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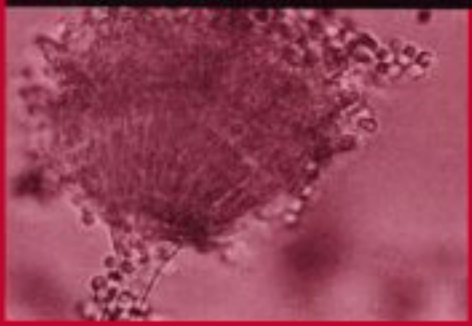

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Foreword

Immunodeficiency—Improving the Deficiency of Knowledge

Rafeul Alam, MD, PhD
Consulting Editor

How many infections are too many before one starts an immunodeficiency work-up? This question has been with us for as long as we started measuring serum immunoglobulins. In the past, many of us considered an immunodeficiency work-up only when someone experienced more than 4 to 5 respiratory infections per year, or more than one infection with an unusual pathogen. This approach is now being challenged. One could argue that a single protracted infection is too many. After all, our immune system is, in theory, prepared to recognize and respond to millions of foreign antigens. Thus, a single protracted infection could indicate the presence of a weak and malfunctioning component in the immune system. This argument has now gained substantial support from many recent discoveries, especially from studies of patients with susceptibility to mycobacterial infections. Interferon-gamma and pathways leading to the production of interferon-gamma play a crucial role in defense against mycobacteria. Thus, any perturbation in this pathway (eg, IL-12/IL-23-STAT4-IFN-gamma) leads to unusual susceptibility to mycobacterial and, in some cases, Salmonella infections. This is just one of many milestone discoveries that have been made in recent years in clinical immunology.

Many immunologists and other clinicians would love to have an ''immune profile'' done on their patients, just like they do the lipid profile to

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predict cardiovascular diseases. At this time, this type of personalized immune profile is a bit premature. We have a long way to go, as the genetic cause of susceptibility to most pathogens still remains unknown. Tests for some of the recently discovered immunodeficiency disorders are commercially available but their cost is prohibitive to most people. Given the advances in technology, it is likely that in the not-so-distant future we will be able to order an immune profile that will accurately predict the susceptibility of our patients to specific pathogens.

This update on immunodeficiency showcases the triumph and victory of immunology research and presents future perspectives. It exemplifies how basic science knowledge helps untangle the mysteries of clinical medicine. Dr. Jordan Orange, one of the young starts in the field, has invited the leaders and ultimate experts to present their recent discoveries. Their writings make us feel proud, joyful, and highly optimistic about the future.

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Preface

Jordan S. Orange, MD, PhD
Guest Editor

It has been 7 years since the last immunodeficiency-dedicated issue of *Immunology and Allergy Clinics of North America* was published. It is difficult to conceive the extraordinary progress that has been made in the field of primary immunodeficiency over this interval. There are now more than 120 primary immunodeficiency disorders that are defined on a genetic level. Additionally, substantial novel mechanistic insight has been obtained in many diseases, which were previously defined but incompletely understood. In this light, publications on the topic of primary immunodeficiency have grown exponentially over the last 7 years (Fig.1). Collectively this progress and these advances are a testament to the global collaboration of dedicated clinicians, patients, basic researchers, and physician scientists. These efforts have not only impacted the patients affected by these diseases, in terms of more specific diagnosis, improved treatment, and better outcome, but have also had broader impact in informing a number of basic scientific fields.

Rather than attempt to provide an unabridged summary of primary immunodeficiency in 2008, the contents of this issue focus upon a select number of innovative topics that underscore some of the tremendous change in the field. The articles loosely follow a path from considerations related to innate immune defenses to adaptive immune defects and conclude with those relevant to diagnosis and treatment.

Innate immunity-oriented works

In recent years, the appreciation of how innate immune defenses participate in combating infection has expanded drastically. Although it would

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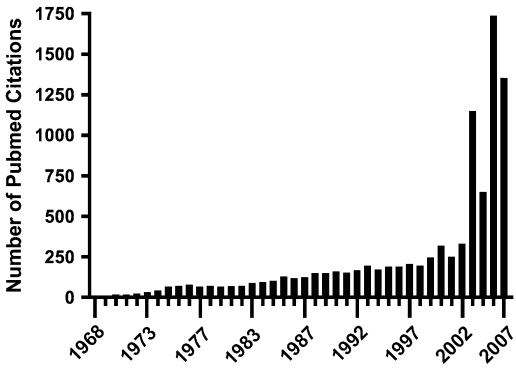

Fig. 1. Annual Pubmed-indexed publications in primary immunodeficiency. The number of publications indexed in Pubmed under the subject header "primary immunodeficiency" was enumerated for each full calendar year from 1968 through 2007. Citations with subject headers "HIV" or "AIDS" were excluded in an effort to maintain specificity for primary immunodeficiency. Each tick on the x-axis represents an individual year.

be easy to construe an entire issue on how primary immunodeficiency has altered our understanding of the role of innate defenses, several topics are presented to address noteworthy advances and concepts. The first article, by Bustamante and colleagues, highlights an evolution in the field of primary immunodeficiency that defines novel infectious phenotypes at a mechanistic immunologic level in a forward genetic manner. It also underscores how variation within known genetic disorders of immunity can provide important insights into immunologic function. This group's consideration of mendelian susceptibilities to mycobacterial disease, invasive pneumococcal disease, and herpes simplex encephalitis has been extraordinarily informative and has provided critical examples with which the field of primary immunodeficiency moves into the future. The second article, by Botzug and colleagues, reviews the recently increased understanding of the broad array of neutropenic disorders that result in impaired host defense. It also recaps their critical discovery of the molecular and genetic mechanism of one of the initially defined primary immunodeficiency disorders, Kostmann's syndrome. The third article, by Freeman and Holland, reviews the hyper-IgE syndromes and describes the magnificent discovery of a genetic explanation for this former diagnostic dilemma. It also elaborates on how this disease, first described in 1966, will now provide years worth of important new basic immunologic insights. The fourth article, by Filipovich, underscores the union of host defense and immunologic regulation that can be defective in primary immunodeficiency diseases. Here, she comprehensively presents the hematophagocytic lymphohistiocytosis and the substantial inroads that have been made on genetic, mechanistic, and therapeutic levels, many of which derive from her own work. Importantly, the deficiency of immunity impairs an ability to fight infection and, in doing so, uncovers an

extraordinary immune dysreregulation. The mechanisms underlying this phenomenon have been especially informative in how certain immunologic cellular processes unfold.

Adaptive immunity-oriented works

Although primary immunodeficiencies affecting the adaptive immune system were among the first to be elucidated molecularly, recent insights have defined new disorders and alternative paradigms. Especially relevant have been the fragile balance between immunologic defense and regulation. Thus, the fifth article, by Torgeson, expands upon the theme of immune dysregulation by underscoring the role of the T regulatory cell in controlling immunologic responses. He describes the primary immunodeficiencies, which have taught us more about the function and development of this critical cell population as well as the devastating conditions associated with their deficiency and dysfunction. The sixth article, by Su and Lenardo, describes the intriguing family of primary immunodeficiency disorders that result from defective cell death. These diseases have many different immunologic signatures, including the abnormal survival of autoreactive T and B cells. The groundbreaking work of these investigators in understanding these diseases has led to critical mechanistic insights into the cellular process of apoptosis as well as its clinical relevance. The seventh article, by Sullivan, describes invaluable clinical and immunologic perspectives, which are derivative from investigation of an extremely large cohort of patients who have DiGeorge syndrome. This experience relates to the basic function of the thymus, the role of a critical transcription factor on the 22nd chromosome and providing optimal clinical care for patients with a complex multisystem disorder. The eighth article, by Yong and colleagues, defines the clinical and immunologic advances relating to the diagnosis, treatment, and pathogenesis of common variable immunodeficiency. Importantly, it recaps the significant progress made in large part by this group in understanding the genetics of this phenotype, which, at one time, were assumed to be purely polygenic.

Diagnosis- and management-oriented works

Fortunately, the scientific advances in the field of primary immunodeficiency have been paralleled by important derivative practical innovations to better the lives of the patients affected by the diseases. One of the areas in which this is most apparent is the ability to provide specific molecular diagnoses to patients who present with particular immunologic and clinical phenotypes. In the ninth article, Morra and colleagues review the application of genetic testing to primary immunodeficiency diseases and discuss key issues relating to methodology and interpretation of results. The increasing availability of these approaches have necessitated that clinicians

have a working knowledge of this subject. The tenth article, by Berger, revisits the original specific treatment for primary immunodeficiency, immunoglobulin replacement. Here, diverse coverage of topics including indication, dose, route, and monitoring for immunoglobulin therapy are provided with particular attention to new developments, as well as important outstanding questions. In the eleventh article, Gennery and Cant comprehensively address the advances made in attempting to cure the deficient immunologic mechanisms in primary immunodeficiency through hematopoietic stem cell transplantation. Numerous specific innovations and options are underscored and include important successes from their own center. Future optimization of outcomes in hematopoietic stem cell therapies are also discussed. Finally, the twelfth article, by Thrasher, provides an update to the blossoming field of treating primary immunodeficiency using gene therapy. The recent successes of his own and other centers as well the innovations and critical reevaluations from the field as a whole are presented. The rapid advancements and critical success provide a realistic optimism for the future of patients who have primary immunodeficiencies.

Dedication

The field of primary immunodeficiency would have not advanced as much, were it not for the commitment and collaboration of the patients affected by these rare diseases. Their quest to better understand their diseases, combined their altruistic ambitions to help advance the field as a whole, have served as a powerful enabling force. It is to them whom this issue is dedicated.

Acknowledgement

I would like to acknowledge the assistance of Ms. Joan Boyd in assembling this issue, and my wife Katie and my daughters Audrey, Marlainia, and Tabitha for their extraordinary support. I would also like to thank all of the contributors to this issue for giving their expertise back to the community and for being a pleasure to work with.

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From Infectious Diseases to Primary Immunodeficiencies

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The field of primary immunodeficiencies (PIDs) is expanding in many directions, including the genetic dissection of infectious diseases [1,2]. PIDs typically confer predisposition to multiple infections, but an increasing number of PIDs have been found to confer a more specific predisposition to one or several infections [3]. Examples include susceptibility to viruses, such as predisposition to Epstein-Barr virus in patients who have X-linked lymphoproliferative syndrome [3–5], predisposition to oncogenic papillomaviruses in patients who have epidermodysplasia verruciformis [6,7], and susceptibility to bacteria, such as predisposition to *Neisseria* in patients who have mutations in the properdin gene or in the genes encoding the terminal components of complement [8]. The authors review here how the exploration of patients who have a selective predisposition to weakly virulent mycobacteria led to the discovery of a new group of PIDs involving impairment of the

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interleukin (IL)-12–interferon (IFN)-γ circuit, how the exploration of children with a predisposition to invasive pneumococcal disease (IPD), either isolated or associated with a predisposition to other infections, led to the discovery of disorders of nuclear factor kappa B (NF-κB), and how the investigation of children with herpes simplex virus (HSV) encephalitis led to the description of disorders of the Toll-like receptor (TLR) 3 pathway.

Mendelian susceptibility to mycobacterial diseases

Mendelian susceptibility to mycobacterial diseases (MSMD) (MIM 209,950) [9] is a rare syndrome characterized by the occurrence of clinical disease caused by weakly pathogenic mycobacteria, such as bacillus Calmette-Guérin (BCG) vaccines and nontuberculous environmental mycobacteria (EM), in otherwise healthy individuals [10]. Patients are also vulnerable to the more virulent species, *Mycobacterium tuberculosis* [11]. They are resistant to most other infections, with the notable exception of systemic nontyphoidal salmonellosis, which is commonly observed [12]. Other infectious diseases have been reported only rarely, mostly in single patients. This syndrome is clinically heterogeneous; the clinical features cover a spectrum ranging from local recurrent to disseminated mycobacterial diseases. In most familial cases, inheritance is autosomal and recessive, but in some kindreds, the syndrome segregates into an autosomal dominant or X-linked recessive (XR) inheritance [13,14]. In recent years, five autosomal genes have been found to be mutated in children with this mendelian genetic disorder: *IFNGR1* [15–19]. *IFNGR2* [20,21], *STAT1* [22,23], *IL12B* [24], and *IL12RB1* (Fig. 1) [25,26]. All are involved in interleukin (IL)-12/23–dependent, IFN-γ mediated immunity. Mutations in *IFNGR1*, *IFNGR2*, and *STAT1* impair the cellular responses to IFN-γ, whereas mutations in *IL12B* and *IL12RB1* impair the IL-12/23–dependent production of IFN-γ. Mutations in *NEMO* have also been identified as responsible for XR form of MSMD (see Fig. 1) [27]. The high level of allelic heterogeneity at these seven loci accounted for the existence of 13 different inheritance disorders, according to the mode of transmission (recessive or dominant), the impact on function (complete or partial), association with a lack of protein expression or the expression of an abnormal protein, and the specific function affected (phosphorylation or DNA binding in the case of STAT-1). However, no genetic cause has yet been identified for about half the patients who have MSMD [28].

Recessive complete IFN-gamma receptor (IFNGR) 1 deficiency was first identified in 1996, in a patient who had a lack of receptor expression [15,19]. Mutations conferring autosomal recessive complete IFNGR1 deficiency with [16] or without cell surface receptor expression have since been reported [15]. A lack of cellular responses to IFN-γ stimulation in vitro has been reported in these patients, who also have high plasma IFN-γ

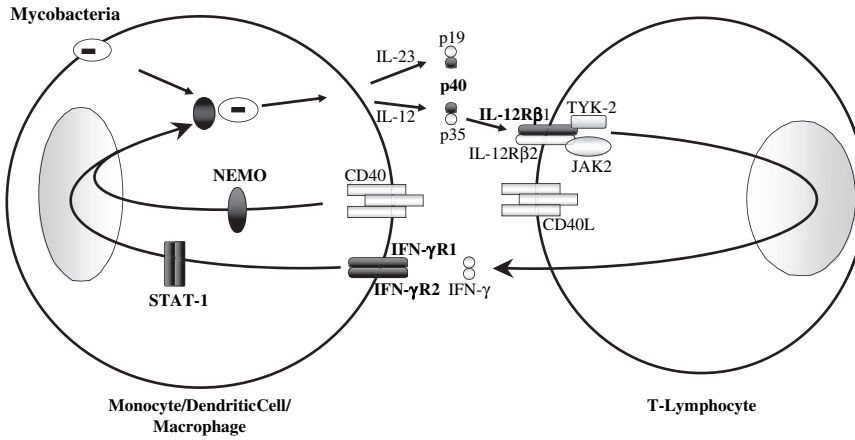

Fig. 1. MSMD-causing gene products in the IL-12/23–IFN-γ pathway. Cytokine production and cooperation between monocytes/macrophages/dendritic cells and natural killer or T cells. Proteins for which mutations in the corresponding genes have been identified and associated with particular susceptibility to mycobacterial diseases are shown in gray. Allelic heterogeneity of the seven genes results in the definition of 13 genetic disorders. The IL-12/23–IFN-γ loop and the CD40L-activated CD40 pathway, mediating cooperation between T cells and monocytes, are crucial for protective immunity to mycobacterial infection in humans. The *NEMO* mutations in the leucine zipper (LZ) domain mostly impair CD40-NEMO–dependent pathways.

concentrations [29]. Mycobacterial infections in patients who have complete IFNGR1 deficiency are associated with an early onset of severe and fatal infectious diseases, lepromatous-like and multibacillary granulomas [30], and a poor prognosis. No asymptomatic patients over the age of 3 years have been reported. *Mycobacterium bovis*–BCG and other EM (eg, *Mycobacterium fortuitum* [16,31], *Mycobacterium chelonae* [18], *Mycobacterium smegmatis* [32,33], *Mycobacterium peregrinum* [34], *Mycobacterium scrofulaceum* [13,35]) frequently cause disease in these patients. All patients vaccinated with BCG developed disseminated BCG infection. Salmonellosis was reported only rarely [15,19,31]. Viral infections caused by human herpes virus 8 [36], cytomegalovirus [37], and other bacterial infections, caused by *Listeria monocytogenes* [38], were documented in single patients. A diagnosis of complete IFNGR1 deficiency should be considered for children with several episodes of mycobacterial disease (BCG, EM) occurring before the age of 3 to 5 years. The outcome is spontaneously fatal and the only potential cure is offered by hematopoietic stem cell transplantation, which is, however, associated with a high rate of graft rejection [39,40], because of the high plasma IFN-γ concentrations in these patients [41]. The oldest surviving patient is now 19 years old and has experienced at least five episodes of mycobacterial disease, caused by five microbial species [35].

Partial IFNGR1 deficiency may be caused by recessive or dominant *IFNGR1* alleles. The recessive form of partial IFNGR1 deficiency results

from a specific mutation, I87T [17,42]. Cells from the patients who have partial deficiency respond to high (but not low) concentrations of IFN-γ in vitro. Clinically, this defect is associated with BCG [17] or EM disease [42]. Affected children had well-delimited granulomas and a favorable outcome. One patient, not vaccinated with BCG, had clinical tuberculosis [11,17]. Dominant partial deficiency in *IFNGR1* is typically associated with the 818del4 mutation, associated with a hotspot for microdeletions, leading to the insertion of a premature stop codon in the proximal intracellular domain [18]. The cellular response to IFN-γ stimulation in vitro is intermediate between that of cells from patients who have complete IFNGR1 deficiency and healthy controls. The severity of the clinical features of patients who have dominant partial IFNGR1 deficiency appears to be intermediate between those of patients who have recessive complete and partial IFNGR1 deficiencies. Patients are susceptible to infections with BCG vaccines and EM (*M peregrinum, M kansasii, M chelonae, M fortuitum,* and *M asiaticum*), although the mycobacterial pathogen most frequently isolated from these patients is *M avium* complex [31,43]. Unifocal or multifocal mycobacterial osteomyelitis is common, and the overall prognosis is good. Some patients were initially diagnosed incorrectly as having Langerhans' cell histiocytosis [44]. In rare cases, these patients present other infections, such as salmonellosis [31]. Viral infection with varicella zoster virus [37] and fungal infection with *Histoplasma capsulatum* have each been observed in one patient [45]. Treatment is based principally on antibiotics and the use of recombinant IFN-γ.

A few patients who have MSMD with autosomal recessive, complete IFNGR2 deficiency have been reported. Two forms of complete IFNGR2 deficiency with [21] and without [20] receptor expression at the cell surface have been documented. The T168N missense mutation in *IFNGR2* creates a new N-glycosylation site caused by the addition of a new glycan branch on the IFNGR2 chain. An *IFNGR2* mutation has also been reported to be dominant in vitro in a healthy heterozygous relative of a patient who had IFNGR2 deficiency [46]. It remains unclear as to whether some *IFNGR2* mutations are clinically dominant in vivo. The criteria for suspicion of complete IFNGR2 deficiency are similar to those for complete IFNGR1 deficiency. Patients who have complete IFNGR2 deficiency display early-onset mycobacterial diseases and have a poor prognosis. No mature granulomas have been observed in these patients. The pathogens causing the infectious diseases included *M bovis* BCG [21], *M avium* [20,21], and *M fortuitum* [20]. The only curative treatment is hematopoietic stem cell transplantation. Partial recessive IFNGR2 deficiency has been reported in only one patient who had infections caused by BCG and *M abscessus* and a relatively mild clinical presentation, similar to that of children with partial recessive IFNGR1 deficiency [47]. Treatment is based on antibiotics and, when needed, recombinant IFN-γ. Overall, the genetic disorders of IFNGR1 and IFNGR2 strongly suggest a tight correlation between the

level of IFN-γ–mediated immunity and the clinical severity of mycobacterial disease [48].

STAT-1 is a critical transducer of cellular responses to type I (IFN-α/β) and type II (IFN-γ) IFNs, and to the less well characterized type III IFNs (IFN-λ) [49]. Patients who have complete STAT-1 deficiency present a syndrome of susceptibility to mycobacteria and viruses because of impaired cellular responses to antimycobacterial IFN-γ and antiviral IFN-α/β and -λ [50,51]. This clinical syndrome, therefore, clearly differs from that of patients who have MSMD, who are normally resistant to most viruses, with a few possible exceptions, as discussed earlier [50,51]. However, some germline mutations in *STAT1* have been shown to be responsible for mycobacterial disease as the sole infectious phenotype in selected patients, owing to the selective impairment of IFN-γ responses mediated by STAT-1 homodimers (gamma-activating factor) activation [22,23]. In these patients, IFN-α responses mediated by STAT-1–STAT-2–interferon regulatory factor (IRF)-9 trimers (interferon-stimulated gamma factor 3), unlike those in patients who have complete STAT-1 deficiency, were entirely normal. Patients who have partial STAT-1 deficiency are heterozygous for these subtle mutations that are intrinsically loss-of-function for IFN-γ and IFN-α/β responses, but dominant for IFN-γ responses only. As a result, these patients have mild clinical features characterized by mycobacterial disease without susceptibility to viruses, resembling those in patients who have partial IFNGR1 deficiency, with a good prognosis. Two types of *STAT1* mutations are dominant, depending on whether the mutation impairs STAT-1 phosphorylation [23] or DNA-binding activity [22]. Clinically, the patients suffered from disseminated BCG infection or *M avium* infection [22,23]. One patient was reported to have *M tuberculosis* infection [22]. Treatment is based on antibiotics and recombinant IFN-γ.

The first genetic defect of the human IL-12 and IL-23 pathways was described in 1998 [24] and indicated that these cytokines were critical for controlling the levels of the antimycobacterial molecule, IFN-γ. Mutations in the *IL12RB1* gene encoding the first chain of the IL-12 receptor (IL-12Rβ1), common to the IL-12 and IL-23 receptors, and the *IL12B* gene encoding IL-12p40, common to IL-12 and IL-23, have been reported (see Fig. 1) [25,52]. All mutations in *IL12B* and *IL12RB1* cause a complete, autosomal recessive defect because they are loss-of-function. IL12RB1 deficiency is the most frequent genetic cause of MSMD affecting families from many different countries [13]. Mycobacterial disease and salmonellosis are the most common infectious diseases for both deficiencies [13]. The clinical phenotype of IL12RB1-deficient patients is similar to that of IL12p40-deficient patients. About one half of all IL12B- and IL12RB1-deficient patients have developed *Salmonella* infection at some time, in contrast to MSMD patients who have mutations affecting IFN-γ signaling immunity [53,54]. These observations suggest that IL-12/-23 plays a key role in the

pathogenesis of salmonellosis, possibly through IL-23–dependent IL-17 induction. Several patients who had *IL12RB1* defects were found to have susceptibility to *M tuberculosis* as their sole infectious phenotype, and one patient who had IL12B deficiency, who had been vaccinated with BCG, had *M tuberculosis* infection, providing the first conclusive evidence that tuberculosis can result from a mendelian predisposition [11,55–57]. Other infectious diseases, such as paracoccidioidomycosis [58], leishmaniasis [59], and nocardiosis [54], have been reported in isolated cases, suggesting that the clinical features of these disorders may expand with the diagnosis of new cases. Treatment is based on antibiotics and IFN-γ.

The recent identification of specific mutations in *NEMO* revealed XR-MSMD–causing gene. *NEMO* encodes the NF-κB essential modulator, also known as IkappaB kinase (IKK)-γ, a regulatory unit of the IKK complex. Mutations in this gene are responsible for various diseases: null mutations lead to incontinentia pigmenti [60] and hypomorphic mutations result in XR anhidrotic ectodermal dysplasia with immunodeficiency, osteopetrosis, and noninflammatory lymphedema (XR-EDA-ID-OL) [61], XR anhidrotic ectodermal dysplasia with immunodeficiency, and osteopetrosis (XR-EDA-ID-O) [61–63], XR anhidrotic ectodermal dysplasia with immunodeficiency (XR-EDA-ID) [61], XR-mild-EDA [64,65], or immunodeficiency (ID) only without ectodermal dysplasia (EDA) [66]. The range of infections varies considerably, and includes viral, fungal, and common pyogenic infections [67]. Specific mutations affecting the leucine zipper domain of NEMO, E315A, and Q319R were recently shown to be associated with mycobacterial infections in three unrelated kindreds with MSMD (XR-MSMD-type 1) [27]. Two mycobacteria were isolated: *M avium* in most patients and *M tuberculosis* in one patient [68]. Another patient probably had tuberculosis [27]. One patient presented mild signs of EDA, limited to conical decidual incisors, whereas the other patients had no overt developmental abnormality. These subtle mutations affect the T-cell–dependent, CD40-dependent activation of c-Rel, resulting in impaired IL-12 induction in monocytes and dendritic cells [27]. The prognosis seems to be unpredictable, varying considerably among patients. Treatment is based on antibiotics and, possibly, IFN-γ.

Mendelian predisposition to invasive pneumococcal disease

IPD is defined by the infection of normally sterile compartments of the body by *Streptococcus pneumoniae* (Fig. 2). In most individuals with IPD, colonization of the upper respiratory tract with *S pneumoniae* is a prerequisite for IPD. Thus, in most cases, IPD is caused by bacteria crossing the mucosal epithelia of the upper respiratory tract and reaching the blood stream. IPD may thus present as sepsis, meningitis, osteomyelitis, or arthritis. The colonization of the upper respiratory tract with *S pneumoniae*

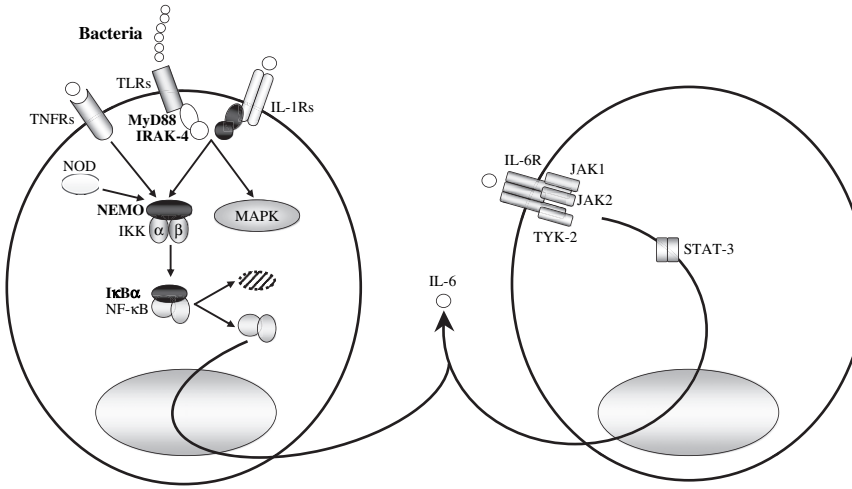

Fig. 2. Mendelian susceptibility to IPDs. Cytokine production and cooperation between phagocytes (polymorphonuclear neutrophils, monocytes, and dendritic cells) and lymphocytes (T, B, and natural killer) recognized by TLRs at the surface of phagocytes. This recognition induces activation of the NF-κB and mitogen-activated protein (MAP) kinase pathways by way of myeloid differentiation factor 88 (MyD88) and interleukin receptor-associated kinase-4 (IRAK-4). These activation pathways induce the production of several proinflammatory cytokines (IL-6, IL-1β, and tumor necrosis factor [TNF]-α). IRAK-4 deficiency is associated with the impaired induction of inflammation by TLR ligands, and the impaired propagation of inflammation by IL-1 and IL-18. NEMO and IκBα deficiencies are associated with the impaired induction of inflammation by TLR ligands and TNF receptor ligands. IRAK-4, NEMO, and IκBα deficiencies are associated with impaired IL-6 production. Proteins for which mutations in the corresponding genes have been identified and shown to be associated with particular susceptibility to IPD are shown in gray.

reaches a peak at the age of 3 years, when more than 50% of children are colonized. Colonization then declines to about 10% by the age of 10 years [69,70]. Before the introduction of a protein-conjugated vaccine against *S pneumoniae*, the prevalence of IPD among children under the age of 2 years ranged from 14/100,000 in Germany, more than 90/100,000 in Spain, and 188/100,000 in the United States [71–73] to estimates as high as 1741/100,000 in sub-Saharan Africa [74]. The introduction of the protein-conjugated vaccine in 2000 has greatly decreased the number of cases of IPD, but the prevalence of this disease remains substantial in children [75–77]. An intact respiratory tract epithelium usually prevents *S pneumoniae* invasion. Having crossed this barrier, opsonization by complement and antibodies is crucial for phagocytosis by neutrophilic granulocytes, monocytes, and, in particular, splenic macrophages. T cells seem to be crucial for the maintenance of a humoral antipneumococcal response. The importance of these components of the immune system is demonstrated by the greater susceptibility to IPD of patients who have mucosal epithelia damaged by flu [78], of patients who have defects of early components of the

classic complement pathway (C1, C4, C2), C3, factors I and D or antibody formation, and of patients who have asplenia or HIV infection [79,80]. Several primarily nonimmunologic conditions also predispose patients to IPD: kidney failure, liver failure, heart failure, sickle cell anemia, leukemia, and fistulae in the cerebroarachnoidal space [80,81]. However, although some cases may be accounted for by these conditions, the cause of IPD remains unknown in most patients.

XR-EDA-ID is a recently defined PID conferring predisposition to multiple infections, including IPD in particular, which has been documented in most patients. In 2001, XR-EDA-ID was shown to be caused by hypomorphic mutations in the gene encoding the NF-κB essential modulator (NEMO) [61–63]. NEMO is a scaffold protein essential for the formation of a protein complex (IKK) catalyzing the phosphorylation of NF-κB-inhibitor proteins (IκBs). IκBs control the balance between the nuclear and cytosolic activities of NF-κB by binding to NF-κB, which is present in the cytosol when bound to IκB. The phosphorylation of IκBs leads to the dissociation of IκBs from NF-κB and an increase in the nuclear activity of NF-κB. Because IKK is the convergence point for several pathways controlling the development of skin appendages (dependent on the receptor for ectodysplasin) and for many pathways mediating immunologic responses (eg, dependent on receptors such as those of the tumor necrosis factor-α-receptor superfamily, the B-cell receptor, and the those of the Toll-like and IL-1 receptor [IL-1R] family [TIR]), most patients who have NEMO deficiency present a clinical combination of ectodermal dysplasia and immunodeficiency (EDA-ID) [61–63], an entity first described by Abinun and colleagues [82,83]. The features of EDA-ID comprise a typical facies (frontal bossing, saddle nose), aberrant development of skin appendages, such as hair (sparse or missing hair and sparse or missing eye brows), teeth (hypodontia, anodontia, conical incisors or delayed eruption of teeth), and sweat glands (anhidrosis and heat intolerance), and strong susceptibility to IPD and other infectious diseases [61–63,82–84].

The ectodermal and immunologic phenotypes of individual patients who have NEMO deficiency may be extremely variable. The degree of EDA ranges from XR-EDA-ID-OL [61,85,86], through XR-EDA-ID-O [63,92], "classic" XR-EDA-ID [61–63,82–84,88–95], and XR-mild-EDA [64,65], to ID only [13,66,84,96,97]. The infectious phenotype is similarly broad. In addition to IPD [61,63–65,83–85,87,90,91], NEMO-deficient patients may present sepsis, meningitis, osteomyelitis, and pneumonia due to other pyogenic bacteria (*S aureus*, *Streptococcus bovis*, *L monocytogenes*, *Streptococcus equii*, *Salmonella enteritidis*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, *Escherichia coli*) [8,61,64,84,86,87,89,90,93–95,97]; lymphadenitis, sepsis, osteomyelitis, and pneumonia caused by mycobacteria (*M abscessus*, *M avium*, *M bovis*, *M kansasii*) (see earlier discussion) [13,61,63,66,84–89,92,96,97]; infections caused by various viruses (chronic molluscum contagiosum; HSV stomatitis; sepsis,

colitis or retinitis due to cytomegalovirus; nonspecified viral meningitis; HSV encephalitis) (see later discussion) [61,63,66,84,86,87,92,96]; sepsis or pneumonia caused by fungi (*Candida albicans*, *Pneumocystis carinii*, *Pneumocystis jiroveci*) [61,84,85,87,93]; or enteritis caused by *Giardia lamblia* [84]. Severe infections may be accompanied by surprisingly low signs of inflammation [84,85,87,93]. In addition to the diversity of possible pathogens, considerable interindividual variation is observed in the frequency and severity of infections, with some patients having a course of disease justifying a bone marrow transplant [85,95] and others doing well on antibiotic prophylaxis only (von Bernuth and Casanova, unpublished data). If the severity of the clinical course justifies a bone marrow transplant, reduced-intensity conditioning and graft-versus-host disease prophylaxis should be considered because severe liver toxicity has been observed [85,95].

Patients who have NEMO deficiency may present a number of comorbid features at an early age, including intractable diarrhea and failure to thrive [84,85,93]. In one family, all patients were reported as born small for gestational age [93]. Some NEMO-deficient patients may present autoimmune phenomena [84,93,96] and impaired natural killer cell–mediated cytotoxicity [84,87,92,95]. In routine immunologic workup, most NEMO-deficient patients present low, high, or fluctuating serum concentrations of the three subtypes of immunoglobulin, in all possible combinations [63,64,83–86,89–93,95,99], leading to the suggested "diagnosis" of "common variable immunodeficiency." Low levels of IgG and IgA combined with high levels of IgM may lead to the "diagnosis" of "Hyper-IgM syndrome" [62,63,66,84,87,93,94,96,99,100]. In some patients, low levels of $CD27^{+}$, IgD^{-} ("class-switched somatically mutated memory") B cells have been observed [66,96,99]. This feature probably results from defective V(D)J recombination in individuals who have hypomorphic mutations in the C-terminal region of the zinc-finger motif [101]. The proliferation of lymphocytes in response to mitogens and antigens may appear normal, but mild impairment of mitogen-dependent and, in particular, T-cell–dependent or antigen-dependent proliferation has been reported in some patients [64,84,85,92,96]. The most consistent immunologic finding among patients who have NEMO deficiency seems to be the absence of antibodies against glycans, such as those of *S pneumoniae,* despite infection or vaccination, and of allohemagglutinins in children over the age of 2 years [63,64,66,83,85,90,91,96].

The discovery that XR EDA-ID was caused by hypomorphic mutations in *NEMO* paved the way for the discovery, in 2003, that a hypermorphic mutation in *IKBA* was the cause of autosomal dominant AD-EDA-ID [102]. A heterozygous dominant-negative missense mutation, S32I, in *IKBA* prevents IκBα phosphorylation at the mutated site, maintaining NF-κB in its IκBα-bound form restricted to the cytosol [102]. The same mutation was described in a second patient who had AD-EDA-ID [103]. A third patient, who had a heterozygous mutation creating a premature

stop codon, W11X, leading to haploinsufficiency of IκBα, was recently reported [104]. The clinical phenotypes of the first two patients were similarly severe, with chronic diarrhea and failure to thrive, recurrent bronchopneumonia, meningitis, and enteritis caused by gram-positive bacteria (*S aureus, Streptococcus pyogenes*), gram-negative bacteria (*Pseudomonas spp*, *K pneumoniae*, *Serratiae spp*, *Salmonella typhimurium*), and fungi (*P jiroveci*). Both patients had a "hyper-IgM phenotype" with low serum concentrations of IgG and IgA, a high serum concentration of IgM, and no antibodies against glycans. Unlike patients who have NEMO deficiency, these patients also showed marked signs of an aberrant T-cell development and impaired T-cell function, with no detectable γ/δ T cells, almost all α/β T cells having the naive CD45RA phenotype, and no antibodies detected in the serum in response to recall antigens, despite vaccination, together with a complete lack of in vitro proliferation in response to CD3-specific antibodies (OKT 3) and recall antigens. Pronounced lymphocytosis was also a typical feature in both patients [102,103]. The third patient described also suffered from multiple bronchopneumonia but with no bacteremia documented to date. In further contrast to the first two patients who had the S32I mutation in *IKBA,* this patient had normal serum IgG and low IgM levels and a less pronounced T-cell phenotype. Antibody responses and in vitro proliferation to recall antigens were normal, as were the percentages of T and B cells, despite marked lymphocytosis [104]. Hematopoietic stem cell transplantation is a curative option for patients who have autosomal dominant EDA-ID [105].

Because NEMO mutations impair multiple pathways and render patients susceptible to a broad range of infections including IPD, the authors hypothesized that a deficiency in one particular pathway upstream from NEMO and IKK might be responsible for cases of IPD without EDA or infections with other pathogens. One obvious candidate was the TIR-dependent pathway, because the extracellular domain of TLRs consists of leucine-rich repeats that sense components of various pathogens, and because the extracellular domain of IL-1Rs consists of immunoglobulin-like domains that recognize IL-1. All human TLRs (1–10) and IL-1Rs (TIRs) signal by way of a structurally similar intracellular domain, the so-called "TIR domain." TIRs, which have no kinase domain, transmit their signal by way of several adapter molecules that bind to the receptors by way of the TIR domain and recruit other molecules to the receptor. One of these adapter molecules, myeloid differentiation factor 88 (MyD88), is used by all TLRs except TLR3 and by all IL-1Rs with a TIR domain. After MyD88, the next molecule to be recruited is interleukin receptor-associated kinase-4 (IRAK-4). Upon binding to MyD88, this kinase is phosphorylated. It then phosphorylates interleukin receptor-associated kinase-1 (IRAK-1), resulting in the degradation of this protein. IRAK-1 activation is essential for the subsequent transmission of the signal to several pathways, including those controlled by mitogen-activated protein (MAP) kinases and IKK,

eventually leading to the translocation of transcription factors activator protein (AP)-1 and NF-κB to the nucleus.

The authors' hypothesis that IPD was caused by defects in this pathway was confirmed by the identification of the first three patients who had a strong, almost specific susceptibility to IPD caused by autosomal recessive complete IRAK-4 deficiency in 2003 [106]. Because IRAK-4 deficiency leads to an isolated impairment of TIR signaling, IRAK-4-deficient patients display no EDA. Despite the major role initially proposed for TLRs in human immunity [107] and the almost complete impairment of TIR signaling (with the exception of TLR3-IFN-β and TLR4-IFN-α/β pathways) in all cell types tested, patients who have IRAK-4 deficiency are not particularly susceptible to mycobacteria, viruses, fungi, or parasites [108,109]. In contrast, IRAK-4 deficiency predisposes patients exclusively to infections caused by pyogenic bacteria, *S pneumoniae* in particular. Meningitis, arthritis, osteomyelitis, and abscess formation in internal organs and just below the skin are the most frequently observed infections. The pyogenic bacteria other than *S pneumoniae* known to have caused severe infections in patients who had IRAK-4 deficiency to date are *S aureus* and *Pseudomona aeruginosa*, with less frequent infections caused by *Clostridium septicum*, *Streptococcus parasanguis*, *Streptococcus milleri*, *E coli*, *Neisseria Meningitidis*, and *Shigella sonnei* [65,106,108–118]. Most patients suffer from IPD and invasive infections caused by other pyogenic bacteria in infancy and early childhood. The prognosis of IRAK-4 deficiency improves significantly with age. No deaths have been reported in patients over the age of 8 and no invasive infection was reported in six patients over the age of 14, even in the absence of antibiotic prophylaxis [108,110,111,114,116]. In most cases, routine immunologic workup gave normal findings for levels of immunoglobulins, antibodies against recall antigens and glycans, percentages of lymphocyte subpopulations, and lymphocyte proliferation assays. Only a few cases of IRAK-4 deficiency associated with low serum levels of antibodies against recall antigens [112] or glycans [108,112] have been reported. A characteristic, but not specific, clinical feature of IRAK-4 deficiency is the prolonged period required for the umbilical cord to detach after birth (>30 days) [118]. As previously reported for a NEMO-deficient patient, most patients who have IRAK-4 deficiency have a delayed or weak inflammatory response (low fever, low C-reactive protein levels), even during severe invasive infection [98,113]. In most patients who have IRAK-4 deficiency, the immunologic routine workup does not reveal any anomaly. Only a few patients displayed low levels of serum antibodies against recall antigens [52] or glycans [52,108]. A defect in TIR-dependent pathways can be suspected in patients whose whole blood cells do not produce IL-6 or whose granulocytes do not shed CD62L after stimulation by TLR agonists [119]. The authors recommend treating patients who have IRAK-4 deficiency with an antibiotic prophylaxis that is directed against pyogenic bacteria, in particular, *S pneumoniae*. Vaccinations against *S pneumoniae* and *N meningitidis* should

be performed and in selected cases, the administration of immunoglobulins may be considered.

