested*	
			High risk	*Low risk*
Unvaccinated	HBIG[†] (one dose) and begin a hepatitis B vaccine series	Begin a hepatitis B vaccine series	Begin a hepatitis B vaccine series	Begin a hepatitis B vaccine series
Known responder[§]	No treatment	No treatment	No treatment	No treatment
Non-responder				
Not revaccinated*	HBIG (one dose) and begin a revaccination series	Begin a revaccination series	HBIG (one dose) and begin a revaccination series	Begin a revaccination series
After revaccination	HBIG (two doses)[†]	No treatment	HBIG (two doses)[†]	No treatment
Antibody response unknown	Treat for anti-HBs[¶] if adequate[§], no treatment if inadequate, HBIG × 1 and vaccine booster	No treatment	Test for anti-BHs[††] if adequate[§], no treatment If inadequate, give vaccine booster and check anti-HBs in 1–2 months	

*Person known to have hepatitis B virus (HBV) infection in the past or who are chronically infected do not require HBIG or vaccine

[†] HBIG (0.06 mL/kg) administered intramuscular (IM)

[§] Adequate response is anti-HBs of at least 10 mIU/mL after vaccination

[¶] Revaccination: Additional three-dose series of hepatitis B vaccine administered after the primary series

[**] First dose as soon as possible after exposure; add the second dose 1 month later

[††] Testing should be done as soon as possible after exposure

Abbreviations: HBsAg, hepatitis B surface antigen; HBIG, hepatitis B immunoglobulin; HBs, hepatitis B surface.

Source: This table was adapted from "Updated US PHS Guidelines for the management of Occupational Exposure to HBV and HIV and recommendations for post-exposure prophylaxis. MMWR Recomm Rep. 2001; 50(No.RR-11): 1-52.

65. A nurse who received the hepatitis B vaccine series more than 10 years ago and had a positive follow-up titer (at least 10 mIU/mL). At present, her titer is negative (<10 mIU/mL). What should be done now?

Nothing. Data show that vaccine-induced anti-HBs levels might decline overtime; however, immune memory (anamnestic anti-HBs response) remains intact indefinitely following immunization. Persons with anti-HBs concentrations that decline to less than 10 mIU/mL are still protected against HBV infection. For HCWs with normal immune status who have demonstrated adequate anti-HBs (at least 10 mIU/mL) following vaccination, booster doses of vaccine or periodic anti-HBs testing is not recommended.

66. A person who is a known nonresponder to hepatitis B vaccine has a percutaneous exposure to HBsAg-positive blood. According to older ACIP recommendations, I have the option to give HBIG × 2 or HBIG × 1 and initiate revaccination. How do I decide which to do?

Current recommendations have been revised. The recommended post-exposure prophylaxis for persons who are nonresponders to hepatitis B vaccine (i.e. have not responded to an initial three-dose series and revaccination with a three-dose series) is to give HBIG as soon as possible after exposure and a second dose of HBIG 1 month later. Exposed persons, who are known not to have responded to a primary vaccine series, but have not been revaccinated with a second three-dose series, should receive a single dose of HBIG and reinitiate the hepatitis B vaccine series with the first dose of hepatitis B vaccine as soon as possible after exposure.

67. Does giving hepatitis B vaccine to a chronically infected person cause any harm?

No, it will neither harm nor help the person.

68. For prevention of HBV infection which is preferred high-risk approach or universal immunization approach?

Considering the high cost of vaccination, initially high-risk approach was followed, wherein individuals at added risk for acquiring infection (such as intravenous drug abusers, patients on chronic hemodialysis therapy, individuals requiring blood and blood products, spouse of an infected or carrier person, infants born to HBsAg-positive mothers, laboratory and health care personnel likely to come to contact with blood and body fluids, sex workers, etc.) were targeted. However, these failed to have any impact on the size of carrier pool in the population.

Universal immunization targets every newborn, child and adult, thereby eliminating all modes of transmission. It also raises the possibility of ultimate eradication of this dreaded virus, as humans are its only reservoirs. Universal immunization may be expensive in the short-term and reduction in carrier.

69. Are there any countries where vaccination against hepatitis B is included as universal immunization?

Yes, USA and most of the European countries have advised universal immunization against hepatitis B.

70. What is future for hepatitis B vaccination?

The available vaccines are quite efficacious. However, any research on developing better vaccines would focus on bringing down the number of doses, making vaccines for non-responders, therapeutic vaccines for chronic cases and exploring the possibility of oral vaccines.

SUGGESTED READING

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Section 3

Non-EPI Vaccines

Chapters

CHAPTER

14

Hepatitis A

Nigam P Narain

1. What is hepatitis A? How is it transmitted?

Hepatitis A is the commonest infection of the liver caused by hepatitis A virus, which is an RNA virus belonging to picornavirus family. Hepatitis A virus spreads mainly through direct person-to-person contact. Transmission less often occurs through food and water contamination or, rarely, through blood. The epidemiology varies according to socioeconomic and sanitary level of the country or area concerned. In some industrialized countries, HAV transmission has practically ceased. In developing countries, HAV infections usually occur early in life resulting in high levels of asymptomatic disease.

Persons from developed countries who travel to under developed parts of the world are at risk of HAV infections. Among those who travel to countries with high or intermediate endemicity and do not receive immunoprophylaxis, the risk is approximately 3 cases per 1000 people per month of stay.

2. What is the epidemiological significance of hepatitis A?

Hepatitis A epidemic occur in two different forms – common source outbreaks and communitywide outbreaks. Communitywide outbreaks are of two different types. In the most common communitywide outbreak, the highest clinical infection rate occurs in those of age 5–14 years, but the main transmitters of HAV are assumed to be asymptomatic infected toddlers. These outbreaks tend to occur periodically. In the other type of communitywide outbreaks, the epidemics are not usually periodic.

Countries are classified as low/medium or highly endemic for hepatitis A. In countries with high endemicity like India, most individuals acquire natural infection in childhood and burden of disease including incidence of outbreaks is low. As a shift occurs towards medium endemicity due to improvements in hygiene and sanitation a certain proportion of children remain susceptible till adulthood. Thus burden of symptomatic disease and incidence of outbreaks paradoxically increase. Epidemiologic data from India though limited, suggests a shift in epidemiology of disease and HAV susceptibility in 30 to 40% of adolescents and adults belonging to the high socioeconomic class.

3. How serious is hepatitis A?

Hepatitis A virus (HAV) infection is a relatively benign infection in young children. As many 85% of children below 2 years and 50% of those between 2 and 5 years infected with HAV are anicteric and may just have nonspecific symptoms like any other viral infection. On the contrary, hepatitis A in adults is symptomatic in 70–95% with a mortality of 1%. The disease severity increases in those with underlying chronic liver disease.

Hepatitis A can be quite serious. Studies show that 35 out of 100 people with hepatitis A are hospitalized, with people age 60 and older more likely to be hospitalized. Many days of work are missed due to hepatitis A, as well. Certain people, such as people with chronic hepatitis C, can get very sick and die from hepatitis A. Death from hepatitis A is fairly rare in healthy young people but more common in people aged 60 years and older.

4. Can HAV cause chronic infection?

No, it causes only acute infection.

5. How long can a person with hepatitis A infection spread HAV?

Generally, duration of spread is two weeks before the appearance of symptoms and one week after the onset of symptoms.

6. Can a person get infected with hepatitis virus more than once?

No. Once a person recovers from infection he develops antibody called anti-HAV that provides life long protection in immunocompetent person.

7. How can it be prevented?

It can be prevented by scrupulous good general hygiene, proper environmental sanitation and immunization by vaccine.

8. Which type of vaccine is used for immunization?

Two types of vaccines are used for immunization. First type is inactivated virus vaccine and second type is live attenuated vaccine made up of H2 strain.

9. What are the different types of inactivated vaccines available in India?

Most of the currently available inactivated vaccines are derived from HM 175/GBM strains and grown on MRC5 human diploid cell lines. The virus is formalin inactivated and adjuvanted with aluminimum hydroxide. The vaccine is stored at 2–8°C.

Another inactivated vaccine is a liposomal adjuvanted vaccine derived from the RG-SB strain, harvested from disrupted MRC-5 cells and inactivated by formalin is now available (Havpur). The liposome adjuvant is immunopotentiating reconstituted influenza virosome (IRIV) composed from an H1N1 strain of influenza virus.

10. What is the vaccine schedule for inactivated hepatitis A vaccine? Is there any difference in efficacy of these vaccines?

The vaccines are given in a two dose schedule, 6 months apart intramuscularly. Two doses are recommended for primary immunization. First dose is given at the age of 12–18 months. Second dose is given after 6–12 months after the first dose.

The adult formulation should be used after the recommended cut-off age of 15 years (Avaxim^{TM}) and 18 years (Havrix^{TM}). Protective antibodies are seen in 95 to 100% 1 month after the 1st dose and almost 100% after the second dose. Serologic correlate of protection has been fixed at 20 mIU/ml. The protective efficacy is around 90–100% and onset of protection is 2 weeks to 1 month after the 1st dose of the vaccine. The vaccine efficacy is lower in the elderly, immunocompromised, those with chronic liver disease, in transplant recipients and those with pre-existing maternal antibodies. The vaccine may be safely given with other childhood vaccines and interchange of brands is permitted though not routinely recommended. Immunity is life-long due to anamnestic response and no boosters are recommended at present in the immunocompetent. Adverse reactions are minor and usually include local pain and swelling.

Both the vaccines have the same efficacy.

11. Can this vaccine be given in second or third year? If yes then which year is the best?

Yes. It can be given at this age, of which second year is the best because:

- It will protect those who do not have maternal antibody.
- It is easier to integrate with existing immunization schedule.
- By the third year many children get immunized by natural subclinical infection.

12. How is hepatitis A vaccine given?

The vaccine is given by an injection into the muscle of anterolateral part of thigh in toddlers and upper part of arm in adults and older children. In patients with bleeding disorders like hemophilia, it has found to be safe and immunogenic when given by subcutaneous route.

13. What are the characteristics of live attenuated hepatitis A vaccine?

This vaccine is derived from the H2 strain of the virus attenuated after serial passage in human diploid cell (KMB 17 cell line). It has been in use in China since the 1990's in mass vaccination programs. The vaccine meets requirements of the

Chinese drug authority and the WHO. It is also now licensed and available in India (Biovac A).

14. What is the dose and schedule of live Hepatitis A vaccine? Is a single dose of live vaccine adequate to protect an individual?

The recommended dose is 1 mm SC ($10^{6.5}$ $CCID_{50}$/ml) in children aged 1–15 years. Immunogenicity studies with single dose show sero conversion rate of more than 98% two months after vaccination and persistence of protective antibodies in more than 80% of vaccines at 10 years follow-up. Uncontrolled studies show an efficacy of almost 100% sustained over 10 years despite decline in seroprotection rate and antibody titers. Recent immunogenicity studies from India have shown 97.3% seroconversion five years following single dose of the vaccine in a multi centric study and 79.3% upto 6 years in another study. Therefore, a single dose of live vaccine is adequate to protect an individual.

15. What are the IAP ACVIP recommendations for use of Hepatitis A vaccine?

The committee has revised its recommendations on administration schedule of live attenuated hepatitis A vaccine, based on the viral H2 strain (Chinese vaccine). Now a single dose of this vaccine is recommended at 12 months of age overriding the previous recommendation of two doses of the same vaccine.

16. Why has IAP changed its earlier recommendation? Do we have adequate data from Indian studies or the committee has relied on old Chinese data?

There are two ongoing studies (uni-centric and multi-centric) from India on the long term persistence of antibodies against Hep-A virus amongst children vaccinated with a single dose of live attenuated Hepatitis A vaccine. The data showed 79.3% of 121 children were seroprotected (considering >20 mIU/mL of anti-HAV antibody titre as seroprotection levels) up to 6 years follow-up in the pivotal single center study whereas 97.3% of 111 children had shown seroprotection after 5 years of follow-up period of multi-centric group. In the multi-centric study, the test subjects maintained good GMT levels even after 5 years of follow-up. The committee had earlier shown its concern on waning of seroprotection in a subgroup of individuals of original single-centre study cohort. However, it was later disclosed that only a handful subjects had shown this phenomenon, and most of these subjects were of comparatively higher age groups than other study subjects. The decision was also facilitated by the SAGE/ WHO recommendations of single dose of live attenuated hepatitis A vaccine. Hence, the committee has now convinced on the long-term immunogenicity and safety of a single dose of live attenuated H2-strain hepatitis A vaccine in healthy Indian children after reviewing the long term follow-up data.

17. Which vaccine should be used and what schedule is recommended by IAP ACVIP?

If a decision to administer the vaccine is taken, any of the licensed vaccines may be used as all have nearly similar efficacy and safety (exception for post exposure prophylaxis/immunocompromised patients where only inactivated vaccines may be used). Two doses 6 months apart are recommended for all inactivated vaccines. A single dose of live attenuated vaccine is sufficient for long-term protection. All Hepatitis A vaccines are licensed for use in children aged 1 year or older. Pre-vaccination screening for Hepatitis A antibody is likely to be cost effective in children older than 10 years at which age the estimated seropositive rates exceed 50%.

18. For how long does a hepatitis A vaccine protect?

Protection after primary immunization ranges from 15 to 20 years in children and longer in adults.

19. How long after first dose we get protection to hepatitis A infection?

It takes 2–4 weeks after first dose. Anti-HAV antibody forms in this duration and helps in protection to HAV infection.

20. Is hepatitis A vaccine safe?

Yes. It has excellent safety profile. No serious side effects have been attributed definitively to HAV vaccine.

21. What are the side effects of hepatitis A vaccine?

Most common side effect is soreness at injection site other side effects are – headache, loss of appetite, low grade fever or tiredness.

22. Can hepatitis A vaccine be administered concurrently with other vaccine?

Yes, it can be safely given with hepatitis B, diphtheria, polio, tetanus, oral typhoid, japanese encephalitis, rabies vaccines and also immunoglobulins can be given at the same time but at different sites.

23. Can a different brand of inactivated Hepatitis A vaccine be used to complete a primary course?

Yes. Any current brand of hepatitis A vaccine can be used to boost another.

24. Does a course of inactivated Hepatitis A vaccine need to be repeated if there has been a long interval between the first and the second dose?

No. An excellent immune response is obtained after a single dose of hepatitis A vaccine, and second dose (if missed) may be given as soon as possible. But there is no need to repeat the first dose.

25. Is there a need for a booster dose inactivated Hepatitis A vaccine after a two dose schedule has been completed?

Not at present. A long-term immunity (5–7 years) is obtained after two doses of vaccine so there is no need for extra dose of vaccine.

26. Is there a vaccine that protects against both HAV and HBV infection?

Yes. Twinrix is a combination of both vaccines, but it is recommended only for persons of above 18 years age. This vaccine is given by a schedule of 0, 1, 6 months.

27. Who should not receive hepatitis A vaccine?

This vaccine should not be given to the following:
- Persons who have hepatitis A infection in past and have recovered from disease
- Persons who are allergic to any component of vaccine
- Children less than one year
- Pregnant and nursing mothers as data regarding safety profile is not available
- Those who are sick or have exanthema (can be delayed).

28. What is the importance of screening before giving this vaccine?

The cost effectiveness of this approach will depend on the likelihood of being anti-HAV positive before getting this vaccination. The general rule is this that if 50% of the target population are anti-HAV positive then screening is cost effective.

29. What is the role of this vaccine in cases of chronic liver disease (CLD)?

Patients with CLD are at increased risk of complications if they are infected with hepatitis A, so these patients should ideally be given this vaccine.

30. What is the role of this vaccine in hepatitis B carriers?

Few studies indicate that if hepatitis B carriers get infected by hepatitis A virus, their disease (hepatitis A) becomes more severe. However, the CDC does not agree with this due to lack of sufficient evidence.

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CHAPTER

15

Varicella

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1. How significant is varicella disease in India?

Varicella caused by the varicella zoster virus (VZV) is a highly contagious disease which in the absence of a vaccination program, affects nearly every person by mid-adulthood in most populations. It is usually a self-limiting and benign illness in children. The mean age of infection is higher in tropical climates as compared to that in the temperate climates, leading to higher number of cases occurring in adolescents and young adults. This could be one of the reasons for a higher morbidity and mortality in the tropical developing countries as compared to the west. One study from India suggests 15% of adults are seronegative and susceptible to varicella. The susceptibility of the unimmunized adults, pregnant women and their infants, and of immunocompromized people from close contacts suffering from varicella is a cause for concern.

2. What is the content of a varicella vaccine?

Varicella vaccine is a live-attenuated vaccine derived from the original Oka strain, grown in human diploid cells. Only one vaccine strain (Oka) is in use throughout the world for manufacture of single antigen and combination vaccines.The minimum infectious virus content should be 1000 plaque units. Clinical studies suggest that the ratio of total viral antigen to total infectious viral particles is important to elicit appropriate immune response to vaccination. Vaccine medium may vary according to the manufacturer but generally contains sucrose and buffering salts. The vaccine is marketed in lyophilized form to improve the stability during prolonged storage. It is reconstituted with sterile distilled water (0.5 mL) according to the instructions provided by the manufacturer. It should be used within 30 minutes of reconstitution.

3. How is the vaccine administered?

The recommended dose is 0.5 mL to be administered subcutaneously. Presently in India it is recommended in all healthy children who can afford the vaccine after one-to-one discussion with the parents. It is to be given as a single dose below 13 years and two doses 4–8 weeks apart in children 13 years or older. All high risk children should however receive two doses 48 weeks apart irrespective of age.

4. What happens if the vaccine is administered intramuscularly?

The vaccine is to be administered subcutaneously and the seroconversion and efficacy data is based on data from individuals administered the vaccine subcutaneously. However inadvertent intramuscular administration does not led to a significant reduction in immunogenicity and seroconversion.

5. What is the efficacy of varicella vaccine and how is it determined?

Immune correlates of protection against varicella vaccine have been studied in the past (Table 15.1) by various serological studies but further research and development is still required.

The FAMA assay is considered the gold standard to which the sensitivity and specificity of other assays of varicella immunity are compared. A seroconversion rate of 100% has been found by the FAMA assay to occur in 1000 children and adults after developing clinical varicella. Since the FAMA test is not widely available, a gp Elisa titre of 5 U/mL or greater at 6 weeks after vaccine is reported to be an "approximate correlate of protection for individual vaccines."

Prelicensure efficacy and postlicensure effectiveness studies have shown the efficacy of a single dose of the vaccine to range from 70 to 90% against any disease and ≥95% against combined moderate and severe disease for 7–10 years after vaccination. Administration of 2 doses three months to 4 years apart improves seroprotection rates to 99% and results in higher GMT's by at least 10-fold. This translates to superior efficacy of 98.3% against any disease/100% against moderate/severe disease and reduces the incidence of breakthrough varicella as compared to single dose by 3.3-fold.

TABLE 15.1 Methods used to determine the correlates of protection against varicella after vaccination

Year	*Parameter of correlate of protection against varicella*	*Efficacy after single dose vaccine*
1982–1991	Anti-VZV (varicella-zoster virus) antibodies at 4-6 weeks after vaccination	96–98% with GMT 1:12 gp Elisa U/mL
2004	Anti-VZV glycoprotein ELISA (gp-ELISA) test with >5 gp Elisa U/mL 6 weeks after vaccination	87.3% with GMT of 12.8 gp ELISA units/mL
2006	A titer of 1:4 of vaccinated fluorescent antibody to membrane antigen (FAMA) at 16 weeks after vaccination	76%

Abbreviations: GMT, Geometric mean titer, ELISA, enzyme-linked immunosorbent assay.

6. What are the side-effects of the vaccine?

It is a very safe vaccine. Local side effects are: pain, redness, swelling in <5% of vaccines. Systemic side effects like fever are rare. Three to seven percent of vaccines can develop varicella like rash, which are very mild with rapid recovery. It can occur up to six weeks after vaccination.

7. Why is it recommended to give two doses of varicella vaccine after 13 years of age?

In prelicensure clinical trials it was noted that the seroconversion in adolescents aged 13–17 years was only 79%, moreover, their GMT was 1:6, half the levels seen in healthy children in this study. These results led to the recommendation of 2 doses of vaccine for children over the age of 13 years.

8. What is breakthrough varicella?

Varicella which occurs in children vaccinated more than 6 weeks before rash onset is called breakthrough varicella and is generally mild and atypical, predominantly maculopaular with little or no fever. But these children should be considered as potentially infectious and isolated like the typical varicella infected children.

9. Why is occurrence of breakthrough varicella a matter of concern?

Approximately 15% of vaccine recipients remain at increased risk of breakthrough disease. These susceptible children may be at risk of severe varicella associated with VZV infection in adolescence and adulthood.

- Studies done in school outbreaks show that schoolchildren with breakthrough disease can serve as the index case for an outbreak
- Because vaccinated cases are mild, recognition or exclusion may be difficult, resulting in more opportunities to infect others. Education of physicians, school officials, and parents about the appearance of varicella in vaccinated persons are important for early recognition of outbreaks
- Varicella infection in immunized population may raise concern regarding vaccine efficacy and a misunderstanding by physicians or parents who may conclude that vaccine efficacy is declining. This misperception can lead to frustration among both parents and physicians and they may lose faith in the varicella vaccine program, especially among people who perceive varicella as a mild illness of childhood
- Because immunized children who experience breakthrough diseases are coinfected with both wild and vaccine strains of varicella virus, they may be at increased risk of zoster from the reactivated wild-type strain later in life, compared with vaccine recipients who do not experience breakthrough disease.

10. What are the causes of vaccine failure?

Primary vaccine failure is defined as failure to mount a protective immune response after a dose of vaccine, and secondary vaccine failure is defined as a gradual loss of immunity after an initial immune response over a period of years after vaccination (waning immunity). The observed vaccine failure after 1 dose of vaccine may be explained in most probability as that immunized children either:

- Do not develop humoral immunity to VZV at all, or
- There is an initial immune "burst" of immunity that is enough to generate a positive gpELISA result but is inadequate to generate a sustained memory T-cell response leading to waning of immunity over a period of time.

11. Does breakthrough varicella occur due to primary vaccine failure or due to waning immunity?

It is still controversial whether the breakthrough varicella occurs due to primary vaccine failure or due to waning immunity.

One study done to estimate primary vaccine failure in 148 healthy child vaccines, found that 113 (76%) seroconverted, and 24% had no detectable VZV FAMA antibodies at 4 months after vaccination. Their data suggested that many cases of varicella in immunized children are due to primary vaccine failure.

The risk of breakthrough varicella and severity of the disease is directly proportionate to the time lapsed from the date of immunization suggesting waning of the immunity acquired by immunization rendering these individual susceptible.

A study involving 11,356 children in California found that breakthrough varicella was twice as likely to be severe (defined as manifesting over 50 skin lesions) in children who became ill more than 5 years after vaccination compared with those who developed breakthrough disease after a short interval. Also it was found that the annual breakthrough rate of disease was 6 times higher in children vaccinated 9 years before compared with those vaccinated 5 years previously. Though this study suggests a waning immunity, the possibility of primary vaccine failure being the causative factor cannot be ruled out.

Studies in Japan indicate persistence of antibodies for at least 20 years; however, these studies were conducted during a period when a substantial amount of wild type VZV was present in the community with many opportunities for natural boosting of immunity by subclinical infection. Thus the available data are inconclusive regarding waning of immunity after one dose of varicella vaccine.

12. Why are the other concerns regarding primary vaccine failure in countries with a good coverage with vaccine?

Primary vaccine failure in just 10% of vaccinees after a single dose could result in progressive accumulation of susceptible individuals over time and lead to an increased incidence of varicella in young adults. Such an increment is potentially dangerous and therefore strongly supports the use of a second dose of vaccine for all children without a history of disease. The unacceptably high rate of primary vaccine failure suggests that the interval between the first and second doses of vaccine should be a matter of months rather than years. Correction of the problem of primary vaccine failure is thus urgent; if done it will probably prevent not only the current phenomenon of isolated outbreaks of breakthrough disease but a subsequent epidemic of serious varicella in vaccinated but unprotected adults.

13. If breakthrough varicella occurs due to primary vaccine failure, why is it milder compared to varicella occurring in unimmunized persons?

It is postulated that an insufficient number of virus specific memory T-cells are generated after one dose vaccination. According to the hypothesis, the immune memory response is less intense after a moderate initial stimulus than after a strong one, because the virus specific effector cell populations are down regulated within weeks or months after the primary contact with the antigen. In this view, the generally moderate course of breakthrough disease indicates that priming has indeed taken place after the single dose of vaccine, but that the initial stimulus did not suffice for complete protection. The fact that a second dose of vaccine brings about a stronger immune memory response supports this hypothesis, as a stronger response would not be expected if a robust primary immune response had already occurred.

14. What are the risk factors for breakthrough varicella?

They include:

- Vaccinating a child less than 16 months of age
- Not observing a gap of 28 days between MMR and varicella vaccination
- Time elapsed since vaccination.

15. Does increasing the virus content in the vaccine help in reducing the risk of breakthrough varicella.

No, because, as the quantity of antigen increases, a plateau effect comes about, so that the immune response probably cannot be made any stronger.

16. If breakthrough varicella occurs due to waning immunity, will the immunity wane after 2nd dose of vaccine?

Quite possible. Waning might also be an issue with the newly recommended two dose vaccination schedule. Sustained surveillance for varicella outbreaks in populations with varicella immunization programs therefore is mandatory.

17. What are the current recommendations of the Indian Academy of Pediatrics Committee of Immunisation (IAPCOI) on varicella vaccine?

The IAPCOI recommends offering the vaccine to healthy children with no prior history of varicella and to children belonging to certain high risk groups as enumerated below:

- Children with humoral immunodeficiencies
- Children with HIV infection but with CD4 counts 15% and above the age related cut-off
- Leukemia but in remission and off chemotherapy for at least 3–6 months
- Children on long-term salicylates. Salicylates should be avoided for at least 6 weeks after vaccination
- Children likely to be on long-term steroid therapy. The vaccine may be given at any time if the children are on low dose steroids/alternate day steroids but only 4 weeks after stopping steroids if the patients have received high dose steroids (2 mg/kg) for 14 days or more
- In household contacts of immunocompromised children
- Adolescents who have not had varicella in past and are known to be varicella IgG negative especially if they are leaving home for studies in a residential school/college
- Children with chronic lung/heart disease
- Seronegative adolescents and adults if they are inmates of or working in the institutional set up, e.g. school teachers, day care center workers, military personnel and health care professionals.

For post exposure prophylaxis in susceptible healthy nonpregnant contacts preferably within 3 days of exposure (efficacy 90%) and potentially up to 5 days of exposure (efficacy 70%, against severe disease 100%).

Now, IAPCOI have also recommended 2nd dose of varicella under 13 years of age just before school entry, i.e. 4–6 years of age.

18. What are the latest recommendations of varicella vaccine by the ACIP?

In June 2005 and June 2006, ACIP adopted new recommendations regarding the use of live, attenuated varicella vaccines for prevention of varicella. This report revises, updates, and replaces the 1996 and 1999 ACIP statements for prevention of varicella.

The new recommendations include:

- Implementation of a routine second dose varicella vaccination program for children, with the first dose administered at age 12–15 months and the second dose at age 4–6 years
- A second dose catchup varicella vaccination for children, adolescents, and adults who previously had received 1 dose; the second dose should be given 3 months after the first dose; however, if the second dose is administered at least 28 days after the first dose, the second dose is considered valid and does not need to be repeated

- Routine vaccination of all healthy persons aged >13 years without evidence of immunity
- Prenatal assessment and postpartum vaccination
- Expanding the use of the varicella vaccine for HIV-infected children with age specific CD4+T lymphocyte percentages of 15–24% and adolescents and adults with CD4+T lymphocyte counts >200 cells/μL, and
- Establishing middle school, high school, and college entry vaccination requirements. ACIP also approved criteria for evidence of immunity to varicella.

19. What is the rationale behind the ACIP recommending the two dose schedule for children?

Despite the successes of the one dose vaccination program in children in US, vaccine effectiveness of 85% has not been sufficient to prevent breakthrough varicella outbreaks which is contagious.

Studies of the immune response after 1 and 2 doses of varicella vaccine demonstrate a greater than tenfold boost in GMTs when measured 6 weeks after the second varicella vaccine dose. A higher proportion (>99%) of children achieve an antibody response of >5 gp ELISA units after the second dose compared with 76–85% of children after a single dose of varicella vaccine.

The efficacy of a single dose versus two doses of vaccine was tested in a randomized clinical study involving 2,216 children who were vaccinated in the USA from 1991 to 1993 (15). Over 10 years of follow-up, the cumulative rate of breakthrough disease in children who had been vaccinated twice was lower by a factor of 3.3 than that of children who had been vaccinated once (2.2% compared to 7.3%; $p < 0.001$). Breakthrough disease most commonly occurred two to five years after vaccination in both groups. Among the children who had been vaccinated twice, no breakthrough disease at all arose during the interval from seven to ten years after vaccination. Hence in June 2006, US implemented a routine 2-dose varicella vaccination program for children, with the first dose administered at age 12–15 months and the second dose at age 4 to 6 years.

20. When implementing a 2-dose schedule below 13 years, what should be the ideal gap between the two doses?

A study where the second dose was given 4–6 years after the first dose found a large increase in the antibody levels during the 7–10 days after the second dose, indicating an anamnestic response (geometric mean titer on the day of second dose: 25.7 and on day 7–10 after second dose: 143.6). However, the antibody levels after 2 doses administered 4–6 years apart were comparable to those levels seen when 2 doses were administered 3 months apart.

The timing of the 2nd dose recommended in US at 4–6 years was partially for compatibility with the MMR dose schedule. But since breakthrough varicella have been observed to have occurred commonly as early as two years, the 2nd dose of varicella can be administered early, i.e. at any time 8 weeks after first dose.

21. How is varicella vaccination related to the incidence of herpes zoster?

Some studies have suggested that the exposure of individuals with latent wild type.

VZV infection (as a result of natural infection) to individuals with varicella reduces the risk for HZ, presumably by externally boosting VZV immunity. After implementation of vaccination program, there is a predicted rise in herpes zoster cases especially for a short to medium term (up to 70 years) although in the long-term a reduction in zoster cases is expected to occur provided that the vaccine recipients have a lower risk of developing zoster than persons who acquire natural infection.

22. Is varicella vaccine recommended for adolescents and adults? What is the Indian scenario of susceptibility of adolescents and adults to varicella?

Yes, healthy adolescents and adults with no evidence of immunity should receive 2 doses of varicella vaccine 4–8 weeks apart. Varicella is a more severe disease in adults. Nearly 100% seropositivity to VZV has been documented by 11–13 years in USA. However, a study from India revealed that the prevalence of antibodies to varicella gradually increases through childhood, adolescence and adulthood. An overall seropositivity rate of >70% was reached between the ages of 11–15 years which increased to nearly 90% at the age of 30 years demonstrating that a significant proportion of adolescents and adults are susceptible to varicella in India.

VZV infection is often more severe in adults than in children, suggesting that India may be at risk of greater morbidity and mortality as a result of later age seroconversion.

23. Can a 30-year-old seronegative/susceptible lady be administered varicella vaccine?

It is not very stringent in India, unlike in countries like US, to furnish an evidence of immunity for varicella to decide whether vaccine is to be given. A history of varicella infection in the past is fairly acceptable. Documentation of age appropriate vaccination with a varicella vaccine, diagnosis or verification of a history of varicella disease/herpes zoster by a healthcare provider, laboratory evidence of immunity or laboratory confirmation of disease are hardcore evidences, but not easily available. In absence of these evidences, it is better to provide the vaccine.

24. Are there some groups of adults for whom varicella vaccination is especially important?

Yes, varicella vaccination is especially important for the following groups of susceptible adults:

- Persons who have close contact with persons at high risk for serious complications from VZV infection; for example, healthcare workers and family members/close contacts of people with impaired immune systems

- Persons who live or work in environments in which VZV transmission is likely: for example, teachers of young children, childcare employees, and residents/staff in institutional settings
- Persons who live or work in places where VZV transmission can readily occur; for example, college students, inmates and staff of correctional institutions, and military personnel
- Nonpregnant women of childbearing age (women should avoid Pregnancy for 1 month following each vaccine dose)
- adolescents and adults living in households with children
- International travellers.

However, all healthy susceptible adults should be vaccinated.

25. When is the vaccine contraindicated?

Varicella vaccine is contraindicated in the following situations:

- In persons allergic to the vaccine or its constituent
- In persons who have any malignant condition, including blood dyscrasias, leukemia, lymphomas of any type, or other malignant neoplasms affecting the bone marrow or lymphatic systems
- Varicella vaccines should not be administered to persons who have a family history of congenital or hereditary immunodeficiency in first-degree relatives (e.g. parents and siblings) unless the immune competence of the potential vaccine recipient has been clinically substantiated or verified by a laboratory
- Varicella vaccines should not be administered to persons receiving high-dose systemic immunosuppressive therapy, including persons on oral steroids >2 mg/kg of body weight or a total of >20 mg/day of prednisone or equivalent for persons who weigh >10 kg, when administered for >2 weeks
- Because the effects of the varicella virus vaccine on the fetus are unknown, pregnant women should not be vaccinated. Nonpregnant women who are vaccinated should avoid becoming pregnant for 1 month after each injection. If a pregnant woman is vaccinated or becomes pregnant within 1 month of vaccination, she should be counselled about potential effects on the fetus. A registry of such patient however has not reported any adverse outcome so far.

26. In which patients should the clinician take a decision to vaccinate with precaution?

Vaccination of persons, who have acute severe illness, including untreated, active tuberculosis, should be postponed until recovery. The decision to delay vaccination depends on the severity of symptoms and on the etiology of the disease.

27. Can a child with ITP be given varicella vaccine?

Cases of thrombocytopenia have been reported after MMR vaccine and after varicella vaccination, but it is not a contradiction for receiving varicella vaccine.

28. Can an individual who has received antibody containing product like whole blood or immunoglobulin be given the vaccine?

Because of the potential inhibition of the response to varicella vaccination by passively transferred antibodies, varicella vaccines should not be administered for the same intervals as measles vaccine (3–11 months, depending on the dosage) after administration of blood (except washed red blood cells), plasma, or IG.

29. Can the vaccine be give to a child receiving salicylates and is salicylate contraindicated after varicella immunization?

No adverse events associated with the use of salicylates after varicella vaccination have been reported; however, the vaccine manufacturer recommends that vaccine recipients avoid using salicylates for 6 weeks after receiving varicella vaccines because of the association between aspirin use and Reye syndrome after varicella. Vaccination with subsequent close monitoring should be considered for children who have rheumatoid arthritis or other conditions requiring therapeutic aspirin. The risk for serious complications associated with aspirin is likely to be greater in children in whom natural varicella develops than it is in children who receive the vaccine containing attenuated VZV.

30. Can varicella vaccine be coadministered with MMR even though both are live vaccines?

Single antigen varicella vaccine is well tolerated and effective in healthy children aged ≥12 months when administered simultaneously with MMR vaccine either at separate sites and with separate syringes or separately ≥4 weeks apart.

31. How does one protect a newborn who is exposed to a case of varicella?

A full term healthy newborn is not at increased risk for complications and does not merit prophylaxis with VZIG/IVIG if exposed to varicella. All neonates born at less than 28 weeks of gestation/ with birth weight less than 1000 gms, exposed in the neonatal period should be administered VZIG. All preterm neonates born at more than 28 weeks of gestation and exposed to varicella in the neonatal period should be administered VZIG only if their mothers are negative for antivaricella IgG.

32. What is the risk to the newborn delivered to a mother having varicella at the time of delivery ?

A maternal rash erupting 5 days before to 2 days after delivery is frequently associated with clinically severe varicella in the newborn, leading to high mortality if untreated. Then the newborn is infectious and must be isolated till scabs are formed. In this setting, acyclovir is generally recommended (60 mg/kg divided every 8 hours). Varicella zoster globulin (VZIG) 125 units/kg IM should be given as soon as possible after delivery.

33. Who should be administered VZIG?

IAPCOI recommends the use of VZIG for post exposure prophylaxis in the following **susceptible individuals** with **significant contact** with varicella/ herpes zoster who are at high risk for severe disease. Here Susceptible is defined as:

- All unvaccinated children who do not have a clinical history of varicella in the past
- All unvaccinated adults who are seronegative for antivaricella IgG. Bone marrow transplant recipients are considered susceptible even if they had disease or received vaccinations prior to transplantation.

A significant contact is defined as any face-to-face contact or stays within the same room for a period greater than 1 hour with a patient with infectious varicella (defined as 1–2 days before the rash till all lesions have crusted) or disseminated herpes zoster.

Patients meeting these two criteria and who are at high risk of developing severe disease merit prophylaxis with VZIG.

34. What is the problem of chickenpox in immunocompromized host?

Varicella can be very severe in immunocompromized host. Chances of complications like encephalitis, pneumonitis, generalized varicella or hemorrhagic varicella are high. 50% of them have secondary bacterial soft tissue infections, 70% develop encephalitis, 40–50% develop pneumonitis. Mortality is 7% in children and 50% in adults.Though only 0.1% cases of all chickenpox cases occur in immunocompromized hosts, it contributes to 25% of mortality due to varicella disease. Hence these children except the ones with severe T cell defects, should be immunized irrespective of age. They should receive 2 doses of the vaccine.

35. How does one protect an immunocompromized child with leukemia (or any other malignancy) on exposure to varicella?

All immunocompromized children especially neoplastic disease, congenital or acquired immunodeficiency or those receiving immunosuppressive therapies should be administered VZIG. Patients who received IVIG @ 400 mg/kg in the past 3 weeks are considered protected and need not be given VZIG.

36. How late can VZIG be administered to a patient? What is the dose and how is it administered?

VZIG should be given as soon as possible but not later than 96 hours following exposure.VZIG reduces risk of disease and complications and the duration of protection lasts for 3 weeks. The currently available VZIG is for intravenous use (Varitect) and is administered at a dose of 0.2–1 mL/kg diluted in normal saline over 1 hour. The efficacy against death in cases where neonatal exposure has occurred is almost 100%. Side effects include allergic reactions and anaphylaxis. Since VZIG prolongs the incubation period, all exposed should be monitored for at least 28 weeks for disease manifestations.

37. Can one use IVIG in place of VZIG ?

The cost of VZIG is prohibitive and is not easily available. If unaffordable/not available the options with uncertain efficacy include IVIG @ 200 mg/kg or oral acyclovir, 80 mg/kg/day beginning from the 7th day of exposure and given for 7–10 days.

38. What should be the interval between administration of VZIG and Varicella Vaccine?

Any patient who receives VZIG to prevent varicella should receive varicella vaccine subsequently, provided the vaccine is not contraindicated. Varicella vaccination should be delayed until 5 months after VZIG administration. Varicella vaccine is not needed if the patient has varicella after administration of VZIG.

39. What is the data on contagiousness of varicella vaccine ?

Transmission of the vaccine virus from vaccinees to contacts is rare especially in the absence of a vaccine related rash in the vaccinees. However, vaccine recipients who develop a rash should avoid contact with persons without evidence of immunity who are at high risk for severe complications.

40. How contagious is breakthrough varicella ?

A study demonstrated that vaccinated persons with varicella with <50 lesions were only one third as contagious as unvaccinated persons with varicella. However, vaccinated persons with varicella who had >50 lesions were as contagious as unvaccinated persons with varicella. Vaccinated persons with varicella tend to have milder disease, and, although they are less contagious than unvaccinated persons with varicella, they might not receive a diagnosis and be isolated. As a result, they might have more opportunities to infect others in community settings, thereby further contributing to VZV transmission. Vaccinated persons with varicella also have been index case-patients in varicella outbreaks.

41. Can the sibling of a child undergoing chemotherapy be given the varicella vaccine?

Yes. IAPCOI, ACIP and AAP recommend that healthy household contacts of immunocompromized persons be vaccinated. This is the most effective way to protect the immunocompromized person from exposure to wild type varicella. However, because of the small risk of household transmission of vaccine virus, vaccinees who develop a vaccine related rash should avoid contact with immunocompromized persons while the rash is present. To date, there have been no documented cases of transmission of varicella vaccine virus to immunocompromized persons. If a susceptible immunocompromized person is inadvertently exposed to a person with a vaccine-related rash, post exposure treatment with varicella zoster immune globulin (VZIG) is not needed because the disease associated with this type of transmission would be expected to be mild. On the basis of available data, the benefit of vaccinating susceptible household contacts of immunocompromised persons outweighs the low potential risk of transmission of vaccine virus to immunocompromised persons.

42. Can persons with HIV/AIDS receive the vaccine?

Screening for HIV infection is not indicated before routine varicella immunization. Vaccine is contraindicated in individuals with clinically manifest HIV. After weighing potential risks and benefits, varcella vaccine should be considered for HIV-infected children in CDC class I with a CDC T lymphocyte percentage of 15%.

HIV-infected children in this group should receive 2 doses of the single-antigen vaccine , separated by 3 months. They are encouraged to return to their healthcare provider if they experience a postvaccination, varicella like rash. Previously, this vaccine was recommended for children in CDC classes N1 and A1 who have age specific CD4 percentages greater than 25%.

Data on the use of varicella vaccine in HIV-infected adolescents and adults are lacking, and the immunogenicity may be lower in this group of HIV-infected individuals. However, based on expert opinion in examining the risk of severe disease from wild varicella infection compared to the benefit of vaccination, vaccination (2 doses administered 3 months apart) of HIV-infected persons >8 years of age who are in CDC clinical class A or B and have CD4+ T-lymphocyte counts greater than or equal to 200 cells/μL may be considered.

If inadvertent vaccination of HIV-infected person results in clinical disease, acyclovir may be used to modify the disease.

43. Can a child on budesunide, budecort metered dose inhaler for asthma since past 6 months receive varicella vaccine?

Yes. The child on MDI can be given varicella vaccine though the data are lacking on whether persons receiving inhaled, nasal, or topical steroids without evidence of immunity can be vaccinated safely. It is recommended that persons without evidence of immunity who are receiving systemic steroids for certain conditions and who are not otherwise immunocompromized can be vaccinated if they are receiving less than 2 mg/kg of body weight or total of less than 20 mg/day of prednisone or its equivalent. Some experts suggest withholding steroids for 2–3 weeks after vaccination if that can be done safely.

44. Is it safe to administer the vaccine to a child of nephrotic syndrome on daily oral steroids (>2 mg/kg/day) since past three weeks?

It is recommended that persons who are receiving high doses of systemic steroids (i.e., greater than or equal to 2 mg/kg prednisone) for greater than or equal to 2 weeks may be vaccinated once steroid therapy has been discontinued for at least 1 month. This child can be given the vaccine once the steroids have been discontinued for at least one month.

45. Can a postpartum lactating woman who is seronegative (susceptible) be administered the varicella vaccine?

Postpartum vaccination of women without evidence of immunity need not be delayed because of breastfeeding. Women who have received varicella vaccination postpartum may continue to breastfeed. The majority of live vaccines are not associated with virus secretion in breast milk. Therefore, single antigen varicella vaccine may be administered to nursing mothers without evidence of immunity.

46. Is there a need to implement a 2-dose varicella schedule <13 years in India?

It has been observed in an Indian study that presently a significant proportion of adolescents and adults are seronegative and are at risk of contracting varicella disease. The Indian scenario also defers from the western world that there is ample opportunity for natural boosting due to wide circulation of the virus due to very low coverage with the vaccine. Hence the recommendations from the western world need to be put in proper perspective and cannot be blindly applied in our setting vaccine efficacy is highly influenced by the vaccine coverage, the disease prevalence and the sociodemographic properties of the population. At the same time we are also seeing breakthrough varicella in children immunized with one dose, in spite of the opportunities of natural boosting. Irrespective of whether there is a possibility of primary vaccine failure or waning immunity or failure of natural boosting, 2 doses of varicella will indeed work better than one dose for the individual protection. This would also help avoid loss of faith in efficacy of the vaccine amongst the public and medical fraternity.

47. What are the challenges if 2-dose schedule is implemented?

As 2-dose immunization schedules are introduced into clinical care and as the vaccine coverage improves, diligent monitoring of breakthrough disease and disease outbreaks will be critical. A 2-dose schedule is anticipated to substantially decrease disease outbreaks and breakthrough disease. If this is the effect, then wild type VZV will circulate to an even lesser extent than it does now, and whether decreased circulation of VZV will contribute to waning immunity over time after receipt of 2 doses of a varicella containing vaccine will need to be monitored.

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CHAPTER

16

Measles, Mumps, and Rubella

Baldev S Prajapati, Sudhir Kumar Choudhary

1. Who should receive MMR vaccine?

The principal strategy to prevent mumps is to achieve and maintain high immunization levels. The Advisory Committee on Immunization Practices (ACIP) recommends that:

- All preschool-aged children 12 months of age and older receive one dose of measles, mumps, and rubella vaccine (MMR)
- All school-aged children receive two doses of MMR
- All adults have evidence of immunity against mumps.

As discussed in detail later, two doses of mumps vaccine are more effective than a single dose. Consequently, during outbreaks and for at-risk populations, ensuring high vaccination coverage with two doses is encouraged. For example, healthcare workers may be at increased risk of acquiring mumps and transmitting it to patients and thus should receive two doses of MMR vaccine or provide proof of immunity. Since vaccination is the cornerstone of mumps prevention, public, and private health entities concerned about spread of mumps in a population can review the vaccination status of populations of interest and work to address gaps in vaccination.

2. How long does it take to develop immunity to mumps after vaccination with MMR?

In one study, 86.6% of vaccinees had evidence of mumps seroconversion at 4 weeks after immunization and 93.3% had evidence of seroconversion after 5 weeks. However, seroconversion may not result in immunity. About 80% of persons who have received one dose can be considered protected and 90% after two doses.

3. Are there any changes to the childhood vaccination schedule for mumps during an outbreak?

No. Any changes would depend on the epidemiology and age groups affected by an outbreak. Unless otherwise advised, children should be vaccinated according to the vaccination schedule. Preschool-aged children should receive the first dose of MMR vaccine as close to age 12 months as possible (i.e. on or after the first birthday). The second dose of MMR vaccine is recommended when children are aged 4 to 6 years (i.e. before a child enters kindergarten or first grade). This recommended timing for the second dose of MMR vaccine has been adopted jointly by ACIP, the American Academy of Pediatrics (AAP), IAP, and the American Academy of Family Physicians (AAFP).

If an outbreak affects the 1 to 4-year-old age group, then those children should receive their second MMR dose, provided 28 days have passed since receipt of their first dose of MMR.

A two dose vaccine schedule for measles vaccine administered as MMR was recommended in 1989. In 1998, states were strongly encouraged to take immediate steps to ensure that, by 2001, all children in grades kindergarten through 12 have received two doses of MMR vaccine. If required, the second MMR dose should be administered as soon as possible, but no sooner than 28 days after the first dose.

4. Why MMR vaccine is not included in EPI schedule?

MMR vaccine is not incorporated in EPI schedule by Government of India due to following reasons:

- Lack of epidemiological data on the incidence of mumps and rubella.
- For the purpose of universal immunization, the vaccine coverage should be at least 80% and should be sustainable for a long time, otherwise it will cause an epidemiological shift and worse situation due to increasing complications following disease in older age group. Measles vaccine coverage in India is 67% and in some states like Bihar and Uttar Pradesh it is < 30%. In this situation, there is fear of paradigm shift of epidemiology of measles infection. It can also cause increase in congenital rubella syndrome.
- Lack of funds is also a big constraint for inclusion of MMR as a routine vaccine in EPI.

5. What is the recommendation of the IAP ACVIP regarding inclusion of MMR vaccine in EPI ?

With at least 80% immunization coverage which is sustainable on a long-term basis, any vaccine can be introduced for the purpose of universal immunization. On this scientific principle, MMR vaccine had been introduced in some Indian states where measles coverage was at least 70%. Simultaneously, a system of surveillance of CRS and catch up immunization for all adolescent girl also needed to be instituted. The IAP has always said that the MMR vaccine in EPI will improve protection against measles by those who have missed measles vaccine or failed to sero convert to the first dose of vaccine. It will also reduce incidence of CRS to some extent and will provide added protection against mumps. Having understood these scientific facts, IAP ACVIP has since long recommended including MMR vaccine in EPI. Now, the Academy has revised its recommendation on Measles and MMR vaccination schedule. The new schedule will have a dose of MMR at 9 months instead of Measles and another dose (2nd) of MMR at 15 months of age. The earlier recommendation of second dose of MMR at 4–6 years of age has been removed now.

6. The last IAP COI recommended second dose of MMR. At what age and why this recommendation was made?

IAPCOI recommended MMR vaccine under category II. Two doses were recommended, one at age of 12 to 15 months and second at school entry (4–6 years) or at any time 8 weeks after the first dose. The reason is that in a child aged 12 months or older who has not received measles vaccines, two doses of MMR at 8 weeks intervals suffices and monovalent measles vaccine is not required. Catch up vaccination with two doses of the vaccine should be given to all those who are not previously immunized. This is specifically indicted for health care workers, adolescent girls and students travelling for studies overseas.

Being non EPI vaccine, MMR vaccine is unlikely to impact the epidemiology of rubella at present. Break through mumps infection has been observed in several children at older age who received the MMR vaccine at very early age. The second dose of MMR vaccine is given to cover those who have missed or have failed to seroconvert with the first dose of both measles and mumps vaccination. It also offers protection against rubella due to primary vaccine failure. It also prevents break through mumps infections and CRS.

7. Why IAP has suddenly changed its recommendations pertaining to MMR vaccine?

The relook becomes unavoidable following the NTAGI Standing Technical Sub-Committee (STSC) recommendation of two doses of MR vaccines in UIP at 9 months and 16–24 months at the time of 1st booster of DTP vaccine. Since the Academy has argued very strongly in favor of MMR instead of MR vaccine in UIP schedule, the revised recommendations will facilitate inclusion of Mumps vaccine in the NIP in near future. Furthermore, it will be more sync with the upcoming UIP schedule.

8. IAP ACVIP has now recommended first dose of MMR at 9 months. Do you think MMR will be effective before 12 months of age? Is there adequate data to support this recommendation?

There are many studies both from India and from other countries demonstrating efficacy and safety of MMR vaccine given at 9 months of age (Table 16.1).

TABLE 16.1 Summary of studies evaluating seroconversion after measles, mumps, and rubella vaccines administered at different ages

Authors	*Place*	*Year*	*Ages/age groups compared*	*Seroconversions at different age groups*		
				Measles	*Mumps*	*Rubella*
Schoub BD, et al.	South Africa	1990	9 and 15 months	Better at 9 months	Similar in both groups	Similar in both groups
Giammanco G, et al.	Italy	1993	10–12 and 15–24 months	Similar, but lower GMTs at 9–12 months	Similar, but lower GMTs at 9–12 months	Similar, Same GMTs
Singh R, et al.	Vellore, India	1994	9, 12, and 15 months	Lower at 9 months (80%) than at 12 and 15 months (95%)	Lower at 9 months (75%) than at 12 and 15 months (92%)	Similar (92%) at all the three age groups
Forleo-Neto E, et al.	Brazil	1997	9 and 15 months	Similar in both groups	Similar in both groups	GMTs higher in 15 months age group
Klinge J, et al.	Germany	2000	9–11, 12–14 or 15–17 months	Lower seroconversion in 1 and 3 groups only (84.8 vs. 100%)	Similar in all the groups	Similar in all the groups
Yadav S, et al.	New Delhi, India	2003	9–10 and 15–18 months	Similar (92% in each group)	Similar (100 vs. 96%)	Similar (98 vs. 94%)
Goh P, et al*.	Singapore	2007	9 and 12 months	Similar (>92% in each group)	Similar	Similar

GMT, geometric mean titers.

*Seroconversion of varicella along with measles, mumps and rubella was also studied. (Adapted FROM IAP Perspectives on Measles and Rubella Elimination Strategies. Indian Academy of Pediatrics, Advisory Committee on Vaccines and Immunization Practices (ACVIP), Vashishtha VM, Yewale VN, Bansal CP, Mehta PJ. Indian Pediatr. 2014;51(9):719-22).

9. Why is a second dose of MMR necessary?

Between 2 and 5% of persons do not develop measles immunity after the first dose of vaccine. This occurs for a variety of reasons. The second dose is to provide another chance to develop measles immunity for persons who did not respond to the first dose.

10. If you can give the second dose of MMR as early as 28 days after the first dose, why do we routinely wait until school entry to give the second dose?

The second dose of MMR may be given as early as a month after the first dose, and be counted as a valid dose if both doses were given after the first birthday. It is convenient to give the second dose at school entry, since the child will have an immunization visit for other school entry vaccines. The second dose is not a "booster"; it is intended to produce immunity in the small number of persons who fail to respond to the first dose.

11. What is the recommended length of time a woman should wait after receiving rubella (or MMR) vaccine before becoming pregnant?

Four weeks. In October 2001, ACIP voted to change its recommendation for the waiting interval following the administration of rubella vaccine. The interval was reduced from 3 months to 4 weeks. The waiting period for measles and mumps vaccine was already one month.

12. What should be done if inadvertent immunization with MMR takes place in early pregnancy?

The rubella component of MMR vaccine can cause teratogenic effect on the fetus and it may result in congenital rubella syndrome as this is the period of organogenesis. Therefore, a female should avoid pregnancy for the 4 weeks after the MMR vaccination. Still inadvertent MMR vaccination in early pregnancy is not an indication for termination of pregnancy. The babies born to women inadvertently vaccinated in pregnancy have been followed up and development of any malformation of congenital rubella syndrome has not observed. Close contact with a pregnant woman is NOT a contraindication to MMR vaccination of the contact. Breastfeeding is NOT a contraindication to vaccination of either the woman or the breastfeeding child.

13. Would you consider a person with 2 documented doses of MMR vaccine to be immune even if their serology for 1 or more of the antigens comes back negative?

There is no ACIP recommendation for this situation. A negative serology would more likely be the result of an insensitive test than of a true vaccine failure. No more doses are necessary.

14. If a pregnant woman had a positive rubella titer in the past, and now has a negative rubella titer, she would not need another MMR vaccination. Doesn't the negative rubella titer mean her immunity has waned and she needs a booster dose?

Rubella antibody levels may decline with time, and may even fall below the level of detection of standard screening tests. However, data from surveillance of rubella and congenital rubella syndrome suggest that waning immunity with increased susceptibility to rubella disease does not occur (MMWR 1998;47[RR-8]:14). Studies of persons who have "lost" detectable rubella antibody indicate that almost all had antibody detectable by more sensitive tests, or demonstrated a booster-type response (absence of IgM antibody and a rapid rise in IgG antibody) after revaccination.

15. If a woman has a negative rubella titer during her first pregnancy, should she be given MMR vaccine or only rubella vaccine alone prior to hospital discharge?

She should be given MMR, unless she has documentation of immunity to measles and mumps (birth before 1957, documented vaccination, or serologic evidence of immunity).

16. In USA, a pregnancy test is required for all 7th graders before giving an MMR. Is this really necessary?

No. ACIP recommends that women of childbearing age be asked if they are currently pregnant or attempting to become pregnant. Vaccination should be deferred for those who answer "yes." Those who answer "no" should be advised to avoid pregnancy for four weeks following vaccination.

17. Should we give an MMR to a 15-month-old whose mother is 2 months pregnant?

Yes. Measles, mumps, and rubella vaccine viruses are not transmitted from the vaccinated person, so MMR does not pose a risk to a pregnant household member.

18. What are the contraindications of MMR vaccination?

MMR is contraindicated in patients with severe immunodeficiency, pregnancy and those with history of serious allergic reaction to vaccine or its components. Pregnancy is considered as an absolute contraindication to MMR vaccination.

19. Which component of MMR vaccine can cause aseptic meningitis? What are its sequelae?

Aseptic meningitis can rarely occur 2–3 weeks following MMR vaccination due to mumps. The peak incidence is in the 3–4 years of age. It is usually mild and fortunately death or long-term sequelae is very rare.

20. Can MMR be given on the same day as other live virus vaccines (e.g., varicella)?

Yes. However, if two live vaccines (e.g., MMR and varicella) are not administered on the same day, they should be separated by an interval of at least 28 days.