Mendelian predisposition to herpes simplex encephalitis

Herpes simplex encephalitis (HSE), which is typically caused by HSV type 1 (HSV-1) in patients older than 3 months [120–122], is the most common sporadic viral encephalitis in the Western world (Fig. 3) [123]. The estimated incidence of HSE is between two and four individuals per million per year [124–126], with approximately one third of all HSE cases caused by primary infections (mostly in children, because about 90% of people are seropositive by the age of 18 years) [122,126,127–129]. The first child with HSE was described in 1941 [130], and the first adult in 1944 [131]. This disease was almost invariably lethal until the advent of acyclovir in the 1980s. Most patients now survive HSE, although many, including young children in particular, present neurologic sequelae [127]. HSE is a devastating disease of unclear pathogenesis, because HSV-1 is widespread and readily transmitted from person to person, with more than 85% of adults between the ages of 20 and 40 serologically positive for HSV-1 in a survey of more than 20 countries [132,133]. Moreover, HSV-1 is benign in most

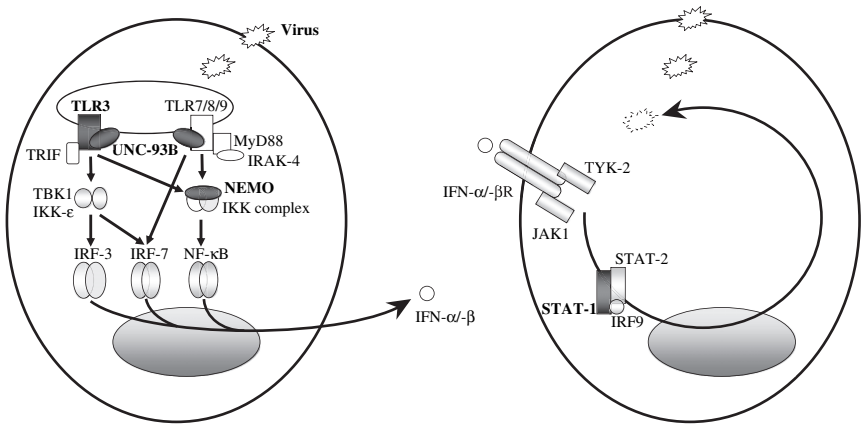

Fig. 3. Mendelian predisposition to HSE. Production and response to IFN-α/β during virus infections. Viruses enter the cells by way of specific receptors, and viral nucleic acids are detected by the various TLRs. This recognition induces activation of the IRF-3, IRF-7, and NF-κB pathways by way of UNC-93B, TRIF, and MyD88, leading to type I IFN production. TLR3, UNC-93B, and NEMO deficiencies are associated with impaired IFN production, particularly during herpes virus infection. The binding of type I IFN to its receptor induces the phosphorylation of JAK1 and TYK-2, activating the signal transduction proteins STAT-1, STAT-2, and IRF9. This complex is translocated as a heterotrimer to the nucleus, where it acts as a transcriptional activator, binding to specific DNA response elements in the promoter region of IFN-inducible genes. STAT-1 deficiencies are associated with impaired IFN responses. Proteins for which genetic mutations have been identified and associated with particular susceptibility to viral diseases are shown in gray.

individuals, infecting the oral mucosa, establishing latency in the trigeminal ganglia, and resulting in asymptomatic infection or recurrent herpes labialis. The pathogenesis of HSE, which affects a few HSV-1–infected individuals, has therefore long remained unclear. No particular risk factors have been definitively demonstrated to be associated with the variation of individual HSV-1 [134–137] or other environmental factors [126,128,129]. The role of human genetic susceptibility in HSE was first suggested by the reports of four unrelated multiplex HSE kindreds [138–141]. Although HSE is sporadic in most cases, the patients from these families belonged to one or two generations and suffered from HSE at several years apart [138–141]. A high incidence of parental consanguinity (14%) was recently documented in French kindreds with HSE [127]. These clinical observations suggest that HSE may result from mendelian predisposition, in at least some children.

The first attempt to document human genetic predisposition to HSV-1 infection was reported in 2001, with apolipoprotein E genotypes associated with HSE in one study [142] but not another [143]. HSE was not reported in patients who had bona fide mendelian PIDs until 2003, when a few patients were reported to lack STAT-1, a critical protein downstream from the receptors to IFN-α/β and IFN-γ [51]. STAT-1–deficient patients suffered from mycobacterial disease caused by their impaired response to IFN-γ [10], and were susceptible to viral diseases, including recurrent HSE in one child [51], presumably because of their impaired IFN-α/β responses [144]. This finding provided the first suggestion that isolated HSE might be caused by inherited immunodeficiencies involving IFN-α/β–mediated immunity. Consistent with this hypothesis, one patient who had a mutation in *NEMO* [66,96] presented recurrent mycobacterial disease and recurrent and lethal HSE. NEMO not only regulates NF-κB activation, it is also involved in the activation of IRF3 after viral infections or virus-associated stimulation [145]. In this patient who had NEMO deficiency, the production of IFN-α, -β, and -λ was impaired and may account for the occurrence of HSE. Although HSE has not been observed in other PIDs, the occurrence of HSE in the STAT1- and NEMO-deficient patients also presenting mycobacterial disease strongly suggests that the human innate immune system is involved in protective immunity to HSV-1 in the central nervous system (CNS), and indicates that the production of, or response to, IFN-α, -β, and -λ is probably important in HSE.

The first genetic cause of isolated HSE was described in 2006, with the identification of homozygous germline mutations in *UNC93B1*, in two unrelated and otherwise healthy patients suffering from HSE, from different consanguineous kindreds [146]. The human and mouse UNC-93B proteins contain 12 transmembrane domains and are found mostly in the endoplasmic reticulum [147,148]. In the mouse, UNC-93B binds to the transmembrane domains of TLR3 and TLR9 [147], and is required for the signaling of intracellular, nucleic acid–sensing TLRs (TLR3, TLR7, and TLR9)

because UNC-93B–deficient mouse macrophages display impaired responses to these TLRs [148]. The identification of human UNC-93B deficiency confirmed the requirement for UNC-93B in human TLR3, TLR7, TLR8, and TLR9 signaling [146]. The 1034del4 and 781G>A mutations in the coding region of *UNC93B1* result in a premature termination codon and lead to a complete loss of UNC-93B expression (caused by nonsense-mediated mRNA decay) and function. TLR3 signaling was abolished in the patients' fibroblasts, as shown by the impaired IFN-β and -λ production following stimulation with poly(I:C). TLR7, TLR8, and TLR9 are expressed in peripheral blood mononuclear cells (PBMCs), and the PBMCs from UNC-93B–deficient patients did not respond to the stimulation of TLR7, TLR8, or TLR9, in terms of the production of IFN-α, -β, and -λ, and other cytokines tested. TLR7-, TLR8-, and TLR9-mediated IFN-α, -β, and -λ production seems to be largely redundant in human antiviral immunity; the IRAK-4–deficient patients [108] displayed impaired IFN-α, -β, and -λ production following the activation of TLR7, TLR8, and TLR9 but were resistant to most common viral infections, HSE in particular. The identification of human UNC-93B deficiency thus provided the first evidence that HSE can result from a monogenic trait, and that impaired TLR3-triggered, UNC-93B–dependent, IFN-α, -β, and -λ induction may be involved in HSE.

In 2007, a germline heterozygous *TLR3* mutation was identified in two other unrelated and otherwise healthy children with HSE from nonconsanguineous families [149]. The identification of the second genetic cause of isolated HSE established that the TLR3-UNC-93B-IFN-α, -β, and -λ signaling pathway is essential for protective immunity to HSV-1 in the CNS, at least in some children, during primary infection. TLR3, one of the 10 members of the human TLR family, recognizes dsRNA, an almost universal viral intermediate potentially generated during most viral infections, and induces IFN production. TLR3 was therefore thought to play a broad role in antiviral immunity. In the two children with autosomal dominant TLR3 deficiency, the same heterozygous 1660C>T mutation (P554S) was found [145]. The 1660C>T mutant allele is loss-of-function and dominant negative for the response to poly(I:C) in fibroblasts, in terms of IFN-β and -λ induction. Different types of TLR3-expressing cells, including fibroblasts, from patients displayed an impaired response to poly(I:C). The production of IFN-β and -λ by fibroblasts was impaired following stimulation with poly(I:C), HSV-1, and vesicular stomatitis virus (VSV), as in UNC-93B–deficient fibroblasts. Moreover, higher viral titers and higher levels of cell death following HSV-1 and VSV infection were observed in TLR3-heterozygous fibroblasts and UNC-93B–deficient fibroblasts complemented with IFN-α and -β, and, less efficiently, with IFN-λ. Impaired TLR3 signaling thus results in abnormally weak IFN-α, -β, and -λ production, enhanced viral replication, and enhanced cell death in patients' fibroblasts. By inference, this provides a plausible mechanism for HSE in the CNS. Indeed, TLR3 is

the most abundantly and widely expressed TLR in human CNS-resident cells, including neurons [150], microglia [151,152], astrocytes [151,152], and oligodendrocytes [151]. HSV-1 replication has been documented in vitro in these cells [150,153,154], and human neurons [150], astrocytes [152], and microglia also produce IFN-β in response to dsRNA.

These findings have provided conclusive evidence that isolated HSE results from a new group of single-gene immunodeficiencies, at least in some children. The production of IFN-α, -β, and -λ by way of TLR3-UNC-93B is critical for protective immunity to HSV-1 in the CNS. These results have important therapeutic implications, because at least some HSE patients would probably benefit from recombinant IFN-α treatment in addition to acyclovir [146]. An interesting unresolved question concerns the role of different IFN-α, -β, and -λ subtypes in anti–HSV-1 immunity. The subtype may be of importance, and may provide further indications for the treatment of HSE patients who have IFNs. HSE patients, including the reported UNC-93B– and TLR3-deficient patients [146,149], are typically not susceptible to other infections, or even to HSV-1 infections outside the CNS [155]. TLR3-independent IFN responses to dsRNA in some cells types, including dendritic cells [149], and TLR3-independent IFN responses to other viral intermediates may confer protection against a wide range of viruses in TLR3- and UNC-93B–deficient patients [156,157]. However, limited by the small number of UNC-93B– and TLR3-deficient patients studied, which resulted in probable strong ascertainment bias, no definitive conclusion can be made concerning the infectious phenotypes of UNC-93B– and TLR3-deficient patients. Five of the seven TLR3-deficient individuals and one of the three UNC-93B–deficient individuals did not develop HSE, despite proved HSV-1 infection [149]. The incomplete clinical penetrance of HSE in TLR3- and UNC-93B–deficient individuals provides an explanation for the sporadic occurrence of HSE. It may be that age at infection is a key factor governing the development of HSE in genetically predisposed individuals, as suggested by the genetic epidemiologic survey of French cases [127]. Finally, the lack of identified genetic deficiencies in most other HSE patients, including adult HSE patients, in whom the pathogenesis of HSE may be different from that in pediatric cases [158], makes it interesting to explore the possible genetic disorders in these patients, whether they concern the TLR3–UNC-93B–IFN-α, -β, and -λ signaling pathway, or other systems. Future genetic studies based on positional cloning, and biologic studies based on functional screens, will undoubtedly provide additional insight into the pathogenesis of HSE, by better defining the molecules and cells involved in immunity to HSV-1 in the CNS.

Summary

From the 1950s onwards, patients who had a selective predisposition to infections caused by weakly virulent mycobacteria (MSMD) were thought

to have a PID, given the occurrence of similar infections in patients who had multiple infections and severe PIDs, such as severe combined ID [159]. It was not until 1996 that the first molecular defects were found, and now we know of 14 genetic causes of MSMD, resulting from mutations in seven genes. Many more genetic causes of MSMD remain to be discovered, and their discovery may help us decipher the genetic basis of pediatric tuberculosis, as suggested by the occurrence of this disease in children with IL12RB1 deficiency [11]. Children with recurrent IPD were also thought, from the 1950s onwards, to suffer from PIDs, because PIDs affecting pneumococcal opsonization, such as X-linked agammaglobulinemia, predispose patients to invasive disease caused by encapsulated bacteria, including pneumococcus [80]. It was not until 2003, with the description of children with IRAK-4 deficiency, based on the prior discovery of *NEMO* and *IKBA* mutations in patients who had a complex PID associated with multiple infections including IPD as the dominant trait, that the first specific genetic cause of recurrent IPD was documented. Most patients who have recurrent IPD and most patients who have a single episode of IPD still have no identified genetic cause. Finally, HSE was not thought to result from a PID until the first two patients who had HSE and mycobacterial disease were identified in 2003, and until a genetic cause of isolated HSE was discovered in 2006 [160]. Most children with a single episode or recurrent HSE still have no identified genetic cause. Research into PIDs will undoubtedly advance through the identification of new genetic causes of MSMD, IPD, and HSE. This expansion may become a paradigm, because other pediatric infectious diseases are also likely to result from novel PIDs.

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Congenital Neutropenia Syndromes

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Congenital neutropenia syndromes comprise a heterogeneous group of inherited disorders. Neutropenia is termed "severe" when absolute neutrophil counts (ANC) are below 0.5×10^9/L. Hereditary conditions associated with low neutrophil counts are persistent and need to be differentiated from neutropenia secondary to autoimmune processes or other pathologic conditions, such as myelodysplasia or leukemia. Clinically, congenital neutropenia is characterized by recurrent bacterial infections, including pneumonia, skin, and deep organ abscesses, as well as septicemia [1,2]. The most common variant is severe congenital neutropenia, characterized by "maturation arrest" at the stage of promyelocytes/myelocytes in bone marrow (Fig. 1), which was described more than 50 years ago by Rolf Kostmann [1]. Recently, several novel genetic defects were described in patients with congenital neutropenia, shedding light on the pathophysiology of these rare diseases [3,4].

Diagnosis of congenital neutropenia

Suspicion of congenital neutropenia is usually raised when an infant presents with severe bacterial or fungal infections, such as omphalitis, soft tissue abscesses, or pneumonia. However, some children may also present later in life with severe and invasive infections. The diagnosis of severe congenital neutropenia requires ANCs below 0.5×10^9/L, determined on several occasions. Cyclic neutropenia, a condition in which neutrophil counts oscillate from normal to very low levels, typically within 18- to 21-day cycles,

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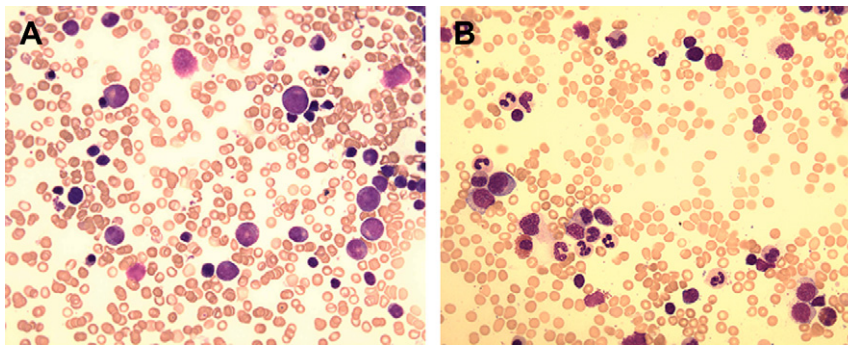

Fig. 1. Bone marrow smear from a severe congenital neutropenia patient and a healthy individual. (*A*) Note the characteristic maturation arrest at the stage of promyelocyte/myelocyte in the bone marrow smear from the severe congenital neutropenia patient. (*B*) In contrast, immature and mature forms of neutrophils are found in a bone marrow smear from a healthy control.

can be determined by regular complete blood counts covering two cycles. The estimated frequency of severe congenital neutropenia is around one to two cases per million, with equal gender distribution. Bone marrow smears show a characteristic picture of "maturation arrest," with few or no cells beyond the promyelocyte/myelocyte stage (see Fig. 1). Congenital neutropenia patients often show elevated numbers of eosinophils and monocytes, most likely as a consequence of the observed arrest of maturation into neutrophils. Furthermore, many patients show elevated immunoglobulin levels (IgG, IgA, IgM) (hypergammaglobulinemia) [5].

Congenital neutropenia syndromes must be differentiated from acquired conditions associated with neutropenia. In newborns and small children, the most common cause of isolated neutropenia is alloimmune and autommune-mediated neutropenia, usually a self-limiting condition without need for specific therapy. Diagnosis can be confirmed by documentation of antigranulocyte antibodies in reference laboratories.

Besides severe congenital neutropenia with myeloid maturation arrest, there are a number of variant forms of congenital neutropenia. These nosologic entities can be subdivided into a group of syndromes associating congenital neutropenia with hypopigmentation and a group of diseases without hypopigmentation (Table 1).

Molecular genetics of congenital neutropenia

In recent years, significant progress has been made in the identification of genetic defects causing severe congenital neutropenia (see Table 1), earlier termed Kostmann's syndrome. A fascinating journey into the exploration of the molecular genetic causes of congenital neutropenia started when Horwitz and colleagues [6] identified heterozygous mutations in *elastase*

Table 1
Congenital neutropenia syndromes

Type of congenital neutropenia	Mutated gene or genes	References
Congenital neutropenia without additional features		
HAX1-deficiency	*HAX1*	[11]
NE-defect	*ELA2*	[7,8]
GFI-1-defect	*GFI-1*	[18]
Wiskott-Aldrich syndrome protein (WASP)-defect	*WASP*	[16,17]
others	Unknown	
Congenital neutropenia associated with hypopigmentation syndromes		
Chédiak Higashi syndrome	*LYST/CHS1*	[29]
Griscelli syndrome, type 2	*RAB27A*	[33]
Hermansky-Pudlak syndrome, type 2 (HPS2)	*AP3B1*	[37]
p14 deficiency (p14, MAPBPIP)	*P14/MAPBPIP*	[40]
Congenital neutropenia as part of other primary immunodeficiency disorders		
X-linked agammaglobulinemia	*BTK*	[122]
Class switch recombination defects	*CD40L, AICDA, CD40, UNG*	[88,123]
Congenital neutropenia associated with metabolic disorders		
Glycogen storage disease, type 1b	*G6PT*	[80,81]
Organic acidurias		[124]
Other circumscribed syndromes associated with neutropenia		
Shwachman Diamond syndrome	*SBDS*	[45]
Warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis (WHIM) syndrome	*CXCR4*	[53]
Cartilage hair hypoplasia	*RMRP*	[60]
Barth syndrome	*G4.5/TAZ*	[65]
Cohen syndrome	*VPS13B/COH*	[71]
Pearson's syndrome	Mutation in mitochondrial genome	[78]

2 (*ELA2*), the gene encoding neutrophil elastase, in patients with autosomal dominant cyclic neutropenia. Subsequently, it was soon recognized that many patients with sporadic or autosomal dominant severe congenital neutropenia have heterozygous mutations in *ELA2* as well [7]. While the exact frequence of *ELA2* mutations remains to be determined, the authors' own data suggest that approximately 40% to 50% of severe congenital neutropenia may be associated with mutations in *ELA2* (International SCN Registry, unpublished data, 2007). Around 50 different mutations in *ELA2* have been found in severe congenital neutropenia patients, the majority of which are missense mutations [8]. Further evidence for the causative role of *ELA2* mutations in autosomal dominant severe congenital neutropenia is derived from a case of mosaic expression of *ELA2* [9] and a recently published study of five unrelated children from healthy mothers, who were retrospectively found to have been impregnated with semen from the same sperm donor carrying an *ELA2* mutation [10].

The genetic cause of autosomal recessive severe congenital neutropenia has remained enigmatic for more than 50 years, since its initial description by Rolf Kostmann [1]. Using genome-wide linkage analysis in three unrelated families of Kurdish descent, the authors identified homozygous mutations in the gene encoding the mitochondrial protein HCLS1-associated X1 (*HAX1*). *HAX1* mutations were also found in affected members of the original Kostmann pedigree. So far, no patient has been identified with mutations in both *ELA2* and *HAX1*, suggesting that these genes define two mutually exclusive groups of severe congenital neutropenia patients [11]. Clinically, and with respect to the morphology of bone marrow smears, *ELA2* and *HAX1* mutants cannot be differentiated.

HAX1 is critical for the control of the inner mitochondrial membrane potential and protects myeloid cells from premature apoptosis [11]. It is also known to be involved in signal transduction and control of cytoskeleton [12]. Recent data suggest that some HAX1-deficient patients have a neurologic phenotype, including developmental delay and epilepsy [13, 14]. Interestingly, mutations in *HAX1* which affect both isoforms A and a splice variant (isoform B) of the molecule [15] are associated with this neurologic phenotype, whereas mutations disrupting exclusively isoform A cause severe congenital neutropenia without a neurologic phenotype [14].

Loss-of-function mutations in the *Wiskott-Aldrich syndrome protein* (*WAS*) gene are associated with Wiskott-Aldrich syndrome or X-linked thrombocytopenia. By contrast, rare cases of X-linked severe congenital neutropenia (XLN) have been reported in which mutations in *WAS* that inhibit its autoinhibitory domain are found [16,17]. In addition to neutropenia, these patients may present with abnormal lymphocyte subsets [16] or myelodysplasia [17]. Recently, Moulding and colleagues have shown that the unregulated actin polymerization in XLN causes defects in mitosis and cytokinesis and, thus, may lead to the phenotype of neutropenia [18].

Autosomal dominant or sporadic mutations in the transcription factor growth factor independent-1 (GFI1) have been associated with severe congenital neutropenia [19]. In contrast to the phenotype described by Kostmann, these patients also showed T and B cell lymphopenia [19]. Murine knockout models suggest that GFI1 is a critical determinant of myeloid differentiation [20–22]. Among many other targets, GFI1 also controls expression of ELA2, a finding that may provide a potential link to a molecule with critical—even though unexplained—importance in the pathophysiology of severe congenital neutropenia [19].

Congenital neutropenia associated with hypopigmentation

Studies of congenital neutropenia syndromes associated with hypopigmentation have provided novel insights into the complex interplay between the biology of lysosomes in pigmentation and immune regulation (reviewed

in [23]). So far, four distinct albinism – neutropenia syndromes have been described: Chédiak-Higashi syndrome (CHS), Griscelli syndrome type 2, Hermansky-Pudlak syndrome type 2, and p14 deficiency. All of these disorders can be distinguished clinically. In contrast to severe congenital neutropenia, mature neutrophils are present in bone marrow smears, while a paucity of neutrophils is found in peripheral blood [24].

Chédiak-Higashi syndrome [25,26] is characterized by hypopigmentation, bleeding diathesis, and a complex immune deficiency caused by defective natural killer cell function and neutropenia [27]. A pathognomonic hallmark are lysosomal inclusion bodies in leukocytes. Severely affected patients often suffer from progressive neurodegeneration [27]. Around 85% to 90% of CHS patients develop a peculiar lymphoproliferative syndrome characterized by lymphohistiocytic infiltrates, fever, hepatosplenomegaly, lymphadenopathy, and pancytopenia (accelerated phase characterized by macrophage activation) [28]. On a molecular level, many CHS patients show mutations in the lysosomal trafficking regulator (*LYST*/*CHS1*) gene [29]; however, some CHS patients do not have mutations in *LYST*/*CHS1*, suggesting that there may be more genetic defects leading to the clinical phenotype of CHS [27]. A study by Karim and colleagues [30] found that patients with severe childhood CHS had null mutant *LYST*/*CHS1* alleles, whereas less severely affected patients had a higher frequency of missense mutations, likely encoding LYST/CHS1 protein with residual function, suggesting genotype-phenotype correlation amongst the different clinical forms of CHS.

Griscelli syndrome, type 2 (GS2), is associated with albinism and transient neutropenia, but in contrast to CHS no lysosomal inclusions are seen [31,32]. Patients suffer from increased susceptibility to infections and develop macrophage activation phases associated with multiorgan lymphohistiocytic infiltrations. GS2 is caused by mutations in *RAB27A* [33], a GTPase with a critical function in exocytosis of secretory vesicles [34]. As a consequence, the coordinated release of cytotoxic granules from cytotoxic lymphocytes is affected in GS2 patients. In addition, RAB27A deficiciency leads to decreased natural killer (NK) cell cytotoxicity, which has recently been linked to dysfunctional NKp30 signaling in NK cells in a GS2 patient [35].

Hermansky-Pudlak syndromes (HPS1–HPS8) are characterized by oculocutaneous albinism, defective thrombocyte granules, and bleeding diathesis. Hermansky-Pudlak syndrome, type 2 (HPS2) is the only subtype associated with congenital neutropenia [23]. Infections tend to be less severe when compared with severe chronic neutropenia (SCN) and there might be a predisposition to macrophage activation syndromes as well [36]. HPS2 is caused by mutations in *AP3B1*, a protein of the heterotetrameric adaptor protein 3 (AP3) complex, which controls intracellular cargo transport in vesicles [37]. HPS2 patients show a decrease in cytotoxicity of NK cells and cytotoxic T cells, because AP3-deficiency affects polarized secretion of cytotoxic granules [38]. AP3-deficient neutrophils express less neutrophil

elastase, yet it remains unknown why AP3 is important for neutrophil homeostasis. AP3 has been shown to mediate intracellular trafficking of neutrophil elastase [39], but it is controversial whether this interaction is of relevance for the pathophysiologic mechanism explaining neutropenia.

Recently, the authors reported on a novel albinism neutropenia syndrome affecting four members of a large consanguineous family. These patients suffered from neutropenia associated with short stature, hypogammaglobulinemia, reduced numbers of class-switched B lymphocytes, and defective cytotoxic T-cell function [40]. Combining a genome-wide linkage study and microarray analysis, the authors identified mutations in the endosomal adaptor molecule *p14* (*MAPBPIP*) gene in these patients. A homozygous mutation in the 3′-untranslated region conferred p14 mRNA instability and consecutively markedly decreased protein expression. P14-deficient neutrophils are characterized by aberrant primary granules and decreased microbicidal activity. P14 is required for proper assembly of late endosomes, because p14 deficient cells show an abnormally scattered distribution of late endosomes and consecutively altered mitogen-activated protien kinase signal transduction [40,41]. Analyses in conditional *p14* knockout mice will further delineate the function of p14 in hematopoiesis and neutrophil function.

Other congenital neutropenia syndromes without hypopigmentation

Shwachman-Diamond syndrome (SDS) is an autosomal recessive disorder characterized by bone marrow failure associated with progressive exocrine pancreatic failure and skeletal abnormalities. Neutropenia may be seen as an isolated finding, but cytopenias often include anemia and thrombocytopenia as well [42–44]. The gene mutated in SDS has been identified and has been termed the *Shwachman-Bodian-Diamond syndrome* (*SBDS*) gene [45]. The corresponding protein SBDS is predominantly localized in nucleoli and plays a role in RNA metabolism [46]. Studies in yeast have shown that the homologous protein Sdo1 is critical for translational activation of ribosomes [47]. Similar to other congenital bone marrow failure syndromes, patients suffering from SDS have a significantly increased risk of developing myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [48,49].

WHIM syndrome has been termed according to its key features: warts, hypogammaglobulinemia, immunodeficiency and myelokathexis [50]. Characteristically, hypersegmented neutrophils with increased apoptosis are found in hypercellular bone marrow, while there are decreased numbers of neutrophils in peripheral blood. In contrast to SCN, severe pyogenic infections secondary to neutropenia appear to be rare, as neutrophils can be released into the periphery in case of infections [51,52]. Most cases of WHIM syndrome follow an autosomal-dominant pattern of inheritance and show heterozygous gain-of-function mutations in chemokine receptor

gene *CXCR4* [53]. Hyperactive CXCR4 signaling may explain the retention of neutrophils in the bone marrow [54]. However, WHIM-like syndromes can also be inherited in an autosomal recessive fashion, but the molecular defects remain to be identified.

Cartilage hair hypoplasia (CHH), initially described by McKusick and colleagues [55], is a multisystem disorder associating short stature and skeletal abnormalities, as well as unusually kinked hair, immunodeficiency, and bone marrow failure [56–58]. CHH is present mainly in Amish and Finnish populations and inherited in an autosomal recessive fashion [55,59]. All patients with CHH display mutations in *RMRP* gene, encoding an endoribonuclease RNase RMRP. RMRP controls mitochondrial DNA synthesis and nucleolar cleaving of pre-rRNA [60]. The neutropenia in CHH appears to be secondary to defective granulopoiesis. Neutropenia in CHH appears to be responsive to treatment with recombinant human (rh)-granulocyte colony-stimulating factor (G-CSF) [61].

Barth syndrome is a rare, X-linked mitochondrial disorder characterized by cardiomyopathy, neutropenia, skeletal myopathy, and growth delay [62]. Affected boys usually present with severe bacterial infections or heart failure. Electron microscopy shows concentric, tightly packed cristae and occasional inclusion bodies in mitochondria [63]. Metabolic alterations include carnitine deficiency and 3-methylglutaconic aciduria [64]. The gene responsible for Barth syndrome, *G4.5* or *TAZ*, was identified in 1996 [65]. Taffazin, the corresponding protein, is important for carnitine synthesis [66–69]. In taffazin deficiency, proper functioning of the electron transport chain across the inner mitochondria membrane is affected. The survival of Barth syndrome patients has improved dramatically since its initial description, most likely because of better recognition and treatment of cardiac defects and neutropenia [68].

Cohen syndrome is an autosomal recessive disorder characterized by microcephaly, a characteristic facial appearance, mental retardation, skeletal abnormalities, and (intermittent) neutropenia [69,70]. Kohlemainen and colleagues [71] identified mutations in the *VPS13B* (*COH1*) gene, which has a suspected role in intracellular vesicle-mediated sorting and protein transport. The disorder is over-represented in the Finnish population [72] and there is a significant heterogeneity with regards to the clinical phenotype and the underlying *COH1* mutations in Cohen syndrome patients [72,73]. The precise molecular pathophysiology underlying neutropenia in Cohen syndrome has not been delineated yet.

Pearson's syndrome is a mitochondriopathy associating exocrine pancreatic insufficiency and pancytopenia. Patients have lactic acidosis and complex organic aciduria [74,75]. Bone marrow has a characteristic appearance with vacuolization of erythroid and myeloid precursors and ring sideroblasts [76]. On a molecular level, Pearson's syndrome is caused by deletions in mitochondrial DNA [77]. There is a polymorphic pattern of the clinical presentation, and the phenotype overlaps with progressive myopathy, external ophthalmoplegia, and Kearns-Sayre syndrome [78,79].

Glycogen storage disease, type 1b, is a complex metabolic disorder caused by mutations in the *glucose-6-phosphate-transporter* (*G6PT*) gene [80,81]. In addition to the typical complications of increased glycogen storage, including hepatosplenomegaly, hypoglycemia, and lactic acidemia, patients are neutropenic and have an increased susceptibility to pyogenic infections and aphtous stomatitis. Neutrophils in GSD1b patients are not only reduced in absolute numbers but also impaired functionally: they display decreased chemotaxis, respiratory burst activity, and phagocytosis [82–86]. Treatment with rh-GCSF is effective in increasing ANCs [83,85].

Neutropenia can be seen in hereditary bone marrow failure syndromes, such as dyskeratosis congenita [87] or Fanconi anemia [48], but usually is not the presenting sign. Several lymphoid primary immunodeficiency syndromes, such as Hyper-IgM-syndrome [88] or X-linked agammaglobulinemia [89], may be associated with neutropenia. Finally, neutropenia is encountered in metabolic disorders, such as propionic acidemia [90,91], methymalonic acidemia [92], or isovaleric acidemia [93].

Leukemic transformation in severe congenital neutropenia

Patients with severe congenital neutropenia are at increased risk to develop myelodysplastic syndrome or acute myeloid leukemia [2,5,94]. This became apparent at the time when rh-GCSF was introduced into clinical practice, because before the availability of rh-GCSF therapy for severe congenital neutropenia, most patients succumbed to infectious complications in infancy. For many years, a vivid and controversial debate has focused on the question of whether rh-GCSF may play a causative role in the evolution of clonal disorders in severe congenital neutropenia. However, there are historic reports of leukemia from before the rh-GCSF era [95,96], suggesting that severe congenital neutropenia may be an intrinsic condition with risk to develop leukemia. Furthermore, the group of cyclic neutropenia patients, even though they often also receive long-term treatment with rh-GCSF appears not to be at increased risk for AML [2].

Data from the Severe Chronic Neutropenia International Registry (SCNIR) have shown that the cumulative risk for severe congenital neutropenia patients to develop MDS and AML is 21% after 10 years of rh-GCSF therapy. The hazard of developing MDS and AML increased during the observation period: from 2.9% per year after 6 years to 8% per year after 12 years. Notably, the risk of developing MDS and AML was significantly higher for the subgroup of patients needing greater than or equal to 8μg/kg body weight per day of rh-GCSF, as compared with the group of patients who received less than or equal to 8μg/kg body weight per day (40% versus 11%, respectively) [97]. The data were interpreted as defining the subgroup of severe congenital neutropenia patients needing higher doses of rh-GCSF being an ''at risk'' group, potentially because of a more severe molecular defect underlying the disease. More recent data from the North American

and Australian Severe Chronic Neutropenia registries have shown similar results, with cumulative incidences for MDS and AML of more than 25% after 15 years of observation [98]. Importantly, there was no significant difference in MDS and AML risk for severe congenital neutropenia patients carrying mutations in *ELA2* versus non-*ELA2* mutated patients. However, the data suggest that specific mutations in *ELA2,* for instance Gly185Arg, may be associated with increased risk, whereas patients with Pro110Leu or Ser97Leu mutations may carry a lower risk of MDS and AML [98]. Further studies are needed to assess the risk of leukemia in defined genetic subgroups of severe congenital neutropenia.

The genetic or epigenetic events involved in leukemogenesis in severe congenital neutropenia patients are largely unknown. The genetic alterations in MDS and AML in severe congenital neutropenia patients are distinct from patients with de novo AML. In the latter group, mutations in the genes encoding different tyrosine kinases such as FMS-like tyrosine 3 (FLT3), KIT, colony-stimulating factor 1 receptor (CSF1R) and janus kinase 2 (JAK2) are common, whereas such mutations are not found in severe congenital neutropenia MDS and AML patients [99]. Conversion to MDS and AML in severe congenital neutropenia patients is usually associated with at least one cellular genetic alteration, such as monosomy 7, trisomsy 21, *RAS* mutation, or granulocyte colony-stimulating factor receptor (*CSF3R*) mutation [100–103]. Point mutations in the gene encoding *CSF3R* were recently found in 78% of severe congenital neutropenia patients with MDS and AML, whereas only 34% of SCN patients without malignant showed these mutations [104]. The data suggest that *CSF3R* mutations may represent an early event during leukemogenesis in severe congenital neutropenia patients. Somatic mutations in *CSF3R* are seen both in *ELA2* and in *HAX1* severe congenital neutropenia, and both subtypes of severe congenital neutropenia are at increased risk for the development of AML. Therefore, some experts recommend yearly bone marrow examinations and cytogenetic studies as well as molecular *CSF3R* analyses.

Toward a unifying disease model for severe congenital neutropenia

The most striking phenotype of severe congenital neutropenia is the maturation arrest in myeloid cell differentiation. Originally, the hypothesis was proposed that defective cytokine signaling may be responsible for this finding. However, the serum of severe congenital neutropenia patients was found to contain normal or increased levels of G-CSF and G-CSF receptors in granulocytes, with no impaired biologic activity of G-CSF [105]. Recently, the idea that defective granulocyte differentiation may be the cause for severe congenital neutropenia has received support. Myeloid progenitor cells from severe congenital neutropenia patients show markedly decreased expression of lymphoid enhancer factor-1 (LEF-1) [106], a transcription factor with multiple target genes including, among others, C/EBPα. C/EBPα

has a critical role in myelopoiesis [107], and ectopic expression of LEF-1 could overcome the maturation arrest in severe congenital neutropenia patient cells [106]. An alternative hypothesis states that the myeloid maturation arrest may rather reflect premature cell death at the level of promyelocyte cell stage of differentiation [108]. Several studies have shown that peripheral neutrophils or bone marrow progenitor cells from patients with severe congenital neutropenia are more susceptible to apoptosis, compared with neutrophils from healthy individuals [11,27,109–111]. The identification of HAX1 deficiency clearly supports this concept, because HAX1 has been defined as an antiapoptotic factor. Independent groups have proposed a model according to which increased cell death may be caused via induction of the unfolded protein response [112,113]. Whether this concept may hold true for the various genetic subtypes of congenital neutropenia remains to be shown. In sum, the detailed molecular mechanisms underlying the "myeloid maturation arrest" remain enigmatic, and it is possible that the hypotheses put forward to date may not be mutually exclusive.

Treatment and prognosis of severe congenital neutropenia

Since 1987, when rh-GCSF became available, quality of life of severe congenital neutropenia patients has markedly improved [114]. In the pre-GCSF era, most severe congenital neutropenia patients succumbed to infectious complications early in life. Today, most patients are able to lead an almost normal life, because treatment with rh-GCSF leads to increased numbers of peripheral neutrophil counts and concomitant decrease in number and severity of infectious episodes in most patients [114].

In 1994, the SCNIR was established and since then has collected data on the clinical course, treatment response, and disease outcome in more than 600 severe congenital neutropenia patients. Meta-analyses show that overall, 90% to 95% of patients suffering from SCN respond to rh-GSCF treatment, with an increase of ANC greater than 1.0×10^9/L and require fewer hospitalizations and antibiotic treatment [5,115–118]. Most patients respond to rh-GCSF doses of 3 μg/kg to 10 μg/kg per day. Treatment usually starts at a dose of 5 μg/kg per day. If no sufficient response is observed, the dose should be increased to 10 μg/kg per day and may be escalated up to a maximum dose of 120 μg/kg per day. Patients with insufficient response to 120 μg/kg per day are defined as nonresponders [102].

Despite of these achievements, prognosis of severe congenital neutropenia patients is limited by two factors: evolution of MDS and AML (see above) and persistent susceptibility to infections. Even though rh-GCSF may reconstitute normal peripheral neutrophil counts, some patients continue to develop invasive infections, suggesting that not only the quantity but also the quality of granulocytes may be affected in severe congenital neutropenia. Several investigators have shown anomalies in the capacity

of neutrophils from severe congenital neutropenia patients to migrate toward a chemotactic gradient or to release [Ca2+] as a second messenger [119]. The expression of bactericidal proteins (such as LL37) [120] or proteases, such as neutrophil elastase [121], is reduced in neutrophils from severe congenital neutropenia patients.

Currently, the only curative therapeutic strategy for severe congenital neutropenia consists of allogeneic hematopoietic stem cell transplantation [56–58]. In view of the effectiveness of symptomatic therapy using rh-GCSF, this procedure is mainly offered to patients who either show progressive signs of clonal hematopoiesis or who do not respond to G-CSF.

Outlook

Marked progress has been made over the last few years with respect to the identification of mutated genes underlying severe congenital neutropenia and other syndromes associated with congenital neutropenia. The identification of genetic causes now allows a classification based on molecular diagnosis and the analysis of genotype-phenotype correlations. This includes the definition of risk factors with respect to susceptibility to infections, responsiveness to therapy, and risk of leukemic evolution. Further insights into the pathophysiology of neutropenia syndromes will undoubtedly also open novel therapeutic concepts. Ultimately, a better understanding of the aberrant pathways and roles of affected genes may pave the way for the development of effective gene-based therapies in the future.

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The Hyper-IgE Syndromes

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The hyper-IgE syndromes (HIES) are rare primary immune deficiencies characterized by elevated serum IgE, dermatitis, and recurrent skin and lung infections. There are two forms of HIES: a dominant form caused by mutations in *STAT3*, and a recessive form, for which a genetic cause is unclear [1–4]. These two different syndromes have distinct presentations, courses, and outcomes and share very little in terms of pathogenesis other than the IgE elevation. The dominant form is characterized by non-immunologic features including skeletal, connective tissue, and pulmonary abnormalities in addition to recurrent infections and eczema. In contrast, the recessive form lacks the somatic features and has marked viral infections and neurologic complications. This article discusses the diagnostic, laboratory, and clinical aspects of these disorders as well as their genetic etiologies.

Autosomal dominant hyper-IgE syndrome (*STAT3* deficiency)

The disease subsequently identified as HIES was described first as "Job's syndrome" by Davis and colleagues [5] in 1966, referring to the Biblical Job, who was "smote with sore boils." In 1972 the syndrome was refined by Buckley and colleagues [6], who recognized extremely high serum IgE levels. Since that time, the classic triad of eczema, recurrent skin and lung infections, and high serum IgE has been expanded to include skeletal, connective tissues, cardiac, and brain abnormalities [1,7,8]. Until 2007, HIES remained the

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last of the major immune deficiencies without either a known genetic etiology or a comprehensive understanding of the associated immune dysfunction. It now is known that *STAT3* mutations are responsible for most, if not all, cases of autosomal dominant HIES, and these mutations have begun to explain the multisystem nature of the disease [2,3]. To distinguish this dominant disease caused by *STAT3* mutation from the recessive forms of HIES and to distinguish this disease from other syndromes of IgE elevation, the authors also refer to this disease as "*STAT3* deficiency."

Clinical manifestations

STAT3 deficiency is a disease of multiorgan dysfunction (Box 1). Although eczema and recurrent infections usually bring patients to initial attention, these individuals have abnormalities in vessels, connective tissue, and skeleton. Before genetic testing, the diagnosis of HIES typically was difficult to confirm until both immunologic and somatic features appeared. A clinical scoring system has been developed that includes both of these categories [9].

Skin

A newborn rash usually is the first manifestation of *STAT3* deficiency [10,11]. Pustular and eczematoid rashes usually begin within the first month

Box 1. Clinical characteristics of *STAT3* deficiency

Immunologic characteristics (% frequency)
- Newborn rash (81%)
- Boils (87%)
- Recurrent pneumonias (87%)
- Pneumatocoeles (77%)
- Eczema (100%)
- Mucocutaneous candidiasis (83%)
- Peak serum IgE > 2000 IU/mL (97%)
- Eosinophilia (93%)
- Increased incidence of lymphoma

Non-immunologic characteristics (% frequency)
- Characteristic face (83%)
- Retained primary teeth (72%)
- Minimal trauma fractures (71%)
- Scoliosis > 10° (63%)
- Hyperextensibility (68%)
- Focal brain hyperintensities (70%)
- Chiari 1 malformations (18%)
- Craniosynostosis (unknown)
- Arterial aneurysms (unknown)

of life, typically first affecting the face and scalp. In a series of 43 patients, 8 babies (19%) were born with the rash, and 23 (53%) acquired the rash within the first week of life [10]. Biopsies typically show an eosinophilic infiltrate, and bacterial culture usually grows *Staphylococcus aureus*. The rash can be quite significant, especially in childhood. To achieve and maintain good control, antistaphylococcal therapies (antibiotics or topical antiseptics, such as bleach) often are essential.

Boils are a classic finding in this disease and are characteristic of the diagnosis. The degree of inflammatory symptoms, such as tenderness and warmth, often is quite variable. The "cold" abscesses initially described by Davis and colleagues [5] are common. Despite the absence of external signs of inflammation, there is frank pus upon aspiration, and *S aureus* usually is cultured. With prophylactic antibiotics, the occurrence of these boils typically diminishes substantially. Trouble areas may persist in intertriginous areas such as the axillae, the inguinal region, or under the breasts.

Lungs

Recurrent pyogenic pneumonias are the rule. Pneumonias typically start in childhood, and the most frequent bacteria isolates are *S aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* (Box 2). Similar to the occurrence of cold abscesses, these pneumonias may present with fewer symptoms (eg, cough, sputum production) than would be expected in a normal person given the extent of disease. This dearth of symptoms and subsequent delay in clinical presentation may contribute to advanced disease and significant tissue damage before identification and initiation of appropriate therapy. Pus is clearly present on sputum inspection or bronchoscopy.