21. If exposed, will the MMR vaccine prevent mumps infection?

Mumps vaccine has not been shown to be effective in preventing mumps in already infected persons.

22. Should an IgG be drawn after two doses of MMR?

No. After vaccination, it is not necessary to test patients for IgG to confirm immunity.

23. A box of MMR vaccine (undiluted) was left at room temperature for 3 hours. Is it okay to use?

If you suspect that this vaccine or any vaccine has been mishandled, you should contact the manufacturer for guidance on its use. This is particularly important for labile live virus vaccines like MMR and varicella. Unfortunately, errors in vaccine storage and handling are common.

24. Once MMR vaccine has been reconstituted with diluent, how soon must it be used?

It is preferable to administer MMR immediately after reconstitution. If reconstituted MMR is not used within 8 hours it must be discarded. MMR should always be refrigerated and should never be left at room temperature.

25. I misplaced the diluent for the MMR dose so I used sterile water instead. Is there any problem with doing this?

Only the diluent supplied with the vaccine should be used to reconstitute any vaccine.

26. Can single antigen preparations for measles and rubella vaccines be mixed together? We have MMR vaccine and single antigen vaccines for those who only need one.

Absolutely not. Vaccines should *never* be mixed except when specifically approved by the FDA. Also, ACIP recommends use of combined MMR whenever one or more of the antigens is indicated, so there is little need to stock single antigen vaccines.

27. Our clinic has given MMR by the wrong route (IM rather than SC) for years. Should these doses be repeated?

All live injected vaccines (MMR, varicella, and yellow fever) are recommended to be given subcutaneously. However, intramuscular administration is not likely to decrease immunogenicity, and doses given IM do not need to be repeated.

28. We often need to give MMR vaccine to large adults. Is a 25-gauge needle with a length of 5/8" sufficient for a subcutaneous injection?

Yes. A 5/8" needle is recommended for subcutaneous injections for people of all sizes.

29. As far as mumps antigen is concerned, there are different strains used to produce different vaccine product. Which strain is the best to use?

Recently, with introduction of a new strain of mumps vaccine- Jeryl-Lynn strain (Priorix, GSK) which is almost 6 to 8 times costlier than available vaccines in Indian market raised a controversy on safety and efficacy of different mumps vaccine virus strains used in MMR vaccines over the years. The other mumps vaccine (Tresivac', marketed by Serum Institute of India) available and widely used in India contains L-Zagreb strain of the virus. The concern raised in the Indian vaccine market is on tendency of L-Zagreb strain to cause higher incidence of aseptic meningitis in the recipients. Before passing any judgment on the safety and efficacy or different strains, let's analyze the various facts associated with different strains (Table 16.2).

- All the strains used in monovalent, bivalent (MM), or trivalent (MMR) vaccines are live, attenuated and contain more than 1000 cell culture infective doses of mumps virus per dose
- The following strains are used for the development of live vaccines:
    a. Jeryl-Lynn strain
    b. Leningard-strain

TABLE 16.2 Comparative efficacy and rates or aseptic meningitis of different strains used in mumps vaccines

Name of strain	*Developed by*	*Efficacy (after single dose)*	*Risk of aseptic meningitis (cases per 1 lac doses)*
Urabe	Japan	92–100%	100
Jeryl-Lynn	USA	75–91%	0.1–1.0
L-Zagreb	Croatia	92–99%	2–90
Leningard-3	Soviet Union	92–99%	20–100
Rubini	Switzerland	6.3–12.4	Not Known

c. L-Zagreb strain
d Urabe strain
e. Rubini strain.

This is clear from above table that all the available mumps virus antigens (except Rubini) are highly efficacious against protecting the disease. The manufacturers of Rubini strain have now recommended two doses of the vaccine instead of one (World Health Organization).

The risk of aseptic meningitis is negligible with all the strains, though it is highest with Urabe strain and lowest with Jeryl-Lynn. Urabe strain though associated with highest incidence of aseptic meningitis but is found to be the most potent of all available strains. In most comparative studies involving with J-L strain.

Urabe strain was shown to provide more sustained protection. On the other hand, waning of protective immunity was noticed with J-L strain over the passing years.

Hence, all the available mumps vaccines (apart from those containing Rubini strain; not available in India) are highly effective and safe. The controversy over association of aseptic meningitis as adverse effect of mumps vaccination is quite inconsequential and unwarranted. So far, the world over more than 500 million doses of the vaccine have been administered and the adverse events are found to be extremely rare and mild. The fears about aseptic meningitis are quite unfounded and unjustified. The aseptic meningitis associated with mumps vaccination is quite benign - asymptomatic, self-limiting and resolve without sequlae within a week. On the other hand, the incidence of aseptic meningitis following natural mumps infection is quite high, and even entails some degree of morbidity. Personally, I might have used more than 6000 doses of MMR vaccine containing Urabe (Morupar) and L-Zagreb (Tresivac) strains and yet to see a single case of aseptic meningitis due to vaccine administration. The more pertinent issue would be how to increase coverage of MMR vaccine. Right now, the usage in pediatric population is not sufficient to make any impact at the epidemiology of the diseases protected by the vaccine.

30. Are there special vaccination recommendations for colleges and other post-high school education institutions?

Risks for transmission of measles, mumps, and rubella at post-high school educational institutions can be high because these institutions bring together large concentrations of persons who may be susceptible to these diseases. Therefore, colleges, universities, technical and vocational schools, and other institutions for post-high school education should require that all undergraduate and graduate students have received two doses of MMR vaccine or have other acceptable evidence of measles, mumps, and rubella immunity before enrollment.

Students who do not have documentation of MMR vaccination or other acceptable evidence of immunity at the time of enrollment should be admitted to classes only after receiving the first dose of MMR vaccine. These students should be administered a second dose of MMR vaccine one month later (but no sooner than 28 days after the first dose).

31. An 18-year-old college student says he had measles and mumps at ages 4 and 5, but never had MMR vaccine. Is rubella vaccine recommended in such a situation?

Actually, this student should receive two doses of MMR, separated by at least 28 days. (It is recommended that all persons attending school receive two doses of MMR vaccine.) A personal history of measles and mumps is NOT acceptable as proof of immunity. Acceptable evidence of measles and mumps immunity includes a positive serologic test for antibody, physician diagnosis of diseases, birth before 1957, or written documentation of vaccination. For rubella, only serologic evidence or documented vaccination should be accepted as proof of immunity. Additionally, persons born prior to 1957 may be considered immune to rubella unless they are women who have the potential to become pregnant.

32. My patient has had two documented doses of MMR. Her rubella titer was nonreactive at a prenatal visit. What should I do?

It is possible that she failed to respond to both doses. It is also possible that she did respond but has a low level of antibody. Failure to respond to two properly timed doses of MMR vaccine would be expected to occur in one or two persons per thousand vaccinees, at most. A small number of people appear to develop a relatively small amount of antibody following vaccination with rubella and other vaccines. This level of antibody may not be detectable on relatively insensitive commercial screening tests. Controlled trials with sensitive tests indicate a response rate of >99% following two doses of rubella-containing vaccine. Ideal way would be to make a note of her documented vaccination and stop testing. Another approach would be to administer one additional dose of MMR. However, there are no data on the administration of additional doses of rubella-containing vaccine in this situation.

33. Can I give MMR to a child whose sibling is receiving chemotherapy for leukemia?

Yes. MMR and varicella vaccines should be given to the healthy household contacts of immunosuppressed children. Oral polio is the only vaccine that should not be given to a healthy child if an immunosuppressed person resides in the household.

34. Is it true that egg allergy is no longer considered a contraindication to MMR vaccine?

Several studies have documented the safety of measles and mumps vaccine (which are grown in chick embryo tissue culture) in children with severe egg allergy. The AAP's "Red Book" Committee no longer considers egg allergy a contraindication to MMR vaccination. The new ACIP statement on MMR also recommends routine vaccination of egg-allergic children without the use of special protocols or desensitization procedures.

35. Is it contraindicated to give MMR to a breastfeeding mother or to a breastfed infant?

No. Breastfeeding does not interfere with the response to MMR vaccine. Vaccination of a woman who is breastfeeding her infant poses no risk to the infant being breastfed. Although, it is believed that rubella vaccine virus, in rare instances, may be transmitted via breast milk, the infection in the infant is asymptomatic.

36. How soon after delivery can MMR be given?

MMR can be administered any time after delivery. The vaccine should be administered to a woman who is susceptible to either measles, mumps, or rubella before hospital discharge, even if she has received RhoGam during the hospital stay, leaves in less than 24 hours, or is breastfeeding.

37. Can a PPD (tuberculin skin test) test be given on the same day as a dose of MMR vaccine?

A PPD can be applied before or on the same day that MMR vaccine is given. However, if MMR vaccine is given on the previous day or earlier, the PPD should be delayed for at least one month. Live measles vaccine given prior to the application of a PPD can reduce the reactivity of the skin test because of mild suppression of the immune system.

38. Is there anything that can be done for unvaccinated people who have already been exposed to measles?

Measles vaccine may be effective if given within the first 3 days (72 hours) after exposure to measles. Immune globulin may be effective for as long as 6 days after exposure.

39. A story on MMR vaccine suggested administering each component of MMR in separate injections to decrease the risk of autism. Is there any reason to do this?

There is no scientific reason for or benefit to separating the antigens. There is no credible evidence that measles vaccine or MMR increases the risk of autism. Separating the doses puts children (and pregnant women who may be exposed to them) at increased risk for these diseases by extending the amount of time children remain unvaccinated. Studies have shown that if parents have to schedule additional appointments for vaccinations, there is an increased risk that their children may not receive all the vaccines they need.

40. Is there any evidence that MMR or thimerosal causes autism?

No. This issue has been studied extensively in recent years, including a thorough review by the independent Institute of Medicine (IOM). The IOM issued a report in 2004 that concluded there is no evidence supporting an association between MMR vaccine or thimerosal-containing vaccines and the development of autism.

41. How likely is it for a person to develop arthritis from rubella vaccine?

Arthralgia (joint pain) and transient arthritis (joint redness or swelling) following rubella vaccination occurs only in persons who were susceptible to rubella at the time of vaccination. Joint symptoms are uncommon in children and in adult males. About 25% of post-pubertal women report joint pain after receiving rubella vaccine, and about 10% report arthritis-like signs and symptoms. When joint symptoms occur, they generally begin 1–3 weeks after vaccination, persist for 1 day to 3 weeks, and rarely recur. Chronic joint symptoms attributable to rubella vaccine are very rare, if they occur at all.

42. If a healthcare worker develops a rash and low-grade fever after MMR vaccine, is she/he infectious?

Approximately 5–15% of susceptible persons who receive MMR vaccine will develop a low-grade fever and/or mild rash 7–12 days after vaccination. However, the person is not infectious, and no special precautions (e.g. exclusion from work) need to be taken.

43. What is the earliest age at which I can give MMR to an infant who will be traveling internationally? Also, which countries pose a high risk to children for contracting measles?

ACIP recommends that children who travel or live abroad should be vaccinated at an earlier age than that recommended for children who reside in the United States. Before their departure from the United States, children age 6 through 11 months should receive 1 dose of monovalent measles vaccine (if available) or MMR. The risk for measles exposure can be high in both developed and developing countries. Consequently, CDC encourages all international travelers to be up to date on their immunizations regardless of their travel destination and to keep a copy of their immunization records with them as they travel.

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CHAPTER

17

Typhoid

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1. What are the epidemiological challenges of typhoid fever faced in our country?

It is estimated that 33–60 million cases and 6 lakh deaths occur annually due to typhoid fever worldwide. As the disease is primarily associated with poor hygienic and sanitary conditions, mainly the developing countries bear the brunt of the disease. Around 5 million cases occur annually in India. Population based studies from urban population in India suggest that the incidence of typhoid fever is 2730 out of per people 10,000 year among 0–4 years old children and 1,170 per 10,000 people every year in 5–19 year age group. Ten percent of cases occur in the infant age group because diagnosis is difficult and hence mortality is higher. A major problem in the last two decades of the 20th century has been the emergence of plasmid encoded multidrug resistance it ranges from 17% to 90% of cores as reported by various studies, drug resistance is seen especially to the quinolones. Children constitute 40–50% cases of multidrug resistant typhoid fever with higher case fatality rates.

2. How can typhoid fever be prevented?

The mode of transmission of typhoid fever is via feco-oral route. Therefore, the best solution would be to provide the entire population with treated , bacteriologically monitored water for drinking and proper sanitation for disposal of human waste without contaminating water. However, such principles of hygiene and public health remain at best a distant reality and the only practical viable option is vaccination.

3. What are the various types of typhoid vaccines manufactured?

Over the years, various types of typhoid vaccines have been manufactured and have been made available for vaccination from time to time. These are:

- *Parenteral whole cell typhoid vaccine:* Various types were invented depending on the process of inactivation namely heat inactivated phenol preserved, acetone inactivated, formol inactivated and alcohol inactivated vaccines. Initially, these were a combination of typhoid and paratyphoid A and paratyphoid B vaccines. However, it was realized that paratyphoid is a less common cause of enteric fever and the vaccines have poor protection while at the same time increasing the adverse effects. Hence, from the TAB vaccine it became TA vaccine and later on T vaccine i.e *S. typhi* vaccine. The efficacy of acetone inactivated preparation (79–88%) is better than the phenol killed (51–66%) vaccine
- *Oral Ty21a live vaccine:* Using mutagenic techniques, a mutant strain of *S. typhi* was produced which has a mutation in the gal E gene and lacks enzyme Uridine diphosphate galactos-4 epimerase. This enzyme is necessary for capsular polysaccharide formation as shown.

$$\text{UDP Glucose} \xrightarrow{\text{UDP Gal-4 epimerase}} \text{UDP Galactose} \longrightarrow \text{Lipopolysaccharide (LPS) capsule}$$

  This results incomplete LPS which doesn't allow the bacteria to multiply beyond one or two generations making it immunogenic but not pathogenic.
- *Capsular Vi polysaccharide vaccine:* This is a new generation subunit vaccine containing highly purified antigenic fraction of Vi antigen of *Salmonella typhi*. Polysaccharide antigen stimulates B cells directly and T helper cells do not get stimulated. Being T cell it independent, can induce only IgM antibody. IgM to TgG switch requires regulation by T helper cells. T independent antigens do not induce the production of memory T cells or memory B cells, so, the serum antibody response is not boosted by administration of additional doses of Vi vaccine. As with other T cell independent purified polysaccharide vaccine, it is not a good immunogen in children less than 2 years of age and most infants fail to respond to this antigen. Two randomized controlled trials suggest an efficacy of this vaccine in the range of 64–72%. However, most of these studies have been done in children above 5 years of age and no data is available for the 2–5 years age group. There have been concerns that as this vaccine does not protect against Vi negative *Salmonella* wide spread use might make it the dominant strain causing typhoid fever, though this concern has been largely baseless scientifically.
- *Conjugate vaccine:* A major breakthrough in the the field of vaccinology has been the method to conjugate polysaccharide vaccines making them T cell dependent leading to immunogenic response even in infants and inducing long and lasting IgG response and T cell memory. After successful development of conjugate Hib, pneumococcal and meningococcal, the latest to be developed is the conjugate polysaccharide typhoid vaccine, the Vi conjugate vaccine.

The first typhoid conjugate polysaccharide vaccine Vi rEPA was developed by Szu et al in which the Vi antigen of typhoid has been conjugated with the nontoxic recombinant exotoxin A of the *Pseudomonas aeruginosa*. Successful field trials in Vietnam on 13,766 children aged 2–4 years have shown the vaccine to be highly immunogenic (100% showing atleast 10-fold increase in Vi antibody levels). The efficacy was as high as 93% at 27 months of age when two doses were given at 6 weeks interval and 89% at 46 months post vaccination. Even with a single dose there was an efficacy of 91% and even the vaccines who developed typhoid had a milder disease. Further, trials are going on to validate these results especially in the infant age group. The side effects of fever and local reactions were seen in less than 2% vaccines making it very safe.

In India Peda Typh™ has been launched by Bio-Med. In this Vi capsular polysaccharide has been conjugated with tetanus toxoid toxin. There is very little field efficacy data for this vaccine. A single multicentric trial that involved 169 volunteers (more than 3 months of age) for safety and 145 volunteers for efficacy was done using a single dose schedule. The control group receiving Vi polysaccharide vaccine in the study was completely unmatched for number and age group. The vaccine in this study was found to be highly immunogenic in infants and children less than 2 years of age. These immunogenic data have been extrapolated to the efficacy data of Vi-rEPA vaccine, but these are two different vaccines, used in different age groups and in different countries. The manufacturers of Peda typh recommended a two-dose schedule from 12 weeks of age and onwards with a booster two and a half years, although, the efficacy trial had a single dose schedule. In the light of these contradictions, it would seem prudent to have large well controlled trials to validate what has been actually claimed by the manufacturers.

A new Vi Polysaccharide conjugate typhoid vaccine by Bharat Biotech is now available after completion of phase III trials done on 981 healthy children. The vaccine uses a dose of 25 μg/0.5 mL of the conjugate Vi Polysaccharide vaccine which is the highest having been used in other trials as well on conjugate vaccine the world over. This vaccine has shown a seroconversion of 98.05% in children from 6 months to 2 years of age and 97.2% in subjects 2–45 years age.

The Indian Academy of Pediatrics recommends this new Vi-PS conjugate vaccine below 1 year of age, preferably between 9 and 12 months (minimum age 6 months). Since the incompatibility data with measles vaccine is not available, it would be prudent to maintain an interval of at least 4 weeks with the former. The IAP also believes that there is a definite need of booster dose during second year of life; however, the available data are insufficient to specify exact timing of the same.

4. What should be the schedule and which type of typhoid vaccine should be used in a 2-year-old child who has not received a primary dose of conjugate typhoid vaccine at 9–12 months of age?

Both conjugate and Vi-PS vaccines can be used at this age group. However, available data favors conjugate vaccine marginally since it has slightly higher seroprotection and GMCs than polysaccharide vaccine and the need to administer boosters at regular intervals can be obviated. Though currently data on long-term persistence of seroprotection after a single dose of conjugate vaccine is lacking, theoretically single booster after 2–3 years of primary dose should ensure long lasting protection.

5. What should be the schedule of booster/s after a primary dose of conjugate typhoid vaccine at or after 2 years of age?

As indicated above, the long-term data on persistence of protective antibodies level against typhoid is lacking, it is difficult to offer a perfect schedule of available typhoid conjugate vaccine. However, based on available data, only a single booster after 2–3 years of primary dose should ensure protection throughout the pediatric age.

6. What are the dose schedules, side effects, and precautions to be considered regarding the various typhoid vaccines?

- *Oral Ty21a vaccine:* This vaccine was available in 3 forms, gelatin sodium bicarbonate capsule, enteric coated capsule and liquid preparation. Though the liquid preparation had best efficacy, it was difficult to produce it in large quantities. Only the enteric coated preparation became commercially available containing 2-6 × 10^9 CFU of Ty21a organisms per capsule. The oral Ty21a leads to good serum and secretory antibody response and even cellular immune response. Seroconversion occurs in 60–70% of the vaccines . Being a live vaccine this vaccine requires stringent temperature maintenance between 2–8°C. Dose consists of one capsule on alternate days for three doses in endemic countries and 4 doses in the U.S. The vaccine has to be swallowed intact and should never be opened and taken which would render it ineffective by the gastric acid juices. This makes intake of the vaccine possible only in children above 6 years. Care should be taken, not to take too much of hot or cold items half an hour before and after the dose. Antibiotics that act against typhoid fever should be avoided for a fortnight starting five days before the start of the first dose. Antimalarial drugs-chloroquine and mefloquine should be avoided for a period of 24 hours following vaccination as they interfere with multiplication of vaccine strain. It can be simultaneously given with any injectable vaccine. However, there should be an interval of 4 weeks between an oral polio vaccine dose and an oral typhoid vaccine dose. The protection begins a week after completion of the dose and lasts for 3–7 years. There are hardly any serious side effects. Nausea, rash, diarrhea, vomiting and fever have been reported in less than 1% of vaccines. Being a live vaccine, it is contraindicated in immunocompromised individuals and during pregnancy.
- *Capsular Vi polysaccharide vaccine:* The vaccine is available for use in children 2 years and above. Each dose contains 25 µg of purified polysaccharide in 0.5 mL of phenolic isotonic buffer. The vaccine has to be stored between 2 to 8°C and can be given intramuscularly or subcutaneously. It protects against drug sensitive as well as multidrug resistant strains of *S. typhi*. The protection begins 2 to 4 weeks after the immunization and lasts for 3–5 years. It offers no protection against *Salmonella paratyphi* A and B. It consists of a single dose which needs to be repeated every three years. The side effects are usually mild in nature ranging from local reactions to systemic in less than 5% of the vaccines. Compared to whole cell killed vaccine reactions are milder and uncommon. As it does not lead to T cell memory, revaccination is required every 3–5 years.

- *Conjugate vaccine:* The schedule for typhoid conjugate vaccine for primary immunization is at 9–12 months of age. The dose is 0.5 mL (25 μg) to be given intramuscular. Since the manufacturer has not yet provided the incompatibility data with measles vaccine, the ACVIP recommends maintaining an interval of at least 4 weeks with the measles vaccine while administering this vaccine. Those who receive a dose of Typbar-TCV® at 9–12 months they can be prescribed booster of either Vi-polysaccharide (Vi-PS) or the Typbar-TCV® vaccine at 2 years of age. The need of further boosters after conjugate vaccine is not yet determined since long-term data is not available, although theoretically, a conjugate vaccine should provide long term, probably lifelong protection owing to formation of memory responses. Catch-up vaccination is recommended throughout the adolescent period, i.e. up to 18 years of age. Below 2 years, only conjugate vaccine is recommended while above 2 years of age any of the two can be employed. The adverse events include fever and pain at the site of injection.

7. What are the recent developments in typhoid vaccine development?

A major breakthrough in the the field of vaccinology has been the method to conjugate polysaccharide vaccines making them T cell dependent leading to immunogenic response even in infants and inducing long and lasting IgG response and T cell memory. After successful development of conjugate Hib, pneumococcal and meningococcal, the latest to be developed is the conjugate polysaccharide typhoid vaccine, the Vi Conjugate Vaccine.

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8. Which conjugate typhoid vaccines are available in our country?

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9. How do the various typhoid vaccines compare with each other in relation to various characteristics?

Comparative features of various typhoid vaccines are discussed in Table 17.1.

TABLE 17.1 Comparison of the various typhoid vaccines

Characteristics	*Whole cell killed vaccines*	*Ty21a capsule vaccine*	*Vi poly-saccharide vaccine*	*Vi polysaccharide conjugate vaccine (Peda Typh™)*	*Vi polysaccharide conjugate vaccine (Typbar-TCV™)*
Type	Killed	Live	Subunit	subunit	Subunit
Route	IM/SC	Oral	IM/SC	IM/SC	IM
Doses	2	3	1	2	1
Revaccination	3–5 years	3–5 years	3 years	?	?
Immunogenicity	++	+	+	+++	+++
Efficacy	51–79%	35–67%	55–72%	91%	97%
Duration of efficacy	65% at 7 years	62% at 7 years	55% at 3 years	91% at 2.3 years	97% at 18 months
Herd immunity	?	Yes	?	?	?
Side effects	++++	+	++	++	++
Age	> 6 months	> 6 years	> 2 years	> 6 weeks	> 6 months
Boosting	–	–	–	++	++
Mass vaccination	Unsuitable	Suitable	Suitable	Suitable	Suitable
Availability	No	No	Yes	Yes (lacks data)	Yes

Abbreviations: IM, intramuscular; SC, subcutaneous.

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CHAPTER

18

Haemophilus influenzae Type b

Jaydeep Choudhury

1. What is the ***H. influenze*** type b disease burden in India?

In India, the estimated *H. influenzae* type b (Hib) incidence is 50–60/100,000 children less than 5 years age. Invasive Bacterial Infections Surveillance (IBIS) study done in India has shown that 76% of Hib occurs before age of 1 year with peak incidence at 6–9 months. From hospital based data in India, 30–45% of cases of pyogenic meningitis and 8–12% of cases of pneumonia in children are due to Hib infection.

2. What is the profile of clinical presentation of invasive Hib disease?

Capsulated *Haemophilus influenzae* has six serotypes of which type b is the most important. *H. influenzae* b (Hib) infection is a common cause of invasive and noninvasive severe bacterial infections in children. Meningitis accounts for over 50% of all recognized cases of invasive Hib disease in infants and children under the age of 5 years. Pneumonia is another serious condition with a high morbidity and mortality in children below 5 years of age. Despite early diagnosis and effective antibiotic therapy, mortality figures are high, about 50% in Hib meningitis and about 20% in Hib pneumonia. Invasive Hib infections may also manifest as bacteremia, septicemia, epiglottitis, cellulitis, pericarditis, osteomyelitis, septic arthritis and peritonitis. Hib may also cause peripartum septicemia in mothers. Noncapsulated Hib disease including, bronchitis, otitis media, sinusitis and some types of pneumonia are not amenable to prevention at present and can occur at all ages.

3. Why cannot Hib infection be treated with antibiotics alone?

Hib infection is often serious and needs prolonged hospitalization and sometimes intensive care management. Since 1970, drug resistant Hib strains have emerged. In India, the initial cases were reported in 1990 from Chandigarh. Subsequently, Vellore has reported 42.5% MDR strains in 1992. IBIS reported 56% Hib resistance

TABLE 18.1 Different formulations of Hib vaccine displaying carrier protein and immunogenicity

Hib vaccine	*Carrier protein*	*Immunogenicity*	*Formulation*
PRP-D	Diphtheria toxoid	Poor, effective only above 12 months age	Lyophilised
HbOC	CRM 197	Late response with good booster effect	Liquid
PRP-OMP	OMP	High for short-term protection, modest booster effect	Not available
PRP-T	Tetanus toxoid	Late response with good booster effect	Lyophilised

(PRP-D or T: Polyribosyl ribitol phosphate (capsular polysaccharide) covalently bound to either diphtheria or tetanus toxoid, CRM 197: Nontoxic mutant of *Corynebacterium diphtheriae* toxin protein, OMP: Outer membrane protein complex of *Neisseria meningitidis*).

to chloramphenicol and 40% resistance to ampicillin in 1999. With increasing spectrum of resistance, Hib infection is better prevented than cured.

4. What are the various formulations of Hib vaccine?

Various formulations of Hib vaccine are depicted in Table 18.1.

5. Why PRP-OMP Hib vaccine is not available?

The PRP-OMP Hib vaccine is unique in producing a dramatic antibody response to the very first dose, even in infants below 6 months of age. Majority of infants achieve anti-PRP antibody levels of >1.0 µg/mL, but it produces minimal further increase in antibody concentration to subsequent doses. After the initial rise, antibody titers decrease significantly over time. Consequently, the final antibody levels are about 30% of those obtained with HbOC or PRP-T. Hence, PRP-OMP was withdrawn.

6. Why is it ideal to use conjugated Hib vaccine?

Majority of Hib infections occur in children less than 18 months of age, with peak incidence of invasive disease at around 6–7 months of age. All Hib vaccines are conjugated vaccines where the Hib capsular polysaccharide (polyribosyl ribitol phosphate or PRP) is conjugated with a protein carrier so as to provide protection in the early years of life when it is most needed.

7. Can we use the Hib vaccines interchangeably?

The Hib vaccines HbOC, PRP-T and PRP-OMP can be used interchangeably even in the primary immunization schedule with good immune response.

8. What is the vaccination schedule of Hib vaccine?

The vaccination schedule for Hib consists of three doses when initiated in children below 6 months age; 2 doses between 6 and 12 months and 1 dose between 12 and 15 months. In every schedule a booster is given at 18 months. The interval between two doses of Hib vaccine should be atleast 4 weeks. For children aged more than 15 months, a single dose may suffice. As Hib disease is essentially confined to infants and young children, catch up vaccination is not recommended for healthy children above 5 years. However the vaccine should be administered to all individuals with functional/ anatomic hyposplenia irrespective of age.

9. Why three doses of Hib vaccines are required in infants below 6 months?

The first dose of the Hib vaccines, HbOC and PRP-T stimulates a very low antibody response in infants below 6 months of age. The infants show a late response and three doses are required to achieve seroprotective antibody levels in infants below 6 months of age. The vaccine may not protect the infants until after the third dose.

10. A six-month-old baby has received BCG, DPT 3 and OPV3 through the public sector. You wish to give Hib immunization. What schedule will you follow and why?

Two doses each one month apart and booster at 15–18 months. The immune response to vaccines improves as the age of the baby advances. In early life, there is limited/ no ability to respond to polysaccharide antigens, lower T cell responses than adults except BCG, lower peak antibody levels, and limited immune memory. Further, there is interference by maternal antibodies also. Hence, only two doses are needed after 6 month of age.

11. Why is booster dose necessary for Hib vaccine?

There is demonstrable waning immunity in general for all types of Hib vaccine formulations available. After the three primary doses of Hib vaccination, >90% vaccinees achieve an antibody titer >1.0 μg/mL which gives protection till 15–18 months of age. In >50% of them, the protective level falls. Being a vaccine intended for boosting the natural immunity and as the prevalence of Hib disease is mainly in children below 5 years, if a booster is given at 18 months of age the titer rises and protects till the age of 5 years.

12. Being a conjugate vaccine (i.e. T-dependent antigen), memory cells are formed during induction phase. Then, why are they not be able to provide protection at the time of natural infection during 2nd year of life and booster is needed?

This is to be noted that presence of memory B cells does not mean protection against a disease which requires reactivation of memory B cells into antibodies secreting plasma cells. This transition usually takes around 4–7 days. However, invasive Hib diseases are characterized by short incubation period, hence, by the time memory B cell transform into plasma cells, the disease already advances and causes morbidity and mortality. This is the reason why boosters are needed for organisms having short incubation period like pneumococcal, influenza, meningococcal, etc., whereas diseases like Hepatitis-B, and A which have long incubation period, boosters are not required and memory is sufficient to accord in protection.

13. Does the tetanus protein in PRP-T Hib immunize against tetanus?

No. Here, the tetanus protein is conjugated to bring the PRP component into contact with the immunologically competent T-lymphocytes. This does not replace the routine dose of tetanus vaccine.

14. What are the available combination Hib vaccines?

Combination Hib vaccines available in India, are:
- DTwP/Hib
- DTwP/Hep B/Hib
- DTap/Hib.

Combination Hib vaccines available in other countries are:
- DTwP/Hib/IPV
- DTwP/Hep B/Mn C/Hib
- DTaP/IPV/Hib
- DTap/Hep B/Hib
- DTap/Hep B/Hib/IPV
- DTap/Hep B/Hib/IPV/Mn C
- Hep B/Hib.

Combination Hib vaccines in the pipeline, are:
- Mn C/Hib
- Pn C/Mn C/Hib.

15. Is it true that when the combination formulation of DTaP/Hib is used, the antibody level of the Hib component is decreased?

Some studies have shown that administering DTP and Hib combination results in reduced mean PRP Hib antibody levels compared to giving the same components separately. The reduction in PRP Hib antibody is more significant in DTaP than DTwP. However, even in these studies the antibody levels are well above the protective titers. Thus reduced immunogenicity of Hib when given in combination with DTP appears to be of no clinical significance. Hence, the DTaP/Hib vaccine formulation can safely be used.

16. Is there any difference between lyophilized and liquid Hib combination vaccines?

Lyophilized or dry powder Hib vaccine has to be mixed with the liquid DTP vaccine which acts as the diluent. Here, PRP-T Hib vaccine is used whereas in liquid combination Hib vaccine HbOC is used where CRM 197 is the carrier protein and the vaccine is in dissolved state. Immunologically, both the forms are equally effective.

17. Is it feasible to combine Hib vaccines ad hoc?

Providers should not create their own ad hoc combinations by mixing separate vaccines in the same syringe unless there is an evidence establishing the stability, safety and immunogenicity of the resultant combinations as reflected in the package inserts.

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CHAPTER

19

Pneumococcal Diseases

Nitin K Shah

1. What is the disease spectrum of pneumococcal disease in children?

Pneumococcus is known to lead to nasopharyngeal (NP) colonization, the rates of which vary in infants from 22.3%, as found in the multi-country Asian Network for Surveillance of Resistant Pathogens (ANSORP) study done in 1998–1999, to 43.5% as seen in India to as high as 80% in the Gambia. It can spread from nasopharynx, locally to middle ear, leading to acute otitis media (AOM) or to sinuses leading to sinusitis. It can spread focally to lungs leading to nonbacteremic pneumonia. Lastly, it can also spread via blood stream leading to invasive pneumococcal disease (IPD) like bacteremia, sepsis, meningitis, or bacteremic pneumonia. Besides, it can also lead to uncommon noninvasive infections like cellulitis, peritonitis, joint infections, etc. The peak incidence of pneumococcal disease in children occurs at 6–24 months of age.

2. What is the pneumococcal disease burden in children?

Incidence of IPD varies from 25–50/100,000 children under 5 years in Europe to 90/100,000 under 5 years in the United States of America (USA) to 500/100,000 under 5 years in the Gambia and Apache Indians. Estimates of IPD incidence for developing countries are difficult to obtain. It is estimated that for every case of meningitis, there are 10 times more cases of bacteremia, 100 times more cases of noninvasive pneumonia and 1,000 times more cases of otitis media. Ninety percent of bacteremia, 30–50% of severe community acquired pneumonia, 30–45% of pyogenic meningitis and 30–60% of all bacterial AOM are estimated to be caused by pneumococcus. The mortality rate of invasive disease is 6–20% and there are long-term sequels like central nervous system (CNS) squeal in survivors of meningitis and deafness in children with recurrent AOM.

According to latest estimates, pneumococcal disease caused about 826,000 deaths (582,000–926,000) in children aged 1–59 months, of which 91,000 (63,000–102,000) were in human immunodeficiency virus (HIV) positive and 735,000 (519,000–825,000) in HIV-negative children. Out of the deaths in HIV-negative children, over 61% (449,000 [316,000–501,000]) occurred in 10 African and Asian countries. Ten countries with the greatest number of pneumococcal deaths in children aged 1–59 months include India (142,000), Nigeria (86,000), Ethiopia (57,000), Democratic Republic of the Congo (51,000), Afghanistan (31,000), China (30,000), Pakistan (27,000), Bangladesh (21,000), Angola (20,000), and Uganda (19,000).

3. What is the burden of pneumococcal pneumonia and mortality?

Table 19.1 shows the distribution of pneumonia disease burden and pneumonia deaths in different WHO regions as on 2011 as published by CHERG (Child Health Epidemiology Reference Group) in 2013. It estimated that as on 2011, of the total 6.8 million under five deaths world over, 18.2% i.e. 1.25 million deaths occurred due to pneumonia of which pneumococcus contributed 32.7% i.e. 0.41 million deaths. It also estimated that there occurred 120 million episodes of 'clinical' pneumonia in 2011 based on the WHO definition. We also know that approximately 24–36% of these clinical pneumonia episodes are radiologically proven (using WHO definition), i.e. 28–42 million episodes; and 6–12% of these clinical pneumonia episodes are severe pneumonia (needing hospitalization), i.e. 7.2–15 million episodes annually. In fact, CHERG estimated that there occurred 12.5 million episodes of severe pneumonia in 2011. Pneumococcus contributed 18.3% of these severe pneumonia episodes i.e. 2.58 million episodes. In India, it was estimated that of 1.4 million under-five deaths every year, 20.3% were caused by pneumonia, i.e. 0.29 million deaths, contributing 23.5% of all pneumonia deaths in the world. India also contributes 40 million episodes of clinical pneumonia. Vaccine probe studies have shown that PCV7 led to 20–30% reduction in radiological pneumonia in the USA and PCV9 led to reduction of 25% in radiological proven pneumonia in non-HIV infected children in South Africa and 35% in the Gambia. This further confirms that pneumococcus is an important cause of severe pneumonia in under-five children.

4. What are the prevalent serotypes of pneumococcus in children in India and how many of them are covered by the PCV?

Invasive Bacterial Infection Surveillance (IBIS) study done in 1993-1997 showed that 10 most common pneumococcus serogroups in children under 5 years were types 1 (13.9%), 6 (21.8%), 19 (10.9%), 14 (7.9%), 4 (5.9%), 5 (5%), 45 (5%), 7 (4%), 33 (4%) and 12 (4%). The same study now continues as SAPNA (South Asian Pneumococcal Alliance) study and the serotype distribution has not much changed with time. Pneumococcal conjugate vaccine 7 (PCV7) covers 55% of serotypes seen in children under 5 years, and PCV10 and PCV13 around 75%. ANSORP data of 2008 shows that 19A is on rise in most Asian countries, as also in India. 19A is covered by PCV13 but not by PCV10. Two recent studies from India have looked at serotype distribution in invasive pneumococcal disease in children <5 years of age. The single centre Pneumonet study done in Bangalore sponsored by Wyeth-Pfizer India showed that PCV10 will cover 63.8% of prevalent serotypes

TABLE 19.1 Global distribution of pneumonia burden in different WHO regions in children < 5 years of age

	Population aged 0–4 years (2014)	*Pneumonia*				
		*Incidence**	*Total episodes ($\times 10^6$)*	*Total severe episodes ($\times 10^6$)*	*Total deaths ($\times 10^6$)*	*Change in mortality from 2010 (%)*
African region	133340762	0.27 (0.14–0.63)	36.4 (18.2–84.4)	4.17 (2.98–12.03)	540.6 (437.8–627.3)	–10.5
American region	76995700	0.08 (0.04–0.18)	6.4 (3.3–14.5)	0.78 (0.57–2.18)	23.9 (22.6–35.6)	–15.7
Eastern Mediterranean region	72151965	0.23 (0.11–0.53)	16.4 (8.2–38.0)	1.88 (1.35–5.42)	168.4 (147.3–217.1)	–16.5
European region	54605243	0.03 (0.02–0.04)	1.6 (1.3–2.1)	0.41 (0.32–0.72)	18.1 (14.7–23.4)	–6.3
Southeast Asian region	179956087	0.26 (0.13–0.61)	47.4 (23.7–109.8)	5.44 (3.81–15.63)	443.8 (336.7–534.2)	–3.8
Western Pacific region	116411580	0.11 (0.05–0.24)	12.2 (6.2–28.2)	1.43 (1.01–4.06)	61.9 (50.7–78.0)	–17.2
World	633461337	0.19 (0.10–0.44)	120.4 (60.8–277.0)	14.11 (10.03–40.04)	1256.8 (1053.2–1482.9)	–10.0

The number of deaths was calculated on the basis of total number of deaths estimates for 2011; proportionate mortality for diarrhea and pneumonia were as reported for 2010. Data in parentheses are uncertainty ranges.

*Incidence = episodes per child-year.

Source: Walker CLF, Liu L, Nair H, et al. Lancet. 2013.381;9875:1405-16, *with permission.*

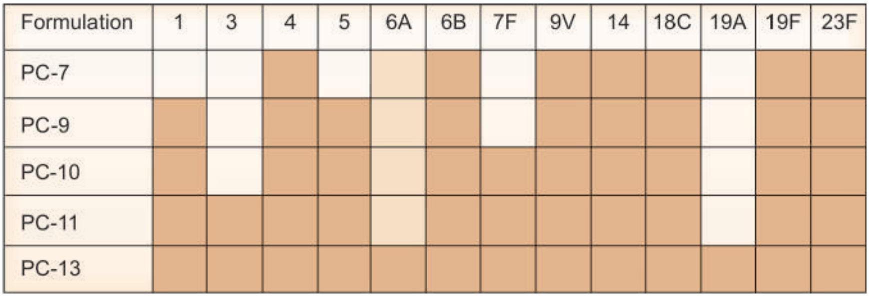
and PCV13 91.6%. ASIP study which was a multi-centric study sponsored by GSK India showed that PCV10 will cover 64.1% of prevalent serotypes and PCV13 74.3%.

5. Which different PCV are available in market? How do they differ?

Different PCV contain different serotypes as shown in figure 19.1. Till recently, only PCV7 (Prevenar®) was available from Wyeth-Pfizer. It covered seven serotypes and contained 2 μg of capsular polysaccharide of 4, 9V, 14, 19F and 23F; 2 μg of oligosaccharide of 14C and 4 μg of capsular polysaccharide of 6B serotypes conjugated with CRM197 carrier protein. PCV13 (prevenar 13) from Wyeth-Pfizer has replaced PCV7 world over including India. It covers six additional serotypes, i.e. 1, 3, 5, 7, 6A and 19A. PCV10 (Synflorix) from GlaxoSmithKline (GSK) is now available internationally, including in India, and covers three additional serotypes besides the PCV7, i.e. 1, 5, and 7. Three different carrier proteins are used and the vaccine contains 1 μg of each capsular polysaccharide for pneumococcal serotypes 1, 5, 6B, 7F, 9V, 14, and 23F and 3 μg for serotype 4 conjugated to nontypable *Haemophilus influenzae* (NTHi) protein D; 3 μg of capsular polysaccharide of serotype 18C conjugated to tetanus toxoid; and 3 μg of capsular polysaccharide of serotype 19F conjugated to diphtheria toxoid. There is significant cross protection between 6B and 6A but only for IPD and not for nasal carriage (and hence probably not for herd effects). There is no cross protection between 19F and 19A. PCV13 has both 6A and 19A and hence is likely to cover more meaningfully 6A and more important 19A which is on rise world over as discussed later.

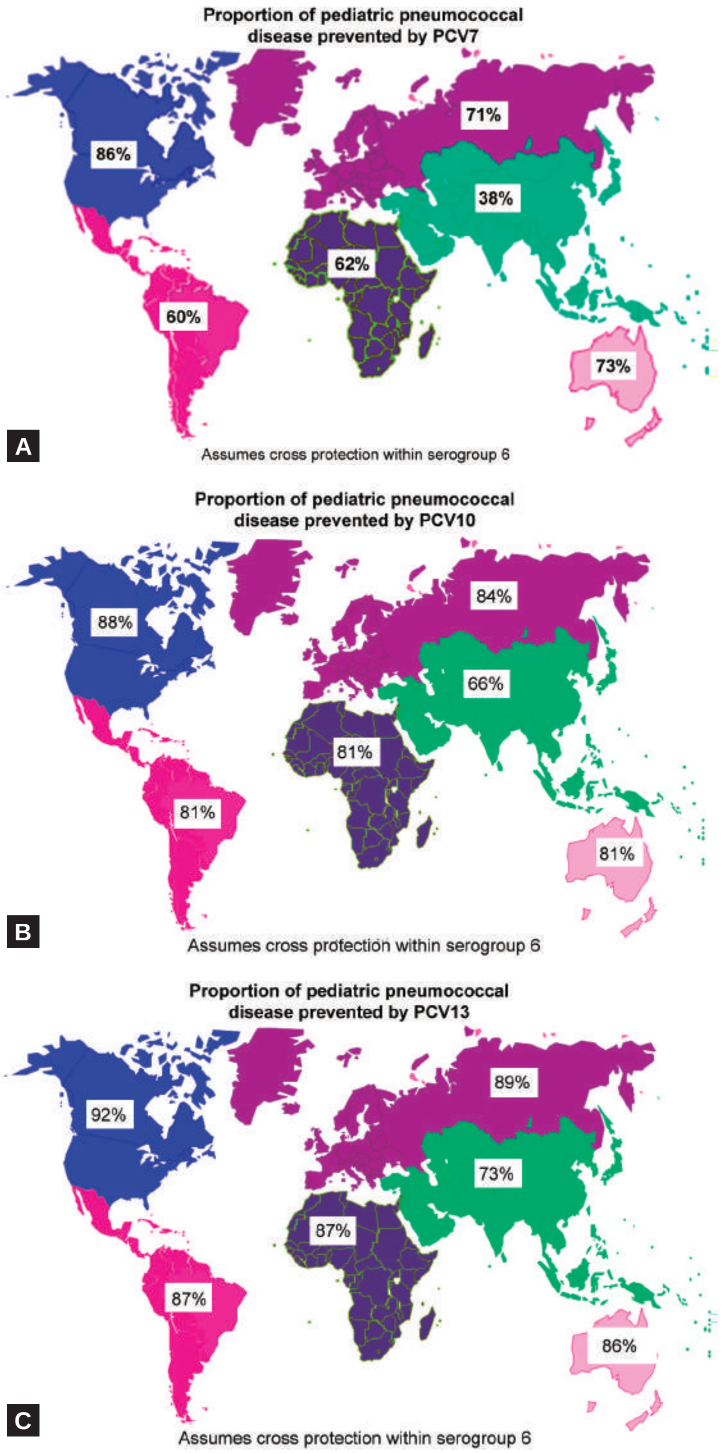
6. What are the coverage of PCV7, PCV10 and PCV13 in different geographic locations?

The serotypes in 7-valent PCV represent over 80% of IPD in North America and Europe, however, going by the latest report on PCV7 coverage in Asia, only 38% of all pediatric pneumococcal disease is prevented by this formulation. On the other hand, PCV10 coverage rates for Asia is around 66% while PCV13 covers 73% of prevailing serotypes according to Pneumococcal vaccines Accelerated Development and Introduction Plan (PneumoADIP) estimates (Figure 19.2).



Formulation	1	3	4	5	6A	6B	7F	9V	14	18C	19A	19F	23F
PC-7			■		▨	■		■	■	■		■	■
PC-9	■		■	■	▨	■		■	■	■		■	■
PC-10	■		■	■	▨	■	■	■	■	■		■	■
PC-11	■	■	■	■	▨	■	■	■	■	■		■	■
PC-13	■	■	■	■	■	■	■	■	■	■	■	■	■

FIGURE 19.1 Serotypes included in pneumococcal conjugate vaccine formulations (PCV-9 and PCV-11 are no more in active production)


Proportion of pediatric pneumococcal disease prevented by PCV7
86%
71%
38%
62%
60%
73%
A
Assumes cross protection within serogroup 6
Proportion of pediatric pneumococcal disease prevented by PCV10
88%
84%
66%
81%
81%
81%
B
Assumes cross protection within serogroup 6
Proportion of pediatric pneumococcal disease prevented by PCV13
92%
89%
73%
87%
87%
86%
C
Assumes cross protection within serogroup 6

FIGURES 19.2A TO C Proportion of pediatric pneumococcal disease prevented by different formulations of pneumococcal conjugate vaccines

(*Source*: www.preventpneumo.org)

7. What is the schedule of PCV?

Healthy children (not at high risk): PCV7/10/13 is given intramuscularly (IM) on the anterolateral aspect of thigh or deltoid region, but never on buttocks as is true for any injection in children. It is available as prefilled syringe in ready to use liquid formulation. The number of doses given depends on the age at presentation for the first dose. If a child presents before 6 months of age, he is given three primary doses at 6, 10 and 14 weeks (developing countries) or 2, 4 and 6 months (Western world) and one booster at 15–18 months of age. If he comes at 6–12 months of age for the first time, he is given two primary doses at 4–8 weeks interval followed by a booster at 15–18 months of age. If he comes at 12–24 months of age for the first time, he receives two doses at 4–8 weeks interval of which the second dose will be at or after 15–18 months of age. For children above 2 years, one dose may be considered till 5 years of age, for healthy children. After the age of 5 years , it is neither licensed nor recommended for healthy children at present in India. The United Kingdom (UK) and many other European countries use two primary dose scheduled at 2–4 months followed by an early booster at 12 months in its National Immunization Program (NIP). Australia uses 3 primary doses at 2-4-6 months without the booster dose thereafter. Lastly GAVI recommends only 3 primary doses at 6-10-14 weeks without the booster thereafter. There is no role of PPSV23 in healthy children.

High risk children: This group includes children with primary immune deficiency syndrome, HIV infected children, patients on prolonged high dose of oral steroids, patients with chronic cardiac or pulmonary diseases, splenectomized children, sickle cell disease (SCD), etc. For these children, one must give age appropriate doses of PCV13 with extended age up to 5- years followed by one dose of PPSV23 after 8 weeks of last dose of PCV13 followed by one more dose of PPSV23 after 5 years. If the child has received complete course of PCV before, he should receive only two doses of PPSV23 as described above. One should avoid giving PPSV23 before giving PCV as there may occur hyporesponsiveness to subsequent PCV. In case a PPSV23 dose is given before PCV, it is better to wait for 1 year if possible before PCV is given to prevent hyporesponsiveness. Recently PCV13 is approved for use >50 years of age in many parts of the world including India for those who are at high risk for developing pneumococcal disease. In certain parts of Europe PCV13 is also approved for 6–19 years age group for at risk persons. Studies are underway for PCV13 in 19–49 years of age for at risk population and once through PCV13 will become a womb to tomb type of vaccine for clinical use in healthy children <5 years and at risk people >5 years of age.

8. What is the efficacy of PCV on invasive pneumococcal disease (IPD)?

Efficacy data against various types of pneumococcal disease is available, largely for PCV7. Prelicensure, Kaiser Permanente® (KP) efficacy trial showed 97% efficacy of PCV7 against vaccine serotype IPD and 89% against all serotype IPD. Subsequent study done in American-Indian population (underserved children of USA) showed efficacy of 77% against vaccine serotype IPD and 51% against all serotype IPD with PCV7. In difficult areas of Alaska too, the efficacy of PCV7 was

90% in natives and 86% in non-natives against vaccine serotype IPD. Several post marketing studies in different countries using PCV7 in their National program using 3+1 schedule, 2+1 schedule and 3+0 schedule have shown that PCV7 was highly effective in reducing vaccine serotype specific IPD in each of these countries with near 100% reduction in vaccine type IPD over 3–5 years of its use.

Experimental vaccine PCV9 (containing serotype 1 and 5 in addition to PCV7 serotypes) was subsequently used in developing countries like South Africa and the Gambia. In South African study, the efficacy against vaccine serotype IPD was 83% and all serotype IPD 42% in non-HIV infected children; and 65% against vaccine serotype IPD and 53% against all serotype IPD in HIV infected children. In Gambia, PCV9 showed 77% efficacy against vaccine serotype IPD and 50% against all cause IPD.

For PCV10, the effectiveness of a two- or three doses in preventing IPD caused by vaccine pneumococcal serotypes was demonstrated in a phase III/IV, cluster-randomized, double-blind trial conducted across Finland. The primary endpoint was vaccine efficacy (VE) against culture-confirmed IPD due to any of the ten vaccine serotypes in children who received at least one PCV10 which was 100% (95% CI 83–100). VE was 92% (95% CI 58–100) for the two-dose primary infant series plus booster schedule. Another large phase III trial COMPAS (Clinical Otitis Media and Pneumonia Study), which evaluated the protective efficacy of PCV10 vis-à-vis hepatitis vaccines in young 24,000 Latin American children (Argentina, Panama and Colombia), showed VE of 100% (95% CI 74.3–100) against IPD caused by vaccine pneumococcal serotypes and VE of 65% (95% CI 11.1–86.2) against any IPD.

PCV13 has no RCT for efficacy but has vast post marketing effectiveness data from countries using PCV13 in their national programs. PCV13 replaced PCV7 in NIP in USA in March 2010. Data from Active Bacterial Core Surveillance study, USA, showed that by 4th quarter 2011, rates of common seven serotypes continued to remain extremely low and six additional serotypes present in PCV13 declined by nearly 90% among children <5 years old ($p < 0.0025$). In Alaska incidence of IPD is particularly high in Alaska Native (AN) children. Introduction of PCV13 in Alaskan region demonstrated that within one year of introduction of PCV13 vaccine, vaccine serotype specific IPD decreased 100% in non-AN and 71% in AN children <5 years of age. Surveillance for IPD conducted by the Health Protection Agency in United Kingdom has demonstrated that the adjusted Vaccine Efficacy (VE) for one dose, two doses under one year of age and one dose over a year of age was 55% (95% CI -11 to 72), 80% (38 to 84) and 73% (37 to 88) respectively showing also significant reduction in major serotypes like 19A and 7F. Similar effectiveness of PCV13 has been shown in Ireland, Spain and Uruguay.

9. What is the efficacy of PCV on NP carriage and otitis media?

Pneumococcal conjugate vaccines have also shown significant efficacy against non-invasive pneumococcal disease. In general, PCV7 results in 50% decrease in NP carriage of vaccine serotypes and simultaneous 50% increase in carriage of nonvaccine serotypes leading to zero net effect on overall NP carriage of pneumococci.

PCV10 has shown reductions in NP carriage of vaccine serotypes in most studies, though its effects on NP carriage of NTHi is less dramatic and non-significant. One study done using PCV10 in Finland showed that NP carriage of vaccine serotypes decreased by 23–38% with a 2+1 schedule and by 19–56% with a 3+1 schedule. COMPAS study with PCV10 also showed reduction of vaccine serotypes NP carriage by PCV10 by 25.6% (95% CI 12.7–36.7),

PCV13 also has shown significant reduction NP carriage of vaccine serotypes in a prospective study in Rochester, New York when compared to PCV7 era. Surveillance of children <5 years of age in Boston, USA following PCV13 introduction showed PCV13 vaccine efficacy of 51% (17–71) in reducing vaccine serotypes NP carriage in fully immunized children compared to unimmunized children. Similar reductions of vaccine serotypes NP carriage was also demonstrated in Atlanta in 6–59 months old children. In France there is an ongoing surveillance program of doing NP carriage study in patients with AOM. A dramatic decrease was observed in NP carriage of 6 additional serotypes from 19.4% in PCV13 non-vaccinated children to 9% in PCV13 vaccinated children, including that of 6C serotype from 8.1% to 3.4% following introduction of PCV13. In Israel, a double blind randomized controlled trial was done in a cohort of newborn babies immunized with PCV7 or PCV13 to see for efficacy of PCV13 as compared to PCV7 against newly acquired NP carriage of various serotypes. The PCV13 vaccine efficacy was found to be 79% against NP carriage of additional six serotpyes. Vaccine efficacy against NP carriage with serotype serotype 1 was 100%, 6A 42%, 7F 73% and 19A 45%, all of which were statistically significant. PCV13 efficacy against NP carriage by 6C was 51% which means 6A in PCV13 (not present in PCV7) cross protects against 6C.

AOM: It is difficult to prove efficacy against AOM as one will have to do middle ear tap and middle fluid culture in each and every case of suspected AOM to establish the etiology which may not be ethically permissible and practically feasible. Hence, only few studies were designed where middle ear tap and culture was routinely done as a standard of care in management of AOM, as was done in Finnish study. PCV7 showed 57% efficacy against vaccine serotype AOM and 34% against all serotype AOM with simultaneous increase in AOM due to non-vaccine serotype pneumococci and AOM due to other pathogens like *H. influenza* and *Moraxella catarrhalis*. Overall there was 8% decrease in all cause AOM, 50% decrease in all cause AOM with effusion, 18% decrease in all cause recurrent AOM and 39% decrease in all cause AOM needing positron emission tomography (PET) insertion on long-term follow-up. The pivotal KP® study using PCV7 showed 8.9% decrease in all cause AOM, 9% decrease in all cause recurrent AOM and 20% decrease in all cause AOM needing PET insertion.

Experimental PCV11 (a different vaccine then the current PCV10 in its contents) was tried in one study for otitis media. The efficacy against vaccine serotype AOM was 57% and against all cause AOM was 33.6%. There was decrease of 35% in NTHi AOM and increase in AOM due to *M. catarrhalis*. However subsequently PCV10 in COMPAS study showed modest decrease of overall clinical AOM by 16.1% (-1.1 to 30.4) with no significant decrease in NTHi AOM.

PCV13 has shown significant effectiveness against AOM in various studies. A prospective study on AOM in Israel after use of PCV13 showed that the incidence of AOM per 1000 children decreased significantly from 12.2 to 6 and that caused by NTHi from 5.7 to 3.8. In Greece PCV13 use led to 47.4% reduction in AOM caused by 6 additional serotypes present in PCV13 compared to PCV7, including AOM caused by serotype 19A, 6A and 3. Similar reduction in AOM have been also shown in USA and Spain.

10. What is the efficacy of PCV against pneumonia?

It is difficult to know the etiology of pneumonia as blood culture is positive in only 10% of cases and lung tap, though is positive in 50% of cases, is unethical and hazardous. Hence, most of the studies have looked at efficacy of vaccines like *H. influenzae* type b (Hib) or PCV against all cause radiological pneumonia which is likely to be bacterial in origin.

Overall PCV7/9 has shown 20–39% efficacy against all cause radiological pneumonia in double-blind randomized controlled trial set-up in several studies in developed and developing countries. In a pivotal study at KP®, USA, PCV7 showed efficacy of 30% (11–46%), in South Africa PCV9 showed efficacy of 25% (4–40%) in HIV noninfected children, and in Gambia PCV9 showed 39% efficacy against radiological pneumonia. In Gambia there was absolute reduction in pneumonia of 15,1000 child-years, 15% reduction in all cause hospitalization and 16% reduction in all cause mortality with PCV9.

PCV10 in double blind randomized vaccine controlled study COMPAS demonstrated that efficacy against first episodes of likely bacterial CAP (B-CAP) in per protocol analysis was 22.0% (7.7–34.2). Similar results were also seen in Finland. PCV10 also is in use in NIP in Brazil since 2010 where interrupted time series analysis for rates of hospitalization for pneumonia in children was conducted in 5 major cities from 2005-2011. PCV10 reduced pneumonia hospitalization by 23.3–28.7% in three cities but there was no reduction in other two cities. Similar interrupted time series analysis from Brazil for reduction in pneumonia mortality rates among infants targeted by PCV10 immunization program 3 years after its introduction showed non-significant reduction by 15.5% (-38.2 to 7.2) in pneumonia mortality rates among children 2–23 months of age.

PCV13 has shown significant effectiveness against pneumonia in children in various studies done in countries like Uruguay, Israel, Argentina, France, United Kingdom, Poland and Nicaragua. Study in Uruguay showed that after PCV13 introduction in 2+1 schedule there was 79% reduction in consolidated pneumonia hospitalization in children after 2nd primary dose and 85% reduction after the booster dose. There was also significant reduction of 69.2% in hospitalizations for empyema and complicated pneumonia. Similar was the experience in North England where the empyema cases were stable during PCV7 era which fell only after introduction of PCV13 indicating that the serotypes causing empyema and complicated pneumonia are more common from the 6 additional serotypes covered by PCV13 like type 1, 3 and 19A. In Israel, PCV13 replaced PCV7 in NIP in end of 2010. The annual incidence of CAP hospitalization rates declined by

14% and 47% in the PCV7 and PCV13 periods, respectively. A similar significant decline was also seen in OPD visits for CAP after introduction of PCV13. In France a group of 8 pediatric hospitals conducted a study to see effects of PCV13 on hospitalization for CAP. They found that there was 31% reduction in CAP hospitalization in children <2 years of age, 51% reduction in those with empyema and 62% in bacteremic pneumococcal pneumonia. Lastly, Nicaragua became the first GAVI-eligible country to introduce PCV13 in NIP using 3+0 schedule. A study looked at pneumonia hospitalization rates and pneumonia OPD visits before and after PCV13 introduction. There was significant 35% reduction in pneumonia hospitalization rates and 23% reduction in OPD visits for pneumonia following PCV13 introduction. There was also significant 19% reduction in pneumonia hospitalization rates in 5–14 years of age suggesting herd effect against CAP of PCV13 in this age group for the first time ever.

11. Will use of PCV lead to replacement disease with non-vaccine serotypes?

As discussed before, there has been a near 100% replacement of vaccine serotypes with non-vaccine serotypes in NP carriage and partial increase in AOM due to non-vaccine serotype pneumococci and other pathogens like *H. influenza* and *M. catarrhalis* after use of PCV7. Hence, such replacement disease in IPD remains a potential risk.

The real challenge came from the study done on Alaskan region where in native Alaskan children under 2 years of age, the incidence of vaccine serotype IPD fell by 265,100,000 population in six years after introduction of PCV7 with simultaneous increase in incidence of non-vaccine serotype IPD by 133,100,000 population during same period nullifying the benefits of vaccine on IPD by 50%. Yet, there was an overall decrease in all cause IPD by 158,100,000 population. There was only small increase in non-vaccine serotype in other age groups of native Alaskan population. There was no significant increase in non-vaccine serotype IPD in any age group of non-native Alaskan population. Also for the rest of the USA, study done by Active Bacterial Core Surveillance (ABCS) group, did not show significant increase in non-vaccine serotype IPD as compared to massive decrease in vaccine serotype IPD in all the age groups. Again no significant increase in non-vaccine serotype IPD is seen in other countries where PCV7 was introduced in NIP including Navajo Americans or even Australian aboriginals.

Serotype 19A is the main culprit for whatever little replacement disease is seen elsewhere in the US where it contributes 50% of IPD seen now. Increase in 19A disease may be due to secular trends, combined with pressure due to continued misuse of antimicrobials in general population coupled with the niche created by reduction in the vaccine serotype colonization due to use of PCV7. Such increase in 19A disease over last few years is also seen in other countries where there is already existing penicillin resistance and where PCV7 vaccine is yet to be used like in Israel or Korea. In any case, minor increase in serotype replacement disease is, in a way, a confirmation of the efficacy of the PCV7 vaccine. Serotype 19A is included in the 13 valent newer pneumococcal vaccine (from Wyeth-Pfizer) but not in the 10 valent newer vaccine (from GSK®).

Fortunately no replacement is seen following use of PCV10 or PCV13 in vaccinated children in countries using these vaccines in NIP since last 4 years.

12. What is the herd effect with PCV against IPD?

Based on the Hib experience, it was expected that some herd effect with PCV will be seen because of reduction in vaccine serotype NP carriage with PCV7. ABCS program is one of the largest surveillance programs that are operational in the USA since 1995. It covers 10 states and nearly 16 million population and collects data on the incidence of invasive bacterial disease. It had an opportunity to look at the impact of PCV7 on the IPD in the general population at all age groups. PCV7 was successfully included in 2000 in the NIP for use in children less than 2 years of age with catch-up program for children up to the age of 5 years. Vaccine uptake rapidly reached national average of 70%. Due to tremendous success of the program and heavy demand for the vaccine, there was shortage of the vaccine for sometime, with the result that some eligible children did not receive the booster dose and some did not receive even complete three primary series. Under this coverage background, data from 1998–1999 was used as pre-PCV7 launch and compared with 2003 data as post PCV7 launch to study the impact of PCV7. The results showed that in 2003, 3 years after successful inclusion of PCV7 in immunization program for infants under 2 years, IPD rates in children under 2 years fell by 96% for vaccine serotypes and 80% for all serotypes. The same for children under 5 years fell by 94% for vaccine serotypes and 75% for all serotypes. What was surprising was its impact on IPD in adult population which was not even the target for vaccination. The IPD rates fell by 41% in 20–39 years age group, by 20% in 40–64 years age group and by 31% in elderly greater than 65 years of age. Even in babies less than 60 days old, who are yet to receive their first dose of the vaccine, the IPD rates fell by 42.5%, and in less than 90 days old (before the first dose can offer any protection) by 39%. Similar experience has been shared by other countries like UK, France, Australia and Canada.

PCV10 is used in NIP in Finland as discussed before. The FinIP study showed reductions in hospital-diagnosed suspected non-confirmed IPD and/or culture-confirmed vaccine-type IPD in older unvaccinated children following PCV10 introduction in NIP. Similar reduction in IPD cases was shown in vaccine ineligible population following PCV10 introduction in Brazil. However a recent study from Finland showed that overall IPD rates after routine infant vaccination with an estimated >90% PCV10 uptake did not change significantly in age groups 50–64 years. Though the incidence of PCV10 serotypes decreases significantly it was offset by 40% and 28.3% increase in non-vaccine serotypes in 50–64 years, and >65 years old respectively. Serotype 19A increased in 18–49 years old and >65 years and serotype 22F in 50–64 years old.

Herd effects with PCV13 are evident from the ABC, USA data after the introduction of PCV13 in 2010. The incidence of 5 extra serotypes in PCV13 (except 6A which any way had already shown herd effects in older population after PCV7 due to cross protection) reduced significantly in 2011–12 as compared to Pre-PCV13 era by 88% in children <5 years, by 59% in 5–17 years old, by 65% in 18–49 years old, by 54% in 50–64 years old and by 47% in >65 years old, all reductions being statistically significant. Similar decreasing trends in IPD caused by additional 6 serotypes covered by PCV13 are also seen in UK for >5 years old after introduction of PCV13. In Uruguay herd effects in form of dramatic decrease in IPD in hospitalized neonates too young to receive vaccination was seen with introduction of PCV13 in NIP.

13. What about herd effect against noninvasive pneumococcal disease?

Noninvasive pneumococcal disease episodes actually outnumber IPD by 10–100 fold and hence even little herd effect on these episodes will lead to significant gain in public. In a study done in the US by Grijalva, et al. the incidence of hospitalization due to all cause clinical pneumonia and pneumococcal pneumonia were compared between 1997–1998 (pre-PCV period) and 2001–2004 (post-PCV7 period). The results showed that incidence of hospitalization went down by 39% (95%, CI 22–52) in children less than 2 years of age ($p < 0.0001$) and by 17% (95%, CI 3–34) in children less than 5 years of age. Similarly, incidence of hospitalization due to pneumococcal pneumonia went down by 65% (95%, CI 47–73) in children less than 2 years of age ($p < 0.0001$) and by 73% (95%, CI 53–85) in children less than 5 years of age ($p < 0.0001$). In age group of 18–39 years, also, there was a similar statistically significant reduction in hospitalization due to all cause clinical pneumonia by 26% (95%, CI 4–43) ($p = 0.021$) and due to pneumococcal pneumonia by 30% (95%, CI 9–47) ($p = 0.008$).

PCV10 has yet not shown herd effects on pneumonia in countries using PCV10 in NIP. As discussed under effectiveness of PCV13 against pneumonia above, use of PCV13 in Nicaragua has shown 19% reduction in pneumonia hospitalization rates in 5–14 years of age (who were vaccine non-eligible) suggesting herd effects of PCV13 against pneumonia.

14. How about the safety of PCV?

With PCV7, mild local reactions are seen in 30–35% of patients and include redness, warmth, pain, induration and tenderness. Severe local reactions greater than 2.5 cm diameter are seen in 5–6% of patients. The reactions are less than those seen with whole-cell, inactivated diphtheria, tetanus and pertussis vaccine (DTwP) and same as seen with DTaP (combined vaccine against diphtheria, tetanus, and pertussis), Hib/measles, mumps, and rubella (MMR) vaccines. There is no increase in side effects with increasing serotypes or number of dose. Fever of greater than 38°C is seen in 25–35% of recipients whereas fever of greater than 39°C is seen in less than 5% of patients. Rare adverse reactions like febrile convulsion, breath holding spasms are same as seen with any other vaccine and are more of a coincidence. Severe adverse reactions are unknown to occur with this vaccine.

15. What is the WHO recommendation for PCV?

World Health Organization, in its March 2007 position paper on pneumococcal vaccine, advises to assess preventable pneumococcal disease burden for each country and when country-specific data is not available, use data from epidemiologically similar populations to make estimates for which technical assistance will be available through WHO and its partners to make such estimates. Introduction of PCV into NIPs is a priority in general and for developing countries, a high priority. PCV can be easily integrated into routine vaccination schedules and should be initiated before 6 months of age and as early as 6 weeks. It also recommends initiating catch-up vaccination for children up to 5 years of age with the first year of introduction. WHO has recommended that PCV should be included with high priority in the National Immunization Schedule for those countries with under-five mortality rate (UFMR) greater than 50 or absolute under-five deaths of greater than 50,000 annually.

16. What is the recommendation for PCV in India?

ACVIP of Indian Academy of Pediatrics recommends to use PCV10/PCV13 in healthy children less than 2 years with catch up for children up to 5 years of age. It also recommends to use PCV13 for high risk children 2–5 years of age.

17. What are the serological correlates of protection for PCVs?

Any new PCV has to meet the following two criteria laid down by the WHO for its licensure: Immunoglobulin G (IgG) (for all common serotyoes collectively and not individually) of equal to or more than 0.35 µg/mL measured by the WHO qualified enzyme-linked immunosorbent assay (ELISA) technique.

Opsonophagocytic activity with opsonophagocytic assay (OPA) titers of 1:8 or higher.

18. How will a new PCV be introduced in market? Will it have to undergo an efficacy trial?

As PCV is recommended for children, it will be unethical to a placebo controlled efficacy trial for any new PCV. Also, WHO has recommended cutoff for minimum protective concentration (C prot.) of IgG following three primary doses that any new PCV should achieve using WHO ELISA technique to make the new vaccine non-inferior to PCV7. This C prot. is established as 0.35 µg/mL of IgG. Any new PCV will have to achieve minimum of this concentration overall for all common serotypes (and not for each and every serotype individually). WHO also recommends use of OPA to prove non-inferiority. In fact, WHO, ELISA, IgG and OPA have been used for licensing PCV10 and PCV13.

19. Can we use 2+1 schedule or 3+0 schedule instead of 3+1 schedule in our practice?

The United Kingdom and many other European countries use PCV13 in their NIP as two primary doses at 2–4 months followed by early booster at 12 months. The studies comparing PCV13 with PCV7 showed that post-primary 2 doses, the seroconversion >0.35 µg/mL by ELISA was poor for 6B and 23F which was corrected following the third dose at 12 months and that seroconversion >0.35 µg/mL by ELISA for type 3 was poor even after third dose at 12 months. However, when you look at OPA, which is the real test of ability of the vaccine to protect against IPD, both seroconversion (greater than 1:8) and GMC for OPA were good for all serotypes including type 3 post-primary two doses and further improved following third dose at 12 months. Effectiveness of one dose of PCV13 was estimated as 48%, two doses as 87% and 2+1 doses as 100%. One dose catch up for toddlers showed 83% effectiveness.

Similarly 3+0 schedule was used in the Gambia study and South African study using PCV9 which showed that efficacy against IPD was highly significant. The efficacy in South African study at mean follow up of 2.3 years was 87% against vaccine serotype IPD in HIV non-infected children which persisted at 78% at mean 6.2 years of follow up. That is why GAVI recommends using PCV in 3+0 schedule in NIP at 6-10-14 weeks in GAVI-eligible countries. However, 2+1 and 3+0 schedules are meant only for NIP and one should refrain from using them for individual in office practice.

20. Can we use PPSV23 in more than 2 year old children for routine immunization instead of PCV?

Twenty valent polysaccharide pneumococcal vaccines (PPSV) being unconjugated vaccine does not induce quality immune response and induces only IgM response which is low in quantity and quality with poor avidity and short lived without memory response. Hence, it is to be used only in high risk patients as discussed before and not for healthy children. Giving PPSV23 before PCV may induce hyporesponsiveness and can be hazardous.

21. What are the characteristics of pneumococcal polysaccharide vaccine (PPSV23)?

The unconjugated PPSV is a 23 valent vaccine (PPSV 23) containing the following serotypes—1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. It is a T cell independent vaccine that is poorly immunogenic below the age of 2 years, has low immune memory, does not reduce NP carriage and does not provide herd immunity. It has at best 70% efficacy against prevention of IPD in the high-risk population but offers no protection against non-bacteremic pneumonia/otitis media. It is stored at 2–8°C and the dose is 0.5 mL subcutaneous/IM. It is a safe vaccine with occasional local side effects. Not more than two lifetime doses are recommended as repeated doses may cause immunologic hyporesponsiveness.

22. What are the recommendations of vaccination for high risk children?

Children at high risk of pneumococcal disease are listed in Table 19.2. It is recommended to offer both PCV and PPSV23 to all high-risk children in schedules discussed below. PPV23 will cover additional 10–15% of serotypes causing IPD that are not covered by PCV10/13. Hence for the common serotypes PCV will induce robust protection; subsequent PPSV23 will provide protection against IPD caused by 11 additional serotypes as well as it will boost up immune response to the common serotypes. If PCV cannot be given due to cost constraint, at least PPSV23 should be given to high-risk children above 2 years of age.

23. What is the vaccination schedule for high-risk children?

Both PCV13 and PPSV23 are recommended for at risk children more then 2 years of age. The order of vaccination should be PCV13 followed by PPSV23 and not the other way as it can lead to hyporesponsivenesss to subsequent PCV13. Children aged greater than 2 years with underlying medical conditions (Table 19.2) should receive PPSV23 after completing all recommended doses of PCV13. These children should be administered one dose of PPSV23 at age greater than 2 years and at least 8 weeks after the most recent dose of PCV followed by one more dose of PPSV23 5 years after the first dose of PPSV23.

TABLE 19.2 Children at high risk for pneumococcal disease

Risk group	*Condition*
Immunocompetent children	Chronic heart disease (particularly cyanotic congenital heart disease and cardiac failure)
	Chronic lung disease (including asthma if treated with prolonged high-dose oral corticosteroids)
	Diabetes mellitus
	Cerebrospinal fluid leak
	Cochlear implant
Children with functional or anatomic asplenia	Sickle cell disease and other hemoglobinopathies
	Congenital or acquired asplenia or splenic dysfunction
Children with immunocompromising conditions	HIV infection
	Chronic renal failure and nephrotic syndrome
	Diseases associated with treatment with immunosuppressive drugs or radiation therapy (e.g. malignant neoplasms, leukemias, lymphomas, and Hodgkin disease, or solid organ transplantation)
	Congenital immunodeficiency includes B (humoral) or T-lymphocyte deficiency; complement deficiencies, particularly C1, C2, C3, and C4 deficiency; and phagocytic disorders (excluding chronic granulomatous disease)

Children who have received PPSV23 previously also should receive recommended PCV13 doses preferably after 1 year since last PPSV23 dose.

Children aged 24–71 months, with underlying medical conditions, who received any incomplete schedule of three doses of PCV/10/13 before age 24 months should receive one dose of PCV13 followed by one dose of PPSV23 administered more than 8 weeks later and a second dose after 5 years.

Children aged 24–71 months, with underlying medical conditions, who received less than 3 doses of PCV7/10/13 before age 24 months should receive a series of two doses of PCV13 at an interval of 8 weeks, followed by one dose of PPSV23 administered more than 8 weeks later and 2nd dose after 5 years.

When elective splenectomy, immunocompromising therapy or cochlear implant placement is being planned; all vaccines including PCV13 and PPSV23 vaccination should be completed at least 2 weeks before surgery or initiation of therapy.

24. What are the recommendations for revaccination with PPSV23 among children at highest risk?

A second dose of PPSV23 is recommended 5 years after the first dose of PPSV23 for children who have anatomic or functional asplenia, including sickle cell disease (SCD), HIV infection, or other immunocompromising condition. No more than two PPSV23 doses are recommended.

25. What is the potential of PCV in NIP in India?

Looking at the enormous pneumonia disease and mortality burden, pneumococcus being the number one cause of these severe pneumonia cases and deaths, and the effectiveness of PCV on pneumonia burden and child mortality, WHO in 2007 recommended to include PCV in the NIP of any country with UFMR greater than 50/1,000 live births or absolute child deaths greater than 50,000 per year. With UFMR of 52 per 1,000 live birth and nearly 1.6 million under-five deaths per year, India merits to include PCV in NIP with high priority. There are 55 GAVI eligible countries which can apply for GAVI support for inclusion of live saving vaccines in NIP at a nominal cost sharing of few US cents per dose by these countries (and also 17 countries that have graduated who can also apply albeit they have to pay the entire GAVI cost of the vaccines). As on 2014, 10 million children have been vaccinated with PCV through GAVI support and 77 million are expected to to immunized with PCV by 2015 that will avert 1.5 million deaths due to pneumococcal disease by 2020. Rawanda was the first country to introduce PCV7 in 2009, and Nicaragua became the first country to introduce PCV13 in Africa. Pakistan became the first Asian country to introduce PCV10 in 2012. Thirty three countries have launched PCV13 and 9 countries have launched PCV10 in NIP through GAVI support so far. India though had shown intent to include PCV in NIP by 2012–13 when PCV10/PCV13 become available, the same is not done so far. Inclusion of PCV (and rotavirus vaccine) is likely to reduce child deaths and help India achieve millennium developmental goal 4, which states that we have to reduce the under-five mortality by two-third by 2015, as compared to 1985. It is up to India to grab the opportunity and apply for GAVI support to include important and life-saving vaccines in the NIP. It is now or never!

26. What are the future pneumococcal vaccines candidates? What role they can play?

Though availability of new PCVs, 10- and 13-valent will broaden the coverage of PCVs, especially in developing countries of Asia, the problem of serotype replacement will still remain unresolved. There are several shortcomings of current conjugate vaccines, such as they are only capable of protecting against infection with bacteria that express polysaccharide capsule types that are included in the vaccine, the potential for replacement disease with non-vaccine serotypes and lastly, the complexity of conjugate vaccines production.

To circumvent these problems, efforts are already on to develop new advanced more refined pneumococcal agents. The options include new components in the conjugate, novel adjuvants and new administration routes. Intranasal immunization with various adjuvants induces strong antibody responses both in serum and on mucous membranes in mice and can protect them against lethal infections. Even though these strategies may circumvent some of the problems of conjugates, they do not address the issues of coverage, replacement, or complexity of vaccine production. Therefore, other types of immunogens, including whole-cell pneumococcal bacteria, DNA (deoxyribonucleic acid) vaccines and protein antigens, are being evaluated as candidates for novel pneumococcal vaccines. However, attempts to develop pneumococcal DNA vaccines are still limited by the generic problems of immunogenicity associated with naked DNA in humans.

Currently, the most promising option that seems quite feasible also is development of protein-based pneumococcal vaccines. Protein-based vaccines are attractive for several reasons. They are expected to be immunogenic in early infancy and in the elderly due to their T-cell-dependent nature and their coverage should be at least, in theory, broader than that of conjugate vaccines. It is also claimed that they would offer stronger protection against colonization and by containing more than one species-specific antigen, they may be able to avoid the replacement phenomenon. In addition, they would be relatively simple to produce because they would have fewer components than multivalent conjugate vaccines; thus protein-based vaccines are expected to be less expensive, allowing wide use in all areas of the world. Already, few companies have initiated development of these novel vaccines and they are in different stages of development. Many philanthropic organizations such as Bill and Melinda Gates foundations are actively involved with generous funding of these projects.

Several pneumococcal proteins such as pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), pneumococcal surface adhesin A (PsaA), pneumolysin (Ply), neuraminidase enzymes (NanA and NanB), pneumococcal histidine-triad proteins, etc. are found to possess immunogenic properties and they have been considered essential for bacterial virulence. Many trials are underway to explore their potential as pneumococcal vaccines—either as such or as carrier proteins for pneumococcal conjugates. However, the best future option would be to develop a protein vaccine that have a combination of more than one such protein antigens, or even better would be to have a combination of a conjugate and a protein vaccine in order to get the best protection and the widest coverage. This type of futuristic pneumococcal vaccine will be most suited for developing countries obviating the need of "redesigning" vaccine every alternate year to reduce the risk of replacement with strains not included in the vaccine.

27. What is the need of the hour?

The issues related to use of PCV, especially their mass use in developing countries scenario are many. There is an urgent need to carry out community-based surveys to establish exact disease burden of various syndromes caused by pneumococci and to establish an effective surveillance system to monitor prevalence of different serotypes. A watch on prevalence of multidrug resistance will also prove fruitful to design future strategies to tackle pneumococcal diseases. Studies are urgently needed to document efficacy of newer broader PCVs and shortened schedule. The decision to introduce currently available conjugate vaccines should be based on exact disease burden, sero-epidemiology of the prevailing strains and the indigenous capability of the developing country to monitor impact of the mass vaccination program, i.e. an effective disease surveillance system. Indigenous production of new pneumococcal vaccines like protein-based vaccines should be explored and pursued aggressively.

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CHAPTER

20

Rabies

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1. Which animals transmit rabies?

All warm blooded animals transmit rabies. Dog is the commonest offender in urban setting. Apart from dog the other culprits are cat, fox, jackals, wild rats, monkeys, horses, sheep, cows, buffaloes, donkeys, and pigs. The domestic animals like cow, buffalo, goat, pig and sheep can transmit rabies after they are bitten and get infected by rabid animals. Monkey transmits rabies if they are infected. Rarely elephants, camels, mongooses and bears may lead to rabies. But rabies deaths due to small rodents have not been reported. Man to man transmission is rare except in cases of cornea transplant from donors with undiagnosed rabies.

Bats infect each other in their roosts and there are few well-established incidences of bats infecting men or other animals. Apart from bites direct infection by the virus in aerial route has been reported. But this mode of transmission has not been reported from India.

Two cycles of rabies exist, sylvatic and urban. Foxes, raccoons, skunks, jackals, mongooses, bats, etc., maintain the sylvatic cycle. Whereas dogs, cats, cattle, horses, sheep, pigs, etc., maintain the urban cycle.

2. What are the various modes of transmission of rabies?

The followings are the modes of transmission of rabies infection: (i) through broken skin— bites and scratches, (ii) lick on damaged skin and intact mucous membrane by rabid animals and (iii) erosol spread (iv) organ transplant—cornea transplant mainly.

- *Through broken skin*: The commonest mode of transmission of virus to human population is the bite from infected dogs. Rabies virus cannot penetrate intact skin. Contamination of broken skin with saliva of rabid animal by simple licking can also become dangerous any time. Scratches by rabid animals are also considered another mode of transmission, which allow virus entry. So biting, scratching and licking of rabid animals on broken skin, all are to be given importance.
- *Direct mucous membrane contact*: Rabies virus can penetrate intact mucous membrane. Drinking of raw milk of infected cow, buffalo or goat is such an

example. Contact of saliva of rabid animals in anal region or mouth cavity, i.e., moist mucous surfaces, is also considered to be dangerous.
- *Erosol infection in bat-infested caves*: This has importance in North American countries.
- *Organ transplants*: Mainly corneal transplant from undiagnosed infected humans can also transmit rabies accidentally.

3. What is the incubation period of rabies in human?

It is highly variable. Incubation period may be as short as 4 days or as long as 3 years. Average incubation period varies between 3 weeks and 3 months. Prolonged incubation period is reported in relatively fewer cases. The size of inoculums of virus and the bites in head and neck region due to proximity to brain, in hands due to excess innervations may have some significance in the early causation of the disease.

4. What are the clinical types of rabies?

Two distinct types of clinical rabies have been described. The common type is furious type, seen in 80% and the other one is paralytic or dumb rabies which in 20%. Both types are common for human and canine rabies. The furious type rabies presents with acute neurological phase characterized by hydrophobia, aerophobia, photophobia, dysphagia, etc. The presenting features are as follows:

Furious Rabies

- Tingling and numbness at bite site
- Nonspecific symptoms like fever, malaise and headache
- Characteristic symptoms are hydrophobia, aerophobia and photophobia related to spasms of gullet
- Aggressiveness
- Death due to cardiac and respiratory failure occurs in 3–5 days.

Paralytic Rabies

- Tingling and numbness at bite site
- Nonspecific symptoms like fever, malaise and headache
- Progressive ascending paralysis. The order of involvement is lower limbs, abdominal muscles, upper limb and thoracic muscles, followed by coma, respiratory failure
- Death due to cardiac and respiratory failure occurs in 7–21 days.

5. What are the various categories of exposure to rabies virus and management?

The WHO recommended management of animal exposure is described in Table 20.1.

TABLE 20.1 Management of animal exposure

Category	*Type of contact with suspected or confirmed domestic or wild animals* or animal unavailable for observation*	*Recommended treatment*
I	Touching or feeding of animals, licks on intact skin	None, if reliable case history is available
II	Nibbling of uncovered skin, minor scratches or abrasions without bleeding, licks on broken skin	Administer vaccine immediately[†] Stop treatment if animal remains healthy throughout an observation period of 10 days[§] Or, if the animal is euthanized and found to be negative for rabies by appropriate laboratory technique
III	Single or multiple transdermal bites or scratches, contamination of mucous membrane with saliva (i.e. licks)	Administer rabies immunoglobulins and vaccine immediately[†] Stop treatment if animal remains healthy throughout an observation period of 10 days[§] Or, if the animal is euthanized and found to be negative for rabies by appropriate laboratory techniques

* Exposure to rabbits, rodents and hares seldom if ever requires specific antirabies treatment.

[†] If an apparently healthy dog or cat in or from a low risk area is placed under observation it may be justified to delay specific treatment.

[§] This observation applies only to dogs and cats.

6. Why are children at a higher risk for rabies?

Children are more at risk of rabies because of the following reasons:

- Short height of the children makes the vulnerable parts of the body like head, face, neck, hands more accessible to the animals. Again due to short height the traversing time of rabies virus from periphery to central nervous system is short
- Tremendous curiosity regarding pets and other animals. Children love to play with dogs and cats and pets love to play with children
- Children do not have any knowledge about the outcome and danger of encounter with animals
- Children often do not report animal bites and scratches to their parents.

7. How to manage a child who has been exposed to an animal?

Exposure to an animal consists of bite, scratch or lick on broken wounds in skin or directly on mucous membrane, i.e. on oral cavity or on anus by an animal. All warm blooded animals are potentially rabid. Treatment following an exposure will consist of the following:

Step I: Proper Wound Management

The most important steps in wound management are:

- Thorough washing of wounds under running tap water for at least 10 minutes with the aim of physical elimination of the viral load and application of soap or detergent for chemical treatment and changing the pH of the wounds
- Application of disinfectants like povidone iodine, spirit, household antiseptics, etc. to remove the remaining virus particles and prevention of secondary infection
- Application of irritants to the wounds, cauterization and suturing for wound closure should be avoided. If suturing is needed for the purpose of hemostasis, it can be done only after administration of RIG.

Step II: Rabies Immunoglobulin

Infiltration of bases of the wound(s) with RIG in all category III exposures. It neutralizes the virus and forms a coat around the virus thus obliterating virus entry into the nerve endings.

Step III: Anti-rabies Vaccination

Post-exposure prophylaxis (PEP) with Antirabies vaccination (ARV) should be initiated and completed the full course.

Step IV: Antitetanus Prophylaxis and Supportive Treatment

Administration of tetanus toxoid (TT or Td) and/or tetanus immunoglobulin (TIG) as required depending on the child's immunization status. Supportive treatment is given for fever and pain with antipyretic and analgesics, local and/or systemic antibiotic as required.

8. How RIG is given? What are the different types of RIG available in the market?

Rabies immunoglobulin provides passive immunity in the form of readymade antibody to tide over the initial phase of the infection. RIG has the property of binding to rabies virus, thereby resulting in neutralization of the virus. Two types of RIGs are available:

- *Equine rabies immunoglobulin (ERIG)*: ERIG is of heterologous source, raised by hyperimmunization of horses. Currently, manufactured ERIGs are highly purified and enzyme refined. The dose of ERIG is 40 IU/kg body weight of patient up to a maximum of 3,000 IU. As per latest recommendations from WHO skin testing prior to ERIG administration is not recommended as skin tests do not accurately predict anaphylaxis risk and ERIG should be given whatever the result of the test
- *Human rabies immunoglobulins (HRIG)*: HRIG is prepared from the serum of people hyperimmunized with rabies vaccines. The dose of HRIG is 20 IU/kg body weight (maximum 1,500 IU). HRIG does not require any prior sensitivity testing.

WHO has recently recommended abolishing the skin sensitivity test as it is considered not predictive of adverse events/reactions to occur. But in India it is obligatory on the part of physician to do it as mentioned in the product insert due to prevailing drug laws and until it is withdrawn.

Rabies immunoglobulin is to be infiltrated as much as possible into and around all the wounds. The remaining RIG, if any, is to be given intramuscularly at a site away from the site of vaccination at deltoid region or anterolateral aspect of thigh. After calculation of the dose of RIG, if it is seen that RIG is insufficient by volume to infiltrate all the wounds, it should be diluted with normal saline to make it two or three times of its volume. If RIG could not be given when antirabies vaccination was initiated, it should be administered as early as possible but no later than the 7th day after the first dose of antirabies vaccine. From the 8th day onward, RIG is not indicated since an antibody response to the vaccine is presumed to have occurred. RIG is also not indicated in individuals who have received preexposure or post-exposure prophylaxis in the past.

9. Can equine RIG be used safely?

Human rabies immunoglobulin is the preferred rabies immunoglobulin, but if not available or unaffordable ERIG may be used. Most of the new ERIG preparations are potent, safe, highly purified and less expensive as compared to HRIG, but do carry a small risk of anaphylaxis.

10. Should RIG be given to all dog bite cases?

All category III exposures to rabies in endemic countries, i.e. in transdermal bites (single or multiple) or scratches, contact of patient's mucous membrane with animal saliva should immediately be treated with RIG along with ARV. If the administration of the RIG is delayed initially, it can be administered up to 7th day after the first dose of vaccine, i.e. along with third dose of vaccine, but at a separate site. RIG need not be given after the 8th day because it results in suppression of the endogenous systemic antibody response. If the volume of RIG is inadequate for infiltration of all wounds, it should be diluted with normal saline up to two- to three-fold to make up sufficient volume.

11. What are the various antirabies vaccines?

Active immunization is done by administration of ARV. In India, nerve tissue vaccine (NTV) (Semple vaccine) was used for post-exposure treatment. The vaccine was highly reactogenic with potential neuroparalyticogenic effect and is no longer used. The currently available vaccines are the modern tissue culture vaccines (MTCV). All tissue culture vaccines have almost equal efficacy and any one of these can be used. These vaccines induce protective antibodies in more than 99% of vaccinees following pre- or post-exposure prophylaxis. The dosage schedule of cell culture rabies vaccine is same irrespective of the body weight or age of the children. The vaccines are available in lyophilized form, are stable for

3 years at 2–8°C. It should be used within 6 hours of reconstitution. The main adverse effects are local pain, swelling and redness and less commonly fever, headache, dizziness and gastrointestinal side effects.

Types of Anti-rabies Vaccines:

- *Cell culture rabies vaccines (CCRV)*:
  - Human diploid cell vaccine (HDCV)
  - Purified chick embryo cell vaccine (PCEC)
  - Purified Vero cell rabies vaccine (PVRV).
- *Purified duck embryo vaccine (PDEV)*: All CCRVs and PDEV used for PEP should have potency, i.e. antigen content greater than 2.5 IU per dose.

Reconstitution and Storage

The lyophilized vaccine should be reconstituted with the diluent provided with the vaccine immediately prior to use. In case of unforeseen delay, it should be used within 6–8 hours of reconstitution.

Presently, liquid HDCV is also available.

12. Which one is the best 'anti-rabies vaccine' available in the market?

All "MTCVs" available in the market are equally effective, highly immunogenic and safe. Though theoretically, HDCV would appear to be the best as it is free from extraneous antigenic protein/s but for all practical purposes there is absolutely nothing to choose between all the available MTCV as far as efficacy and safety is concerned. WHO has now recommended abandoning old, crude "NTV" and replace it by MTCV. Even in India, Supreme Court has also recently issued a directive to phase out NTV immediately from the country.

13. What is the schedule of pre-exposure prophylaxis of rabies vaccination?

Pre-exposure of prophylaxis is indicated for those people who are having high professional risk of acquiring rabies infection. It may be advisable to vaccinate children after they attain the age of 3 years and start playing in the streets and a may come in contact with street or pet dogs.

Pre-exposure prophylaxis is recommended for certain high-risk groups enumerated below.

- *Continuous exposure*: Laboratory personnel involved with rabies research and production of rabies biologics
- *Frequent exposure*: Veterinarians, laboratory personnel involved with rabies diagnosis, medical and paramedical staff treating rabies patients, dog catchers, zoo keepers, and forest staff
- *Infrequent exposure*: Postmen, municipality workers, policemen, courier boys, travelers to rabies endemic countries, particularly those who intend to trek
- *Pets:* Children having pets in home
- *Higher threat:* Children perceived with higher threat of being bitten by dogs.

Schedule of Pre-exposure Vaccination

- *Intramuscular (IM)*: Three doses of any CCRV (1 mL or 0.5 mL depending on the brand) administered on the anterolateral thigh or deltoid region on days 0, 7 and 28 (day 21 if time is limited)
- *Intradermal (ID)*: The dose (0.1 mL) is same for all vaccine brands and 0.1 mL is administered intradermally over the deltoid on days 0, 7 and 28.

Routine assessment of antirabies antibody titer after completion of vaccination is not recommended unless the person is immunocompromized. It is desirable to monitor antibody titer every 6 months in those with continuous exposure and every year in those with frequent exposure. A booster is recommended if antibody levels fall below 0.5 IU/mL. When serologic testing is not available booster vaccination every 5 years is an acceptable alternative.

It has been shown in several studies that preexposure vaccination elicit a good immune response and the memory cells generated lasts for many years. If such people are exposed to rabies by animal bites, two booster doses given on day 0 and on day 3 will elicit a rapid and stronger secondary immune response which will neutralize the virus. There is no need for administration of rabies immunoglobulin in patients who had taken a complete course of preexposure prophylaxis.

14. Is there some change in new IAP ACVIP 2014 recommendations pertaining to rabies?

Yes, now the committee has recommended that practically all children need vaccination against rabies and following two situations to be included in 'high-risk category of children' for rabies vaccination:

1. Children having pets in home;
2. Children perceived with higher threat of being bitten by dogs such as hostellers, risk of stray dog menace while going outdoor.

These groups of children should be offered 'pre-exposure prophylaxis (Pre-EP)' against rabies. This must be preceded by a one to one discussion with the parents. However, the 'Pre-EP' is not included in the IAP immunization schedule for all children. There is no change in the IAP recommendations for 'post-exposure prophylaxis (PEP)' of rabies.

15. A four-year old boy is bitten by a stray dog. He has received a full course (five doses) of post-exposure prophylaxis only 3 months back. Does he need any shot of anti-rabies vaccine?

Yes, he should be offered two doses of anti-rabies vaccine at 0 and 3 days. There are studies to show that good antibody levels persist up to 10 years even after a 3 dose pre-exposure prophylaxis followed by a booster at one year. However, on account of the nature of the disease, for re-exposure at any point of time after completed and documented, pre- or post-exposure prophylaxis, two doses are to be given on days 0 and 3. Rabies immunoglobulins (RIG) is not needed in these children.

16. What are the benefits of 'Pre-exposure prophylaxis (Pre-EP)' against rabies?

The advantages of the Pre-EP include elimination of the need for RIG, reduction in the number of vaccine doses on exposure and provision of immunity to individuals whose post-exposure prophylaxis is delayed. Further, the likelihood of lack of documentation of a dog bite amongst young children who may not report scratches and small playful bites from dogs and cats are other reasons why Pre-EP would be useful.

17. If there are so many benefits of 'Pre-EP immunization' against Rabies, why IAP ACVIP has not included it in its routine Immunization schedule?

The committee is of the opinion that inclusion of Pre-EP against rabies in only IAP schedule for office practice would not serve the desired purpose since majority of deaths occur among children belonging to low socioeconomic strata and those living in remote areas. The committee has strongly recommended to GoI to include Pre-EP against rabies in their UIP.

18. What are WHO recommendations in this regard?

Though WHO has not yet given a recommendation to include Pre-EP immunization in NIP of the countries having a high burden of rabies, yet they have encouraged the implementation of carefully designed studies on the feasibility, cost-effectiveness and long-term impact of incorporating 'Cell Culture Vaccines and Embryonated egg-based vaccines' (CCEEVs) into the immunization programs of infants and children where canine rabies is a public health problem.

19. What is PEP?

Post-exposure prophylaxis is a medical emergency. PEP should be initiated as soon as possible and should not be delayed till results of laboratory tests or animal observation is available. Infancy, pregnancy and lactation are never contraindications for PEP. Persons presenting several days, months or even years after the bite should be managed in a similar manner as a person who has been bitten recently (with RIG if indicated) as rabies may have a long incubation period and the window of opportunity for prevention remains.

All category II and III animal bites merit rabies vaccine. Any of the MTCV may be used intramuscularly in anterolateral thigh or the deltoid depending on the age. Rabies vaccine should never be injected in the gluteal region. The dose is same at all ages and is 1 mL IM for HDCV, PCEV, PDEV and 0.5 mL for PVRV. The standard schedule, which is known as Essen protocol is five doses on days 0, 3, 7, 14 and 30, with day "0" being the day of commencement of vaccination.

Following rabies vaccine administration on days 0, 3, 7 if the animal remains healthy over a 10 day observation period, further vaccination may be discontinued.

It is, however, desirable to administer one more dose on day 28 in order to convert the initial PEP schedule to pre-exposure prophylaxis schedule.

20. How many doses of MTCV should be administered for PEP—five or six? To whom the sixth dose is required?

The most widely used and also adopted by WHO (Known as "Essen protocol") schedule comprises five IM injections on days 0, 3, 7, 14, 28/30. The sixth dose on day 90 is optional, which is administered in selected cases such as in immunocompromized patients, neoplastic disorder patients, in extremes of ages, in malnourished cases, patients undergoing antimalarial treatment, suffering from viral hepatitis and those on long-term steroid therapy.

21. Is there any alternate route of antirabies vaccine administration?

The ID schedules have been used successfully in many countries. ID regimen consists of administration of a fraction of IM dose of CCRVs on multiple sites in the layers of dermis of skin. The use of ID route leads to considerable savings in terms of total amount of vaccine needed for full pre- or post-exposure vaccination, thereby reducing the cost of active immunization. But the regimen will not make any economic sense when one or two doses are used in private clinics. It is ideally used in antirabies clinics and centers (ARCs) where many cases of animal bites and scratches come for management.

Purified chick embryo cell vaccines and PVCV have been approved by Drug Controller General of India (DCGI) for use by ID route. As purified duck embryo cell vaccine is a suspension, its use is not recommended for ID administration, similarly the liquid and adjuvanted human cell culture vaccine is not suitable for ID use irrespective of reconstituted volume 0.1 mL of vaccine, is administered per ID site as per schedule below. But the staff of the ARCs should be adept in administering ID injections.

- *Updated Thai Red Cross (Updated TRC-ID) schedule (2-2-2-0-2)*: This is the most ideal ID schedule. This involves injection of 0.1 mL of reconstituted vaccine per ID site and on two such ID sites per visit (one on each deltoid area, an inch above the insertion of deltoid muscle) on days 0, 3, 7 and 28. The day 0 is the day of first dose administration of IDRV and may not be the day of rabies exposure or animal bite. No vaccine is given on day 14.
- *Thai Red Cross (TRC-ID) schedule (2-2-2-0-1-1)*: Same as the above schedule except 0.1 mL single dose is given on day 28 and day 90, but as the chance of drop out will increase the first regimen is preferred.

22. Should initial dose of MTCV be doubled if RIG is not available?

Yes, in certain circumstances such as category III bite near head and neck and multiple bites where RIG is very much indicated but is not available or affordable, the first dose of MTCV in Essen protocol is doubled (two doses) or trebled (three doses). The rational behind using this high dose is to increase the antigen load anticipating an early antibody response. This is the usual practice while dealing patients with immunocompromised state, those on antimitotic or antimalarial drugs, patients with chronic underlying illnesses (e.g. cirrhosis), and in severely malnourished patients.

The first dose should always be doubled for patients coming for treatment after a delay 48 hours or more.

23. Is observing the dog for 10 days without initiating treatment risky or justifiable?

Whether to observe the dog for 10 days following exposure or to start vaccination immediately after exposure is still a matter of controversy. However, in a hyperendemic country like India, it would be a sane approach to start treatment and discontinue or convert it to "pre-exposure schedule" after the dog remains healthy, or has been found laboratory negative. For example, if the dog is well on day 7 (by which time the third vaccine dose is due), the day 7 vaccine is postponed to day 8; if the dog is well on day 8, it is postponed to day 9 and so on; if the dog is well on day 10 no further vaccine is given and post-exposure schedule, is converted into a pre-exposure schedule, i.e. the 3rd dose is given on day 28.

24. What should be the treatment protocol for a patient who has had exposure, but only goes for treatment after considerable delay (weeks to months)?

Since prolonged incubation period have been noted, persons who present themselves for evaluation and treatment even months after having been bitten should be dealt in the same manner as if the contact occurred recently.

25. What will be rabies post-exposure treatment of previously vaccinated persons?

Patient who has been previously received pre- or post-exposure prophylaxis with rabies vaccine should receive the treatment protocol as follows:

- Local treatment of wound
- *Vaccination schedule*: One dose immediately and second on day 3, no RIG should be applied
- However, full treatment should be given to persons who have received pre- or post-exposure treatment with vaccines of unproven potency, with old nerve tissue vaccine or people who have not demonstrated acceptable rabies neutralizing antibody titer.

26. What should be the management if a child is bitten by a vaccinated pet dog?

One should judge the situation carefully. It is always safe to administer the vaccine than take risk of not administering the vaccine. PEP may be deferred only if the pet at the origin of exposure is more than a year old and has a vaccination certificate indicating that it has received at least two doses of a potent vaccine, the first not earlier than three months of age and the second within 6–12 months of the first dose and in the past 1 year. If vaccination is deferred, the pet should be observed for 10 days; if the dog shows any sign of illness during the observation period, the patient should receive full rabies PEP urgently.

27. Can a vaccinated dog transmits rabies?

A dog effectively vaccinated against rabies cannot suffer and transmit the disease. Still, it is very difficult to say with certainty that a particular dog immunized with a specific vaccine is immune against rabies. If a tissue culture vaccine is regularly given to a healthy dog, it should develop sufficient protection. But, in a recent survey, 6% of dogs having a reliable history of rabies vaccination were found rabid.

28. Can rabies be transmitted from man to man?

Theoretically, the saliva of a patient with hydrophobia is infectious and occasionally such transmissions have been reported. But, the well- documented transmission is through corneal transplantation. Hence, confirmation of cause of death particularly in neurological cases is very important before corneal transplantation. Besides, it is also important to avoid contact with saliva and secretions of a patient diagnosed as having rabies.

29. Do modern rabies tissue culture vaccines interfere with other commonly used childhood vaccines?

No. They can safely be given with other childhood vaccines.

30. Is it possible to include rabies vaccines in Expanded Program on Immunization (EPI) schedule?

Yes. In fact rabies vaccines had been administered along with DTP-IPV in Vietnamese infants at 6, 10, 14 weeks of age and has been found to be safe and efficacious. This could lead to future integration of pre-exposure rabies vaccination in to EPI of countries where rabies is enzootic and endemic for humans.

31. How are rabies neutralizing antibody titers estimated? Where in India are such facilities available?

Rabies neutralizing antibody titers are estimated utilizing the rapid fluorescent focus inhibition test (RFFIT). Routine estimations are not required if the WHO recommendations are followed and the five injections are taken on schedule. However, in certain circumstances (e.g. considerable delay in first dose, incomplete treatment, miscalculation of scheduled days of administration, etc.), it may be necessary to ensure that adequate antibody titers have been achieved.

Following centers in India are estimating these titers at:
- National Institute for Communicable Diseases, Delhi
- Central Research Institute, Kasauli
- National Institute of Virology, Pune
- Pasteur Institute, Coonor
- National Institute of Mental Health and Neurosciences, Banglore.

32. If the patient has consumed milk of a cow or buffalo bitten by a rabid dog, is it necessary to give antirabies vaccine?

Unboiled milk of a rabid animal may certain rabies virus, which may be dangerous as the virus may be absorbed through the mucous membrane of the mouth or any abrasion in the esophagus. The boiling of the milk inactivates the virus. Virus present in unboiled milk used in making tea or coffee also gets inactivated since it cannot withstand such temperatures. If the virus were not absorbed from oropharynx, the gastric juices with a highly acidic pH would definitely kill the virus. Hence, the possibility of a man contracting rabies after having consumed unboiled milk of a rabid animal does exist. The chances are however remote, but for a 100% fatal disease like rabies out of abundant caution, it is always better to overtreat.

33. Is there a carrier state of rabies in dogs?

There have been stray reports of carrier state of rabies in dogs. However, no report has convincingly demonstrated a carrier state in dogs and for all practical purposes this can be taken as non-existent.

34. In the event of a rat bite, do we need to give antirabies vaccine?

Most rodents in India have been found to be free of rabies. These include mice and squirrels. Mongooses have been shown to be suffering from rabies and it is possible for these animals to transmit the infection to other rodents and man. Cases of bites by rodents that are usually aggressive must be viewed with suspicion. Theoretically, all warm-blooded mammals are capable of suffering from rabies and transmitting it to man.

35. If an individual has been vaccinated with a cell culture vaccine (CCV) by ID route and later exposed again to rabies, what should be the booster schedule?

If an individual has been vaccinated with a CCV by IM route and later exposed again to rabies, two booster injections of a vaccine are recommended because one may not always be sufficient. Patients who received vaccine intradermally for their primary series are particularly at risk of a slow response to booster. Another suggestion from Thailand is to give four simultaneous ID vaccinations as a single booster. However, subjects who previously received CCV uniformly have long-lasting immunologic memory, and there is no evidence that more than two boosters are necessary. Patients who gave a history of vaccination with NTVs responded poorly to boosters in 18% of cases, and they should therefore receive a full primary regimen unless antibodies were previously shown to be present.

36. What are the contraindications to rabies vaccination? How should allergic reactions to rabies vaccines be handled?

Because rabies is a lethal disease, any contraindication to post-exposure treatment should be considered carefully before disqualifying an individual for PEP.

Individuals with histories of severe allergies are more prone to develop allergic reactions to rabies vaccine. When those individuals are vaccinated, prophylactic antihistamines should be given and epinephrine should be available. If an allergic reaction occurs, one may give an alternative vaccine of different tissue origin, e.g. PVRV or Purified Chick Embryo Cell Vaccine (PCECV) in the case of a reaction to HDCV. A similar strategy was applied in allergic individuals in whom brain tissue vaccine caused symptoms of central nervous system involvement during the course of injections. Administration of nerve tissue vaccine was interrupted immediately, and the series was completed with vaccine produced in tissue other than brain.

However, only severe reactions not controlled with premedication are grounds for interruption of rabies vaccination. Treatment with steroids may control allergy but may also inhibit viral neutralizing antibodies (VNA) responses. Accordingly, VNA titers should be determined after the last dose if steroids have been used. Similarly, patients receiving immunosuppressive medications for other diseases should have VNA levels checked after immunization to verify an adequate response to the vaccine. If titers are inadequate, a booster dose should be administered.

37. Is pregnancy a contraindication to rabies vaccination?

No, pregnancy is not a contraindication to rabies vaccination. Follow-up of 202 Thai women vaccinated during pregnancy revealed no excess of medical complications or abnormal births. Vaccination of the newborn is probably unnecessary, but has been carried out successfully.

38. What is the current status of new generation (fourth generation) rabies vaccines?

Trials are going on on the “vector-based” rabies vaccines. Both vaccinia and canarypox vectors containing the rabies G protein have been prepared and tested in humans. With both vectors, two injections at 1-month intervals raised levels of rabies VNAs, although less than two injections of HDCV given at the same interval. A third dose of the vectors gave striking booster effects, both to those who had received the vectors previously and to those who had received the HDCV previously. Other vectors that have been constructed include poxviruses, human and chimpanzee adenoviruses, and nonpathogenic rhabdoviruses.

A rabies virus was deleted for the M gene and although unable to replicate was immunogenic.

Plasmid vaccines containing the complementary DNA of the gene for the rabies virus G glycoprotein have been constructed and tested in animals. Protection on challenge with wild virus was demonstrated in mice, dogs, cats and monkeys. In a recent study, a single injection of 100 µg of a DNA expressing the rabies G protein protected dogs challenged 1 year later. DNA-vaccinated monkeys were also protected against challenge 1 year after a single IM injection.

All of the above experimental vaccines protected mice against rabies virus challenge, but unfortunately none except canarypox rabies has been tested in humans. The latter did induce antibodies, as mentioned above.

Vaccines based on N protein of rabies virus are also being pursued. The rabies virus N protein also may be important in protection. Although the G protein alone is protective in experimental animals, so is the N protein, but without the induction of VNAs. After vaccination with a CCV, antibodies appear to both N and G proteins. The function of N may be to induce protective cellular immune responses, but it also appears to enhance antibody responses to G. The N protein might be used to sensitize hosts to rabies proteins, so that subsequent injection of CCV would result in prompt development of high titers of VNAs. However, the N protein does not appear to broaden the neutralizing responses against rabies-related viruses. The N protein can be produced in a baculovirus vector, which is a potential source of antigen for large-scale preexposure immunization of humans and animals. Large amounts of G protein also can be produced by baculovirus vectors.

Synthetic peptides have been constructed that produce antibodies in laboratory animals, but none of the peptides has so far induced either development of VNAs or protection against rabies virus challenge. Anti-idiotypic antibodies as vaccines are still experimental. It may be possible to genetically engineer attenuated rabies strains and to have them serve as vectors of foreign genes. Engineering involving reverse genetics also may allow for the construction of species-specific rabies vaccines.

39. What are the new technologies for production of antirabies vaccines?

Two potentially inexpensive technologies for rabies vaccine production are expression of the G protein in genetically modified plants and the generation of recombinant plant viruses, both of which could be administered orally to humans. Genetically modified plants could also be the substrate for production of parenterally administered rabies antigen production. However, the concentrations of antigen in plants may not be adequate for protective and long-lasting immunity to rabies.

40. What is the status of use monoclonal antibodies directed against rabies virus as a substitute for HRIG or ERIG?

The availability of HRIG or ERIG in developing countries remains a problem. Safety issues concerning the use of human globulin plus its limited availability have generated attempts to find other means of providing rabies antibodies. Although ERIG has been a useful substitute, it too has safety issues and is not always available. Moreover, consistency of production has been a problem. Monoclonal antibodies that neutralize rabies virus have been produced in human-mouse hybrid cells, and might be an alternative to the scarce HRIG. A cocktail of three monoclonals was created by investigators in Philadelphia and at CDC. The Crucell Corporation developed two antibodies that were complementary to each other because they are directed against two different epitopes on the G protein. These two antibodies, called CR57 and CR4098, have been combined and tested against rabies in Syrian hamsters, in comparison with HRIG. The monoclonals efficiently neutralized 26 rabies street viruses, did not block the immunogenicity of concomitant vaccination, and protected hamsters against a challenge with a virulent rabies virus.

Escape mutants are also neutralized by the combination. Monoclonal antibodies may soon come into commercial use. The expression of monoclonal antibodies as single chain or double chain antibodies in plants could make production less expensive.

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CHAPTER

21

Japanese Encephalitis

Vipin M Vashishtha

1. What is Japanese encephalitis (JE)?

Japanese encephalitis (JE) is a serious infection caused by a virus. It occurs in certain rural parts of Asia. JE spreads through the bite of infected mosquitoes. It cannot spread directly from one person to another. Japanese encephalitis can cause:

- Mild infections with fever and headache.
- Severe infections with encephalitis. About one in four of such cases results in death. Symptoms of more severe infection are headache, high fever, neck stiffness, stupor, disorientation, abnormal movements, occasional convulsions (especially in infants), coma and paralysis.

2. How is JE transmitted?

By rice field breeding mosquitoes (primarily the *Culex tritaeniorhynchus* group) that become infected with Japanese encephalitis virus (a flavivirus antigenically related to St. Louis encephalitis virus).

3. How do people get JE?

By the bite of mosquitoes infected with the Japanese encephalitis virus (JEV).

4. What is the basic transmission cycle?

Mosquitoes become infected by feeding on domestic pigs and wild birds infected with the JEV. Infected mosquitoes then transmit the JEV to humans and animals during the feeding process. The JEV is amplified in the blood systems of domestic pigs and wild birds.

5. Could you get the JE from another person?

No, JEV is not transmitted from person-to-person. For example, you cannot get the virus from touching or kissing a person who has the disease, or from a health care worker who has treated someone with the disease.

6. Could you get JE from animals other than domestic pigs, or from insects other than mosquitoes?

No. Only domestic pigs and wild birds are carriers of the JEV.

7. What are the symptoms of JE?

Mild infections occur without apparent symptoms other than fever with headache. More severe infection is marked by quick onset, headache, high fever, neck stiffness, stupor, disorientation, coma, tremors, occasional convulsions (especially in infants) and spastic (but rarely flaccid) paralysis.