Box 2. Pathogens of *STAT3* deficiency

Frequent pathogens
- *Staphylococcus aureus* (lung and skin)
- *Streptococcus pneumoniae* (lung)
- *Haemophilus influenzae* (lung)
- *Candida albicans* (mucocutaneous)

Secondary pathogens of lung
- *Pseudomonas aeruginosa*
- *Aspergillus* species
- *Scedosporium* species
- Nontuberculous mycobacteria

Less frequently seen pathogens
- *Pneumocystis jiroveci* (lung)
- *Histoplasma* species (gastrointestinal tract)
- *Cryptococcus* species (brain and gastrointestinal tract)

Although the pneumonias typically respond promptly to appropriate antimicrobial therapy, the healing of the lungs is aberrant. Pneumatocoeles and bronchiectasis form during the healing process and usually persist once the infection has cleared. These persistent structural abnormalities, which can be quite significant, predispose the patient to gram-negative bacterial infection (typically *Pseudomonas*) and fungal infections (typically *Aspergillus* or *Scedosporium* species) in addition to the primary pathogens in *STAT3* deficiency (Fig. 1). The secondary infections typically are indolent and difficult to clear. These long-term infections are more frequently associated with mortality than the acute pyogenic infections, causing rupture into large pulmonary vessels with life-threatening hemoptysis or fungal dissemination to the brain [12].

Other infections

Mucocutaneous candidiasis is common in *STAT3* deficiency, manifesting typically as oral thrush, vaginal candidiasis, or onychomycosis [1]. Systemic *Candida* infections are very rare and most likely are nosocomial in origin (eg, an indwelling catheter infection). Disseminated *Cryptococcus* and *Histoplasma* infections also occur, although less frequently than candidiasis. These uncommon yeast infections often are localized (eg, histoplasmosis of the tongue or intestinal cryptococcal infection) [13,14]. *Pneumocystis jiroveci* pneumonia also occurs, albeit infrequently, and its presentation in infants may be similar to the initial presentation of *Pneumocystis jiroveci* pneumonia in infants infected with HIV [15].

Musculoskeletal abnormalities

Skeletal abnormalities in *STAT3* deficiency include scoliosis, osteopenia, minimal trauma fractures, hyperextensibility, and degenerative joint disease. Scoliosis occurs in about 75% of patients, typically emerging during

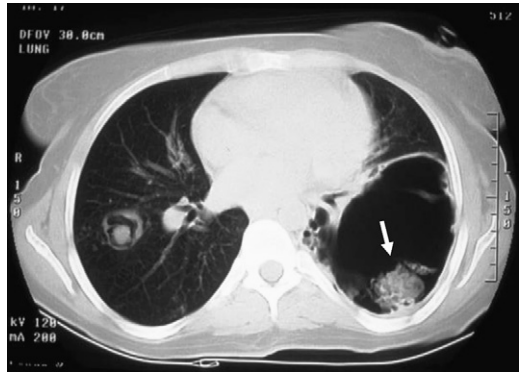

Fig. 1. Chest CT of an individual who has *STAT3* deficiency showing the characteristic pneumatoceles. The pneumatoceles are prone to infection with fungi and gram-negative bacteria. Arrow indicates an aspergilloma.

adolescence in a pattern similar to that of idiopathic scoliosis [1]. In some patients, there is associated leg length discrepancy; in others scoliosis develops or worsens after thoracotomy for lung infections. The scoliosis varies in severity, and some individuals have required surgical stabilization or correction.

Hyperextensibility of both the large and small joints is frequent and may be related to the early development of severe degenerative joint disease, particularly of the spine, that the authors have seen in several patients in their 20s to 40s. Several patients have required stabilization procedures as early as in their third decade, and many suffer from chronic pain caused by extensive arthritis. Minimal trauma fractures and decreased bone mineral density also are common but may occur independently of one another [1]. Fractures tend to be of the long bones, ribs, and pelvic bones. Bone resorption has been shown to be abnormally increased in patients who have HIES because of abnormalities in the prostaglandin synthetic pathway and is responsive to nonsteroidal agents [16,17]. Healing seems to be normal after surgery or fractures.

Cranial abnormalities

Craniosynostosis of varying degrees occurs but typically does not require surgical repair [18,19]. Chiari 1 malformations also occur fairly frequently; in one study of 50 individuals, 9 (18%) had Chiari 1 malformation on brain MRI [7]. The Chiari malformations observed typically do not require surgical repair and usually are incidental findings.

Dental abnormalities

Most individuals who have *STAT3* deficiency retain at least some, if not all, of their primary teeth past the age of normal primary dental exfoliation [1,20]. This manifestation seems to be a failure of the primary teeth to exfoliate, not of the secondary teeth to develop or to erupt. Once the primary teeth do exfoliate, whether by dental extraction or naturally, the secondary teeth, which are normally formed, emerge. At times, layers of both primary and secondary teeth are present simultaneously when the secondary teeth emerge although the primaries have not fallen out. There also are characteristic findings of the oral mucosa, tongue, roof of the mouth, and cheeks [21]. Central depressions in the tongue (central rhomboid glossitis) may be caused by or become secondarily infected with *Candida*. The palate typically has a high arch with varying degrees of a central band-type protrusion. Abnormally prominent wrinkles often are observed on the oral mucosa.

Vascular abnormalities

Arterial aneurysms are an important recently appreciated aspect of *STAT3* deficiency [8]. Bilateral berry aneurysms of the internal carotid arteries and mycotic aneurysm have been reported in an autopsy series of HIES [12]. A large aneurysm in the left anterior descending coronary artery

resulted in myocardial infarction in one adult male patient [8]. That case prompted the authors to look more closely at other adult patients who had HIES. The authors were surprised at the frequency of coronary artery anomalies, including arterial tortuosity, dilation, and aneurysms. Of 18 individuals studied by either cardiac CT or MRI, 14 had one of these abnormalities, with tortuosity and dilation predominating and aneurysms being present in only 4 patients (Freeman AF, Holland SM, Gharib A, unpublished data, 2008). Significant atherosclerosis is uncommon in the individuals studied by CT and MR angiography, despite these patients having risk factors for coronary artery disease. One of the patients had a myocardial infarction as the result of an aneurysm, clearly indicating that these findings can be medically important. Whether and when these aneurysms require therapy, and if so with what, is unclear. Whether the coronary and extracoronary aneurysms are reflections of the same underlying pathophysiology also remains to be determined.

Lacunar infarcts have been reported at a younger age than is typical and have had varying clinical consequences, ranging from thalamic infarction to no symptoms [7]. T2-weighted hyperintensities seen on brain MRI are similar to incidental findings that accumulate in otherwise healthy adults as they age but are seen at much younger ages than in the general population [7]. Similar hyperintensities in elderly, otherwise healthy, adults are thought to reflect small-vessel abnormalities, which may be signs of subtle vascular abnormalities, or demyelination. Gross neurologic abnormalities are not detected in the majority of patients with these findings.

Face

The characteristic facial appearance that individuals who have *STAT3* deficiency share may make patients resemble one another more than their family members [1,22,23]. Facial asymmetry, broad nose, and deep-set eyes with a prominent forehead are common. The facial skin has a rough appearance with exaggerated pore size. This characteristic appearance typically develops during childhood and adolescence.

Malignancies

An increased risk of malignancy is associated with *STAT3* deficiency [24–26]. Both Hodgkin's and non-Hodgkin's lymphoma have been described, with the majority of the non-Hodgkin's lymphomas being of B-cell origin with aggressive histology. Individuals have been treated successfully and apparently cured with chemotherapy. In one study of 11 individuals, 7 died, but 2 deaths were for reasons other than lymphoma [24]. The increased mortality may reflect a delay in diagnosis. Other cancers described in HIES include leukemia and cancers of the vulva, liver, and lung [26].

Laboratory abnormalities of STAT3 *deficiency*

The two most consistent laboratory findings of Job's syndrome are elevated serum IgE and eosinophilia; otherwise, there is a lack of pathognomic

laboratory signs. Therefore, the diagnosis historically has been more syndromic than laboratory based. The serum IgE typically peaks above 2000 IU/mL and usually is elevated even at the time of birth. It is important to keep in mind the natural change in IgE levels over time: they usually are undetectable in cord blood and rise to the adult range slowly over the years. In adulthood the IgE level may diminish over time in some individuals and actually can normalize, despite persistence of the clinical abnormalities of *STAT3* deficiency. (In one report, IgE levels normalized in 20% of patients) [1]. Eosinophilia almost always is present in these patients, at least at some point, but is not correlated with the serum IgE level.

Other laboratory findings are variable in *STAT3* deficiency. Total white blood cell counts typically are normal but may not increase appropriately during acute infection. Neutropenia has been reported but is uncommon. Serum IgG, IgA, and IgM typically are normal, although some individuals have deficiencies in one or more of these immunoglobulins.

Immunology of STAT3 *deficiency*

The mechanisms of the immune deficiencies in *STAT3* deficiency remain elusive. Multiple reports with small numbers of patients have conflicted regarding whether a neutrophil chemotactic defect is present and whether there is a T-helper 1/T-helper 2 cytokine imbalance [27–32].

STAT3 is a key regulator of many immunologic pathways. Mouse Stat3 homozygous knockouts die in utero, reflecting the absolute necessity of some Stat3 function for survival. Therefore, most experiments in mice have used organ-specific or conditional knockout animals. Animals with a myeloid-specific deletion of *Stat3* had increased expression of tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) and decreased expression of interleukin (IL)-6 and IL-10 [33,34]. Consistent with these mouse data, microarray analysis of leukocytes from *STAT3*-deficient humans showed significant up-regulation of proinflammatory genes at rest or after stimulation [2]. Similarly, patients showed excessive proinflammatory cytokine production in response to innate immune agonists, consistent with the impaired regulation of these cytokines by *STAT3* [2]. After incubation with lipopolysaccharide, levels of TNF-α and IL-12 were elevated. IFN-γ also was increased after the mitogen phytohemagglutinin. As expected for a defect in *STAT3*, upon which the signaling of IL-6 and IL-10 depend, there are impaired responses to these cytokines. Monocyte chemoattractant protein-1 levels are diminished after IL-6 stimulation of leukocytes from patients who have HIES [2].

Many of the specific immune defects of *STAT3* deficiency remain somewhat unexplained. Infections of the lung and skin may predominate because *STAT3* is a key regulator of beta-defensins of the skin and lung through IL-22 signaling [35]. The high IgE may result from defects in *STAT3*-mediated IL-21 receptor signaling, because heterozygous IL-21 receptor knockout

mice have increased IgE [36]. HIES is not exclusively a disorder of too little inflammation and resulting inability to control invading micro-organisms. It is also, surprisingly, a disorder of too much inflammation. The increased inflammation is evident in the lung, where tissue breakdown leading to pneumatocoeles may be a consequence of exuberant inflammation. In contrast, there are aspects of *STAT3* deficiency that are more consistent with too little inflammation, such as the frequent "cold" abscesses and the relative paucity of symptoms compared with the extent of disease. Exploration and charting of these areas of aberrant regulation, both too little and too much, should be highly informative about the nature of the early and late aspects of inflammation.

Genetics of STAT3 *deficiency*

STAT3 mutations cause most, if not all, cases of autosomal dominant HIES. So far, mutations have been found in only two regions of the *STAT3* gene: the SH2 domain (mediating protein–protein interaction) and the DNA-binding domain (mediating the interaction of protein with DNA). There are mutational hot spots in both the SH2 and DNA-binding domains, as shown by multiple independent families carrying the same mutation [2,3]. Many of the same mutations have been found in multiple ethnic groups among whites, Africans, and Asians, indicating recurrent de novo mutations. All mutations found thus far have been missense mutations or in-frame deletions, allowing the production of full-length mutant STAT3 protein able to exert a dominant negative effect. The importance of the production of mutant protein with dominant negative effect is shown by the fact that mice with heterozygous deletions of Stat3 have no apparent phenotype, suggesting that having a single copy of a functional gene is adequate for most functions. This finding suggests that the development of human HIES requires an inhibition of function below that afforded by a single allele. The mutations have no intrinsic function on their own but act in a dominant-negative manner to inhibit the function of the normal allele [3]. Consistent with this in vitro phenotype, HIES is transmitted in an autosomal dominant manner. Importantly, somatic mosaicism has been recognized in a man who had an intermediate HIES score but had two HIES-affected children. His peripheral blood showed evidence for two populations of cells: one population with the mutant allele, and one population without it [2]. Efforts to define the existence of somatic mutations that cause intermediate types of disease are underway. Despite the existence of only two mutated domains of *STAT3* that are thought to mediate such different effects as DNA and protein binding, genotype/phenotype correlations have yet to be determined.

STAT3 is a major signal transduction protein involved in diverse pathways including wound healing, angiogenesis, immune pathways, and cancer. Homozygous *STAT3* knockout mice are not viable, but organ- and

tissue-specific knockouts are and have been informative. In mice with *STAT3* deficiency of the pulmonary epithelium, there is excessive inflammation and airspace enlargement when exposed to hyperoxia, reminiscent of the pneumatoceles that form following bacterial pneumonia in patients who have HIES [37]. Mice with hematopoiesis-specific *STAT3* deficiency develop osteopenia and increased osteoclast generation, reminiscent of the osteopenia and fractures of HIES patients [38]. Mice with brain-specific *STAT3* have increased inflammation, demyelination, and astrocytosis, reminiscent of the hyperintensities seen in brain MRIs of patients who have HIES [7,39]. *STAT3* also is involved in vascular remodeling and atherosclerosis, which may be relevant to the coronary artery aneurysms and lack of atherosclerosis in patients who have HIES [40].

Therapy of hyper-IgE syndromes

Only now are specific therapies for *STAT3* deficiency being developed, but successful supportive care has been well honed. Effective skin care often depends on control of both superficial and invasive *S aureus* infection. Bleach baths (120 mL bleach in a tub of water, soaked in for 15 minutes three times weekly) and swimming in chlorinated pools are highly effective. Systemic immune suppression (eg, with corticosteroids) to treat the eczema usually is not necessary, because there typically is an excellent response to antimicrobials, but topical steroids help in difficult cases. Antimicrobial prophylaxis to prevent *S aureus* skin and lung infection (eg, 2.5 mg/kg of the trimethoprim component twice daily) may be broadened if gram-negative lung infections occur. Antifungal prophylaxis to prevent pulmonary aspergillosis remains attractive but unproven, but it is highly effective in treating and preventing mucocutaneous candidiasis. Ideally, treatment of pneumonia is guided by the etiologic agent. Bronchoscopy is helpful to recover the pathogen and to assist with clearance of mucus and pus, because these patients often do not have an adequate cough response. Because *STAT3*-deficient patients often feel well and have minimal fever despite significant infection, it is good to have a low threshold for investigating slight changes, such as new cough, chest discomfort, or fatigue, even in the absence of fever. The decision to resect the large pneumatoceles that sometimes form following pneumonia is complex. These large cysts may become secondarily infected and be a source of infection, bleeding, and possibly death. On the other hand, thoracic surgery can be complicated by poor expansion of the remaining lung after surgery, often resulting in thoracoplasty.

Before *STAT3* deficiency was identified as the cause of HIES, there were only a few immunomodulator trials. Levamisole is an unusual antihelminthic drug that also stimulates T-cell and natural killer–cell function. In a blinded, randomized study, however, levamisole was found to be inferior to placebo [41]. IFN-γ has been used with mixed results. In vitro it improved neutrophil chemotaxis, but in vivo it had inconsistent effects on IgE levels

[42]. Intravenous immunoglobulin may decrease the number of infections for some patients [43]. Case reports and small case series have extolled cyclosporine and histamine-2 receptor blockade [44,45]. Omalizumab (the monoclonal antibody against IgE) has not yet been studied, and it is unclear whether there may be any benefit.

Two bone marrow transplantations in HIES patients have been reported [46,47]. An adult died 6 months after transplantation [47]. His death was thought to have been caused by complications of the transplantation, but his serum IgE level decreased and his improvement in HIES-related symptoms in the posttransplantation interval. A 7-year-old girl underwent transplantation because of severe HIES. Although she initially had a good response, when posttransplant immune suppression was weaned, her serum IgE once again became elevated, and infections recurred despite engraftment of the donor cells [46]. Subsequently she seems to be doing well, leaving open issues of short- and long-term benefits of transplantation (A. Cant, personal communication, 2007).

The role of bisphosphonates in treating the osteoporosis and minimal-trauma fractures is undefined. Although the authors have treated several patients with bisphosphonates leading to improved bone mineral density without adverse events, it is unclear whether this improvement will translate into fewer fractures. Possible adverse dental events from bisphosphonates also remain unclear. The proper therapy for coronary artery aneurysms or other blood vessel abnormalities, if any, is undefined. Coronary artery aneurysms from Kawasaki disease typically are treated with anticoagulation depending on the size of aneurysm (from aspirin to warfarin). The development, progression, and significance of the arterial abnormalities in *STAT3* deficiency are unknown, however. The hypothetical benefits of anticoagulation must be weighed against the real risks of pulmonary hemorrhage. Close attention to blood pressure and other cardiovascular risk factors seems sensible.

A complex multisystem disease like *STAT3* deficiency requires a sophisticated multidisciplinary approach. In addition to close management of infectious disease, other subspecialists often are required. The orchestrated expertise of orthopedists for scoliosis, fractures, and degenerative joints; dentists to address the retained primary teeth; and pulmonologists for diagnostic and therapeutic bronchoscopy and pulmonary toilet is necessary.

Autosomal recessive hyper-IgE syndrome

In 2004, Renner and colleagues [4] described 13 patients from six consanguineous families who had a disease similar to, but distinct from, what now is known to be autosomal dominant HIES (*STAT3* deficiency). Their patients had elevated serum IgE, eczema, and recurrent skin and cutaneous viral infections but lacked the connective tissue and skeletal findings characteristic of *STAT3* deficiency. One patient has been described as having

a recessive disease similar to autosomal recessive (AR)-HIES caused by a homozygous mutation in tyrosine kinase 2 (*Tyk2*), a major signal-transducing molecule for IL-12, IL-6, and IFN-α [48]. *Tyk2* deficiency, however, is distinct from AR-HIES, and patients who have AR-HIES have normal *Tyk2* and *STAT3* sequences [49].

Clinical manifestations

The predominant clinical manifestations of AR-HIES are severe eczema and recurrent skin and lung infections (Box 3) [4]. All of the patients described as having AR-HIES have had severe eczematoid rashes, starting early in life, although not necessarily in the newborn period. Skin abscesses do occur, typically caused by *S aureus*. The skin disease of AR-HIES differs from *STAT3* deficiency in the much higher incidence of cutaneous viral infections, including Molluscum contagiosum, herpes simplex, and varicella zoster virus infections. Patients who have AR-HIES and those who have *STAT3* deficiency share the propensity for developing mucocutaneous candidiasis. Sinopulmonary infections are common in AR-HIES; causative agents include *S aureus*, *H influenzae*, *Proteus mirabilis*, *P aeruginosa*, and *Cryptococcus*. Unlike *STAT3*-deficient patients, in whom pneumatoceles complicate pneumonias, patients who have AR-HIES heal their lung infections without pneumatoceles. Fatal sepsis occurs in AR-HIES from both gram-positive and gram-negative bacteria.

Patients who have AR-HIES have more symptomatic neurologic disease than those who have *STAT3* deficiency [4]. In the Renner series [4], seven patients had neurologic symptoms, ranging from facial paralysis to hemiplegia. The etiology of the neurologic complications was not clear for all patients, but one had a cerebral cryptococcoma with meningitis, and others had severe central nervous system vasculitis.

Box 3. Clinical characteristics of autosomal recessive hyper-IgE syndrome

Eczema
Boils
Recurrent pneumonia without pneumatoceles
Sepsis
Mucocutaneous candidiasis
Skin viral infections
Neurologic symptoms
Vasculitis
Increased serum IgE
Eosinophilia

AR-HIES lacks the connective tissue and skeletal abnormalities of *STAT3* deficiency. Patients who have AR-HIES have normal primary tooth exfoliation, no tendency for mild-trauma fractures, and normal facies.

Immunologic manifestations

Patients who have AR-HIES have high serum IgE levels, comparable to those who have *STAT3* deficiency [4]. Their eosinophilia typically is higher than in *STAT3* deficiency. Autoimmune phenomena, including hemolytic anemia, may occur. Lymphocyte phenotyping and function are inconsistent in AR-HIES, although decreased lymphocyte proliferation was seen in response to a staphylococcal antigen. Neutrophil chemotaxis and nitroblue-tetrazolium reduction are normal.

Genetics of autosomal recessive hyper-IgE syndrome

The patients who had AR-HIES and *Tyk2* deficiency were products of consanguineous unions [4,48]. The *Tyk2*-deficient patient shares features of AR-HIES including high serum IgE levels, eczematoid rash, and recurrent skin and sinopulmonary bacterial and viral infections. He had Bacille Calmette-Guerin and salmonella infections, however, which are seen more commonly in defects of the IFN-γ/IL-12 axis. Indeed, upon cytokine stimulation of the peripheral blood mononuclear cells, defects were found in IL-12 and IFN-α signaling. This *Tyk2*-deficient patient had a homozygous mutation leading to a four-nucleotide deletion and resulting in a premature stop codon. His related parents were heterozygous for the same deletion and were healthy.

Mutations of *Tyk2* are absent in AR-HIES [48]. Therefore, although it is possible that *Tyk2* mutations may cause some cases of AR-HIES disease, AR-HIES probably is heterogeneous, with more than one gene contributing to its etiology. Because *STAT3* is the genetic deficiency in autosomal dominant HIES, related genes in these pathways may cause some of these undefined diseases.

Therapy of autosomal recessive hyper-IgE syndrome

Therapy of AR-HIES remains supportive and has been less explored than therapy for *STAT3* deficiency. Prophylactic antimicrobials probably help, with antistaphylococcal agents, antivirals if needed, and antifungals if mucocutaneous candidiasis or invasive fungal disease occurs. Aggressive skin care may help prevent invasive bacterial infection.

Disease in AR-HIES often is more severe than in *STAT3* deficiency. Immunomodulatory therapies and bone marrow transplantation need further exploration.

Summary

Hyper-IgE syndromes were described first in 1966 and until recently remained one of the few primary immunodeficiencies without a genetic etiology. Now, however, two genetic defects have been described: *STAT3* mutations act in a dominant negative manner to cause of autosomal dominant HIES, and *Tyk2* deficiency acted in a recessive manner to cause one of the cases of AR-HIES. Investigators now need to focus on understanding the pathogenesis of these complicated diseases. Understanding how *STAT3* deficiency leads to the many facets of this disease may help investigators understand diseases that are more common, such as idiopathic scoliosis, atopic dermatitis, staphylococcal skin abscesses, and the coronary artery aneurysms of Kawasaki disease. Understanding the pathogenesis of *STAT3* deficiency will make it possible to create better therapies to prevent the morbidity and mortality of many diseases, including *STAT3* deficiency.

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Hemophagocytic Lymphohistiocytosis and Other Hemophagocytic Disorders

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Hemophagocytic disorders result when critical regulatory pathways responsible for the natural termination of immune/inflammatory responses are disrupted or overwhelmed. Hemophagocytic disorders reflect pathologic defects that alter the normal crosstalk between innate and adaptive immune responses and compromise homeostatic removal of cells that are superfluous or dangerous to the organism. Although hemophagocytic disorders are considered rare, increased awareness of these conditions has led to more frequent diagnoses, more rapid initiation of life-saving treatments, and new insights into the molecules and pathways involved in natural immune down-regulation. Furthermore, improved understanding of the immunologic abnormalities revealed by hemophagocytic disorders informs potential new treatments for life-threatening multisystem organ dysfunction related to sepsis in the ICU setting, and severe cases of viral infections in previously healthy individuals.

The term hemophagocytic lymphohistiocytosis (HLH) was formally adopted by the international Histiocyte Society to describe a lethal autosomal recessive defect associated with overwhelming systemic inflammation [1]. Characteristic features of HLH were described as hectic or prolonged fevers, hepatosplenomegaly, and cytopenias, typically occurring in infancy or early childhood [2]. Farquhar and Claireux [3], at the University of Edinburgh, are credited with the first description (in 1952) of HLH, which they named "familial hemophagocytic reticulosis." Before the description of specific genetic defects underlying HLH, sporadic cases of HLH, particularly in older children and adults, were often referred to as "secondary." Secondary cases of HLH were attributed to infectious agents identified at the time of disease, to other circumstances such as immune suppression for management of rheumatoid disorders, or to malignancies. In recent

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years, many more cases of hemophagocytic disorders have been identified, especially in the context of severe inflammatory reactions to viral exposure, including HIV [4] and avian influenza [5].

Currently, HLH (FHL:OMIM 267,700, 603,553) includes the autosomal recessive genetic disorders, with an estimated prevalence of 1/50,000 live births, as well as the secondary forms.

Pathogenesis of hemophagocytic lymphohistiocytosis and other histiocytic disorders

HLH is characterized by multisystem inflammation, a reactive process resulting from prolonged and intense activation of antigen-presenting cells (macrophages, histiocytes) and CD8 + T cells, and excessive proliferation and ectopic migration of T cells.

Normal functions of histiocytes, a major population of cells within the innate immune system, include phagocytosis, antigen presentation, and activation of the adaptive immune system through contact and cytokine signaling.

Abnormalities in the function (but rarely the quantity) of natural killer (NK) cells have been observed in patients with all forms of HLH. NK cells play significant roles at many stages of the immune response to pathogens. They are a frontline of defense against intracellular pathogens such as viruses, which infect nonlymphoid tissues early, on entry into the organism (cytotoxic and cytokine-mediated mechanisms). NK cells modulate the initial responses of antigen-presenting cells to incoming pathogens (likely through cytokine signaling), attenuating the signal communicated to antigen-specific T cells. NK cells likely also play a role in culling activated T cells and histiocytes in the later stages of antigen-driven activation, contributing to the natural contraction of the immune response.

NK cells, and natural killer T (NKT) cells in particular, play a major role in maintaining a healthy threshold of immune responsiveness to noxious external stimuli, and are critical to the prevention and control of autoimmune conditions and severe reactions to viral infections.

Also critical to the contraction process of active T-cell populations is the mechanism of activation-induced apoptosis. Like NK cell cytotoxicity, this process involves granule-mediated cytotoxicity.

Studies of cytokine levels in blood and tissues have indicated persistently elevated circulating levels of multiple proinflammatory cytokines during symptomatic disease [6]. Published results vary from study to study, and levels of proinflammatory cytokines appear to be particularly elevated in Epstein-Barr virus (EBV)-driven HLH in Asian populations. It is currently believed that ''hypercytokinemia,'' and possibly ''hyperchemokinemia,'' generated by uncontrolled activation of histiocytes (antigen-presenting cells and T cells), underlies the progressive organ dysfunction that eventually leads to death in affected patients. Symptoms and signs of HLH include

fevers, hyperlipidemia, endothelial activation/coagulopathy, hepatitic triaditis, central nervous system (CNS) vasculitis and demyelination, inflammatory lung disease with acute respiratory distress syndrome, and marrow hyperplasia or aplasia. Hemophagocytosis is a hallmark of cytokine-driven macrophages/histiocytes.

Ongoing hypercytokinemia is a reflection of the failure of natural immune down-regulation due to defective NK and cytotoxic T lymphocyte (CTL) function.

Clinical presentation of hemophagocytic lymphohistiocytosis

Until recently, it was widely believed that the symptoms of familial HLH (also referred to as FHL in the literature) generally arose during infancy and early childhood. With the more widespread availability of genetic testing, it is apparent that the first significant episode of HLH can occur throughout life [7,8], including in utero [9]. HLH should be considered as part of the differential diagnosis of nonimmune hydrops fetalis [9]. In infants, significant symptomatic overlap has been reported with neonatal sepsis [10,11], acute hepatic failure, and neonatal hemochromatosis [12].

Despite attempts to differentiate primary from secondary HLH, or the reactive forms, the symptomatic presentations are highly overlapping. In the most typical form of primary HLH, the clinical course is characterized by prolonged fever (>7 days) and hepatosplenomegaly. Neurologic symptoms may dominate the initial clinical course, with seizures or ataxia. Neurologic findings may be highly variable and can include irritability, hypo- or hypertonia, cranial nerve palsies, meningismus, and signs of increased intracranial pressure and altered consciousness.

Rash, lymphadenopathy, and diarrhea are less frequently observed. Standard blood testing typically reveals cytopenias, especially anemia and thrombocytopenia, liver dysfunction, hypofibrinogenemia, hypertriglyceridemia, hypoalbuminemia, and hyponatremia. In the early days to months of the disease, symptoms may improve spontaneously, followed by clinical exacerbations. Importantly, frank hemophagocytosis may not be obvious on bone marrow biopsy examination early in the course of the disease [13].

Genetics of hemophagocytic lymphohistiocytosis and other histiocytic disorders

To date, all described genetic defects associated with HLH and other histiocytic disorders appear to be related to one another in the pathway of granule-mediated cytotoxicity. These genetic defects interrupt the mechanisms responsible for triggered apoptosis (mediated by cytotoxic cells on the target cell) or activation-induced apoptosis (putative suicide of activated T cells). The first gene linked to HLH was perforin [14], a soluble, pore-forming cytolytic protein synthesized in cytolytic lymphocytes and

sequestered, along with Granzyme serine proteases, in secretory cytotoxic granules. When cytotoxic cells contact their targets, the intracellular cytoskeletal scaffold (the microtubule organizing center [MTOC]) is rotated to focus on the contact site where the cytotoxic immunologic synapse forms. Cytotoxic granules are carried along the MTOC toward the immunologic synapse, where they degranulate, allowing perforin and Granzyme B to enter the contact zone, permeabilization of the target membrane, and delivery of Granzyme B into the target cell. Once internalized, Granzyme B initiates caspase-dependant and caspase-independent apoptotic pathways, killing the target cell.

A murine model of perforin deficiency published in 1994 revealed that perforin was the crucial effector molecule in T- and NK-cell–mediated cytolysis [15]. This model, in a specific murine strain infected with lymphocytic choriomeningitis virus (LCMV), has been used to recapitulate the HLH scenario, revealing a key role of high gamma-interferon production and excessive activation of antigen-presenting cells in the symptomatic evolution of murine disease [16]. Perforin deficiency accounts for 15% to 20% of HLH cases in several geographic areas (also referred to as FHL2). HLH due to perforin deficiency has been reported to occur at a few weeks of age, and in older children and adults (49- and 62-years-old) [17]. Patient series have been examined to identify putative genotype/phenotype correlations [18]. These indicate that the most deleterious mutations associated with virtually no production of protein are typically identified in patients affected during infancy, whereas compound heterozygous missense mutations are more commonly identified in older patients. Mutant perforin sequences capable of generating near-normal quantities of mature perforin protein result in milder clinical phenotypes [19]. In contrast, nonrandom homozygosity of severe PRF1 mutations, such as 50delT, L17fsX22, observed in patients of African descent, is associated with mean age at onset of 2 months [20]. Heterozygosity for C272T (A91V) in PRF1 is the most frequently observed missense mutation in Caucasian patients who had FHL2, and was found to exceed the prevalence in the general population by threefold in patients from Italy [21] and North America [22] who had HLH. Once considered a benign polymorphism, A91V has more recently been proposed as a disease-modifying gene. Structural and functional studies of the human A91V perforin protein indicate decreased activity compared with native perforin [19,23]. In the North American experience, a substantial proportion of patients who have HLH and who carry this genotype have also been found to have either other biallelic disease–causing mutations in PRF1, biallelic or heterozygous deleterious mutations in MUNC 13-4, or absent NK function, suggesting the presence of other gene defects that contribute to the pathogenesis of HLH [22].

No defects in Granzyme B have been identified in association with human HLH.

A second gene responsible for HLH, MUNC 13-4, was reported in 2003 [24]. MUNC 13-4 was described as essential for cytolytic granule fusion with

other structures related to the cytoplasmic membrane in the process of degranulation. Recently, a murine model of this disease has been developed, *Jinx*, a murine cytomega 6 virus (MCMV) susceptibility phenotype caused by disruption of the murine homolog, *Unc13d* [25]. As with perforin-deficient mice, *Jinx* mice do not spontaneously develop HLH, but require infection with LCMV, which stimulates hyperactivation of CTLs and antigen-presenting cells in the context of inadequate restriction of viral proliferation. MUNC 13-4 deficiency, also referred to as FHL3, has a worldwide distribution and accounts for 15% to 20% of HLH cases.

The gene defect responsible for FHL4 is syntaxin 11 [26], which has been shown, as in MUNC 13-4 deficiency, to result in defective degranulation [27]. Syntaxin 11 is a member of the SNARE protein family that facilitates fusion in intracellular membrane trafficking events. It was recently shown to be expressed in NK cells and activated CTLs [28]. To date, the few HLH cases attributed to syntaxin 11 deficiency have been found almost exclusively in Turkish families. In some families, a more indolent course, and findings consistent with myelodysplasia and myeloid malignancy, have been seen [29,30]. Extensive study of patients from the Far East and North America has not revealed additional similar patients.

The genetic defect responsible for FHL1 linked to chromosome 9 [31] in a study of two extended Pakistani families has not yet been discovered.

Related hemophagocytic disorders occur with significant frequency in five other genetic diseases that have been linked with defective cytotoxic function. Three distinct immunodeficiencies associated with pseudoalbinism due to defects in lysosomal trafficking have been associated with life-threatening episodes of HLH: Chediak Higashi syndrome (LYST, or CHS1) [32], Griscelli syndrome (Rab27A) [33], and Hermansky-Pudlak syndrome type II (AP3B1) [34]. Rab27a, a small Rho GTPase, interacts directly with MUNC 13-4 and is thought to play a role in docking the cytotoxic granules on the MTOC. These diagnoses can be suspected on physical examination in the presence of very fair or grayish hair, and when distinctive laboratory abnormalities of the neutrophil and platelet compartments have been detected.

HLH following exposure to EBV and, less commonly, to other viruses, termed fulminant infectious mononucleosis [35], is the most frequent life-threatening complication of X-linked lymphoproliferative syndrome (XLP). XLP is caused by hemizygous mutations in SH2D1A encoding SLAM-associated protein (SAP), which lead to abnormal NK cell responses and NKT cell deficiency. XLP2, due to hemizygous mutations in X-linked inhibitor-of-apoptosis (XIAP or BIRC4), was recently described in three families with multiple related males who developed EBV-associated HLH [36]. Similar to XLP, marked decreases in NKT cell numbers have been demonstrated in patients who have XIAP deficiency. In addition to HLH complications, patients who have XLP and XLP2 may demonstrate features of common variable immunodeficiency, autoimmune disease, and lymphoproliferative disorders, even without prior exposure to EBV. Patients who

have XLP and XLP2 may survive into adulthood in good health before succumbing to a serious complication of their underlying disease. Thus, lack of prior significant medical history should not exclude these diagnoses. Several murine models of XLP have been created [37]. Following infection with LCMV, but also leishmania and toxoplasma, SAP-deficient mice develop aggressive CD8 T-cell populations, generating high levels of gamma-interferon, leading to life-threatening tissue inflammation. As with other murine populations harboring defects in genes that affect cytotoxicity or apoptosis, noninfected XIAP-deficient mice do not express a pathologic phenotype.

Taken together, the nine genetic disorders described above still account for less than one half of the diagnosed cases of HLH in children, including many familial cases still awaiting molecular definition.

Hemophagocytic disorders associated with other immunodeficiencies and infections

The clinical syndrome of HLH has occasionally been observed in patients who have other underlying primary immunodeficiencies, including some with restricted T repertoires and function, such as the del22q11 syndrome, or DiGeorge syndrome [38], and severe combined immunodeficiency. Sporadic cases of hemophagocytic complications in patients who have chronic granulomatous disease [39] and X-linked agammaglobulinemia, and the X-linked NEMO [40] defect have also been reported. In these case reports, various infections, including Burkholderia cepacia and adenovirus, have been temporally associated with the development of the hemophagocytic symptomatic complex.

Although it is clear that many genetic forms of HLH may be triggered by viral or parasitic infections, leishmaniasis, in particular, has been cited as an HLH mimic. HLH associated with leishmaniasis is curable by specific treatment of the infection itself without need for anti-inflammatory or cytotoxic therapies (see later discussion), but is potentially fatal without antibiosis [41,42]. Visceral leishmaniasis is a strongly immunosuppressive, generalized infection of the reticuloendothelial system manifested by intermittent fevers, massive hepatosplenomegaly, pancytopenia, fatigue, and hepatitis. Hemophagocytosis is often obvious on bone marrow biopsy. Leishmanial amastigotes may also be seen on the marrow biopsy. Today, molecular diagnostics to identify this infection are widely available, and should be applied to individuals originating from endemic areas bordering the Mediterranean basin, as part of the differential diagnosis of HLH. Although the genetic make-up of patients who develop life-threatening leishmaniasis has received little study, it appears that this condition represents a clear example of secondary HLH.

In contrast, other infection-associated cases of HLH should remain suspect for underlying genetic predispositions. Most commonly, viral infections have been reported in association with HLH, especially involving the herpes virus group. EBV has long been recognized in Asia as a major instigating

infection. EBV-associated HLH in Japanese series appears to differ from typical EBV infections and HLH associated with EBV in the West, in two regards [43,44]. Principally, in Japanese cases of HLH, EBV is commonly found as an ectopic infection of NK and T cells, often associated with clonal expansion. In contrast, this phenomenon has been reported to a much lesser extent in the West. Indeed, EB viral load typically decreases with anti–B-cell therapy (eg, Rituxan) when used in combination with cytotoxic therapy outside of Asia, suggesting that the major niche for EBV (B cells) is being destroyed. Secondly, many cases of HLH in Japan respond to a short course of therapy and do not require hematopoietic cell transplant to achieve prolonged remission or cure. This result is less commonly seen in the West.

Other herpes group viruses that appear to trigger HLH include cytomegalovirus [45], varicella zoster virus [46], human herpesvirus (HHV) 6 [47], and HHV8 [48]. Anecdotally, Varivax has been described to precipitate HLH in genetically susceptible individuals. Avian influenza is a particularly potent stimulus for hemophagocytic reactions in Asia, which appear to be associated with the high mortality seen in that infection. Rubella [49], adenovirus [50], parvovirus [47], and hepatitis B virus [51] have all been reported in connection with HLH.

HLH is a recognized complication of HIV infection. In some cases, it is diagnosed in the context of associated opportunistic infections or lymphoma. It can present at almost any time along the course of the HIV disease, from acute seroconversion to end-stage disease, or during highly active antiretroviral therapy. In some cases, it has been hypothesized that a strong antiviral cytotoxic response may be responsible for the symptoms of HLH.

Other microbial pathogens include Mycobacterium tuberculosis [52], Serratia marcesans [11], Burkholderia cepacia [39], and fungal infections such as Candida [53], aspergillus [54], and histoplasmosis [55].

In most cases of infection-associated HLH, cytotoxic therapy in combination with antimicrobial therapy is required to control the disease process.

Hemophagocytic disorders associated with autoimmune disorders

The most commonly recognized association of hemophagocytic syndrome with rheumatoid disorders is the macrophage activation syndrome (MAS) [56,57]. MAS, most often associated with systemic onset juvenile inflammatory arthritis (soJIA), can be the first presenting feature of soJIA. MAS, and particularly recurrent MAS, bears a close resemblance symptomatically and immunologically to HLH. Many cases of MAS are characterized by low NK-cell function and deficient perforin expression in cytotoxic cells [58]. More recently, mutations in MUNC 13-4 have been identified in MAS associated with soJIA [59].

MAS is less frequently reported in other rheumatologic disorders such as pauciarticular rheumatoid arthritis and systemic lupus erythematosus [60]. MAS has also been described in association with Behcet's syndrome [61].

An HLH-like syndrome can complicate inflammatory bowel disease [62], especially when immune suppressive therapy precedes activation of EBV or histoplasmosis, especially in the setting of EBV or histoplasma infections during immune suppression of affected individuals.

Hemophagocytic disorders associated with malignancies

The association of hemophagocytic complications with malignancies, typically lymphoid malignancies, including T-cell and NK-cell tumors, peppers the literature [44]. In many cases, HLH represents a proximate life-threatening complication of the tumor or its therapy, and is associated with a poor prognosis. Until recently, these malignancies and hemophagocytic reactions have been viewed as side effects, perhaps the consequence of abnormal cytokine generation by the malignant cells. However, more recent findings linking underlying genetic defects (such as PRF1 and SH2D1A) suggest that pre-existing genetic susceptibility, as in murine models, which predict increased risk of lymphomagenesis [63], may play a role in the evolution of lymphoid tumors in humans and a predisposition to severe inflammatory reactions subsequent to therapy.

A retrospective study of 29 archival lymphoma samples revealed that tumors from eight individuals harbored mutations in PRF1 (four biallelic, four heterozygous) [64]. Furthermore, in a sample of 100 cases of childhood acute lymphoblastic leukemia (ALL), the incidence of heterozygosity for A91V mutation in perforin was more than twice as frequent in children with leukemias as in the general population [65]. A much larger study prompted by the occurrences of HLH during remission of childhood ALL with the current treatment protocol in the United States, examined 2272 children with ALL and 655 normal controls. Allele frequencies in Caucasian controls and patients who had ALL were similar, except for a significantly higher frequency in the small subgroup of BCR-ABL–positive ALL [66]. The study of correlations and causality between mutations in perforin and malignancies is still nascent.

Diagnosis of hemophagocytic lymphohistiocytosis

To assist with the rapid diagnosis of HLH, the Histiocyte Society has developed a set of diagnostic guidelines that encompass clinical and laboratory findings (Box 1) [2]. In the absence of a family history or specific genetic diagnosis, an assemblage of five or more of the eight diagnostic criteria are needed for a provisional diagnosis of HLH and initiation of therapy. These include persistent fevers without clear cause, splenomegaly, bicytopenia, hypertriglyceridemia or hypofibrinogenemia, and hemophagocytosis on bone marrow biopsy. Hemophagocytosis may not be clearly apparent in the initial bone marrow biopsy early in the disease process (Fig. 1). However, autopsy studies of more advanced cases repeatedly show that

Box 1. Diagnostic guidelines for hemophagocytic lymphohistiocytosis (patterned after the HLH 2004 protocol)

1. A molecular diagnosis consistent with HLH
2. Diagnostic criteria for HLH fulfilled (five or more out of the eight criteria below)
   - Fever
   - Splenomegaly
   - Cytopenias (affecting two of three lineages in the peripheral blood: hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L), platelets <100 109/L, neutrophils <1.0 109/L)
   - Hypertriglyceridemia or hypofibrinogenemia: fasting triglycerides >3.0 mmol/L (>265 mg/dL), fibrinogen <1.5 g/L
   - Hemophagocytosis in bone marrow, spleen, lymph nodes, or cerebrospinal fluid: no evidence of malignancy
   - Low or absent NK cell activity (according to local laboratory reference)
   - Elevated ferritin (>500 mg/L)
   - Soluble CD25 (ie, soluble interleukin-2 receptor) above normal limits for age

The diagnosis of HLH can be established by one or both of the criteria described.

hemophagocytosis is present throughout the organs of the reticuloendothelial system, and most other organs, including the lung, testes, pancreas, kidney, intestine, and even brain [67]. Diagnostic liver biopsies, often performed early in the disease for diagnosis of hepatitis, rarely reveal hemophagocytosis; rather, perivascular lymphoid infiltrates and triaditis with lymphoid infiltration are commonly seen. This latter finding should not decrease suspicion for HLH if other clinical findings point to the diagnosis.