8. What is the incubation period for JE?

Usually 5–15 days.

9. What is the mortality rate of JE?

Cases fatality rates range from 0.3 to 60%.

10. What is the epidemiology of JE? How many cases of JE occur in the world?

Japanese encephalitis is the most common cause of viral encephalitis in the Asian Pacific region. The virus exists in a transmission cycle between mosquitoes and pigs and/or water birds such as herons and egrets which are the main host reservoir. JE is therefore a mosquito-borne zoonotic viral infection, the reservoir of which is water birds and in which pigs play the role of amplifying host in rural areas.

Japanese encephalitis used to be prevalent in countries with a temperate climate, including Japan, but data from tropical countries (Thailand, Cambodia, Indonesia) show that these zones also are favorable for JE transmission. Indeed, JE can now be found from the extreme south-eastern part of Russia to the North of Australia and Papua New Guinea, and from Japan to the west of India Figure 21.1. However, less than one case per year is reported in US civilians and military personnel traveling to and living in Asia. Rare outbreaks in US territories in Western Pacific have occurred. Some 50,000 cases of JE occur annually, with 25–35% case fatality rates, and more than 30% severe long-term disabilities in survivors.

11. Where do JE outbreaks occur?

Japanese encephalitis outbreaks are usually circumscribed and do not cover large areas. They usually do not last more than a couple of months, dying out after the majority of the pig amplifying hosts have become infected. Birds are the natural hosts for JE. Epidemics occur when the virus is brought into the peridomestic environment by mosquito bridge vectors where there are pigs, which serve as amplification hosts, infecting more mosquitoes which then may infect humans. Countries which had major epidemics in the past, but which have controlled

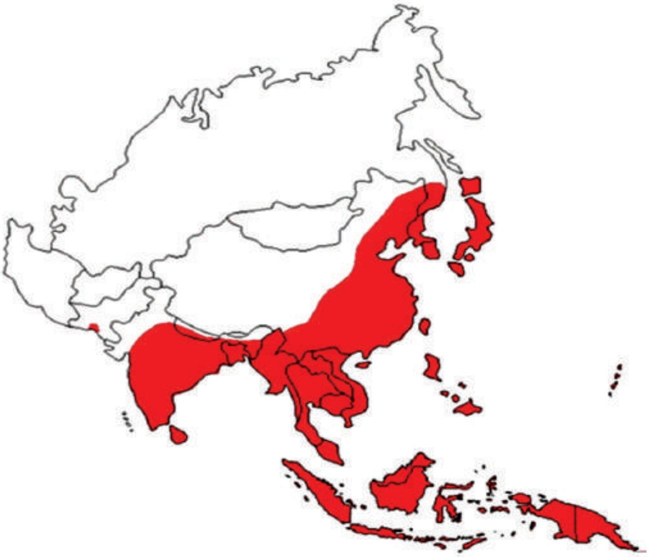

FIGURE 21.1 Distribution of Japanese encephalitis in Asia 1970–1998

the disease primarily by vaccination, include China, Korea, Japan, Taiwan, and Thailand. Other countries that still have periodic epidemics include Vietnam, Cambodia, Myanmar, India, Nepal, and Malaysia.

12. What is the epidemiology of JE in India?

Japanese encephalitis has been reported from all states and union territories in India except Arunachal, Dadra, Daman, Diu, Gujarat, Himachal, Jammu, Kashmir, Lakshadweep, Meghalaya, Nagar Haveli, Orissa, Punjab, Rajasthan, and Sikkim. Highly endemic states include West Bengal, Bihar, Karnataka, Tamil Nadu, Andhra Pradesh, Assam, Uttar Pradesh, Manipur, and Goa. There are around 2,000–3,000 cases annually with around 500–600 deaths. The risk is highest in children aged between 1–15 years, in rural areas and in the monsoon and post monsoon season. Periods of greatest risk are May to October in Goa, October to January in Tamil Nadu, August to December in Karnataka (second peak, April to June in Mandya District), September to December in Andhra Pradesh, and July to December in Northern States. Urban cases have been reported from Lucknow.

13. Is the disease seasonal in its occurrence? What seasonal pattern is seen in India?

Seasonality of the illness varies by country, and patterns of JE transmission vary within individual country. In India, the transmission pattern is different in northern and southern states. While, July to December is the high transmission season for the north, May to October is the season for the South.

14. Who is at risk for getting JE?

Residents of rural areas in endemic locations, active duty military deployed to endemic areas, and expatriates who visit rural areas. JE does not usually occur in urban areas.

15. What are the personal preventive measures against JE?

As with any disease transmitted by mosquitoes, you can prevent exposure to JE virus by:

- Remaining in well screened areas.
- Wearing clothes that cover most of the body, and
- Using an effective insect repellent, such as those containing up to 30% N, N-diethyl-metatoluamide (DEET) on skin and clothing. Use of permethrin on clothing will also help prevent mosquito bites.

Japanese encephalitis vaccine can prevent JE; however, JE vaccine is not 100% effective and is not a substitute for mosquito precautions.

16. How can JE be controlled at community level?

The control of JE is based essentially on three interventions:

1. Mosquito control
2. Avoiding human exposure to mosquitoes
3. Immunization.

Mosquito control has been very difficult to achieve in rural settings and avoidance of exposure is difficult as culex mosquitoes bite during day time. Immunization is the only effective method for sustainable control. Routine immunization of school-age children is currently in use in Korea, Japan, China, Thailand and Taiwan. The introduction of the JE vaccine into the Expanded Program on Immunization has helped curb the disease in countries like Thailand, Vietnam, Sri Lanka and China.

17. What is the current status of JE vaccines?

Licensed JE vaccines include inactivated mouse brain-derived vaccines, a Vero cell-derived inactivated vaccine, and a live attenuated SA14-14-2 vaccine produced in primary hamster kidney cells. Currently, there is no JE vaccine which is WHO-prequalified. Recognizing the burden of disease, more countries are introducing JE vaccination into their routine pediatric schedules. The inactivated mouse brain-derived vaccine is gradually being replaced by the live attenuated SA14-14-2 vaccine. In addition, several candidate vaccines are in late stage development, and are expected to be licensed in the near future. With improved vaccines becoming available, the global demand for JE vaccines is projected to more than double by 2012.

18. What are the different inactivated mouse brain-derived JE vaccines available in the world ?

Among the currently available vaccines is a formalin-inactivated vaccine derived from mouse brain-grown JEV strain Nakayama, which still is produced by manufacturers in Korea, Thailand and Vietnam. The vaccine is relatively expensive, requires three doses on days 0, 7 and 30, followed by a booster at 1 year and thereafter at intervals of 3 years. The vaccine can often generate neurological adverse reactions. In addition to local and systemic side effects, individual cases of generalized urticaria and angioedema were reported in about 1 case per 1,000 vaccinees after vaccination of travelers from Western countries.

Another formalin-inactivated JE vaccine is prepared in China using the JEV P3 strain propagated in primary hamster kidney-cell cultures. The vaccine appears to be more immunogenic than that based on the Nakayama strain and can be integrated into the routine childhood immunization schedule but is not distributed outside of China. It is now largely being replaced by the live attenuated vaccine.

19. What is the current status of new more refined inactivated JE vaccines?

Several attempts are in progress to prepare inactivated JEV vaccines starting from virus grown in controlled cell line cultures. Several manufacturers are developing Vero cell-derived purified inactivated JE vaccines, either using the virulent Nakayama strain, as done by Japanese manufacturers, or starting from the attenuated SA14-14-2 JEV strain, as done by the Austrian biotech company Intercell. Phase I and phase II clinical trials have shown that the vaccine was safe and immunogenic and a phase III trial was recently completed. The Japanese vaccine candidates have been recently licensed in Japan, while the Intercell vaccine, IXIARO® was licensed by the US FDA for adults. A two-dose rapid immunization schedule has been worked up for administration to travelers. Most people immunized with the Intercell vaccine developed protective neutralizing antibody levels that lasted for at least one year and the vaccine was well tolerated. The company pursues a separate clinical development for pediatric indication for endemic countries in a joint venture with Biological E, an Indian manufacturer. A large pediatric phase IIb trial is currently taking place in endemic settings in India. Similarly, a Vero cell inactivated vaccine is now being produced in China by the Beijing Institute of Biological Products.

20. What vaccines are available against JE in India?

Two types of JE vaccines are available in India:

- *Mouse brain-derived inactivated JE vaccine (commercially available):* It is based on Beijing or Nakayama strain of the virus. It is given subcutaneously 0.5 mL in children 1–3 years, and 1 mL in older children. Primary immunization consists of three doses given on 0, 7 and 30 days – 0 being the elected date (abbreviated schedule at 0, 7 and 14 days may be given in certain circumstances). A booster dose is recommended after 1 year and subsequently at 3 year intervals.

Common adverse reactions include fever, malaise, local tenderness and redness in 20% of recipients. Anaphylactic reactions are known to occur with this vaccine. Acute encephalitis temporally linked to JE vaccination has also been reported in 1–2.3 per million vaccinees. Advantage of the vaccine is high stability. Drawbacks include cost, complicated dosing schedule requirement of numerous doses, concerns about side effects and reliance neurological tissue for production and thus limited supply.

- *Cell culture derived live SA-14-14-2 vaccine:* This vaccine is based on a stable neuro-attenuated strain of JE virus (SA-14-14-2). It was first licensed for use in 1988 in People's Republic of China and now it is also licensed for use in Nepal, S. Korea, India and Sri lanka. Dose is 0.5 mL SC at all ages preferably given at 9 months with measles or 15 months with MMR. Recent studies have shown efficacy reaching 99% even with single dose. As per a WHO report no serious adverse effects (other than anaphylaxis) have been reported over 20-year period 1979–1998 and with 200 million doses. This vaccine is not available in commercial market in India, however it has been used for public health by Government of India in 2006.

21. What are the target groups for JE vaccination in India?

The IAPCOI recommends the inclusion of JE vaccine in the immunization schedule of children living in districts highly endemic for JE.

Japanese encephalitis vaccination should also be considered for travelers to India who are likely to visit the rural areas of districts endemic for JE during the JE season, who are likely to stay outdoors and whose stay will exceed more than a month.

The vaccine should not be used as an outbreak control vaccine during which time other preventive measures such as mosquito control and control of reservoirs should be employed.

22. Who should NOT get JE vaccine?

Anyone who has ever had a life-threatening reaction to mouse protein, thimerosal, or to a previous dose of JE vaccine. Following groups of individuals should consult a doctor before administering JE vaccine:

- Have severe allergies, especially a history of allergic rash (hives) or wheezing after a wasp sting or taking medications
- Are pregnant, or are a nursing mother
- Will be traveling for fewer than 30 days, especially if in major urban areas. (A person may be at lower risk for JE and not need the vaccine period).

23. Is it safe to administer JE vaccine (mouse brain-derived inactivated) to a pregnant mother?

No specific information is available on the safety of JE vaccine in pregnancy. Vaccination poses an unknown but theoretical risk to the developing fetus, hence the vaccine should not be routinely administered during pregnancy. Pregnant women who must travel to an area where risk of JE is high should be vaccinated when the theoretical risks of immunization are outweighed by the risk of infection to the mother and developing fetus.

24. What are the side effects of mouse brain-derived inactivated JE vaccine?

In general, the mouse brain-derived vaccines are considered relatively safe although local side effects such as tenderness, redness and swelling occur in about 20% of the vaccines. However, the vaccine can also rarely leads to severe neurological complications like acute encephalitis. These side effects are grouped as follows:

Mild Side Effects:

- Soreness, redness, or swelling where the shot was given (about one person in five)
- Fever, headache, muscle pain, abdominal pain, rash, chills, nausea/vomiting, dizziness (about one person in 10)
- If these problems occur, they usually begin soon after the shot and last for a couple of days.

Moderate or Severe Side Effects:

- Serious allergic reactions including rash; swelling of the hands and feet, face, or lips; and breathing difficulty. These have occurred within minutes to as long as 10–17 days after receiving the vaccine, usually about 48 hours after the vaccination. (About 60 per 10,000 people vaccinated have had allergic reactions to JE vaccine).
- The vaccine can often generate neurological adverse reactions. In addition to local and systemic side effects, individual cases of generalized urticaria and angioedema were reported in about 1 case per 1,000 vaccinees after vaccination of travelers from western countries.
- Other severe problems, such as seizures or nervous system problems, have been reported. These are rare (probably less than 1 per 50,000 people vaccinated).

25. What are the drawbacks of mouse brain-derived inactivated JE vaccine?

Low seroconversion, high cost, requirement of numerous doses, and reliance on a neurological tissue substrate make the mouse brain-derived JE vaccines unacceptable for future large scale vaccination programs. In a few western studies, seroconversion was noted in only 80% of vaccines following the previously used two-dose schedule and in 90% of them, the titers of neutralizing antibodies declined below protective level within 6–12 months. Recent reports of acute encephalitis linked with mouse brain derived vaccine, have further raised concerns on the safety of this vaccine in long run.

Not with standing these drawbacks, the mouse brain-derived vaccines are generally considered safe and another plus is their high stability even at higher temperature; following reconstitution it retains its original potency for at least 2 weeks at 22°C while at 37°C, potency is still 85% after 2 weeks.

26. Tell us more about the new live attenuated SA14-14-2 vaccine.

The live attenuated JE vaccine strain, SA14-14-2, which was obtained after 11 passages in weaning mice followed by 100 passages in primary hamster kidney cells, has been developed and used in China since 1988. The vaccine, which is produced by the Chengdu Institute of Biological Products in China, was licensed in recent years in several Asian countries and was extensively used from 2006 to 2008 in mass immunization campaigns in India.

Although the product is not WHO prequalified at this time, much investment and efforts have been made to bring the production and quality control to international standards. The vaccine is produced on primary hamster kidney cells, lyophilized, and administered to children at one year of age and again at two years, in annual spring campaigns.

27. What are the current estimates of efficacy of live attenuated SA14-14-2 vaccine?

Initial observational studies in southern China involving more than 200,000 children had demonstrated the vaccine safety, immunogenicity (99–100% seroconversion rate in nonimmune subjects) and protective efficacy over 5 years. The short-term effectiveness of a single dose of SA14-2-14 was demonstrated in 2001 in a case control study on Nepalese children where an efficacy of 99.3% was reported. A five year follow-up study found the single-dose efficacy was maintained at 96.2%. Another five year follow-up study showed that neutralizing antibody persistence was close to 90% at 4 years and 64% at 5 years after a single dose of the vaccine in adult volunteers. Recent studies in the Philippines have demonstrated the safety and efficacy of the vaccine even when coadministered with measles vaccine at 9 months of age. Similar studies in Sri Lanka and Indonesia will help confirm these findings in other Asian settings.

Currently, more than 30 million doses of the live SA14-2-14 vaccine are distributed annually in Southern and Western China and exported to Nepal, India and Korea.

28. How efficacious was the live SA-14-14-2 JE vaccine in Indian settings?

So far, there is no authentic published data on performance of this vaccine in India. However, preliminary findings of a recent study suggest that the JE vaccine efficacy has been around 60% in Uttar Pradesh and around 70% in Assam which is quite contrary to the Nepal-based study, on the basis of which JE vaccine was introduced in India.

The study, which began around a year ago to ascertain the efficacy of the JE vaccine in parts of Uttar Pradesh and Assam, has been completed and the data is being compiled. The draft of the research reflects that these findings are contrary to the findings of the Nepal-based study.

The JE vaccine was introduced in India in 2006 but there was no zero-surveillance study to know its efficacy on Indian children. The vaccine was introduced in India by taking the Nepal-based study as the base, according to which JE vaccine provided around 96% immunity to children. With the need increasingly being felt that India should have its own study on vaccine efficacy, National Technical Advisory Group on Immunization recommended the Central government the same in 2008.

After some delay, the study, undertaken by the Indian Council for Medical Research, began in 2009, studying around 1,000 children in Uttar Pradesh and Assam in the JE affected high-risk areas. While 750 children were covered in Uttar Pradesh, including 150 suspected of positive cases, and four controls against each case, 250 children were covered in Assam with 50 cases and 200 controls.

In Uttar Pradesh, Gorakhpur based National Institute of Virology, Pune wing, in support of BRD Gorakhpur has pursued the study. In Assam, the study was undertaken by Regional Medical Research Centre of ICMR along with the Army Medical College.

The study is very significant as for a long time experts have been raising questions over vaccine efficacy in India and have demanded at least two dozes of JE vaccine within the first 2 years to provide the right kind of immunity.

However, these are just the preliminary findings. It is feared that once the report comes out in print, the efficacy in some parts of eastern Uttar Pradesh would come to be around 50%.

29. What is the Indian experience with the live attenuated SA-14-14-2 vaccine?

Recognizing the need to control JE in highly endemic districts, Government of India had initiated a pilot project of immunizing children from hyper endemic districts against JE in 2006. Seven districts in UP, two in Assam and one each in West Bengal and Karnataka were targeted. SA-14-14-2 live JE vaccine was used manufactured by Chengdu institute of Biological Products, Chengdu, China. It was given in a campaign mode to children aged 1–15 years as single dose SC using AD syringe. The whole campaign was carried out from 15th May to 15th July 2006. 11 million children were targeted as beneficiaries and 9.3 million children actually received the vaccine, i.e. nearly 86% of the target was achieved. UP recorded 96% coverage against all expectations. There were 504 adverse effects following the campaign of which 482 were minor adverse effects. Twenty-two deaths were reported but none were causally related to the vaccine as cleared by an expert committee set up to monitor the adverse effects. The severe adverse events and critical press coverage nevertheless had a deep negative impact on vaccine acceptance in the rest of the country, highlighting the need for proper safety monitoring and case investigations.

30. What is the WHO stand on live attenuated SA-14-14-2 JE vaccine?

Currently, there is no JE vaccine which is WHO prequalified. WHO's Global Advisory Committee on Vaccine Safety (GACVS) has reviewed safety aspects of this vaccine at two of its meetings (12th held on 9–10 June, 2005, and 15th, held on 29–30 November, 2006). GACVS reviewed data related to the safety, immunogenicity and efficacy of the vaccine, and scrutinized data on coadministration with measles vaccine.

Global Advisory Committee on Vaccine Safety concluded that the short-term safety profile of live JE vaccine appears satisfactory and that there appears to be a high level of vaccine efficacy after the administration of a single dose. The vaccine is immunogenic in infants from the age of eight months of age, and can be safely administered with measles vaccine from nine months. However, a modest reduction in both seroconversion and geometric mean titer (GMT) titre has been observed for measles vaccine when coadministered with JE vaccine. Current data suggest, however, that the interference is only temporary, and co-administration of live JE vaccine and measles vaccine is acceptable. Further studies to confirm that measles vaccine effectiveness remains undiminished were encouraged.

In relation to serious adverse events reported after mass vaccination campaigns in India during 2006, no direct causality has been established between the reported illnesses and the SA14-14-2 JE vaccine. Nevertheless, GACVS recommended that in future, potential vaccine related serious adverse events should be better investigated. Furthermore, more investigations are required to assess the possible risk of low frequency adverse events (especially neurological). Since live JE vaccine is currently used in "catch-up" campaigns on many millions of children in Asian countries, the opportunity should be taken to examine whether the vaccine safety profile remains valid in large study populations.

31. Recently Japan suspended routine vaccination with inactivated mouse brain-derived JE vaccine. What is the WHO's stand on this vaccine?

In May 2005, the Government of Japan announced a suspension of routine vaccination with the mouse brain-derived inactivated JE vaccine. This decision followed a review by the national advisory committee on vaccine adverse events of a single case of acute disseminated encephalomyelitis following JE vaccination and that committee's conclusion was that it could not rule out a causal link with the vaccine. The national authority recommended continuation of JE vaccination in high risk areas only and for travel to endemic regions, and plans to reconsider routine vaccination when newer, possibly safer, inactivated JE vaccines become available.

World Health Organization's GACVS noted that there is no definite evidence of an increased risk of acute disseminated encephalomyelitis temporally associated with JE vaccine, and that a causal link has not been demonstrated.

The committee concluded, on the basis of the information presently available, that there is no need for WHO and national immunization programs to change the current recommendations for JE vaccination for residents in and travelers to

JE endemic regions. The Committee will continue to maintain a watch over the issue and will review the situation if further information becomes available.

32. What are the novel approaches to produce new generation of JE vaccines?

Recent advances in recombinant DNA technology have made it possible to explore a novel approach for developing live attenuated flavivirus vaccines against other flaviviruses. Full length complementary DNA (cDNA) clones allow construction of infectious virus bearing attenuating mutations or deletions in corporated in the viral genome.

It is also possible to create chimeric flavivirus in which the structural protein genes for the target antigens of a flavivirus are replaced by the corresponding genes of another flavivirus. By combining these molecular techniques, the DNA sequences of Dengue virus 4 (DEN4) strain 814669, Dengue virus 2 (DEN2) PDK-53 candidate vaccine and YF 17 D vaccines have been used as the genetic backbone to construct chameric flaviviruses with the required attenuation phenotype and expression of the target antigens. Encouraging results from preclinical and clinical studies have shown that several chemeric falivivirus vaccines have the safety profile and satisfactory immunogenicity and protective efficacy to warrant further evaluation in humans. The chemeric flavivirus strategy has led to the rapid development of novel live attenuated vaccines against Dengue, tick-born encephalitis (TBE), JE, and West Nile viruses.

Many arthropod-born flaviviruses are important human pathogens responsible for diverse illnesses, including dengue, JE, yellow fewer and TBE. Live and attenuated vaccines have afforded the most effective and economical means of prevention and control, as illustrated by YF 17D and JE SA 14-14-2 vaccines.

Other approaches to JE vaccines including naked DNA, oral vaccination, and recombinant subunit vaccines are also being currently reviewed. Vaxfectin, a recently developed adjuvant, was found to have an ability to enhance immunogenicity of DNA vaccines in experimental models.

The three promising approaches/candidates for future JE vaccines include chimeric vaccines, recombinant JE vaccines and DNA multivalent vaccines.

33. Tell us about new generation of future JE vaccines?

Broadly, three types of new JE vaccines are undergoing clinical trials. They include:

1. *Recombinant JE vaccines:* Recombinant JE vaccines using pox vectors expressing the premembrane, envelop, NS1 and NS2 A protein genes have been tested in monkeys and in humans. Vaccinia and avipox vectored vaccines have been tested clinically, but are no longer being pursed due to restricted effectiveness mediated by antivector immunity and this vaccine approach has been stopped.
2. *DNA multivalent vaccines:* A single intramuscular immunization of DNA vaccine of Japanese encephalitis and West Nile viruses protected mice and horses from virus challenge. The use of DNA vaccines in multivalent and/or combination vaccines designed to immunize against multiple flaviviruses is

thus a promising area of development, although the immunogenicity of DNA vaccines in humans has yet to be improved.

3. *Chimeric vaccine:* A new live attenuated vaccine (Chimeri Vax-JE, developed by Acambis) that uses a reliable flavivirus vaccine yellow fewer 17D as a live vector for the envelop genes of SA 14-14-2 virus has completed phase 1 trial and appears to be well-tolerated and immunogenic after a single dose.

34. What is a future JE Chimeric vaccine?

A promising approach for a future JE vaccine has been the construction of a yellow fever (YF)-JE chimera based on the attenuated 17D YF virus genome, in which the YFV sequences encoding viral structural proteins prM and E were replaced by the corresponding prM and E sequences from JEV strain SA14-2-14. The resulting YF-JE chimeric virus, ChimeriVax-JETM, developed by Acambis and now licensed to Sanofi Pasteur, was grown on Vero cells and shown to elicit JEV neutralizing antibodies as well as protection against nasal and intracerebral virus challenge in rhesus monkeys. The vaccine was tested in human adult volunteers in the USA, showing good safety and immunogenicity, with 94% of the vaccinees in the phase II trial developing protective neutralizing antibody levels after a single dose. The chimeric virus was shown not to replicate in mosquitoes which were fed the Chimerivax-JE vaccine, a further proof of attenuation. The vaccine has been undergoing Phase III clinical trials in the USA and Australia for adult indication, whereas a parallel pediatric development program has been launched in Thailand by Sanofi Pasteur.

35. What is the status of live recombinant vaccines against JE?

Replication defective canarypox (ALVAC) and the highly attenuated vaccinia virus strain NYVAC were used as vectors to express the pr-M, E, NS1 and NS2a gene from JEV. The vaccine candidates were found to be well tolerated but their immunogenicity was too weak, especially in nonvaccinia immune volunteers, to warrant further development.

36. Should vaccination against JE be included in National Immunization Program in India?

Various Asian countries like China, South and North Korea, Japan, Thailand, Vietnam, Taiwan practice childhood immunization against JE whereas some other countries like India, Nepal, Malaysia, Bangladesh, Mayanmar, Indonesia, Philippines, Laos and Cambodia despite having significant burden of the disease, do not have any routine vaccination policy against this disease. Thailand was the first country to successfully incorporate JE vaccination in the National immunization program.

The widespread occurrence of the disease (virtually every state and union territory has reported cases of JE), a very high rate of morbidity and mortality, non availability of a specific therapy, and availability of an effective and safe vaccine all make a strong case for inclusion of vaccination against JE in the National Immunization Program. Considering the logistic involved, it is not desirable to have universal immunization with JE vaccine all over the country but surely the time has come to formulate certain area specific guidelines and policy on childhood immunization against JE especially for those children residing in the endemic areas or where an epidemic of JE has recently been reported. There is a need for a proper pilot trial on JE vaccination by involving three to four hyper endemic districts for making a policy on mass JE vaccination in the community. As of now, India is importing the new SA-14-14-2 vaccine from China for mass immunization in campaigns at select hyperendemic districts only. There is no control or check on the quality of vaccine supplied by the Chinese manufacturers. Since, the mouse brain-derived vaccine has got several limitations and drawbacks; it would be prudent to explore feasibility of indigenous production of newer JE and other flavivirus vaccines to meet the enormous demand. Furthermore, the development of newer JE vaccines which are not only inexpensive and safer in comparison to conventional mouse brain killed vaccines but can also be produced in large quantities to meet the local demand. In fact, many Indian vaccine manufacturers are already conducting trials on newer JE and other flavivirus vaccines.

37. What are the recommendations of IAP on the use of different JE vaccines?

Three vaccines are available and the IAP recommendations for use are as follows:

- *Live attenuated SA-14-14-2 vaccine:* This vaccine is not available for office practise. Queries have often been raised about the need of second (booster) dose of the vaccine. Conventionally, boosters are unlikely to be required as with most live, attenuated vaccines, one dose will provide lifelong protection. Studies have already documented ongoing protection from a single dose for a minimum of 5 years in a JE-endemic area. However, after analyzing recent Indian efficacy/effectiveness data, the academy thinks there is need of a second dose of the vaccine to provide more complete and more sustained protection. First dose of the vaccine can be administered at 9 months along with measles vaccine and second at 16–18 months at the time of first booster of DTP vaccine.
- *Inactivated Vero cell culture derived SA 14-14-2 JE vaccine (JE-VC), (JEEV®):* This is an inactivated vaccine (JE-VC) derived from the attenuated SA 14-14-2 JEV strain propagated in Vero cells. The parent vaccine, IXIARO® by Intercell AG, Austria, has been evaluated several clinical studies in adults and children in India and in several other countries. Primary series involves two doses administered 28 days apart. Need of boosters is undetermined but the Advisory Committee on Immunization Practices recommends a booster dose in adults.
- *Inactivated Vero cell culture derived Kolar strain, 821564, JE vaccine:* The committee reviewed the data provided by the manufacturer on the clinical trials of the new JE vaccine, JENVAC® in India. Although it lacks the experience of multinational trials of IXIARO® in different country settings, nevertheless the results of a pivotal phase II/III study conducted in India appear satisfactory

for issuing recommendations for clinical use. The vaccine seems immunogenic and safe even in pediatric age group.

The committee recommends two doses of the vaccine (0.5 mL each) administered intramuscularly at 4 weeks interval for the primary immunization series for office practice starting from 1 year of age onward. Since appreciable waning was noted in both seroconversion and seroprotection rates, and GMTs were also waned significantly, there is definitely a need of booster dose at later stage. The exact timing of the booster along with feasibility of single dose for primary series can be determined only after obtaining the long-term follow-up data.

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CHAPTER

22

Rotavirus

Nitin K Shah

1. How common is rotavirus disease in children?

Every child develops at least one episode of rotavirus infection by the age of 5 years. Many patients develop more than one episode, of which the first infection is the most severe and symptomatic with potential to lead to dehydration and hospitalization and may also be fatal. Rotavirus infection is not controlled by measures of personal hygiene. Hence, it is a democratic virus in the sense that incidence of the rotavirus infection is almost the same world over whether developed countries or developing countries. What differs is the outcome of severe rotavirus infection.

2. What is the rotavirus disease burden in children in the world?

Rotavirus is responsible for around 527,000 annual deaths, worldwide. It is estimated that by the age of 5 years, 1:1 child develops rotavirus diarrhea, 1:5 will need medical attention and treatment, 1:50 will get hospitalized and 1:210 will die of the same. In actual numbers it would mean that nearly 130 million episodes of rotavirus diarrhea occur annually world over in children less than 5 years of age, of which 25 million need outdoor visit, 2.5 million need hospitalization and nearly 0.5–0.6 million die meaning a death due to rotavirus diarrhea almost every minute! 53% of these deaths occur in Asia and 42% in Africa. The chances of death following rotavirus diarrhea is estimated to be 1:50,000 in USA and 1:200 in developing countries.

3. What is the disease burden of rotavirus disease in India?

2010 publication of Recent Child Health Epidemiology Reference Group (CHERG) of World Health Organization (WHO) and United Nations International Children's Emergency Fund (UNICEF) estimates that as of 2008, approximately 8.795 million children under the age of 5 years die world over every year of which 20.8%, i.e. 1.829 million deaths occur in India alone. Fifteen percent of these deaths are due to diarrhea, i.e. 1.31 million diarrhea deaths in the world and 0.274 million diarrhea deaths in India alone. Recent studies have shown that 39% of severe diarrhea cases needing hospitalization are caused by rotavirus in India. Hence, similar percentage of diarrhea deaths are likely to occur due to rotavirus, i.e. nearly 100,000 deaths every year.

4. Which rotavirus vaccines (RVs) are available in the market? How are they different?

There are 2 rotavirus vaccines available internationally for human use, one is the live pentavalent vaccine RotaTeq® developed and tried by Merck that contains human G1, G2, G3, G4 and P8 strains reassorted with WC3 bovine strain, and the other is a live monovalent attenuated human Rotavirus G1P8 strain vaccine Rotarix® developed by GSK. Of these, the GSK vaccine is at present available in India and the Merck vaccine is likely to be available soon. Another vaccine containing monovalent lamb strain G1P10 is licensed in China, however not much data is available for this vaccine.

5. What is the schedule of rotavirus vaccine? Can they be interchanged?

Rotavirus vaccines are given at an interval of 4–8 weeks starting at 6–8 weeks of age. RotaTeq® is given in the schedule of three doses and Rotarix® as two doses. Both are given orally and they should not be injected. Rotateq® comes as ready to use liquid vaccine in a single dose tube. Rotarix® comes as single dose tube containing lyophilized vaccine with a separate syringe containing diluent and an adapter to connect the syringe to the tube. The vaccine is reconstituted by pushing the diluent from the syringe in the tube using the adapter just before administration. Rare cases of injecting this vaccine intramuscularly have been reported even though syringe is made such way that no needle will actually fit into it! Soon even Rotarix® will come as ready to use liquid vaccine in single dose tube.

One should not interchange brands of any vaccine knowingly. However, in case one does not know the brand used in previous vaccination, one has to complete total of three doses using any of the brands for subsequent doses.

6. What is the upper age limit for rotavirus vaccines and why?

As per Advisory Committee on Immunization Practices (ACIP) guidelines, Rotavirus vaccines should not be given after the age of 15 weeks for the first dose and 32 weeks for the second dose. This age limits are set because actual ages of vaccination in pivotal studies were 6–12 weeks for RotaTeq® and 6–14 weeks (Latin America) or 6–15 weeks (Europe) for Rotarix®. The maximum age for last dose was 32 weeks for RotaTeq® and 24 weeks 6 days for Rotarix®. This is a tight time frame and many children who come late for vaccination in developing countries will miss out this important vaccine. The product insert of Rotarix® mentions that both doses should be over by 32 weeks and does not mention about upper age limit for the first dose.

7. What if the child vomits after being given rotavirus vaccine?

If the child vomits, the manufacturers recommend to repeat the dose, however most experts world over believe that this is not required as the subsequent doses will take care.

8. What was the efficacy of rotavirus vaccines in pivotal studies?

A multicentric pivotal study using RotaTeq® was done in 11 developed countries including USA using three doses at 6–8 weeks interval starting at 6–8 weeks of age. The efficacy against emergency care visit or hospitalization for severe Rotavirus Gastroenteritis (RVGE) was 94.5% during the first season of rotavirus disease, against hospitalization due to any cause of diarrhea was 58.9% and in the intention to treat analysis efficacy of 88% after the first dose against severe RVGE. In the second year the efficacy was 62.6% against any rotavirus diarrhea and 88% against severe rotavirus diarrhea.

Two pivotal studies were conducted using Rotarix® where two doses were given at 6–8 weeks interval starting at 6–8 weeks of age. Latin American study showed 85% efficacy against severe RVGE in first year which was maintained at 81% in combined 2 years follow-up. Efficacy against all cause diarrhea hospitalization was 40% in first year and 39% in the second year. In European study efficacy against severe RVGE was 96% in first year and 90% in second year, against all cause diarrhea hospitalization was 75% in first year and 72% in second year, and against any severity RVGE was 79%.

9. What about safety regarding intussusception with current rotavirus vaccines?

Rotashiled®, a quadrivalent Rhesus-human reassortant vaccine used in past in USA was voluntarily withdrawn due to increased incidence of intussusception with rate ratios (RR) of 37 for the first 3–7 days following first dose in vaccines compared to placebo. In the safety cohort followed up for 1 year in the pivotal Rotavirus Efficacy and Safety Trial (REST) study using RotaTeq®, there were 11 cases of intussusception within 42 days after any dose of which 6 were in vaccinees and 5 in placebo with RR of 1.6 (95% CI, 0.4, 6.4). Hence, there was no significant increase in incidence of intussusception with RotaTeq®. This is also confirmed with postmarketing surveillance done in USA with 207,621 doses studied 42% first dose, 33% second dose. There were 5 cases of suspected cases of intussusception indentified following any dose as against expected 6 cases for RR of 0.64 and that following first dose were 2 cases against expected 1.41 cases for RR of 1.41 which was not significant. Similarly in pivotal study using Rotarix®, safety cohort was followed for 1 year. In the first 31 days following any dose, there were 6 cases of intussusception in vaccinees and 7 in placebo group for RR of 0.85 (0.30 ; 2.42). In the efficacy cohort followed for 1 year, there were 4 cases of intussusception in vaccines as compared to 14 in placebo for RR of = 0.28 (0.1 ; 0.81). Hence again for Rotarix® too there was no increase in the incidence of intussusception. All these data for both the vaccines is very reassuring that the current vaccines are extremely safe.

10. What are the concerns while recommending rotavirus vaccines in developing countries?

Live oral viral vaccines are known to have lower efficacy in developing countries for various reasons including widely prevalent malnutrition in children, use of vaccines in Expanded Program on Immunization (EPI) schedule, interference when coadministered with oral polio vaccine (OPV), effect of breastfeeding and most important being live oral viral vaccines interference by prevalent multiple gastrointestinal (GI) infections. One such example is OPV which does not work as well in developing countries especially India as in the Western world. This is the reason why WHO insisted on efficacy data with rotavirus vaccines from at least one developing country from Asia and Africa before these vaccines are recommended in national immunization program (NIP) of all the countries, including Asia and Africa.

11. What then is efficacy of rotavirus vaccines in developing countries?

Both the rotavirus vaccines have undergone efficacy trials in developing countries of Asia and Africa where rotavirus disease and mortality burden are high. OPV was administered simultaneously and breastfeeding was allowed at the time of vaccination. Rotarix® has been tried in South Africa and Malawi; and RotaTeq® in Bangladesh, Ghana, Kenya, Mali, and Vietnam.

Rotarix® was studied in South Africa and Malawi where human immunodeficiency virus (HIV) infection rates are 5% in children. It was given as 2 (10–14 weeks) or 3 (6–10–14 weeks) doses. There was not much deference in efficacy between two and three doses. The pooled vaccine efficacy using two or three doses at 1 year follow-up against severe RVGE was 61.2% (44–73.2%) overall, 76.9% (56–88.4%) in South Africa and 49.5% (19.2–68.3%) in Malawi. Efficacy against all cause severe gastroenteritis (GE) was 30.2% (15.0–42.6%) overall.

RotaTeq® has been tried in 2 studies in developing countries. Study done in Bangladesh and Vietnam used three doses of vaccine in 6-10-14 weeks schedule. HIV-infected children were not excluded. Overall combined efficacy against severe RVGE at 2 years was 48.3% (22.3–66.1%). In Bangladesh the efficacy was 42.7% (10.4–63.9%) and in Vietnam 63.9% (7.6–90.9%). Similar studies done in African countries (Ghana, Kenya and Mali) using three doses of vaccine at 6-10-14 weeks schedule showed overall efficacy of 39.3% (19.1–54.7) at mean 21 months follow-up.

All these studies show that the effcicacy was lower in developing countries of Asia and Africa as compared to that in developed countries.

12. Will rotavirus vaccine have impact in developing countries when the efficacy is much lower than in developed countries?

Though the efficacy in developing countries was not as high as seen in Latin America, European or US study it was significant and reassuring as overall 5 episodes/100 infant year of severe rotavirus disease needing hospitalization were prevented by vaccine in the African study using Rotarix®, as compared to estimated 1 episode/100 infants-year prevented in most of the western trials. Similarly RotaTeq® in Bangladesh and Vietnam prevented 3 episodes/100 infants-year and 2 episodes/100 infants-year in African study. This is due to higher burden of disease in community in developing countries as compared to developed world.

13. How will a monovalent rotavirus vaccine (RV1) work against different rotavirus serotypes?

A study was done in Mexico, to analysis the natural history of symptomatic and asymptomatic Rotavirus infection, a cohort of newborns were followed with testing of stools for Rotavirus every week and in between whenever the child developed diarrhea for the first 2 years of life. The results showed that every child developed at least one Rotavirus infection by 2 years of age, 65% developed two infections, 40% developed 3 infections, 20% developed 4 infections and 5–10% developed 5 infections with Rotavirus by 2 years of age. What was more interesting was that the first infection was usually moderate to severe, second infection was mild and 3rd, 4th and 5th infections were asymptomatic. The repeated infections due to Rotavirus were caused by different serotypes in most of the children. It means that 1st Rotavirus infection protects against severe subsequent Rotavirus infections even when caused by different serotypes. Monovalent human rotavirus vaccine mimics exactly the same, i.e. protection against severe Rotavirus infections, however with even the first infection (caused by the vaccine virus) being asymptomatic.

14. Will monovalent rotavirus vaccine work even against G2P4 serotype when the vaccine strain does not share any antigen with G2P4?

G2P4 causes significant proportion of severe rotavirus diarrhea cases world over. As monovalent human rotavirus vaccine Rotarix® does not share P or G antigens with G2P4, doubts are created as to whether it will work against G2P4 and if not whether use of Rotarix® will lead to replacement by G2P4? In a pivotal study done in Latin America the efficacy of Rotarix® against G2P4 was 41% in first year and 39% at the end of 2 years; and that in European study was 75% (-336-100%) at 1 year, both of which were not statistically significant. Only 2 year efficacy against G2P4 in European study [86% (24–98%)] reached statistical significance. Rotarix® was introduced in Brazil in 2006 when 15% of rotavirus disease was caused by G2P4 which went up to 70% in 2007 and 100% in 2008 again raising doubts about replacement. However, the same also gave opportunity to study effectiveness of Rotarix® against G2P4. A case controlled study done in a major hospital in Recife, Brazil from 2006–2008 when 88% of hospitalized rotavirus cases were caused by G2P4. Effectiveness of Rotarix® against severe G2P4 RVGE was 77% (42–81%) and against G2P4 RVGE hospitalization was 85% (54–85%). This data shows that the effectiveness of Rotarix® was as high against G2P4 as against serotypes sharing some antigen with vaccine virus.

15. Will rotavirus vaccine work in malnourished babies?

A subset analysis was done in malnourished children in a study using three different formulations of Rotarix® differing in contents of virus particles per dose where overall 17% of the subjects were malnourished showed that the vaccine efficacy was 73% (11.2–92.3) in malnourished children as compared to 74.1% (52.2–86.2) in well nourished children.

16. Can we give OPV with rotavirus vaccines?

Two live oral vaccines can interfere with one another's uptake and efficacy and accordingly there is concern for the same while administering OPV simultaneously with rotavirus vaccine. In Bangladesh Rotarix® was co-administered with or after 15 days of OPV at 12 and 16 weeks. The antirota IgA seroconversions were 56.5% (44.0–68.4%) when co-administered with OPV and 66.7% (54.0–77.8%), when Rotarix® was administered after 15 days and geometric mean antibody concentrations (GMCs) were 46.6 (30.3–71.7) and 75.3 (47.5–119.4) respectively showing no statistically significant difference. Similarly there was no effect on the seroconversion or GMCs for either polio vaccine virus by the rotavirus vaccine when co-administered with OPV.

17. Can breastfeeding be given immediately before/after rotavirus vaccine?

Breastfeeding is a norm at least in the early age in developing countries. We know that breast milk does not interfere with other live viral vaccines like OPV. In European study using Rotarix® breastfeeding was allowed by the mothers of the subjects. Subset analysis showed that efficacy against severe RVGE in breastfeeding population was 95.7% (88.2–98.9) and in formula fed population was 96.2% (74.1–99.9) and that against any RVGE was 86% (76.9–91.9) in breastfed subjects and 90.8% (72.5–97.7) in formula fed subjects, again showing that breastfeeding did not interfere with the efficacy of the vaccine.

18. What is the data on effectiveness of rotavirus vaccine in countries where it is used in the National Immunization Program?

RotaTeq® was recommended for routine use in infants in USA in February 2006. Studies in subsequent years have shown vaccine effectiveness of 85–95% against severe RVGE. Centers for Disease Control and Prevention (CDC) data shows that rotavirus diarrhea season was delayed by 2–4 months to February instead of mid-November as for the previous 15 rotavirus seasons. Proportion of all diarrhea tests that were positive for rotavirus fell from 41% in previous 15 seasons to 11–17% during 2007–2008, which was lower than the lowest ever seen in previous 15 years. Similarly, the total number of tests performed for rotavirus fell by 37% and positive for rotavirus fell by 78.5% in 2007–2008 compared with previous seven seasons. Mean coverage with three doses of rotavirus vaccine among children aged 13 months at the sentinel sites was 3.4% (range: 0–11.0%) in May 2007 and 33.7% (range: 1.1%–53.0%) in March 2008. It is obvious that the reduction in cases of rotavirus disease was far more than can be explained by the direct benefits to vaccinees alone, suggesting possibility of herd effects of vaccine in a population with significant coverage. Similarly in Nicaragua the effectiveness of RotaTeq® was 52–63% against severe RVGE and 73–86% against very severe RVGE. In neighboring El Salvador effectiveness of Rotarix® was found to be 74% against severe RVGE and 88% against very severe RVGE.

Rotarix® has been used in Mexico since 2006. By 2008 there was reduction of 41% in all cause diarrhea mortality in children less than 12 months (age group eligible for Rotavirus vaccine in previous year) which was further sustained at 62% in next year. This vaccine is also used in Brazil since 2006 and in one year there was 48% reduction in all cause diarrhea hospitalization and 67% reduction in rotavirus outbreaks. Similar results were obtained in Belgium where Rotarix® is used since 2006.

19. What is the WHO recommendation on use of rotavirus vaccines?

Rotavirus vaccines have shown significant efficacy even in developing countries where disease and mortality burden due to rotavirus is very high. Hence, WHO now recommends including rotavirus vaccines in the national immunization programs of all countries including developing countries of Asia and Africa.

20. What is the recommendation in India?

It is a well-known fact that first dose of monovalent rotavirus vaccine (RV1) administered at 6 weeks along with OPV is non-immunogenic. In a study conducted in South Africa, the seroconversion of first dose of RV1 when administered at 6 weeks along with OPV was found to be only 13%, whereas when the same dose was administered at 10 weeks along with Inactivated polio vaccine (IPV), the seroconversion rose to 43%. In the same study, the antirotavirus IgA antibody seroconversion rates were higher for the 10–14 weeks schedule (55–61%) compared to the 6–10 weeks schedule (36–43%) which was of course not statistically significant.

In Africa trial, the two dose and three dose schedule of RV1 starting at 6 weeks of age showed that vaccine efficacy against severe rotavirus diarrhea for the first year with two dose schedule was 58.7 [95% confidence interval (CI) 35.7–74] while for three dose schedule the same was 63.7 (95% CI 42.4–77.8). There was no difference on the first year efficacy of both the schedules in Malawi, but a definite gradient favoring three dose schedule in South Africa (81.5, 95% CI, 55.1–93.7 for three-dose vs. 72.2, 95% CI, 40.4–88.3) which was again not statistically significant. However, when second year efficacy against severe rotavirus diarrhea was considered, there was significant difference in the efficacy of the two schedules in both the countries (85% for three-dose vs. 32% for two-dose in South Africa, and 49% vs. 18%, respectively in Malawi).

Most of the comparative studies with two dose versus three dose schedule have employed RV1 in 10 weeks, 14 weeks instead of recommended 6 weeks, 10 weeks schedule. An immunogenicity study from India has shown that RV1 given in a two dose schedule, with first dose between 8 weeks and 10 weeks and second dose between 12 weeks and 16 weeks is immunogenic and well tolerated in healthy Indian infants. Pentavalent vaccine given in three doses has shown adequate immunogenicity in Indian infants when started at 6 weeks of age. A three dose schedule of RV 116E starting at 8 weeks demonstrated a robust immune response. To improve the immunogenicity, and in turn hoping that it will also improve the efficacy of Rotarix in developing countries, researchers have tried delaying the first dose from 6 weeks to 10 weeks or use 3 or more doses instead of standard

2 dose schedule. Two different studies, one in Pakistan and other in Ghana gave Rotarix in 3 schedules; 2 doses in 6–10 weeks schedule, 2 doses in 10–14 weeks schedule, and 3 doses in 6–10–14 weeks schedule. In both these studies there was no statistical difference in immune response between 6–10 weeks schedule or 10–14 weeks schedule, and though in Ghana there was significantly better immune response with 3 doses than 2 doses, the same was not true for Pakistan study. In fact Ghana uses Rotarix in NIP as 2 doses given as 6–10 weeks schedule. Another study done in Vellore compared 3 doses vs. 5 doses of Rotarix and found no statistical difference in immune response between these schedules. Hence there is no evidence from recent studies that delaying first dose from 6 weeks to 10 weeks will help improve immune response significantly and also for increasing the number of doses from 2 to 3 there is conflicting evidence from different studies. So to finalize a perfect administration schedule of Rotarix, there is need to find a better 'correlate of protection' than the existing one and more studies are needed in this regards, especially field efficacy study. Till then all international agencies conclude that there is insufficient evidence to either add another dose or delay the first dose while using Rotarix in developing countries.

Considering all the above mentioned facts, the Indian Academy of Pediatrics (IAP) Advisory Committee on Vaccines & Immunization Practice (ACVIP) is of the opinion that if RV1 vaccine is to be administered in a two dose schedule, the first dose should start at 10 weeks of age instead of 6 weeks in order to achieve better immune response. The second dose can be administered at 14 weeks to fit with existing national immunization schedule. However, three dose schedule of any rotavirus vaccine can start at 6 weeks of age with minimum interval of 4 weeks between the doses. However, all other international agencies including WHO and the manufacturer recommend two doses of Rotarix at minimum 4 weeks interval which can be started at 6 weeks of age onwards.

21. What is the potential of rotavirus vaccine in developing countries if included in NIP?

World over nearly 600,000 deaths in under five years age group occur due to rotavirus of which 90% occur in developing countries. As nearly 60% of these cases of severe rotavirus diarrhea are preventable by the current rotavirus vaccines, it has the potential to save 0.3–0.4 million (under 5 years) children world over. Most of the high disease burden countries including India are eligible for Global Alliance for Vaccines and Immunization (GAVI) support to include such vaccines in NIP with nominal cost sharing of few US cents per dose by these countries.

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CHAPTER

23

Human Papillomavirus

Jaydeep Choudhury

1. What is the cause of cervical cancer?

Human papillomavirus (HPV) has a predilection for mammalian epithelial cells. Until recently it was assumed that there was only one HPV that was responsible for various warty lesions. With the advent of recombinant DNA technology it was known that there are multiple HPV genotypes with a tropism for cutaneous or mucosal squamous surfaces. A subset of it infects the anogenital tract are true human carcinogens and cause carcinoma of cervix.

These viruses are classified on the basis of their DNA sequence into various genotypes. One group is responsible for most genital warts and are known as "low risk" as they do not cause cancer. This group is typified by the closely related species HPV 6 and HPV 11. There is another group of 30 oncogenic or high risk HPV which are responsible for cervical cancer. Of this HPV 16 and HPV 18 accounts for 70% cases and along with HPV 31, 33 and 45 more than 80% cases.

2. How does HPV cause cervical cancer?

HPV infects the transformation zone which lies between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix. The virus infects basal keratinocytes probably via microabrasions of the epithelial surface that leaves the basal lamina intact. The subsequent event in the viral cycle is linked with the differentiation of the keratinocytes as it moves up through the epithelium. This intraepithelial life cycle causes no inflammation thus no danger signals are sent to alert the innate immunity. HPV does not kill the infected cells – the life cycle is carried out in the keratinocytes which are destined for death from natural cause. Also there is no viremia hence the adaptive immunity is also not stimulated.

In the cervix, persistent infection can cause dysplastic changes that are termed as low grade squamous intraepithelial lesion (LSIL) or high grade squamous intraepithelial lesion (HSIL) on cytology and cervical intraepithelial neoplasia (CIN I) and CIN II/III on histopathology respectively. These precancerous lesions may take 2 to 5 years to progress. Cervical cancer is a late consequence of persistent HPV infection and may take over 10-20 years to develop. The most common form is the squamous cell carcinoma followed by the adenocarcinoma (10%) and adenosquamous carcinoma (2%).

HPV is known to infect women in the prime of their reproductive age though the disease spectrum may unfold in the later years. It is believed that women are likely to be infected with the virus in certain period of their reproductive life but they may remain asymptomatic and be cleared of the infection over a period of 2 years or they may develop neoplastic changes over more than a decade later.

Also 80 to 90% of genital HPV infections resolve with time about 10 to 20% of the individuals do not become HPV DNA negative and develop persistent infection. During persistent high risk HPV infection there is expression of the oncogenes E6 and E7 that leads to uncontrolled growth of host cells leading to carcinogenesis.

The following co-factors help in causing persistent infection.
- Young age of sexual initiation, particularly below 25 years
- Multiple sex partners
- Multiple pregnancies
- Smoking
- Oral contraceptive use
- Lack of circumcision of male partner.

The human papilloma virus is known to be associated with neoplastic changes in the anus, cervix, vulva and vagina. In addition they can predispose to esophageal cancer, penile cancer and recurrent respiratory papillomatosis.

3. How does HPV get transmitted?

Oncogenic HPV can spread via skin-to-skin genital contact and does not necessarily require penetrative sexual intercourse. Thus unlike other STDs condom cannot prevent HPV infection.

The various modes of transmission are as follows:

Sexual Contact
- Through sexual intercourse
- Genital-genital, manual-genital, oral-genital
- Genital HPV infection in virgins is rare, but may result from non-penetrative sexual contact
- Proper condom use may help reduce the risk, but is not fully protective against infection.

Nonsexual Routes
- Mother to newborn (vertical transmission)
- Fomites (e.g., undergarments, surgical gloves, biopsy forceps).

These are hypothesized but not well-documented.

4. Is cervical cancer a real problem in India?

Cervical cancer is the most frequent cancer in women in India followed by breast cancer. The crude incidence rate of cervical cancer in India is 26.2 per 100,000 women/year compared to 16.5 per 100,000 women/year for breast cancer. India has a population of 0.3 billion women aged 15 years and older who are at a risk of developing cervical cancer. Current estimates indicate that every year 134,420 women are diagnosed with cervical cancer and 72,825 die from the disease. About 7.9% of women in the general population are estimated to harbor cervical HPV infection at a given time, and 82.5% of invasive cervical cancers are attributed to HPVs 16 or 18. The incidence rate of cervical cancer in India is 23.5 per 100,000 women. Overall HPV prevalence in India was similar to the high-risk areas in Latin America, but lower than that observed in some parts of sub-Saharan Africa. One out of 4 women who die due to cervical cancer in the world is an Indian.

5. Will everybody who gets HPV infection develop cervical cancer?

Over 80% of HPV infections are transient, asymptomatic and resolve spontaneously. For every 1 million women infected with HPV, 100,000 will develop precancerous changes (cervical dysplasia), 8000 will develop carcinoma in situ (CIS), and 1600 will develop invasive cervical cancer if dysplasia and CIS are not detected or treated. Cervical cancer is a relatively rare outcome of a common oncogenic HPV infection.

6. What are the common HPV serotypes that affect human?

HPV 16 and 18 account for 70% of squamous cell carcinoma and high grade invasive cancer worldwide. Of the two, HPV16 prevalence is 57% in Asia and 58% in Europe. HPV 18 is the second most common cause of cervical cancer. Other HPV cause less than 5% of cervical cancers. Six most common high risk types are 31, 33, 35, 45, 52 and 58. Together these 8 serotypes account for 90% of cervical cancers. HPV 16/18 is slightly higher in Australia, Europe and North America (74–79%) than in Africa, South and Central America and Asia (65–70%). HPV 52 and 58 are more prevalent in Asia than elsewhere. In a study on the prevalence of high risk HPV (HR-HPV) infection among apparently healthy populations in various regions of India, the most common HPV types reported were (in descending order) HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

7. Cervical cancer is a problem of middle-aged women. Then why are young girls vaccinated?

The lag period between infection with oncogenic HPV and invasive cervical cancer is 15-20 years. Since protection is seen only when the vaccine is given before infection with HPV, the vaccine should be given prior to beginning of sexual activity. The recommended age for initiation of vaccination is 10 to 12 years.

8. Does natural infection with HPV give immunity? How does HPV vaccine provide protection against cervical cancer?

Natural HPV infection induces a weak immune response. It could be argued that the mechanism by which HPV infection at the squamo-columnar junction is prevented is due to antibody in cervical mucus. There is no viremia following HPV infection and no local inflammation. Antibody concentrations in cervical secretion are usually very low, 10-1000 times less than those in the serum and likely to be undetectable in many subjects 18-24 months postvaccination. HPV binding to the basement membrane is prevented by antibodies to HPV. Available data shows that HPV 16 neutralize at very low concentrations. This implies that much lower concentrations of neutralizing antibody would be needed for protection.

Primarily HPV infection requires a microabrasion of the genital epithelium that results in epithelial denudation but retention to the epithelium basement membrane. HPV initially binds to this exposed basement membrane by a primary receptor in L1 before binding to and entering keratinocytes. This occurs presumably when the keratinocytes migrate along basement membrane to re-epithelialize the small wound. Rapid serous exudation occurs in the wound which contains serum proteins like immunoglobulins and various immunocytes. This results in rapid virus neutralization and also, it provides an opportunity to encounter with circulating B memory cells to initiate memory response.

Virus-neutralizing antibodies to L1 also act by binding to the receptor or by binding to the capsid and preventing the initial conformational distortion essential initiate the complex virus entry process.

9. What are the currently available vaccines against HPV? How do they differ?

Presently two HPV vaccines are available. Both are manufactured by recombinant DNA technology that produces noninfectious virus-like particles (VLP) comprising of the HPV L1 protein, the major capsid protein of HPV. The quadrivalent vaccine is a mixture of L1 proteins of HPV serotypes 16, 18, 6 and 11 with aluminum containing adjuvant. The other is a bivalent vaccine. It is a mixture of L1 proteins of HPV serotypes 16 and 18 with AS04 as an adjuvant. Both are manufactured by recombinant DNA technology that produces non-infectious virus like particles (VLP) comprising of the HPV L1 protein, the major capsid protein of HPV. Clinical trials with both vaccines have used efficacy against cervical intraepithelial neoplasia (CIN) 2/3 and adenocarcinoma *in situ* (AIS) caused by HPV strains contained in the concerned vaccine as primary end points. Both the vaccine covers the two commonest oncogenic HPV serotypes 16 and 18. The former contains 6 and 11 HPV serotypes in addition which protects against genital warts.

10. Will someone be protected against HPV-related diseases if they do not get all 3 doses?

No studies so far have shown whether or not 1 or 2 doses protect as well as getting 3 doses, so it is very important to get all 3 doses.

11. What are the recommendations for males?

HPV can cause genital warts and penile and anal cancer in men. Males can also carry HPV, which can be transmitted to their sexual partners. The 3-dose series of quadrivalent HPV vaccine may be given to males through 26 years of age to reduce the risk of acquiring genital warts. The VFC Program resolution allows permissive use of quadrivalent HPV vaccine for VFC-eligible males, through 18 years of age.

12. On comparing the quadrivalent vaccine with the bivalent vaccine, which one is superior?

Both the available vaccines are equally efficacious and safe for protection against cervical cancer and precancerous lesions as of currently available data. The quadrivalent vaccine additionally protects against anogenital warts.

While, the quadrivalent vaccine contains more (four) serotypes in comparison to the bivalent vaccine, the latter contains a better adjuvant namely ASO4. The quadrivalent vaccine also protects against most genital warts.

Efficacy studies of bivalent vaccine gives 100% CIN 2/3 protection caused by HPV 16/18, 68% CIN 2/3 protection irrespective of HPV type, 88% HPV 45 related incident infection protection and 54% HPV 31 related incident infection protection. All these for at least 8.4 years in a naïve population as per the ongoing PATRICIA II study.

Efficacy studies of the quadrivalent vaccine gives 100% CIN 2/3 protection caused by HPV 16/18 for at least 5 years in a naïve population, 44% CIN 2/3 protection caused by HPV 16/18 over 3 years in a mixed population, and 17% CIN 2/3 protection caused by any HPV after 3 years in a mixed population. HPV 45 protection was not demonstrated. Further more 75% HPV 31 CIN 2+ protection has seen for at least 5 years in a naïve population as per FUTURE II study.

Immunogenicity studies with the bivalent vaccine show 100% seroconversion with HPV 16, 18, 45 and 31 with a seropositivity >98% at 6.4 years. Immunogenicity studies with the quadrivalent vaccine show 100% seroconversion with HPV 16 and 18. Seropositivity with HPV 16 remains >98% at 5 years and with HPV 18 it drops after 2 years. There is some evidence of anti-HPV 45 and 31. Both vaccines do not protect against the serotype with which infection has already occurred before vaccination.

13. Should screening be continued in women who have received HPV vaccine?

Vaccination is primary prevention while screening is secondary prevention. Hence need for both should be stressed. The available HPV vaccines protect against HPV types 16 and 18 which are responsible for about 70% of cervical cancer. Periodic screening for cervical cancer has to be continued even in vaccinated women because women may get infected with other oncogenic HPV types. The integration of the vaccination of women against HPV types 16 and 18, with cervical screening at three-yearly intervals, could reduce the incidence of cervical cancer by 94% compared with no intervention.

Hence cervical cancer screening should be continued in women who have received HPV vaccines.

14. What special precautions are to be followed for HPV vaccine administration?

As a precaution against syncope following any vaccine in adolescents, the vaccinee should be counseled prior to vaccination, vaccine should be administered in sitting or lying down position and the patient observed for 15 minutes postvaccination. Both vaccines are contraindicated in those with history of previous hypersensitivity to any vaccine component and should be avoided in pregnancy. The vaccines may be administered in the immunocompromised but immunogenicity and efficacy may be lower.

15. Are boosters needed?

At present, there is no data to support use of boosters.

16. Are there other HPV diseases that the two vaccines may prevent?

Studies have shown that the quadrivalent vaccine prevents cancers of the vagina and vulva, which like cervical cancer, can be caused by HPV types 16 and 18. Studies of the bivalent vaccine have not specifically looked at protection against vaginal and vulvar cancers.

Published studies have not looked at other health problems that might be prevented by HPV vaccines. It is possible that HPV vaccines will also prevent cancers of the head and neck, penis, and anus due to HPV 16 or 18. The quadrivalent vaccine might prevent recurrent respiratory papillomatosis (RRP), a rare condition caused by HPV 6 or 11 in which warts grow in the throat.

17. Should pregnant women be vaccinated?

Pregnant women are not included in the recommendations for HPV vaccines. Studies show neither vaccine caused problems for babies born to women who got the HPV vaccine while they were pregnant. Getting the HPV vaccine when pregnant is not a reason to consider ending a pregnancy. But, to be on the safer side until even more is known, a pregnant woman should not get any doses of either HPV vaccine until completion of pregnancy.

18. What should a woman do if she realizes she received HPV vaccination while pregnant?

If a woman realizes that she got any shots of an HPV vaccine while pregnant, she should wait until after her pregnancy to finish the remaining HPV vaccine doses.

19. Can one use both these vaccines interchangeably?

Whenever feasible, the same HPV vaccine should be used for the entire vaccination series. No studies address interchangeability of HPV vaccines. However, if the vaccine provider does not know or does not have the HPV vaccine product administered previously, either HPV vaccine can be used to complete the series to provide protection against HPV 16 and 18. For protection against HPV 6 or 11-related genital warts, a vaccination series with less than 3 doses of HPV4 might provide less protection against genital warts than a complete 3-dose HPV4 series.

20. What is the IAP ACVIP recommendation regarding use of HPV vaccination?

The IAP ACVIP has included HPV vaccination in routine immunization. Minimum age for vaccination is 9 years. As of current licensing regulations in India, catch up vaccination is up to the age of 45 years.

21. What are the new ACVIP recommendations on use of HPV vaccines in office practice?

According to new 2014 recommendations, two doses of HPV vaccine are advised for adolescent/preadolescent girls aged 9-14 years while for girls 15 years and older, current 3 dose schedule will continue. For two-dose schedule, the minimum interval between doses should be 6 months. The interval between the first and second dose may be extended up to 12 months, should this facilitate administration-say in school settings.

22. Is this recommendation valid for both the vaccine brands available in the market?

Yes.

23. Can we give two-doses of these vaccines when drug inserts of these vaccines do still mention three doses?

Yes, of course. Even WHO has also approved two doses of both these vaccine for the adolescent girls aged 9–14 years of age. The manufacturer of bivalent vaccine has started the process of revising the drug inserts of their brand. Even the manufacturer of quadrivalent vaccine is working on the same line.

24. How many doses of HPV vaccine a 15 year 4 month-old girl should take now? She has received her first HPV shot when she was 14 years 10 month-old.

Only one dose. According to both ACVIP and WHO, for girls, primed before the age of 15 years, even if older at the time of boosting (second dose), a 2-dose schedule will be applicable.

25. Why IAP ACVIP has suddenly changed its recommendations on HPV vaccination?

The move to revise HPV vaccine immunization schedule for adolescent girls from existing 3 to 2 doses would not only be cost saving, but would also simplify logistics like increased flexibility of the intervals and annual doses for school-based delivery. Hence, the revised recommendations may help in improving acceptance, facilitating delivery, and enhancing coverage of the vaccine.

26. Is the two-dose recommendation valid for certain high risk group of adolescent girls also?

No, for immuno-compromised individuals, including those known to be HIV-infected, the three-dose schedule is recommended irrespective of age.

27. Is there enough evidence for this change in recommendations?

Yes, there is now enough evidence emanating from various countries and different trials favoring this shortened schedule. The main source of evidence for the revised recommendations is provided by a systematic review commissioned by WHO-SAGE WG. The systematic review has identified various studies that include both randomized and non-randomized trials of both the vaccines, bivalent and quadrivalent, from various high income group countries like Canada, Australia, Sweden, Denmark, Germany, & LMI countries like Uganda, Mexico, and India. The other sources include non-systematic review of the data from observational studies on 2 versus 3 dose schedule, and proceedings of an Ad hoc Expert Consultation on HPV vaccine schedules organized in Geneva, 2013. Even the European Medicines Agency's (EMA) has also approved 2 doses for pre-adolescent and adolescent girls aged 9–14 years for the bivalent HPV vaccine and also offered positive opinion for a similar schedule for quadrivalent vaccine. Many countries that include Canada (Quebec), Switzerland, South Africa, UK, France, The Netherlands, Chile, Spain, etc. have either already adopted or are planning to adopt a 2-dose schedule. Few countries like Brazil, Mexico, Columbia, British Columbia are running an extended schedule of (2+1, i.e. 0, 6, 60 months) where the last dose at 5 years depends on follow-up assessment of the need. In Costa Rica, strong 4 year protection was reported in women who received just one dose of bivalent HPV vaccine. Above all, even the WHO has now approved this schedule in their October 2014 position paper on use of HPV vaccines.

28. Do we have some Indian data also on the shortened schedule?

Yes, there is a large ongoing multi-centric RCT on alternative dosing schedule of quadrivalent HPV vaccine in India. In this trial, comparisons favored the 2-dose schedule and the ratio of antibody levels was higher in the 2-dose group than in 3-dose group. The GMCs for HPV18 in the 2-dose group were non-inferior to that in the 3-dose group.

29. The recommendations of shortened schedule are based entirely on comparisons of immunogenicity and GMC levels achieved after vaccination. We still do not have long-term clinical efficacy data. How prudent would it be to assume the higher antibodies level will translate into real-time protection against cervical cancer?

Yes, it is true that only limited data on efficacy/effectiveness is available on clinical efficacy/outcome of the vaccine in the two schedules. The assumption is that the mechanism of protection afforded by the HPV vaccines is neutralizing antibody-mediated. This assumption is supported by animal models that demonstrate protection against viral challenge in animals immunized by passive transfer of hyperimmune serum from donors immunized with L1 VLPs. Circulating antibodies generated by L1 VLP (HPV) vaccination are thought to reach the site of infection by active IgG transudation at least in the female genital tract, and by passive exudation at sites of trauma that are believed to be required for initiation of HPV infection.

Thus, HPV antibody titers represent a valid marker to compare the expected clinical efficacy of various vaccines and schedules. VLP vaccines elicit very high antibody concentrations. Therefore, when different schedules are compared non-inferiority of antibody concentration must be achieved for alternative schedules if it is expected that the clinical efficacy will be equivalent. Strong 4 year protection was reported in Costa Rican women who received just one dose of bivalent vaccine.

The antibody responses are different after natural infection compared with HPV L1 VLP vaccination. After natural infection, 70–80% of women seroconvert and their antibody responses are typically slow, weak and of low avidity. But this is sufficient for antibodies generated in natural infections to be usually protective against subsequent incident infection. Following HPV L1 VLP vaccination, in contrast, close to 100% of women seroconvert after the first vaccine dose (priming). Peak antibody titers reach levels 10-1000 times greater than in natural infections and are of much higher avidity – i.e. protective capacity. Neutralizing antibodies persist for >9 years post immunization (longer time point assessed) in women. These high-level and high-avidity antibody responses persist such that unquestionable to date, vaccine failures have not yet been identified in clinical studies, precluding the identification of a minimal antibody threshold level that correlates with the protection. No specific immune correlate is thus yet available.

HPV vaccines were licensed based upon the demonstration of their clinical efficacy in young adult women.

The age extension for adolescent girls, in whom efficacy trials would not be feasible, was granted because studies demonstrated that antibody responses in adolescent girls were not inferior to those elicited in women ("immunological bridging"). Alternative adolescent vaccine schedules should thus demonstrate that their immunogenicity is similarly non-inferior. To seek licensing, a Phase III immunogenicity study of the quadrivalent HPV vaccine was conducted in adolescents with the objective of bridging the efficacy findings in young women to pre-adolescents and adolescents. The neutralizing anti-HPV GMTs at month

7 were non-inferior in adolescents - and indeed 1.7–2.7 fold higher than in the group of 16–23-year-old females in whom efficacy was demonstrated. Similar observations were made for the bivalent vaccine and for the nonavalent vaccines currently in clinical development.

The magnitude of the vaccine response is determined by the age at the first dose. The review of different trials have shown that 100% adolescents can be primed with a single dose of the vaccine and the second dose after 6 months results in higher (almost twice) peak titers in adolescents than in adults. These antibodies then plateau for about 12 months after the peak and decline very slowly providing a long lasting protection.

Hence, there are enough evidence to justify correlation of antibodies level with ultimate clinical protection against HPV disease.

30. Can we have shorter, e.g. 2 months interval for two-dose schedule?

No, the available data strongly favor long interval between two doses. In a recent RCT, different intervals (0, 2, vs. 0, 6 vs. 0, 12 months) between doses of HPV vaccine were compared. The results revealed that the 6-month interval resulted in superior GMCs compared with the 2-month interval one month after the last vaccine dose in all the age groups enrolled (9–14, 15–19, 20–25 years). There are no data as yet publically available from the other trial comparing 0,6 months with 0, 12 months interval.

31. What is the immunological basis of efficacy of long interval schedule?

The immunological basis of this can be explained by the following. In addition to quantity and quality, kinetics are critically important for HPV-vaccine induced protection: (1) memory B cells elicited by the first vaccine dose require at least 4–6 months to mature and differentiate into high-affinity B cells. This implies that any immunization schedule must include at least a 4 month interval before the last dose (prime-boost) to efficiently reactivate memory B cells. Two dose schedules with shorter intervals (prime-prime) might not allow this affinity maturation and are expected to be less immunogenic/protective. (2) antibody persistence, i.e. the plateau of antibodies produced by long-lived plasma cells, is best estimated at least 6 months and preferably 12–18 months after the last immunization.

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CHAPTER 24

Influenza

Shobha Sharma

1. What is influenza?

Influenza, commonly known as "the flu," is an infectious disease of the respiratory tract caused by ribonucleic acid (RNA) viruses of the family Orthomyxoviridae, the influenza viruses. Flu is highly contagious and is usually spread by the coughs and sneezes of a person who is infected. Adults are contagious 1 day before getting symptoms and up to 7 days after becoming ill.

2. What are the causes of flu?

Influenza viruses cause flu and are divided into three types designated A, B and C. Influenza type A is responsible for most of the seasonal and epidemics of respiratory illness that occur almost every winter, whereas type B is responsible for lesser numbers. Type C infection usually causes either a very mild respiratory illness or no symptoms at all; it does not cause epidemics.

3. What do the terms "antigenic shift" and "antigenic drift" means?

Influenza viruses have cell envelope glycoproteins hemagglutinin (HA) and neuraminidase (NA). Peculiarity of influenza viruses (especially A type) is the highly unstable genetic constitution which is very sensitive to mutation and reassortment while replicating in host cells. More commonly, it undergoes point mutations that occurs over time and causes a gradual evolution of the virus. This is called "antigenic drift". In addition, there may be abrupt and complete change in the HA and/or the NA proteins due to complete genetic reassortment between different strains of infecting viruses and host viruses. This is called "antigenic shift". In this case, a new subtype of the virus suddenly emerges. Type A viruses undergo both kinds of changes; influenza type B viruses change only by antigenic drift and therefore do not cause pandemics.

4. What are the clinical implications of the phenomenon described in the previous question?

Antigenic drift is expected phenomenon and is monitored closely by laboratories all over the world for any changes in genetic constitution, and expected emerging strain designated by number of subserotype of HA and NA proteins, and is also responsible for directions for new vaccine strains to be used in upcoming season.

Antigenic shift is sudden unexpected and complete change in virus strain due to mixing of different genetic material in host cell (usually avian, swine or other mammals). If shifted virus is capable of efficient human-to-human transmission, the stage is set for pandemic as humans are not prepared immunologically for it. Highly contagious nature of infection results in influenza pandemics occurring form time to time, most recent being 2009 flu pandemic by H1N1 strain. H1N1 swine flu has an RNA genome that contains five RNA strands derived from various swine flu strains, two RNA strands from bird flu (also termed avian flu) strains, and only one RNA strand from human flu strains.

5. What are the symptoms of flu?

Typical clinical features of influenza or influenza like illness are fever (usually 100–103°F in adults and often even higher in children), chills, headache, muscle aches and fatigue (sometimes extreme).

Systemic features are more common in influenza virus compared to other respiratory viruses. Respiratory symptoms are mainly localized in upper tract such as cough (more often in adults), sore throat (more often in adults), runny or stuffy nose (especially in children). Although nausea, vomiting and diarrhea can sometimes accompany influenza infection; in children, gastrointestinal symptoms are rarely present.

6. How serious is flu?

In most cases (90%), it is a self-limiting benign respiratory febrile illness with duration of 1–2 weeks, but some high-risk groups may show more severe illness with high case fatality due to acute hemorrhagic pneumonia, acute respiratory distress syndrome (ARDS), febrile encephalopathy, myocarditis, superimposed bacterial infection causing complicated pneumonia, toxic shock syndrome, etc. These high-risk groups are age over 65 years, very young child below 2 years, pregnant females, underlying cardiovascular disease, chest problem such as asthma or bronchitis, kidney disease including nephrotic syndrome, diabetic persons, those on long-term steroids or undergoing treatment for cancer.

7. What are emergency or danger signs of flu in children?

Any sick child who has fast or troubled breathing, bluish or gray skin color, not drinking enough fluids, severe or persistent vomiting, not waking up or not interacting, being so irritable that the child does not want to be held.

8. What is the epidemiological trend of influenza and how is it monitored across the world?

Influenza viruses' transmission peak infection rate varies in different regions. Countries are divided into Northern and Southern Hemispheres and further classified into temperate and tropical zones.

World health organization's (WHO's) Global Influenza Surveillance and Response System (GISRS) through its network of laboratories across the world monitors the evolution of influenza viruses and provides recommendations in areas including diagnostics, vaccines, antiviral susceptibility and risk assessment on weekly and twice-weekly basis. According to latest WHO report, India continues to report mainly influenza A(H1N1)pdm09, A(H3N2) and influenza B although at low levels of circulation.

9. What are virological and epidemiological differences between seasonal and pandemic flu?

As described previously, seasonal flu is caused by different serotypes in different regions as determined by expected antigenic drift in viral genome which changes with time, in India recent seasonal flu strains are influenza A(H1N1)pdm09, influenza A(H3N2) and influenza B; whereas, pandemic influenza is result of sudden antigenic shift and cause by same viral subserotype all over world, latest being influenza A(H1N1)pdm09. Epidemiologically seasonal flu transmission peaks at certain season which vary at different regions (divided into Northern and Southern Hemispheres). In India, seasonal flu circulates at lower levels throughout the year with peak in rainy season which is June to August in Northern India and October to December in Southern India. Pandemic flu cases were reported maximally in months of August and September. Risk factors for severe disease in seasonal flu are described previously with extremes of ages showing maximum case fatality, whereas pandemic flu of 2009 showed maximum case fatality in 20–39 years age group without any risk factors.

10. How can influenza be diagnosed in patients suffering from flu like illness?

Diagnosis of influenza depends on epidemiological, clinical and laboratory considerations. During epidemic, any young child with fever without focus and having respiratory symptoms with systemic features, diagnosis can be made to a certain degree clinically and treatment started. In seasonal flu laboratory diagnosis of suspected cases is based on virus isolation from nasopharynx in early stages. Reverse transcription polymerase chain reaction (RT-PCR) is other method for confirmation of diagnosis. Direct fluorescence, enzyme-linked immunosorbent assay and paired sera testing by hemagglutination assay are other reliable methods for diagnosis.

11. What types of influenza vaccines are available?

Currently available seasonal influenza vaccines include trivalent inactivated vaccine (TIV) and live attenuated influenza vaccines (LAIV).

Trivalent inactivated vaccine has two strain subtype of influenza A and an influenza B strain and is approved by US Food and Drug Administration (FDA) for use in children above 6 months. Vaccine formulation (of representative subtypes) is prescribed by the WHO twice every year before the peak influenza season in the Southern and Northern Hemispheres. Inactivated vaccine can be whole cell or newer types of split virion vaccine or subunit vaccine with lesser reactogenecity and higher immunogenicity. For children (6 months to 10 years), two doses and for all above 10 years, a single dose has been recommended. From June 2010, monovalent influenza A(H1N1)pdm09 vaccine is available in India, the vaccine is recommended for 18 years and above, and should be administered by a single 0.5 mL intramuscular (IM) preferably in deltoid region.

Although LAIV are more efficacious, have relative ease of administration and require lesser doses, they are not available in India.

Other newer vaccines are adjuvant vaccine and quadrivalent vaccine.

12. What are the guidelines for influenza vaccination?

The American Academy of Pediatrics (AAP) recommends annual seasonal influenza immunization for all people, including all children and adolescents, 6 months of age and older during the 2013–2014 influenza season.

The Indian Academy of Pediatrics (IAP) has now (in 2013) formulated recommendations regarding flu vaccination. Adequate data on burden of disease in India is not known. According to published data, 5–10% of all cases of acute respiratory infections (ARIs) are due to flu. Reported incidence of influenza upper respiratory infection (URI) is 10/100 child years and that of acute lower respiratory infection (ALRI) only 0.4/100 child years. There is an Indian review reporting 1.5–14% of all ARI cases due to influenza.

The IAP has recommended seasonal influenza vaccine [including the earlier monovalent A (H1N1) vaccine] only for the category of "high-risk children". This category includes the following:

- Chronic cardiac, pulmonary (excluding asthma), hematologic and renal (including nephrotic syndrome) condition, chronic liver diseases and diabetes mellitus
- Congenital or acquired immunodeficiency [including human immunodeficiency virus (HIV) infection]
- Children on long-term salicylates therapy
- Laboratory personnel and healthcare workers.

13. What is "Target group prioritization"?

Indian academy of pediatrics recommends vaccination to prevent mortality and morbidity in vulnerable high-risk group. Prioritization is based on: contribution of risk group to the overall influenza disease burden in population, disease severity within individual risk group, and vaccine effectiveness in different age groups and categories.

Prioritization of target groups (1-highest priority, 4-lowest priority):

1. Elderly individuals (>65 years) and nursing home residents (the elderly or disabled)
2. Individuals with chronic medical conditions including individuals with HIV/acquired immunodeficiency syndrome (AIDS) and pregnant women (especially to protect infants 0–6 months)
3. Other groups: healthcare workers including professionals, individuals with asthma and children from ages 6 months to 2 years
4. Children aged 2–5 years and 6–18 years, and healthy young adults.

Amongst pediatric population, apart from the children with chronic medical conditions (see prioritization of target groups), the children below 2 years of age should be considered a target group for influenza immunization because of a high burden of severe disease in this group.

14. What is the ideal time for influenza vaccination in India?

The best time for offering influenza vaccine is just before the rainy season. The data on seasonality of influenza in India suggests the need for staggered approach in vaccination timing. Therefore, for individual residing in southern states, it would be before October while for the rest of the country, it should be June. Although, India lies within the Northern Hemisphere, parts of the country has distinct tropical environment being located closer to the equator and behaves much like Southern Hemisphere seasonality with almost year round circulation and monsoon months. Hence, IAP recommends that it would not be prudent to stick to strain formulations recommended for one hemisphere, but one should use the vaccine that has the most recent strains irrespective of the hemisphere-specific formulation.

15. What is the protocol for administering influenza vaccines?

Trivalent inactivated vaccine is administered by the IM route (usually deltoid) to most children and adults. For infants and young children, the anterolateral aspect of the thigh is a preferred site. The dose is 0.25 mL for infants 6–35 months of age and 0.50 mL for other children and adults. Two doses are administered 4 weeks apart. Each year, a single dose of the latest recommended vaccine has to be administered. TIV should be stored at 2–8°C; and should not be frozen. Vaccine prepared for a previous influenza season should not be administered to provide protection for any subsequent season.

Live attenuated influenza vaccine is designed for intranasal administration only and cannot be given by any other route. Half the content of a pre-filled syringe is sprayed into each nostril with the child positioned upright. The sprayer (device) has a dose-divider clip which ensures that more than half the dose cannot be inadvertently sprayed into any nostril. The vaccine is stored at 2–8°C.

16. What is the place of monovalent vaccine?

Monovalent vaccine has influenza A(H1N1)pdm09 strain which is the most common strain circulating in India, but influenza B and other A strains are not covered by it. So it might be useful in epidemic period but in this postpandemic state TIV is recommended, as it contains viral strain which is antigenically similar to A(H1N1)pdm09 strain and children vaccinated with TIV do not need to go for separate "swine flu vaccine".

17. Who should not be given the live vaccine?

- The viruses used for the production of vaccines are grown in eggs, hence should not be used in children with severe allergy to egg protein or chicken
- Children younger than 2 years and adults more than 50 years
- Pregnant women
- Long-term health problem such as heart disease, kidney or liver disease, lung disease, metabolic disease such as diabetes, asthma, anemia and other blood disorders
- Children younger than 5 years with asthma or one or more episodes of wheezing during the past year
- Anyone with certain muscle or nerve disorders (such as cerebral palsy) that can lead to breathing or swallowing problems
- Anyone in close contact with a person with a severely weakened immune system (requiring care in a protected environment, such as a bone marrow transplant unit)
- A patient of Guillain-Barré syndrome (GBS).

18. What are the adverse effects following immunization with TIV?

Trivalent inactivated vaccine (TIV) are generally safe with reports of transient local reactions at the injection site (>1/100), fever, malaise, myalgia, and other systemic adverse events in persons without previous exposure to the influenza vaccine antigens. There are sporadic reports of increased incidence of GBS, febrile seizures in some countries following use of TIV and monovalent pandemic 2009 vaccine, but seen more if given simultaneously with pneumococcal vaccine. Children allergic to egg proteins can have severe adverse events like anaphylaxis, angioedema, allergic [immunoglobulin E (IgE) mediated] bronchial asthma and urticaria.

19. What are the problems in formulating universal recommendation for influenza vaccination in India?

There are various factors which prevent formulation of universal recommendation in India like:
- Exact burden of disease regarding morbidity and mortality is not known
- Attack rates are highest in young children but fatality more in elderly population, so target population cannot be defined well

- Limited data on effectiveness of vaccine in our country; moreover, optimum timing and optimum strain of vaccination is often not clear.

20. What drugs are available for treatment?

Antiviral treatment is recommended for suspected or confirmed influenza with severe, complicated or progressive illness or those who require hospitalization. Treatment is also recommended in outpatient with confirmed or suspected influenza if they are at risk for complication based on age (less than 2 year or more than 65 year), with underlying chronic medical condition, pregnancy and immunocompromized.

Two group of drug adamantane (amanatadine and rimantadine) and newer NA inhibitors (oseltamivir, zanamivir, peramivir) are recommended although there are reports of decreased sensitivity with adamantane group of drugs.

21. What drugs are used for prophylaxis?

Oseltamivir or zanamivir is used for prophylaxis indicated for at risk individual who may develop complication, who have been suspected or confirmed H1N1 influenza, healthcare worker who come in close unprotected contact with diagnosed case of H1N1.

22. What are the key messages?

- Influenza is a seasonal respiratory febrile viral illness which in most cases is self-limiting
- Certain high-risk groups vulnerable for complications should be identified and should be offered prevention and treatment on priority basis
- Effective trivalent and quadrivalent vaccine options are available
- There is need for a more extensive, region-specific surveillance in our country to determine optimum strains, timings and target group for vaccination.

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CHAPTER

25

Swine Influenza

Vipin M Vashishtha, CP Bansal

1. What is swine flu (novel H1N1 influenza A swine flu)?

Swine flu (swine influenza) is a respiratory disease caused by influenza viruses that infect the respiratory tract of pigs and result in nasal secretions, a barking-like cough, decreased appetite, and listless behavior. Swine flu produces most of the same symptoms in pigs as human flu produces in people. Swine flu can last about one to two weeks in pigs that survive. Swine influenza virus was first isolated from pigs in 1930 in the US and has been recognized by pork producers and veterinarians to cause infections in pigs worldwide. In a number of instances, people have developed the swine flu infection when they are closely associated with pigs (for example, farmers, pork processors), and likewise, pig populations have occasionally been infected with the human flu infection. In most instances, the cross-species infections (swine virus to man; human flu virus to pigs) have remained in local areas and have not caused national or worldwide infections in either pigs or humans. Unfortunately, this cross-species situation with influenza viruses has had the potential to change. Investigators think the 2009 swine flu strain, first seen in Mexico, should be termed novel H1N1 flu since it is mainly found infecting people and exhibits two main surface antigens, H1 (hemagglutinin type 1) and N1 (neuraminidase type 1). Recent investigations show the eight RNA strands from novel H1N1 flu have one strand derived from human flu strains, two from avian (bird) strains, and five from swine strains.

2. Why is swine flu (H1N1) now infecting humans?

Many researchers now consider that two main series of events can lead to swine flu (and also avian or bird flu) becoming a major cause for influenza illness in humans.

First, the influenza viruses (types A, B, C) are enveloped RNA viruses with a segmented genome; this means the viral RNA genetic code is not a single strand of RNA but exists as eight different RNA segments in the influenza viruses. A human (or bird) influenza virus can infect a pig respiratory cell at the same time as a swine influenza virus; some of the replicating RNA strands from the human virus can get mistakenly enclosed inside the enveloped swine influenza virus. For example, one cell could contain eight swine flu and eight human flu RNA segments. The total number of RNA types in one cell would be 16; four swine and four human flu RNA segments could be incorporated into one particle, making a viable eight RNA segmented flu virus from the 16 available segment types. Various combinations of RNA segments can result in a new subtype of virus (known as antigenic shift) that may have the ability to preferentially infect humans but still show characteristics unique to the swine influenza virus. It is even possible to include RNA strands from birds, swine, and human influenza viruses into one virus if a cell becomes infected with all three types of influenza (for example, two bird flu, three swine flu, and three human flu RNA segments to produce a viable eight-segment new type of flu viral genome). Formation of a new viral type is considered to be antigenic shift; small changes in an individual RNA segment in flu viruses are termed antigenic drift and result in minor changes in the virus. However, these can accumulate over time to produce enough minor changes that cumulatively change the virus' antigenic makeup over time (usually years).

Second, pigs can play a unique role as an intermediary host to new flu types because pig respiratory cells can be infected directly with bird, human, and other mammalian flu viruses. Consequently, pig respiratory cells are able to be infected with many types of flu and can function as a "mixing pot" for flu RNA segments. Bird flu viruses, which usually infect the gastrointestinal cells of many bird species, are shed in bird feces. Pigs can pick these viruses up from the environment and seem to be the major way that bird flu virus RNA segments enter the mammalian flu virus population.

3. How do people become infected with influenza A(H1N1)?

Outbreaks in humans are now occurring from human-to-human transmission. When infected people cough or sneeze, infected droplets get on their hands, drop onto surfaces, or are dispersed into the air. Contacts get infected by breathing contaminated air, or touching infected hands or surfaces.

4. What are the signs and symptoms of infection?

Early signs of influenza A (H1N1) are flu-like, including fever, cough, headache, muscle and joint pain, sore throat and runny nose, and sometimes vomiting or diarrhea. Like seasonal flu, swine flu may cause a worsening of underlying chronic medical conditions.

5. How long someone with the flu can infect someone else?

Infected people may be able to infect others beginning one day before symptoms develop and up to seven or more days after becoming sick.

6. What surfaces are most likely to be the sources of contamination?

Germs can be spread when a person touches something that is contaminated with germs and then touches his or her eyes, nose, or mouth. Droplets from a cough or sneeze of an infected person move through the air.

7. How long can viruses live outside the body?

We know that some viruses and bacteria can live two hours or longer on surfaces like cafeteria tables, doorknobs, and desks. Frequent hand washing will help you reduce the chance of getting contamination from these common surfaces.

8. Are there medicines to treat swine flu?

Yes. Oseltamivir is the recommended antiviral drug for the treatment and/or prevention of infection with the influenza A H1N1. If you get sick, antiviral drugs can make your illness milder and make you feel better faster. They may also prevent serious flu complications. For treatment, antiviral drugs work best if started soon after getting sick (within 2 days of symptoms). Government has adequate stock and the drug is made available for free through government hospitals at the time of outbreak. The drug is to be administered under supervision of clinicians.

9. What should I do if I get sick?

If you live in areas where influenza A H1N1 cases have been identified and become ill with influenza like symptoms, e.g. fever, body aches, runny nose, sore throat, nausea, or vomiting or diarrhea, you may contact your healthcare provider, particularly if you are worried about your symptoms. Your healthcare provider will determine whether influenza testing or treatment is needed. If you are sick, you should stay home and avoid contact with other people as much as possible to keep from spreading your illness to others. If you become ill and experience any of the following warning signs, seek emergency medical care.

In children, emergency warning signs that need urgent medical attention include:

- Fast breathing or trouble breathing
- Bluish skin color
- Not drinking enough fluids/eating food
- Not waking up or not interacting
- Being so irritable that the child does not want to be held
- Flu-like symptoms improve but then return with fever and worse cough
- Fever with a rash.

In adults, emergency warning signs that need urgent medical attention include:

- Difficulty breathing or shortness of breath
- Pain or pressure in the chest or abdomen
- Sudden dizziness
- Confusion
- Severe or persistent vomiting.

10. Can I get influenza A H1N1 from eating or preparing pork?

No. Swine influenza viruses are not spread by food. Properly handled and cooked pork products are safe.

11. What is the status of swine flu (H1N1 2009) pandemic?

Novel H1N1 2009 influenza pandemic has created widespread concern across the country. The virus continues to circulate and cause waves of infections leading to hospitalization and complication in different parts of our country even this year. There have been at least 2,241 deaths and 41,234 laboratory confirmed cases (testing is done only for severe cases hospitalized) till 5th September 2010; actual cases may be much higher as many are not tested. 1,257 confirmed cases with 128 deaths have occurred in the week ending on 5th September 2010. Since April 2010, Maharashtra, Kerala, Karnataka, Gujarat and some other cities like Kolkata have shown rise in confirmed cases due to novel H1N1 2009 influenza. This means that though WHO has declared that the world is in postpandemic phase, it does not seem to be the case in India at present.

12. What is the current WHO statement on H1N1 2009 pandemic?

In many parts of world, novel 2009 H1N1 virus transmission peak is over and WHO has declared that we are in postpandemic phase. In this regard, WHO Statement dated 10th August 2010 on pandemic influenza is relevant and reproduced below.

The world is now in the postpandemic period. Based on knowledge about past pandemics, the H1N1 (2009) virus is expected to continue to circulate as a seasonal virus for some years to come. While the level of concern is now greatly diminished, vigilance on the part of national health authorities remains important. Such vigilance is especially critical in the immediate postpandemic period, when the behavior of the H1N1 (2009) virus as a seasonal virus cannot be reliably predicted.

For example, it is likely that the virus will continue to disproportionately affect a younger age group, at least in the immediate postpandemic period. Groups identified during the pandemic as at higher risk of severe or fatal illness will probably remain at heightened risk, though the number of such cases could diminish. In addition, a small proportion of people infected during the pandemic developed a severe form of primary viral pneumonia that is not commonly seen during seasonal epidemics and is especially difficult to treat. It is not known whether this pattern will continue during the postpandemic period, further emphasizing the need for vigilance.

WHO is today issuing guidance on recommended activities during the postpandemic period, including advice on epidemiological and virological monitoring, vaccination, and the clinical management of cases. National health authorities are reminded that cases and local outbreaks of H1N1 (2009) infection will continue to occur, and in some locations, such outbreaks could have a substantial impact on communities.

13. Is the situation still alarming?

Though there is lot of awareness and anxiety amongst the people about the disease and the vaccine/s available, present situation does not warrant a panic situation. Seasonal influenza, which has in past caused morbidity and mortality all these years and for which vaccine have been available did not create such hype. Case fatality rate in pandemic influenza is not very high and it is estimated to be between 0.05 to 0.2%. Though the virus has high transmissibility it is not highly virulent. Ironically, people in high-income countries used their vaccine stockpiles reluctantly. Around 80% of people in the UK chose not to be vaccinated, many because they doubted they were at serious risk. However this is a perception and not a scientific fact.

14. What are the different vaccines against swine flu available in India?

Various monovalent novel H1N1 vaccines were made available in the world and India and these vaccines have been used in the developed world since last 9 months. These vaccines are safe and as such should not be denied to any one demanding it. One can consider the local epidemiology of the H1N1 2009 and use it on the patient after one to one discussion and informed consent. Now trivalent inactivated influenza vaccines are available in India (and world of course) and one live trivalent vaccine in USA. These vaccines cover Novel H1N1 component and seasonal flu strains recommended for year 10–11 season.

Monovalent provide protection only against H1N1 2009 Strain while the Trivalent provides protection against three strains which are commonly notorious in SE Region and the mortality and morbidity is associated with these strains as well, hence the protection by Trivalent certainly is much wider.

The 2010–2011 vaccines provide protection against A/H1N1 (pandemic-2009 California Strain) influenza and two other influenza viruses—influenza A/H3N2 and influenza B. It will not prevent illness caused by other viruses. It takes up to 2 weeks for protection to develop after the shot. Protection lasts about a year. A "high-dose" inactivated influenza vaccine is available for people 65 years of age and older.

Dose and Route of Administration

- *Live vaccine*: 0.25 mL of the reconstituted vaccine in each nostril with the syringe and adaptor provided with the vaccine vail.
- *Inactivated vaccine*: 0.5 mL intramuscular (0.25 mL for a child between 6 months and 3 years).
- *Number of doses*: One dose above 9 years.
  (2 doses 28 days apart in less than 9 years old who is vaccinated for the 1st time).

Seasonal Influenza 2010 and Novel H1N1 Influenza Vaccines Available in India

- A live monovalent novel H1N1 vaccine (Serum Institute of India). Immunogenicity, and safety of the vaccine has been established in pre-licensure animal and human studies.

- The inactivated novel H1N1 monovalent vaccine of Zydus is licensed for use only in adults above the age of 18 years.
- Trivalent inactivated vaccine containing the novel H1N1 strain are available now (Chiron, Solvay, Lupin, GSK and Sanofi pasteur). The 2010-11 trivalent vaccines contain A/California/7/2009 (H1N1)-like, A/Perth/16/2009 (H3N2)-like, and B/Brisbane/60/2008-like antigens. The influenza A (H1N1) vaccine virus is derived from a 2009 pandemic influenza A (H1N1) virus.

15. Who should be administered the H1N1 vaccine?

The following high risk individuals should be vaccinated against novel H1N1(ACIP recommendations):

When vaccine supply is limited, vaccination efforts should focus on delivering vaccination to persons who:

a. are aged 6 months–4 years (59 months)
b. are aged 50 years and older
c. have chronic pulmonary (including asthma), cardiovascular (except hypertension), renal, hepatic, neurologic, hematologic, or metabolic disorders (including diabetes mellitus)
d. are immunosuppressed (including immunosuppression caused by medications or by human immunodeficiency virus)
e. are or will be pregnant during the influenza season
f. are aged 6 months–18 years and receiving long-term aspirin therapy and who therefore might be at risk for experiencing Reye syndrome after influenza virus infection
g. are residents of nursing homes and other chronic-care facilities; are morbidly obese (body-mass index is 40 or greater)
h. are healthcare personnel; are household contacts and caregivers of children aged younger than 5 years and adults aged 50 years and older, with particular emphasis on vaccinating contacts of children aged younger than 6 months; and are household contacts and caregivers of persons with medical conditions that put them at higher risk for severe complications from influenza.

16. What are the recommendations of Indian Academy of Pediatrics (IAP) on seasonal influenza vaccination?

The novel 2009 H1N1 infection, hospitalization and mortality have been disproportionately high in healthy young children and adults (unlike the past seasonal influenza outbreaks). Hence IAP recommends to offer 2010 trivalent vaccine (inactivated or live) to high risk children as given below and any one who desires it (live influenza vaccines are contraindicated in these group of individuals).

At risk individuals include:

- Congenital or acquired immunodeficiency
- Chronic cardiac, pulmonary, hematologic, renal, liver disease and diabetes mellitus

- Children on long-term aspirin therapy
- Any neurologic disease that might cause respiratory compromise or impair ability to handle secretions
- Asthma requiring oral steroids
- Elderly aged more than 65 years.

17. Who should not be given the live vaccine?

2009 H1N1 LAIV should not be given to the following groups:
- Children younger than 3 years and adults 50 years and older
- Pregnant women
- Anyone with a weakened immune system
- Anyone with a long-term health problem such as heart disease – kidney or liver disease – lung disease – metabolic disease such as diabetes – asthma – anemia and other blood disorders
- Children younger than 5 years with asthma or one or more episodes of wheezing during the past year
- Anyone with certain muscle or nerve disorders (such as cerebral palsy) that can lead to breathing or swallowing problems
- Anyone in close contact with a person with a severely weakened immune system (requiring care in a protected environment, such as a bone marrow transplant unit)
- Children or adolescents on long-term aspirin treatment. Individuals who are moderately or severely ill (might be advised to wait until complete recovery from the illness)
- Guillain-Barré syndrome H1N1 LAIV and seasonal LAIV should not be given together.

18. Who should not be vaccinated with TIV?

Those younger than 6 months; those who have a moderate to severe febrile illness; those who have a history of hypersensitivity, including anaphylaxis, to eggs, to any previous influenza vaccine dose, or to any of the vaccine components; and those who are known to have experienced Guillain-Barre´ syndrome (GBS) within 6 weeks after a previous influenza vaccination (whether influenza vaccination specifically might increase the risk for recurrence of GBS is unknown).

19. How many doses need to be given?

In previously unvaccinated persons aged <9 years, 2 doses of seasonal influenza vaccine are required to induce immunity because young children typically have had limited exposure to influenza viruses and are not immunologically primed (i.e., they do not have preexisting antibodies). Even those children (<9 years) who have already received two doses of seasonal flu vaccine in preceding year should be given two doses of current vaccine. The lack of preexisting antibody cross-reactive with the novel influenza A (H1N1) virus among children and younger adults raises the possibility that 2 doses of vaccine (typically separated by >21 days) also will be needed to provide protection for persons in these age groups. Ongoing studies will provide additional information about the immune response vaccine, including which groups might need 2 doses.

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CHAPTER

26

Avian Influenza

Vipin M Vashishtha

1. What is avian influenza?

Avian influenza, or "bird flu", is a contagious disease of animals caused by viruses that normally infect only birds and, less commonly, pigs. Avian influenza viruses are highly species-specific, but have, on rare occasions, crossed the species barrier to infect humans.

In domestic poultry, infection with avian influenza viruses causes two main forms of disease, distinguished by low and high extremes of virulence. The so-called "low pathogenic" form commonly causes only mild symptoms (ruffled feathers, a drop in egg production) and may easily go undetected. The highly pathogenic form is far more dramatic. It spreads very rapidly through poultry flocks, causes disease affecting multiple internal organs, and has a mortality that can approach 100%, often within 48 hours.

2. Which viruses cause highly pathogenic disease?

Influenza A viruses have 16 H subtypes and 9 N subtypes. Only viruses of the H5 and H7 subtypes are known to cause the highly pathogenic form of the disease. However, not all viruses of the H5 and H7 subtypes are highly pathogenic and not all will cause severe disease in poultry.

H5 and H7 viruses are introduced to poultry flocks in their low pathogenic form, when allowed to circulate in poultry populations, the viruses can mutate, usually within a few months, into the highly pathogenic form. This is why the presence of an H5 or H7 virus in poultry is always cause for concern, even when the initial signs of infection are mild.

3. Do migratory birds spread highly pathogenic avian influenza viruses?

The role of migratory birds in the spread of highly pathogenic avian influenza is not fully understood. Wild waterfowl are considered the natural reservoir of all influenza A viruses. They have probably carried influenza viruses, with no apparent harm, for centuries. They are known to carry viruses of the H5 and H7 subtypes, but usually in the low pathogenic form. Considerable circumstantial evidence suggests that migratory birds can introduce low pathogenic H5 and H7 viruses to poultry flocks, which then mutate to the highly pathogenic form.

In the past, highly pathogenic viruses have been isolated from migratory birds on very rare occasions involving a few birds, usually found dead within the flight range of a poultry outbreak. These observations suggested that wild waterfowl are not agents for the onward transmission of these viruses.

Recent events make it likely that some migratory birds are now directly spreading the H5N1 virus in its highly pathogenic form.

4. What is special about the recent outbreaks in poultry?

The recent outbreaks of highly pathogenic avian influenza, which began in South-East Asia in mid-2003, are the largest and most severe on record. Never before in the history of this disease have so many countries been simultaneously affected, resulting in the loss of so many birds.

The causative agent, the H5N1 virus, has proved to be especially tenacious. Despite the death or destruction of an estimated 150 million birds, the virus is now considered endemic in many parts of Indonesia and Vietnam and in some parts of Cambodia, China, Thailand, and possibly also the Lao People's Democratic Republic. Control of the disease in poultry is expected to take several years. The H5N1 virus is also of particular concern for human health.

5. What are the implications of avain influenza for human health?

The widespread persistence of H5N1 in poultry populations poses two main risks for human health.

The first is the risk of direct infection when the virus passes from poultry to humans, resulting in very severe disease. Of the few avian influenza viruses that have crossed the species barrier to infect humans, H5N1 has caused the largest number of cases of severe disease and death in humans. Unlike normal seasonal influenza, where infection causes only mild respiratory symptoms in most people, the disease caused by H5N1 follows an unusually aggressive clinical course, with rapid deterioration and high fatality. Primary viral pneumonia and multiorgan failure are common. In the present outbreak, more than half of those infected with the virus have died. Most cases have occurred in previously healthy children and young adults.

A second risk, of even greater concern, is that the virus—if given enough opportunities—will change into a form that is highly infectious for humans and spreads easily from person to person. Such a change could mark the start of a global outbreak (a pandemic).

6. Where have human cases occurred?

In the last outbreak, laboratory-confirmed human cases have been reported in four countries: Cambodia, Indonesia, Thailand, and Vietnam.

Hong Kong has experienced two outbreaks in the past. In 1997, in the first recorded instance of human infection with H5N1, the virus infected 18 people and killed 6 of them. In early 2003, the virus caused two infections, with one death, in a Hong Kong family with a recent travel history to Southern China.

7. How do people become infected?

Direct contact with infected poultry, or surfaces and objects contaminated by their feces, is presently considered the main route of human infection. To date, most human cases have occurred in rural or peri-urban areas where many households keep small poultry flocks, which often roam freely, sometimes entering homes or sharing outdoor areas where children play. As infected birds shed large quantities of virus in their feces, opportunities for exposure to infected droppings or to environments contaminated by the virus are abundant under such conditions. Moreover, because many households in Asia depend on poultry for income and food, many families sell or slaughter and consume birds when signs of illness appear in a flock, and this practice has proved difficult to change. Exposure is considered most likely during slaughter, defeathering, butchering, and preparation of poultry for cooking.

8. What changes are needed for H5N1 or another avian influenza virus to cause a pandemic?

Three conditions must be met for a pandemic to start:

- A new influenza virus subtype must emerge for which there is little or no human immunity
- It must infect humans and causes illness; and
- It must spread easily and sustainably (continue without interruption) among humans.

The H5N1 virus in Asia and Europe meets the first two conditions: it is a new virus for humans (H5N1 viruses have never circulated widely among people), and it has infected more than 190 humans, killing over half of them.

However, the third condition, the establishment of efficient and sustained human-to-human transmission of the virus, has not occurred. For this to take place, the H5N1 virus would need to improve its transmissibility among humans. This could occur either by "reassortment" or adaptive mutation.

Reassortment occurs when genetic material is exchanged between human and avian viruses during coinfection (infection with both viruses at the same time) of a human or another mammal. The result could be a fully transmissible pandemic virus — that is, a virus that can spread easily and directly between humans. A more gradual process is adaptive mutation, where the capability of a virus to bind to human cells increases during infections of humans.

9. What drugs are available for treatment?

Two drugs (in the neuraminidase inhibitors class), oseltamivir (commercially known as Tamiflu) and zanamivir (commercially known as Relenza) can reduce the severity and duration of illness caused by seasonal influenza. The efficacy of the neuraminidase inhibitors depends, among others, on their early administration (within 48 hours after symptom onset). For cases of human infection with H5N1, the drugs may improve prospects of survival, if administered early, but clinical data are limited. The H5N1 virus is expected to be susceptible to the neuraminidase inhibitors. Antiviral resistance to neuraminidase inhibitors has been clinically negligible so far but is likely to be detected during widespread use during a pandemic.

An older class of antiviral drugs, the M2 inhibitors amantadine and rimantadine, could potentially be used against pandemic influenza, but resistance to these drugs can develop rapidly and this could significantly limit their effectiveness against pandemic influenza. Some currently circulating H5N1 strains are fully resistant to these the M2 inhibitors. However, should a new virus emerge through reassortment, the M2 inhibitors might be effective.

10. What are the constraints of using neuraminidase inhibitors at mass level? Will antibiotics be needed during pandemic influenza?

For the neuraminidase inhibitors, the main constraints — which are substantial — involve limited production capacity and a price that is prohibitively high for many countries. At present manufacturing capacity, which has recently quadrupled, it will take a decade to produce enough oseltamivir to treat 20% of the world's population. The manufacturing process for oseltamivir is complex and time-consuming, and is not easily transferred to other facilities.

So far, most fatal pneumonia seen in cases of H5N1 infection has resulted from the effects of the virus, and cannot be treated with antibiotics. Nonetheless, since influenza is often complicated by secondary bacterial infection of the lungs, antibiotics could be life-saving in the case of late-onset pneumonia. WHO regards it as prudent for countries to ensure adequate supplies of antibiotics in advance.

11. What is the status of vaccines against pandemic influenza?

Vaccination is a vital part of the strategy to mitigate morbidity and mortality caused by influenza pandemics and is integral to the WHO global influenza preparedness plan. Pandemic vaccines are produced as soon as a pandemic is declared using the specific pandemic viral strain. However, these vaccines will only be available several months after the onset of the pandemic due to the length of time required for their manufacture. The efficacy of prepandemic vaccines, which are produced in advance of a pandemic, relies on the vaccine's ability to provide a breadth of protection against different, related strains, as it is not possible to predict exactly the strain that will cause such an outbreak in advance due to the progressive accumulation of antigenic changes.

12. Is there a vaccine to protect people from some strains of the H5N1 virus?

Vaccines have been formulated against several of the avian H5N1 influenza varieties. Vaccination of poultry against the ongoing H5N1 epizootic is widespread in certain countries. Some vaccines also exist for use in humans, and others are in testing, but none have been made available to civilian populations, nor produced in quantities sufficient to protect more than a tiny fraction of the Earth's population in the event of an H5N1 pandemic.

Three H5N1 vaccines for humans have been licensed:

- Sanofi Pasteur's vaccine approved by the United States in April 2007
- GlaxoSmithKline's vaccine Pandemrix approved by the European Union in May 2008
- CSL Limited's vaccine approved by Australia in June 2008.

All are produced in eggs and would require many months to be altered to a pandemic version.

13. Which is the first vaccine approved by US FDA against pandemic H5N1?

On April 17, 2007, the US Food and Drug Administration (FDA) announced its approval of the first vaccine to prevent human infection with one strain of the avian influenza (bird flu) H5N1 virus. The vaccine, produced by Sanofi Pasteur Inc., has been purchased by the federal government for the US Strategic National Stockpile; it will be distributed by public-health officials if needed. This vaccine will not be made commercially available to the general public. Other H5N1 vaccines are being developed by other companies against different H5N1 strains.

A second adjuvanted H5N1 vaccine was approved for prepandemic use in the European Union in 2008.

Production of vaccine has been hampered by manufacturing difficulties and modest immunogenicity in humans. High priority research goals include improving production speed and increasing quantity of vaccine. Areas of research development include use of cell culture systems, dose-sparing approaches (e.g., whole virion formulation, intradermal administration) and use of adjuvants and live attenuated viruses to get better antigenic responses.

14. What are "prepandemic vaccines"?

H5N1 continually mutates, meaning vaccines based on current samples of avian H5N1 cannot be depended upon to work in the case of a future pandemic of H5N1. While there can be some cross-protection against related flu strains, the best protection would be from a vaccine specifically produced for any future pandemic flu virus strain. "Prepandemic vaccines" have been created; are being refined and tested; and do have some promise both in furthering research and preparedness for the next pandemic. Vaccine manufacturing companies are being encouraged to increase capacity so that if a pandemic vaccine is needed, facilities will be available for rapid production of large amounts of a vaccine specific to a new pandemic strain.

15. What are the problems with H5N1 vaccine production?

Problems with H5N1 vaccine production include:

- Lack of overall production capacity
- Lack of surge production capacity (it is impractical to develop a system that depends on hundreds of millions of 11-day old specialized eggs on a standby basis)
- The pandemic H5N1 might be lethal to chickens.

Cell culture (cell-based) manufacturing technology can be applied to influenza vaccines as they are with most viral vaccines and thereby solve the problems associated with creating flu vaccines using chicken eggs as is currently done.

Currently, influenza vaccine for the annual, seasonal influenza program comes from four manufacturers. However, only a single manufacturer produces the annual vaccine entirely within the US. Thus, if a pandemic occurred and existing US-based influenza vaccine manufacturing capacity was completely diverted to producing a pandemic vaccine, supply would be severely limited. Moreover, because the annual influenza manufacturing process takes place during most of the year, the time and capacity to produce vaccine against potential pandemic viruses for a stockpile, while continuing annual influenza vaccine production, is limited. The US government has purchased from Sanofi Pasteur and Chiron Corporation several million doses of vaccine meant to be used in case of an influenza pandemic of H5N1 avian influenza and is conducting clinical trials with these vaccines. Researchers at the University of Pittsburgh have had success with a genetically engineered vaccine that took only a month to make and completely protected chickens from the highly pathogenic H5N1 virus.

16. What is the benefit of the FDA-approved H5N1 vaccine produced by Sanofi Pasteur Inc?

The H5N1 vaccine approved by the US Food and Drug Administration (FDA) on April 17 2007, was developed as a safeguard against the possible emergence of an H5N1 pandemic virus. However, the H5N1 virus is not a pandemic virus because it does not transmit efficiently from person to person, so the H5N1 vaccine is being held in stockpiles rather than being used by the general public. This vaccine aids H5N1 preparedness efforts in case an H5N1 pandemic virus were to emerge.

17. What is a universal flu vaccine?

Many groups worldwide are working on a universal flu vaccine that will not need changing each year, as the sector has been viewed as “increasingly hot”. Companies pursuing the vaccine as of 2009 and 2010 include Theraclone, Dynavax Technologies Corporation, VaxInnate, Crucell NV, and Inovio Pharmaceuticals. In 2010, the National Institute of Allergy and Infectious Diseases (NIAID) of the

US NIH announced a breakthrough; the effort targets the stem, which mutates less often than the head of the virus. In 2009, the Wistar Institute received a patent for using "a variety of peptites" in a flu vaccine, and announced it was seeking a corporate partner.

Some universal flu vaccines have started early stage clinical trials. It may be possible to target the less variable stalk of the hemagglutinin molecule as done by BiondVax with Multimeric-001. This is aimed at Type A (inc H1N1) and Type B influenza and has started a phase IIa study. Dynavax have developed a vaccine N8295 based on two highly conserved antigens NP and M2e and their TLR9 agonist, and started clinical trials in June 2010. In 2008 Acambis announced work on a universal flu vaccine (ACAM-FLU-ATM) based on the less variable M2 protein component of the flu virus shell.

DNA vaccines such as VGX-3400X (aimed at multiple H5N1 strains) contain DNA fragments (plasmids). Inovios SynCon DNA vaccines include H5N1 and H1N1 subtypes. Other vaccines are polypeptide based.

Based on the results of animal studies, a universal flu vaccine may use a two-step vaccination strategy — priming with a DNA-based HA vaccine followed by a second dose with an inactivated, attenuated, or adenovirus-vector–based vaccine.