Symptoms of CNS dysfunction, cerebrospinal fluid pleocytosis, or findings of foci of inflammation by CNS MRI scanning, are found in more than one half of patients who have HLH at initial clinical presentation. Characteristic findings on MRI, as summarized recently [68], include

- Multiple nodular or ring-enhancing parenchymal lesions
- A laminated pattern of nodular parenchymal lesions on T2-weighted images
- Leptomeningeal enhancement
- Confluent parenchymal lesions
- Mild ventriculomegaly
- Brain edema

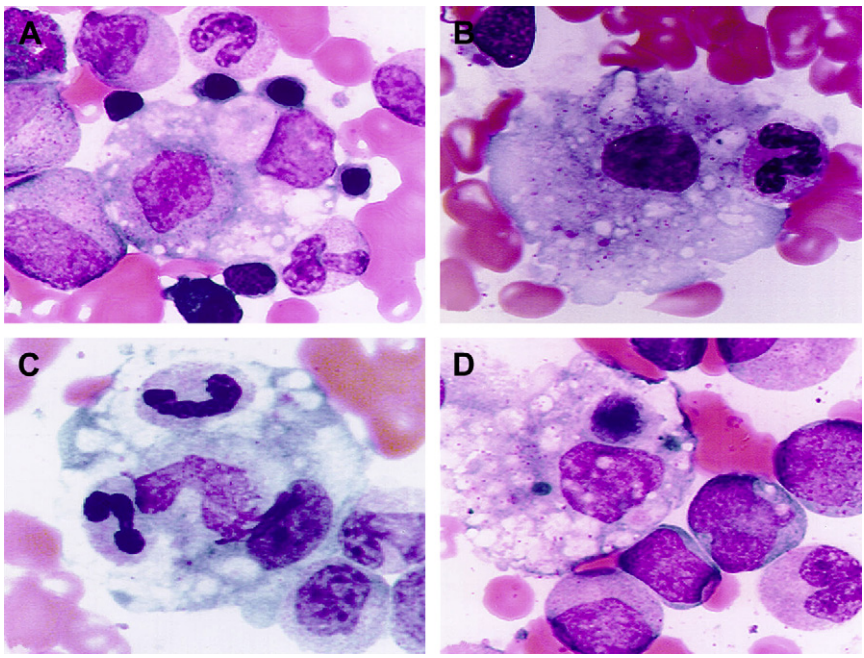

Fig. 1. Several examples of hemophagocytosis observed in the bone marrow of patients with HLH. (*A*) Attachment of hematopoietic cells to the surface of an activated histiocyte prior to internalization. This attachment is believed to be facilitated by the up-regulation of heme receptors CD163. (*B–D*) Clear examples of hemophagocytosis, showing polymorphonuclear cells, red cell precursors, and platelet debris in the cytoplasm of activated histiocytes. (*Courtesy of* Dr. A. Grom, Division of Rheumatology, Cincinnati Children's Hospital, Cincinnati, OH.)

Examination of the spinal fluid typically reveals a predominance of lymphocytes and monocytes, elevated protein content, and sometimes, hemophagocytosis on cytospin. Generalized cerebral atrophy may be apparent on brain scans at diagnosis and can worsen with prolonged steroid treatment.

Additional criteria for provisional diagnosis of HLH include elevated levels of ferritin [69,70] and soluble IL2Rα (sCD25) [71], both markers of generalized inflammation. A recent review of charts from patients who had elevated ferritin results from a large pediatric hospital concluded that a ferritin level of more than 10,000 mcg/dL was 90% sensitive and 96% specific for HLH [69]. However, this measurement does not discriminate between genetic or secondary forms of HLH. Synthesis of ferritin and interleukin (IL)-10 is induced during the protective anti-inflammatory process of macrophage scavenging of heme through the CD163 receptor [72].

High levels of sIL2Ra are almost never seen outside HLH. Normal ranges for levels of sIL2Ra vary with age (highest in infants and lower in teens and adults) (Fig. 2A, B). When interpreting results of sIL2Ra levels, it is important to know the relevant normal age ranges and the method

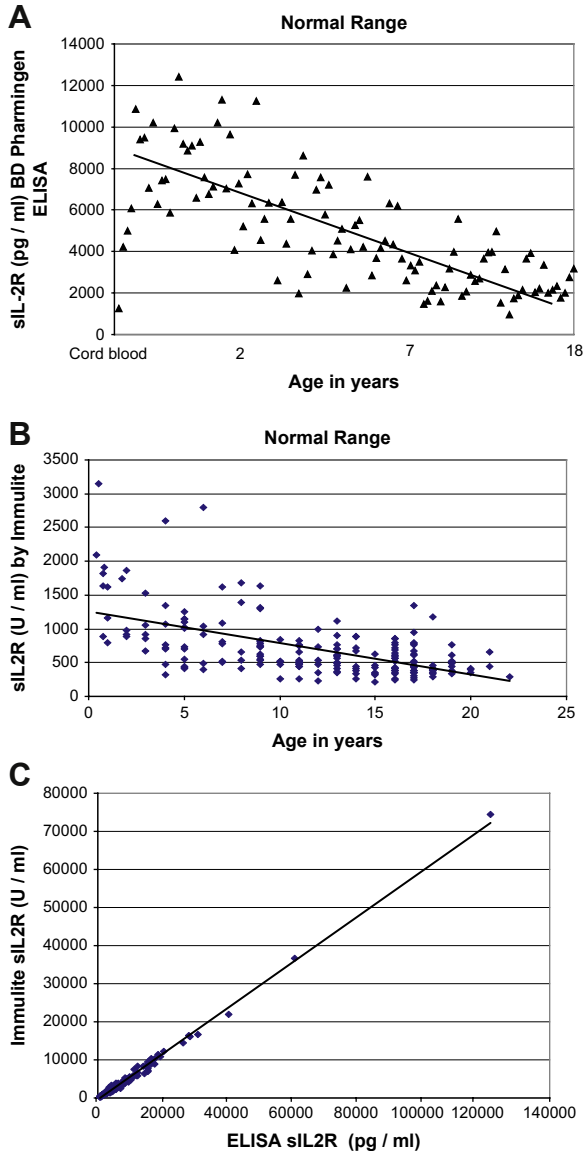

Fig. 2. (*A*) Plasma levels of sIL2R from normal controls, as determined by the BD Pharmingen ELISA method, displayed according to age of subjects. Note that plasma levels in younger subjects are higher than in adults. (*B*) Plasma levels of sIL2R from normal subjects, as determined by the Immulite chemiluminescent method, displayed according to age of subjects. (*C*) Plasma levels of sIL2R from the same control samples, analyzed by two different methods (BD Pharmingen ELISA and Immulite). The levels reveal different absolute values, but perfect correlation between the two methods.

used for analysis. Although trends of sIL2Ra levels are correlated, the quantities reported can vary, depending on the method used (Fig. 2C). Another disease marker is soluble CD163, an anti-inflammatory molecule shed on engagement of Toll-like receptors on monocyte/macrophages [72]. CD163 attaches hematopoietic cells to monocyte/macrophages, facilitating hemophagocytosis. Examination of bone marrow and liver biopsies for CD163 expression in hemophagocytic syndromes frequently reveals extensive activation of resident or infiltrating monocyte/macrophages, far exceeding the microscopic findings of hemophagocytosis (Fig. 3). In some cases, plasma proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interferon-γ, IL-1, IL-6, and IL-18, are also elevated, but these are not specific for the diagnosis of HLH; rather, trends in circulating cytokine levels may be more informative in predicting response to therapy. Recently, soluble TWEAK (a TNF-related inducer of apoptosis) has also been suggested as a biomarker for HLH [73].

In most familial cases of HLH, NK function is low or absent, although the numbers of circulating NK cells (CD56+/16+) are generally normal. However, the finding of NK function within normal limits, especially during active symptomatic disease, should not preclude a diagnosis of primary or secondary HLH. A recent review of patients diagnosed with HLH due to mutations in perforin or MUNC 13-4, through the Molecular Genetics Laboratory at Cincinnati Children's Hospital Medical Center, found that 24% of patients who had perforin deficiency (17/69) and 35% of patients who had MUNC 13-4 defects (14/41) demonstrated normal NK function in a standard chromium release assay. In another group of patients who had HLH but normal sequences of PRF1 and MUNC 13-4, only 59% demonstrated decreased NK function.

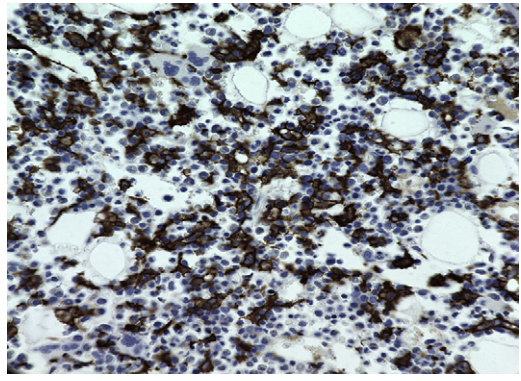

Fig. 3. In situ staining for CD163 expression on cells from a bone marrow biopsy of a patient with HLH. Abundant histiocytes expressing CD163 are seen. (*Courtesy of* Dr. J. Mo, Division of Pathology, Cincinnati Children's Hospital, Cincinnati, OH.)

Screening assays using flow cytometry of cytotoxic cells have been developed to assist the rapid diagnosis of distinct genetic subtypes of HLH. Intracellular staining for perforin [74] and SAP [75] and BIRC4 (Filipovich, personal observation) can identify patients who have FHL2, XLP, and XIAP deficiency, respectively. Recently, it has been proposed that decreased surface expression of CD107a (LAMP 1) [27,76] on NK cells can predict the presence of mutations in MUNC 13-4 and syntaxin 11 genes. CD107a is a molecule expressed on the membrane of the secretory granules in cytotoxic T cells and NK cells. When degranulation occurs, after fusion of the granules' molecular structures with the cytoplasmic membrane of the cytotoxic cell, CD107a is transiently detectable on the surface of the cytotoxic cell and can be quantitated by surface staining. This approach can serve as an initial screen for FHL3 and FHL4, for which no rapid screening tests have yet been developed, and may potentially help identify patients who have related genes causal for HLH (Fig. 4).

The goals of the diagnostic evaluation are

- To exclude other underlying conditions (eg, malignant disease)
- To identify coexisting infections
- To establish the extent of the disease (eg, CNS involvement)
- To collect materials for future studies (eg, genetic testing)

Treatment of hemophagocytic lymphohistiocytosis and related disorders

A retrospective review of HLH 25 years ago described mean survival of less than a month after symptomatic onset and 5% overall survival at 1 year

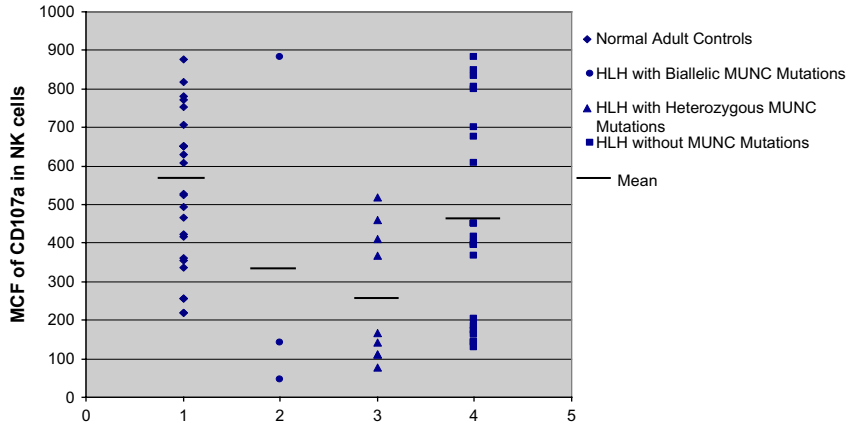

Fig. 4. Results of studies examining the proportion of NK cells expressing CD107a by flow cytometry 6 hours after exposure to K562 target cells. Results from normal controls on the left are compared with results observed with NK cells from patients who have HLH, patients who carry biallelic or heterozygous mutations in MUNC 13-4, and patients who have HLH for whom the genetic cause has not been determined.

after diagnosis [77]. Today, effective initial therapy of HLH consists of combinations of proapoptotic chemotherapy and immunosuppressive drugs targeting the hyperactivated T cells and histiocytes. Currently, definitive treatment of, and a potential cure for, FLH is only achieved by hematopoietic cell therapy (HCT). Projected survival rates, 5 years from diagnosis, range from 50% to 70% [44].

Because HLH can be rapidly fatal without specific intervention, it is recommended that treatment be started when a high clinical suspicion exists, even when results of diagnostic studies are still pending. Effective treatments for HLH have included therapies that target activated macrophages/histiocytes (etoposide, steroids, high-dose intravenous IgG) or activated T cells (steroids, cyclosporine A, antithymocyte globulins, 2 CdA). Generally, combinations of drugs that interrupt the ''vicious cycle'' of immune activation in HLH are most effective. Anecdotal reports of the use of anti-TNF antibodies, Campath-1H (a humanized anti-CD52 antibody that targets antigen-presenting cells and T cells), and abatacept (CTLA4 agonist) have claimed benefit in symptom control. The need to treat coexisting infections, potential triggers of HLH, is obvious.

Results of the international consensus protocol sponsored by the Histiocyte Society for treatment of patients newly diagnosed with HLH (HLH-94) were published in 2005 [78]. The goals of the trial were to achieve clinical remission of the life-threatening inflammation and to provide potentially curative therapy with allogenic HCT. Initial treatment combined intravenous etoposide and intravenous or oral dexamethasone (for CNS penetration), with or without intrathecal methotrexate, followed by a maintenance therapy with the addition of cyclosporine A. Approximately 75% of eligible patients who had de novo HLH who started HLH-94 therapy achieved satisfactory clinical remission after 8 weeks of treatment. However, nearly a quarter of patients on protocol experienced persistent or recrudescent symptoms and ultimately died from complications of HLH. Overall, results with HLH-94 show a 55% HLH-free survival at 3 years. A minor proportion of patients came ''off therapy'' after 8 weeks because they were symptom free and lacked features of familial disease; some have remained healthy for several years off therapy. HLH-94–like therapy has been effective in control of hemophagocytic reactions in patients who have XLP, CHS, Griscelli syndrome, viral-associated HLH (especially EBV), and MAS.

Some patients have continued maintenance treatment with episodic etoposide for a year or more after diagnosis of HLH; such prolonged exposure to etoposide has been associated with evolution to acute myeloid leukemia [78]. Because no genetic studies related to defects potentially underlying HLH have been published in such cases, it is not clear whether predisposition to malignant transformation is inherent or acquired.

HLH 2004 (which represents a modest modification of HLH-94) is currently enrolling patients.

Patients who proceeded to HCT after initial treatment with HLH-94 experienced a 3-year disease-free survival of 63% [39]. HCT recipients of matched related or matched unrelated donor grafts achieved a 3-year disease-free survival of approximately 70%, in contrast to that of recipients of mismatched related or unrelated donor grafts (50%) [77]. Similarly, promising results with HCT have been published from centers in Europe [79–81], and with the use of reduced-intensity pretransplant conditioning [82]. Results of posttransplant chimerism studies reported in the French transplant series confirm that stable mixed chimerism can be associated with freedom from HLH relapse [80], although the relevant cell types contributed by the donor graft have not been fully characterized.

The best results with HCT have been observed in children who experienced prompt and complete response to the HLH-94 before transplant, and were free of significant CNS involvement [83].

Several posttransplant complications are reported with significant frequency, including severe inflammatory reactions during the early engraftment period, often mimicking HLH! Acute graft-versus-host disease has occurred in 30% to 60% of patients who had HLH treated with conventional chemotherapy, and posttransplant autoimmune hemolytic anemia, and, to a lesser degree, other immune cytopenias, are commonly seen. Late complications of prior CNS damage can manifest months to years after HCT with neurocognitive deficits. Fortunately, long-term follow-up of survivors of HCT for HLH indicates that most children return to a normal or near-normal quality of life [84].

Frequently asked questions about hemophagocytic lymphohistiocytosis

1. Why is the disease named hemophagocytic lymphohistiocytosis versus macrophage activation syndrome or lymphohistiocytic activation syndrome?

Hemophagocytosis (typically sought on bone marrow biopsy) is not a universal finding of this group of disorders, even in the most fulminant forms. Indeed, some controversy exists as to whether the phenomenon recognized by the findings of hematopoietic cells engulfed by activated monocyte/macrophages represents phagocytosis, pinocytosis, or some related mechanism. Perhaps in the future a new name for this group of disorders will be adopted.

2. How common is natural killer cell dysfunction in hemophagocytic lymphohistiocytosis?

The HLH 2004 protocol, developed by the working party of the Histiocyte Society, indicated that decreased or absent NK function showed high sensitivity and specificity for the diagnosis of HLH. Recent studies from North America indicate that substantial numbers of patients who have

known genetic defects associated with HLH demonstrate normal NK function during active disease (up to 40%) (see text). The likely reason for the discrepancy between this newer data and that used for the analysis to develop diagnostic markers for HLH 2004 is the greater heterogeneity in ethnic backgrounds and diversity of underlying genetic causes represented in the much larger North American series than that available at a few selected European centers for the earlier analysis.

3. Is hemophagocytic lymphohistiocytosis predominantly a disease of infants?

With the description of several genetic defects causing life-threatening histiocytic disorders, it has been become clear that HLH can present at any time, from intrauterine development into the seventh decade of life. "Null" mutations of HLH-related genes are associated with younger age at symptomatic onset, whereas mutations resulting in some synthesis of mature protein, often associated with missense mutations, may result in symptomatic disease at older ages.

4. Is evidence of hemophagocytosis required for the diagnosis of hemophagocytic lymphohistiocytosis?

Although "hemophagocytosis" is a hallmark of activated histiocytes, the finding of hemophagocytosis on bone marrow biopsy (the most readily accessible tissue for differential diagnosis of pancytopenia) is not a prerequisite for the clinical diagnosis of HLH. Hemophagocytosis may not be evident even during fulminant disease. Other noninvasive assays supporting the presence of highly activated histiocytes are being developed, such as the detection of soluble CD163 in plasma samples. Recent work suggests that engulfment of hematopoietic cells by activated histiocytes requires expression of specific surface receptors, among them CD163, which is involved in the pathway of ferritin-related hemoglobin salvage, a putative homeostatic anti-inflammatory pathway. Autopsy studies indicate that hemophagocytosis is more commonly observed in the reticuloendothelial organs (liver, spleen, lymph nodes) than in bone marrow or other tissues. Hemophagocytosis can also be detected on cytospin slides of cerebrospinal fluid, when the CNS is involved with disease. Recent studies staining bone marrow and liver biopsies for CD163 expression of histiocytes in HLH and related disorders indicate a high level of activated cell populations, even when hemophagocytosis is scant or not apparent.

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Immune Dysregulation in Primary Immunodeficiency Disorders

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The traditional dogma that patients who have primary immunodeficiency disorders (PIDD) are defined by their susceptibility to infections has recently been expanded to include a new group of syndromes defined primarily by susceptibility to autoimmunity. These new syndromes of "immune dysregulation," including immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), have helped to expand our understanding of basic immune tolerance mechanisms [1,2]. They have also raised our awareness and understanding of the autoimmunity/immune dysregulation that can complicate more traditional immunodeficiency disorders.

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome

IPEX syndrome (OMIM #304930) is the prototypic example of the clinical outcome resulting from unregulated or severely dysregulated immune responses. It is a testament to the destructive power of effector T cells and to the equally potent regulatory activity of regulatory T cells (T_{REG}).

The basic clinical triad of IPEX includes autoimmune enteropathy, early-onset endocrinopathy, and dermatitis [3–5]. The enteropathy typically presents early in life as watery (and at times bloody) diarrhea, frequently resulting in malnutrition and failure to thrive. Type I diabetes is the most common endocrinopathy but clinical or laboratory evidence of thyroiditis is also common. Eczema is the most common dermatitis observed in patients, but erythroderma, psoriasiform dermatitis, and pemphigoid nodularis have also been observed [2,6,7].

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In addition to the "IPEX-triad," most patients who have mutations in the *FOXP3* gene also have associated autoimmune disorders, including autoimmune hemolytic anemia, thrombocytopenia, neutropenia, nephropathy, or hepatic disease (Troy R. Torgerson and Hans D. Ochs, unpublished data, 2008). These other autoimmune symptoms contribute substantially to the morbidity of patients who have IPEX and increase the risk of death from disease. Patients who have the classic form of the disease typically die before the age of two, secondary to malnutrition, electrolyte imbalances, or infection, if not treated with aggressive immunosuppression. A more extensive review of the clinical features of IPEX has been published recently [2].

From a clinical laboratory standpoint, the most consistent abnormality among patients who have IPEX is markedly elevated IgE, present in most cases. IgA was also modestly elevated in more than 50% of patients in the cohort that the author and his colleagues evaluated. Consistent abnormalities do not exist in absolute lymphocyte numbers or in numbers of $CD4^+$, $CD8^+$, $CD19^+$, or $CD16/56^+$ cells. T cells usually respond normally to proliferative stimuli with mitogens or antigens (Troy R. Torgerson and Hans D. Ochs, unpublished data, 2008).

IPEX is caused by mutations in the forkhead DNA-binding protein FOXP3, a 431 amino acid protein expressed primarily by $CD4^+CD25^{high}$ T_{REG} [8–10]. Recent studies have demonstrated that FOXP3 is required for T_{REG} cells to develop suppressor function [11,12]. In two separate knock-in mouse models, cells destined to become T_{REG} were prevented from expressing functional FOXP3 but were marked with green fluorescent protein (GFP). These cells acquired the expected cell surface phenotype of a T_{REG} ($CD25^{high}$CTLA-$4^{high}GITR^{high}$) but they had no suppressive function. Instead, they developed a gene expression profile suggestive of an effector/cytotoxic T cell, and the animals developed evidence of systemic autoimmunity similar to *Scurfy* mice, the murine ortholog of IPEX [12].

Under quiescent conditions, FOXP3 expression is restricted primarily to T_{REG} cells; however, recent data have demonstrated that FOXP3 can be inducibly expressed in virtually any activated human T cell [13–16]. Although it was originally shown to be a transcriptional repressor acting on key cytokine genes [17–19], recent genome-wide screening approaches suggest that FOXP3 functions more commonly as a transcriptional enhancer [20,21]. A great deal of work remains to be done to identify the key gene or genes that are regulated by FOXP3 to confer suppressor function on FOXP3-expressing cells.

Structure/function analysis of FOXP3 has determined that three key structural domains of the protein are critical for its function, and most confirmed pathologic mutations cluster in these domains [22]. The key structural domains include a highly conserved C-terminal forkhead DNA-binding domain, which is required for interaction with DNA and for nuclear import; a leucine zipper in the central part of the molecule, which is required for homo- and possibly heterodimerization of the protein; and a repression

domain in the N-terminal portion of FOXP3, which has been shown to interact with a large protein complex to regulate transcription [22]. Recent studies suggest that FOXP3 physically and functionally interacts with other transcription factors, including NFAT, NF-κB, and AML-1/RUNX1, to modulate gene transcription at key cytokine promoters [18,19,23].

A diagnosis of IPEX is generally suspected in any patient who demonstrates at least two of the three features of the "IPEX triad" or has evidence of additional autoimmune disease. Elevated IgE, present in almost all cases of IPEX, is a helpful clue. Flow cytometry using intracellular staining for FOXP3 protein to identify $FOXP3^+$ T_{REG} cells is a valuable tool to rapidly screen for the absence of T_{REG}. Approximately 5% to 7% of the $CD4^+$ T-cell population is positive for FOXP3 expression in normal individuals, and a marked decrease suggests a diagnosis of IPEX, which may be confirmed by sequencing of the *FOXP3* gene. Currently, the gold standard for diagnosis is identification of a mutation in *FOXP3*; however, in the cohort of patients that the author and his colleagues have evaluated, mutations have been identified in only 25% to 30% of those in whom disease is clinically suspected (Troy R. Torgerson, unpublished data, 2008).

Adoptive transfer studies have demonstrated that the $CD4^+$ T cells from an affected male *Scurfy* mouse are capable of recapitulating the entire disease phenotype in a lymphopenic recipient [24]. Treatment of IPEX has therefore focused primarily on suppression of unregulated, autoaggressive T cells, using cyclosporine A, tacrolimus (FK506), or sirolimus (rapamycin) [4,5,25,26]. These drugs are often combined with steroids or other immunomodulatory agents, including methotrexate or azathioprine, in an effort to control symptoms [26,27]. In cases with evidence of pathogenic autoantibodies, rituximab (anti-CD20) has proven effective [7]. These therapies are often effective initially and one case was reported of a patient who had classic IPEX being maintained for a prolonged period with aggressive immunosuppression; however, most patients ultimately seem to fail therapy [28]. At this point, bone marrow transplant holds the only hope for a long-term cure [29–31]. Complete and reduced-intensity conditioning protocols have been reported as successful, although reduced-intensity regimens seem to be associated with better survival [30,31]. In many of the cases transplanted thus far, patients are successfully engrafted and have resolution of all IPEX symptoms, except for type I diabetes. Rapid diagnosis and transplant early in the course of disease, before the pancreatic islets are destroyed, should therefore be the goal, to avoid the long-term sequelae of diabetes in these patients.

Defects of interleukin-2 signaling

CD25 deficiency

Only a portion of the patients who have symptoms that suggest a diagnosis of IPEX actually have mutations in the *FOXP3* gene, suggesting that

other defects may lead to a similar phenotype. Murine models that are phenotypically similar to the *Scurfy* mouse have provided some clues to other defects that may be present in this "IPEX-like" cohort. CD25-deficient mice were among the most interesting of these models.

Two unrelated patients who had CD25 deficiency (OMIM #606367) have now been described in the literature, and one recent report highlighted the IPEX-like phenotype of one of these [32]. Both affected patients developed severe, chronic diarrhea and villous atrophy in infancy (one at 6 weeks and the other at 8 months of age), similar to the clinical manifestations of IPEX [32,33]. One also developed early-onset insulin-dependent diabetes and later also developed eczema [32]. Subsequently, both patients developed autoantibodies, hepatosplenomegaly, lymphadenopathy, and lymphocytic infiltrates in various organs (gut, liver, and so forth), indicative of ongoing immune dysregulation [32–34]. Unlike IPEX patients, serum IgE levels were only mildly elevated in one patient and were normal in the other [32,33].

In addition to autoimmune features, both CD25-deficient patients had infectious complications suggestive of a more extensive defect in cellular immunity. Most prominent in both patients was early-onset, recurrent cytomegalovirus pneumonitis, although persistent thrush, candidal esophagitis, chronic gastroenteritis, and Epstein-Barr virus infection were also seen [32,33]. One patient failed to reject an allogeneic skin graft [35].

Immunophenotyping demonstrated either normal or modestly decreased T lymphocyte numbers (particularly $CD4^+$ T cells). T-cell proliferative responses to mitogens and anti-CD3 stimulation were markedly decreased ($<20\%$ of the control) but could be restored with high-dose IL-2 or IL-15 [32,33]. Evaluation of the T_{REG} compartment in human CD25 deficiency was limited, showing only that the percentage of $FOXP3^+$ cells in the $CD4^+$ T-cell population was comparable to a normal control but slightly lower than that reported in the literature for other normal individuals ($\sim 4\%$ compared with 6%–7% for other studies) [13,32,36].

In each case, inheritance was found to be homozygous recessive, leading to a complete lack of CD25 protein expression on activated T cells. One patient, the product of consanguineous parents, was homozygous for a four-base-pair deletion in the coding region of *CD25* that caused a frameshift and early termination codon within the CD25 protein [33,35]. The other patient had compound heterozygous mutations in the *CD25* gene with a frameshift on one allele and a premature stop codon on the other [32].

Because T_{REG} cells from CD25-deficient patients lack the major cell-surface marker used to differentiate and isolate intact T_{REG}, it is difficult to assess their suppressive function in typical in vitro assays. It is therefore unknown whether the $CD4^+FOXP3^+$ T_{REG} from these patients have normal suppressive capacity.

Mice lacking the α chain of the IL-2 receptor (CD25) have a dramatic phenotype with splenomegaly, lymphadenopathy, severe colitis, and autoimmune hemolytic anemia that is reminiscent of the *Scurfy* mouse [37].

Recent studies in CD25-deficient mice have demonstrated that T_{REG} development is normal in the thymus and that $CD4^+$ $FOXP3^+$ T cells from these animals have normal suppressive function in vitro. However, the survival, maintenance, and competitive fitness of mature T_{REG} cells have a defect, which is thought to be the cause of the immune dysregulation observed [38,39]. It is hoped that future efforts to assess the maturity of the $CD4^+FOXP3^+$ T_{REG} pool in CD25-deficient patients will help determine whether a similar mechanism is at play in humans.

Both patients identified and described to date exhibit autosomal recessive disease in which both alleles of CD25 are affected, resulting in complete absence of CD25 expression on T cells. Flow cytometry is therefore an effective screening tool for identifying CD25-negative patients, but sequencing of the *CD25* gene is recommended to confirm the diagnosis.

Because of the "severe combined immunodeficiency (SCID)-like" features of this syndrome, one patient underwent a successful bone marrow transplant from a matched sibling donor and has done well [34,35]. It is possible, however, that patients may respond well to IL-2 therapy because the T-cell proliferative defect was able to be overcome in one patient by treatment of the cells with high-dose IL-2 or IL-15. Exogenous IL-2 may provide enough stimulus through the remaining "low-affinity" IL-2 receptor beta chain to allow T_{REG} cells to survive and control autoreactive effector T cells.

Signal transducer and activator of transcription 5b deficiency

Deficiency of signal transducer and activator of transcription (STAT) 5b (OMIM #245590) causes a rare autosomal recessive disorder reported in only a handful of individuals [40–42]. The most notable clinical feature of the syndrome is dwarfism associated with a normal serum growth hormone level but low insulin-like growth factor-1 (IGF-1) levels [41,42]. Other physical features include a prominent forehead, a saddle nose, and a high-pitched voice. Most patients also have a marked immunodeficiency, with recurrent varicella virus, herpes virus, and *Pneumocystis jiroveci* infections, suggesting a defect in natural killer or cytotoxic T cells [41,42]. Immunophenotyping of affected patients has shown low γδ T cell and natural killer cell numbers, and modest T-cell lymphopenia with a normal CD4/CD8 ratio [41,43].

In addition to frank immunodeficiency, most patients who lack functional STAT5b also have symptoms suggestive of immune dysregulation, including chronic, early-onset diarrhea; eczema; and lymphocytic interstitial pneumonitis [40–42]. Murine models have demonstrated that Stat5b is a critical transducer of the IL-2–mediated signals required to sustain FOXP3 expression in T_{REG} and to maintain T_{REG} cells themselves [44,45]. Mice lacking Stat5b have a significant reduction in the number of $FOXP3^+$ T_{REG} cells in the thymus and spleen and, as a consequence, have a marked increase of activated T cells in the periphery [45,46].

STAT5b is a member of the STAT family of proteins, which regulates gene transcription in response to various cytokines and growth factors. It plays a particularly important role in T cells, where it is a key mediator of IL-2–induced responses. Like other STAT proteins, STAT5b is present as a monomer in the cytoplasm of quiescent cells. It is recruited to the activated IL-2 receptor, where it is phosphorylated by receptor-associated tyrosine kinases. The phosphorylated subunits dimerize through their SH2 domains, translocate to the nucleus, and bind DNA to regulate gene transcription [47].

To evaluate the effect of STAT5b deficiency on T_{REG} cells in humans, two patients have been studied, one with a homozygous missense mutation (A630P) in the SH2 domain of STAT5b, and the second with a homozygous nonsense mutation (R152X). Both result in markedly reduced or absent STAT5b protein expression [41,43]. In both cases, patients had significantly fewer $CD4^{+}CD25^{high}$ cells than normal individuals and the cells that were present expressed much less FOXP3 than normal $CD4^{+}CD25^{high}$ T_{REG} [41,43]. Consequently, isolated $CD4^{+}CD25^{high}$ T cells from a STAT5b-deficient patient had no suppressive activity on either autologous or allogeneic responder T cells in an in vitro suppression assay [43]. Decreased CD25 expression ($\sim$20% of normal) on the patients' T cells in response to activation was shown to be a direct consequence of STAT5b deficiency and is thought to synergize with the underlying STAT5b mutation to effectively abrogate the IL-2 signals required for the maintenance of FOXP3 expression and T_{REG} function. The signaling pathways required for IL-2–induced expression of other effector molecules, such as perforin, remained intact in STAT5b-deficient T cells [43].

Defects of autoimmune regulator function

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy

APECED (OMIM #240300) is an autosomal recessive disorder characterized by systemic autoimmunity affecting primarily the endocrine organs, including the parathyroid glands, adrenal glands, and pancreas [1,48]. Hypoparathyroidism, adrenal insufficiency, and chronic mucocutaneous candidiasis typically characterize the syndrome, but patients may also have type 1 diabetes, gonadal failure, and pernicious anemia (secondary to atrophy of gastric parietal cells) [1,48].

APECED is caused by mutations in the autoimmune regulator (AIRE), a transcription factor that plays a role in ectopic expression of tissue-specific antigens on medullary thymic epithelial cells. In mice, AIRE-mediated self-antigen expression in the thymus has been shown to play a significant role in negative selection of autoreactive T-cell clones, thus preventing their escape into the periphery where they may cause autoimmunity [49,50]. The mechanism by which AIRE causes expression of a wide range of tissue-specific

gene products is unknown, but recent evidence suggests that it may do so through regulating large-scale access to chromatin.

Because naturally arising T_{REG} cells are also thymically derived, it was hypothesized that AIRE may also play a role in the generation of T_{REG}, but various studies in AIRE-deficient mice failed to show a significant deficit in bulk T_{REG} numbers or function [49,51,52]. Recent investigations using a transgenic mouse model with a monospecific T-cell receptor demonstrated that expression of the transgenic antigen (hemagglutinin) on $AIRE^{+}$ medullary thymic epithelial cells resulted in effective generation of antigen-specific $CD4^{+}CD25^{+}$ $FOXP3^{+}$ T_{REG} that had potent suppressive activity in vitro [53]. These data suggest that the autoimmunity seen in $AIRE^{-/-}$ mice is the result of both defective negative selection of autoreactive effector T cells and defective generation of antigen-specific T_{REG}.

Because the pace and severity of autoimmune disease in $AIRE^{-/-}$ mice is mild compared with that seen in patients who have APECED, it was hypothesized that in humans, AIRE may play a more significant role in the generation and function of T_{REG}. To address this possibility, two studies evaluated patients who had APECED by flow cytometry and quantitative real-time polymerase chain reaction, for the presence of $FOXP3^{+}$ T_{REG} cells in the peripheral blood. In each study, the patients were found to have a decreased percentage of $CD4^{+}CD25^{high}$ T_{REG} cells within the $CD4^{+}$ T-cell population. In addition, $CD4^{+}CD25^{high}$ T cells from these patients expressed less FOXP3 than similar cells from normal controls [54,55]. Coincident with the decreased FOXP3 expression in the $CD4^{+}CD25^{high}$ cells, isolated T_{REG} from APECED patients had a decreased ability to suppress proliferation of effector T cells in response to anti-CD3 or mitogen phytohemaglutinin (PHA) [54]. These data support a role for AIRE in the generation of functional T_{REG} in humans.

Omenn's syndrome

Omenn's syndrome is a variant phenotype of SCID in which a limited repertoire of T cells are able to overcome a molecular block in development and undergo explosive expansion in a lymphopenic environment. Many of the T-cell clones that expand appear to be autoreactive, which almost certainly contributes to the dramatic phenotype of this disorder [56]. Omenn's syndrome has been associated most frequently with hypomorphic mutations in the recombinase activating proteins RAG1 and RAG2 but has also been reported in IL-7 receptor deficiency, ARTEMIS deficiency, adenosine deaminase deficiency, and cartilage hair hypoplasia [57–61]. Clinically, patients often present with diarrhea, a severe erythematous rash, lymphadenopathy, marked eosinophilia, and elevated IgE, a clinical picture not unlike patients who have severe IPEX [56,62].

Studies evaluating thymic tissue from patients who have Omenn's syndrome suggest that one potential explanation for the large expansion of

autoreactive T cells in Omenn's syndrome is a marked reduction of AIRE expression in thymic epithelial cells, relative to controls [63]. This reduction is hypothesized to decrease negative selection and deletion of potently autoreactive T cell clones, and to decrease positive selection and development of T_{REG} in the thymus. It is unknown whether these findings are applicable to all defects associated with Omenn's syndrome or if they are specific to defects in RAG1 or RAG2.

Defects of the cytoskeleton and other signaling pathways

Wiskott-Aldrich syndrome

Wiskott-Aldrich syndrome (WAS) (OMIM #301000) is a rare X-linked disorder characterized by thrombocytopenia, small platelets, eczema, recurrent infections, immunodeficiency, and a high incidence of autoimmune disease and malignancies. It is caused by mutations of the WAS protein (*WASP*) gene [64]. WASP has five well-defined functional domains and is a key regulator of actin polymerization in hematopoietic cells, where it is involved in signaling, cell locomotion, and immune synapse formation.

Because 40% to 70% of WAS patients develop autoimmune disease [65,66], several recent studies have focused on the quantity and quality of T_{REG} cells in murine models and in patients lacking functional WASP [67–69]. In mice, WASP appears to play no role in thymic T_{REG} production but is required for peripheral T_{REG} expansion and survival [68,69]. Specifically, $WASP^{-/-}$ T_{REG}, compared with wild-type T_{REG}, showed decreased competitive fitness in three independent in vivo murine models, including skewing of T_{REG} in heterozygous female carriers, and progressive loss of $WASP^{-/-}$ T_{REG} in recipients of chimeric bone marrow and in *Scurfy* mice that received $WASP^{-/-}$ T_{REG} by way of adoptive transfer [68]. Although WASP deficiency in humans is not associated with a significant decrease in the percentage of T_{REG} in the peripheral blood, the function of T_{REG} was found to be consistently reduced in WAS patients, as demonstrated by the inability to suppress proliferation of effector T cells.

Consistent with the observations in mice, a strong selective advantage for $WASP^{+}$ T_{REG} in vivo was observed in a patient who had a revertant mutation, leading to the expression of WASP in a significant percentage of newly generated T_{REG}. It appeared that in this patient, the appearance of a population of T_{REG} cells expressing WASP correlated with decreased autoimmune disease activity and improved clinical condition [68].

Common variable immunodeficiency

The most apparent clinical presentation of common variable immunodeficiency (CVID) is one of abnormal humoral immunity, with recurrent sinopulmonary infections resulting from immunoglobulin deficiency [70].

However, a significant proportion of patients also have abnormalities in T-cell number and function, suggesting the presence of defects that lead to a combined immunodeficiency [71]. Approximately one third of patients develop various autoimmune phenomena and 20% develop an impressive clinical presentation marked by lymphadenopathy, splenomegaly, and pulmonary infiltrates [72]. One recent study investigated FOXP3 mRNA expression levels and numbers of $CD4^+FOXP3^+$ T_{REG} cells in patients who had CVID, and found low FOXP3 expression levels and low numbers of T_{REG} cells in affected patients. Patients who had splenomegaly had the lowest FOXP3 expression levels and T_{REG} numbers, suggesting a correlation with increased disease severity [73].

A handful of molecular defects have been associated with CVID in various cohorts of patients. Among these is the T-cell inductible costimulatory (ICOS) molecule [74,75]. Recent investigations in a murine model of ICOS deficiency have suggested a mechanism for decreased $FOXP3^+$ T_{REG} cell numbers in this form of CVID. In this murine model, was found that ICOS plays a significant role in sustaining the survival and expansion of effector T cells following antigen-specific T-cell activation and that this effect also extends to $FOXP3^+$ T_{REG} cells. ICOS-deficient animals exhibit decreased numbers of effector T cells and T_{REG}, suggesting that ICOS plays a role in regulating the size of the effector T-cell and T_{REG} pools in vivo [76]. It is hoped that further studies to investigate T_{REG} numbers and function in ICOS-deficient patients will provide additional insight into this mechanism.

Summary

In recent years, identification of the gene defects in a handful of clinical syndromes with congenital systemic autoimmunity has led to the definition of a new class of PIDD that is defined by abnormal immune tolerance. Lessons learned about T_{REG} from these syndromes are now being applied to older, more traditional PIDD to identify new molecular pathways that affect T_{REG} development, maintenance, and function. This area will undoubtedly continue to provide a fertile field for investigation over the coming years as we strive to uncover the basic mechanisms of immune regulation.

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Genetic Defects of Apoptosis and Primary Immunodeficiency

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Programmed cell death (reviewed in [1]) is a physiologically important process in which lymphocytes are triggered to undergo suicide by various stimuli, including death receptor ligation, restimulation of antigen receptors (often termed "activation-induced cell death"), or withdrawal of trophic factors such as interleukin (IL)-2. One form of programmed cell death is apoptosis, which is characterized ultrastructurally by cell shrinkage, nuclear condensation and fragmentation, membrane blebbing, and shedding of apoptotic bodies containing relatively intact organelles [2]. Biochemically, apoptosis is marked by activation of caspases, intracellular enzymes that degrade cellular components including structural proteins and cell-maintenance enzymes [2]. In vivo, apoptotic cells are engulfed rapidly by phagocytic cells while eliciting minimal inflammatory sequelae. Necrosis and autophagy are other forms of programmed cell death [2] that can occur during intracellular infections with pathogens, but their physiologic relevance in humans is generally less well understood.