18. What are the newer developments in the field of cell-based influenza vaccines?

In addition to supporting basic research on cell-based influenza vaccine development, United States Department of Health and Human Services (HHS) is currently supporting a number of vaccine manufacturers in the advanced development of cell-based influenza vaccines with the goal of developing licensed cell-based influenza vaccines produced in the United States.

Dose-sparing technologies: Current US licensed vaccines stimulate an immune response based on the quantity of HA (hemagglutinin) antigen included in the dose. Methods to stimulate a strong immune response using less HA antigen are being studied in H5N1 and H9N2 vaccine trials. These include changing the mode of delivery from intramuscular to intradermal and the addition of immune-enhancing adjuvant to the vaccine formulation. Additionally, HHS is soliciting contract proposals from manufacturers of vaccines, adjuvants, and medical devices for the development and licensure of influenza vaccines that will provide dose-sparing alternative strategies.

Chiron Corporation is now recertified and under contract with the National Institutes of Health to produce 8,000 to 10,000 investigational doses of Avian Flu (H5N1) vaccine. MedImmune and Aventis Pasteur are under similar contracts. The United States government hopes to obtain enough vaccine in 2006 to treat 4 million people. However, it is unclear whether this vaccine would be effective against a hypothetical mutated strain that would be easily transmitted through human populations, and the shelf life of stockpiled doses has yet to be determined.

19. What are different H5N1 'clades'? What is their significance to vaccine production?

Clades denote genetic groups based on their prevalence in different geographic regions. On August 18 2006, the WHO changed the H5N1 strains recommended for candidate vaccines for the first time since 2004. The WHO's new prototype strains, prepared by reverse genetics, include three new H5N1 subclades. The hemagglutinin sequences of most of the H5N1 avian influenza viruses circulating in the past few years fall into two genetic groups, or clades:

Clade 1 includes human and bird isolates from Vietnam, Thailand, and Cambodia and bird isolates from Laos and Malaysia.

Clade 2 viruses were first identified in bird isolates from China, Indonesia, Japan, and South Korea before spreading westward to the Middle East, Europe, and Africa.

The clade 2 viruses have been primarily responsible for human H5N1 infections that have occurred during late 2005 and 2006, according to WHO. Genetic analysis has identified six subclades of clade 2, three of which have a distinct geographic distribution and have been implicated in human infections:

- Subclade 1, Indonesia
- Subclade 2, Middle East, Europe, and Africa
- Subclade 3, China.

On the basis of the three subclades, the WHO is offering companies and other groups that are interested in pandemic vaccine development these three new prototype strains:

- An A/Indonesia/2/2005-like virus
- An A/Bar headed goose/Quinghai/1A/2005-like virus
- An A/Anhui/1/2005-like virus.

Until now, researchers have been working on prepandemic vaccines for H5N1 viruses in clade 1. In March, the first clinical trial of a US vaccine for H5N1 showed modest results. In May, French researchers showed somewhat better results in a clinical trial of an H5N1 vaccine that included an adjuvant. Vaccine experts are not sure if a vaccine effective against known H5N1 viral strains would be effective against future strains. Although, the new viruses will now be available for vaccine research, WHO said clinical trials using the clade 1 viruses should continue as an essential step in pandemic preparedness, because the trials yield useful information on priming, cross-reactivity, and cross-protection by vaccine viruses from different clades and subclades.

20. Can a pandemic be prevented?

No one knows with certainty. The best way to prevent a pandemic would be to eliminate the virus from birds, but it has become increasingly doubtful if this can be achieved within the near future.

Following a donation by industry, WHO will have a stockpile of antiviral medications, sufficient for 3 million treatment courses, by early 2006. Recent studies, based on mathematical modeling, suggest that these drugs could be used prophylactically near the start of a pandemic to reduce the risk that a fully transmissible virus will emerge or atleast to delay its international spread, thus gaining time to augment vaccine supplies.

The success of this strategy, which has never been tested, depends on several assumptions about the early behavior of a pandemic virus, which cannot be known in advance. Success also depends on excellent surveillance and logistics capacity in the initially affected areas, combined with an ability to enforce movement restrictions in and out of the affected area. To increase the likelihood that early intervention using the WHO rapid-intervention stockpile of antiviral drugs will be successful, surveillance in affected countries needs to improve, particularly concerning the capacity to detect clusters of cases closely related in time and place.

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CHAPTER

27

Meningococcal Vaccines

Parang N Mehta

1. What are meningococci?

Meningococci, or *Neisseria meningitidis*, are Gram-negative cocci. They are associated with some very serious illnesses. Chief among them are meningitis, purpura fulminans, shock, and sepsis. Thirty percent or more of purulent meningitis in children is caused by meningococci. Meningococci also cause focal disease like arthritis and pneumonia.

An important feature of meningococci is the presence of a polysaccharide capsule. This capsule protects them against destruction by the host's complement system, as well as from phagocytosis.

Meningococcal disease is endemic in most parts of our country, but occurs in deadly epidemics also. The disease is most common in infants 3–12 months of age. Case fatality rates are 9–12% with invasive meningococcal disease, but can be as high as 30% with purpura fulminans. Meningitis also has a high mortality, and survivors have a significant incidence of sequelae.

2. Are there different types of meningococci?

Yes, there are thirteen known serotypes of meningococci. Of them, only five commonly cause human disease (types A, B, C, W-135, and Y). In India, meningococcal disease is endemic, and most of it is caused by type B. Meningococcal disease also occurs in epidemics sometimes, and these are usually type A.

Type B is the problem type all over the world. It causes about 50% of meningococcal cases, and effective vaccines against it are not yet readily available. Not only are the strains variable antigenically, but there is molecular similarity between some of them and human nervous system components.

3. Are effective vaccines available?

An unconjugated polysaccharide vaccine has been available for many years. Like pneumococci, meningococci also have a capsular polysaccharide, which makes this vaccine effective only in children above the age of 2 years. This vaccine, the unconjugated meningococcal polysaccharide vaccine (MPSV-4), covers four types of meningococci (A, C, W-135 and Y) types. These vaccines are available in lyophilized form, to be kept at 2–8°C and reconstituted with water just before use. Administration is subcutaneous.

These vaccines induce antibodies to the included serotypes in 7–10 days. Serotype A can sometimes induce antibodies in infants as young as 3 months, but type C is poorly immunogenic below 2 years. All four serotype vaccines are safe and immunogenic. Protection rates of 85–90% have been shown with types A and C. In young children, antibody levels decrease after 3 years.

These vaccines are T-cell independent, which means they are ineffective in children younger than 2 years. Antibodies induced are only immunoglobulin M (IgM) type, and levels fall after some years. Another problem with these vaccines is that they do not induce immunological memory. There is no booster effect of another dose. Where the conjugated vaccine is available, MPSV-4 is not recommended for general use.

4. Is there a better vaccine?

Conjugated meningococcal vaccines have recently become available. Meningococcal antigens are conjugated with proteins like the CRM 197 or tetanus toxoid, which induces a T-cell response.

Such vaccines are effective in younger children also, and are approved for use from 9 months onwards. Considering the high incidence of meningococcal disease in infants, this vaccine can do much good. Conjugate vaccines are more immunogenic than unconjugated polysaccharide vaccines, and are the recommended choice. About 98% of recipients achieve protective antibody titers with conjugate vaccines. Conjugate vaccines are also expected to provide herd effect, and reduce nasopharyngeal carriage of meningococci. The conjugated vaccines also provide protection against four types of meningococci: types A, C, W-135, and Y. There is still no conjugate vaccine against type B, which is the prevalent type in India.

5. What are the side-effects with these vaccines?

Most adverse reactions are mild and short lived. Local reactions consist of pain, redness and swelling, and are rarely severe. Fever, headache and malaise occur in a small proportion of recipients in the first few days. Only symptomatic treatment is required.

6. Are there any serious side-effects?

Some cases of Guillian-Barré syndrome (GBS) have been reported after the use of meningococcal conjugate vaccine. The incidence is low, and it is difficult to say whether it is higher than the background incidence of this rare disease.

7. Are there any contraindications to vaccination?

Children who have had a severe allergic (anaphylactic) reaction to the vaccine, or a component of it, in the past, must not receive it again. In children with moderate or severe acute illness, the vaccine should be deferred. Breast feeding and immunosuppression are not contraindications.

8. Is meningococcal vaccine recommended in India?

As usual, we have very little data on the incidence and prevalence of disease. We do know that most endemic cases are caused by type B meningococci, against which a vaccine is not yet available.

Though the conjugated meningococcal vaccine is now available, IAP recommends this vaccine only for:

- High risk group of children
- During disease outbreaks.
- International travelers, including people going for Haj, and students going abroad for studies. Travelers to certain African countries where meningococcal meningitis is endemic must also be vaccinated.

At present the meningococcal vaccine is not recommended for general use in children in India.

9. Who are considered children at risk?

- Children with asplenia, functional or anatomic.
- Children with congenital or acquired immunodeficiencies, particularly children with terminal complement component deficiencies.
- Children with cerebrospinal fluid (CSF) leaks, cochlear implants, or malignancies.
- Children on long term steroid, salicylate, immunosuppressive, or radiation therapy.

It is also recommended for:

- Together with chemoprophylaxis in close contacts of patients with meningococcal disease (Household contacts, school and daycare contacts, healthcare workers).
- Laboratory personnel and healthcare workers who are exposed routinely to *N. meningitidis*.

10. Why should chemoprophylaxis and vaccine both be given to contacts of cases?

The vaccine is not immediately protective. Development of an antibody response takes 7–10 days, and the person is at risk of developing a dangerous disease during this period. Contacts should therefore be given both chemoprophylaxis and the vaccine. Chemoprophylaxis alone is an acceptable solution, but vaccination alone is not.

11. Are meningococcal vaccines effective in preventing or controlling epidemics?

Many factors need to be considered in answering this question. The type of the organism, the age group mainly involved, and the population density all have a bearing.

If adults and older children are mainly affected, then mass vaccination is likely to yield some benefit. Among infants, vaccination may yield some benefit if the organism is Type A. Against serotypes C, W-135, and Y, the currently available vaccine is ineffective before age 2 years. No vaccine is useful against meningococci type B.

12. If vaccine has been given once, is revaccination needed?

Yes. Immunity induced by both polysaccharide and conjugated meningococcal vaccines wanes over time. Revaccination is recommended 3–5 years after the primary dose. Vaccination is not recommended after the age of 21 years.

CHAPTER

28

Cholera

Nupur Ganguly

1. What is cholera?

Cholera is an acute gastroenteritis caused by *Vibrio cholerae* infection leading to profuse watery diarrhea. Before the development of rehydration therapy with oral and intravenous fluid, the case fatality rate exceeded to more than 40%. Cholera remains an important cause of death in developing countries, especially Asia, Africa and South and Central America. The disease is also a risk to travelers to these areas of the world. *V. cholerae* spreads by feco-oral route. Transmission occurs through consumption of contaminated food and water. If contaminated food is kept at room temperature *V. cholerae* will multiply and can give rise to an outbreak. The environmental water becomes contaminated with human feces during epidemic and contamination further spreads the epidemic. If sanitation is adequate, secondary cases do not occur. In the environment, the vibrios also survive in association with certain plankton chitinous and shelled animals (e.g. crabs, prawns, shrimps and other shellfish) many of which are eaten partially cooked. This remains an important cause of cholera in developed countries. Severe cholera is characterized by acute diarrhea, passing rice watery stools and vomiting, giving rise to moderate to profound dehydration within 4–18 hours. However, not all patients with cholera will have severe diarrhea and may remain asymptomatic or have only mild diarrhea. The case-to-infection ratio (number of symptomatic cases per asymptomatic people infected) ranges from 1:3 to 1:100 depending upon the geographical region, biotype, phase of epidemic and size of inoculums. In epidemic situation, the case to infection ratio increases. Establishment of adequate personal hygiene, food safety and sanitation along with vaccination can bring down the incidence of asymptomatic cases and control this life-threatening disease. Vaccination against cholera was first tested in the 19th century and played a role in controlling epidemics. Injected (parenteral) whole-cell vaccines were used in the 1960s and 1970s, but they went out of favor as their efficacy was thought to be low and short-lived, high titers of serum vibriocidal antibodies were thought not to provide sufficient intestinal immunity to prevent infection, and they were said to have a high rate of adverse effects. Cholera epidemics have shown that there is a requirement for an effective vaccine against this major disease. Oral vaccines have been under development since the 1980s, understanding the importance of stimulating local intestinal immunity in the prevention of the disease. Both killed and live oral vaccines are now licensed, but the injected vaccine is now superseded with oral vaccines and is withdrawn.

2. What is the disease burden of cholera in India?

There is a lack of proper data of disease burden in India due to under reporting. Cholera occurs over a wider geographic area in India. National Institute of Cholera and Enteric Diseases (NICED), Kolkata, West Bengal, India, is a World Health Organization (WHO) Collaborating Center for Diarrheal Diseases Research and Training. It receives about more than 1,500 strains of *V. cholerae* every year from about 30–40 institutions from India and a few from outside the country for biotyping, serotyping and phage typing. In this center, one of the largest studies (18 year study) was conducted from 1990 to 2007. During this period, 24 of the 35 states and union territories in India had sent strains at least once. Andhra Pradesh, Delhi, Goa, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu and West Bengal had sent strains for 3 consecutive years. Highest number of strains were received from Maharashtra, followed by West Bengal. No strains were submitted from Puducherry during this period. From 2004 onward, strains were also received from Kerala and Sikkim. Of the total strains received, 96.5% strains were serotyped as Ogawa and the remaining 3.5% were Inaba. From 1997 to 2006, there were 68 outbreaks in 18 states, and 222,038 cases were detected overall in 7 out of the 10 years. This figure is about six times higher than the number reported to WHO (37,783) over the same period. The states of Orissa, West Bengal, Andaman and Nicobar Islands, Assam and Chhattisgarh accounted for 91% of all outbreak-related cases as per the analysis report of NICED. It shows there is considerable disease burden throughout the country, and the annual number of cholera cases reported to WHO by the government was several times lower than the numbers obtained through strains received at the phage typing unit. This may be due to lack of surveillance as well as proper laboratory.

3. In which countries cholera infection is prevalent?

In 2001, 58 countries officially notified WHO of a total of 184,311 cases and 2,728 deaths, but due to considerable underreporting, the true global figures are estimated as closer to 1 million cases. Estimates of global cholera-specific mortality are believed to be 100,000–130,000 deaths per year, with most of the deaths occurring in Asia and Africa. Case fatality rates (CFRs) vary greatly from country to country. For example, very low CFRs were recorded in South Africa (0.22%) whereas rates up to 30% have been observed in other parts of Africa. Sporadic cases occur along the US, Gulf coast and are due to undercooked shellfish especially crabs.

4. What are the various types of causative organisms of cholera?

There are over 200 distinct serological groups of *V. cholerae*, classified on the basis of the "O" antigen present on the cell surface, of which only two are known to cause epidemics serogroups O1 and O139. *V. cholerae* O1 can be further classified into two biotypes; classical and El Tor, each of which can be divided into three serotypes: Ogawa, Inaba and Hikojima. The epidemic strains currently in circulation worldwide are the El Tor biotype of *V. cholerae* O1, which was first recognized in Indonesia in 1961 and has now spread to many other countries in Asia, Europe, Africa and Latin America; and the Bengal strain of *V. cholerae* O139 which began in 1992 in India and Bangladesh, remains restricted to Asia. The classical biotype of *V. cholerae* O1 is also known to cause epidemics, though these are now uncommon, and non-O1/non-O139 strains occasionally cause sporadic cases of gastroenteritis.

5. How is the protective immunity in cholera mediated? What type of vaccine will be helpful—oral or parenteral?

The main virulence factor for cholera is mediated through cholera toxins (CTs). It is responsible for massive watery diarrhea characteristic of Cholera. Mainly antibodies produced locally in the intestinal mucosal surface mediate protective immunity in cholera. These antibodies are directed against bacterial components including CT, and protect by inhibiting bacterial colonization and multiplication and by blocking toxin action. Immunoglobulin A (IgA), IgG and IgM antibodies to cholera antigens have been demonstrated in the intestinal lumen; although in terms of protective immunity, intestinal IgA antibodies are the most important one. Oral vaccine is always preferable as it has been seen that the efficacy of injectable vaccine is around 50% only, and moreover it does not prevent introduction of cholera into a country or interrupt transmission (no herd immunity). Also, it cannot prevent development of carrier state. For all these reasons, parenteral vaccine is not recommended by WHO for controlling epidemics.

6. Why old killed parenteral whole-cell cholera vaccine is withdrawn?

During 1960s, different controlled studies from India, Bangladesh, Philippines and Indonesia showed that the vaccine had only 50% efficacy, duration of protection lasted for only 6 months and it had to be given every 6–12 months to maintain clinically significant protection. It had higher rates of side effects and it did not prevent transmission. In 1970s, it was withdrawn.

7. What is CVD 103-HgR vaccine? How efficacious and safe it is?

CVD 103-HgR (Orochol or Mutachol) is a single dose live attenuated oral vaccine consisting of genetically manipulated classical *V. cholerae* O1 strain available since 1994. It is safe and safety profile is due to poor colonization in the human intestine. As the vaccine strain is live, maintenance of cold chain is needed to preserve the bacteria. The bacteria must also be protected from stomach acid which is achieved by formulating and packaging of the vaccine with a buffer. In a randomized, placebo-controlled field trial in Indonesia; a single dose conferred 60% protection in first 6 months, only 24% during the first year. However, it does not confer significant long-term protection during 4 years of observation. Being a live vaccine it is not recommended for immunocompromized individual. Moreover, it does not confer any protection against O139 strain.

8. What is whole-cell recombinant B-subunit (WC/rBS) vaccine? What is its schedule and what is your opinion on its effectivity and safety? Can this vaccine help to prevent cholera in our country?

It is a newer generation oral vaccine, heat or formalin killed. It is marketed since 1990. It contains both Ogawa and Inaba serotypyes of classical and El Tor biotypes with a recombinant B-subunit of oral CT. Field trial in Bangladesh and Peru showed that it provides 80–90% protection during first 6 months in all age groups after administration of two doses 1–2 weeks apart. Because heat labile toxin of enterotoxigenic *Escherichia coli* (ETEC) cross reacts with CT, the vaccine provides short-term protection against ETEC, which is an added benefit for the travelers. It is currently licensed in 60 industrialized countries and mostly used by Western tourists. The limitation of the vaccine is that the vaccine is supplied with a buffer [as cholera toxin B subunit (CTB) is sensitive to stomach acid] which is to be dissolved in a glass of water. This needs safe drinking water and it also needs to be stored at 2–8°C. In developing country with poor sanitation safe drinking water may not be easily available. Further, this vaccine does not have O139 strain has been found as a causative pathogen causing epidemic in West Bengal in 1994, this vaccine is not recommended in India.

9. What are the other WHO prequalified oral cholera vaccines?

The other WHO approved oral cholera vaccine available is WC/rBS (Dukoral®), a double-dose Swedish vaccine, which costs about Rs. 1,250 a dose and needs to be coadministered with a relatively large volume of buffer solution. This Swedish vaccine is prohibitively expensive for use in developing countries, requires a buffer during administration, and due to its B-subunit component has strict cold chain requirements.

10. Which cholera vaccine has been launched recently in India?

An effective inactivated whole-cell bivalent cholera vaccine against *V. cholerae* O1 and O139 was produced in Vietnam and was found to be safe and confer significant protection against El Tor cholera in both children and adults. Since 1990 it is being used in Vietnam to protect against cholera and implemented for public health purposes. This vaccine has further been reformulated by the International Vaccine Institute (IVI) to meet WHO requirements. Large phase III study of the vaccine was carried out in Kolkata, India, in over 120,000 participants aged from 1 year and above. Results of the study showed vaccine gives over 60% protection against cholera. The vibriocidal antibody response rate was 80% in children and 53% in adults. The reformulated vaccine has been shown to be safe and immunogenic in Indian children as well as adults. In India, the vaccine is produced in the name of Shanchol™ and it has been licensed in India in April 2009. It is approved by Drug Controller General of India (DCGI), Central Drug Laboratory, Kasauli, India, to be used in children above 1 year. It received WHO prequalification in November 2011. The vaccine is now being marketed in India and is available at an affordable price for the developing countries. Shanchol™ is recommended for children above 1 year in two doses. Two doses are given 15 days apart. A booster dose is recommended after 2 years. Duration of immunity and protection has been demonstrated to last for 3 years. A booster dose is recommended after 2 years. As per WHO position paper all age groups are vulnerable to cholera. Where resources are limited, immunization should be targeted at high-risk individuals above 1 year. In India, children aged 1–5 years are at highest risk of cholera in endemic setting.

11. Is herd immunity conferred by killed oral cholera vaccines?

Analyzing data from a field trial in Bangladesh and Peru to ascertain the evidence of indirect protection from killed whole-cell oral cholera vaccines and B-subunit killed whole-cell vaccine in children and adult women showed in addition to providing direct protection to vaccine recipients; killed oral cholera vaccines confer significant herd protection to neighboring nonvaccinated individuals. This effect was attributed to vaccine-induced reduction of fecal excretion of vibrios in vaccinated individuals giving rise to less environmental contamination and thus reducing feco-oral transmission. Use of these vaccines could have a major effect on the burden of cholera in endemic settings.

12. What is the safety and immunogenicity of the reformulated WHO prequalified Vietnamese vaccine (Shanchol™)?

Following immunization, 53% of adult and 80% of children vaccines showed four-fold rise in serum *V. cholerae* O1 vibriocidal antibody titers. A less pronounced response to *V. cholerae* O139 vibriocidal antibody titers postimmunization was noted among vaccinees. No serious adverse event was noted with vaccine.

13. What are the differences between WC/rBS which is a killed, whole-cell vaccine and CVD 103-HgR (Orochol or Mutachol) which is a live attenuated oral vaccine?

The main difference between the vaccines is the level and duration of protection they confer. The WC/rBS is a double-dose vaccine to be administered 14 days apart and it shows protection 7 days after last vaccination and also shows effectiveness against ETEC. It confers protection up to 60% for 3 years, whereas the CVD103-HgR is a single-dose vaccine conferring protection for at least 6 months and shows protection within 8 days of vaccination. This vaccine is indicated in travelers to a developing country where cholera is endemic. This vaccine may also be useful in prevention of cholera before or during epidemic.

14. What is the role of cholera vaccine in epidemic situation?

Experience shows that, once a cholera outbreak has begun, a reactive vaccination campaign with a two-dose vaccine is almost impossible; a single-dose vaccine requiring no buffer and no cold chain, easy to administer and providing long-term protection would provide the ideal solution. Limitations of the currently available and internationally licensed two-dose vaccine is that it needs administration in two doses 10–14 days apart which needs to reach the same population twice. The need for buffer solution in case of WC/rBS vaccine means a need for safe water. Thus, there are important logistic constraints (clean water, cold chain, weight and volume of vaccines) and implementation difficulties in emergencies, where the population at risk is constantly moving and often situated in areas with limited access. However, efforts should be made to find ways of overcoming the limitations of the currently available vaccine. A mass vaccination campaign cannot be improvised at the last moment; it needs careful advance preparation and needs to be given beforehand for proper protection. If an outbreak is about to start or has already started, oral cholera vaccine use may not be appropriate. Vaccination with the current internationally available prequalified vaccine is not recommended once a cholera outbreak has started.

15. What are the future vaccines in pipeline?

A number of other live oral vaccines are under development in the USA (Peru 15, CVD 110, 111 and 112) and in Cuba (Cuban 638 strain). Results are promising and phase II and III trials are planned. Safety and immunogenicity study has also been conducted among Bangladeshi adults. Live oral vaccine against O139 is also undergoing safety and efficacy trials. A research on polysaccharide conjugate vaccine for cholera is currently being conducted in France and USA for parenteral use and evaluations are planned in countries with endemic cholera.

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CHAPTER

29

Combination Vaccines

Narendra Rathi

1. What was the need to develop combination vaccines?

Congestion of pediatric vaccination schedule is increasing day-by-day with the development of newer vaccines. Economics, covenience and manpower are other factors stimulating the efforts to provide new combination vaccines. However, developing combination vaccine involves many complex issues.

2. What do you mean by combination vaccine? How they are different from simultaneous or concurrent vaccination?

A combination vaccine is defined as a vaccine containing more than one immunogen that have been physically combined in a single preparation. On the other hand, simultaneous vaccines are those which are administered concurrently but are physically separate. Simultaneous vaccines are either injected at separate sites or are administered by separate routes. Simultaneous vaccination is as effective and as safe as administering various vaccines alone.

3. What are the types of combination vaccines?

Combination vaccines are either single pathogen or multiple pathogen vaccines. Single pathogen vaccines contain various antigens or serotypes of a pathogen while multiple pathogen vaccines contain various antigens or serotypes from multiple pathogens.

No.	*Type of vaccine*	*Examples*
1.	Single pathogen	Oral polio, IPV, Influenza, Pneumococcal, Rotavirus, HPV, Meningococcal
2.	Multiple pathogen	DTwP, DTaP, DT, Td, Tdap, MMR, DTwP+Hib, DTwP+Hep B, DTwP+Hib+Hep B, DTaP+Hib, DTaP+Hib+IPV, Hep A + Hep B

Abbreviations : IPV, inactivated poliovirus vaccine; HPV, human papillomavirus (caccine); DTwP/DTaP, diptheria-tetanus-pertussis (vaccine); Td, tetanus-dipthesia; Tdap, tetanus-diptheria-perterssis; Hiby haemophilus influenzae type b; Hep B, Hepatitis B, Hep A, Hepatitis A, MMR, measles-mumps-rubella (vaccine).

4. What about adverse events after combination vaccines as compaired to monocomponent vaccines?

As far as local adverse events are concerned, these occur more commonly and are more severe at the injection site. But this increase is offset by the absence of injections and hence the absence of local adverse events in other limb. On the other hand, systemic adverse events are increased only modestly, if at all. Given the numerous advantages combination vaccines offers, a modest increase in minor adverse reactions is considered acceptable.

5. What are the advantages of combination vaccines?

There are various advantages of combination vaccines from the viewpoint of consumer as well as health planner of a country.

Advantages to consumer	*Advantages to health planner*
1. Reduced number of needle sticks	1. Reduced burden on shipping, handling and storage of vaccines
2. Reduced parental and patient anxiety	2. Decreased possibility of errors
3. Reduced vaccination visits	3. Reduced paper work
4. Decreased likelihood of missed vaccinations	4. Increased compliance of vaccination program
5. Increased satisfaction	5. Economic benefit from 1 (above), reduced cost for labor and supplies, reduced vaccination visits
6. Timely completion of indicated vaccinations	

6. What are the various immunological problems involved in mixing multiple antigens in the same syringe? What are the immunological issues concerning the production of combination vaccines?

There are various challenges in developing the combination vaccines.

- *Antibody levels:* Antibody levels to individual antigen in combination may be different as compared to antibody levels induced by those component antigens if given separately, for example:
  - Whole cell pertussis vaccine is a potent adjuvant and combining three antigens in DTwP actually improves the immunogenicity of the toxoids as compaired to separate administration.
  - Administering DTwP and Hib in combination result in reduced mean PRP antibody levels compaired to giving these components separately. This fact has no clinical relevance, as levels were still higher than those needed for protection from the Hib disease.

  - The addition of HepB to DTwP results in significantly increased mean HepB antibody levels.
  - Combining DTaP with Hib tends to reduce, often markedly, the Hib antibody response. But this is a relevant issue only in the context of an immunization schedule that fails to provide a booster dose in the second year of life.
  - Competition amongst viruses can lead to altered immune responses in case of combination vaccines containing live attenuated viruses. This problem is tackled either by increasing the number of doses of vaccine (e.g. OPV) or increasing the concentration of the individual viral strain (e.g. increased varicella component in MMRV).
  - Live vaccines can interfere immunologically with each other by one vaccine stimulating interferon production which inhibits replication of another viral vaccine strain, e.g. in case of OPV, type 2 strain replicated faster than type 1 and 3 inducing interferon and hence interfered with growth of the latter viruses. To overcome this problem, the quantity of type 1 and 3 viruses was increased in mixure. This led to partial success but the administration of multiple doses became necessary to ensure optimal intake of all three serotypes.
  - Chemical interactions can sometimes leads to reduced immunogenicity, e.g. thiomersal present in some DTwP and Hib decreased the potency of IPV vaccine with more effect on type 1 poliovirus than on types 2 and 3. Unlike the situation with OPV, there is no evidence of interference between the inactivated vaccine strains themselves. To overcome this problem of chemical interaction, other preservatives were substituted and enhanced potency IPV was introduced.
- Carrier induced epitopic suppression: Simultaneous exposure to multiple conjugate antigens (i.e. polyvalent conjugate vaccine) results in either enhanced or diminished immune response. Antibody response to haptens presented on a carrier are inhibited by prior immunization with the specific carrier and this phenomenon is known as carrier induced epitopic suppression. Dose, route, choice of carrier protein and presence of adjuvant are the factors which determine whether epitopic suppression or enhancement will occur. Suppression is likely to occur if large amount of carrier protein are used for priming, high level of anticarrier antibodies are present and two conjugate vaccines employing same carrier protein are administered concurrently. Infants given vaccine containing Hib-PRP conjugated to tetanus toxoid (PRP-T) plus quadrivalent pnemococcal vaccine conjugated to tetanus toxoid demonstrated inferior antibody levels as compaired to pnemococcal vaccine conjugated to diphtheria toxoid. Thus, it is clear that effect of concomitant administration of carrier protein in conjugate vaccines is unpredictable and should be evaluated for each and every combination vaccine.
- Chemical and physical interaction among vaccine components: these interactions can result into altered immune response to vaccine components. If one vaccine that is administered with adjuvant is combined with a vaccine without adjuvant, the adjuvant might get displaced from first antigen and may get combined with second antigen. This would result into reduced

immunogenicity of first antigen and altered immune response to second antigen. Other components (like buffers, stabilizers, excipients) in one vaccine may interfere with components of second vaccine, e.g. thiomersal can significantly reduce the potency of IPV.

- Multiple antigens and immune overload: some parents are concerned that normal immune system of infants is capable of becoming overloaded and multiple antigens in combination vaccines may induce such overload. It should be clearly and strongly communicated to them that, there is no scientific evidence to support such an assertion and there is much evidence to refute it. It should be explained that newborn is naturally exposed to thousands of antigens in first few months of life and simultaneous exposure to multiple vaccine antigens will not overwhelm the infant immune system. In fact infant immune system requires fairly intense challenge to develop normally and insufficient stimulation leads to increased risk of autoimmune disorders.
- Relationship of multiple immunizations to type I diabetes and allergic diseases like asthama. There is evidence to reject the causal relationship between these diseases and multiple immunizations.

7. What are the practical issues concerning the administration of combination vaccines?

There are various issues regarding administration of combination vaccines.

- *Administration of superfluous antigen:* As numerous combination vaccines are available, one may have to give an extra dose of an antigen which patient may not need (as he has already received the recommended doses of that antigen). It has been demonstrated for many antigens that giving an extra dose involves no adverse consequence. Low reactogenicity of Hib, IPV and Hep B makes it unlikely that giving an extra dose would cause a problem. When patients have received the recommended immunizations for some of the components in a combination vaccine, administering the extra antigen(s) in the combination vaccine is permissible if they are not contraindicated and doing so will reduce the number of injections required. One should be careful about some antigens which are known to be associated with increased adverse effects if administered too frequently, e.g. diphtheria and tetanus toxoid leading to extensive local reactions.
- *Brand interchangeability:* The question of whether vaccines from different manufacturers can be used interchangeably also applies to monocomponent vaccines. It is reassuring to know that interchanging one brand of vaccine for another has never been shown to result in performance that is outside the range expected for the vaccine in question. The Advisory Committee on Immunisation Practices has recognized DTwP (and it is individual components), IPV, OPV, Hib (as long as one uses complete three doses) and HepB as interchangeable. For the vaccines for which robust data on the interchangeability is not available (e.g. DTaP, newer combination vaccines), this committee has recommended that same product be used throughout the primary series. Still it is recommended that the opportunity to administer a vaccine, for which the child is eligible, should not be missed even if earlier brand is not available or it is identity is not known.

- *Ad hoc combinations:* Healthcare practitioners should not create their own ad hoc combinations by mixing separate vaccines in same syringe unless there is evidence establishing the stability, safety and immunogenicity of the resultant combination, as reflected in the package inserts.
- *Cost issues:* At the time of commercial introduction of new combination vaccine, it is component vaccines are already available and would continue to be used instead of combination, should the combination's price exceed the amount which buyers are willing to pay for the convenience the combination represents. Hence the price of the combination is effectively capped and it is costs of research and development must be recoverable within that cap, otherwise the combination will not be developed. The premium pricing of the combination vaccines or reduced practitioner reimbursement (as result of reduced number of injections/visits) might inhibit use of combination vaccines. But given the various advantages of combination vaccines (discussed above in Que. 3), combination vaccines should be preferred over monocomponent vaccines.

8. What is meant by second shot combination vaccines?

Combination vaccines that incorporate conjugate pneumococcal and conjugate meningococcal antigens are known as second shot combination vaccines.

9. What are various multiple pathogen combination vaccines available today?

- Combination vaccines currently licenced in India are:
  - DTwP+Hib, DTwP+Hep B, DTwP+Hib+Hep B: These are either available as ready to use or lyophilized form. Though antibody response to Hib is reduced, there is no reduced efficacy as most subjects achieve the seroprotective level of 1 ug/ml. They have good immunogenicity and safety profile for both primary and booster immunization.
  - DTaP+Hib, DTaP+Hib+IPV: The primary concern in these combination is reduced Hib immunogenicity noted specially for primary immunization and when vaccines were administered earlier in life and in premature babies. This lower immunogenicity to Hib was conclusively attributed mainly to nonadministration of booster dose at 18 months. The US FDA and ACIP has approved this pentavalent vaccine for primary immunization.
  - Hep A + Hep B: Available in both pediatric and adult (for those aged 18 and above) formulations. Dosing schedule is 0, 1 and 6 months (3 doses).
- Combination vaccines available internationally are:
  - DTwP+IPV, DTwP+IPV+Hib: These vaccines would have immense importance in the Indian EPI to facilitate a shift from OPV to IPV as polio eradication nears.
  - DTaP+IPV, DTaP+Hep B, DTaP+IPV+Hep B, DTaP + Hib + Hep B, DTaP + IPV + Hep B + Hib: Hepatitis B antibody titers following primary immunization are lower than when Hepatitis B is administered alone. This is due to close spacing of doses at 1 month interval rather than immune interference.

- MMRV: The antigen content of varicella is more in MMRV vaccine as compaired to monocomponent varicella vaccine. Though MMRV has equivalent immunogenicity and efficacy, it has greater side effects in the form of fever, rash and increased risk of febrile seizures.
- Hepatitis A+Typhoid
- Hepatitis B+Hib.

10. What are IAPCOI recommendations for use of combination vaccines?

IAPCOI concludes that all currently licensed combination vaccines in India have an immunogenicity, efficacy and safety profile comparable to separately administered vaccines as of currently available data. However, it recommends strict observance of manufacturer's instructions regarding mixing of vaccines in same syringe.

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Section 4

Vaccines in the Pipeline

Chapters

CHAPTER

30

Dengue

Vipin M Vashishtha, Ajay Kalra

1. What are the challenges in the development of vaccines against dengue?

- There are four antigenically distinct serotypes of dengue. They are antigenically very similar but different enough to elicit only transient partial cross-protection after infection by any of them. Therefore, any vaccine against dengue has to be tetravalent to protect against all four serotypes. As there is no cross-protection between the four dengue virus (DV) serotypes and because of fear of immune enhancement by heterotypic DV antibodies, only a tetravalent vaccine will be acceptable.
- Poor growth of dengue virus in cell culture is a further constraint.
- The lack of an animal model for the disease, an *in vitro* correlate of protection and a surrogate for vaccine efficacy. The efficacy and long-term safety of candidate vaccines have to be demonstrated before use.
- Also, the number of doses that would confer immunity is a vital issue.

2. Would a dengue vaccine be able to prevent only the mild form of dengue or the serious one as well?

Clinical studies have shown that the quantity of virus circulating in the body of patients who develop DHF and DSS is higher by around 1 to 2 logs compared with patient suffering form milder forms of Dengue. This observation suggests that progress to serious dengue and adverse outcomes may be reversed by administering potent and safe compounds that target essential steps in virus replication earlier during the disease, thereby lowering the viral load and thus preventing serious forms of dengue.

3. What is the present status of development of dengue vaccines?

Progress in DV vaccine development has been relatively slow, mainly because DV grows poorly in cell culture and there is no reliable animal model for DHF. Also, tetravalent DV vaccines have often generated disappointing immunogenicity results as compared to monovalent vaccines, due to a phenomenon of interference among the 4 strains, the identity of the dominant serotype(s) depending on the nature and composition of the vaccine. The application of infectious clones technology to dengue vaccine development has greatly stimulated the development of candidate vaccines and the current pipeline of dengue vaccines is diverse and overall promising.

Four types of vaccines are under development and trials:

- Live attenuated virus (LAV)
- Chimeric live attenuated virus vaccine
- Live recombinant, DNA, and subunit vaccines
- Inactivated vaccines.

4. Tell us more about live attenuated vaccines (LAVs).

The initially favored strategy has been to attenuate DV strains by repeated passage of wild type DV in cell culture in order to prepare a vaccine based on live attenuated virus strains. LAVs can induce durable humoral and cellular immune response since they most closely mimic a natural infection.

5. What are the difficulties in production of live attenuated vaccines?

Live attenuated vaccines face the difficulty to define a correct balance between insufficient attenuation and overattenuation of the candidate vaccine strains, the lack of correlation between *in vitro* markers of attenuation such as small plaque phenotype or thermosensitivity, and *in vivo* attenuation, and the phenomenon of immunological interference among the four DV serotypes. Unacceptably, higher reactogenicity of tetravalent vaccine in few trials was another hindrance.

Over a period of 12 years, a series of bivalent, trivalent and tetravalent trials were carried out in Thailand in young adults and in children, both immune and nonimmune. While results were generally acceptable, considerable effort was directed towards determining the best formulation of the four attenuated candidate vaccine viruses to provide the best immunogenicity for all four serotypes. The DEN3-GMK33-FRhL-2 virus, however, remained problematic; producing low neutralizing antibody titres in most recipients of the tetravalent formulation. Moreover, not all vaccine recipients seroconverted to all four vaccine serotypes. At the end, however, the tetravalent vaccine was judged to be safe and of acceptable immunicity in children 5 to 12 years of age.

The Mahidol tetravalent dengue vaccine was tested in nonimmune adult volunteers in the United States in 1995, with a high rate of reactogenicity. This increased reactogenicity was due to the DEN3-GMK33-FRhL-2 virus overgrowing the other serotypes; the neutralizing antibodies in these volunteers were primarily DENV-3, with low or undetectable titres against the other three serotypes. Interestingly, a subsequent trial of the tetravalent vaccine in Thailand using different formulations of the four monovalent candidates resulted in an improved safety profile.

The development of a tetravalent live attenuated vaccine was eventually taken over by Sanofi Pasteur before being put on hold. Despite these results, in 2000 Sanofi Pasteur contracted with the Division of Vector-Borne Infectious Diseases

(DVBID), Centers for Disease Control and Prevention (CDC) in Fort Collins, Colorado, United States, to re-derive the DENV-3 candidate vaccine. It was found that the DEN3-GMK33-FRhL-2 vaccine virus did not revert to a virulent form; instead, this strain contained two subpopulations, one of them attenuated and one still the wild type virulent virus. Interestingly, the peer review panel had speculated that this might be the case in 1991 (WHO, 1991). For reasons that are not fully understood, the wild type virulent population overgrew the attenuated population when it was put into non-immune humans. The attenuation markers were used in the original development by the CDC group to derive a new candidate vaccine strain of DENV-3 from the original Mahidol candidate vaccine. This monovalent candidate was tested for safety and immunogenicity in 2004 among medical students in Hong Kong SAR. Unfortunately, it was still highly reactogenic and was deemed unacceptable as a vaccine. At this point, Sanofi Pasteur decided to shelve the tetravalent Mahidol live attenuated dengue vaccine.

6. What is the current status of development of LAVs based on Mahidol University strain?

Although, the original tetravalent vaccine candidate developed at Mahidol University was not successful, the initiative in the early days was directly responsible for the subsequent progress that has been made in developing a tetravalent vaccine for this complicated disease. Thus, while the initial candidate failed, the concept and the overall programme was an unequivocal success, to the point today that two of the largest pharmaceutical companies in the world are involved in dengue vaccine development. Currently, there are at-least five candidate vaccines that are in or ready to be taken into clinical and safety trials. Phase III efficacy trials on at-least one of these candidates in 2008. Finally, the Bill and Melinda Gates Foundation has funded the Pediatric Dengue Vaccine initiative to facilitate the development and introduction of a safe, effective and economical dengue vaccine.

Another measure of success of the programme is that the original Mahidol monovalent DEN2-PDK53 vaccine was an excellent vaccine and is currently being used as the backbone to produce a second-generation chimeric tetravalent vaccine that shows great promise.

7. What are the other LAV strains involved in the production of dengue vaccine?

The two other institutions, namely, Walter Reed US Army Institute of Research (WRAIR) and the US National Institutes of Health (NIH) are currently involved in developing new LAV vaccines.

The Walter Reed US Army Institute of Research (WRAIR) succeeded in developing a tetravalent live attenuated vaccine by serial passage of all four DV strains in dog kidney cells, and tested various tetravalent formulations in adult volunteers, then in Thai children. The vaccine was then licensed to GSK, which continued clinical trials and is presently going through Phase II trials with the live attenuated tetravalent vaccine candidate.

The US National Institutes of Health (NIH) also have developed attenuated DV strains, using reverse genetics to create an attenuating 30-nucleotide deletion (?30) in the 3' untranslated region of the genome of the four DV strains. Two of the resulting virus strains, DV1-?30 and DV4-?30, were found to be attenuated in rhesus monkeys and safe and highly immunogenic at a dose of 103 PFU/vaccinee in Phase I/II clinical trials in human volunteers. However, this strategy did not yield suitable candidates for serotypes 2 and 3, which had to be generated by replacing the sequence coding for M and E structural proteins in the attenuated DV4-?30 genome by the corresponding sequences from DV2 or DV3, thus yielding intertypic chimeric viruses DV2/4-?30 and DV3/4-?30, respectively. The DV 2/4-?30 virus strain was tested in humans and appeared safe and strongly immunogenic at the dose of 103 PFU/dose. The replication of these chimeric viruses was also attenuated for Ae aegypti, indicating that they would likely manifest decreased transmissibility by mosquitoes. The DV3/4-?30 virus was evaluated in clinical trials but immunogenicity appeared to be weak. The four attenuated virus strains should eventually be combined together and tested as a tetravalent live attenuated candidate vaccine.

8. What are the different chimeric vaccines under production against dengue? What are their current statuses?

A homotypic chimeric virus approach has been also applied by the US CDC to engineer DV2 chimeras by inserting the structural protein genes from DV1, DV3, and DV4 into an attenuated PDK53 DV2 genome that had been attenuated by replacing a portion of the DV terminal 3' stem and loop structure with that of West Nile virus. The clinical development of these DV2-based chimeras is being carried by Inviragen, in collaboration with the US CDC and Shantha Biotechnic.

Another approach, the engineering of heterotypic chimeric viruses, was initiated in the 1990s by Acambis, using the YFV 17D vaccine strain as the genetic background and replacing the M and E structural protein-coding sequences in the YFV genome with those from either the four DV serotypes, or from JEV, or from WNV. The DEN-YF chimeras were developed by Acambis and licensed to Sanofi Pasteur. When injected to monkeys for safety and protective efficacy tests, the monkeys developed a brief viremia followed by a DV neutralizing antibody response and were protected against challenge with DV.

The chimeric viruses were tested and found to be safe and immunogenic in humans. A tetravalent combination of the four DEN-YF chimeras, ChimeriVax-Den vaccine, was shown to induce a transient and low-grade viremia in nonhuman primate and in human volunteers, followed by a solid immune response against the four serotypes with some strains showing dominant immunogenicity. A dose adjustment for the DV2 chimera resulted in a more balanced response. The chimeric viruses also were found to replicate and disseminate poorly in the body of mosquitoes, indicating that the risk of infection and transmission by mosquitoes in nature would be minimal. The Phase II trial is taking place in the USA and Latin America and the Phase IIb pediatric trial has been launched by Sanofi Pasteur in early 2009 in Thailand.

The primary objective of the phase IIb trials was to assess the efficacy of CYD-TDV in preventing dengue disease, after the completion of vaccination schedule of three doses given 6 months apart. Additional objectives include the evaluation of the vaccine safety and immunogenicity. The study population consisted of 4,002 children aged 4–11 years in Ratchaburi Province, Thailand. Efficacy, safety and immunogenicity results, as available 1 year after completion of the vaccination schedule, were published in September, 2012. The study protocol included a follow-up period of additional 2 years, which is currently ongoing to investigate protective efficacy in children began late in 2009 and phase III trials have been in progress since 2010.

The Strategic Advisory Group (SAGE) on immunization from WHO met from 9th April to 11th April 2013 in Geneva, Switzerland. They reviewed the results of the phase IIb trial of this lead vaccine candidate, a tetravalent live attenuated vaccine. The vaccine was shown to be safe and immunogenic against DENV-1, -2, -3, and -4. The overall vaccine efficacy was 30.2% (95% confidence interval: −13.4 to 56.6). The exploratory intention to treat analysis suggested efficacy for DENV-1, -3 and -4. No efficacy was demonstrated for DENV-2.

Phase III efficacy studies of CYD-TDV are currently underway in 31,000 children and adolescents in ten countries in Asia and Latin America. These large scale, multicenter studies in a variety of epidemiological settings will be important to obtain pivotal efficacy results, additional safety data, and further insight into the relationship between vaccine-induced immune responses and protection against dengue disease.

9. What is the progress in the development of recombinant DNA vaccine for dengue?

The Naval Medical Research Center is pursuing a DNA-based vaccine approach, using a Biojector device for immunization. Evaluation of the Phase I study is going on.

In this technique, DV genes were inserted at the Naval Medical Research Center into a new, nonreplicative adenovirus vector (Ad5) to engineer double recombinants expressing the prM and E sequences from both DV1 and DV2, and DV3 and DV4, respectively. The pair of recombinants was tested in mice and shown to induce neutralizing antibodies to the four DV serotypes. It also was tested in macaques, in which it induced significant protection against challenge with all four DV serotypes.

10. What are different subunit vaccines in development?

Several groups are developing subunit vaccines based on domain III of the DV E protein, a strategy that is aimed at reducing the induction of cross-reactive antibodies.

A subunit dengue vaccine has been developed by Hawaii Biotech Inc using the truncated amino-terminal 80% of the E glycoprotein from each serotype plus the entire NS1 protein from DV2 formulated in a proprietary adjuvant. The candidate vaccine elicited robust immune responses in nonhuman primates and clinical trials are envisaged in the near future. A novel dengue subunit vaccine candidate was developed using a consensus dengue virus envelope protein domain III (cEDIII). BALB/c mice immunized with the recombinant cEDIII in the presence of aluminium phosphate developed long-lasting neutralizing antibodies against all four serotypes of dengue virus.

11. At last, what is the status of inactivated dengue vaccine?

Another approach has used inactivated virus. A purified inactivated DV2 candidate vaccine has been tested and shown to elicit a good level of neutralizing antibody and protection in nonhuman primates.

12. What is pediatric dengue vaccine initiative?

For the first time in 60 years, an opportunity exists to introduce newly developed dengue vaccines into the field quickly. In July 2003, the Bill and Melinda Gates Foundation funded the Pediatric Dengue Vaccine Initiative with 55 million dollars for 5 years. The Pediatric Dengue Vaccine Initiative has been collaborating with vaccine manufacturers, governments, and the WHO to fund a 4-point program to accelerate the development and field-testing of dengue vaccines. This program will (1) better define the global burden of dengue; (2) elucidate the social and economic costs of dengue, particularly in countries that may introduce dengue vaccines; (3) identify phase III vaccine test sites in different countries where dengue is endemic, as well as build local capacity for dengue research; and (4) fund basic research on how dengue vaccines can be administered safely.

13. When can one expect a safe effective dengue vaccine available for clinical use?

Though, it is still not certain but two of the world's topmost vaccine developers (GSK and Sanofi Pasteur) are seriously involved in this field. With the involvement of Bill and Melinda Gates Foundation and other vaccine institutions like WHO, IVI Korea and formation of a Pediatric Dengue Vaccine Initiative all have paved the way for an early availability of dengue vaccine ready for clinical use.

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CHAPTER

31

Malaria

Arun Kumar, Ajay Kalra

1. What is the need to have a malaria vaccine?

Malaria is the cause of huge morbidity and mortality the world over. WHO has estimated 300 to 500 million cases annually with 0.7 to 2.7 million global deaths. Over 15 lacs malaria cases were reported in 2009 in our country. Diagnosis is difficult due to lack of trained technicians. Moreover, there is an increasing drug resistance by *Plasmodium falciparum*. In view of this prevention of malaria by use of vaccine is of paramount importance.

2. Do we have a malaria vaccine till now?

Not yet.

3. Is it possible to have a vaccine?

Yes. Several lines of evidence suggest that a prophylactic malaria vaccine for humans is feasible. At first, naturally acquired immunity builds up during the first two decades of life in people living in malaria-endemic countries. This naturally acquired immunity is, however, partial and short-lived, and appears to depend on continuous antigenic stimulation, waning when antigen exposure ceases. Protection has been elicited by passive transfer of immunoglobulins from malaria-immune adults to malaria-naïve human volunteers. There is also experimental evidence that immunization of humans and animals with irradiated sporozoites results in partial or complete protection from an experimental infection with viable sporozoites, which might pave the way to a genetically attenuated live sporozoite vaccine. But when an effective vaccine would be available, may be difficult to predict, may be a decade or even more.

4. What is the reason of not having one till now?

Malaria is a protozoal parasite, bigger and considerably more complex than viruses or bacteria. The parasite gets into human cells very quickly and hides itself from the immune system. Once inside cells, certain proteins appear on its surface. Although vaccines can be designed to recognize these proteins, the parasite varies these 'signals.' Not only different parasites have different protein coats, but each parasite can also vary the particular signals it displays. The immune response to the parasite is thus complex. While the body mounts a variety of responses, it is still unclear which actually helps to get rid the body of the parasite.

One can not use the whole malaria parasite to make a vaccine that means we have to design a subunit vaccine, which is difficult. In this case, the major problem is to induce a big enough immune response to kill the parasite. Hence, the multiple obstacles to the development of a malaria vaccine remain, such as the lack of general agreement on possible immune correlates of protection, the lack of predictive animal models and assays, and the multiple stages and antigenic diversity and variability of the parasite. Furthermore, the genetic complexity of the parasite remains a significant challenge. Plasmodia have more than 5000 genes and finding which ones code for appropriate candidate vaccine antigens may be a quandary.

5. What is the progress in development of the vaccine?

It is extremely difficult to kill the parasite by vaccine induced immunogenicity. Different vaccines are being developed that target the different stages of the parasite life cycle:

- Pre-erythrocytic (sporozoite and liver stage)
- Erythrocytic (blood stage)
- Gametocyte (sexual stages).

Pre-erythrocytic stage vaccine strategies aim to generate an antibody response that will prevent sporozoites from invading hepatocytes as well as to elicit a cell-mediated immune response that will inhibit intrahepatic parasite development. This type of vaccine would be ideal for travelers because it would prevent infection and the advent of clinical disease.

Erythrocytic stage vaccine strategies aim to elicit antibodies that will target merozoite antigens and/or antigens expressed on the surface of infected RBCs. These vaccines ideally should be able to induce antibody-mediated cellular toxicity and/or complement lysis, as well as T-cell responses that will inhibit the development of merozoites in RBCs. This type of vaccine is hypothesized to allow parasite densities to be controlled at levels which would minimise morbidity and would therefore be suitable for residents of endemic countries for morbidity reduction but not for prevention of infection.

Finally, vaccines targeting the sexual stage of the parasite do not aim to prevent infection or disease but to prevent the transmission of the parasite to new hosts. Efficacious transmission blocking vaccines are thought to be highly desirable in pre-elimination settings where interruption of transmission becomes a key aim of an immunization programme. It is postulated that the antibodies produced by these vaccines will reach into the mosquitoes through the gametocytes by the blood meal. These antibodies will destroy the antigens (PFS 25, PVS 25) within the mosquitoes and thus neutralize the sexual stage. Such a vaccine would not only protect the vaccinee but also the other persons bitten by the mosquitoes.

Almost all of the vaccines under development are directed at *P. falciparum,* which is responsible for the vast majority of severe malaria disease and deaths.

6. Is there any ray of hope in present scenario?

RTS,S is the most advanced candidate vaccine against human malaria. During its remarkable journey from conception and design in the early 1980s to the multicenter Phase III trial currently underway across sub-Saharan Africa, RTS,S has overcome tremendous challenges and disproved established vaccine paradigms. In the last several years, Phase II studies conducted in infants and children in endemic areas have established the efficacy of RTS,S for reducing morbidity due to clinical malaria. If the results are realized in the Phase III trial, the chances for licensure in the near future appear high.

7. Please tell us more about this vaccine. What are the results of different clinical trials of this vaccine?

This vaccine is known as RTS,S/AS01—the most advanced malaria vaccine candidate at this time, which is based on the circum-sporozoite protein (CSP) that is the predominant surface antigen of the sporozoites and is expressed early on infected hepatocytes. This vaccine, RTS,S/AS01, is made of recombinant chimeric viruslike particles (VLPs) produced in *Saccharomyces cerevisiae* combining the Hepatitis B surface antigen (HBsAg) with the C-terminus portion (aa 207-395) of *P. falciparum* CSP. The candidate vaccine has shown 40% protective efficacy in multiple clinical challenge studies conducted in partnership with the US Military Malaria Vaccine Program. A randomized controlled field efficacy trials in Gambian adults demonstrated 71% efficacy against time to infection for the first 9 weeks after vaccination, but low efficacy for the subsequent 6 weeks. Efficacy over the entire 15 week period was 34% and it has been proposed that heterogeneity of risk may partly or wholly account for the apparent waning seen in this study. The vaccine was found to be safe and well tolerated in adults in a hyperendemic region of western Kenya as well as in 1 to 4 years-old children in Mozambique in whom it induced a strong antibody response to both the CSP and to HBsAg and a TH1 CD4$^+$ T cell response. The paediatric clinical development of RTS,S has occurred as a partnership between the PATH Malaria Vaccine Initiative and GSK.

A randomized phase IIb trial in 2022 Mozambiquan children aged 1 to 4 years showed 30% efficacy (95%CI 11-45%) over six months against time to first or only clinical malaria episode and 57% efficacy (95%CI 16-80%) against severe malaria. Vaccine efficacy over an extended 18-month followup period was 35.3% against time to first or only episode of malaria and 48.6% against severe disease. In a study in Bagamoyo, Tanzania, 170 infants were immunized at 8, 12, and 16 weeks of age with RTS,S in coadministration with a DTPw/Hib vaccine. Seroconversion to CSP was 98.6%, and the efficacy of the RTS,S vaccine against first infection with

P. falciparum at sixth month after vaccination was 65.2%, but GMT to diphtheria and tetanus vaccines were lower in coadministration with RTS,S.

All studies cited to date had used AS02 adjuvant, an oil-in-water emulsion with added MPL and QS21. Paediatric development has since shifted to AS01, which consists of a liposomal preparation with MPL and QS21. This adjuvant is more immunogenic for both IgG and $CD4^+$ T cell responses. A randomised controlled study with about 850 children aged 5 to 17 months who were followed for 8 months in Kilifi, Kenya and Korogwe, Tanzania, showed an efficacy of 55% in terms of the rate of all episodes of malaria for RTS,S/AS01. The magnitude of the anti-CSP antibody responses in that study was substantially higher than in children that had received the RTS,S/AS02 vaccine previously. Whether the higher antibody titers associated with the use of AS01 will translate into a longer duration of protective efficacy for the RTS,S vaccine remains to be demonstrated.

Other clinical trials of the RTS,S vaccine are under way in combination with other candidate malaria vaccines. A pivotal phase III trial design was planned to start in the second quarter of 2009 and is intended to enrol up to 16,000 children in 11 different sites in Burkina Faso, Ghana, Gabon, Malawi, Mozambique, Kenya, and Tanzania, covering different transmission patterns.

8. What are the other 'pre-erythrocytic stage' vaccines under development?

Another CSP-based candidate vaccine was developed by Dictagen, Inc, in collaboration with the University of Lausanne, Switzerland, that contained a 102–amino acid synthetic peptide representing the C terminal portion of the CSP antigen formulated with Montanide ISA 720. The formulation was found to be safe in human volunteers and to elicit both an antibody response and a cellular immune response. The vaccine currently is in Phase IIa clinical trials.

The US Military Malaria Vaccine Program (USMMVP), in collaboration with Vical, Inc, developed a candidate DNA vaccine for malaria by mixing five plasmids that encoded five different *Plasmodium falciparum* antigens. The vaccine, however, only showed modest immunogenicity in nonhuman primates and elicited little protection against sporozoite challenge in human volunteers.

The vaccine potential of *P. falciparum* LSA-3 was further investigated in nonhuman primates: both a DNA vaccine and a long LSA-3 synthetic peptide vaccine were able to elicit sterilizing immunity against sporozoite challenges in chimpanzees and Aotus monkeys, respectively.

Another liver stage antigen, the TRAP antigen, was developed as a candidate vaccine by the Oxford University Malaria Vaccine Clinical Trials Group, which conducted studies of a DNA, a fowlpoxvirus (FPV) and a MVA-based vaccines expressing the TRAP antigen. The recombinant FPV and MVA vaccines were tested independently or in prime-boost combinations with or without DNA vaccine and found to be well tolerated and highly immunogenic in human volunteers in the UK in terms of induction of specific $CD4^+$ T cells. Trials in Gambia and in Kenya failed however to demonstrate protective efficacy of these approaches.

Live recombinant vaccines expressing CSP determinants also were developed by Oxford University using a chimpanzee Adenovirus (AdCh63), MVA or FPV as vectors. These vaccines are in early clinical development.

9. What is the status of development of 'erythrocytic stage' vaccines?

Various groups, focusing on different merozoite antigens and different regions of the proteins, and using different antigen expression systems and formulations are developing a variety of erythrocytic stage malaria vaccine candidates. Results of preliminary efficacy trials involving some of these candidates should be made available over the next couple of years. However, the choice of clinical case definitions and end-points in efficacy trials of erythrocytics vaccines, which aim to decrease morbidity by reducing parasitemia, still remains a difficult issue.

Blood stage vaccine candidates currently in early clinical trials are the merozoite surface proteins 1 (MSP-1), 2 (MSP-2), and 3 (MSP-3), apical membrane antigen 1 (AMA-1), the glutamate-rich protein (GLURP) and the serine repeat antigen (SERA) protein. Anti-MSP-1 antibodies have been reported to strongly correlate with reduced risk of clinical malaria in Ghanaian children.

The combination B vaccine which combined the merozoite surface proteins MSP-1 and MSP-2 with the ring-stage infected erythrocyte surface antigen RESA and induced a 62% reduction in parasitemia in the Phase II trial on 5 to 9 years old children in Papua New Guinea , but this effect was restricted to parasites expressing one of the two allelic forms of MSP-2. A biallele MSP-2 vaccine is now under development and a phase I trial of this vaccine recently took place in Australia.

Another promising vaccine in clinical development is an MSP-3-based vaccine. The vaccine was tested in Phase I trials and found to elicit antibodies able to block multiplication of *P. falciparum* in red blood cells *in vitro* in a monocytedependent manner. There are many other eythrocytic stage vaccines undergoing phase I trials in different countries.

Despite encouraging progress, the lack of immune correlates of protection together with the high polymorphism of many of the erythrocytic stage antigens constitute major obstacles to the development of vaccines that target the blood stage of the parasite cycle. In contrast with pre-erythrocytic stage vaccine candidates, erythrocytic vaccine candidates lack an appropriate human artificial challenge model and have had to rely on natural transmission in the field to provide a proof-of-concept of their efficacy. Their development is therefore slower and necessitates major commitment, intensive collaboration as well as high-level coordination supported by adequate funding.

10. What are 'transmission-blocking vaccines'?

Transmission-blocking vaccines aim to prevent onward transmission to humans by targeting the sexual stages of *P. falciparum* and blocking sexual mating so as to prevent the development of sporozoites in Anopheles mosquitoes. Antibodies against gametocytes could act directly in humans, or at a later stage in mosquitoes.

This approach has the advantage of having robust *in vitro* assays that could be used to demonstrate proof-of-concept, as well as a relatively clear effector immune response. Several candidates are in clinical development, including vaccines that target the Pfs25 or Pvs25 and Pvs 28 surface proteins, but the ISA51 formulation of these vaccines turned out to be unacceptably reactogenic. Also, a major challenge for this vaccine approach is proving true field efficacy.

11. What does 'pregnancy malaria vaccines' signify?

In Africa, where an estimated 50 million women become pregnant each year, maternal malaria causes untold numbers of abortions, stillbirths and over 10,000 maternal deaths. In addition, malaria infection causes more than 2,00,000 low-birthweight babies to die within their first year of life. The possibility of developing a placental malaria vaccine by targeting Plasmodium antigens expressed on the surface of infected erythrocytes that attach to placental proteins is the subject of promising R&D efforts.

12. What is the current status of *P. vivax* vaccines?

If overall malaria disease burden continues to decrease then it is likely that *P. vivax* control will become a high priority within the malaria community. *P. vivax* disease already accounts for a rising proportion of cases in co-endemic areas. The WHO has taken a leading role in providing guidance on the *P. vivax* vaccine R&D agenda. Phase I trials of Pvs25 and *P. vivax* CSP-based peptide vaccines have been the only *P. vivax* clinical trials in recent years. A potentially promising approach based on *P. vivax* Duffy Binding Protein is under development by a group in India.

13. How expensive would be the effort to develop and deploy 'malaria vaccines' to combat the disease?

It has been estimated that the cost for supporting the minimal set of malaria interventions required to effectively control malaria is around US$ 3.2 billion per year for 82 countries with the highest burden of disease (US$ 1.9 billion for Africa alone). Increased commitment and financial support, through programs such as the Global Fund to Fight AIDS, Tuberculosis and Malaria which disbursed more than US$ 200 million in 2003–2004 to 28 countries, will be needed to support control strategies in an effective and sustainable way. The next 5-year is likely for the first time to witness the submission of a malaria vaccine for possible registration (or "positive scientific opinion"—the EMEA article 58 equivalent). The malaria community will then need to consider the role of a partially efficacious pre-erythrocytic malaria vaccine as an addition to the current complement of malaria control measures. Generation of appropriate clinical trial data to allow assessment of public health impact of vaccination in the context of existing control measures will be a crucial component of this process.

14. What about the intravenous malaria vaccine?

Most malaria vaccines so far have been subunit vaccines. Michael Hoffman used whole sporozoite and not just subunit components to make a vaccine. An initial trial in 2011 using intradermal vaccination with this vaccine protected only 2 of the 80 who are exposed to *Plasmodium*. According to the results of a recent trial published in Science, 2013, when this vaccine was given intravenously, six patients who got five doses of the vaccine were 100% protected when exposed to *Plasmodium* while five of the six who were unvaccinated developed malaria. However, there are still logistic difficulties with this vaccine. It needs five intravenous doses and has to be preserved in liquid nitrogen.

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CHAPTER

32

Newer TB Vaccines

Sangeeta Sharma

1. Why is there a need for a new BCG vaccine?

Conventional BCG vaccine has important limitations regarding the control of tuberculosis. The current BCG vaccine is not very effective in preventing pulmonary TB, the most common and infectious form of the disease. Efficient drug therapy exists but the treatment is long and case detection rates are low for making the development of a better vaccine as an important goal.

2. What is the current status of the conventional BCG vaccines being used today?

Cochrane database analyses have shown that BCG vaccine being used today can only protect against development of severe and disseminated forms of tuberculosis amongst children, i.e., tubercular meningitis and miliary tuberculosis and is not very effective at preventing pulmonary TB. Also, it cannot prevent primary infection to occur. This has an important implication in countries like Africa where a huge proportion of TB "carriers" are co-infected with HIV. This dramatically increases the likelihood of degenerating from latent into active TB.

This protection wanes off in 10 to 15 years. The implication of this is that BCG has very limited role in preventing a reinfection or reactivation during adolescents. However, the conventional BCG does not boost the immune response to a level that it can prevent reactivation/reinfection TB. Therefore, there are only 5 countries in the world practicing revaccination with a second dose of BCG, but this practice is fast loosing ground as Brazil has recently stopped this practice based on their countrywide Government-funded trial.

3. What should be the characteristics of an ideal BCG Vaccine?

The following issues must be taken into consideration during the development of new vaccine candidates:

An ideal vaccine should be the one which would not only prevent the progression of TB disease, as is done by the conventional BCG vaccine, but it should also be able to prevent the primary infection, its transmission and re-activation. Further, it should be safe, not interfere with the diagnosis and if required also be used as an adjunct to chemotherapy. The greatest need is to develop a vaccine that works either before or after infection has occurred, Mucosal delivery would again be of a great help and low cost is always a major consideration. It should also not need frequent boosters.

4. What are the challenges in development of the newer BCG vaccine?

A number of challenges have been identified and some of these are:

- Lack of any good "protective markers" requires that randomized tests be carried out to evaluate the efficacy of candidate vaccines.
- Animal studies/models provide limited information and cannot be extrapolated on potential human vaccine candidates.
- Most human beings have already been immunized with BCG or exposed to atypical environmental mycobacteria. This interferes with the vaccine uptake. Therefore, it may not be possible to find a clean human population for the trial of a new vaccine.
- Moreover, the level of immunity cannot be increased any further in these already immunized or environmental mycobacteria exposed individuals. Thus, the new vaccine might not seem to give the expected results in these cases.
- There have been concerns on the other hand that induction of a strong immune response in some infected individuals might produce immunopathology and that it would not be possible to exclude such individuals from vaccination settings.

5. What are the other non-TB usage of BCG vaccine?

The non-TB uses of BCG have been described in the following conditions:

- Leprosy
- Buruli ulcer
- As immune modulator in atopy
- As immune modulator for some types of cancers especially bladder cancer, and
- Ancylostomiasis and other helminthic infections.

6. What are the main strategies for developing these newer vaccines?

The main strategies for developing these vaccines are either to boost or modify the already existing BCG or find an alternative to BCG, which are summarized below:

- Modify BCG – Ag, Cytokine, MHC-1 presentation Recombinant and Auxotrophic
- Attenuate M. TB – Auxotrophic
- Naturally attenuated/Avirulent – *M. vaccae*, *M. microtim*, etc.
- Subunit – natural, recombinant, synthetic
- DNA – CpG motifs, cationic lipids, heterologus host prime boosters
- Non-mycobacterial living vaccine vectors – *Salmonella*, vaccinia virus, adenovirus

- Vaccines with live, attenuated mycobacteria—these consist of BCG vaccines that have been genetically modified to contain the protective antigens of the *M. tuberculosis* or attenuated mutant strains obtained through genetic engineering. These may possibly be used as a booster to the BCG
- Subunit vaccines (DNA, Recombinant Natural, Synthetic virus vector vaccines or recombinant protein vaccines)—capable of carrying one or more immunodominant antigen the *M. tuberculosis*, that can have a protective effect. These may possibly be used to substitute the BCG vaccine. Some of the vaccine candidates showed promising results in studies that were carried out using animal models. These are still at phase I of the clinical tests and include:
- MVA85A (Recombinant Modified Vaccinia Ankara expressing Antigen 85A), Recombinant virus vector vaccine (attenuated vaccinia virus), expressing Ag 85 A in the *M. tuberculosis*. This is considered to be safe, and displayed good results in a population of noninfected and also previously BCG received guinea-pigs. In 2003, phase II of the study began in South Africa
- rBCG30—recombinant vaccine (Ag 85 B)
  In 2004, phase I of the study began in the United States
- Mtb72F—recombinant vaccine that includes polyproteins, obtained by combining two antigens (Mtb32 and Mtb39) that are recognized by the immune system of the infected patient. In 2004, phase I of studies began in the United States
- ESAT6 and Ag85B—recombinant vaccines that express polyproteins. In 2005, phase I of studies began in Europe.

7. What is the recent status of the clinical trials on leading new TB vaccines?

Results of the phase I trial of a leading new TB vaccine, MVA85A, appeared in the American Journal of Respiratory and Critical Care Medicine. MVA85A have shown that it is effective, safe, does not induce any immunopathology and is also immunogenic in the BCG-vaccinated individuals. This is the first subunit TB vaccine to enter clinical trials in *M. tuberculosis* infected subjects.

In 2009, atleast six different TB vaccine candidates both live and subunit vaccines, have completed initial phase I clinical trials, three are currently in phase II trials (Table 32.1). Most of the new vaccines will atleast initially be given as booster doses on top of a priming BCG vaccination. A virus vectored booster vaccine candidate has recently entered an early efficacy (phase IIb) trial and we would soon know if this vaccine provides an advantage over BCG. In an optimistic scenario, i.e., assuming that atleast one of the first generation candidates successfully completes phase III (efficacy) evaluation, licensure of a new TB vaccines is anticipated around 2014 to 2015. Until that time, extensive resources are needed to conduct reliable clinical trials in developing country settings. As with other vaccines targeting diseases of poverty, particular efforts in the areas of financing, production capacity logistics, etc., must be made to ensure that once a new TB vaccine is available, it can be implemented rapidly and up to high coverage rates.

TABLE 32.1 Selected promising TB vaccines

Vaccine candidate	*Example*	*Comment*	*Current status*	*Reference*
Subunit vaccine				
Antigen and adjuvent	Mtb 72 F	Skewed Ag profile	Ph I clinical trial in	J Immunol, 2004
	Fusion protein: Ag85-ESAT-6	Mild side effects	Healthy volunteer GMP production	Infect Immun, 2001
Naked DNA	Ag85 mTB 72f	CD4 and CD8 stimulation, Safety concern	Clinical Ph II started	Nat Med, 1996
Recombinant carrier expressing antigen	r-MVA expressing Ag85	CD4 and CD8 stimulation, Safety concern	Chemical PhI trial	Nat Med, 2004
Viable mycobacterial vaccine				
Mtb deletion mutant	PhoP/PhoQ-Mtb	CD4 and CD8 stimulation, Safety concern	Ph I trial completed	Nature, 2000
Auxotrophic mutants	Met, leu, ilv - BCG	Improved safety, Reduced efficacy	Used in HIV patients	Nat Med, 1996
Recombitant BCG expressing cytolysin	Aurease r-BCG expressing listerolysin	CD4 and CD8 stimulation, Safety concern	Clinical PhI started	Curr hiv Res, 2004
Recombitant BCG over expressing Ag	r-BCG-Ag85	Improved antigenicity esp CD4 cells	Clinical PhI ongoing	Proc Natl Acad Sci, 2000
Prime boost vaccination				
Based on BCG prime	BCG/protein (Ag85)r-MVA (Ag85)/Leprae	Improve efficacy of BCG prime		Infect Immun, 2001
Naturally attenuated NTM	*M. vaccae* *M. microti* *M. smegmatis*		Heat-inactivaed used in HIV patients	Indian J Pediatr, 2000
Non-Mtb vector	*Salmonella*, Vaccinia and Adenovirus	Improve efficacy of BCG prim		Int J Tub Lung Dis, 1999

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CHAPTER

33

Human Immunodeficiency Virus

Arun Kumar

1. What is the need to have an HIV vaccine?

It has been estimated that more than 33.4 million people are living with HIV/AIDS, with more than 2.5 million in India. HIV/AIDS is the world's leading infectious killer and was responsible for an estimated 2 million deaths in 2008. The extent of prevalence and fatality, therefore, warrants a necessity of the vaccine.

2. Do we already have a vaccine or any possibility to have one in future? Is there a reason for hope?

Currently, none is available but it is possible to have one as the virus is limited to a human host with no known animal reservoir, it is not highly infectious, and natural infection generates both antibody and T-cell responses (body's innate defense mechanisms which can be instigated/augmented by vaccine).

However, HIV also has many characteristics that make vaccine development difficult: subclinical cases and a carrier state, a long-term infection process, a nonspecific acute clinical disease, significant antigenic variation, viral DNA integration into the host genome, transmission through infected cells, and destruction or alteration of immunoregulatory cell function.

3. What is the reason for not having one till now?

Reasons—high genetic variability of the virus, the lack of immune correlates of protection, limitations with the existing animal models and logistical problems associated with the conduct of multiple clinical trials.

Also, there is a number of factors that cause development of an HIV vaccine to differ from the development of other classic vaccines:

- Classic vaccines mimic natural immunity against reinfection generally seen in individuals recovered from infection; there are almost no recovered AIDS patients
- Most vaccines protect against disease, not against infection; HIV infection may remain latent for a long-period before causing AIDS

- Most effective vaccines are whole-killed or live-attenuated organisms; killed HIV-1 does not retain antigenicity and the use of a live retrovirus vaccine raises safety issues
- Most vaccines protect against infections that are infrequently encountered; HIV may be encountered daily by individuals at high risk
- Most vaccines protect against infections through mucosal surfaces of the respiratory or gastrointestinal tract; the great majority of HIV infection is through the genital tract.

4. What is the progress in the development of HIV vaccine?

More than 35 vaccine candidates have been tested in Phase I/II clinical trials and two Phase III trials have been completed. Multiple vaccine concepts and vaccination strategies have been tested, including DNA vaccines, subunit vaccines, live vectored recombinant vaccines, and various prime-boost vaccine combinations.

Over the past year, scientists have identified neutralising antibodies against HIV, which are a fundamental building block for developing a vaccine. In addition, the Thai vaccine trial was a major breakthrough; for the first time ever, it was illustrated that we could have vaccine protection against HIV in humans. These are both game-changing developments.

5. Tell us more about this Thai HIV vaccine trial.

The Phase III HIV vaccine trial, also known as RV144, was the largest HIV vaccine study ever conducted in humans and involved more than 16,000 volunteers in Thailand. The trial tested a "prime-boost" combination of two vaccines: ALVAC® HIV vaccine (the prime), and AIDSVAX®B/E vaccine (the boost). The vaccine combination was based on HIV strains that commonly circulate in Thailand. Results of the trial show that this (ALVAC-HIV and AIDSVAX B/E) vaccine regimen may reduce the risk of HIV infection in a community-based population. The regimen was found to be safe and 31.2% effective at preventing HIV infection. While this is a modest level of efficacy, it represents a major step forward for HIV vaccines, providing the first evidence that development of a safe and effective preventive HIV vaccine is possible.

However, on the flip side, vaccination did not affect the viral load or CD4$^+$ count after HIV infection, and no immune correlates of protection (neutralizing antibodies, or IFN-gamma producing T cells) could be found.

6. How ethical are today's HIV vaccine trials?

Prevention trials are complex in nature — you are enrolling people at risk of HIV who are HIV-negative. But overall, today's trials are conducted incredibly well. UNAids and the World Health Organization have come up with good guidance on conducting clinical trials.

7. What is the way forward? Is it all despair and no hope?

Since HIV-1 was identified, development of a preventive vaccine has been a major goal. An effective HIV vaccine is a global health priority. Recently, four HIV vaccine trials failed to demonstrate efficacy and one that showed modest protection as a pathway forward. For the first time, an AIDS vaccine has shown modest protective efficacy in a human clinical trial.

Recent findings: The Merck Ad5 phase IIb T-cell vaccine failed to show efficacy and might have increased the risk of HIV acquisition in men who have sex with men. Although, VaxGen gp120 alone was not efficacious in groups at high risk for HIV-1 infection, the RV144 ALVAC prime and gp120 boost regimen showed 31% efficacy in low-incidence heterosexuals. All trials demonstrated the limitations of available laboratory and animal models to assess relevant vaccine induced immune responses and predict clinical trial outcome. Analysis of innate and adaptive responses induced in RV144 will guide future trial design.

Significant progress toward that goal has been made by 2010. In macaques, a vigorous T-effector cell response has protected some animals from disease caused by simian immunodeficiency virus (SIV). Broadly, neutralizing human anti-HIV antibodies have been isolated and their structures, and targets are rapidly being elucidated.

Future HIV vaccine trials should define the RV144 immune responses relevant to protection, improve durability and level of protection, and assess efficacy in diverse risk groups. New strategies examining heterologous vector prime-boost, universal inserts, replicating vectors, and novel protein or adjuvant immunogens should be explored to induce T-cell and antibody responses. HIV vaccine development requires innovative ideas and a sustained long-term commitment of scientists, governments, and the community.

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CHAPTER

34

Alzheimer's Disease

Vipin M Vashishtha, Ajay Kalra

1. What is Alzheimer's disease?

Alzheimer's disease (AD) is the most common form of dementia, affecting approximately 26 million people worldwide. While the major clinical symptoms include progressive memory loss, personality changes, language problems, and confusion, it is believed that the onset of two major pathological hallmarks of AD, extracellular amyloid-beta (Aβ) plaques, and intracellular neurofibrillary tangles containing hyperphosphorylated tau, precedes clinical symptoms by years.

In addition, to plaques and tangles, AD brain is characterized by gliosis, inflammation, neuritic dystrophy, neuron loss, and changes in neurotransmitter levels. Aβ protein, a 40- to 42-amino acid protein, is generated by proteolytic cleavage from the β-amyloid precursor protein (β-APP) by beta-secretase at its amino-terminus and by gamma-secretase at its carboxyterminus.

Currently, there are no effective treatments and no known means to prevent this devastating neurological disease. However, Aβ is now a major therapeutic target for AD because genetic mutations in APP and presenilin proteins 1 and 2 (PS1 and PS2), part of the gamma-secretase complex, are associated with AD in a small number of families.

2. What are the strategies to prevent AD or treat already established disease? What do the term 'immunotherapy against AD' denotes?

Current therapeutic strategies aim to lower production of Aβ by inhibiting or modulating beta- or gamma-secretase, prevent formation of Aβ aggregates and/or dissolve preformed aggregates, and enhance clearance of Aβ from the brain.

Aβ immunotherapy, one of the strategies under intense investigation, uses anti-Aβ antibodies by either active or passive vaccination to reduce Aβ deposition in the brain and enhance Aβ clearance. Hence, use of monoclonal antibodies against the end terminus of Aβ referred as passive immunotherapy while use of vaccines in generation of antibodies against amyloid-β is called active immunotherapy.

Attempts have been made to form a vaccine comprising of molecular compounds capable of disassembling the misfolded amyloid clumps to form smaller amyloid pieces which can then be cleansed from the brain more easily.

3. What was the outcome of first AD vaccine that underwent clinical human trials in 2001–02?

The first clinical trial of Aβ active immunotherapy, sponsored by ELAN/Wyeth, involved an active vaccine, AN1792, that contained Aβ1-42 synthetic peptide and a strong, T helper 1 (Th1)- biased adjuvant, QS-21. Although the phase I and IIa trials were deemed safe, the multisite phase IIb trial was halted in 2002 due to the occurrence of meningoencephalitis in approximately 6% (18/300) of AD patients worldwide. The cause of these adverse events is unknown but many have speculated that they may have been due to an autoimmune-like T cell response to Aβ, a self-antigen. Other possibilities include reformulation of the vaccine in polysorbate 80 and a strong proinflammatory Th1 response due to the adjuvant, QS-21. Only 19% of the immunized patients generated an Aβ antibody response, perhaps due to the limited dosing (1–3 inoculations). Importantly, the occurrence of meningoencephalitis was independent of antibody response.

The Aβ antibodies generated by AN1792 were shown to bind to the amino-terminus of Aβ and to human Aβ plaques and vascular amyloid but not soluble Aβ42. Tau levels in CSF were lower in antibody responders. Transient cortical shrinkage was observed by MRI in these same patients, although brain volumes resumed baseline levels after approximately 1 year following vaccination. In most of the patients who have come to autopsy since the initiation of the trial, plaque deposition was focally and regionally reduced. Some slowing of cognitive decline has been observed in antibody responders (International Conference on Alzheimer's Disease, Chicago, IL, 2008). However, two patients from an earlier Phase I AN1792 trial who came to autopsy several years later, generated Aβ antibodies, had very few plaques (suggestive of plaque clearance, and yet had severe dementia. A possible explanation for this finding may be that at the time of vaccination, the patients may have had substantial cerebral pathology, including neuron loss and neuritic plaques, which could not be reversed by the removal of Aβ plaques. Thus, the need for early intervention, possibly before the onset of symptoms, is critical in moving forward with Aβ-lowering treatments.

4. What was the experience with first passive immunotherapy trial against AD?

The first passive immunotherapy trial with bapineuzumab, a humanized monoclonal antibody against the end terminus of Aβ, also encountered some dose dependent adverse events during the phase II portion of the study, vasogenic edema in 12 cases, which were significantly over represented in ApoE4 carriers. The proposed remedy is to treat future patients with lower doses, particularly in the ApoE4 carriers.

5. What is the current status of passive immunotherapy trials against AD?

Several passive Aβ immunization trials are currently underway. The ELAN/Wyeth Phase II clinical trial results were reported at the 11th International Conference on Alzheimer's Disease (ICAD) in Chicago in July 2008. Intravenous administration of a humanized monoclonal antibody, bapineuzimab, recognizing the amino-terminus of Aβ showed a nonsignificant trend for cognitive stabilization in mild-to-moderate AD patients. Post-hoc analysis demonstrated significant cognitive benefits in multiple tests in Apo E4 noncarriers but only a trend in Apo E4 carriers, possibly due to accelerated pathogenesis in E4 carriers (ICAD, 2008). A Phase III trial is currently underway. Eli Lilly is currently testing a humanized monoclonal antibody that recognizes the mid-region of Aβ, and binds soluble Aβ protein.

In all, there are at least five ongoing anti-Aβ immunotherapy clinical trials. Three of the clinical trials use humanized monoclonal antibodies, which are expensive and require repeated dosing to maintain therapeutic levels of the antibodies in the patient. However, in the event of an adverse response to the passive therapy antibody delivery can simply be halted, which may provide a resolution to the problem. Because at this point we cannot readily identify individuals in the preclinical or prodromal stages of AD pathogenesis, passive immunotherapy is reserved for those that already have clinical symptoms. Unfortunately those individuals have by that point accumulated substantial neuropathology in affected regions of the brain. Moreover, if Aβ pathology drives tau pathology as reported in several transgenic animal models, and once established if tau pathology can become self propagating, then early intervention with anti-Aβ immunotherapy may be critical for favorable clinical outcomes.

6. What are the merits/demerits of active and passive immunization against AD?

Although, the first clinical trial for an active Aβ vaccine ended prematurely due to adverse events, preclinical and, to some degree, clinical studies indicate potential for this therapeutic strategy to prevent AD or stop it in early stages. A modified active vaccine would be less costly to prepare than humanized monoclonal antibodies, would require fewer doctor visits, and would be more feasible than passive immunization for the large population of individuals with or at risk for AD. The effects of an active vaccine would be longer lasting than passive administration of anti-Aβ antibodies whose half-life is typically around 30 days. However, the cellular immune response to an active vaccine may be difficult to stop, should problems arise, once it is underway. Thus, going forward, many labs have sought to develop second-generation active Aβ vaccines that target the B cell epitope in the Aβ amino-terminus and avoid an Aβ-specific T cell response directed at the mid-region or carboxyl-terminus of Aβ. Three such vaccines are currently being tested now.

7. What are the different types of AD vaccines currently under production?

Though active immunization with AN-1792, a mixture of Aβ1-42 peptide and adjuvant QS21 induced autoimmune encephalitis in humans. Surprisingly, although AN-1792 cleared senile plaque amyloid, it showed no benefit in humans. It is speculated that AN-1792 failed in deleting more toxic forms of Ab such as oligomers and intracellular Ab, suggesting that newly developing vaccines should delete these toxic molecules. Since, T cell epitopes exist mainly in the C-terminal portion of Aβ, vaccines using shorter N-terminal peptides are under development. In addition, since T helper 1 (Th1) immune responses activate encephalitogenic T cells and induce continuous inflammation in the central nervous system, vaccines inducing Th2 immune responses seem to be more promising. Improvement of vaccines will be also achieved by the administration method, because Th2 immune responses are mainly induced by mucosal or transcutaneous immunizations.

Many active Aβ vaccines now target the amino-terminus of Aβ to generate a strong humoral response and avoid an Aβ-specific T cell response, thought to account for the adverse effects in the AN1792 trial. As more and more preclinical and clinical data are collected, it appears that Aβ immunotherapy, like other Aβ-lowering therapies, may have its best efficacy if given before or in the early stages of cognitive decline, prior to massive neuritic dystrophy and neuronal loss.

So, currently vaccine candidates against AD can be categorized in the following four groups:

- Aβ peptide and non-Ab peptide vaccines (N-terminal short Ab peptides with Th2 adjuvant or Th2-stimulating molecules)*
- DNA vaccines**
- Recombinant viral vector vaccines
- Recombinant vegetables, bacteria, and phage vaccines.

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*These immunogens include dendrimeric A β-15 (16 copies of A β1-15 on a lysine antigen tree), 2xA β1-15 (a tandem repeat of two lysine-linked A β1-15 peptides), and 2xA β1-15 with the addition of a three amino acid RGD motif (R-2xA β1-15). Intranasal immunization with short A β fragment immunogens and a mucosal adjuvant, mutant Escherichia coli heat-labile enterotoxin LT(R192G), resulted in reduced cerebral A β levels, plaque deposition, and gliosis, as well as increased plasma A β levels and improved cognition in a transgenic mouse model of AD.

** The DNA vaccine does not contain beta-amyloid itself but instead a piece of the beta-amyloid gene that codes for the protein. In a recent study, the researchers coated tiny gold beads with the beta-amyloid DNA and injected them into the skin of the animals' ears. Once in the body, the DNA stimulated an immune response, including antibodies to beta-amyloid.

8. What are the pre-requisites of an ideal vaccine candidate against AD?

The future next generation Aβ vaccines should fulfill the following criteria:

- They should not induce auto-immune encephalitis, in other words they should activate Th2 T cells rather than Th1 T cells.
- They should be useful in prevention of the disease if given at an early stage.
- They should modify disease course and hopefully improve cognitive functions, if given after the disease progression mechanisms have started.
- They should be efficient in elderly people whose immune functions have deteriorated.
- They should not be painful and have good compliance.
- They should not be expensive.

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Section 5

Novel Approaches to Vaccine Formulations and Delivery Systems

Chapters

CHAPTER

35

DNA Vaccines

Puneet Kumar

1. What are DNA vaccines?

DNA vaccines are a new approach to immunization and immunotherapy in which, rather than a live or inactivated organism (or a subunit thereof), one or more genes that encode proteins of the pathogen are delivered. They actually contain DNA plasmids encoding the required antigenic proteins. When injected into the recipient, the plasmid DNA directly transfect the animal cells in vivo and elicit protective antibody and cell-mediated immune responses. These are also called "genetic-, nucleic acid-, naked DNA- or third generation-vaccines". The main differentiating feature of these vaccines from conventional vaccines is that there is endogenous synthesis of the encoded antigen rather than exogenous administration.

2. How DNA vaccines are developed and what are their mechanisms of action?

Using genetic engineering, the genes encoding the antigen of choice (and the necessary regulatory elements to express them) are inserted into a bacterial plasmids, which are readily and economically amplified in bacteria and recovered with a high degree of purity. When the plasmid is injected into the host, it transfect the host cells and expresses the required antigen *in vivo*. Thus, the antigen is presented to the host's immune system in a natural form, similar to live attenuated vaccine. This method of producing the protein antigens of interest directly in host cells can provide immunogenic proteins with correct posttranslational modification, conformation and oligomerization. This ensures the integrity of epitopes that stimulate conformationally specific neutralizing antibody (B cell) responses. Further, DNA (or RNA) immunization is exceptionally potent in stimulating T cell responses because antigenic peptides are efficiently generated in processing pathways without interference by viral proteins. Thus, DNA vaccines induce a full spectrum of immune responses that include cytotoxic T cells, T helper cells and antibodies. The immune response to DNA vaccines can be further enhanced by genetic engineering of the antigen to facilitate its presentation to B and T cells. Furthermore, the immune response can be modulated by genetic adjuvants in the form of vectors expressing biologically active determinants or by more traditional adjuvants that facilitate uptake of DNA into cells. The immune response of these vaccines can also be modulated by altering the route or method of DNA administration and by coadministration of cytokine genes with the gene encoding the antigen of interest.

There is another interesting property of plasmid DNA that adds to the efficacy of these DNA vaccines. Plasmid DNA has innate immunostimulatory activity of its own. Because it is made in bacteria, plasmid DNA has sequences known as *CpG motifs* that are recognized by the human immune system as foreign. Hence, plasmid itself stimulates nonspecific production of cytokines that increases the specific immune response directed against the encoded antigen.

The DNA plasmid is retained in the transfected cells in an unintegrated form for the life of the cell. The majority of transfected cells are eliminated, but residual expression has been detected for longer periods.

3. What are the advantages of DNA vaccines over conventional vaccines?

Following are the areas where DNA vaccines score over the conventional ones:

- Good immune response similar to live-attenuated vaccination but *without the possibility of contamination with undesirable agents*. In conventional vaccines, some viral proteins have undesired effects (for example, they downregulate immune responses) or act as decoys for the immune system (for example, regions of HIV Env that generate nonfunctional or highly strain-specific immune responses). On the other hand, the DNA vaccines, by providing only the genes that encode antigens against which an immune response is desired, can *induce immune responses in a more specific manner* than can a live virus vaccine.
- DNA vaccines induce both humoral and cellular immune response, unlike conventional vaccines that primarily induce humoral response only. Actually, DNA itself acts as a powerful “built-in” adjuvant that triggers several innate immune receptors to initiate the production of immune-stimulatory molecules, the maturation of antigen-presenting cells (APCs) and the induction of protective adaptive immunity. Thus, these vaccines are more likely to be effective for intracellular microorganisms (since only cell-mediated immunity can tackle them), such as the agents of tuberculosis, malaria, leishmaniasis, toxoplasmosis, brucellosis, trypanosomiasis, and AIDS. Induction of such antigen-specific cytolytic T-cell responses by DNA vaccines has been demonstrated in small animals like mice, nonhuman primates and more recently in human clinical trials.
- Since cytolytic T cells recognize epitopes derived from proteins, including conserved (often internal) proteins, a vaccine that *generates cytolytic T cells may protect against different strains.* This is very helpful in development of vaccines against pathogens like influenza with several strains and those like HIV that have great variability of outer (envelop) proteins. Ulmer et al. demonstrated

that vaccination of mice with DNA vaccine (derived from 1934 H1N1 strain) protected them from a lethal challenge of a strain that was not only of different subtype but also had arisen 34 years later (1968 H3N2 strain).

- The humoral immune response to a DNA vaccine is qualitatively better than that of conventional vaccine, since the antigen synthesized in vivo in the immunized host would more likely have the conformation and post-translational modifications made by that host during infection by the pathogen. For example, the glycosylation pattern of proteins produced recombinantly in bacterial or yeast cells might differ from the glycosylation pattern of proteins made in mammalian cells. Thus, DNA vaccine would induce more *specific humoral response.* In addition, a transmembrane protein (such as the gp160 Env protein of HIV) cannot be readily produced and purified in a recombinant manner, but can be produced *in situ* by the immunized host after DNA immunization.
- With live-attenuated vaccines, there is some risk of reversion of attenuated virus to pathogenic form. A prime example is reversion of live-attenuated polio vaccine, which can cause wild-type polio. An experimental example is the death of infant monkeys immunized with an attenuated simian immunodeficiency virus that had been safe and effective in adult monkeys. Such risks are obviously absent in DNA vaccines.
- Newborns are known to mount less vigorous adaptive immune responses than older children and adults to antigens and infectious agents with conventional vaccines. This delays the administration of many currently available vaccines until late infancy or early childhood. However, there is some evidence that DNA vaccines can *induce both humoral and cell-mediated antimicrobial immunity when administered within hours of birth*. Further, immune response induced by single DNA vaccination soon after birth is not transient, but is long-lasting: there is no need for secondary immunization (boosting).
- Combinations of DNA-encoded antigens and/or cytokines can be administered: One vaccine can potentially address multiple infections.
- Genetic stability.
- *Potential speed of making new vaccines* with genetic identity: Once a manufacturing and purification process for the plasmid DNA has been established, a similar process can be applied to a different DNA vaccine, since only the inserted gene will be different. In contrast, each attenuated, inactivated, or recombinant protein vaccine requires a unique manufacturing and purification process that is time- and labor-intensive. Thus, the average time required to develop a new DNA vaccine is cut down from 14–20 years to 8–10 years. Once the vaccine is developed and tested, the time to develop a new one against new strains (for example, in response to influenza pandemic) is just a few weeks. This makes these vaccines further cost-effective and ideal for situations like epidemics/pandemics and bioterrorist attacks.
- DNA vaccines can result in more long-term production of an antigenic protein when introduced into a relatively nondividing tissue, such as muscle. Thus, *"boosters" may not be required* as in case of conventional vaccines.
- Vaccines for *agents that cannot be grown in culture* can be developed with this technology.

- In most of the DNA vaccines, there is *no need for a cold chain*. Thus, DNA vaccines may be more suitable for worldwide distribution and can save lot of resources of the countries because of simplified logistics.
- Simplicity of manufacture facilitates construction of *vaccines against disease subsets or even for individual patients.*
- These vaccines allow modulation of the immune response, thus making "*therapeutic vaccines*" (*vide infra,* Q. 6*)* possible. With DNA vaccines, not only bacterial and viral infections, but also parasitic infections and noninfectious conditions like allergies, autoimmunity, Alzheimer's disease, atherosclerosis, chronic kidney disease, and malignancies, are in reach of these vaccines.
- DNA vaccines are also being used as a *laboratory tool* for a variety of applications ranging from proteomics to understanding antigenic presentation and cross-priming.

4. What are the disadvantages of DNA vaccines as compared with conventional vaccines?

Following are the potential disadvantages of DNA vaccines:

- Since this technology can introduce only those antigens that can be encoded by DNA, vaccines against carbohydrate antigens cannot be prepared with this technique.
- The success of DNA vaccination is not as straight-forward as that of conventional vaccines. The success of DNA immunization depends on a variety of parameters (e.g. type and number of antigen(s), route, method and site of application and usage of chemical and/or genetic adjuvants). Therefore, different strategies need to be explored to modulate the induced immune response with respect to the requirements necessary to protect against a specific pathogen (e.g. induction of mucosal or cell-mediated immunity).
- There are concerns with regard to the *persistence and distribution of inoculated plasmid* DNA in vivo.
- There is a *risk of inappropriate expression of antigens*. For example, if the antibiotic resistance genes present in plasmid DNA used for vaccination are not removed, it can be a major trouble.
- There is *theoretical risk of integration of plasmid* into the host genome and thus unforeseen consequences (However, the risk is miniscule, below that of spontaneous mutation frequency in humans). To obviate even this miniscule risk, "RNA replicon vaccines" are also being developed. These vaccines are self-replicating and self-limiting and may be administered as either RNA or DNA, which is then transcribed into RNA replicons in transfected cells or *in vivo*. DNA-based self-replicating RNA replicons eventually cause lysis (apoptotic death) of transfected cells. Thus these are called *suicidal DNA vaccines.*
- There is a risk of *induction of anti-DNA antibodies and auto-immunity*.
- Another risk is that of *induction of tolerance* (due to persistent expression of a foreign antigen), not seen with conventional vaccines. The suicidal DNA vaccines (*vide supra*) circumvent this risk, as expression mediated by these is

transient and lytic (as compared to classical DNA vaccines where the vectors are persistent and the expression constitutive).

5. What are second generation DNA vaccines?

Although the preclinical research successfully demonstrated the immunologic mechanisms of action and efficacy of DNA vaccines, the results in primates or large animals have not been as good as in small animals. The clinical results have been disappointing in the breadth and depth of the immune response and have shown that relatively high doses of plasmid DNA are needed to elicit a response. To overcome this hurdle, several strategies have been devised and tested. These strategies can broadly be grouped under five categories:

1. Enhancing in vivo delivery and transfection of DNA vaccine vectors
2. Improving DNA vaccine construct immunogenicity
3. Using chemical adjuvants to modulate and skew immune responses
4. Using DNA vaccines in combination with other types of vaccines
5. Modifying professional APCs to enhance DNA vaccine potency

DNA vaccines developed using above strategies are called *Second Generation DNA Vaccines.*

- Enhancing *in vivo* delivery and transfection of DNA vaccine vectors
  - One technique is *in vivo electroporation (EP) (also called electro-gene-transfer or electropermeabilization)* where an externally applied electrical field is used to increase the permeability of the cell membrane. This has been found to be highly effective in delivering DNA vaccines intracellularly. Although the mechanism remains unknown, it is speculated that transient pores on the cell surface could lead to enhanced antigen expression and transient tissue damage may lead to the recruitment of inflammatory cells and production of cytokines. Electroporation has also been used as "prime/boost" strategy for naked DNA vaccination, where naked DNA vaccination followed by EP dramatically increased antibody levels.
  - Another technique is to coinject a *particulate adjuvant*, like micron-size gold particles (which do not bind or adsorb DNA) or other cationic microparticles along with the DNA vaccine to improve the delivery of vaccine. Immunization is done by bombardment of skin with plasmid DNA coated onto microscopic gold particles using a gene gun. The underlying mechanism is possibly particles acting as attractant for immune cells, especially APCs. This reduces the immune response time and may increase vaccine efficacy.
  - Epidermis is known to be rich in rich in both professional APCs and accessory cells, which are capable of producing immunostimulatory cytokines. However, administration of vaccines into the epidermis is difficult to achieve using conventional vaccine delivery methods employing a needle and syringe, and vaccine antigens are too large to be effectively delivered using standard topical formulations. Thus, a needle-free vaccine delivery system has been developed that efficiently delivers powdered DNA vaccines into the epidermal tissue. A number of such systems have been

developed. In *epidermal powder immunization*, the protein antigens are formulated into a powder and delivered into the extracellular environment where they are picked up by APCs. Other systems to deliver these vaccines effectively intradermally are CO_2-powered Biojector® device, various types of microneedles and even skin-tattooing device.

- Mucosa is an important barrier to infections, as it is a common portal of entry of most pathogens. The "mucosal immune system" is comprised of specialized mucosal immune cells that accumulate or are in transit between various mucosa-associated lymphoid tissues (MALT). The MALT is a highly compartmentalized immunological system that functions independently of the systemic immune apparatus. It is comprised of anatomically defined sites that serve as mucosal inductive sites for initiation of immune responses. Because these sites are anatomically separated but functionally connected, the induction of an immune response at one site can lead to an effector response at a different mucosal site mediated by homing receptors on induced memory T and B cells. This feature is important for the development of vaccines against mucosal pathogens, such as HIV, since effective immune responses able to protect from infection and control viral replication may be needed at different anatomical locations as well as at systemic sites of virus spreading and replication. Thus, mucosal route (oral, intranasal, rectal, and vaginal) is also being tried as an alternative for better delivery of DNA vaccines.
- Another technique to enhance immune response to DNA vaccines is "*cluster intradermal DNA vaccination*". It has been shown that cluster (short-interval) DNA vaccination regimen generates potent immune responses in a minimal time frame.

- Improving DNA vaccine construct immunogenicity

Three complementary strategies have been used to improve and modulate the immune response induced by DNA vaccines: (i) supplementing DNA vaccines with plasmids encoding cytokines; (ii) targeting the antigen encoded by DNA vaccine through genetically fusing the antigen to molecules binding cell surface receptors; and (iii) targeting multiple antigens.

Cytokines like interferon (IFN)-alpha, IL-2, IL-6, IL-7, IL-12, IL-15, or IL-22 and costimulatory molecules like B7-1, HSP70, ICAM-1, GM-CSF, and LIGHT [a new member of tumor-necrosis factor (TNF) superfamily] have been evaluated as adjuvants for DNA vaccines because they influence the host immune response favorably.

To overcome the problem of poor delivery of the antigen into the class I antigen presentation pathway, some authors have attempted a novel approach. They used completely engineered peptide/major histocompatibility complex (MHC) class I complexes whereby all three components (class I heavy chain, beta(2)m, and peptide) were attached by flexible linkers and expressed as a single polypeptide (single chain trimers or SCT). They found such genetically engineered vaccines co-expressing a universal CD4 epitope are highly effective in generating immune responses to cancer antigens, which, being "self-antigens" rather than foreign ones, are otherwise poorly processed and presented. This approach was also successful in inducing protective CD8(+) T cell responses against lethal West Nile virus infection. Similarly, vaccines can be developed targeting MHC class II in case of infections like influenza, where antibody response is more important than T-cell response.

Another approach used for improving immunogenicity of vaccines against malignancies is to develop "*DNA fusion vaccines*" in which DNA encoding tumor antigens (or any other desired antigen) is fused to pathogen-derived sequences (for example, that of tetanus toxoid, which acts like a *"genetic adjuvant"*). This strategy activates high levels of T-cell help, the key to induction and maintenance of effective immunity. Similar approach has been used to develop better DNA vaccines against infectious agents also. DNA encoding the required antigen is fused to one encoding an IgG Fc fragment. After vaccination, the DNA is taken up by cells that produce and secrete the antigen-Fc fusion proteins. The secreted fusion proteins, in addition to inducing B cells, are efficiently captured and processed by dendritic cells via receptor-mediated endocytosis and then presented to the MHC class II and as -I (cross-priming), thus making the vaccine highly immunogenic. In yet another approach, DNA encoding the desired antigen is fused to sequences derived from antibodies to surface antigen(s) of APCs that helps in targeted delivery and hence better immune response of the vaccine.

Addition of a DNA vaccine capable of generating high numbers of pan-HLA-DR reactive epitope (PADRE)-specific CD4(+) T cells also improves immune response to tumor antigens significantly. Similarly, intramuscular coadministration of DNA encoding xenogenic MHC class-I can further improve the antigen-specific immune responses, as well as antitumor effects generated by DNA vaccines through enhancement of cross-priming mechanisms.

It has also been shown that DNA vaccine encoding multiple surface antigens or a combination of surface antigen and nonstructural protein antigen has better immunogenicity than DNA vaccines encoding single antigen. In pathogens like *Plasmodium,* which has several different stages in life cycle in humans, constructing DNA vaccine encoding antigens of different stages (or a mixture of DNA vaccines encoding different antigens) also improves immune response of the vaccine.

With advances in genetic engineering and molecular biology, more and more complex "designer" DNA vaccines are now being developed. For example, Meerak et al. incorporated an autophagy inducing system (autophagy is a physiologic process involved in removing cytoplasmic constituents, including organelles and intracellular pathogens) in DNA vaccine against *Mycobacterium tuberculosis*. This resulted in significantly improved the immunogenicity of the vaccine. Kong et al. specifically developed recombinant attenuated *Salmonella* vaccine (RASV) strain to be used as a delivery vehicle for DNA vaccines. The strain was further modified genetically to induce delayed attenuation and programmed self-destruction of the bacteria so as that the bacterial cell contents are released after lysis in the lymphoid tissue: thus deliver the vaccine effectively.

- *Using chemical adjuvants to modulate and skew immune responses*

A number of adjuvants have been designed to improve plasmid DNA immunogenicity, either by directly stimulating the immune system or by enhancing plasmid DNA expression. Formulation of plasmid DNA with *cationic lipids, monophosphoryl lipid A*, or *QS-21, encapsulated in poly(lactide-coglycolide)* *microparticles,, macroaggregated polyethyleneimine-albumin conjugates, biodegradable alginate microspheres or alginate-coated chitosan nanoparticles* enhance the expression and immunogenicity of plasmid DNA vaccines. They also induce significant secretory IgA responses at the mucosal sites.

Some pathogen-derived proteins have also been used as novel adjuvants.

In addition to above, cimetidine and levamisole are two pharmacologic agents that are known to enhance humoral and cellular responses to DNA vaccines via different mechanisms, including inhibition of suppressor T cells. Hence, they have been used as adjuvants to DNA vaccines, especially for hepatitis B vaccine.

- *Using DNA vaccines in combination with other types of vaccines*

In recent years, the heterologous prime-boost vaccination strategy has demonstrated considerable advantages over the classical vaccine strategies based on homologous prime-boost strategies. Thus, recent studies have focused on using DNA vaccines in combination with other types of vaccines. Such an approach is called "mixed-modality vaccine". If a DNA vaccine encoding an antigen is given as a prime, followed by a different type of vaccine as a boost (for example, a viral vector encoding the same protein), the immune responses or protection observed may be enhanced compared with use of either vaccine alone. Such a strategy can elicit immune responses that differ in magnitude, quality, and balance of cellular and humoral responses from those elicited by single components and thus provide further enhancement for DNA immunizations. For example, when monkeys were first given plasmid DNA encoding HIV Env, then were boosted with recombinant Env protein, they were protected against subsequent infection with a chimeric simian–human immunodeficiency virus. Mice that were immunized with DNA encoding malarial antigens and then boosted with recombinant vaccinia (a strain known as *modified vaccinia virus Ankara*) expressing the same antigens had 100% survival against a subsequent challenge with the parasite. In contrast, the challenge caused substantial mortality among animals given the DNA vaccine alone, the modified vaccinia virus Ankara vector vaccine alone, or the combination of these vaccines in the reverse order. Similarly, increased protective efficacy of DNA vaccines has been demonstrated in other animal studies when used in combination with modified vaccinia virus Ankara or another poxvirus vectors. This has further been proved recently in a randomized, double-blind, placebo-controlled phase I trial in healthy human volunteers. He et al. have recently demonstrated utility of DNA vaccine in augmenting the efficacy of a different type of vaccine in mouse model of melanoma. Studies, including human clinical trials, are now being conducted to further fine-tune this strategy of use of combination of DNA vaccine with other vaccines for most optimal immune response. One of such human trials of HIV candidate vaccine has even been conducted in India (Chennai). However, the concerns that still need to be addressed are: (i) how long are these responses maintained after the MVA boost; and (ii) whether continued boosting will be required for sustaining a sufficient number of cells to confer protection.

Another strategy in focus in recent years is *DNA prime-protein boost strategy.* Many workers have succeeded in boosting the initial immune response of DNA vaccines by administering corresponding recombinant protein subsequently.

In an alternative approach, a different type of vaccine can be coadministered with DNA vaccine to improve immunogenicity of latter. For example, oral live attenuated *Salmonella* vaccine has been coadministered with DNA vaccines to improve their immunogenicity.

Tretyakova et al. developed yet another strategy combining the benefits of DNA vaccine and live attenuated vaccines. They termed this as "*iDNA vaccine technology*". The iDNA vaccine is based on transcription of the full-length genomic RNA of the live-attenuated virus from plasmid DNA *in vivo*. The *in vivo* generated viral RNA initiates limited replication of the vaccine virus, which in turn leads to efficient immunization. The authors applied this technology for vaccination of mice against infection with Venezuelan equine encephalitis virus (VEEV) and obtained excellent results.

- *Modifying professional antigen-presenting cells to enhance DNA vaccine potency*

These approaches include strategies to increase the numbers of antigen-expressing DCs, strategies to enhance MHC class I and/or II presentation of the encoded antigen and strategies to prolong the life of antigen-expressing DCs to enhance DNA vaccine potency.

6. How do DNA vaccines help in TREATMENT of chronic diseases?

DNA vaccines have taken vaccinology beyond prevention of infectious diseases, as they have the potential to have therapeutic effect in many chronic conditions like chronic infections, allergies, autoimmune disorders, graft-versus-host disease (GVHD), multiple sclerosis, and malignancies. The concept behind these "therapeutic vaccines" is that these vaccines induce a full spectrum of immune responses that include cytolytic T cells, T helper cells and antibodies, and the immune response can be manipulated as per requirement with relative ease. Further, they have the advantage of being simple to construct, produce and deliver. Thus, these vaccines represent a practical means of manipulating inappropriate or ineffective immune response occurring during immune-inflammatory diseases.

These vaccines are obviously helpful in chronic infections due to persisting virus genomes, such as recurrent herpes (HSV-1 and HSV-2), pre-AIDS (HIV-1), chronic hepatitis B (HBV), human papillomavirus (HPV) and tuberculosis as focused killing of infected cells is possible with these vaccines. In these, even the problem of introducing foreign viral DNA may not be of crucial importance, since the recipient is already a viral DNA (or provirus) carrier.

Pathogens (viral/bacterial/fungal/parasitic) causing chronic infections have one thing in common: all of these have developed one or the other way to withstand immune attack and establish chronic infections by suppressing host immunity. Thus, it's difficult to develop "classical vaccines" against these organisms. Vaccines against these pathogens need to counter parasite-driven "immunomodulation" to be effective. One such approach is to develop a DNA vaccine against the immunomodulatory molecules expressed by the pathogen to escape the immune attack. Proof of this concept has recently been demonstrated successfully by Babayan et al. who developed such a DNA vaccine against Filarial nematodes. The vaccination resulted not only in suppression of parasite-driven immunosuppression, but also enhancing Th2 immune responses and parasite antigen presentation by dendritic cells.

Similarly, these vaccines have been used to generate protective immunity against tumors in a variety of experimental models. The favorite target antigens have been those that are frequently expressed by human tumors, such as p53, phosphatase of regenerating liver (PRL)-3, carcinoembryonic antigen (CEA), ErbB2/neu, melanoma-associated antigens and androgen receptor. Compared to other nucleic acid vectors, DNA vaccines are usually devoid of viral or bacterial antigens and can be designed to deliver only the target tumor antigen(s). This is likely to be important when priming a response against weak tumor antigens. DNA vaccines have been more effective in rodents than in larger mammals or humans, since the tumour antigens are usually weakly immunogenic. However, there are a large number of methods that can be applied clinically to enhance immunogenicity of these vaccines. The prerequisite for a successful antitumor vaccination is breaking tolerance to tumor-associated antigens, which represent "self-antigens." Currently, immunization with xenogeneic DNA to induce immune responses against self-molecules is under intensive investigation. Tumor cells can develop immune escape mechanisms by generation of antigen loss variants, therefore, it may be necessary that DNA vaccines contain more than one tumor antigen. Polyimmunization with a mixture of tumor-associated antigen genes may have a synergistic effect in tumor treatment. Other techniques to enhance immunogenicity of DNA vaccines include *in vivo* electroporation, and/or inclusion of various immunostimulatory molecules, antigen-cytokine fusion genes, agents that improve antigen uptake or presentation, and molecules that activate innate immunity mechanisms. In addition, CpG motifs carried by plasmids can overcome the negative effects of regulatory T cells (For details, *vide supra,* Q. 5). Some candidate vaccines using these strategies are now undergoing human trials with encouraging results.

The ideal immunological target for cancer vaccine development should meet the criteria of tumor specificity, immunogenicity and vital dependency of the tumor on the functional activities of the antigenic target so as to avoid antigenic loss by mutation. Brother of the regulator of imprinted sites (BORIS) is one such "universal" tumour antigen on which a lot of research is focused today. BORIS was previously described as a transcription factor for epigenetic reprogramming the expression of which is strictly confined to germ cells of adult testes but is aberrantly activated in the vast majority of neoplastic cells. Potent anticancer CD8(+)-cytotoxic lymphocytes have been demonstrated after immunization with the DNA-based BORIS vaccine. These cytolytic responses were observed across a wide range of different mouse cancers including mammary adenocarcinoma, glioma, leukemia, and mastocytoma.

DNA vaccines may also be useful in the prevention of tumors with genetic predisposition. By immunization against chronic infections, these vaccines would also help in preventing virus-induced malignancies (like hepatocellular carcinoma secondary to chronic hepatitis B infection).