Although apoptosis takes place in different tissues and during embryonic development, it serves a particularly important role in the immune system. Apoptosis eliminates strongly self-reactive immature T cells during thymic development (central tolerance). Self-reactive T cells that have escaped

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This article is dedicated to the memory of the late Stephen Straus, who led the NIH ALPS group to many new discoveries.

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this negative selection or autoreactive B cells can undergo apoptosis in the periphery as mature lymphocytes (peripheral deletional tolerance). Additionally, apoptosis serves to limit the magnitude and duration of the immune response to foreign antigens. Mature lymphocytes normally respond to these antigens by becoming activated and proliferating. Later, their numbers return to baseline, except for a small pool of memory cells that persists. This decrease is achieved through a combination of death receptor– dependent, antigen receptor restimulation– dependent, and cytokine withdrawal–dependent death mechanisms that occur at or after the height of the immune response. Although naive lymphocytes start out relatively resistant to apoptosis, entry into the cell cycle sensitizes them to die. Thus, activated, cycling T and B cells are governed by a negative feedback control mechanism termed ''propriocidal regulation'' [3]. This mechanism is crucial for maintaining normal overall lymphocyte homeostasis over the course of a person's life.

The prototypic disorder of impaired apoptosis in humans is autoimmune lymphoproliferative syndrome (ALPS), which has been described extensively in a previous volume [4]. This article focuses on new advances in understanding ALPS and a related primary immunodeficiency with a genetic defect in apoptosis called ''caspase-8 deficiency state'' (CEDS). In addition, it briefly discusses a variant of ALPS called ''autoimmune lymphoproliferative disease'' (ALD) and another primary immunodeficiency featuring apoptosis abnormalities, X-linked lymphoproliferative syndrome (XLP). Related disorders with hemophagocytic lymphohistiocytosis, which impair cytotoxic granule formation and the perforin-granzyme death pathway, are beyond the scope of this article [5]. For further details on all these disorders, the reader is referred to several other recent reviews [1,6,7].

Autoimmune lymphoproliferative syndrome

Clinical features

Patients who have ALPS typically present in infancy or early childhood (median age of 24 months) with persistent lymph node and/or splenic enlargement [8]. There often is accompanying hepatomegaly without liver disease and occasional thymic enlargement on CT scan [9]. Patients lack the constitutional symptoms that would suggest infectious or neoplastic etiologies. Most patients have T- and B-cell lymphocytosis as well as polyclonal hypergammaglobulinemia [10–13]. Eosinophilia or monocytosis may be noted [14,15]. Up to 80% of patients have detectable autoantibodies, most commonly anticardiolipin or direct Coombs antibodies [8,16–18]. By contrast, only half of these have actual autoimmune disease, usually autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, or autoimmune neutropenia [8]. Glomerulonephritis, optic neuritis or uveitis, Guillain-Barré syndrome, primary biliary cirrhosis, coagulopathy with

factor VIII inhibitor, autoimmune hepatitis, vasculitis, and linear IgA dermopathy have been reported in patients who have ALPS; however, these autoimmune diseases targeting non-hematopoietic organ systems are uncommon [8,19,20]. By contrast, the MRL-*lpr* or *gld* mouse counterparts of ALPS often are considered animal models of systemic lupus erythematosus (SLE) because of characteristic autoantibodies (anti-dsDNA, anti-Sm, anti-immunoglobulin) and prominent non-hematopoietic autoimmune disease, including glomerulonephritis, polyarteritis, and sialoadenitis [21].

Diagnosis

The differential diagnosis for ALPS is extensive (Box 1).

Several diagnostic criteria have been proposed for ALPS: (1) chronic nonmalignant lymphadenopathy or splenomegaly, (2) expansion of $CD4^-$ $CD8^-$ T-cell receptor (TCR) α/β rearranged (DNT) cells, and (3) defective lymphocyte apoptosis [1,8]. Supporting criteria include autoimmune manifestations, characteristic histopathologic findings, relevant gene mutations (see later discussion), and family history. Of note, ALD, a variant of ALPS, lacks DNT expansion but shares lymphoid organ enlargement, autoimmunity, and defective lymphocyte apoptosis [26,27]. The diagnostic

Box 1. Differential diagnosis for autoimmune lymphoproliferative syndrome (not exclusive)

- Infections
  - Epstein-Barr virus
  - Cytomegalovirus
  - Tuberculosis
  - Toxoplasmosis
  - Hepatitis C
  - HIV
- Medications
- Benign lymphoproliferative disorders
  - Sinus histiocytosis with massive lymphadenopathy (also known as Rosai-Dorfman disease) [22]
  - Progressive transformation of germinal centers
- Leukemia
- Lymphoma
- Hematologic disorders
  - Evans syndrome [23]
  - Hereditary spherocytosis
- Autoimmune diseases
  - Systemic lupus erythematosus
  - Rheumatoid arthritis (Felty syndrome) [24,25]

criteria for ALPS should be qualified in light of recent findings discussed in the following sections.

Lymphoaccumulation

Lymphadenopathy in ALPS typically is nontender and generalized, especially involving the neck and axillary regions. At the National Institutes of Health (NIH), lymphadenopathy is scored as 2+ if 1 to 2 cm in diameter, 3+ if visible without palpation, or 4+ if normal anatomic landmarks are distorted [11]. Splenomegaly is measured in the craniocaudal dimension and is monitored for changes over time. CT scans also are used to assess for intrathoracic or intra-abdominal lymphadenopathy in equivocal cases with borderline lymphadenopathy or splenomegaly on physical examination. Lymph nodes and spleen sizes remain relatively stable over years but may decrease somewhat with age [9,28,29].

Expansion of $CD4^-$ $CD8^-$ T-cell receptor α/β rearranged cells

Patients who have ALPS have lymphocytosis that affects T and B but not natural killer (NK) cells [11,12]. There is expansion of $CD8^+$ T cells that express CD57, but there is no net expansion of CD4 T cells because of low $CD4^+$ $CD25^+$ numbers. T-cell expression of HLA-DR is increased. Both total B cells and $CD5^+$ B cells are increased. A distinctive but not pathognomonic feature of ALPS, which also is seen in the *lpr* or *gld* mouse models, is the expansion of an unusual polyclonal population of mature DNT cells that expresses rearranged TCRα/β [12,30]. DNT cells are thought to be senescent T cells that have reduced CD4 or CD8 coreceptors. They express CD57, CD27, and HLA-DR, as well as the CD45R B220 isoform usually found on B cells but not on T cells [31]. It is important to distinguish DNT cells from TCRγ/δ rearranged cells, which normally are $CD4^-$ $CD8^-$, because the latter can be increased nonspecifically in ALPS and in other conditions (eg, infection, autoimmunity, and malignancy, including T-cell large granular lymphocyte leukemia). At the NIH Clinical Center Laboratory, DNT cells constitute less than 1% of peripheral lymphocytes, or less than 18 cells/μL^3 in healthy adults. By contrast, they can constitute more than 40% of lymphocytes in patients who have ALPS [32], although most patients exhibit a more modest and sometimes inconsistent DNT cell elevation. The authors and others have observed mildly elevated DNT cell levels in children who do not fulfill other criteria of ALPS or who have other primary immunodeficiencies such as DiGeorge syndrome (Jack Bleesing, MD, PhD, personal communication, 2007). Thus establishing age-dependent normal ranges for DNT cells in diagnostic flow cytometry laboratories will aid in interpreting borderline DNT cell elevation. Given this confusion, it is not clear whether patients who have ALD, who reportedly lack DNT cell expansion but who otherwise fulfill criteria for ALPS, actually have a forme fruste of ALPS [26,27]. Supporting this possibility, mice with conditional Fas deletions in B cells or dendritic cells lacked DNT cell expansion but developed

other markers of ALPS (ie, lymphoaccumulation, hypergammaglobulinemia, and autoimmunity) [33,34]. Alternatively, ALD may represent a related but separate entity having a genetic defect distinct from ALPS.

Defective lymphocyte apoptosis

Because the clinical features of this disease stem from defective apoptosis, testing for this functional abnormality remains a sine qua non for diagnosing ALPS. T cells are activated and cycled in IL-2 to render them susceptible to apoptosis. Alternatively, herpesvirus saimiri– or Epstein-Barr virus–immortalized T- or B-cell lines can be tested, although any defects should be confirmed in primary lymphocytes [13,35]. Although initial studies demonstrating an apoptotic defect tested death in response to TCR restimulation, results are more reproducible when cells are stimulated directly through the Fas death receptor (also known as CD95/APO1) [28,36]. Induction of cell death is tested after overnight stimulation with cross-linked APO1.3 antibodies [15]. The numbers of living cells excluding the dye propidium iodide are counted using a flow cytometer. Treated samples are compared with untreated samples to calculate the percentage of cell loss. A difference in percentage of cell loss (defined as [1-(number live cells in presence of apoptotic stimulus/number of live cells in absence of apoptotic stimulus)] $\times$ 100) exceeding 50% compared with normal, healthy controls indicates an apoptosis defect [15]. Lesser but reproducible differences still may indicate a milder apoptosis defect.

The standard killing assay using anti-Fas agonistic antibodies to trigger Fas signaling reveals defects in most patients who have ALPS, including disease caused by germline Fas mutations or caspase-10 mutations, which together account for up to approximately 65% of ALPS cases. To reveal apoptotic defects caused by Fas ligand (FasL) mutations, killing of a Fas-expressing cell line by blasts of T-cells from the patient (which have been activated to express FasL) can be tested [37]. Alternatively, killing of the Fas-expressing cell line by cells transfected with mutant FasL constructs is measured [38,39]. When results of the first-line apoptosis screening are normal, the authors assess IL-2 withdrawal–induced death in primary T cells after washing cells extensively and resuspending them in fresh media supplemented without, as compared to IL-2 [40]. Whereas death can be seen after a day of anti-Fas stimulation of normal T cells, IL-2 withdrawal–induced death takes about 3 days to become evident. Research testing is available to assess pathways of apoptosis induced by other stimuli such as γ-irradiation or certain drugs.

A diagnostic limitation of apoptosis testing is that it can be assessed reliably only in activated, cycling T cells. Approximately 2% of patients who have ALPS have somatic Fas mutations that are found consistently only in the DNT cells [41,42]. These cases are problematic, because DNT cells cannot be maintained in culture. Despite their expansion in vivo, DNT cells do not respond to stimuli that normally activate or cause proliferation of

conventional T cells [10,41,43,44]. Thus, upon initial TCR stimulation, conventional T cells expand to take over the culture, but DNT cells do not [41]. Most of these DNT cells are not mutated and have no apoptotic defect, producing a readout of apparent normal apoptosis. In all cases, apoptosis can be evaluated properly only if cells are proliferating well. Lymphocytes that do not cycle well may not have received appropriate signals needed to render them susceptible to propriocidal death [3]. Therefore, in the setting of immunodeficiency, apparent apoptotic defects should be interpreted with caution.

Finally, apoptotic defects also can be measured in dendritic cells isolated from patients who have ALPS [32]. Dendritic cells function as potent antigen-presenting cells for T cells, and recent studies in mice indicate that impaired dendritic cell apoptosis contributes to increased lymphoproliferative and autoimmune responses [34,45]. This finding suggests a similar contribution to disease pathogenesis in patients who have ALPS.

Supporting diagnostic tests

Both histopathology and cytokine profile are distinct for ALPS, but these features generally correlate with the extent of peripheral blood DNT cell expansion [46]. Concern for lymphoma often prompts biopsy of enlarged lymph nodes. Histopathology reveals a characteristic paracortical expansion marked by increased DNT cells, accompanied by follicular hyperplasia and plasmacytosis [46]. These early lymphocyte expansions are nonmalignant and differ from the lymphomas that can develop later in some patients who have ALPS. DNT cell infiltration also can be seen in both splenic and liver histopathology [46]. In some cases, lymph node histology also can display parafollicular histiocyte proliferation with emperipolesis suggestive of sinus histiocytosis with massive lymphadenopathy [22]. Patients who have ALPS also have increased serum IL-10, which largely results from increased production by the expanded DNT cells, as well as a skewed T-helper type 2 cytokine profile [47–49].

Elevated serum cobalamin (vitamin B_{12}) can be used as an additional screening test for ALPS [1,50]. Although serum B_{12} also is increased with liver disease, myeloproliferative disorders, hypereosinophilic syndrome, and metastatic cancers, accompanying signs and symptoms help differentiate these diseases from ALPS.

Genetic testing

Given the technical demands of lymphocyte apoptosis assays and the limited availability of this testing outside of specialized laboratories such as at the NIH, gene sequencing has been increasingly used to diagnose ALPS as well as other diseases. Genetic analysis requires understanding that the human genome normally exhibits considerable sequence variability. Variants existing at a population frequency of 1% or less are designated as mutations, and those occurring at a population frequency greater than 1% are designated as polymorphisms. The discovery of a mutation in

a patient suggests but does not necessarily mean that the mutation is involved with disease. Furthermore, some polymorphisms can be functional, meaning that they can influence disease. Molecular studies must demonstrate that any given DNA sequence alteration significantly changes the coding sequence or its level of expression and that these changes deleteriously affect cellular function for disease phenotype. Thus, when using genetic analysis as a stand-in for apoptosis assays, it is important to determine whether any given sequence alteration is meaningful. Doing so requires reviewing previously published literature or databases for evidence establishing the pathogenicity of a polymorphism.

For ALPS, more sophisticated analyses also may be required to reveal somatic mutations when the mutated cells make up less than 20% of the peripheral blood mononuclear cell population [41]. Such analysis entails physically isolating DNT cells by fluorescence or magnetic cell-sorting technologies before preparing genomic DNA for sequencing. Diagnostic algorithms have been proposed to incorporate this step where clinical suspicion for ALPS remains high despite normal apoptosis testing (see earlier discussion) [42,51].

Prognosis and treatment

In ALPS, autoimmune and lymphoproliferative disease can follow a variable course but often improve over a span of decades [9,28]. For severe autoimmune disease, a corticosteroid pulse (methylprednisolone, 5–30 mg/kg intravenously) can help control autoimmune disease rapidly. This pulse is followed by a daily course of oral prednisone (1–2 mg/kg) for up to several months [15]. Patients also may benefit from simultaneous treatment with intravenous immunoglobulin (1–2 g/kg) for hemolytic anemia or thrombocytopenia, or granulocyte colony-stimulating factor (1–2 μg/kg subcutaneously once daily or three times a week) for neutropenia [15]. Patients whose autoimmune disease recurs after tapering and stopping corticosteroids may need to be kept on low doses every other day. For long-term maintenance, mycophenolate mofetil (approximately 600 mg/m^2 orally, twice daily) can be used to avoid side effects of corticosteroids or splenectomy [52,53]. If this treatment is not successful, cytotoxic or biologic agents such as cyclophosphamide, azathioprine, vincristine, anti-thymocyte globulin, or rituximab (375 mg/M^2/wk for 4 weeks) have been tried on a case-by-case basis [15,54,55]. Rapamycin can reduce autoimmune disease, lymphadenopathy, and splenomegaly in *lpr* mice but has not yet been tested in patients who have ALPS [56]. Allogeneic bone marrow transplantation has been used as a last resort for severe intractable disease caused by homozygous Fas mutations, but matched unrelated-donor transplants are associated with considerable morbidity and mortality [57–59].

Lymphoproliferative disease can be disfiguring but by itself usually is not treated, because it tends to recur after discontinuing immunosuppressants

(eg, corticosteroids, mycophenolate mofetil, cyclosporine A, or azathioprine) [15]. Despite initial promising case reports, pyrimethamine, with or without sulfadoxine, has no efficacy in shrinking lymph nodes or spleen or in controlling autoimmune disease either in patients who have ALPS or in MRL-*lpr* mice [60]. Although hypersplenism increases the risk for splenic rupture, it generally is not treated. When hypersplenism contributes to chronic refractory severe cytopenias, splenectomy may be considered, but splenectomy increases risks for postsplenectomy-associated pneumococcal sepsis and death [15]. Before splenectomy patients should receive immunizations to *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*, and titers should be used to guide postsplenectomy re-immunizations [15]. Splenectomized patients need to be maintained on lifelong amoxicillin or fluoroquinolones for pneumococcal prophylaxis, and patients developing significant fevers should be treated for potential bacteremia [15].

Although lymphoproliferation is benign initially, approximately 10% of patients who have type 1A ALPS develop lymphoma [8]. Patients are at 51-fold increased risk for developing Hodgkin's lymphoma at a median age of 11 years and a 14-fold increased risk for developing non-Hodgkin's lymphoma at a median age of 21 years. The average age for diagnosis of ALPS is 5 years and for diagnosis of lymphoma is 28 years [61]. The increased predisposition may result from differential signaling thresholds for heterozygous Fas mutations, with continued Fas-dependent signaling promoting growth despite impaired apoptosis induction [62]. Lymphomas are primarily of B-cell origin but can also be of T-cell origin and display diverse histologic types [61]. Most cases of ALPS-associated lymphoma have developed in patients who have type 1A ALPS, bearing intracellular death domain mutations in Fas; no patients who have FasL or caspase-10 mutations have yet developed lymphoma. Therefore, lymphomas are characteristic of type 1A but not necessarily of types IB, II, or III ALPS; however, the number of patients is too small for a definitive assessment. By contrast, the ALPS patient who had neuroblastoma ras sarcoma oncogene homolog (*NRAS*) mutation (ALPS type IV) had childhood leukemia and developed non-Hodgkin's lymphoma at age 32 years [40]. Because of the propensity for developing lymphoma, lymph nodes should be monitored carefully over time. Such monitoring can be accomplished effectively by periodic CT scans or ultrasound [9]. Suspicious lymph nodes should be biopsied for clonality and chromosomal studies. Positron emission tomography to assess lymphoma-associated increased glucose uptake also can aid in distinguishing lymphoma from the benign lymphadenopathy in ALPS [63]. ALPS patients do respond to conventional treatment for lymphomas, probably because the lymphomas exhibit no loss of heterozygosity and remain sensitive to other apoptosis inducers, although they retain resistance to Fas-induced apoptosis [15,61].

Finally, in vitro studies suggest that rat sarcoma viral oncogene homolog (*RAS*) antagonists such as farnesyltransferase inhibitors might be useful in treating ALPS type IV [40]. These experimental agents currently are being

tested against cancers that bear somatic mutations in *KRAS* and *NRAS*. Studies testing these agents in ALPS will become feasible as additional patients are identified.

Genetics and molecular mechanisms

ALPS can be classified by the underlying genetic mutation (Table 1) [1]. Most mutations affect components of the death-inducing signaling complex (DISC), and understanding mechanistically how the DISC works is key to understanding how these mutations exert their effects (Fig. 1) [6]. In brief, FasL and Fas death receptors each exist as preformed homotrimers. When FasL engages Fas, Fas recruits the Fas-associated death domain (FADD) adapter molecule, which in turn recruits initiator procaspase-8 or -10. Assembly of these components into the DISC occurs through homotypic interactions of death domains (for Fas and FADD) or death effector domains (for FADD and procaspase-8/10). The fully assembled DISC oligomerizes procaspase-8/10, a step required for caspase-8/10 activation. The caspases then cleave each other, converting themselves into a highly stable, soluble form that can reach effector caspases. When effector caspases are cleaved and activated, they in turn cleave downstream substrates to cause cell death. Thus, the DISC temporally and spatially converts extracellular signals received through death receptors into an intracellular proteolytic cascade that degrades essential cellular components.

ALPS type IA results from mutations in the Fas death receptor [13,36,64]. More than half of these mutations occur in exon 9, within the death domain that is important for FADD binding (see ALPSbase at http://research.nhgri.nih.gov/ALPS/). Affected individuals possess heterozygous mutations, which exert a dominantly interfering effect on apoptosis induction [65]. For this effect to occur, an extracellular region of Fas called the "preligand assembly domain," which directs Fas self-association, must be intact [66]. The defective protein poisons the receptor, which has three Fas chains; the presence of even one bad chain compromises further

Table 1
Autoimmune lymphoproliferative syndrome classification by gene mutation

ALPS type	Gene mutation	Results of apoptosis testing: Fas-induced apoptosis	Results of apoptosis testing: IL-2 withdrawal–induced apoptosis
IA	*TNFRSF6* (FAS)	defective	normal
IB	*TNFSF6* (FASLG)	defective when patient cells used as stimulus to kill Fas-expressing cell line	normal
I_m	somatic *TNFRSF6* (FAS)	normal	normal
II	*CASP10*	defective	normal
IV	*NRAS*	normal	defective
III	unknown	defective to at least one stimulus	

ARIS
DISC
MHC:peptide
CD28
TCR
PDK1
PKCθ
BCL10
MALT1
CARMA1
IKK complex
NF-κB
ACTIVATION
DD
DED
CARD
FAS ligand
ALPS type IB
FAS
ALPS types IA, Im
FADD
procaspase-8
CEDS
caspase-8 (or caspase-10)
Bid
Apaf-1
caspase-3
APOPTOSIS
procaspase-10
ALPS type II
Cyt.c
caspase-9
Apoptosome
Bim
Bim
Bim
cytokine withdrawal
X
ALPS type III
?
NRAS
ALPS type IV
RAF
MEK
ERK

DISC assembly. Defective recruitment of DISC components leads to impaired oligomerization, which prevents activation of initiator caspases and interrupts signal propagation [65]. In some cases, Fas mutations outside the death domain may not affect DISC assembly but may hinder the effective formation of higher-order signaling protein oligomerization transduction structures further downstream [67]. These structures contribute to optimal caspase activation, probably by increasing their local concentration for activation and cleavage. In a minority of cases, heterozygous, compound loss-of-function Fas mutations may exert their effects through haploinsufficiency [68,69]. In general, mutations in the Fas death domain are more severe in terms of functional apoptotic defects and their clinical consequences [70]. Additionally, homozygous Fas mutations with Fas deficiency, sometimes termed "ALPS type 0," are associated with more severe disease [71,72].

A variant of ALPS type 1, termed "type I_m" (for mosaic), has heterozygous somatic Fas mutations only in the DNT population of blood cells [41,42]. In any individual patient, the mutation apparently arises in a committed hematopoietic stem cell such that the DNTs and perhaps a proportion of other hematopoietically derived cells also may carry the mutation, but the

Fig. 1. Autoimmune lymphoproliferative syndrome (ALPS) and caspase-8 deficiency state (CEDS) illustrate signaling pathways for lymphocyte apoptosis and activation. In the death-inducing signaling complex (DISC) (*center of figure*), death receptor stimulation, here typified by trimerized Fas, leads to recruitment of Fas-associated death domain (FADD) adapter molecules, which in turn recruit the initiator caspase-8 (and/or caspase-10). Oligomerization causes caspase-8/10 activation and cleavage of downstream effector caspases. Caspase-8 cleavage of BID, a proapoptotic member of the B-cell leukemia/lymphoma 2 (BCL-2) family, feeds into a mitochondrial amplification loop in which apoptosome formation oligomerizes and activates caspase-9. During cytokine withdrawal–induced apoptosis (*left side of figure*), interruption of interleukin (IL)-2-stimulated signaling leads to decreased activation of the RAF/MEK/ERK pathway. This decrease causes increased expression of BIM, another proapoptotic member of the BCL-2 family, which in turn triggers mitochondrial depolarization. Cytochrome c (cytc) released from depolarized mitochondria assembles with apoptotic protease activating factor 1 (Apaf-1) and caspase-9 to form the apoptosome. Both the death receptor–induced and cytokine withdrawal–induced apoptosis pathways converge following activation of initiator caspases: these cleave and activate downstream effector caspases, which in turn cleave intracellular substrates, causing cell death. In the activation receptor–induced signalosome (ARIS) (*right side of figure*), immunoreceptor stimulation leads to caspase-8–dependent recruitment of the CARMA1-BCL10-MALT1 (CBM) complex with the inhibitor of κB (IKK) complex. Caspase-8 linker and enzymatic function are required for optimal downstream nuclear factor-kappa B (NF-κB) induction for lymphocyte activation. Mutations in the indicated signaling components that cause ALPS or CEDS are indicated. Death domains (DD), death effector domains (DED), and caspase recruitment domains (CARD) in relevant proteins containing these structures are as indicated. BCL10, B cell CLL/lymphoma 10; BID, BH3 interacting domain death agonist; BIM, Bcl-2 interacting mediator of cell death; ERK, extracellular signal regulated kinase; FADD, Fas (TNFRSF6)-associated via death domain; MALT, mucosa associated lymphoid tissue lymphoma translocation gene 1; MEK, mitogen-activated protein kinase (MAPK) kinase; MHC, major histocompatibility complex; NRAS. neuroblastoma rat sarcoma viral oncogene homolog; PDK1, 3-phosphoinositide-dependent kinase 1; TCR, T cell receptor; PKC, protein kinase C; RAF, rapidly accelerated fibrosarcoma.

DNT cells are the only population in which all cells are mutated. In patients who have type I_m ALPS, lymphocytosis may be limited to DNT cells, and certain flow cytometric features such as increased HLA-DR expression on T cells may be absent [41,42]. Patients who have type I_m ALPS, however, display salient features of lymphadenopathy and splenomegaly, characteristic histopathology, DNT cell expansion, increased IL-10 production, hypergammaglobulinemia, and gradual improvement of disease with age. Some patients also have autoimmune cytopenias. Together, these associations suggest a key role for DNT cells in disease pathogenesis.

Patients who have type IB ALPS have mutations in FasL. The first individual identified with a FasL mutation (an 84–base pair deletion in exon 4) had SLE, with lymphadenopathy, splenomegaly, and defective TCR-restimulation–induced apoptosis, but no DNT cell expansion [37]. Two subsequent individuals fulfilling diagnostic criteria for ALPS have homozygous (A247E) or heterozygous FasL (R156G) mutations, which affect the extracellular portion thought to participate in FasL trimerization or Fas binding, respectively [38,39]. The latter patient also has unexplained common variable immunodeficiency (CVID), with hypogammaglobulinemia and granulomatous infiltration of lymph nodes, spleen, and liver.

Patients who have ALPS type II have mutations in caspase-10 [1]. Two ALPS patients have heterozygous I406L mutations [73], and one has a heterozygous L285F mutation [32], both in the large enzyme subunit. Interestingly, one of the two patients who has I406L mutation also fulfills diagnostic criteria for SLE, and the patient who has L285F has anti-nuclear autoantibodies (more commonly associated with SLE than with ALPS). These caspase-10 mutations dominantly interfere with apoptosis, as demonstrated when introduced by transfection into normal T-cell lines, as does L285F when introduced into dendritic cells [32,73]. By contrast, the roles in disease pathogenesis of a number of other caspase-10 variants identified in patients who have ALPS (or ALD) are less clear [27,32,69,73,74]. An earlier study suggested V410I mutations were pathogenic, based in part on experiments in epithelial cells demonstrating dominant interference [32]. This interference did not occur when T-cell lines were used, however, and reanalysis indicates that V410I is a polymorphism found at a frequency of approximately 5% to 10% in the normal population [73,74]. Another variant, Y446C, exhibited decreased apoptotic function when overexpressed in caspase-8/10–deficient T cells but not in wild-type T cells, suggesting that Y446C may act as a functional polymorphism depending upon the genetic context [73]. A possible example may be a patient who has ALPS and who has both the Y446C variant and a predicted haploinsufficient Fas mutation [69]. Additionally, a recently identified patient who has ALPS has a known haploinsufficient Fas mutation and a caspase-10 P501L mutation [69]. The P501L mutation exhibited decreased caspase-10 activity but no dominant interfering effect when introduced into epithelial cells [69]. The mother carried the P501L mutation, but not

Fas mutation, and her T cells also displayed an apoptotic defect; however, she herself was healthy. Overall, these studies indicate that the caspase-10 I406L and L285F mutations clearly cause ALPS, whereas the V410I and Y446C polymorphisms might contribute to but are not sufficient for disease. Identifying additional patients will help clarify the pathogenicity of the P501L mutation.

ALPS type IV is a new designation that has been proposed for ALPS patients who have *NRAS* mutations [1,40]. Unlike ALPS type I and II, this form of ALPS lacks Fas death receptor–induced apoptosis abnormalities but instead manifests a specific apoptosis defect triggered by IL-2 deprivation. This defect reflects the use of a different upstream pathway for apoptosis induction following cytokine withdrawal (see Fig. 1) [1,6]. Here, pro- and antiapoptotic sensors of the B-cell leukemia/lymphoma 2 (BCL2) family, especially the proapoptotic member Bcl-2 interacting mediator of cell death (BIM), control mitochondrial stability. The balance of these factors determines whether mitochondria become depolarized, leading to formation of a mitochondria-derived complex called the "apoptosome" that in turn promotes caspase activation for cell death. To date, one patient has been identified with ALPS type IV, which is caused by a germline heterozygous G13D gain-of-function mutation in *NRAS* [40]. This mutation decreases intrinsic GTPase activity by keeping bound RAS proteins in an active GTP-bound state. Hyperactivation of the mitogen-activated protein kinase (MAPK) (rapidly accelerated fibrosarcoma [RAF]/MAPK kinase [MEK]/extracellular signal regulated kinase [ERK]) pathway suppresses induction of the BIM apoptosis regulatory protein, which in turn impairs apoptosis induced upon cytokine withdrawal [40]. Although this patient clearly fulfilled all three diagnostic criteria for ALPS, he also had several atypical features, including a history of childhood leukemia, lymph node histology showing sinus histiocytosis without prominent DNT cell expansion, and no increased T cells expressing HLA-DR or CD57 by flow cytometry [40].

Patients who have ALPS type III have defects in apoptosis but lack mutations in Fas, FasL, caspase-10, caspase-8, or FADD [1]. Approximately 33% of patients who have ALPS are classified as having type III [39]. ALPS type III probably represents a heterogeneous group of patients with different molecular etiologies. It is not known whether any of these patients have somatic mutations in genes other than *Fas*. Also, there is a range of patients who have the ALPS phenotype but who have modest or no apoptosis defects; this group is classified as having "ALPS spectrum," which is not yet understood at the molecular level.

Although ALPS can be caused by single gene mutations (as discussed previously), it is clearly a complex human disease with variable disease penetrance and severity [28,39]. Several factors contribute to this variability. First, a strong determinant is the mutation and its location. Disease is more likely to occur and to be more severe when intracellular (especially death domain) rather than extracellular *Fas* mutations are present [29,70].

For instance, lymphoma develops primarily in patients who have ALPS with FADD mutations and in a few who have intracellular mutations but does not develop in patients who have extracellular mutations [61]. Other family members who bear identical mutations in death receptor components may display apoptotic defects but manifest no disease or incomplete disease [28,75]. Moreover, inbred genetic background affects disease expression in the *lpr* or *gld* murine counterparts of ALPS types IA or IB in humans [21]. Together, these observations suggest the contribution of other genetic modifiers.

Potential genetic modifiers include those that participate in various death pathways and also those that contribute to coexisting autoimmune and inflammatory conditions. In ALPS (or ALD), perforin, tumor necrosis factor receptor (TNFR) type I, pyrin, and osteopontin have been implicated as genetic modifiers. Perforin is an essential component in granule-mediated cytotoxic death for eliminating virally infected cells and has been suggested to compensate at least partially for Fas deficiency in T-cell death during repeated antigenic stimulation [76]. Thus, alterations in genes involved in cytotoxic granule-mediated, Fas-mediated, TCR-restimulated, or other pathways of apoptosis may cooperatively influence disease phenotype. Consistent with this possibility, certain perforin variants (N252S, A91V) were more highly represented in patients who had ALPS or ALD than in the general population, although the number of patients was too small for statistical significance, and the effects on perforin function were controversial [77–79]. Similarly, several patients who had ALPS were discovered also to have variants in genes known to be associated with autoimmune or inflammatory conditions. The TNFR type I R92Q variant is a polymorphism found at higher frequency in patients who have TNFR-associated periodic fever syndrome or rheumatoid arthritis than in the general population; it is therefore considered to be functional, although the mechanism by which it contributes to disease still is unclear [80]. TNFR1 R92Q has been found in several patients who have ALPS, including one who also has a homozygous caspase-10 V410I polymorphism [6]. The pyrin E148Q functional polymorphism associated with familial Mediterranean fever also was discovered in a patient who had ALPS who had a caspase-10 polymorphism [6]. Certain polymorphisms of osteopontin, a cytokine secreted during chronic inflammatory and autoimmune conditions, were seen more often in patients who had the ALPS variant, ALD [81]. In contrast, other genes, such as HLA alleles or CTLA-4 polymorphisms, which are highly associated with certain autoimmune diseases, have shown no relationship to ALPS [6]. It is likely, however, that additional genetic modifiers will be found that influence ALPS disease penetrance and severity.

In summary, approximately 60% to 70% of patients who have ALPS have known monogenic causes for ALPS. These causes include Fas, FasL, caspase-10, and *NRAS*. ALPS, however, is clearly a complex disease, and its penetrance and severity probably are influenced by many genes that

influence different pathways of programmed cell death and contribute to other coexisting autoimmune or inflammatory conditions.

Caspase-8 deficiency state

Clinical features

A brother and a sister who had CEDS were identified with a distinct clinical entity that prominently features combined lymphocyte immunodeficiency [82]. Unlike patients who have ALPS, who lack primary immunodeficiency, these patients who have CEDS have recurrent sinopulmonary infections, with mild bronchiectasis in one patient. Both patients also have recurrent mucocutaneous herpesvirus infections with Bell's palsy. They have hypogammaglobulinemia and make poor antibody responses to pneumococcal polysaccharide antigens. Although they possess normal numbers of lymphocyte subsets, their B cells, T cells, and NK cells do not activate well to stimuli including antigen receptors and various immunoreceptors [82,83].

Counterintuitively, the immunodeficiency of CEDS is accompanied by lymphoaccumulation, autoimmunity, and impaired lymphocyte apoptosis resembling that seen in ALPS. Both patients have lymphadenopathy and mild splenomegaly, which have improved gradually as they have entered early adulthood. They exhibited direct Coombs-positive antibodies with a compensated mild hemolytic anemia, as well as anti-thyroid antibodies without thyroid disease. One patient has lupus anticoagulant with prolonged partial thromboplastin time but no history of thrombotic disease. DNT cell numbers have been normal or borderline-high, in a range that is not clearly interpretable at the present time (see previous discussion).

These patients who have CEDS have several other clinical features distinct from ALPS. Although they have NF-κB activation defects (as discussed later), they lack certain features characteristic of immunodeficiency patients who have mutations in nuclear factor-kappa B (NF-κB) essential modulator (NEMO) (also known as inhibitor of NF-κB, kinase, gamma subunit [IKKγ]) or in NF-κB inhibitor, alpha (IκBα). Both patients have ichthyosis. This condition is more typical than hypohidrotic ectodermal dysplasia in patients who have NEMO (Maria Turner, MD, personal communication, 2005). Neither CEDS patient has ectodermal dysplasia or abnormal teeth. Both patients also have multiple splenic cysts consistent with isolated splenic peliosis, which have remained stable over years. The presence of this extremely rare finding of unknown etiology in both CEDS patients suggests a role for caspase-8 in mesenchymal or vascular development.

Given the limited number of humans who have CEDS and the potential modulation of disease spectrum by genetic background, the phenotypes of mice rendered genetically deficient in caspase-8 can be informative. Although complete deficiency in mice is embryonically lethal, [84] in humans it is compatible with embryonic development, postnatal survival, and

successful maternal pregnancy. Conditional deletion of caspase-8 in T or B cells results in immunodeficiency resembling that in humans [85,86]. The mice also develop an ALPS-like condition with lymphadenopathy, splenomegaly, but without DNT cell expansion or autoantibodies, and with an unusual lymphocyte infiltration of lungs, liver, and kidneys [87]. These findings indicate that lymphocyte immunodeficiency is a consistent feature of caspase-8 deficiency, whereas the ALPS-like features of lymphoaccumulation, autoimmunity, and DNT cell expansion are variable.

Diagnosis

The differential diagnosis for CEDS includes ALPS, CVID, Wiskott-Aldrich syndrome, and NEMO or IκBa mutations. CVIDs, especially those caused by transmembrane activator and calcium-modulator and cyclophilin ligand interactor (*TACI*) mutations, frequently are associated with benign lymphoproliferation and autoimmune disease affecting the hematopoietic system [88]. Wiskott-Aldrich syndrome features thrombocytopenia with small platelets, autoimmune disease, and variable immune defects [89]. Antibody levels and function can be decreased, as well as T-cell and NK-cell function. NEMO or IκBa mutations variably impair B-, T-, and NK-cell function but often can be distinguished by characteristic skin or dental abnormalities [90].

Clinical features suggesting CEDS should be investigated by immunologic studies assessing serum immunoglobulin levels, antibody function, and lymphocyte activation. Lymphocyte apoptosis testing reveals defects but must be performed carefully to avoid potentially confounding factors of impaired lymphocyte proliferation. For these reasons, CEDS is diagnosed more easily by caspase-8 gene sequencing.

Prognosis and treatment

The prognosis is uncertain. Patients who have CEDS generally have done well while maintained on intravenous immunoglobulin and prophylactic acyclovir to decrease sinopulmonary infections and mucocutaneous herpesvirus outbreaks. Lymphoaccumulation seems to improve gradually with age, but mice conditionally deficient in caspase-8 within T cells developed lethal lymphocytic infiltrative disease of the lungs, liver, and kidneys when they got older [87]. Lymphoma is a potential complication, based on the authors' experience with patients who have ALPS. They have seen no evidence of either lymphocytic infiltrative disease or lymphoma in the CEDS patients described, however.

Genetics and molecular pathogenesis

Disease is inherited in an autosomal recessive manner [82]. The two reported patients, who are siblings, have consanguineous homozygous C → T

transitions that cause R248W loss-of-function mutations in caspase-8 [82]. The substitution lies in the large enzyme subunit, which affects an extended loop structure that normally stabilizes substrate when bound. The mutations render the caspase-8 protein enzymatically inactive and unstable, leading to functional caspase-8 deficiency [82]. Heterozygous carriers are healthy and lack immune-function abnormalities [82].

Caspase-8 deficiency impairs lymphocyte activation because of failure to induce the gene transcription factor NF-κB when various immunoreceptors are stimulated. These include antigen receptors on T and B cells, as well as innate immune receptors such as toll-like receptors -2, -3, and -4 on B cells or Fc gamma receptor type III (FcγRIII) (also known as CD16) found on NK cells [83,86]. By contrast, NF-κB activation is normal in response to the cytokine tumor necrosis factor-α in T cells, or CD40 ligand in B cells [83]. NF-κB typically is found in the cytosol, complexed to IκBα. Stimulation through certain immunoreceptors activates the inhibitor of κB kinase (IKK), leading to IκBα degradation. This process frees NF-κB to translocate to the nucleus, where it binds to NF-κB DNA–binding motifs in promoters to turn on transcription of various genes. In the case of antigen receptors, caspase-8 functions to link IKK with an upstream signaling complex. This linkage occurs in what is termed the "activation-receptor induced signalosome" (see Fig. 1) [83]. The adapter function of caspase-8, as well as its enzymatic activity, is required for optimal IKK activation and downstream NF-κB activation. The specific defect, elicited by certain immune stimuli, is consistent with the less severe and more immune-restricted clinical phenotype of patients who have CEDS as compared with patients who have NEMO or IκBα mutations.

Caspase-8 also normally participates in an entirely different signaling complex, the DISC (see Fig. 1) [6]. As discussed earlier, upon death receptor ligation, oligomerization of caspase-8 in this complex renders it enzymatically active and capable of cleaving various cellular substrates upon release from the DISC. Thus, caspase-8 deficiency blocks the initiating events necessary to turn on the caspase cascade that is crucial for propagation of signaling for cell death. In this context, it is not surprising that patients who have caspase-8 mutation have defective apoptosis and ALPS-like clinical features.

Other immunodeficiencies with apoptosis abnormalities

Associations with increased basal apoptosis of lymphocytes have been reported for virtually all primary immunodeficiencies, but how specific gene mutations responsible for these immunodeficiencies mechanistically affect apoptosis is not understood adequately. Some articles also have reported apoptosis defects but lack careful assessment to exclude confounding factors such as impaired cell cycling that may exist with immunodeficiencies.

Immunodeficiencies characterized by concomitant immune dysregulation with lymphoproliferative features may reflect impaired apoptosis. One such

disorder is XLP, which features lymphoproliferation including B-cell lymphoma, hypogammaglobulinemia, and a fulminant and fatal infectious mononucleosis, often associated with impaired 2B4-dependent NK-cell and CD8 T-cell cytotoxicity against cells infected with Epstein-Barr virus [7]. Most cases are caused by mutations in *SH2D1A* (SAP), an adapter molecule that interacts with the signaling lymphocyte activation molecule family of immunoreceptors. Although the precise function of this molecule is still being elucidated, the authors recently have discovered that, as in SAP-deficient mice, *SH2D1A* is required for TCR restimulation–induced apoptosis in humans [91,92]. A profound decrease in TCR restimulation apoptosis with unabated immune activation may explain the lymphoproliferative burst in XLP. Thus, XLP now joins ALPS and CEDS (impaired death receptor or cytokine-withdrawal apoptosis) and familial hemophagocytic lymphohistiocytosis and related disorders (with impaired perforin/granzyme-mediated killing) in a group of immune homeostasis disorders featuring impaired programmed cell death.

Summary

Humans who have ALPS have defective lymphocyte apoptosis leading to lymphoid organ enlargement, autoimmune disease, and lymphoid malignancy. These manifestations result from impaired DISC formation caused by mutations in Fas, FasL, or caspase-10. Recently, an ALPS patient was discovered who has a cytokine withdrawal–induced apoptotic defect caused by a gain-of-function mutation in *NRAS* that blocks BIM induction. Genetic defects in apoptosis also occur in primary immunodeficiencies that share clinical features with ALPS. Patients who have CEDS caused by mutations in the caspase-8 gene have impaired lymphocyte activation as well as impaired apoptosis. The immunodeficiency is caused by failure to form a caspase-8–dependent NF-κB gene transcription factor signaling complex distinct from the DISC. Additionally, a SAP-specific defect in antigen receptor restimulation–induced apoptosis can contribute to disease pathogenesis in XLP. These primary immunodeficiencies highlight molecular mechanisms by which signaling for lymphocyte activation and apoptosis are regulated coordinately.