DNA vaccine encoding a major house dust mite allergen (Der p 2) has been shown to prevent the development of house dust mite allergy through Th1-skewed immune response characterized by the drastic reduction of allergen-specific

IgE, interleukin (IL)-5 and lung inflammation together with the induction of strong specific IgG2a titers and IFNg secretion.

Bronchial asthma is yet another noninfectious condition, which might be amenable to DNA vaccines in future. Song et al.recently successfully demonstrated a DNA vaccine against calcium-activated chloride channel (CLCA), which are upregulated in asthmatics and play a significant role in its pathogenesis. The vaccination resulted in development of autoantibodies against CLCA and ultimately led to reduction in pathological changes in lungs, including a remarkable reduction in the air pressure-time index of the trachea, the number of eosinophils and mast cells in the bronchoalveolar lavage fluid in mouse model of asthma.

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CHAPTER

36

Chimeric Vaccines

Puneet Kumar

1. What are chimeric vaccines?

Chimeric vaccines are an emerging class of genetically engineered vaccines, developed using a *chimeric gene*. A chimeric gene is an artificial gene constructed by juxtaposition of fragments of unrelated genes or other DNA segments, which may themselves have been altered.

2. How does this technology help in developing better vaccines?

This technology has been used in at least five different ways to develop new, better vaccines:

1. A well known, safe and effective vaccine virus is used as a vector to carry genes of a lesser immunogenic virus, so as to improve the efficacy of vaccine against latter. For example, a chimeric virus (ChimeriVax-JE virus) has been constructed using yellow fever (YF) 17D vaccine virus with heterologous genes from Japanese encephalitis virus (JEV). ChimeriVax-JE virus has been used to develop a very effective vaccine against JE.
2. This technology has also been used to develop chimeric genes that express epitopes of more than one antigen of the same pathogen, in cases where there is antigenic variability. For example, factor H-binding protein (fHbp) is a novel meningococcal vaccine candidate, but one limitation of fHbp as a vaccine candidate is antigenic variability, since antibodies to fHbp in the variant 1 (v.1) antigenic group do not protect against strains expressing v.2 or v.3 proteins, and vice versa. Hence, recombinant chimeric protein has been developed that expressed epitopes from all three variant groups.
3. Chimerization has also been used to achieve attenuation of live vaccine candidates as in tetravalent dengue vaccine.
4. Vectors for vector-based vaccines have also been engineered using chimerization so as to overcome the problem of pre-existing anti-vector immunity in these vaccines.

5. Chimerization, as a technology, has also been used to study to track antigen specific immune responses, to assess their protective capacity and address other questions related to immune response to a specific pathogen. This ultimately helps in development of better, “targeted” vaccines.

3. What are the agents against which chimeric vaccines are being developed?

Most of the work on chimeric vaccines has been done on using Yellow fever (YF) 17D vaccine virus to develop effective vaccines against other medically important flavi viruses like Japanese Encephalitis (JE), Dengue fever, West Nile Fever and tick borne fever.

For *JE,* a chimeric was constructed in 1999 by replacing the genes encoding two structural proteins (prM and E) of yellow fever 17D virus with the corresponding genes of an attenuated strain of JEV, SA14-14-2. Since the prM and E proteins contain antigens conferring protective humoral and cellular immunity, the immune response to vaccination with progeny virus (named ChimeriVax-JE virus) is directed principally at JE. It has been demonstrated to be genetically stable and is able to induce a rapid humoral immune response and to protect against a very severe, direct intracerebral JE virus challenge in mice and non-human primates. It appears safer (lower neurovirulence) than yellow fever 17D vaccine but has a similar profile of immunogenicity and protective efficacy. ChimeriVax-JE, now known as JE-CV or IMOJEV® and manufactured by Acambis/Sanofi-Aventis has proven safe, immunogenic and effective in a number of human trials. Most of initial trials involved adult volunteers, but now has been tested in both adults and children more than 9 months of age. This vaccine has recently obtained marketing authorization in some countries in South-East Asia and Australia. It has an indication as a one-dose vaccine, and booster requirements remain to be determined. Another chimeric vaccine against JE is being developed using a new type of flavivirus vaccine, a pseudoinfectious virus (RepliVAX WN) that prevents West Nile virus (WNV)-induced disease. A chimeric RepliVAX (RepliVAX JE) has been developed by replacing the WNV prM/E genes with those of JEV. The initial prototype RepliVAX JE failed, but was genetically modified further to develop a second-generation RepliVAX (RepliVAX JE.2). RepliVAX JE.2 elicited neutralizing antibodies in both mice and hamsters and provided 100% protection from a lethal challenge with JEV or WNV, respectively. RepliVAX JE.2 has been further improved by replacing WNV NS1 gene in RepliVAX JE with that of JEV (producing TripliVAX JE). This has elicited higher anti-E immunity and displayed better efficacy in mice than RepliVAX JE in initial trials. Furthermore, TripliVAX JE displayed reduced immune interference caused by pre-existing anti-NS1 immunity.

Using conventional technologies for development of vaccine against *dengue fever* is fraught with numerous challenges: absence of an animal model; need to develop a live attenuated vaccine; existence of 4 antigenically distinct serotypes with the resulting risk of competition between vaccine strains; immunologic risks related to antibody-dependent enhancement that has been hypothesized to be the cause of severe forms of the illness; absence of a well defined correlate of protection and preexisting vaccine; complexity associated with industrial production of a tetravalent vaccine. To overcome some of these problems, researchers have used an approach identical to that of ChimeriVax-JE virus vaccine to construct chimeric virus ChimeriVax-D2. This vaccine is currently undergoing phase III clinical trials. Other strategies that have been tried for construction of better dengue vaccine are:

a. Using recombinant dengue viruses and achieving attenuation by antigenic chimerization between two related dengue viruses (DENV-DENV Chimera, Inviragen)
b. Using Dengue 2 PDK-53 virus as a chimeric carrier (DENVax vaccine). This vaccine has shown promising results in mice and non-human primates.
c. Using DENV-4 containing a 30-nucleotide 32 non-coding region (NCR) deletion.
d. Using RepliVAX technology to produce a dengue vaccine by replacing the prM/E genes of RepliVAX WN (a WNV RepliVAX) with the same genes of dengue virus type 2 (DENV2). Here also, the initial prototype RepliVAX D2 failed, but was genetically engineered to develop a second-generation RepliVAX D2 (designated RepliVAX D2.2) and subsequent results have been promising.

For *Meningococcal disease,* fHbp is a novel target for vaccine development. It is a principal antigen in a multicomponent meningococcal vaccine recently licensed in Europe (4CMenB vaccine: Bexsero®) for prevention of serogroup B diseases. The protein specifically binds human complement factor H (fH), which downregulates complement activation on the bacterial surface and enables the organism to evade host defenses. Anti-fHbp antibodies can bind to meningococci and elicit complement-mediated bactericidal activity directly. The antibodies also can block binding of the human complement down-regulator, factor H (fH). However, one limitation of fHbp as a vaccine candidate is antigenic variability, since antibodies to fHbp in the variant 1 (v.1) antigenic group do not protect against strains expressing v.2 or v.3 proteins, and vice versa. Further research on fHbp molecule has given lot of information on the locations of the fHbp epitopes important for eliciting bactericidal antibodies. It has been found that one epitope expressed by nearly all v.1 proteins mapped to the B domain, while epitopes expressed by fHbp v.2 or v.3 mapped to the C domain. Thus, a chimeric fHbp molecule has been engineered that contains the A domain (which is conserved across all variant groups), a portion of the B domain of a v.1 protein, and the carboxyl-terminal portion of the B domain and the C domain of a v.2 protein. The resulting recombinant chimeric proteins expressed epitopes from all three variant groups. In mice, the chimeric vaccines elicited serum antibodies with bactericidal activity against a panel of genetically diverse strains expressing fHbp v.1, v.2, or v.3. The data demonstrate the feasibility of preparing a meningococcal vaccine from a single recombinant protein that elicits broad bactericidal activity, including group B strains. Work is underway to design a second generation chimeric fHbp that may elicit higher antibody responses than the first generation chimeric vaccine. More than 610 fHbp amino acid sequence variants have been identified, and efforts are underway to find out the best one for vaccine development. Another strategy being tried is construction of mutants of *Neisseria meningitidis* that express chimeric fHbp, which can be used to prepare native OMV (Native Outer Membrane Vesicle) vaccines that may elicit higher and broader anti-fHbp bactericidal antibody responses than the recombinant chimeric fHbp vaccines.

Human cytomegalovirus (HCMV) is another pathogen against which a successful vaccine formulation using conventional technologies remains elusive. Live attenuated HCMV candidate vaccines based on the Towne strain are well-tolerated but have demonstrated only moderate efficacy in clinical settings. Hence, Heineman et al. attempted using chimeric technology to produce live HCMV vaccine candidates that retain the excellent safety profile of the Towne strain but are more immunogenic. They recombined genomes of the Towne strain and the unattenuated HCMV Toledo strain to yield four independent chimeric vaccine candidates. The initial results of these Towne/Toledo chimeric vaccine candidates have been encouraging. More recently, Zhong et al. have tried another strategy. They designed a vaccine which included the extracellular domain of HCMV-encoded glycoprotein-B (gB) covalently linked to multiple HLA class I- and class II-restricted T-cell epitopes from multiple HCMV antigens as a contiguous polypeptide in a replication-deficient adenoviral vector Ad5/F35 (referred to as Ad-gBCMVpoly). This vaccine induced both humoral and cellular immune response following a single dose by generating strong gB-specific neutralizing antibody and a broad range of HCMV-specific pluripotent CD8+ and CD4+ T-cells.

For *Leishmaniasis* also, a chimeric therapeutic vaccine is being developed using three recombinant leishmanial antigens (LeIF, LmSTI-1 and TSA) in the form of a fusion protein combined with monophosphoryl lipid A in squalene oil as adjuvant. Phase I trials of this vaccine in healthy volunteers in the USA and initial efficacy testing as a therapeutic vaccine in patients in Latin America suggest the safety and immunogenicity of the vaccine.

Chimerization is one of the approaches being used to develop vaccine against human immunodeficiency virus (HIV). Researchers have used major antigenic component in influenza, hemagglutinin (HA) to construct phenotypically mixed, chimeric influenza HA/SHIV 89.6 VLPs. These chimeric virus like particles were used to induce immune responses in CD4(+) T cell knockout (CD4 KO) mice. The results suggested a potential approach for mucosal immunization for prevention of HIV infection, novel adjuvant role of influenza HA and a new strategy to develop more effective therapeutic vaccines for acquired immunedeficiency syndrome (AIDS) patients with low CD4(+) T cell counts.

This technology has also been used to develop DNA vaccines for even non-infective conditions like *Alzheimer's disease* and *malignancies* like B-cell lymphoma and multiple myeloma.

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CHAPTER

37

Edible Vaccines

Puneet Kumar

1. What are edible vaccines?

Edible vaccines are emerging class of genetically engineered subunit vaccines, in which transgenic plants are used not only as a protein production system, but as an antigen transportation system as well, being capable of delivering antigens to the mucosal immune targets.

2. What is the concept behind edible vaccines?

Vaccination is the most effective and affordable strategy to prevent, control and eradicate infectious diseases. Conventional vaccines, based on live-attenuated or inactivated pathogens, despite being the most effective currently available tools are fraught with several disadvantages like need of strict cold chain, risks associated with incomplete inactivation or reversion to pathogenic form, limited production capacity, risks and logistic problems associated with injections and high cost. Although subunit vaccines developed in the last century circumvent some of these problems, there are still some issues with each of them. For example, the antigens expressed by prokaryote systems may lack native conformation or post-translational modification and there is always a risk associated with use of live vectors. From parents' perspective, the worst thing about vaccines is the need of being injected to their healthy little ones.

Edible vaccines are an interesting concept in this regard. Stable integration of a gene into the plant nuclear or chloroplast genome can transform higher plants (e.g. tobacco, potato, tomato and banana) into bioreactors for the production of subunit vaccines for oral or parental administration. This can also be achieved by using recombinant plant viruses as transient expression vectors in infected plants. It has been shown that such antigens retain their native immunogenic properties. For example, transgenic potato tubers expressing a bacterial antigen [binding subunit of *Escherichia coli* heat-labile enterotoxin B subunit (LT-B)] stimulated humoral and mucosal immune responses when they were provided as food to mice. These results provide "proof of concept" for the use of plants as a vehicle to produce vaccines. These vaccines have been shown to be safe and immunogenic without the need for a buffer or vehicle other than the plant cell. Improved understanding of plant molecular biology and consequent refinement in the genetic engineering techniques have led to designing approaches for high level expression of vaccine antigens in plants. In addition, adjuvants and targeting proteins have increased the immunogenicity of mucosally administered plant-made vaccines. During the last decade, several efficient plant-based expression systems have been examined and more than 100 recombinant proteins including plant-derived vaccine antigens have been expressed in different plant tissues. Some of these have now been tested in animals and even human beings.

Oral vaccines must be formulated in such a way that antigens are protected as they pass through the adverse environment of the stomach and are delivered to the mucosal inductive sites. Thus, dose levels for protein subunit vaccines are likely to be very high and the antigen may need to be protected from proteolysis for oral delivery to be efficacious. In edible vaccines, the rigid walls of the plant cell (bioencapsulation) protect antigenic proteins from the acidic environment of the stomach; enabling intact antigen to reach the gut associated lymphoid tissue. Thus, expression of candidate vaccine antigens in edible recombinant plant material offers an inexpensive means to deliver large doses of vaccines in encapsulated forms.

3. What are the advantages of edible vaccines over conventional subunit vaccines?

These vaccines offer following advantages:

- *Lower cost of production and delivery*: Edible vaccines are significantly cheaper and easier to produce, distribute and consume. As the fruits of this technology are realized, the whole landscape of vaccination and infectious diseases may change. For example, in case of malaria, conventional wisdom suggests that the immune mechanisms responsible for protection will require a multiple (possibly 10–15) antigen targets for proper protection against various stages of malarial infection. By standard vaccination protocols, such a large number of targets would not be appropriate to be used for vaccination as a single dose due to antigenic competition. It would be almost impossible to immunize over 2 billion individuals who live in malaria susceptible areas with several carefully crafted immunization schedules delivered 4–6 weeks apart in the form of two different antigens as a single dose. Besides, if immunization schedules could be arranged, the stability of vaccines carrying different malarial antigens, their transport and the logistics of vaccination would be an almost impossible task to achieve under the current fiscal constraints. Chowdhury et al. have proposed a unique way to circumvent these logistical difficulties to deliver the malaria vaccines to every susceptible home at a small fraction of a cost. They hypothesized that the anti-malaria edible vaccines may be developed using transgenic tomato plants where different transgenic plants expressing different antigenic type(s). Immunizing individuals against two to three antigens and against each stage of the lifecycle of the multistage parasites would be an efficient, inexpensive and safe way of vaccination. Tomatoes with varying sizes, shapes and colors carrying different antigens would make the vaccines easily identifiable by lay individuals.

- *Easily scalable production*: Estimates suggest that it may become possible to obtain antigen sufficient for vaccinating millions of individuals from 1 acre crop by expressing the antigen in seeds of an edible legume, like peanut or soybean. In particular, plant virus-based transient expression systems are suitable for rapid engineering, ease of scale-up and cost-effective production of target antigens and provide an ideal approach for producing large quantities of vaccine antigens in a short period of time. Thus, they are ideal for tool to counteract accidental or intended release of bio-threat agents, such as Bacillus anthracis, Yersinia pestis and variola virus, to control natural outbreaks and for rapid-response vaccines for emerging viruses with pandemic potential.
- *Convenience of delivery*: Edible vaccines eliminate risks and logistic difficulties associated with injections. For example, the currently available vaccines against diphtheria, pertussis and tetanus are of proven efficacy, but six injections are required for every child as per immunization schedule recommended by Indian Academy of Pediatrics. One can well imagine the benefits to the society if edible vaccine becomes available. Results of early trials on such a vaccine developed in transgenic tobacco, tomato and carrot plants are encouraging.
- No need of cold chain
- *Induction of humoral and cell-mediated responses*: Since mucosal immunization induces cell-mediated immune response in addition to humoral response, these vaccines are more likely to be effective for intracellular microorganisms (that require cell-mediated immunity) such as the agents of tuberculosis, malaria, leishmaniasis and human immunodeficiency virus (HIV) infection. Most pathogenic microorganisms enter their host via the mucosal surfaces lining the digestive, respiratory and urino-reproductive tracts of the body. The apparent linked nature of the mucosal immune system allows delivery to any mucosal surface to potentially induce immunity at others. Thus, mucosal immunization would be at an advantageous position to confer protection against infectious diseases. For example, development of a mucosal vaccine would be beneficial against sexually transmitted infections like hepatitis B virus (HBV) and human papillomavirus (HPV). Currently used subunit HBV vaccines based on hepatitis B surface antigen (HBsAg) synthesized in yeasts or mammalian cell culture have already proved their worth. Similarly, currently available prophylactic vaccines for HPV (based on the L1 major capsid protein) are also effective. Development of plant-based HBV and HPV vaccines is already underway with encouraging results.

4. What are the potential disadvantages of edible vaccines?

These vaccines have some limitations as compared to other types of subunit vaccines:

- *Inconsistent dosage*: Edible vaccines are not a "purified product" and thus may suffer from inconsistencies in dosage they deliver
- *Oral intolerance*: The bioavailability, and thus efficacy of vaccine may suffer if somebody does not tolerate the oral product

- *Allergenicity*: The chances of allergic reactions might be more with these vaccines as compared to a purified product
- *Worker exposure*: The risk of exposure to the antigens or proteins to workers in the field cultivating the transgenic plants needs to be studied and taken care of
- *Unintended exposure in the food chain*: This is another problem unique to cultivation of these crops
- *Risks to the environment*: Risk of gene transfer and exposure to antigens or selectable marker proteins.

These risks are controllable through appropriate regulatory measures at all stages of production and distribution of a potential plant-made vaccine. Currently, the production of pharmaceutical proteins in transgenic plants is tightly regulated in countries like USA, with the US Department of Agriculture focusing on containment of recombinant material and the US Food and Drug Administration focusing on the production system as it relates to manufacture of the drug or vaccine. Current regulations for the production of plant-made pharmaceuticals are to prevent recombinant proteins from entering the food chain or from persisting in the environment, and to guard against recombinant nucleic acid sequences entering genomes of food or feed crops, or wild species.

5. What is the difference between "stable" and "transient" transformation of plants for production of vaccines?

"Stable" and "transient" transformations are two types of plant-expression systems used for production of vaccines. In stable transformation, the required gene(s) is permanently incorporated into a host plant's nuclear or plastid genome. On the other hand, transient transformation implies that the expression of a foreign DNA can not be inherited, but it is still transcribed within the host cell transiently. Table 37.1 lists the essential differences among the two.

Chloroplast genetic engineering to produce transgenic plants offers several advantages, including high levels of transgene expression, transgene containment due to lack of pollen transmission, and multi-gene expression in a single transformation event. Oral delivery is facilitated by hyperexpression of vaccine antigens against cholera, tetanus, anthrax, plague or canine parvovirus [4–31% of total soluble protein (TSP)] in transgenic chloroplasts (leaves) or chromoplasts

TABLE 37.1 Essential differences between stable and transient transformation of plants

	Stable transformation	*Transient transformation*
Expression location	Whole plant	Local or systemic
Time required	Longer (months)	Shorter (days)
Horizontal gene transfer	Yes	No
Expression level	Low	High
Delivery	Edible or purified	Edible or purified
Method	Transformation	Virus or *Agrobacterium tumefaciens* infection

(fruits/roots) as well as the availability of antibiotic free selectable markers or the ability to excise selectable marker genes. Also, their prokaryotic nature to express native bacterial genes (up to 46.1% total leaf protein) is attractive feature for therapeutic protein production. Functionality of chloroplast-derived vaccine antigens has been demonstrated by several assays, including the macrophage lysis assay, GM1-ganglioside binding assay, protection of HeLA cells or human lung carcinoma cells against encephalomyocarditis virus, systemic immune response, protection against pathogen challenge, and growth or inhibition of cell cultures.

Plant viral vectors, on the other hand, have small genome that is easier to manipulate and recombinant proteins can be produced more quickly and in greater yields. Thus, the transient expression methods are currently preferred over any other transgenic system for the exploitation of large and unrestricted numbers of plants in a contained environment. By designing optimal constructs and related means of delivery into plant cells, the overall technology plan considers scenarios that envisage high yield of bioproducts and ease in monitoring the whole spectrum of upstream production, before entering good manufacturing practice facilities. Several viruses have been extensively developed for vaccine production, including tobacco mosaic virus, potato virus X and cowpea mosaic virus. Agrobacterium-mediated expression has an added advantage that it allows the production of complex proteins (assembled from subunits) in plants.

6. What are the pathogens or diseases against which edible vaccines are being developed?

This technology has been shown to be a promising approach to develop vaccines against many pathogens against no vaccine is currently available, including protozoan diseases like malaria and amebiasis, viral infections like dengue and Crimean-Congo hemorrhagic fever bacteria like *Helicobacter pylori* and noninfective condition like atherosclerosis, bronchial asthma and Alzheimer's disease for which no vaccines are currently available. In addition, better vaccines against HPV and HBV are also under advanced stage of development.

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CHAPTER

38

Newer Adjuvants

Puneet Kumar

1. What are adjuvants?

Adjuvants, in context of vaccines, are formulations that increase and/or optimize immune response to an antigen *in vivo* as per the need.

2. What are the functions of an adjuvant?

Classically, adjuvants were added to the vaccine formulations just to increase the immune response (antibody titer) to the antigen. These adjuvants were developed using empirical methods, thus these are not optimal for many of the challenges in vaccination today. However, with advancements in immunology and allied fields, adjuvants are being developed and used to perform various other *specific* functions so as to *optimize/fine-tune* immune response as per the need:

- Increase immunogenicity (seroconversion rate) in populations with reduced responsiveness because of age (in young infants/preterm babies and the elderly), disease, or therapeutic interventions. For example an adjuvant, MF59, enhances the response of influenza vaccine in elderly
- To help induce robust mucosal immune response
- Facilitate the use of smaller doses of antigen, thus making the vaccine production faster and more cost-effective
- Reduce number of doses of a vaccine and/or obviate need of boosters that simplifies logistics and improves patient compliance
- *Accelerate* immune response that may be of critical importance in certain situations like vaccination in response to outbreak, bioterrorist attack, etc.
- To achieve qualitative alteration of the immune response. Newer adjuvants are increasingly developed to help induce functionally appropriate types of immune response (e.g., Th1 versus Th2 cell, $CD8^+$ versus $CD4^+$ T-cells, specific antibody isotypes), as per the requirement. For example, for vaccines against chronic infections [e.g., HIV, hepatitis C virus (HCV), tuberculosis and herpes simplex virus (HSV)], intracellular pathogens and malignancies, cellular immune responses is more important than humoral immune response. Further, specific adjuvants may also increase the generation of memory; especially

T-cell memory and alter the breadth, specificity, or affinity of the response, as per the need.

3. What are the features of an ideal adjuvant?

Ideal adjuvant should be stable with long shelf-life, biodegradable, cheap to produce, not induce immune responses against themselves and promote an appropriate immune response (i.e. cellular or humoral immunity depending on requirements for protection).

4. What are their mechanisms of action?

Adjuvants act through one or more of many modes of action including the delivery of antigen, recruitment of specific immune cells to the site of immunization, activation of these cells to create an inflammatory microenvironment, and maturation of antigen-presenting cells (APC) for enhancement of antigen-uptake and antigen-presentation in secondary lymphoid tissues.

Particulate adjuvants (e.g., emulsions, microparticles, iscoms, and liposomes) act by increasing the antigen availability and uptake by immune cells. Antigen-carrying vehicles (like liposomes and microfluidized squalene or squalane emulsions) target antigens to antigen-presenting cells, including dendritic cells (DC), follicular dendritic cells (FDC) and B-lymphocytes. They also activate innate immunity pathways in vivo, thus generating an immunocompetent environment at injection site. Modern adjuvant formulations contain small molecules with immunomodulating properties. These immunostimulatory adjuvants are derived predominantly from pathogens and often represent pathogen-associated molecular patterns (e.g., lipopolysaccaride, monophosphoryl lipid A, CpG DNA) which activate cells of the innate immune system. Thus, cytokine cascades are triggered. The cytokines elicit cell-mediated immune responses and the formation of antibodies of protective isotypes, such as IgG1. Antibodies of these isotypes activate complement and collaborate with antibody-dependent effector cells in protective immune responses.

Some adjuvants like chitosan-adjuvate nanoparticles act by preventing degradation of the antigen, thus potentiating its immunogenicity. This is in contrast to classical aluminum salt adjuvants, which in fact can destabilize certain protein antigens.

Although aluminum-containing adjuvants are one of the oldest and the most widely used adjuvants, their mechanism of action is still not fully delineated. It was only in 2008 that it was realized that alum adjuvant triggers an ancient pathway of innate recognition of crystals in monocytes and triggers them to become immunogenic dendritic cells, nature’s adjuvant. It is also clear now that adjuvants trigger the stromal cells at the site of injection, leading to the necessary chemokines that attract the innate immune cells to the site of injection.

With increasing insight of various mechanisms of actions of new/emerging adjuvants, adjuvants are now classified into five major types depending on their principle mechanism of action:

- Immunomodulation (modification of cytokine networks)
- Presentation (maintenance of antigen confirmation)
- Cytotoxic T-lymphocytes (CTL) induction
- Targeting specific cells
- Depot generation.

Of course, an adjuvant can have multiple modes of action, and a vaccine can have more than one adjuvant for desired immunogenicity.

5. With advances in molecular biology and genetic engineering, we have more and more specific antigens. Do we still need new or better adjuvants?

Yes, and in fact, the need of better adjuvants is even more! Newer vaccines based on highly purified antigens better reactogenicity and safety profile than some of the early whole-pathogen vaccines. However, the purity of these subunit antigens and the absence of the self-adjuvanting immunomodulatory components associated with attenuated or killed vaccines often result in *weaker* immunogenicity. Thus, with these vaccines, novel adjuvant formulations need to be developed that can use the interplay between innate and adaptive immune systems and the central role played by APC to *enhance* the immune response. In fact, adjuvants can now be "designed" to *optimize* immune response as per the need. For example, T-cell immunity can be triggered if the vaccine is against intracellular pathogen or cancer. In addition, new adjuvants may also allow vaccines to be delivered mucosally. Currently, adjuvants are selected on the basis of the route of administration and the type of immune response (antibody, cell-mediated, or mucosal immunity) that is desired for a particular vaccine. In fact, the choice of adjuvant or immune-enhancer determines whether the immune response is effective, ineffective or damaging. Vaccine design has therefore now become more tailored and emerging vaccines are based on the use of innovative adjuvants combined with careful antigen selection. This, in turn, has opened up the potential of extending the field of vaccinology to develop better prophylactic and *therapeutic* vaccines against chronic infectious [e.g., herpes simplex virus (HSV), human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), human papillomavirus (HPV), or *Helicobacter pylori*] and even *noninfectious diseases* such as malignancies, tumors (e.g., melanoma, breast, or colon cancer), Alzheimer disease, multiple sclerosis, insulin-dependent diabetes, rheumatoid arthritis, allergy and other immune-mediated disorders.

6. What are the different approaches that are currently being tried to develop newer adjuvants?

Developing efficient and safe adjuvants for use in human vaccines remains both a challenge and a necessity. The development of vaccine adjuvants for human use has been one of the slowest processes in the history of medicine. This is because of a number of knowledge gaps, the most important of which is the complexity involved in designing adjuvants that are both potent and well-tolerated. Alum adjuvants, consisting of aluminum salts, first described in the 1920s, were the only licensed adjuvants till late 1990s. Despite decades of research and hundreds of preclinical candidates, even today only a handful of adjuvants are approved for human use (e.g., aluminum salts, micro-fluidized squalene-in-water emulsion MF59® and monophosphoryl lipid A). MF59 was developed in 1990's and has been shown to increase in antibody levels in the elderly but no significant difference in younger individuals when compared to unadjuvanted vaccine. Thus, it is being used in a seasonal influenza vaccine (Fluad®) licensed for use in elderly since 1997. MF59 has also been shown to be superior to alum in inducing antibody responses to hepatitis B vaccine in baboons and humans. It is also being tested currently as a part of Aflunov® (an investigational prepandemic influenza vaccine) and two H1N1 pandemic vaccines (Focetria® and Celtura®).

Recent advances in our understanding of innate immunity have led to the identification of immune pathways and adjuvant formulations more suitable for clinical advancement. Ligands of the innate recognition systems thus emerge as new adjuvants for vaccine design, whereas manipulation of the signaling pathways offers new avenues for fine tuning immune responses and optimizing immunotherapies.

Many adjuvants appear to be ligands for toll-like receptors (TLR). TLRs are a family of conserved pattern recognition receptors that recognizes specific microbial patterns and allow the cell to distinguish between self and nonself materials. The very property of the TLRs to link innate and adaptive immunity offers a novel prospect to develop vaccines engaging TLR signaling. Thus, *TLR agonists* are being developed as adjuvants and are showing promise as vaccine adjuvants and even as stand-alone products capable of eliciting nonspecific protection against a wide range of infectious pathogens. However, recent evidence suggests that some adjuvants activate the innate immune system in a TLR-independent manner possibly through other pattern recognition receptors and signaling machinery. In particular, newly identified intracellular retinoic-acid-inducible gene (RIG)-like receptors, NOD-like receptors, or even as yet unknown recognition machinery for the adjuvant may regulate TLR-independent vaccine immunogenicity. By understanding more about TLR-dependent and TLR-independent innate immune activation we would be able to devise novel adjuvants and thus control the consequent adaptive immune responses to vaccine.

It is known that particulate adjuvants, like mineral salts, oil-in-water emulsions, and microparticles, do not activate dendritic cells (DCs) directly, but their mechanism of action is poorly characterized. In the last 2 years it has been revealed that particulate adjuvants induce chemokine production in accessory cells like macrophages, monocytes, and granulocytes, leading to cell recruitment at injection site followed by the differentiation of monocytes into activated DCs. The *NLRP3 inflammasome complex* is one of the molecular targets of particulate adjuvants and it is required for alum adjuvanticity. Thus, it is another pathway that can be exploited for development of better adjuvants.

Some *heat shock proteins (HSPs)* like HSP70 and Gp96, in addition to maintaining cell homeostasis under physiological and stress conditions, are potent inducers of immunity. They activate dendritic cells partly through toll-like receptors, activate natural killer cells, increase presentation of antigens to effector cells and augment T-cell and humoral immune responses against the peptides bound to HSPs, but not against HSPs per se. Thus, they are highly effective carrier molecules for cross-presentation. Moreover, they have peptide-independent immunomodulatory capacity also. Their roles in priming multiple host defense pathways are being exploited in adjuvant development for vaccines against cancer and infectious diseases. The antigenic peptide in the vaccine is complexed with either tissue derived or recombinant HSPs in vitro to generate HSP-peptide complexes as peptide-specific vaccine.

A synthetic glycolipid *α-galactosylceramide* (α-GalCer) represents a new class of immune stimulators and vaccine adjuvants that activate type I natural killer T (NKT) cells to swiftly release cytokines and to exert helper functions for acquired immune responses. This is specially being tried in anticancer and antiviral vaccines with encouraging results. *β-mannosylceramide* is another novel adjuvant in this category.

Also among the newer adjuvants are immunostimulatory complex (ISCOM) and ISCOMATRIX**.** ISCOMs are particulate antigen delivery systems composed of antigen, cholesterol, phospholipid and saponin, while ISCOMATRIX is a particulate adjuvant comprising cholesterol, phospholipid and saponin but without antigen. ISCOMs and ISCOMATRIX combine the advantages of a particulate carrier system with the presence of an in-built adjuvant (Quil A) and consequently have been found to be more immunogenic, while removing its hemolytic activity of the saponin, producing less toxicity. ISCOMs and ISCOMATRIX vaccines have now been shown to induce strong antigen-specific cellular or humoral immune responses to a broad range of antigens of viral, bacterial, parasite origin or tumor in a number of animal species including nonhuman primates and humans. *Biodegradable micro or nanoparticles* that have been extensively studied and used as carrier or delivery systems for various medications are now being tried as vaccine-delivery systems. In these formulations, antigen can either be entrapped or adsorbed to the surface of the particles. These can act as depot from which the encapsulated antigen is gradually released. Additionally, the polymeric particles may offer protection to encapsulate the antigens delivered and facilitate uptake by M-cells in the mucosal-associated lymphoid tissue (MALT), thus serving as a vehicle for mucosal immunization. Thus, these delivery systems act as adjuvants for vaccine formulations. Similarly, *nonbiodegradable nanoparticles,* like gold, latex, silica and polystyrene, have also been used as vaccine delivery/adjuvant systems either alone or in combination with other strategies like electroporation. *Virosomes, Virus-like particles (VLPs)* and *Virus vectors* are another group of vaccine delivery systems being explored as adjuvants in modern vaccine formulations (*vide infra, Q. 8)*

Most conventional adjuvants are poorly defined, complex substances that fail to meet the stringent criteria for safety and efficacy desired in new generation vaccines. Adjuvants for newer vaccines need to be more focused and safer. Modern adjuvant formulation is often *composed of multiple adjuvants* which can potentially act synergistically and are specifically adapted to each target and to the relevant correlate(s) of protection. Such "second generation adjuvant systems" are already in use with licensed vaccines like Cervarix® (vaccine against HPV) and Fendrix® (new hepatitis B vaccine) and in development are vaccines against malaria and genital herpes. The pre-pandemic vaccines also use new generation of adjuvanted systems.

One emerging class of newer adjuvants is *carbohydrate-based adjuvants*. *γ-inulin* is a carbohydrate derived from the plant roots of the *Compositae* family. It is a potent adjuvant inducing humoral and cellular immunity without the toxicity. γ-inulin can be combined with a variety of other adjuvant components, e.g., aluminum hydroxide, to produce a range of adjuvants with varying degree of Th1 and Th2 activity. They principally stimulate the innate immune system through their ability to activate the alternative complement pathway have proven ability to induce both cellular and humoral immunity. Thus, they use a "natural" mechanism and the biochemical basis of their action is well-understood in general terms. With their excellent tolerability, long shelf-life, low cost and easy manufacture, they offer great potential for use in a broad range of prophylactic and therapeutic vaccines. Based on successful animal studies in a broad range of species, human trials are about to get underway to validate the use of inulin-based adjuvants in prophylactic vaccines against hepatitis B, malaria and other pathogens. If such trials are successful, then it is possible that inulin-derived adjuvants might one day replace alum as the adjuvant of choice in most human prophylactic vaccines. Other carbohydrates that have been studied as adjuvants are glucans, dextrans, lentanans, glucomannans, galactomannans and acemannan.

It is known that parasitic infections can alter host immune responses. Among parasitic infections, helminth infection often leads to systemic immune suppression or anergy, allowing their long-term survival in the host and restricting pathology. Thus, persons who are chronically infected with helminths have lesser incidence of allergies and autoimmune diseases. Helminth infection or helminth extracts drive CD4+ T-helper (Th) cell responses towards Th2 type and activate APCs such that these cells express an anti-inflammatory phenotype. Among the myriad molecules present on or secreted by helminth parasites, *glycans* have been shown to be the key in inducing Th2-type and anti-inflammatory immune responses. New insights into these pathways could be useful to antagonize suppression and hence boost vaccine efficacy or to optimize suppression induced by helminth derived molecules and control inflammatory diseases. There is another adjuvant under development that is derived from a helminth. It is called *activation-associated protein-1 (ASP-1)* and is derived from a helminth, *Onchocerca volvulus*. It has potent adjuvant activity and, unlike alum adjuvant, is able to induce both Th1 and Th2-associated humoral responses and Th1 cellular responses. Thus, it can be further developed as a promising adjuvant for subunit-based and inactivated vaccines.

Among the newer approaches is to design "*mucosal adjuvant*" that can induce maximal protective mucosal immunity to the antigen (*vide infra,* Q. 7).

Another emerging approach is to use "*cellular adjuvants*". For example, recent research has shed light on pivotal role of dendritic cells in the initiation and regulation of the immune response. Subsequent preclinical studies and pilot clinical trials have provided some evidence on the potential advantages of using dendritic cells as cellular adjuvants. Current research efforts are focused on the definition of optimal protocols for dendritic cell-based therapies in patients.

Recent advances in basic immunology have revealed central role of cytokines in linking the innate and adaptive immunity through their action on dendritic cells. Thus, *cytokines* are being increasingly used as adjuvants, especially for therapeutic vaccines against chronic infections and cancer-vaccines. The initial results have been encouraging.

7. What are mucosal adjuvants?

A large proportion of pathogens either invades through, or cause disease at mucosal surfaces. This can be prevented by induction of immunity in the common mucosal immune system (CMIS), which interconnects inductive tissues, including Peyer's patches (PPs) and nasopharyngeal-associated lymphoreticular tissue (NALT), and effector tissues of the intestinal and respiratory tracts. However, mucosal vaccines have to overcome several formidable barriers in the form of significant dilution and dispersion; competition with a myriad of various live replicating bacteria, viruses, inert food and dust particles; enzymatic degradation; and low pH before reaching the target immune cells. Thus, vaccination through mucosal membranes requires potent adjuvants to enhance immunogenicity, as well as delivery systems to decrease the rate of dilution and degradation and to target the vaccine to the site of immune function.

When vaccine antigen is administered together with mucosal adjuvant, antigen-specific T-helper (Th) 1 and Th2 cells, cytotoxic T lymphocytes (CTLs) and IgA B cell responses are effectively induced by oral or nasal routes via the CMIS. In the early stages of induction of mucosal immune response, the uptake of orally or nasally administered antigens is achieved through a unique set of antigen-sampling cells, M cells located in follicle-associated epithelium (FAE) of inductive sites. After successful uptake, the antigens are immediately processed and presented by the underlying dendritic cells (DCs). Elucidation of the molecular/cellular characteristics of M cells and mucosal DCs is very likely to facilitate the design of a new generation of effective mucosal adjuvants and of a vaccine delivery vehicle that maximizes the use of the CMIS. Numerous such candidate adjuvants have been studied: *mutants of heat labile enterotoxin from Escherichia coli,* mutants of *cholera toxin from Vibrio cholerae*, a chimeric protein formed by genetic combination of mutant cholera toxin A subunit and heat labile toxin B subunit, another chimeric protein combining enzymatically active CTA1 gene from whole cholera toxin to a gene encoding a synthetic analogue of *Staphylococcus aureus* protein A (with a high affinity towards B cells), Monophosphoryl lipid A (MPL) [derived from the lipopolysaccaride (LPS) of *Salmonella minnesota*, a Gram-negative bacteria], N-acetylmuramyl-L-alanyl-D-isoglutamine (*Muramyl dipeptide*) derived from cell wall of mycobacteria, Proteosomes (multi-molecular preparations of meningococcal outer membrane protein, CpG motif-containing DNA, saponins like QS-21, cytokines like interleukin IL-1, IL-12 chemoattractant lymphotactin, regulated upon activation, normal T-expressed and secreted (RANTES) and defensins and recently, nanoparticles.

8. What are virosomes, virus-like particles and virus vectors? What are their advantages over other conventional adjuvants?

A virosome is a drug or vaccine delivery mechanism consisting of unilamellar phospholipid bilayer vesicle incorporating virus derived proteins to allow the virosomes to fuse with target cells. Virosomes are not able to replicate but are pure fusion-active vesicles. They are approximately 150 nm in diameter, at least 100 times smaller than the particles in aluminum-adjuvanted vaccines. (Fig. 38.1).

Virosome technology has been used to induce immunity to a variety of antigens without the adverse reactions associated with other adjuvants. The novelty of this biodegradable delivery system lies in the natural presentation of antigens and the stimulation of a specific immune response, induced by the active targeting of antigens to immunocompetent cells. Virosomes have been shown to elicit both cell-mediated and humoral immune responses.

Virosome technology has been most advanced in influenza and hepatitis A, in association with protein or peptides, but it is rapidly being used for other antigens as well. A potential advantage of this technology is to take advantage of the physical properties of virosomes in terms of uptake by APCs, as well as the chemical composition, and compatibility with adjuvant molecules derived from lipid-A. Some vaccines like Epaxal® (for hepatitis A) and Invivac® (for influenza) are already licensed in some countries.

Virus-like particles are essentially noninfective virus consisting of self-assembled viral envelope proteins without accompanying the genetic material. They maintain a morphology and cell-penetrating ability similar to infective viral particles and can stimulate both cellular and humoral immunity. The first licensed

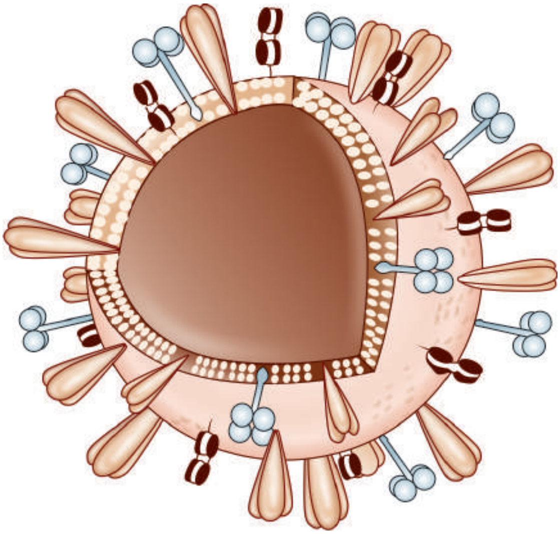

FIGURE 38.1 Hemagglutinin (large trimers), neuraminidase (black tetramers), as well as Fab′ fragments with a spacerarm are anchored in the lipid bilayer of virosomes

recombinant HBV vaccines, Recombivan® and Energin-B®, were composed of the viral small envelope protein which upon expression in yeast formed 22 nm VLPs. While effective, these had relatively low immunogenicity (85–90%). They were further improved upon and a potential third generation hepatitis B vaccine, Bio-Hep-B®, is a VLP-based vaccine with 100% immunogenicity and is licensed in Israel. Gardasil®, the currently available HPV vaccine is a VLP-based vaccine. Most recently, this approach is being tried to develop vaccine against human bocavirus (HBoV), a recently identified pathogen with a worldwide distribution is closely related to pediatric acute respiratory infection and gastroenteritis.

Viral-vectored vaccines consist of a nonreplicating virus that contains some defined genetic material from the pathogen to which immunity is desired. Advantages of viral-vectored vaccines include their ease of manufacture, good immune response and potential for mucosal immunization. The most common viruses used for this purpose are adenovirus and modified vaccinia virus Ankara (MVA), although other viruses like proxy-viruses, measles virus, vesicular stomatitis virus, HSV and alpha virus have also being studied.

9. What are the limitations of adjuvants?

To be successful, the adjuvants being developed need to overcome some crucial limitations like problems related to stability, bioavailability and cost. Not only stability of the adjuvant, but stability of the antigen and its epitope configuration needs to be considered, since addition of protein antigen to the adjuvant formulation or vice versa can impact the epitopes of the antigen. The adverse effects related to the adjuvant *per se* can affect the overall safety profile of the vaccine formulation. Potential increase in immune toxicity from improved immunogenicity provided by vaccine adjuvants is also of concern. Furthermore, since each adjuvant generates a characteristic immune response (e.g., Th1 or Th2 bias), there is no "universal" adjuvant for all vaccine formulations.

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Section 6

Therapeutic Vaccines

Chapter

CHAPTER

39

Therapeutic Vaccines

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1. What do you mean by a therapeutic vaccine?

A vaccine is a medical product developed to stimulate your body's immune system in order to prevent or control an infection. An effective vaccine trains your immune system to fight a particular microorganism so that, it can not make you sick. Traditionally vaccines have been thought as preventive vaccines which are so designed so as to prevent the occurrence of the infection in people who do not have that infection. A therapeutic vaccine (also known as a treatment vaccine) is a vaccine used in the treatment of an infected person. Therapeutic vaccines are designed to boost the body's immune response to that particular infection in order to better control the infection.

2. What type of diseases would therapeutic vaccines be most likely to be effective against?

Therapeutic vaccines are more likely to be effective against persistent infections which tend to have a chronic course. This is so because the time sequence to boost the body's own immune response against the disease would render such a therapy redundant in more acute diseases or infectious conditions. Besides infections other chronic diseases where the immune system plays a role in the pathogenesis such as autoimmune diseases, tumors are also potential candidates where the "therapeutic vaccine" concept is likely to benefit.

3. What is the current status of therapeutic vaccines in clinical medicine?

Conventional preventive vaccines against infectious diseases have been highly effective at reducing drastically the incidence and morbidity of many life-threatening plagues, such as smallpox and viral poliomyelitis. Preexposure vaccination quite opposite to the concept of a "therapeutic vaccine" is essential for most infectious diseases caused by viruses or bacteria. A single exception is the **rabies vaccine**, developed by Pasteur more than 100 years ago, which is administered only after exposure to the virus. The mechanism for vaccine efficacy is believed to be that of induction of antibody that suppresses viral migration through the nerve axons.

Recently, **Copolymer 1 (glatiramer acetate)** has been developed to be used as a therapeutic vaccine against multiple sclerosis and has shown excellent results in decreasing the relapse rate in this chronic debilitating disease. This is a good example of a beneficial treatment for autoimmune diseases, based on its similarity to the myelin basic protein, one of the putative causes of multiple sclerosis. This finding could lead to therapeutic vaccines against other autoimmune diseases such as myasthenia gravis, systemic lupus erythematosus, and rheumatoid arthritis.

US biotech Antigenics has won Russian approval to market **Oncophage** to treat kidney cancer. It has been a long haul since the discovery in 1991 of the first tumour antigen, coupled with increased understanding of the immune system, inspired attempts to make the body recognize cancer cells as foreign and deal with them accordingly. Oncophage exemplifies many of the issues that have dogged the progress of cancer vaccines, from the initial hype, to clinical setbacks and investor scepticism. After 11 years in development the product is yet to win approval from the US Food and Drug Administration (FDA) because the data from the Phase III trial, completed in 2006 did not show sufficient efficacy. But in April, Russian regulators approved the drug on the basis of a subset of that Phase III data, relating to patients with less advanced cancer who mounted a more effective response to the treatment.

In April 2010, the FDA approved the first cancer treatment vaccine. This vaccine, **sipuleucel-T Provenge®,** manufactured by Dendreon), is approved for use in some men with metastatic prostate cancer. It is designed to stimulate an immune response to prostatic acid phosphatase (PAP), an antigen present on most prostate cancers. In a clinical trial, sipuleucel-T increased the survival of men with a certain type of metastatic prostate cancer by about 4 months. Unlike some other cancer treatment vaccines under development, sipuleucel-T is customized to each patient. The vaccine is created by isolating immune system cells called antigen-presenting cells (APCs) from a patient's blood through a procedure called leukapheresis. The APCs are sent to Dendreon, where they are cultured with a protein called PAP-GM-CSF. This protein consists of PAP linked to another protein called granulocyte-macrophage colony-stimulating factor (GM-CSF). The latter protein stimulates the immune system and enhances antigen presentation. APC cells cultured with PAP-GM-CSF constitute the active component of sipuleucel-T. Each patient's cells are returned to the patient's treating physician and infused into the patient. Patients receive three treatments, usually 2 weeks apart, with each round of treatment requiring the same manufacturing process. Although the precise mechanism of action of sipuleucel-T is not known, it appears that the APCs that have taken up PAP-GM-CSF stimulate T-cells of the immune system to kill tumor cells that express PAP.

Great effort is being devoted to developing therapeutic vaccines against infections like AIDS, hepatitis B, hepatitis C, tuberculosis, malaria, HPV and possibly against the bacteria that cause gastric ulcers. Furthermore, current studies raise hope for vaccines against prion diseases, bovine spongiform encephalitis, and Huntington's disease. Passive antibodies against a peptide derived from β-amyloid plaques might be able to degrade plaques and be used as a therapeutic vaccine against Alzheimer's disease.

4. What are the difficulties faced in the development of therapeutic vaccines?

Conventional vaccines are for healthy people, to prevent them from getting sick. So perhaps the expression "therapeutic vaccine" is an oxymoron. The purpose of adding antigen to an already infected person may be tantamount to giving in to the spurious notion that "like cures like." Besides to develop therapeutic vaccines one would have to develop newer and applicable immunologic principles which would benefit the host already compromised with the disease process. One such example is that of breaking immune tolerance in cancer through application of antigen bound to heat shock proteins that gain uptake through the CD91 receptor by dendritic cells where both cytotoxic T-cells and T-helper cell responses are evoked. Another lies in breaking the chain of continuing infection of new cells with HIV virus by substances that block viral entry into the cell. Another lies in the nonimmunologic intervention through specific gene (viral) silencing by RNAi interference through use of short interfering RNA.

5. Therapeutic vaccines against which diseases are most likely to come in the near future?

Trials of therapeutic vaccines are currently underway for some diseases and are most likely to come in the near future as shown below:

Human Pappiloma Virus

Prophylactic vaccines cannot make an immediate impact on the prevalence of cervical cancer, which usually takes 20 years or longer to develop after initial infection with HPV. In contrast, a therapeutic vaccine theoretically could help women who are already infected with HPV. Therefore, medical researchers also are investigating therapeutic vaccines for use as an adjunct to standard therapies. Such a vaccine could:

- Help prevent low-grade disease from progressing
- Cause existing lesions to regress
- Control the spread of metastatic cancer, and/or
- Prevent recurrence of cervical cancer after treatment.

Hepatitis C Virus Infection

The rationale for Hepatitis C therapeutic vaccine is many:

- Patients with insufficient immune control progress towards chronic hepatitis C
- Specific T-cell responses and IFN-a are generally absent in patients who progress; non-specific T cells cause inflammation and fibrosis

TABLE 39.1 Therapeutic hepatitis C vaccines under development and patient groups most likely to benefit

Company	*Antigen*	*Indication*	*Phase*
Schering	Low-dose PEG-IFN	IFN-NR	III
NIH Roche	Low-dose PEG-IFN	IFN-NR	III
Intermune	IFN-cγ	IFN-NR	IIa/stopped
Inercell	CTL peptides	IFN-NR	IIa
Chiron CSL	Core/ISCOM	HLA class A2	I

Abbreviations: IFN-NR, interferon non-responders; ISCOM, immune-stimulating complex; Hepatitis C E1 (antifibrotic approaches) and poly-epitope therapeutic vaccines.

- Redirection of immune response to control chronic hepatitis C could halt or reverse liver fibrosis progression
- Immune responses against the E1 envelope protein especially are weak (antibodies) or absent (T-cells), therefore the E1 envelope is a prime candidate. Development of poly-epitope vaccine has the potential advantages of targeting all patient segments and lower costs
- There are numerous hepatitis C therapeutic vaccines under development, targeting different antigens and patient groups (Table 39.1).

Human Immunodeficiency Virus Infection

HIV is a chronic debilitating disease which slowly destroys the immune system of the infected individual. There have been numerous attempts to make preventive vaccines which have largely failed. Current stay of treatment is antiretroviral drugs which do not completely suppress the disease. Besides a number of adverse effects these drugs also leave patients with a level of harmful immune activation which can lead to premature ageing. It is believed that the development of therapeutic HIV vaccines would help in decreasing the reliance on these antiretroviral drugs besides controlling the infection better.

A number of such vaccines are currently being developed and in various phases of trials (Table 39.2). Clinical trials of therapeutic vaccines are recruiting

TABLE 39.2 Therapeutic HIV vaccines under development currently

Study Group	*Antigen*	*Phase of trial*
Geovax labs	HIV DNA	I
Imperial college, London	HIV DNA+ IL-2+ GM-CSF+ Growth hormone	I
University Pittsburg/French National Agency	Dendritic cell	I/II
Baylor Research Institute	Dendritic cell	Ia/II
Instituto Superiore di Sanito	HIV tat protein	II
FIT biotech	HIV protein (6 fragments)	II

volunteers with CD4 counts greater than 250 cells/mm^3, and most studies require a CD4 count greater than 350 cells/mm^3. People with weaker immune systems may be unable to produce a good immune response to a therapeutic HIV vaccine, and are therefore not eligible for these trials. Most trials require that therapeutic vaccine recipients continue taking antiretroviral drugs during the study.

6. What is the current status of therapeutic cancer vaccines?

They are an emerging, experimental type of therapy designed to stimulate the immune system––the body's natural defenses––to find and fight cancer cells. herapeutic cancer vaccines are often now studied in combination with other cancer treatments, including:

Surgery, to remove tumors, radiation, which kills cancer cells with high-energy X-rays, chemotherapy, systemic drug therapy that destroys cancer cells (and unfortunately often healthy cells as well), monoclonal antibody therapy, a type of immunotherapy using proteins made in the laboratory, antiangiogenesis therapy, which stops tumors from making new blood vessels and thereby slows their growth. In addition to combining therapeutic vaccines with other anticancer treatments, researchers are looking to enhance them by adding substances called adjuvants, which act to increase the immune response. Although therapeutic cancer vaccines are not expected to cure cancer on their own, researchers believe they may help by slowing the progression of existing cancers, delaying the recurrence of cancers, or destroying remaining cancer cells not killed by prior treatments, while maintaining a favorable safety profile. The task of a therapeutic cancer vaccine is to stimulate the immune system to recognize these molecules as foreign, and to generate a response to find and attack cells that express them. Once the immune system is able to identify cancer-specific antigens, it can specifically attack and destroy any cells containing these antigens without damaging normal cells. Therapeutic cancer vaccines are considered active immunotherapy since they are designed to activate the body's own immune system to respond.

Types of therapeutic cancer vaccines being studied today include the following:

- Tumor cell vaccines, also called whole cell vaccines. These consist of cancer cells removed during surgery and then injected into the patient. Tumor cell vaccines may be either autologous, where cells are taken from the patient's own tumor, or allogeneic, where cells are taken from other patients' tumors
- Antigen vaccines use tumor-specific antigens, rather than the whole tumor cells
- Dendritic cell vaccines use dendritic cells, which are special cells that breakdown the antigens on cancer cells into smaller pieces and thereby help the T-cells identify them. Similar to autologous cell vaccines, dendritic cells used in therapeutic vaccines must be specifically prepared in the lab for each individual patient
- Anti-idiotype vaccines use anti-idiotypes, antibodies that look like cancer-specific antigens. They trick the body into attacking both the anti-idiotypes and the antigens that exist in the cancer cells
- DNA vaccines use small pieces of DNA that, when injected into the body, instruct cells to keep making certain antigens. The more antigens are produced, the more T-cells the immune system makes to fight them off.

Vector-based vaccines make use of certain viruses, bacteria, or yeast cells to stimulate the immune system. These organisms are modified to ensure that they cannot cause disease, and they are used to carry the tumor-specific antigens into the body. The vaccine used in the MEL11 study, a phase II study exploring a new treatment approach to advanced-stage melanoma, is a vector-based vaccine.

Therapeutic cancer vaccine besides the ones mentioned earlier which are already in clinical use, which are likely in the near future are ones against melanoma (Phase II/III), leukemia, non-Hodgkin's lymphoma (Phase II/III), non-small cell lung cancer (Phase II), colorectal cancer (Phase II), and cancers of the breast, kidney, pancreas, and prostate (Phase II/III).

7. What are the side effects of therapeutic vaccines encountered so far?

As regards to the side-effects there have been no serious ones encountered in the various trials undergoing. Side effects observed so far have been similar to the side effects that occur with FDA-approved vaccines. These side-effects include Soreness, swelling, redness, or pain at the site of injection and mild flu-like symptoms (fever, chills, muscle pain or weakness, nausea, headache, and dizziness).

8. Will therapeutic vaccines be able to cure the disease targeted?

Probably not. If therapeutic vaccines are effective, they may be able to help keep the diseases under control. However, most researchers do not think therapeutic vaccines will be able to completely eliminate infection because the viruses hide in certain cells of the body where it can last for decades. The primary benefit might be in better controlling the disease progressions, preventing relapses and decreasing the exposure to the standard therapeutic drugs with concomitant decrease in their side-effects.

SUGGESTED READING

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