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Chromosome 22q11.2 Deletion Syndrome: DiGeorge Syndrome/ Velocardiofacial Syndrome

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Velocardiofacial syndrome, DiGeorge syndrome, and a variety of other clinical syndromes have a hemizygous deletion of chromosome 22q11.2 as their cause. This deletion syndrome is extremely common, with nearly 1 in 3000 children being affected. This pattern of malformations was recognized as early as 1671, when Nicolai Stensen described a patient with cleft palate and truncus arteriosus. In 1829 L. H. Harrington described a patient with a hypoplastic thymus and hypoplastic parathyroid glands. In the modern era, Eva Sedlackova described a syndrome of velopharyngeal insufficiency and developmental delay, and David Lobdell reported a patient with a hypoplastic thymus and hypoplastic parathyroid glands in 1959. "DiGeorge syndrome" came to be named in 1965 when Angelo DiGeorge described the common embryologic derivation of the heart, thymus and parathyroid glands as the explanation for their joint malformation in patients, and "velocardiofacial syndrome" was named in 1978 by Robert Shprintzen. Despite the extensive history of this disorder, the management of patients remains a challenge. The complex medical care of these patients requires a multidisciplinary approach, and each patient has unique clinical features, requiring a tailored approach. This article focuses on the immune system, but patients require a holistic approach to their needs.

"Chromosome 22q11.2 deletion syndrome" is a term applied to patients who have a hemizygous deletion of chromosome 22q11.2. Approximately 90% of patients carrying the clinical diagnosis of DiGeorge syndrome and 80% of patients carrying the clinical diagnosis of velocardiofacial syndrome

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carry the hemizygous deletion. The deletion also has been demonstrated in additional patients who have coloboma, heart anomaly, choanal atresia, retardation, and genital and ear anomalies (CHARGE syndrome), conotruncal anomaly face syndrome, and cat eye syndrome. There can be confusion in the nomenclature, because there are patients who have the deletion who do not fall into the category of a clinically defined syndrome, and there are patients who have DiGeorge syndrome who do not carry the deletion. The term ''DiGeorge syndrome'' historically has referred to patients who have a cardiac anomaly, hypocalcemia, and poor T-cell production. In this article, the term ''chromosome 22q11.2 deletion syndrome'' is used when describing studies of patients known to have the deletion, and specific syndromic nomenclature is used when discussing studies that relied on clinical features.

Chromosome 22q11.2 deletion syndrome is estimated to occur in nearly 1 in 3000 children, and patients typically are born with a conotruncal cardiac anomaly and a mild to moderate immune deficiency (Table 1) [1–11]. Developmental delay, palatal dysfunction, and feeding problems also are seen in most of these infants. A frustrating and as yet incompletely understood aspect of this syndrome is the enormous phenotypic heterogeneity.

Diagnosis

The deletion arises as a result of an aberrant meiotic exchange event caused by low copy number repeats that bracket the commonly deleted region [12]. Most deletions are spontaneous, and from epidemiologic studies the spontaneous mutation rate is estimated to be between 1 in 4000 and 1 in 6000. The deletion of chromosome 22q11.2 is at least 10-fold more common a spontaneous deletion than the next most frequent human deletion syndrome, suggesting that the low copy number repeats in this region lead to more substantial genomic instability.

The estimate of an incidence of 1 in 3000 births is derived from the spontaneous mutation rate plus the growing number of familial cases. Before the mid-1980s patients who had severe cardiac anomalies did not survive. Now there is a large cohort of adults who have these anomalies, and they are raising their own families. The hemizygous deletion is inherited in an autosomal dominant fashion; thus affected parents have a substantial risk of passing on the deletion to a child.

Identification of an affected infant or an older child in the absence of a family history relies on the recognition of a single feature seen commonly in patients who have the deletion, such as interrupted aortic arch, or a combination of features that individually are not strongly predictive of a deletion but in aggregate raise the suspicion (Table 2).

The diagnosis currently relies on the fluorescence in situ hybridization (FISH) method, which is extremely accurate but time-consuming and expensive. Efforts to develop a rapid polymerase chain reaction–based method are underway and soon may yield a commercial test [13–15]. Certain patients

Table 1
Clinical findings in patients who have chromosome 22q11.2 deletion syndrome

Finding	Percentage of patients affected
Cardiac anomalies	49–83
Tetralogy of Fallot	17–22
Interrupted aortic arch	14–15
Ventriculoseptal defect	13–14
Truncus arteriosus	7–9
Hypocalcemia	17–60
Growth hormone deficiency	4
Palatal anomalies	69–100
Cleft palate	9–11
Submucous cleft palate	5–16
Velopharyngeal insufficiency	27–32
Bifid uvula	5
Renal anomalies	36–37
Absent/dysplastic	17
Obstruction	10
Reflux	4
Ophthalmologic abnormalities	7–70
Tortuous retinal vessels	58
Posterior embryotoxon (anterior segment dysgenesis)	69
Neurologic	8
Cerebral atrophy	1
Cerebellar hypoplasia	0.4
Dental: delayed eruption, enamel hypoplasia	2.5
Skeletal abnormalities	17–19
Cervical spine anomalies	40–50
Vertebral anomalies	19
Lower extremity anomalies	15
Speech delay	79–84
Developmental delay in infancy	75
Developmental delay in childhood	45
Behavior/psychiatric problems	9–50
Attention deficit hyperactivity disorder	25
Schizophrenia	6–30

Data from [1–11].

who have classic features but no evidence of a deletion by FISH can represent a diagnostic dilemma. Point mutations in the T-box 1 gene (*TBX1*) [16,17], a very small deletion not detected by standard FISH, or a non–chromosome 22 basis could account for the clinical features. In particular, deletions of chromosome 10, mutations in the chromodomain helicase DNA-binding protein gene *CHD7*, and prenatal exposure to teratogens such as isotretinoin or glucose should be sought as potential explanations [18–22]. In spite of best efforts, no clear etiologic basis currently can be found in some patients who have a clinical picture of DiGeorge syndrome. This issue is a clinically significant, because the risk of recurrence in these kindreds is not known.

Table 2
The frequency of the chromosome 22q11.2 deletion in various patient populations

Phenotypic featuren	Frequency of deletion (%)
Any cardiac lesion	1.1
Conotruncal cardiac anomaly	7–50
Interrupted aortic arch	50–60
Pulmonary atresia	33–45
Aberrant subclavian	25
Tetralogy of Fallot	11–17
Velopharyngeal insufficiency	64
Velopharyngeal insufficiency postadenoidectomy	37
Neonatal hypocalcemia	74
Schizophrenia	0.3–6.4

The genetic basis of the phenotype

Within the commonly deleted region of chromosome 22q11.2, there are more than 35 genes. A continuing question is which of the genes within the deleted region contribute to the phenotype. A series of clever LoxP Cre deletions were made in mice mimicking the deletions in humans and uncovered the transcription factor *TBX1* as the most likely gene contributing to the cardiac phenotype [23–26]. The murine models have revealed two surprising features. All embryos with a deletion show abnormalities of the branchial arch, which is the precursor to the heart and thymus, but only a subset of mice have cardiac anomalies at birth. This ability to recover from the early branchial arch artery defect is intriguing and raises the question of whether an in utero intervention could be developed to mitigate the effects of the deletion. The second surprise was the magnitude of the effect of the background genes [27]. A parathyroid or thymus phenotype was not seen initially. When the deletion mice were backcrossed onto other strains, the parathyroid and thymic phenotypes were more obvious. Data on background gene effects are difficult to identify in humans. Series of patients from the United States and Europe agree largely on the phenotypic manifestations, but patient cohorts from Chile and China have slightly different profiles that could represent ascertainment bias or true phenotypic differences related to background gene effects [28,29]. Studies of multiplex kindreds, in which background gene effects would be expected to be minimized, show significant phenotypic heterogeneity, suggesting that background genes contribute to the phenotype. Other factors, however, are probably substantial source of variability.

Based on the murine studies, haplosufficiency for *TBX1* seems to be the major determinant of cardiac, thymus, and parathyroid phenotypes, probably because of the role *TBX1* plays in the development of branchial arch structures. *TBX1* contributes to the endodermal proliferation in the branchial arches, with haplosufficiency leading to compromised formation,

particularly of the arch arteries. Secondary effects are seen because *TBX1* drives the expression of fibroblast growth factors that contribute to the growth of surrounding cells and also regulates the expression of myogenic growth factors that are important for branchiomeric muscle development. Thus, haplosufficiency for the transcription factor *TBX1* leads to direct compromise of branchial arch structures and has a wealth of secondary effects. Patients who have chromosome 22q11.2 deletion syndrome also have a variety of malformations that do not map to branchial arch structures. Central nervous system changes such as developmental delay and psychiatric problems are common, and skeletal anomalies and renal anomalies are seen also—features that are difficult to attribute to dysfunction in branchial arch development. *TBX1* is expressed somewhat in the developing brain mesoderm and in the sclerotome that gives rise to various structures in the spinal column [30]. Although its role in these sites is not understood, haplosufficiency in these regions may contribute to the other phenotypic features. Confirming the importance of *TBX1* in brain development, patients who have a mutation in *TBX1* have the same developmental delay phenotype as patients who have the deletion [16].

Because the role of *TBX1* primarily involves embryologic development, interventions directed at preventing or treating the biologic effects of haplosufficiency probably would need to be instituted in utero. Recent advances in understanding the regulation of *TBX1* have led to the possibility of regulating its expression through the retinoic acid pathway. Isotretinoin exposure causes a syndrome with similarities to chromosome 22q11.2 deletion syndrome [31]. Retinoic acid is known to be a repressor of *TBX1* expression [32]. Manipulation of this pathway could normalize levels of *TBX1* in haplosufficient babies if detected early enough. There also is intense interest in identifying modifier genes, either within the deleted region or in background genes, with the hope that these genes could provide a framework for developing meaningful interventions [33,34]. Recently vascular endothelial growth factor was identified as a modifier of the cardiac phenotype [34].

Management—overview

There are few prospective studies to support a specific management style for patients who have chromosome 22q11.2 deletion syndrome. A coordinated approach to the many subspecialty needs and a recognition that patient phenotypes and outcomes vary tremendously are essential. Most patients receive their diagnosis shortly after birth because of the presence of a cardiac anomaly. In infants, an approach to identify medical problems such as cardiac anomalies, hypocalcemia, severe immunodeficiency, or intestinal malrotation, which could lead to severe morbidity, should be instituted as soon as possible. Feeding problems can compromise development, and frank nutritional compromise delays healing and contributes to defects in

host defense [35]. The toddler years require attention to development and speech; the school-age years require additional attention to cognitive development and growth. Behavioral issues are more likely to become a problem with increasing age, and frank psychiatric disorders are seen in teenagers and adults. Fig. 1 gives a sense of the dynamic nature of the needs of patients who have chromosome 22q11.2 deletion syndrome. Education of parents regarding the future potential requirements of the patient is difficult because of the variations among patients and the current inability to predict psychiatric needs of patients.

Management—neonatal

Cardiac anomalies are seen in approximately 75% of all patients who have chromosome 22q11.2 deletion syndrome and are the major cause of death. An early initial echocardiographic evaluation is important for infants diagnosed shortly after birth, because not all cardiac anomalies are obvious. For patients who require early cardiac surgery, there are several questions regarding management from the immunologist's perspective. Low T-cell numbers are seen in 75% to 80% of infants who have chromosome 22q11.2 deletion syndrome. In most cases this decrement is mild or moderate and does not impact the procedure. It is not known whether postoperative infections are increased in this population. Less than 1% of patients who have the deletion lack T cells, but these patients represent a special category at the time of cardiac surgery [5]. These patients require protection from infection and blood products. Blood products can induce graft-versus-host disease in patients without T cells, and graft-versus-host disease from transfusions almost always is fatal. Definitive therapy for patients lacking T cells is discussed later.

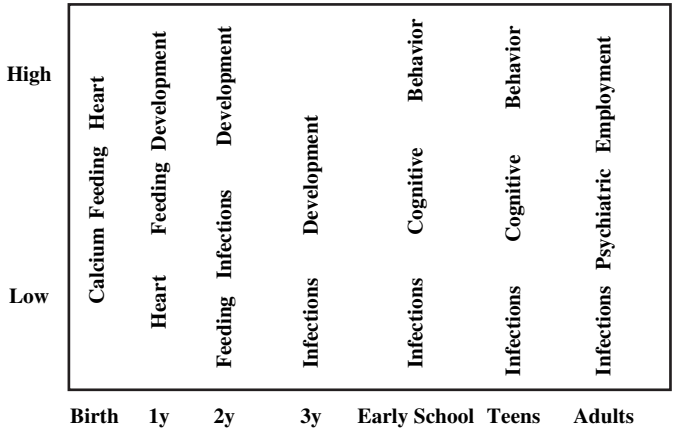

Fig. 1. Changing needs of patients who have chromosome 22q11.2 deletion syndrome. The y-axis indicates the level of attention.

Often patients require cardiac surgery before definitive information regarding the immune system is available. Many large centers in the United States irradiate all blood products provided to infants less than 1 year of age to prevent graft-versus-host disease. Another strategy is to stratify risk according to the absolute lymphocyte count as a surrogate for a T-cell count, but this strategy lacks both specificity and sensitivity.

Hypocalcemia often presents in the neonatal period and is exacerbated by cardiac surgery. Calcium supplementation typically is sufficient in the immediate postoperative period. Persisting hypocalcemia requires management by an endocrinologist to balance vitamin D requirements, calcium, and phosphorus. Oversupplementation leads to nephrocalcinosis, and a balance is required. Most children do not require prolonged calcium supplementation, although rare cases of late onset or recurrence of hypocalcemia have been described.

Feeding issues can be extremely difficult for parents in early infancy. Feeding and swallowing problems seem to arise from poor coordination of the pharyngeal muscles, tongue, and esophageal muscles [35]. Patients who have cardiac defects also might have shortness of breath, leading to poor feeding, and breastfeeding is known to be difficult for patients who have palatal clefting. Thus, many variables may contribute to poor feeding.

Management—development

Speech is one of the most troubling aspects for most parents, because communication is fundamental to bonding. Defects in phonation, language development, and comprehension are fairly common [36]. Phonation problems can be caused by laryngeal webs, velopharyngeal insufficiency, or vocal cord paralysis. Surgery can correct these abnormalities, but phonation typically remains problematic [37,38]. Speech is more delayed than receptive skills, and social language skills typically are even more delayed. Early receptive language skills seem to correlate with overall function [39]. This pattern of skills and weaknesses is almost unique to patients who have chromosome 22q11.2 deletion syndrome [40]. Optimal management for speech delay is not known. Advocates of sign language believe that the ability to communicate is critical and that signing allows the child to progress developmentally [40,41]. Advocates of speech therapy believe that signing delays language acquisition [36]. There have been no direct comparisons of the two methods, and parents who have used both strategies report satisfaction with the method. Most patients learn to speak and communicate effectively.

The mean full-scale IQ is approximately 70, with a range from normal to moderately disabled [1,4,7,42]. Visuoperceptual abilities and planning tend to be the weakest areas [4,43]. This pattern of nonverbal learning disability is not unique to chromosome 22q11.2 deletion syndrome and is seen in other syndromes that involve developmental delay. In fact, learning disability

occasionally is the only manifestation of chromosome 22q11.2 deletion syndrome [44]. Successful school-based interventions have been developed for children who have nonverbal learning disabilities and typically are used for patients who have chromosome 22q11.2 deletion syndrome, although no interventions targeted for this specific group of patients have been developed.

The central nervous system manifestations of chromosome 22q11.2 deletion include structural defects such as microcephaly and functional aspects such as attention deficit hyperactivity disorder, poor social interaction skills, impulsivity, and bland affects [45–48]. Ten percent to 30% of older patients experience bipolar disorder, autistic spectrum disorder, or schizophrenia/schizoaffective disorder. Psychiatric disorders are common in all patients who have developmental delay, but there is a significant increase in psychiatric disturbances in this syndrome.

Management—immunodeficiency

Most patients who have chromosome 22q11.2 deletion syndrome have diminished T-cell numbers as a consequence of thymic hypoplasia. Approximately 20% of the patients have no evidence of diminished T cells [49], and less than 1% have true thymic aplasia requiring a transplant [5]. Most patients have mildly or moderately diminished circulating T cells. The next two subsections describe management for both the common patients who have a mild or moderate decrement in T-cell numbers and the rare patient who has complete thymic aplasia.

Management—mild or moderate immune deficiency

Children who have chromosome 22q11.2 deletion syndrome and a mild to moderate decrement in the T-cell count have largely normal immunoglobulin levels and T-cell proliferative responses [49,50]. T-cell numbers decline with age in all children, but the decline in patients who have chromosome 22q11.2 deletion syndrome seems to be slower. In fact, adults who have chromosome 22q11.2 deletion syndrome have normal T-cell numbers for the most part. The slower age-determined decline in T-cell numbers in patients compared with controls is caused by the homeostatic proliferation of existing T cells. The initial clue that homeostatic expansion occurs was a study describing the differences in naive and memory T cells in an early childhood population [51]. Early changes in the T-cell repertoire also were seen, and, although mild, they indicated that the T-cell compartment was altered by the early lymphopenia. A study of adults confirmed that the changes in naive and memory T cells progress further with aging, as do the defects in repertoire (deletions, oligoclonality) [52,53]. Telomere length was found to be shorter even within the naive T-cell population in patients [53]. Based on these findings, compromise in T-cell function would be expected in adult patients. A compromised repertoire would restrict the ability

of the T cells to respond to pathogens, and shortened telomeres would be expected to compromise the proliferative ability of T cells in response to infection. There are few data from adult patients addressing this issue, but modest defects in function have been seen in children. The T-cell receptor rearrangement excision circles (TREC) count, a marker of proliferative history, was found to correlate with proliferation of memory T cells, suggesting that extensive shortening of telomeres compromises T-cell function as measured by proliferation [54]. Supportive of the studies documenting compromised proliferative ability in at least a subset of cells is the finding that spontaneous apoptosis is increased in patients compared with controls [55]. Because cells with short telomeres undergo spontaneous apoptosis, this finding may reflect the inherent limitations of the T cells. Despite these findings, cytokine production is normal, and global proliferative ability is intact in children [54,56].

The humoral immune system has been examined in several studies. Although the humoral immune system is largely intact in patients who have chromosome 22q11.2 deletion syndrome, as one would expect for a defect in thymic development [57], there are data demonstrating infrequent humoral dysfunction. It is an uncommon finding in the patients who have the deletion, but the frequency is clearly greater than in the general population. IgA deficiency, impaired responses to vaccines, and frank hypogammaglobulinemia have been described [58–60]. A pattern of more severe infection correlated with immunoglobulin abnormalities [58,59].

Compared with an HIV population with similar T-cell counts, patients who have the deletion have much better immunologic function. Opportunistic infections are very infrequent [5], the most common infection being an upper respiratory tract infection. The frequency of these infections does not correlate with T-cell counts, suggesting anatomy may be the major contributor to upper respiratory tract infections [61]. Nevertheless, there are long-term clinical consequences of thymic hypoplasia. Autoimmune disease is significantly increased, with juvenile rheumatoid arthritis and hematologic autoimmune diseases being the most common [49,62–64]. Idiopathic thrombocytopenia purpura is the most common of the autoimmune diseases, although platelet size and number are slightly aberrant at baseline in most patients who have the deletion [33]. Celiac disease was recently described in 1 of 48 patients who had chromosome 22q11.2 deletion, which seems to be increased over the frequency in the general population [65]. The mechanism underlying the susceptibility to autoimmune disease is not well established, but homeostatic expansion selects for self-reactive or low-affinity T cells. A decrease in regulatory T cells also has been seen [66] and could contribute to the predisposition to autoimmunity. An increase in allergic diseases also is seen in chromosome 22q11.2 deletion syndrome, contributing to the infection pattern, and this predisposition to allergy also may be related to the homeostatic expansion because T-helper type 2 differentiation seems to be the default pathway in homeostatic proliferation in mice [67].

Clinical studies show that most patients do not demonstrate a susceptibility to opportunistic infections. Concordantly, the risk of using live viral vaccines in infants seems to be low except for patients who have thymic aplasia and/or very low T-cell counts [68]. Both the measles, mumps, rubella and the varicella vaccine were found to be safe and efficacious in children who had the deletion and who had mild to moderate T-cell compromise [69,70]. It would not be appropriate to give live viral vaccines to patients who have severe T-cell compromise.

Management—thymic aplasia

Patients who have true thymic aplasia and absent T cells represent a very specific group. The genetic etiologies are slightly different, with chromosome 22q11.2 deletion found in approximately half of these patients [71]. A spectrum of T-cell counts is seen in thymic aplasia, ranging from a T-cell count of zero to a normal T-cell count (see discussion below). These features may make it difficult to identify patients who require a transplant.

A thymus transplant, fully matched peripheral blood transplant, or donor lymphocyte infusions are required for patients who have thymic aplasia. It is not always clear at which point a thymus transplant or a fully matched T-cell transplant would be appropriate. An evaluation of the naive T-cell count in early infancy can be used to estimate the potential for thymic production of T cells, but the counts can change substantially over a few months. The two interventions with data to support them are a thymic transplant and a fully matched transplant of T cells (so that thymic education is not required) [72,73]. For a thymus transplant, the donor thymic tissue is harvested and cultured to ensure that mature T cells capable of causing graft-versus-host disease have been eliminated [74]. Thin slices of the cultured thymus are implanted in the quadriceps muscle. Although partial HLA matching is desirable, it is not necessary [75]. Functional T cells appear approximately 3 to 4 months after transplantation, and the T-cell repertoire after transplantation is normal initially, suggesting that the graft is capable of supporting normal T-cell development [76]. The implanted thymus involutes rapidly and does not sustain prolonged production of T cells, but sufficient numbers are produced to provide adequate host defense, and patients do well clinically [71]. Follow-up studies will define the long-term fate of the patients who have undergone transplantation.

Approximately one third of infants who have thymic aplasia caused by DiGeorge syndrome or chromosome 22q11.2 deletion syndrome have a dramatic oligoclonal expansion of a few founding T cells [77]. In this setting, the T-cell counts do not reflect the adequacy of the T-cell compartment because they are expanded from a very small number of functional T cells. Fortunately, there are several clues to this phenomenon. Often the infants have erythroderma, similar to that seen in Omenn's syndrome. The T cells are predominantly or almost exclusively of a memory phenotype (CD4/CD45RO

or TREC negative), and the repertoire is oligoclonal [77,78]. Repertoire and TREC studies are not widely available, but the combination of an almost exclusively memory T-cell phenotype and erythroderma should lead to the suspicion of either Omenn's syndrome or oligoclonal expansion in the setting of thymic aplasia.

Summary

The immunologist typically is the care coordinator for patients who have DiGeorge syndrome or chromosome 22q11.2 deletion syndrome. If not the coordinator, the immunologist often is one of the first clinicians to discuss the syndrome with the family. A clear understanding of the multidisciplinary needs as well as a strategy to prioritize urgent needs is valuable in the infancy period, when many issues may arise. Anticipatory guidance is valuable for the family, and a comprehensive approach to the consequences of the immune deficit can improve the quality of life markedly.

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Common Variable Immunodeficiency: An Update on Etiology and Management

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Common variable immunodeficiency (CVID) is the commonest symptomatic primary immunodeficiency and represents a heterogeneous group of primary antibody deficiency disorders that on the whole do not yet have an identified molecular or genetic basis. Patients who have CVID are prone to recurrent infections primarily affecting the respiratory tract and gut, although other atypical presentations and unusual organisms have been reported. In addition, a significant proportion of patients also manifest features of immune dysregulation, including autoimmune disease and granulomatous inflammation, as well as malignant disease. Although the principal defect is thought to result in failure of B-cell differentiation and antibody secretion, multiple abnormalities in almost all other components of the immune system have been described.

Janeway and colleagues [1] have been credited with the first description of CVID in 1953. In more recent years, significant advances in elucidating some of the underlying genetic defects and molecular mechanisms in CVID have contributed to the understanding of human immunology and are likely to have implications beyond the scope of primary immunodeficiency diseases. Clinical care of these patients has also improved with attempts to classify these patients more accurately to help guide prognosis, better treatment modalities, and a push toward greater awareness of the condition and the need for early diagnosis to prevent complications.

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The exact prevalence of CVID is unknown, although estimates of between 1:10,000 and 1:50,000 have been made [2]. CVID has been defined clinically by the presence of recurrent infection and a reduction in IgG (of at least 2 SD below the mean) and at least one other immunoglobulin isotype, as well as by a failure to mount a significant specific antibody response to challenge with vaccination or natural infection [2,3]. For diagnosis, other known causes of hypogammaglobulinemia—genetic or acquired—must be excluded [3].

The disease occurs equally in both genders, and onset of symptoms can occur at any age, with peaks in the first and third decades [4]. There usually is a significant interval of 4 to 9 years from onset of symptoms to diagnosis [4–6], potentially contributing to increased morbidity and poorer outcomes. CVID usually is sporadic, although familial clustering has been documented in approximately 10% of patients [7]. In addition, it has also been noted that IgA deficiency occurs in family members of patients who have CVID [8], consistent with the observation that IgA deficiency can progress to CVID [9].

Genetic advances

There has been intensive research to identify genetic defects in CVID. To date, mutations in five genes have been identified that are associated with a CVID phenotype. Not surprisingly, all are molecules involved in some element of B-cell biology. The genetic mutations identified to date result in defective inducible costimulator (ICOS) on T cells [10], transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI, encoded by *TNFRSF13B*) [11,12], CD19 [13], and B-cell activating factor receptor (BAFF-R, encoded by *TNFRSF13C*) [14] on B cells; as well as a deficiency in *MSH5* [MutS homolog 5 (E. Coli)], a gene encoded in the major histocompatibility (MHC) class III region that is involved in regulating meiotic homologous recombination and also contributes to class switch recombination [15].

Inducible costimulator deficiency

ICOS is a member of the CD28 family of immunoglobulin-like costimulatory surface molecules and is expressed on activated T cells. One of its roles is in the superinduction of interleukin (IL)-10, which is necessary for terminal B-cell differentiation into memory and plasma cells [16]. ICOS binds to ICOS ligand (ICOS-L), which is constitutively expressed on antigen-presenting cells, including naive B cells [17]. This signaling pathway has a significant role in T-helper cell activation as well as providing B-cell help for T-dependent antibody responses [16].

ICOS deficiency was first reported in humans in 2003 [10]. To date, nine individuals from four families have been identified with the same

homozygous mutation, which contains a deletion affecting exons 2 and 3 resulting in a frameshift and truncated protein of 28 amino acids [18]. It is thought that all four families are descended from a common founder and either migrated along the Danube River or are linked by the House of Habsburg which ruled from Graz to Freiburg between the 1500s to the 1700s, because all the affected patients have the same homozygous haplotype at the D2S2289 locus near the *ICOS* gene [18].

Patients who have ICOS deficiency have reduced immunoglobulin levels as well as reduced B-cell numbers, particularly in the IgM memory and switched memory B-cell subsets, suggesting abnormalities in T-dependent B-cell maturation in germinal centers and further confirming the role of ICOS in late B-cell differentiation, class-switching, and memory B-cell development [10]. Interestingly during infection, patients can mount IgM responses.

Transmembrane activator and calcium-modulator and cyclophilin ligand interactor deficiency

TACI belongs to the tumor necrosis factor receptor superfamily (TNFRSF) and is part of a group of TNFRSF receptors on B cells that play important roles in B-cell survival, development, and antibody production [19]. Other members in this group are B-cell maturation antigen (BCMA) and B-cell activating factor receptor (BAFF-R). The ligands for TACI and BCMA are B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL). TACI, similar to other members of the TNFRSF, requires ligand-induced trimerization for effective intracellular signaling via binding to tumor necrosis factor (TNF)-associated factors (TRAFs) 2, 5, and 6, resulting in nuclear factor-kappa B (NF-κB) and Jun amino terminal kinase activation [20]. Intracellular signaling also occurs through calcium-modulator and cyclophilin ligand (CAML) which up-regulates calcineurin and activates nuclear factor of activated T cells transcription factor (NF-AT) dephosphorylation and nuclear translocation [21].

In murine models, TACI deficiency results in increased B cells and autoimmunity, with systemic lupus erythematosus-like features and lymphoproliferation [22]. The human phenotype seems to be different, however. In 2005, two separate groups identified mutations in TACI resulting in either CVID or IgA deficiency [11,12]. For CVID, there is a complex pattern of inheritance with homozygous, heterozygous, and compound heterozygous mutations identified. Mutations were described in the extracellular (C104R, S144X), transmembrane (A181E), and intracellular (S194X, R202H, Ins204) portions of the molecule [11,12]. Both the homozygous and heterozygous mutations were associated with antibody deficiency, suggesting autosomal dominant and recessive forms of inheritance. Data accumulated from screening a large cohort of more than 500 patients suggest that TACI mutations are present in 8% to 10% of patients who have CVID [23].

The role of heterozygous mutations in the pathogenesis of CVID is less clear, however. Subsequent studies published after the original discovery of TACI mutations in patients who had CVID demonstrated that unaffected family members could carry the same TACI mutation as their affected relatives [24]. In addition, another large study showed that the C104R and A181E (but not the R202H) mutations occurred with greater frequency in patients who had CVID than in the general population [25,26]. These findings raised the possibility that TACI mutations (at least in the heterozygous state) might be disease-susceptibility rather than disease-causing genes.

Although there is a suggestion that TACI mutations predispose the bearer toward autoimmunity and lymphoid hyperplasia in CVID [24], this possibility has not been clearly borne out in other large studies in which there have been no clear genotype–phenotype correlations [23]. Further molecular studies will be required to determine exactly how TACI mutations influence the clinical phenotype.

CD19 deficiency

CD19 is a cell-surface molecule present on mature B cells that forms a co-receptor complex with CD21, CD81, and CD225 and serves to reduce the signaling threshold following antigen recognition by the B-cell receptor [27]. In addition, this co-receptor complex plays a role in linking the innate and adaptive immune system. The CD21 component of this complex can bind C3d bound to antigen, linking complement recognition to the CD19 signaling function [28]. Human CD19 deficiency has been described in four patients with a CVID phenotype from two unrelated consanguineous families [13]. All patients presented in childhood with recurrent infections and were found to have low immunoglobulin levels. One patient had a single–base pair (bp) insertion resulting in a frameshift and premature stop codon in the proximal region of the intracellular domain. The other three patients had a homozygous 2-bp deletion, also causing a frameshift and premature stop codon resulting in the deletion of a large portion of the intracellular domain. Patients had normal numbers of B cells in the periphery but reduced numbers of CD5+ and memory B cells. Germinal center formation was normal, but patients had poor secondary antibody responses to rabies vaccination. These data suggested that homozygous CD19 deficiency resulted in relatively normal B-cell development but poor responses to antigen, without any significant features of autoimmunity.

B-cell activating factor receptor deficiency

In view of the discovery of TACI mutations and the understanding of the role of BAFF and APRIL in B-cell development, defects were sought in the BAFF-R. To date, only a single patient, a 60-year-old man, has been

identified with a defect. The findings have been presented only in abstract form [14]. A 24-bp homozygous deletion was found in exon 2, which codes for the transmembrane portion of the receptor. On B-cell phenotyping, there was a block at the transitional B-cell stage, consistent with the role of the BAFF–BAFF-R axis in peripheral B-cell survival.

MSH5 mutations

MSH5, a gene encoded in the MHC class III region and known to have a role in homologous recombination in meiosis, was found also to play a part in class switch recombination [15]. In MRL/lpr mice with a congenic H-$2^{b/b}$ MHC interval, there were low levels of *MSH5* expression and abnormalities in class switch recombination, including a profound IgG3 deficiency and long microhomologies at Ig switch (S) joints. Subsequent genotyping of *MSH5* in 420 patients who had IgA deficiency and 370 patients who had CVID identified several nonsynonymous single-nucleotide polymorphisms. These were present in greater frequency in IgA deficiency (C580G, L85F/P786S, rs3131378) and CVID (Q292H, rs3131378) [15]. With the L85F/P786S allele, *MSH5* was shown to have reduced binding affinity to MSH4, its heterodimerization partner. In addition, patients who had CVID with heterozygous nonsynonymous *MSH5* polymorphisms had Sμ-Sα1joints with increased microhomology, a reduced mutation rate, and increased in-phase alignment of pentamer repeats at junctions, similar to those seen in the mouse phenotype. Immunoglobulin deficiencies were not observed in controls with heterozygous *MSH5* polymorphisms, although there were some subtle changes in the S-joint phenotype. Although the study did identify an increased frequency of certain alleles in CVID and IgA deficiency, it was difficult to be certain if these mutations were pathogenic in themselves or merely conferred disease susceptibility [15].

Immunopathology

In more than 90% of patients who have CVID, no genetic defect has been identified, although various abnormalities have been described affecting both the innate and the adaptive immune systems [29–42]. It is likely that at least some of these abnormal findings represent epiphenomena rather than being pathogenic.

Innate immune dysfunction

Older studies have described abnormalities in monocytes in patients who have CVID [29]. More recently, several groups have shown in vitro abnormalities in monocyte-derived dendritic cells [30–32], although their findings have not been consistent, reflecting the heterogeneity of the underlying

disease. Subsequently, it was demonstrated that the numbers of myeloid and plasmacytoid dendritic cells are reduced in patients who have CVID [33]. Signaling defects in the Toll-like receptor 9 pathway in plasmacytoid dendritic cells and B cells also have been reported [34]. In view of the help provided by dendritic cells for T-cell activation, their direct role in B-cell support, and their secretion of soluble factors such as BAFF and APRIL [43], the data so far suggest that defects in the innate immune system might account for some cases of CVID.

Adaptive immune dysfunction

Significantly more abnormalities have been described in the adaptive immune system. Various T-cell defects, including reduced proliferation following stimulation with antigen and mitogens [4], impaired cytokine production [35], failure to generate antigen-specific T cells following vaccination [36], reduced expression of cell surface molecules (CD40L, attractin) [37,39], and an increased rate of T-cell apoptosis [38], have been described. More recently, elevated levels of serum IL-7, a cytokine involved in homeostatic proliferation of lymphocytes, have been found in a subgroup of patients who have CVID, with increased numbers of circulating CD8+ T cells with decreased apoptosis [40]. These patients had a higher incidence of splenomegaly and autoimmunity, and there was a poor in vitro response to IL-7 stimulation (in terms of proliferation, interferon-γ, and transforming growth factor-β secretion), suggesting a disordered IL-7 feedback loop.

Recent work on T-cell receptor signaling has shown defects in Vav, a cytoplasmic guanine nucleotide exchange factor for Rho-family GTPases. Previously, defective recruitment and activation of zeta-chain–associated protein kinase 70 (ZAP-70) caused by impaired CD3ζ phosphorylation had been shown [41]. Subsequently, in these patients, reduced Vav1 mRNA levels were found, resulting in defective f-actin polymerization and CD28/T-cell receptor (TCR) up-regulation of lipid rafts [42]. Mutations in Vav and its promoter were not found, and it remains to be seen if the lack of Vav expression results in failure of ZAP-70 recruitment or a common defect lies upstream of both.

Classification schemes

In view of the heterogeneity present in CVID, various efforts have been made to develop classification schemes to divide patients into different groups to help facilitate research as well as to offer further information on prognosis. Most of the classification schemes have focused primarily on B cells, although one group recently has published a potential classification scheme using T cells [44].

They investigated multiple phenotypic and functional T-cell parameters in 60 patients who had CVID, including absolute T-cell counts, naive

T cells, activated T cells, thymic output, apoptosis studies, cytokine production, CDR3 spectratyping, and differential gene expression. On the basis of their study, they were able to divide patients who had CVID into three groups based on the numbers of naive T cells. Reductions in naive T-cell numbers reflected the severity of abnormalities in the multiple parameters they investigated [44]. The patients who had the lowest numbers of naive T cells had increased T-cell activation, proliferation, apoptosis, and disruption of TCR repertoires and had severe immunodeficiency with splenomegaly. Those who had normal levels of naive T cells had mild clinical symptoms with no/mild splenomegaly. The final group represented an intermediate phenotype. Naive CD4+ T-cell levels also were found to correlate with the switched memory B-cell classification by Warnatz and colleagues [45], although concordance did not exceed 58.8%.

Most of the prior work on classifying patients who had CVID had looked at B-cell phenotype. One of the earlier classifications by Bryant and colleagues [46] divided CVID patients into three groups based on the ability of peripheral blood lymphocytes to produce immunoglobulins on stimulation with *Staphylococcus aureus* Cowan I plus IL-2 or anti-IgM plus IL-2. The patients in group A failed to produce any immunoglobulin in vitro; those in group B were able to produce only IgM; and those in group C were similar to healthy individuals. This method of classification was not widely adopted, however, because it was time consuming to perform and did not always correlate with the clinical phenotype.

Subsequently, two groups published CVID classification systems based on flow cytometric phenotyping of memory B cells (defined by the presence of CD27 and lack of IgD/M to determine isotype switch) [45,47]. These techniques originally were done using separated lymphocytes but subsequently were modified for use of whole blood, making them easier to use in a routine laboratory context [48]. Generally, the patients with the lowest proportion of switched memory B cells had the most severe clinical phenotype. There were some differences in the methods, however. Warnatz and colleagues [45] used memory B cells as a percentage of peripheral blood lymphocytes and had an additional subdivision based on the quantity of immature CD21 B cells, whereas Piqueras and colleagues [47] used memory B cells as a percentage of total B cells. There also were some differences as to which complications occurred significantly more frequently in the groups with the lowest numbers of memory B cells. In addition, there are patients who have CVID who have virtually no B cells and who probably have an early defect in B-cell maturation [49]; these patients were not included in these schemes.

In view of these differences and the small number of patients in both those studies, a large European trial with 303 patients subsequently was undertaken to unify the existing schemes and improve classification [50]. Based on the results, the authors proposed an improved scheme (EUROClass) which separately classified patients who had nearly absent B cells ($< 1\%$ of lymphocytes), severely reduced switched memory B cells ($< 2\%$ of total

B cells) and expansion of transitional B cells (> 9% of total B cells) or increased $CD21^{low}$ B cells (> 10% of total B cells). Those who had absent B cells represented an early B-cell defect; those with reduced switched memory B cells represented a germinal center defect, and those with increased transitional or $CD21^{low}$ B cells have defects that remain to be explicated. Splenomegaly and granulomas were found more commonly in patients who had reduced switched memory B cells and in those who had elevated $CD21^{low}$ B cells, whereas lymphadenopathy was more common in patients who had elevated transitional B cells.

Although there is a degree of correlation with memory B-cell phenotype, the current classification schemes are relatively imprecise with regard to the clinical phenotype, because complications can occur in all groups of patients and because the monogenetic defects of *ICOS* and TACI/*TNFRSF13B* do not fall into only one category. Further work remains to be done, and it might be that a combined T- and B-cell scheme, reflecting the various abnormalities seen in both compartments in CVID, might provide better segregation of patients and their complications.

Clinical aspects

Infections

Recurrent infections are the most frequent complication of CVID. Sinopulmonary infection is seen most commonly in these patients, occurring in up to 98% of patients in one series [4]. Gastrointestinal infection particularly with *Giardia lamblia*, *Campylobacter jejuni*, and *Salmonella* species is relatively common as well. As a whole, opportunistic infections with *Pneumocystis jiroveci* and atypical mycobacteria are rare. Patients who have CVID handle most viral infections without problems but have a particular predisposition to several unusual infections, which include mycoplasma infection of the joints [51] and enteroviral meningoencephalitis [52].

Bronchiectasis and chronic respiratory tract infections

The long-term sequelae of repeated respiratory tract infection—chronic sinusitis, hearing loss, and bronchiectasis—are the major substrate of morbidity and (along with lymphoma) mortality in antibody-deficient patients [4]. In a recent CVID cohort, bronchiectasis affected about a third of patients at baseline, with another 12.2% developing this feared complication during follow-up despite appropriate treatment [6]. Chronic lung disease at diagnosis is a strong predictor of early mortality, whereas early diagnosis and timely intervention predict good outcome [53].

Different subsets of patients identified with flow cytometric memory B-cell phenotyping seem to be prone to the development of lung disease. Patients who have reduced levels of IgM memory B cells [54] and/or switched memory B cells [55] may be at greater risk of respiratory infection and its sequelae.

Interstitial lung disease, granulomata, and lymphoproliferation

Patients who have CVID may develop inflammatory disease characterized by tissue infiltration with polyclonal lymphocytes. The lungs, liver, spleen, and gut are the most common target organs, but skin, renal, ophthalmic, and neurologic involvement are well described. Lung involvement is manifested as interstitial lung disease; the described histologic variants of CVID-associated interstitial lung disease include a granulomatous pattern (also known as "granulomatous lymphocytic interstitial lung disease," GLILD), lymphocytic interstitial pneumonitis, lymphoid hyperplasia, and follicular bronchiolitis [56]. Because these patterns may co-exist on biopsy sample [56,57], they may represent variants of the same disease. The granulomatous pattern is observed most frequently, in 8% to 22% of patients [4,56,57], often with multisystem involvement (granulomatous disease has been described in lymph nodes, spleen, liver, parotid glands, meninges, and bone marrow [58]), and is associated with increased morbidity and poor prognosis [56]. In addition, in one cohort of patients with granulomatous lung disease, two of nine patients developed frank B-cell lymphomas [59]. High-resolution CT (HRCT) findings include mediastinal lymphadenopathy; multiple ill-defined parenchymal nodules, usually with bronchocentric distribution; ground-glass opacities; and interseptal lines [60–62]. The presence of normal pulmonary function tests does not exclude the diagnosis, but in the absence of more generalized granulomatous disease, treatment generally is not indicated in this setting.

The etiology of granulomatous disease is unclear. T-cell dysregulation has been proposed to contribute [58], possibly arising from chronic viral infection; human herpesvirus 8 (HHV8) in the lung [59] and cytomegalovirus in the gut [63] have been implicated, but the data are inconclusive. The HHV8 study is striking, in that six of nine CVID patients who had GLILD were HHV8 positive, compared with 1 of 21 patients who did not have GLILD [59]; to date, however, this study has not been replicated elsewhere.

Gut complications

Helicobacter pylori infection is commonly found in patients who have CVID and may account for the high frequency of chronic gastritis found in almost a third of patients [6]. Because *H pylori* infection is associated with gastric carcinoma and lymphoma [64], it would seem prudent to conduct routine screening and eradication therapy as appropriate.

In addition, about 20% of patients who have CVID have gut symptoms without an infectious cause [65]. Symptoms of CVID enteropathy range from mild discomfort, bloating, and diarrhea to more severe profuse diarrhea, malabsorption, and weight loss. CVID enteropathy differs from Crohn's disease in having normal C-reactive protein levels and a different T-helper type 1 cytokine profile with excess IL-12 but not IL-23 [66]. It is unclear if CVID enteropathy is caused by genetic or environmental factors, but gut inflammation has been found in asymptomatic patients who have

CVID, and the incidence of diarrhea increases with time [6], suggesting that CVID enteropathy is a progressive condition.

There are two types of enteropathy, one exclusively affecting the large bowel and the other predominantly affecting small bowel with malabsorption [67]. In the former, patients present with frequent watery stools but no systemic effects. One published report of a patient with the large-bowel form of CVID colitis had a positive cytomegalovirus test (by polymerase chain reaction of large-bowel biopsy), which responded to ganciclovir and infliximab [63]. The small-bowel form of CVID enteropathy is more severe, because malabsorption and weight loss can be marked. Some workers have considered this entity to be Crohn's disease, because the ileum often is involved, and stricturing can occur [68]. Although small-bowel biopsy often shows villous atrophy and crypt hyperplasia, similar to findings in celiac disease, CVID enteropathy does not improve with gluten-free diet [69]. Patients often respond to budesonide and prednisolone, but steroid dependence can increase with time. Recently infliximab has been used in three such patients who responded with benefit in quality of life, although there was no histologic improvement [69]. There are scant published data on other steroid-sparing agents and mesalazine in CVID enteropathy.

Autoimmunity

Autoimmune disease is common in CVID, with 20% to 25% of patients developing some form of autoimmunity during follow-up. Autoimmune cytopenias (particularly autoimmune thrombocytopenia and hemolytic anemia) are the most commonly reported [4,6]. In addition, various other autoimmune diseases, including rheumatoid arthritis, sicca syndrome, pernicious anemia, and systemic lupus erythematosus, have been described [4]. It should be noted that autoimmune thrombocytopenia preceded the development of hypogammaglobulinemia in up to 62% of cases in one series [70]. Consequently, CVID should be considered in the differential diagnosis of all patients who have autoimmune thrombocytopenia. In this study, replacement immunoglobulin therapy did not prevent the thrombocytopenia, although standard therapy with steroids and splenectomy seemed efficacious. Clinical observation suggests that subcutaneous immunoglobulin is less efficient in controlling autoimmune thrombocytopenia than intravenous immunoglobulin (IVIG). Obviously, additional vigilance is required when using immunosuppressants in an already immunodeficient individual.

Malignancy

There is a significantly greater risk of all cancers, and of gastric carcinoma and non-Hodgkin's lymphoma in particular, in patients who have CVID [4,6]. Various other cancers have been described including colorectal cancer, multiple myeloma, breast cancer, ovarian cancer, and Waldenstrom's macroglobulinemia, although the numbers in the study were too

small to determine if these reports represented a true increase above population levels.

The increased incidence of gastric carcinoma and non-Hodgkin's lymphoma is striking, with rates between 10 to 16 and 18 times higher, respectively, than in healthy individuals. There were wide confidence intervals for these rates, however [6,71]. Patients who have atrophic gastritis are probably at even greater risk of gastric carcinoma, and annual routine endoscopy may detect malignancy at an earlier stage.

Monitoring of patients

At present there is limited evidence to guide monitoring protocols in CVID. Recommendations have been drawn mostly from expert consensus and extrapolated from non-immunodeficient patients who have similar complications.

For respiratory monitoring, lung imaging and pulmonary function tests provide baseline information for identifying patients who have progressive disease who might benefit from intensified therapy. Patients who have CVID frequently have abnormalities of pulmonary function [72,73]. Pulmonary function tests are inferior to HRCT imaging for both the detection and monitoring of early lung disease [56,73] but are useful for monitoring chronic lung disease and assessing treatment requirements. The authors collect lung function data at baseline and annually thereafter.

Plain chest radiography is of limited value in CVID. HRCT is the modality of choice for the detection of interstitial lung disease and bronchiectasis, but consensus on the indications has not been achieved [72,74]. It is likely that HRCT will be performed increasingly often in patients who have CVID, mirroring practice in the cystic fibrosis setting. Baseline scans in all adult patients who have CVID and interval scans at least every 5 years are performed in the authors' institution, although in one cohort study, HRCT scanning was performed every 4 years [6]. Given the radiation exposure from serial imaging, reduced-dose HRCT protocols that may provide adequate information should be considered.

Optimal monitoring for gastrointestinal disease, lymphoproliferation, and splenomegaly is less clear. One study routinely performed biennial upper gastrointestinal endoscopy and annual ultrasound screening [6]. The authors perform annual abdominal ultrasound scans and routine *H pylori* antigen screening in feces. Gastrointestinal endoscopy is performed at diagnosis and follow-up depending on the initial findings (eg, nodular lymphoid hyperplasia, atrophic gastritis). It remains to be seen whether earlier detection of pathology improves outcomes for patients who have CVID.

Treatment

The mainstays of treatment for CVID remain replacement immunoglobulin and antibiotics for infections and appropriate treatment for the

noninfectious complications. In addition, in more recent years, biologic agents, including rituximab and infliximab, have been used for various complications (gut disease and autoimmune thrombocytopenia) [69,75].

Prevention of infection

Although there are no placebo-controlled studies of immunoglobulin replacement in patients who have CVID, data available from its use in various antibody-deficiency syndromes are suggestive of benefit. IVIG reduces both the rate of acute and chronic infections and their long-term complications in agammaglobulinemia [76–78], CVID, [79–83] and hyper-IgM syndrome [84–86]. Although administration by the intravenous and subcutaneous routes seems to be similarly efficacious [87], the intramuscular route is clearly inferior and has been abandoned [88]. Home therapy programs are well established in Europe and have been shown to be cost effective and popular with patients. Immunoglobulin is administered intravenously every 2 to 4 weeks or subcutaneously on a weekly basis, with a usual starting dose of 0.4 to 0.6 g/kg/mo. The complex and variable pharmacokinetics, however, necessitate individualized dosing based on IgG trough levels [89]. The optimum trough level is not established. Original recommendations were for a trough IgG level of 5 g/L, but there is some evidence for improved outcome with higher levels [78,81,83,90]. The authors aim for an IgG trough level of 7 g/L.

Antimicrobial prophylaxis is prescribed widely in CVID, but the evidence base is poor. In the authors' practice, frequent (generally more than three per year) or severe infections trigger consideration of prophylaxis. The choice of antibiotic should be guided by previous microbiology results and patient factors.

Treatment of acute respiratory infections

A zero-tolerance approach to respiratory infections is advocated to prevent structural damage. Whenever possible, sputum should be collected before commencing treatment, but empiric therapy generally should not be delayed until results are available. The most common respiratory pathogens in CVID are *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* [4,53,65,91,92], and empiric therapy should be directed appropriately according to local protocols. Although formal evidence is lacking, extended treatment courses (eg, 10–14 days for bronchitis) often are required to prevent relapse.

Management of bronchiectasis

Bronchiectasis affects 17% to 76% of antibody-deficient patients in various cohorts [61,65,72,73,91–96]. There are few data specific to the management of bronchiectasis in CVID, and protocols are extrapolated from the cystic fibrosis (CF) and non-CF settings. Some physicians advocate higher

IgG trough levels in this setting. Patients who have recurrent infections and/or progressive lung damage despite adequate IVIG replacement may benefit from regular suppressive antimicrobials. There is evidence that macrolides, which have anti-inflammatory as well as antimicrobial properties, may reduce exacerbation frequency and preserve lung function in CF and non-CF bronchiectasis [97–102]. Although unusual in CVID, pseudomonas in the CF setting heralds declining lung function, and eradication of first growth [103] and suppressive treatment of colonized patients with aerosolized antibiotics may be helpful [104–106].

Physical therapies (including postural drainage, inspiratory muscle training, and pulmonary rehabilitation programs) have some evidence base in a variety of non-CVID respiratory diseases, including bronchiectasis. These measures are widely utilized [107] and are strongly supported by the authors. Inhaled corticosteroids may improve lung function in bronchiectasis [108]. Surgical management of selected bronchiectatic patients with highly localized disease remains an option [109], although patients who have CVID tend to have more generalized lung damage. Finally, patients who have CVID and intractable lung disease may be candidates for lung transplantation [110,111].

Granulomatous and interstitial lung disease

Immunoglobulin replacement has no effect on interstitial lung disease [72]. The optimal treatment is unknown. In the authors' experience, the restrictive ventilatory defect of CVID-associated interstitial lung disease generally is steroid responsive, but toxicity is a limiting factor, because high doses may be required, and inflammation returns on tailing. There is limited data on steroid-sparing agents. Cyclosporin has been recommended [112] but has poor tolerability. Methotrexate is supported by some evidence in the sarcoidosis setting [113]; in addition to methotrexate, the authors have had positive experience with the use of azathioprine and mycophenolate mofetil. In case reports, the anti-TNFα monoclonal antibody infliximab was reported to be efficacious in sarcoidosis and granulomatous CVID [114], but the clinical benefits were highly doubtful in a formal trial of patients who had pulmonary sarcoidosis [115]. Whether these results can be extrapolated to CVID is unclear.

Biologic agents and other therapies

There is a paucity of data regarding best treatment for noninfectious complications in CVID. Standard immunosuppressive therapies have been used for inflammatory and autoimmune complications, in most instances with varying degrees of efficacy. There now have emerged a number of case reports using biologic agents in CVID, including rituximab for autoimmune thrombocytopenia [75] and infliximab for CVID enteropathy [69] and granulomatous disease [114]. It remains to be seen whether these more targeted agents are safer and more effective in CVID. In addition, allogeneic

stem cell transplantation has been undertaken in a limited number of patients (unpublished data, presented at the UK Primary Immunodeficiency Network forum 2007), and a full report is awaited. Obviously, it is early days, and the indications and success of this therapy need to be elucidated more fully.

Quality of life

There also has been an increased focus on the quality of life of patients who have CVID. Studies have shown that CVID affects quality of life as much as other chronic diseases such as cancer, congestive heart disease, psoriasis, and arthritis [116,117]. Of note, home subcutaneous immunoglobulin replacement, which previously had been shown to be as safe and effective as intravenous immunoglobulin [87], was shown to result in better quality of life for patients, particularly those who had been treated with intravenous immunoglobulin in a hospital setting [118].

Survival

There is a relatively high mortality rate with CVID. A large cohort study published in 1999 showed a 23% mortality rate after 7 years of follow-up [4]. When compared with the expected survival in the general United States population, this report showed that the probability of a male surviving 20 years after diagnosis of CVID was 64% (67% for females) compared with 92% (94% for females) in the general population. A more recent Italian cohort study published in 2007 does suggest an improvement in survival rates with 6% mortality after 11 years of follow-up [6]. This difference was thought to result from the greater use of immunoglobulin as well as better diagnostic and therapeutic strategies. These two studies are the largest cohorts reported. The primary immunodeficiency database registry in Europe now is building momentum, and it will be interesting to analyze the data generated from this registry [5].

Summary

Recent exciting genetic advances in CVID have increased the understanding of this heterogeneous group of disorders. These advances have potential implications outside the field of primary immunodeficiency, because these "knockouts" provide an in vivo human model of the role of various components of the immune system that could be useful in developing drugs for other diseases, including malignancy, autoimmunity, and allergy. The management of CVID has evolved as well, although not as quickly, with some evidence that patients now live longer and have an improved quality of life. Classification schemes have been developed to allow better characterization of patients for research and prognostication. There remains, however,

a need for earlier diagnosis and better education about CVID, because there still are prolonged diagnostic delays resulting in significant morbidity and mortality. It is likely that increasing numbers of genes will be discovered in the future, allowing greater understanding of the immune system and potentially enabling more tailored treatment for individual patients based on a greater knowledge of their molecular defect.

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Genetic Diagnosis of Primary Immune Deficiencies

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The increasing availability of genetic testing for many disorders is facilitating improvements in patient management [1,2]. Such testing can establish or confirm a suspected diagnosis and also may predict future disease risk in advance of clinical signs and symptoms, inform reproductive decision making, and guide clinicians in selecting the most appropriate therapeutic options. Traditionally, geneticists familiar with the nuances of rare disorders have orchestrated complex genetic testing through intimate relationships with academic laboratories accustomed to low-volume, high-complexity testing. This boutique laboratory approach may be effective for rare "single-gene" disorders but may falter as testing complexity increases. The expanding knowledge of the molecular pathogenesis of many human disorders has revealed new complexities of "nonclassic" single-gene disorders. Moreover, interest in genetic testing has expanded to medical specialties beyond medical genetics [3]. In coming years, knowledge of the complex genetic

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foundation of many common polygenic disorders (eg, diabetes and hypertension) will be used to establish a definitive clinical diagnosis. Thus, genetic medicine is transitioning rapidly from the academic-based genetics clinic to a service routinely used by other medical specialties and community-based primary care providers [1,3].

The field of primary immune deficiencies (PIDs) has seen a similar transition of testing from the research to the clinical setting [4]. Much progress has been made since Bruton [5] presented the first example of human PID in 1952 by using the then-new technology of serum electrophoresis. Since then, many novel laboratory methodologies have been developed and adopted in the clinical diagnosis of PIDs. Although historically the molecular diagnosis of PID has occurred in leading academic centers [6–16], commercial clinical diagnostic laboratories now perform an increasing proportion of genetic testing for PIDs. Moreover, the field of PIDs currently is experiencing a paradigm shift in the definition of PIDs from rare monogenic disorders leading to severe infections in infancy to much more common diseases [17,18]. One in 1200 persons in the United States is diagnosed as having some form of PID [19], and population-wide newborn screening currently is being considered for severe combined immunodeficiency (SCID) [20].

With the emerging understanding that many individuals may suffer from at least one of a multitude of PIDs [21], community-based primary care providers must be empowered to share the responsibility for diagnosis and treatment of PIDs with academic medical centers. To increase awareness of PIDs among health care providers, patients, and families, outreach programs have been established by agencies and organizations such as the US National Institutes of Health, the Mount Sinai Hospital (New York City, NY), the Jeffrey Modell Foundation (http://www.info4pi.org/), the Immune Deficiency Foundation (http://www.primaryimmune.org/), and the National Organization for Rare Disorders (http://www.rarediseases.org/). To facilitate more widespread use of genetic testing as a diagnostic tool, providers must have easy access to testing and know when genetic testing is indicated. Accuracy of test results must be assured, and the interpretations provided by clinical laboratories must be standardized and easy to understand.

In the past, such testing lacked specific national standards for quality assurance, quality control, test accessioning and reporting, or proficiency evaluation. Scientific, clinical, and regulatory efforts are underway to ensure the responsible expansion of genetic testing for PIDs [4]. The Clinical Laboratory Improvement Amendments of 1988 (CLIA; http://www.fda.gov/cdrh/clia/), the American College of Medical Genetics (ACMG; http://www.acmg.net/), and molecular diagnostics guidelines published by the Clinical and Laboratory Standards Institute (CLSI; http://www.nccls.org/) have hastened the transition of genetic testing for PID from academic to clinical laboratories. The Human Variome Project also has recently published guidelines designed to improve genetic testing [22,23]. In this article, some of the advances and challenges currently encountered in the clinical

molecular genetic diagnosis of PIDs are discussed, based on the authors' experience and a review of the published literature.

Clinical utility of genetic testing in the field of primary immune deficiencies

Practice parameters and guidelines for diagnosis and management of PIDs include genetic testing as an integral component [24–26]. Patients who have PID can benefit from early diagnosis and improved clinical management based on the definitive diagnosis that genetic testing can provide [27]. Currently, however, many clinicians view genetic testing more as a confirmatory test than as a screening test. Some of the major impediments to widespread adoption of genetic testing for PIDs include (1) relative costs and related concerns about insurance coverage; (2) difficulties in identifying an appropriate clinical laboratory; (3) the length of time between the request for a test and the delivery of a result; and (4) lack of clinical standardization at the laboratory level. Increased use of genetic testing in clinical practice will be accelerated by educating primary care providers [27] and developing patient-centered screening protocols designed for non-immunologists [26] as well as by the availability of validated clinical genetic testing services. Table 1 provides a nonexhaustive list of PIDs for which a responsible gene has been identified (see Geha and colleagues [28] for more details). A complete list of genetic tests, for both clinical use and research, is provided by the GeneTests Laboratory Directory [2].

Clinical utility of genetic testing for severe combined immunodeficiency

SCID subtypes often are classified based on counts of individual cell populations. This information is a valuable guide to clinicians attempting to select the most appropriate genetic test [24,29]. Because distinct mutations within the same gene can lead to different immunologic phenotypes [30], a pitfall of this method of classification is that a clinical diagnosis based on an immunologic phenotype could obscure a definitive genetic diagnosis. Of note, genetic testing can provide a definitive diagnosis of SCID and SCID subtype, especially when clinical and immunologic diagnosis based on counts of individual lymphocyte populations is complicated by engraftment of maternally derived lymphocytes. Muller and colleagues [31] detected maternal T cells in 40% of infants who had SCID. In eight patients who had prominent skin graft-versus-host disease (GVHD), the underlying SCID presentation was characterized only by the absence of B cells, although maternal T-cell levels did not correlate well with GVHD intensity and skin manifestations [31], and maternal engraftment might not occur in certain SCID subtypes [31]. Consequently, the significant heterogeneity of clinical and immunologic findings present in SCID must be considered when attempting a diagnosis. Testing peripheral blood for maternal T cells should be a routine part of both the pretransplantation compatibility

Table 1
Primary immune deficiencies for which a responsible gene has been identified

Disease	Inheritance	Genetic defects	Gene symbol
Combined T-cell and B-cell immunodeficiencies			
T^-B^+ severe combined immunodeficiency			
γc deficiency	XL	Defect in γ chain of receptors for IL-2, -4, -7, -9, -15, -21	*IL2RG*
JAK3 deficiency	AR	Defect in Janus-associated signaling kinase 3	*JAK3*
IL7Rα deficiency	AR	Defect in IL-7 receptor α chain	*IL7RA*
CD45 deficiency	AR	Defect in CD45	*CD45*
CD3δ/CD3ϵ deficiency	AR	Defect in CD3δ or CD3ϵ chains of T-cell antigen receptor	*CD3D, CD3E*
T^-B^- severe combined immunodeficiency			
RAG 1/2 deficiency	AR	Complete defect of Recombinase activating gene 1 or 2	*RAG1, RAG2*
DCLRE1C (Artemis) deficiency	AR	Defect in Artemis DNA recombinase-repair protein	*DCLRE1C*
ADA deficiency	AR	Mutations in the Adenosine deaminase gene	*ADA*
Omenn syndrome	AR	Missense mutations allowing residual activity	*RAG1, RAG2, DCLRE1C, IL7RA*
CD40 ligand deficiency	XL	Defects in CD40 ligand (CD40L) protein	*CD40L/TNFSF5*
CD40 deficiency	AR	Defects in CD40 protein	*CD40/TNFRSF5*
ZAP-70 deficiency	AR	Defects in the Zeta-associated protein of 70-kD signaling kinase	*ZAP70*
Predominantly antibody deficiencies			
Severe reduction in all serum Ig isotypes with severely decreased or absent B cells			
BTK deficiency	XL	Mutations in the Bruton tyrosine kinase gene	*BTK*
Other gene deficiencies	AR	Mutations in μ heavy chain, λ5, Igα, IgB, the BLNK adapter	*IGHM, λ5, Igα, IgB, BLNK*

Severe reduction in serum IgG and IgA with normal, low or very low numbers of B cells			
ICOS deficiency	AR	Mutation in the Inducible costimulator gene	*ICOS*
CVID	AD (AR)	Mutation in the Transmembrane activator and calcium-modulator and cyclophilin ligand interactor gene, and in other genes	*TNFRSF13B, other genes*
X-linked lymphoproliferative syndrome 1	XL	Mutations in the SH2 domain protein 1A gene	*SH2D1A*
Severe reduction in serum IgG and IgA with normal or elevated IgM and normal numbers of B cells			
CD40 ligand deficiency	XL	Defects in CD40 ligand (CD40L) protein	*CD40L/TNFSF5*
CD40 deficiency	AR	Defects in CD40 protein	*CD40/TNFRSF5*
AICDA deficiency	AR	Mutation in the Activation-Induced Cytidine Deaminase gene	*AICDA*
UNG deficiency	AR	Mutation in the Uracil-DNA glycosylase gene	*UNG*
Other well-defined PIDs			
Wiskott-Aldrich syndrome	XL	Mutations in the Wiskott-Aldrich syndrome protein gene	*WASP*
Other DNA repair defects			
Ataxia-telangiectasia	AR	Mutation in the Ataxia-telangiectasia gene	*ATM*
Nijmegen breakage syndrome	AR	Mutation in the Nibrin gene	*NBS1*
Bloom syndrome	AR	Mutation in Helicase gene	*BLM*
Hyper-IgE syndrome (HIES)			
Job syndrome	AD	Mutations in the Signal transducer and activator of transcription 3 gene	*STAT3*
AR HIES with mycobacterial and viral infections	AR	Mutation in Tyrosine kinase 2 gene	*TYK2*

(*continued on next page*)

Table 1 (*continued*)

Disease	Inheritance	Genetic defects	Gene symbol
Diseases of immune dysregulation			
Immunodeficiency with hypopigmentation			
Chediak-Higashi syndrome	AR	Mutations in the Lysosomal trafficking regulator gene	*LYST*
Griscelli Syndrome, type 2	AR	Mutations in the Rab protein 27A gene	*RAB27A*
Hermansky-Pudlak syndrome, type 2	AR	Mutations in the B subunit of the Adaptor protein-3 complex gene	*AP3B1*
Familial hemophagocytic lymphohistiocytosis syndromes			
Perforin deficiency	AR	Mutations in the Perforin 1 gene	*PRF1*
Munc 13-D deficiency	AR	Defects in MUNC13D	*MUNC13D*
X-linked lymphoproliferative syndrome (XLP)			
XLP1	XL	Mutations in the SH2 domain protein 1A gene	*SH2D1A*
XLP2	XL	Mutations in X-linked inhibitor of apoptosis protein (XIAP)	*XIAP*
Syndromes with autoimmunity			
Autoimmune lymphoproliferative syndrome			
type 1a	AD (AR)	Mutations in the CD95 (Fas) gene	*TNFRSF6*
type 1b	AD, AR	Mutations in the CD95L (Fas ligand) gene	*TNFSF6*
type 2a	AD	Mutations in the Caspase 10 gene	*CASP10*
type 2b	AD	Mutations in the Caspase 8 gene	*CASP8*
Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (APECED)	AR	Mutations in the Autoimmune regulator gene	*AIRE*
Immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX)	XL	Mutations in the Forkhead box protein 3 gene	*FOXP3*
Congenital defects of phagocyte number, function, or both			
Severe congenital neutropenias	AD	Multiple gene defects	*ELA2, GFI1, G-CSFR*
Kostmann disease	AR	Mutations in the HCLS1-associated protein x1 gene	*HAX1*
Cyclic neutropenia	AD	Mutations in the Elastase 2 gene	*ELA2*

Leukocyte adhesion deficiency (LAD)			
type 1	AR	Mutations in the adhesion protein INTG2	*ITGB2*
type 2	AR	Mutations in the Fucose transporter 1 gene	*FUCT1*
Rac 2 deficiency	AD	Mutations in the Regulation of actin cytoskeleton gene	*RAC2*
B-Actin deficiency	AD	Mutations in the cytoplasmic actin B gene	*ACTB*
Localized juvenile periodontitis	AR	Mutations in the Chemokine receptor FPR1	*FPR1*
Papillon-Lefevre syndrome	AR	Mutations in the Cathepsin C activation of serine proteases gene	*CTSC*
Shwachman-Diamond syndrome	AR	Mutations in the Schwachman-Bodan-Diamond syndrome gene	*SBDS*
Chronic granulomatous disease			
X-linked	XL	Mutations in the Electron transport protein (gp91phox) gene	*CYBB*
Autosomal	AR	Multiple gene defects	*CYBA, NCF1, NCF2*
IL-12 and IL-23 receptor B1	AR	Mutations in the IL-12 and IL-23 receptor B1-chain gene	*IL-12RB1*
IL-12p40 deficiency	AR	Mutations in the gene of the IL-12p40 subunit of IL12/IL23	*IL-12p40*
IFN-γ receptor 1 deficiency	AR/AD	Mutations in the IFN-γ receptor 1 gene	*IFN-γR1*
IFN-γ receptor 2 deficiency	AR	Mutations in the IFN-γ receptor 2 gene	*IFN-γR2*
STAT1 deficiency	AR/AD	Mutations in the signal transducer activator of transcription 1 gene	*STAT1*
Defects in innate immunity			
Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID)	XL	Mutations in the NF-κB essential modulator (NEMO) gene	*IKBKG*
	AD	Mutations in the NF-κB inhibitor, alpha (IκBα) gene	*IKBA*
IL-1 receptor–associated kinase 4 (IRAK4) deficiency	AR	Mutations in the IL-1 receptor–associated kinase 4 gene	*IRAK4*
WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome	AD	Mutations in the Chemokine receptor CXCR4 gene	*CXCR4*

(*continued on next page*)

Table 1 (*continued*)

Disease	Inheritance	Genetic defects	Gene symbol
Epidermodysplasia verruciformis	AR	Mutations in the Epidermodysplasia verruciformis genes 1 or 2	*EVER1, EVER2*
Herpes simplex encephalitis	AR	Mutations in the *UNC93B1* gene	*UNC93B1*
	AD	Mutations in the Toll-like receptor 3 gene	*TLR3*
Autoinflammatory disorders			
Familial Mediterranean fever	AR	Mutations in the Mediterranean fever gene	*MEFV*
TNF receptor–associated periodic syndrome (TRAPS)	AD	Mutations of the Tumor necrosis factor receptor soluble factor 1A gene	*TNFRSF1A*
Hyper-IgD syndrome	AR	Mutations in the Mevalonate kinase gene	*MVK*
Muckle-Wells syndrome	AD	Mutations in the cryopyrin (PYPAF1/NALP3/CIAS1) gene	*CIAS1*
Familial cold autoinflammatory syndrome	AD	Mutations in the cryopyrin (PYPAF1/NALP3/CIAS1) gene	*CIAS1*
Neonatal-onset multisystem inflammatory disease (NOMID)	AD	Mutations in the cryopyrin (PYPAF1/NALP3/CIAS1) gene	*CIAS1*
Pyogenic sterile arthritis, pyoderma gangrenosum, acne (PAPA) syndrome	AD	Mutations in the Proline/serine/threonine phosphatase-interacting protein 1 gene	*PSTPIP1*
Blau syndrome	AD	Mutations of the Nucleotide-binding oligomerization domain protein 2 gene	*NOD2 (CARD15)*
Majeed syndrome	AR	Mutations in the Lipin-2 gene	*LPIN2*
Complement deficiencies			
Complement gene deficiencies	AR/AD/XL	Multiple gene defects	

Table 1 is based on the PID classification of the 2007 Update from the International Union of Immunologic Societies Primary Immunodeficiency Diseases Classification Committee, which contains further details and a complete list of PIDs. A full list of genetic testing available for PIDs and laboratories contact information also is available at: www.genetest.org.

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; CVID, common variable immunodeficiency; IL, interleukin; IFN, interferon; NF-κB, nuclear factor-kappa B; SCID, severe combined immunodeficiency; XL, X-linked.

From Geha RS, Notarangelo LD, Casanova JL, et al. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. J Allergy Clin Immunol 2007;120(4):779; with permission.

assessment [32] and genetic test selection and results interpretation for patients who have SCID.

Use of full-gene sequencing to detect mutations

Full-gene sequencing is considered the reference standard in molecular genetic diagnostics, because it can identify both previously characterized and novel sequence variations with little or no ambiguity. Some diseases are caused by only a few common mutations in a gene and therefore can be detected by targeted screening for these particular mutations. Such cases are unusual, however, and often are dependent on the ethnicity of the individuals tested. For most genes, searching for previously characterized sequence variation may not be informative. For example, about half of all SCID-associated alleles of the adenosine deaminase (*ADA*) gene deficiency are unique to single families, and most patients are compound heterozygotes with a different mutation on each allele [33]. This observation also is supported by data derived from the authors' genetic testing service across the spectrum of PIDs and other disorders.

Among the currently available sequencing methods, dideoxy chain termination sequencing (Sanger sequencing) [34] remains the standard in sensitivity and specificity. Its initially high cost has been reduced significantly through the introduction of automation, so that cost no longer justifies the use of alternative mutation scanning methods such as single-stranded conformation polymorphism, heteroduplex analysis, denaturing gradient gel electrophoresis, or denaturing high-performance liquid chromatography, which are less sensitive methods of mutation detection [35]. Because direct sequencing of complementary DNA (cDNA) is limited by messenger RNA (mRNA) instability and inability to detect intronic variation, sequencing usually is performed on polymerase chain reaction (PCR)-amplified genomic DNA. Using genomic DNA as template allows determination of both protein-coding regions (exons) and noncoding regions (introns and 5′ and 3′ untranslated regions). Typically only 20 to 50 nucleotides flanking each exonic region are sequenced, because known functional elements generally are located within this interval and the clinical significance of mutations deeper into the introns usually is unclear. Therefore, testing of additional noncoding sequences would be considered only when the functional significance of these sequences has been demonstrated previously.

Because of the average size and number of exons per gene [36], the development of affordable high-throughput sequencing technology is necessary. Several alternatives to standard dideoxy chain termination sequencing [34] now are available, including pyrosequencing [37], combinatorial sequencing by hybridization [38], oligonucleotide resequencing arrays [39], multiplex polymerase colony or polony sequencing [40], and sequencing-by-synthesis [41]. These alternatives may revolutionize genetic testing further by drastically reducing cost and turnaround time and allowing a full-genomic sequencing

approach. In current clinical practice, dideoxy chain termination sequencing remains the most sensitive and is still considered the standard.

Limitations of full-gene sequencing for mutation detection

Mutation detection by gene sequencing, although highly sensitive and specific, is subject to a number of limitations. For example, gene sequencing cannot distinguish if two heterozygous mutations are located on the same or on different alleles. For a recessive disease such as *ADA*-related SCID, the former case would be a carrier, and the latter would be affected. These possibilities can be determined by testing the proband's biologic parents. If the two heterozygous variants are located on the same allele, they have been inherited from the same parent.

Another important limitation of gene sequencing is its inability to detect certain types of pathogenic mutations such as large deletions, insertions, duplications, and inversions. For example, failure to amplify an X-linked gene in a male may be the result of a gene deletion, and large deletions of a single allele for autosomal genes may go undetected, leading to false-negative results. Genomic copy number variation is widespread in healthy individuals [42] and may complicate the interpretation of sequence-based results. Because failure to amplify one allele of an autosomal gene will lead to apparent homozygosity of all variants located in the corresponding region of the amplified allele, homozygosity for a rare variant in a non-consanguineous family always should raise suspicion. In this case, true homozygosity can be distinguished from hemizygosity caused by non-amplification of one allele by testing the proband's biologic parents for the presence of the rare variant. Finally, variants in a PCR primer site may lead to PCR failure of the variant-containing allele, a phenomenon known as "allele drop-out" [43]. This technical failure can result in erroneous interpretation of a disease variant's zygosity or erroneous reporting of sequence data reflective of only one copy of a given chromosome. In diseases in which a significant proportion of cases may be caused by deletions or other types of mutations not detectable by Sanger sequencing, complementary methods such as Southern blot, fluorescent in situ hybridization, real-time quantitative PCR, multiplex ligation and probe amplification, or comparative genomic hybridization may be required. The details of these techniques are reviewed elsewhere [44].

Other limitations of genetic testing may depend on the sample type. When testing patients who have SCID, for example, the use of DNA derived from peripheral blood could lead to contamination of proband sequences with maternal sequences. Maternal–fetal transfusion occurs commonly among patients who have SCID [31] and can lead to maternally derived engrafted cells. Sequence data preferentially derived from the maternal DNA could obscure a pathogenic variant or lead to improper interpretation of a variant's zygosity. Sequencing of DNA isolated from a buccal swab can avoid this pitfall. Detection of variations that are present only in some

cell types obviously depends on the sample type being tested and is another important consideration, because variants present at low levels of somatic mosaicism may be very hard to detect. Selective expansion of rare revertant lymphoid cells also has been described [45,46]. In at least one published case [47], a "second site" mutation restoring normal *ADA* mRNA splicing seems to have occurred in a common progenitor of T and B cells but not in non-lymphoid cells. Thus, the possibility of mosaicism must be considered when interpreting gene-sequencing results, particularly when test results do not correlate with the clinical picture.

In general, the technical limitations of each genetic test must be brought to the attention of ordering physicians and genetic counselors. These providers also must be aware that a negative genetic test result cannot exclude a diagnosis and must be prepared to inform the patient of these limitations (both technical and scientific), ideally through a process of informed consent initiated before the test is performed. Thus, gene-sequencing results always should be considered carefully in the context of the clinical presentation and should not be considered an isolated diagnostic determinant.

Gene testing and quality assurance

Clinical mutation detection requires a comprehensive approach to provide the highest level of accuracy and reliability. A single genetic test performed once on a patient can lead to a life-long diagnosis. Clinical decisions made as a result of genetic testing may be irrevocable. Both false-positive and false-negative results could have a devastating impact on the lives of patients. A false-positive result would lead to an inappropriate intervention, whereas a false-negative result would lead to false reassurance and potential missed intervention.

The analytic validity of a gene test is determined by a number of elements, such as analytic sensitivity, analytic specificity, and overall assay robustness. In the authors' practice, all three parameters are validated before clinical implementation. Robustness and specificity of the assay are demonstrated by reproducibly obtaining sequences for the desired gene regions from a defined number of normal samples. Sensitivity and specificity of mutation detection are ascertained by detecting known mutations in a group of blinded samples, in which all mutations must be detected on both DNA strands. Importantly, the assay must be able to discriminate the target gene sequences from pseudogenes and functional homologous genes with highly similar or even identical sequences. Also, because DNA sequencing has an inherent error rate, it is important that pathogenic mutations are reported only when found by two sequencing reactions (ie, sequencing of both forward and reverse DNA strands).

Laboratory quality control protocols also are essential in maintaining assay reliability. Periodic internal proficiency testing should be performed, consisting of unannounced inclusion of blinded samples of known genotype

into the workflow. Continuous monitoring of processes and results serves to identify developing issues, such as primer degradation or unanticipated occurrence of normal variation underneath the primer site, preventing amplification of alleles containing such variation (allele drop-out) [48]. Well-documented laboratory quality control procedures, including personnel training and periodic proficiency evaluations, are essential elements for laboratories performing clinical genetic testing.

An additional key factor in minimizing human error during clinical performance is automation, which facilitates reproducibility of results and tracking of errors in processes that otherwise are susceptible to error (eg, setting up reactions for PCR amplification or registering sequence analysis data). Records of automated processes facilitate monitoring and quality assurance. For example, the authors' laboratory has developed an integrated laboratory-information management system for genetic testing that automates large portions of the genetic testing process from test performance through result reporting. As the availability and awareness of genetic testing continues to expand, genetic testing laboratories will depend increasingly on automation and informatics to support high test volume with short turnaround times, low costs, and, most importantly, accurate and reliable results.

Clinical interpretation of gene-sequencing results

Advances in molecular genetic techniques have made gene testing for PIDs increasingly available in clinical practice with more timely results. At the same time, such testing detects more and more variants that do not obviously alter the gene structure and function and are difficult to interpret in the clinical setting. Nonsense mutations, frame-shifting mutations, deletions, gene rearrangements, and obvious splice-junction mutation are relatively easy to interpret. In contrast, missense amino acid changes and most intronic and promoter variants often are difficult to interpret.

Interpretation of genetic testing results must consider each individual variant as well as the interpretation of the overall set of variants identified. Genetic variants are interpreted based on predictions of the effect on protein structure and function and potentially on published descriptions of the functional effect of a variant in an experimental system. The frequency of a variant in the population may be a clue to pathogenicity. Common variants are less likely to be pathogenic.

Standardization is highly necessary, considering the complexity of gene testing results. Because sequence variations are described best at the DNA level, the adoption by all clinical laboratories of the gene variant syntax and nomenclature recommended by the Human Variation Genome Society is an important step [49]. Importantly, the use of the appropriate cDNA reference sequence for naming mutations avoids dangerous confusion by

guarding against historical changes in the numbering of introns and communication errors. In contrast, the phenotypic classification of genetic variants is not currently unified. Different laboratories apply different variant classification algorithms. Moreover, the clinical interpretation and the threshold criteria for classifying variants of truly unknown significance versus pathogenic and normal variants largely reflect the protocols and specific experience of the clinical laboratory that has performed the PID genetic test. This lack of standardization ultimately represents a major impediment to the effective communication of genetic test results to physicians and genetic counselors. The following sections provide a brief overview of some principles guiding the clinical interpretation of gene variants.

Interpretation of novel variants

A novel variant is defined commonly as a variant not previously described in the published literature in association with a disease phenotype. Identification of a novel genetic variant in a patient is of no clinical use unless it is ''clinically classified'' based on available biochemical and genetic factors. In the absence of empiric data, the clinical laboratory may attempt to predict a variant's biochemical and functional consequences by considering (1) mutation type; and (2) location within the gene (in a coding sequence versus a noncoding sequence). A missense mutation causes an amino acid change, but not all missense changes are pathogenic. The laboratory considers whether the amino acid change is conserved across species and whether it affects known functional domains or motifs within the protein. Even without considering functional implications, the laboratory may infer the likelihood of pathogenicity based on the allele's frequency in a control population of healthy individuals. Variants occurring commonly in healthy individuals are very unlikely to be pathogenic. Cosegregation of the variant in families with disease, as quantified by the overall number of genotype–phenotype concordant versus discordant individuals, is another consideration. The exact weighting of each factor in the ultimate equation is debatable but can be incorporated into a rational and reproducible approach to predict pathogenicity.

The ultimate goal of any clinical laboratory is classification of variants into defined categories ranging from disease-causing mutations to normal variants (Fig. 1). A variant classification system must be flexible enough to assimilate novel scientific knowledge and data. A substantial proportion of identified variants will be of uncertain clinical significance but may be defined more easily in the future. To address all of these needs, the authors' laboratory has developed its own standardized algorithm to classify each variant for probability of association with disease and to store the results in an adaptable database (GeneExplorer, Correlagen Diagnostics, Waltham, MA). A number of integrated algorithms and statistical approaches also have been reported previously [50–53]. In general, many algorithms

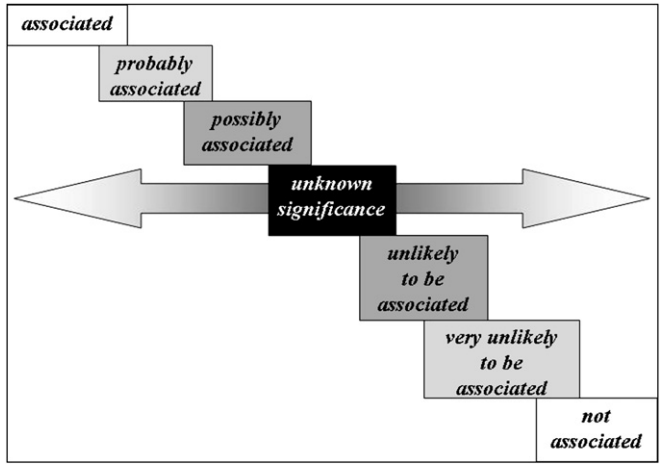

Fig. 1. Clinical classification of gene variants into defined categories. After its identification in a patient's DNA, a gene variant is evaluated for its clinical significance. A standardized algorithm based on both genetic and biochemical information is applied. This approach allows the classification of each variant for probability of association with disease, ranging from disease-causing mutations (*left side of the panel*) to normal variants (*right side of the panel*). A certain proportion of identified variants will be of unknown significance (*center of the panel*) but may be defined more easily in the future when additional information could become available.

incorporate segregation data, species conservation, biochemical analysis, and functional data to allow the most accurate and efficient classification of genetic variants.

Interpretation of previously published variants

Clinical testing often is performed on isolated individuals or small families, and the classification of typically rare variants in small pedigrees presents a substantial challenge. Although predictive biochemical and genetic tools can help, empiric data from previous patients often are very helpful. This information usually is catalogued in locus-specific databases or reported in peer-reviewed publications. The compendium on genetic disorders and genes "The Mendelian Inheritance in Man (MIM)" [54] represented the first attempt at summarizing and cataloguing the clinical effects of genetic variation. Highly curated databases of published and unpublished mutations in PID genes are publicly available [55,56]. Although typically accurate, such online databases may be incomplete or only sporadically updated with the most recent peer-reviewed literature. Databases with direct links to electronic copies of supporting literature are desirable but virtually nonexistent.

To meet these needs, the authors' laboratory has attempted to capture and classify such published information into comprehensive and dynamic

gene-specific databases, as described previously. Sophisticated data-mining algorithms and monitoring mechanisms are used to guarantee database curation through constant integration of the latest scientific information. The interfacing of this variant repository with a rules-based reporting system (RightReport, Correlagen Diagnostics, Waltham, MA) is key in assuring consistency of reporting and interpretation of variants, based on the most updated variant information. Of note, although ideally the wealth of published genetic variant information would be incorporated into public databases [57], much key data currently reside in proprietary laboratory databases or individual patient records around the world. Impediments to well-curated databases include lack of available resources, privacy concerns, and the lack of generally acknowledged means to report or publish these data [22,23].

Several publicly available general resources, such as the Single Nucleotide Polymorphism Database (dbSNP; http://www.ncbi.nlm.nih.gov/projects/SNP/) [58], the HAPMAP Data Coordination Center (http://www.hapmap.org/) [59], and the Human Genome Variation Database (HGVbase; http://hgvbase.cgb.ki.se) [60] also may assist in variant classification. Such databases do not attempt to determine explicit causative relationships between genotype and phenotype but are helpful in determining allele frequencies in individuals from different ethnic groups. In conclusion, complete, accurate, and standardized variant repositories are a prerequisite for variant classification algorithms seeking to classify correctly variants identified in an isolated patient, variants that otherwise would, improperly, be considered ''novel'' variations.

Interpretation of variants that affect messenger RNA splicing

The proper clinical classification of previously uncharacterized single-nucleotide intronic variants may be particularly challenging. Intronic variants at the consensus splice donor and acceptor sites can be classified definitively as pathogenic, but outside those positions the interpretation typically is unclear. Within coding regions, synonymous mutations that do not change an amino acid may be clinically relevant because of their effects on mRNA stability or because they create cryptic splice sites that alter the mRNA sequence [61]. Although a number of predictive tools are available [62,63], in vitro mRNA transcription studies often are the only way to determine the effect of these variants. Although molecular assays can determine the mutation's impact on mRNA message splicing [64], because causative intronic variants usually are removed from the mRNA message by splicing, it is important to associate the mutation to the allele that does not contribute to the normal mRNA, which can only be done using haplotyping [53]. In clinical practice, the allele frequency of a sequence variant in a population of unaffected individuals often is the only factor suggesting that an intronic variant is more or less likely to be pathogenic. Allele frequencies vary

across ethnic groups, however, necessitating variant prevalence studies in specific populations (eg, the Human Population Collection samples obtained from the Coriell Institute for Medical Research, Camden, NJ). The Single Nucleotide Polymorphism Database [58] and the HAPMAP Data Coordination Center [59] often are very helpful in this process, although data accuracy and validation always must be taken into consideration, particularly when data contamination by pseudogenes may be an issue.

Interpretation of missense variants

The clinical classification of novel, previously unreported missense variants also can be particularly challenging [65]. A number of biochemical algorithms and software tools designed to predict in silico the biochemical effect of amino acid changes may be helpful but must be used cautiously. Some examples are (1) BLOSUM (Blocks Substitution Matrix) [66]; (2) the GRANTHAM matrix [67]; (3). SNPper Amino Acid Variation [68]; (4) SIFT (Sorting Intolerant From Tolerant) [69]; and (5) PolyPhen (Polymorphism Phenotyping) [70]. Many of these predictive algorithms are based on the analysis of multiple protein sequence alignments and comparative interspecies analysis in combination with chemical differences.

Predictive methods are not as reliable as empiric data in the interpretation of clinical genetic test results, and when these algorithms are tested in parallel a high false-positive prediction rate generally is seen [71]. In one large study [72] the authors concluded that position of deleterious missense substitutions is biased strongly toward invariant residues and tends to have relatively high chemical difference index scores. Indeed, the majority of nonsynonymous single-nucleotide polymorphisms probably are neutral and do not have an effect on molecular function, phenotype, or fitness [70].

As indicated by comparative genomics studies, approximately 10% of deviations of a nonhuman protein from its human orthologue are compensated pathogenic deviations caused by a missense mutation that, at the given site, would be pathogenic to humans [73]. The Encyclopedia of DNA Elements (ENCODE) project [74] recently has suggested the possibility of a large genomic pool of neutral elements that are biochemically active and act as a ''warehouse'' for natural selection. Therefore, because certain missense changes in conserved positions could help by creating functional specificity for diverging gene paralogues, analyses of sequence alignments is best performed if restricted to true gene orthologues. Notably, by comparing computational methods that use evolutionary conservation alone, chemical change, or a combination of the two, Chan and colleagues [75] has shown a high predictive value for methods that use evolutionary sequence conservation, with or without considering protein structural change. Moreover, a higher biochemical predictive value can be achieved if different computational biology tools are used simultaneously [75].

Interpretation of genetic variants based on data from functional assays

Biochemical, functional, or in vitro cellular assays with informative phenotype readouts are available for many PID genes, often based on flow cytometry or Western blotting techniques [24,25,76–80]. Ideally, high-throughput assays would be designed to characterize any genetic variant identified within a PID gene, especially when variants of uncertain clinical significance are encountered. In actual practice, biochemical assay data may be available from peer-reviewed publications and can be taken into consideration when providing a clinical interpretation of genetic test results. In vitro experimental follow-up, if available, generally is recommended when novel gene variation of uncertain significance is identified by gene testing. Of note, in vitro tests are not immune to pitfalls and limitations. Many proteins related to PID lie at the cell surface, and many amino acid substitutions with a clinical consequence are located in the protein's cytoplasmic domain, thus affecting intracellular signaling but not cell membrane expression. Thus, mutated cell receptors may be detected as normal by flow cytometry [77]. Also, missense mutations of cytoplasmic adapters may affect signal transduction without impairing s protein's stability and expression [81,82]. For example, in a biochemical functional study of 10 different *SH2D1A* missense mutations identified in patients who have X-linked lymphoproliferative (XLP) syndrome [83], selected mutants (eg, p.Thr53Ile and p.Thr68Ile) impaired the SH2D1A ability to bind to receptors of members of the signaling lymphocytic activation molecule (SLAM; CD150) family, although they did not affect protein stability or half-life. Gene sequencing may obviate certain pitfalls of biochemical studies or aid by strengthening the interpretation of such studies.

Standardization of criteria for interpretation and reporting of genetic variants

In conclusion, clinical testing may identify genetic variants of uncertain significance, either novel coding mutations or intronic variation. Nonsense mutations, specific missense mutations, and splice-site mutations are recognized as deleterious based on empiric observations in high-risk families, the absence of such variants in control populations (except at carrier frequency), comparative genomics predictive algorithms, and/or functional molecular or biochemical evidence. The optimization of computational algorithms integrating this information is sorely needed; because of advances in technology, the detection of mutations has outpaced the ability to interpret them. Although generic American College of Medical Genetics guidelines for sequence variant interpretation are helpful [84], standardized algorithms and approaches for clinical interpretation of the gene variants identified by gene sequencing are a requirement for a cost-effective use of genetic testing in patient management [22,23]. Moreover, result reports for genetic tests should include all test results and also should provide clear and standardized

interpretations, to be consistent across patients and over time and also to allow revisions as new data on variant significance emerge.

Genetic variants and phenotypic manifestations of primary immune deficiencies

Genotype–phenotype correlations

Establishing a correlation between a specific pathogenic variant and disease severity or selective PID subphenotypes often is impossible. Allelic heterogeneity (distinct mutations within the same gene) can lead to different phenotypes, and distinct phenotypes can arise from a single mutation [30]. The same PID phenotype also may arise through locus heterogeneity (ie, mutations in different causative genes) [85,86]. Few examples are known of strict genotype–phenotype correlation for PID genes [33,56], although some weaker correlations seem to be more common [87]. The inability to correlate genotype with phenotype may arise from interaction between disease-causing mutations and genetic variants elsewhere in the genome [88]. Additionally, host–environment interactions could account, at least in part, for the variable penetrance of autosomal dominant PIDs [10]. For example, individuals who have single heterozygous mutations in the tumor necrosis factor receptor superfamily member 13B (*TNFRSF13B*; *TACI*) have variable phenotypes ranging from unaffected to overt common variable immunodeficiency [89–91], suggesting a role for unidentified genetic modifiers and redundant signaling pathways. Complex host–environment interactions play an additional role, as demonstrated by some PIDs that arise in infancy and resolve spontaneously in adulthood, probably because of compensation by adaptive immunity [17,21].

Clinical, immunologic, and genetic diagnosis of primary immune deficiencies

The weak genotype–phenotype correlation for PIDs ultimately translates into difficulties in clinical classification and diagnosis. Historically, classification of PIDs has evolved as an ''immunologic'' classification as discoveries of these disorders has shaped and furthered the understanding of many immune system functions [6,27,92–94]. In particular, a combination of immunologic phenotypes and modes of inheritance has been the ruling principle for the International Union of Immunological Societies Committee Classifications of PIDs [28,94]. Because of the recent definition at the molecular genetic level of selective susceptibility to a number of specific pathogens, an updated clinical criteria-based classification of PIDs also has been proposed [21].

Ultimately, any classification system serving clinical purposes must be primarily phenotypic [17,21]. Because clinical presentation can vary and is dependent on environmental effects, deciphering the complexity of mutations

and genes involved in PIDs ultimately will allow the best operative approach to diagnosis, prognosis, and patient management. For example, in the case of XLP, identifying a pathogenic mutation in the *SH2D1A* gene provides a definitive diagnosis, overcoming clinical diagnostic difficulties caused by the pleomorphic clinical presentations of this disorder (Fig. 2) [82,95] and allowing therapeutic intervention before the patient contracts a potentially fatal Epstein-Barr virus infection. Additionally, among B-cell deficiencies, nomenclature of the hyper-IgM syndromes recently has been amended with a recommendation to reference the gene defect directly [85]. With respect to the immunologic diagnosis, gene testing offers the most definitive diagnosis in immunoglobulin-deficient patients, overcoming the variability of serum IgM levels in these gene diseases. Continued improvements in understanding the complex genetic basis of PIDs will lead to operational changes in the immunologic and phenotypic classification.

Role of hypomorphic mutations

Milder or atypical mutations, also known as "hypomorphic mutations," in PID genes or related genes in a pathway also could be responsible for more common conditions, such as autoimmune, allergic, or inflammatory disorders. Supporting this notion, different experimental studies have linked the XLP1 gene *SH2D1A* and its receptors, the *SLAM/CD150* family member genes, with selective susceptibility to infections and autoimmune disorders [96–99]. Because of the key role that SH2D1A plays in host immune defenses [82,100,101] and the striking homology between SH2D1A and the EAT-2 (EWS/FLI1 activated transcript 2) adapter [99,102,103], it is tempting to speculate that mutations in the *EAT-2* gene and its ligands also could be involved in autoimmune disorders or human PIDs. Notably, these genes map within the lupus susceptibility *Sle1b* locus [98,102].

Genetic diagnosis by gene sequencing identifies benign and disease-causing genetic variations and variants of uncertain significance. Variants that currently are difficult to interpret may be understood more clearly in the future. New information may lead to reclassification of genetic variants, and patients must be made aware of these changes through updated results reports. Therefore, the authors' laboratory reports all genetic variants identified by gene testing, because patients may only be tested once, and because clinical genetic test reports should be as informative as possible. Because of the exponential increase in genetic and functional data acquired both experimentally and in the context of the clinical genetic practice, flexible and adaptable databases are needed to collect the wealth of knowledge produced by clinical genetic testing [57]. At the present time, variant interpretation criteria primarily are relevant only to monogenic disorders. As time goes on, genetic test interpretation will be able to consider variable penetrance caused by modifier genes and hypomorphic genomic variation. A complete understanding of PIDs will require combined analysis of Mendelian and common

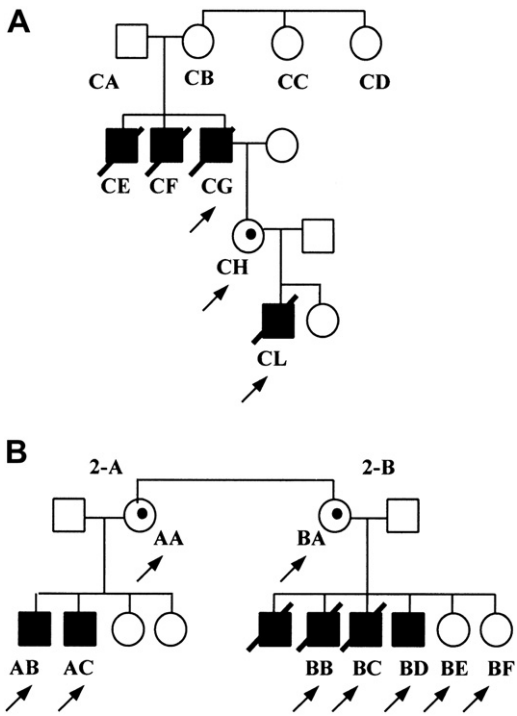

Fig. 2. Variability of clinical phenotypic manifestations in family members affected by X-linked lymphoproliferative-1 syndrome (XLP1) carrying the same *SH2D1A* mutation. Three main clinical phenotypes can characterize the clinical presentation of XLP1: fatal infectious mononucleosis (FIM), lymphomas, and immunoglobulin deficiencies. Defects in the XLP1 gene, *SH2D1A* were investigated in two unrelated families in which male members were affected by common variable immune deficiency (CVID). Arrows indicate patients analyzed for the presence of *SH2D1A* gene mutations. The genetic trees of the two families are shown in panels A and B, respectively. Patients are labeled with numbers and letters; boxes indicate males; circles indicate females. A line through the box or circle indicates that the patient is deceased. A solid box indicates an affected male; a dot within a circle indicates a carrier; open boxes and circles indicate unaffected subjects. (*A*) Three brothers (CE, CF, and CG) were examined for recurrent respiratory infections. A grandson (CL) died of a severe *Aspergillus* infection secondary to progressive immunoglobulin deficiency, FIM, aplastic anemia, and B-cell lymphoma. (*B*) Two brothers had B lymphocytopenia and immunoglobulin deficiencies (AB and AC). The presentation of FIM in another male relative (BC) led to the investigation of the *SH2D1A* gene. Pathogenic *SH2D1A* mutations were detected in both branches of the family. These results highlight the difficulties in establishing a diagnosis of X-linked lymphoproliferative disease (XLP) based on a clinical presentation and the relevance of gene testing in establishing a definitive diagnosis. Notably, the genetic diagnosis of XLP allows the selection of appropriate therapy (such as bone marrow transplantation) because the prognosis for XLP1 is much worse than for CVID syndrome in general. (*From* Morra M, Silander O, Calpe S, et al. Alterations of the X-linked lymphoproliferative disease gene *SH2D1A* in common variable immunodeficiency syndrome. Blood 2001;98(5):1323; with permission. Copyright © 2001, American Society of Hematology.)

Box 1. Key points

1. When ordering a genetic test, one should be aware of the testing methodology and its limitations and properly inform and counsel the patient in advance.
2. Gene sequencing, although the reference standard for molecular diagnostics, has a number of technical limitations, as described in the text.
3. Some genetic variants currently identified by complete gene sequencing are difficult or impossible to interpret and are called ''variants of unknown significance.''
4. The clinical understanding of variants of unknown significance will improve as new genetic information becomes available, requiring updated clinical reports and communication with patients and their families.
5. Genetic testing of family members is recommended in many cases because it might help provide a better understanding of a novel gene variation identified in an affected proband.
6. One always should test for the presence of maternal cells in the blood of a patient who has SCID. Detection of DNA sequences from maternal cells might obscure the identification of genetic variants in DNA isolated from peripheral blood of such a patient.
7. Although genetic testing can be performed on samples other than blood (eg, buccal swabs), one always should archive a blood sample from a patient undergoing bone marrow transplantation.
8. One always should discuss genetic testing results with the clinical laboratory that has performed the test if there are any uncertainties.

genetic variation. Eventually the utility of genetic testing will extend beyond highly penetrant disorders to predictive tests for low-penetrance and genetically heterogeneous disorders. Because the clinical presentation of PIDs often is nonspecific, with sometimes mild or absent clinical signs, the majority of PID patients may be undiagnosed [87]. Many mild disease-causing mutations probably are involved in these cases, and the definitive diagnosis will depend on genetic testing.

Summary

Box 1 lists key points in the clinical use of gene testing.

Clinical genetic testing laboratories are growing contributors to the field of PIDs, both in terms of knowledge being accumulated and patient care.

PID gene testing now is performed outside the traditional domain of academic, research-oriented laboratories and is being used by physicians who have a range of clinical expertise. The traditional separation between PIDs and more common infectious, autoimmune, and inflammatory diseases is fading as knowledge accumulates. In the future, genetic testing will become an increasingly important diagnostic tool in immunology clinics. Clinical diagnostic laboratories must adhere to the highest practice standards to ensure the quality of genetic testing for PIDs. Adoption of automation, laboratory informatics interfaces, and quality assurance protocols will improve the reliability of genetic test results. High-throughput gene-sequencing approaches will speed the process while maintaining quality. Optimization and standardization of approaches to variant interpretation will increase further the usefulness of genetic testing results by minimizing the reporting of variants of unknown significance. Clinical diagnostic laboratories are playing an increasing role in providing genetic health care and personalized medicine to patients who have PID.

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Principles of and Advances in Immunoglobulin Replacement Therapy for Primary Immunodeficiency

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Historical perspective

Today it seems quite straightforward to give IgG to patients who have immune deficiencies involving decreased antibody production. It is interesting reflect that, although serum therapy was used in the early 1900s, that treatment generally involved the use of serum from convalescent patients or from horses immunized with specific bacteria or toxins [1]. Primary immune deficiency (PID) had not yet been recognized, and penicillin had not yet been discovered; high-titered serum was the only specific therapy for common infections such as pneumococcal pneumonia. Not until World War II did concentrates of human immune globulin became available for widespread use, and it was not until 1952 that Bruton [2] published the first report of the use of immune serum globulin (ISG) in treating a patient who had PID. The studies of the Working Group on Hypogammaglobulinemia in the United Kingdom firmly established the benefit of regular ISG injections in the treatment of this family of illnesses and set the dosage at 25 mg/kg/week [3]. Not until the early 1980s were preparations of IgG that could be safely given intravenously licensed in the United States. Since that time, more purified and better-tolerated IgG preparations have become available, and there has been widespread interest in subcutaneous rather than intravenous administration. The doses of IgG used in patients who have PID have escalated steadily, with increasingly ambitious goals for prevention of infection and end-organ damage. Unfortunately, there also have been a number of reminders that treatment with blood products carries real and potential risks, including transmission of bloodborne infections. More

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recently, the use of high-dose intravenous immunoglobulin (IGIV) for its anti-inflammatory effects in diseases such as Kawasaki syndrome and for its immunomodulatory effects in autoimmune diseases has increased the demand for this precious commodity. To simplify the discussion of products that usually are denoted by their route of administration (ie, intravenous [IGIV], subcutaneous [IGSC], or intramuscular [IGIM]), this article uses the inclusive abbreviation "ISG" to refer to all polyclonal human immune globulin preparations.

What is immune serum globulin?

Stringent safety standards, the desire to provide antibodies against a wide range of pathogens, and the need to produce products with consistent tolerability and efficacy have led to large-scale industrial production of IgG concentrates which may contain the antibodies from 40,000 to 50,000 units of plasma per batch. These goals may seem to be at odds with the desire to assure safety by using a limited number of well-characterized, usually related, plasma donors for individual patients. The decades since IGIV was introduced have witnessed the recognition of HIV as a bloodborne virus, an outbreak of hepatitis C transmission by IGIV and other blood products in the early 1990s, and increasing concerns about the transmission of prions, which cause spongiform encephalopathies. Therefore, reducing the risk of transmission of known as well as possibly emerging bloodborne diseases has become one of the most important considerations for government regulators (the Food and Drug Administration [FDA] in the United States) and for the plasma fractionation and protein therapeutics industry. Multiple safety steps are used during the purification of therapeutic IgG concentrates from blood. These steps can be summarized as falling into four major categories, summarized in Box 1.

Most of the plasma used for production of therapeutic proteins such as ISG is obtained specifically for this purpose by plasmapheresis and is termed "source" plasma, although some plasma from donations of whole blood (recovered plasma) still is used as well. All products marketed in the United States must be made from plasma obtained in the United States. FDA regulations governing donor selection and plasma collection are available on the Internet [4,5]. Donors must complete a questionnaire, undergo a physical examination, and have normal blood counts and liver function tests before use of their plasma is considered. Units of plasma are tested for serologic markers of known bloodborne diseases and are discarded if found positive. Plasma services use a "quarantine" or "inventory hold" procedure in which an individual unit, even if it tests negative for all know pathogens, is stored separately until the donor returns and provides another unit of plasma. Only when the second (and subsequent donations) also tests negative can a previously obtained unit be used. Computerized databases enable the tracking of all products derived even in part from any given plasma donation. Patients

Box 1. Steps used to minimize risk of transmission of bloodborne diseases by immune serum globulin

Plasma collection
Food and Drug Administration supervision of donor centers
Donor screening/deferral
Donor testing (liver function tests)
Inventory hold

Manufacturing process
Good manufacturing practices and quality assurance
Process validation
"Minipool" testing
Food and Drug Administration approval of lot release

Specific steps for viral inactivation/removal
Cold ethanol precipitation and depth filtration
Heat (pasteurization at 60°C)
Low pH
Treatment with pepsin or other proteases
Fatty alcohol/fatty acid treatment
Solvent/detergent treatment
Nanofiltration

Record keeping/recall notification

receiving blood-derived proteins are encouraged to keep careful records of the lot numbers and names of all products they receive. If a donor ever is determined to have been incubating an undetected, potentially bloodborne disease (eg, a slow-virus encephalopathy or emerging viruses such as hepatitis C in the early 1990s, or West Nile virus more recently), "lookback" programs can be activated so that any patient who received a product containing plasma from that donor can be identified, examined and tested, and treated if necessary.

The manufacturing processes of all ISG products currently marketed in the United States include at least one step of the cold-alcohol fractionation process developed by Cohn and his colleagues in the early days of World War II [6]. Different manufacturers then use different purification steps, including differing column chromatography protocols, to produce final products that are highly enriched (> 95%) in IgG. Most products are essentially free from IgM, but all contain at least small amounts of IgA. The properties of ISG products currently marketed in the United States are summarized in Table 1. All products currently available in the United States contain more than 95% IgG, and all contain all subclasses of IgG in approximately the same ratio of concentrations as in normal plasma. Testing for the spectrum

Table 1
Properties of polyclonal immune serum globulin products currently marketed in the United States

Product	Manufacturer	IgG concentration	IgA concentration	Excipients	Viral safety
Products intended for intravenous use					
Carimmune NF	CSL-Behring	powder[a]	0.72 mg/mL	sucrose	low pH, pepsin, 35 nm NF
Flebogamma DIF	Grifols	5%	< 0.05 mg/mL	50 mg/mL sorbitol; < 3 mg/mL PEG	pasteurization, S/D, 20 nm NF
Gammagard S/D	Baxter	powder[b]	< 2.2 μg/mL	3 mg/mL albumin; 22.5 mg/mL glycine; 20 mg/mL glucose; 2 mg/mL PEG; 8.5 mg/mL NaCl	S/D
Gammagard Liq.	Baxter	10%	37 μg/mL	250 mM glycine	pH 4, S/D, NF
Gamunex	Talecris	10%	46 μg/mL	200 mM glycine	pH4, caprylate
Octagam	Octapharma	5%	< 0.2 mg/mL	10% maltose	S/D, pH 4
Privigen	CSL-Behring	10%	< 25 μg/mL	250 mM L-proline	pH4, NF
Products intended for subcutaneous use					
Vivaglobin	CSL-Behring	16%	< 1.7 mg/mL	0.3 g/L NaCl 250 mM glycine	pasteurization fatty alcohol/low pH
Products Intended for intramuscular use					
Gamastan	Talecris	16%	NL	300 mM glycine	S/D

Abbreviations: NF, nanofiltered; NL, not listed; PEG, polyethylene glycol; S/D, solvent/detergent.

[a] May be reconstituted to 3%, 6%, 9%, or 12% solution.

[b] May be reconstituted to 5% or 10% solution. Data given for 5% solution.

and titer of multiple different specific antibodies in any given lot of a product is not required; federal standards mandate only that a minimal titer of antibody against measles virus be present. The current FDA guidelines for licensing IGIV preparations are available on the Internet [7]. Similar standards have been applied for the licensure of ISG intended for administration by the intramuscular and subcutaneous routes. Most of the products currently marketed in the United States have been licensed while these guidelines have been in effect.

IgG molecules in concentrated solutions tend to aggregate, which brings their crystallizable fragments (Fc) into close proximity. These Fc portions can activate complement and cross-link Fcγ-receptors, leading to the production of mediators that cause adverse reactions during IgG infusions. All the currently available ISG products contain excipients such as amino acids or sugars that are included to minimize the formation of aggregates and preserve the IgG in its monomeric state. These excipients differ in different products, as listed in Table 1. The use of products with certain excipients may be inadvisable in specific patients (eg, sucrose in patients at risk for renal damage [8], products containing proline in patients who have disorders of metabolism of that amino acid, products containing maltose in diabetic patients whose glucose monitors might give false readings because of that sugar) [9]. Many products are treated at low pH, and some are bottled at low pH as well. The buffering capacity of the low-pH solutions is limited, however, and low pH has not been problematic. None of the products currently marketed in the United States, even those intended for intramuscular or subcutaneous use, contain thimerosal or any other preservative. Some products require refrigerated storage, but others have been shown to have satisfactory stability at room temperature. All should be brought to room temperature before administration. The content of salt and the concentration of IgG itself vary as well, and not all products are licensed for administration by all routes. Thus, the selection of the most appropriate product must be individualized for each patient. Despite the use of large donor pools, the spectrum of antibodies, concentrations of certain specific antibodies, and the presence of trace amounts of other plasma proteins in different preparations also differ. These differences may lead to unpredictable and idiosyncratic differences in tolerance of different products by individual patients. Therefore, IgG products should not be considered as interchangeable generics. Physicians should be notified and may want to slow the rate of infusion, administer premedications, and/or give the IgG under close observation if a patient must be given a product he or she has not received previously.

Minimizing the risk of bloodborne pathogen transmission

A dilemma arises in assuring the absence of bloodborne pathogens in IgG preparations, because the IgG itself may complex with and impair detection

of viruses and other pathogens without actually inactivating them. Complexes of virus particles with bound IgG usually have chemical properties that differ from the viruses themselves. Thus, the presence of antibodies against a virus may cause partitioning of the virus-antibody complex away from the product during purification steps that would not remove the uncomplexed virus itself. This phenomenon probably contributed to the transmission of hepatitis C when plasma containing antibodies to that virus was excluded from pools used to manufacture ISG at a time when sensitive tests for the virus itself were not available. To avoid this problem, animal viruses and prions that have properties similar to important human pathogens but against which humans are not likely to have antibodies are "spiked" into units of donor plasma that then are run through scaled-down production steps to assess the ability of each step to remove and/or inactivate the virus [10]. These viruses are listed in Table 2 together with the human viruses they are intended to simulate. Note that significant removal of many intact viruses can be accomplished by the Cohn cold-alcohol precipitation steps used for the initial purification of the IgG-containing fraction of plasma. The two major targets for viral inactivation are the protein coat of nonenveloped viruses and the lipid envelope of enveloped viruses. In general, surface proteins of nonenveloped viruses are more sensitive to inactivation by low pH, proteolytic enzymes, and heat (pasteurization) than are the lipid and protein structures of enveloped viruses. Inactivation of enveloped viruses generally requires dissolution of the lipid envelope, which is accomplished in some products by treatment with fatty acids, fatty alcohols, or solvent/detergent combinations such as tri-(*N*-butyl) phosphate/Triton X-100 [11]. Many products also are processed by passage through filters with nanometer pore sizes that can remove many virus particles by size, regardless of their chemical characteristics.

Table 2
Viruses used to test efficacy of viral removal/inactivation steps during IgG preparation

Virus	Nucleic acid	Size (nm)	Human virus modeled
Enveloped viruses			
HIV-1	RNA	80–100	HIV 1 & 2
Pseudorabies	DNA	120–200	Herpes
Bovine viral diarrhea	RNA	50–70	Hepatitis C
West Nile virus	RNA	50–70	West Nile
Nonenveloped viruses			
Encephalomyocarditis	RNA	25–30	Hepatitis A
Minute virus of mice	DNA	18–24	Parvovirus B19
Reovirus	RNA	70	Rotavirus
Porcine or bovine parvovirus	DNA	20–30	Parvovirus B19
Prion (agent of Creutzfeldt-Jacob disease)			
Rodent-adapted hamster scrapie			

Who needs it?

Assessing the need for IgG replacement or augmentation in any given patient usually requires an in-depth understanding of the patient's condition and underlying diagnosis. In patients who have confirmed diagnoses known to result in severe antibody deficiency, such as severe combined immunodeficiency, X-linked agammaglobulinemia, Wiskott-Aldrich syndrome, and hyper-IgM syndromes, the diagnosis alone may provide sufficient grounds to initiate IgG replacement therapy as soon as it is established. The pattern of infections, family history, physical examination, and flow cytometry usually will lead readily to a diagnosis that can be confirmed by molecular analysis of the patient's mutation [12,13]. For patients who present with recurrent infections or with symptom complexes that may or may not be caused by infection, the decision to start IgG therapy should be based on laboratory data demonstrating the antibody deficiency and a deficient response to appropriate vaccines, as well as on evidence for increased morbidity caused by infection. The use of measurements of specific antibody titers and vaccine responses to help determine whether IgG supplementation may be indicated for a given patient has been discussed extensively elsewhere [14–16] and is beyond the scope of this article. Guidelines for the diagnosis and management of PID and the use of IgG replacement in antibody-deficiency diseases have been promulgated by the Immune Deficiency Foundation and the Joint Council on Allergy, Asthma and Allergy in the United States (JCAAI) [12,13] and presented at a meeting of the European Society for Immune Deficiency [17]. A very helpful scheme abbreviated from the latter is reproduced in Box 2.

In many patients the antibody deficiency may be only transient. These patients include very low birth weight babies, infants with delayed development of the full spectrum of necessary humoral responses, older children and adults who have been given cytotoxic chemotherapy for cancer, and patients who have been given cytotoxic or immunosuppressive therapy for autoimmune disease and/or to prevent transplant rejection. Nearly all patients who receive hematopoietic stem cell transplants for severe combined

Box 2. Guidelines for IgG replacement/augmentation presented at the 2006 meeting of the European Society for Immune Deficiency

When is IgG replacement/supplementation indicated?

IgG < 200 mg/dL: All patients
IgG 200–500 mg/dL: If a specific antibody deficiency is identified and frequent infections are documented.
IgG > 500: If a specific antibody deficiency identified and severe/recurrent infections are documented

immunodeficiency require IgG supplementation for at least a year. Many recipients of stem cell transplants have poor B-cell engraftment or function and require replacement therapy for life. Patients, particularly children under 5 years of age, who do not have confirmed genetic diagnoses of PID disease should be re-evaluated periodically to determine if IgG replacement/augmentation is still necessary. Re-evaluation typically is performed after the patient has been off IgG therapy for at least several months and usually involves measuring the specific antibody response after administration of protein and polysaccharide vaccines. There are no commercially available preparations of enriched IgA or IgM, so patients who have isolated IgA deficiency generally are not considered candidates for IgG supplementation. Patients who have significant morbidity associated with low or absent IgA levels should be checked for specific IgG antibodies and vaccine responses, however, because these conditions may coexist as part of a broader immune deficiency. Because most laboratories use very broad ranges for ''normal'' IgG levels, patients who do not meet rigorous criteria for common variable hypogammaglobulinemia (also termed ''common variable immune deficiency'') still may benefit from IgG supplementation. Conversely, patients who have IgG levels below a laboratory's normal ranges for the age of the patient do not necessarily require IgG replacement therapy if they have satisfactory specific antibody concentrations and vaccine responses.

Many patients who do not have antibody-deficiency diseases receive IGIV for its immunomodulatory and/or anti-inflammatory properties. The most common such condition for which IGIV is used in pediatrics is Kawasaki syndrome [18]. IGIV also is used in toxigenic bacterial infections and in idiopathic thrombocytopenic purpura (ITP), Guillain-Barre syndrome, chronic idiopathic demyelinating polyneuropathy (multifocal motor neuropathy), and other autoimmune diseases. A recent evaluation of the evidence for the use of IGIV in different conditions is available elsewhere [19] and is beyond the scope of this article.

Alternatives to immune serum globulin therapy

Patients who have confirmed antibody deficiency may be maintained without IgG replacement for periods of time, using relative isolation and/or prophylactic antibiotics. If exposed or potentially exposed to viral pathogens (eg, in a local outbreak of chickenpox or mumps or when traveling to a developing nation), such patients may be given hyperimmune (eg, Varicella-zoster immune globulin) or standard intramuscular ISG preparations. This approach may be satisfactory for patients who reasonably can be expected to overcome a developmental delay in antibody production or to recover from the effects of a treatment regimen that has been completed. This approach should not be considered a long-term strategy for patients who have clearly diagnosed PID disease. IGIV has been found to be helpful in certain HIV-infected infants, but with prenatal treatment reducing the

maternal transmission of HIV and improved antiviral chemotherapy, this setting is not a major use of ISG. In a previous era, plasma, often from a parent or relative, was used for antibody replacement in children who had PID disease. This approach is rarely used in the United States, mainly because of the time and effort required to be sure that the donor plasma is free from any bloodborne pathogens (which maybe clinically unapparent) and the desire to provide a broad range of protective antibodies.

How to administer immune serum globulin: intravenously or subcutaneously?

Although individual doses of ISG may be given intramuscularly, the pain and risks associated with deep intramuscular injections limit the amount that can be administered this way. Routine therapy by the intramuscular route is rarely used now, although it was the mainstay of treatment for PID disease for more than 30 years. Most IgG replacement/augmentation regimens now employ intravenous or subcutaneous administration. In general, doses are in the range of 300 to 800 mg/kg per month (see next section). Intravenous infusions of IgG generally are well tolerated. The introduction of ISG preparations that could be given safely by the intravenous route in the early 1980s was a major advance in the care of patients who have PID disease. Obstacles that had to be overcome included stabilizing the IgG molecules so they would not aggregate in solution and purification to remove traces of proinflammatory molecules such as activators of the kallikrein-kinin and clotting cascades. As noted earlier, a major breakthrough was the recognition of the importance of including excipients such as amino acids and/or sugars in the final preparations. Most adverse reactions to IGIV are related to the rate of infusion. Patients who are naive to IgG replacement, who have had interruptions in their therapy, and/or who are actively or chronically infected have an increased risk of infusion-related adverse effects. These effects may be related, in part, to the formation of antigen-antibody complexes while the IgG is being given and/or the rapid release of lipopolysaccharide or other components of pathogens already present in the recipient. The risk of these reactions may be reduced by making sure patients are afebrile and that active infections are being treated with antibiotics before beginning IGIV therapy or giving any scheduled infusion. Some studies have shown that the incidence of reactions is increased when patients already receiving therapy are given a different brand of IGIV [20]. To minimize rate-related adverse effects, infusions should be started slowly, at rates not above 0.01 mL/kg/minute (equaling 0.5 mg/kg/minute of 5% solution or 1 mg/kg/minute of 10% solution). Vital signs should be checked frequently during IGIV infusions, particularly in naive patients. If the patient remains comfortable and stable, the rate of the infusion may be increased in a stepwise manner, usually at 15- to 30-minute intervals, up to the maximum tolerated by the patient. Most preparations are labeled

for administration at a maximum rate of 0.08 mL/kg/minute (4 or 8 mg/kg/minute of 5% or 10% solution, respectively). Infusion-related adverse reactions frequently mimic the signs of infection, including chills and even rigors, arthralgias and/or myalgias, and headache. Because most of these symptoms are related to the rate of infusion, slowing or temporarily stopping the infusion may allow the symptoms to subside; then the infusion can be resumed at the previously tolerated rate. If these precautions fail to prevent these symptoms, premedication with antipyretics, antihistamines, and/or corticosteroids may help ameliorate the symptoms. During prolonged infusions, such medications may be repeated if necessary. In some cases, patients report systemic reactions including back pain, chest tightness, and a feeling of anxiety or a sense of impending doom, frequently in association with flushing and tachycardia. This type of reaction may resemble anaphylaxis but usually does not involve IgE. Therefore, this type of reaction has been termed ''anaphylactoid.'' A key difference between the anaphylactoid reactions that accompany IGIV infusions and true IgE-mediated anaphylaxis is that the former usually are associated with hypertension, rather than hypotension, as would be expected in true anaphylaxis. True anaphylaxis may occur in patients receiving IGIV, particularly in those who are deficient in IgA but still have the capacity to produce IgE [21]. True anaphylaxis occurs very rarely but may be life threatening. Therefore, any practitioner or facility that administers IGIV should be equipped to treat anaphylaxis if it occurs. The risk of true anaphylaxis can be minimized by screening patients for complete IgA deficiency, by starting initial infusions extremely slowly, by using products with the lowest concentration of IgA, and by testing the patient for IgE-antibodies against IgA if this is a concern. Many IgA-deficient patients also are deficient in other immunoglobulin isotypes and/or in specific IgG antibodies against important pathogens. Such patients should not be denied ISG therapy because of the IgA deficiency, but caution should be used in its administration.

Headaches may occur during or after intravenous infusions and sometimes repeatedly follow the infusions by as much as 48 to 72 hours. These headaches may have the character of migraines and are more common in patients who suffer from migraines independently of their IGIV infusions. In rare cases, headaches following IGIV infusions may be accompanied by meningismus, and aseptic meningitis has been well documented. Headaches sometimes may be prevented by the use of corticosteroids as premedication or for a day or two following the infusion, or by the use of triptans or other migraine treatments. Additional, rare complications of IGIV therapy include transfusion-related acute lung injury, renal failure, and thromboses. A comprehensive review of the adverse effects and complications of IGIV infusions is available and should be read by everyone who administers this form of therapy [22]. Quite often, patients who experience headaches or other adverse effects with one brand of IGIV may tolerate another with no problem. Therefore, switching products may be indicated to obviate the

adverse effects. Conversely, because not all products are tolerated equally by a given patient, substitutions should be made carefully, and under supervision. Generally when switching products, slow infusion rates should be used, at least initially.

The pharmacokinetics of IGIV have been well described [23]. By the end of an intravenous bolus of IgG, the IgG is mostly intravascular, and its concentration can be expected to rise by 100 to 200 mg/dL for every 100 mg/kg given. It thus is common for peak serum IgG concentrations to rise by as much as 1000 mg/dL following doses in the conventional replacement range (300–800 mg/kg). Over the next 48 to 72 hours, the IgG becomes distributed into the total extracellular fluid volume, and the serum concentration may drop by 25% to 40%. After this re-equilibration, IgG is catabolized with first-order kinetics and has a half-life of around 22 days. Currently marketed ISGs contain intact IgG molecules that have not undergone any chemical modification. Thus, the distribution and half-life of intravenously administered IgG is essentially identical to that of endogenously produced native IgG [24–27]. For this reason, most intravenous regimens repeat doses at 21- to 28-day intervals. Depending on the dose and whether there is any endogenous IgG production at all, dosing intervals of 28 days or longer frequently leave patients with serum IgG concentrations in the range of 400 to 500 mg/dL, or even less, at the end of the dosing interval. Many patients report flulike symptoms, increased fatigue, and/or malaise when the trough serum IgG level falls so low, and they may have increased susceptibility to infection at that time. In that situation, the dose may be increased or the interval shortened. Higher doses and/or shorter intervals also will be necessary in patients who have gastrointestinal or renal protein loss and/or increased catabolism of IgG, as discussed later.

Although many patients tolerate long-term intravenous IgG replacement regimens with no problems, surveys show that a high proportion of patients describe the conditions under which they receive their infusions as inconvenient, because they must travel to a hospital or infusion center, miss school or work, and/or experience difficulty with intravenous access. Subcutaneous regimens offer freedom from those concerns. Bruton treated the first agammaglobulinemic patient to be reported with subcutaneous injections of ISG, but other investigators used intramuscular injections, which became the standard of care for nearly 3 decades. In the early 1980s, the use of small, battery-powered syringe driver pumps to give subcutaneous infusions of 16% ISG intended for intramuscular use over several hours was described [28–30]. This method of administration is free from the pain associated with the deep intramuscular injections and was much better tolerated by the patients, so higher doses could be administered routinely [31]. In general, 25- to 27-gauge needles extending 6 to 11 mm under the skin are inserted perpendicularly. A wide variety of special needles and infusion sets are now available specifically for IGSC therapy [32]. Depending on the size of the patient, 5 to 20 mL are delivered per site, usually over 2 hours or

so. Several sites may be used for each infusion of ISG, so weekly dosing is a common regimen. Frequently used sites include the abdomen, inner or anterior thighs, and the backs of the arms. In general any site at which one can "pinch an inch" is acceptable for subcutaneous infusions of 10 to 20 mL of ISG. A very nice illustration of potential subcutaneous infusion sites and good technique for inserting subcutaneous needles is available on-line from the nursing staff of the National Institutes of Health Clinical Center [33]. The author and colleagues have found that the size of the patient and the time over which the infusion is given are important factors in determining the maximum volume that can be infused at any given site. Thus, some patients may give themselves 40 to 60 mL of ISG in a single site, over a period of time as long as 8 hours or more. Some patients prefer this type of regimen and take their IGSC while they sleep. In contrast, other patients may prefer to take small doses of IGSC on a daily basis (eg, by pushing 10 mL into a single site over several minutes). In a series of 20 patients using 15% or 16% ISG preparations reported from UH/Rainbow Babies & Children's Hospital, most of the regimens were within the parameters of 0.12 to 0.24 mL/kg/site/hour [34] Basically, once the monthly dose of IGSC has been calculated, a wide variety of schedules may be used to tailor the infusion regimen to the patient's preferences.

Although most physicians and patients in the United States adopted the intravenous route when preparations that could be administered intravenously became available, patients in other countries and those who experience severe adverse effects from intravenous preparations continue to use the subcutaneous route [35,36]. Serious systemic adverse effects are rarely a problem with subcutaneous infusions; most studies have reported that less than 1% of infusions are associated with systemic adverse events [37]. Because the expertise and experience necessary to establish and maintain intravenous access are not required for subcutaneous administration, and because of the low risk of serious reactions, subcutaneous infusions usually are administered at home by the patients themselves or their parents or partners. The major disadvantage of subcutaneous infusions is the limitation on the volume of ISG that can be given at one time. Because the infusions can be given at home and/or while the patient is performing other activities, most patients readily adapt to weekly or more frequent infusions. The division of the monthly dose of ISG into weekly or even more frequent infusions and the slower absorption of IgG into the circulation from the subcutaneous site than from intravenous boluses tends to flatten out the curve of serum IgG concentration over time, as seen in Fig. 1. Elimination of the high peaks and low troughs associated with intermittent intravenous boluses ameliorates most infusion-related adverse effects and also the feelings of malaise and fatigue associated with the low troughs. Subcutaneous infusions frequently are associated with local swelling, redness, and an itching or burning sensation, but these effects are rarely serious and usually subside over several hours. If the patient continues with subcutaneous infusions, these local

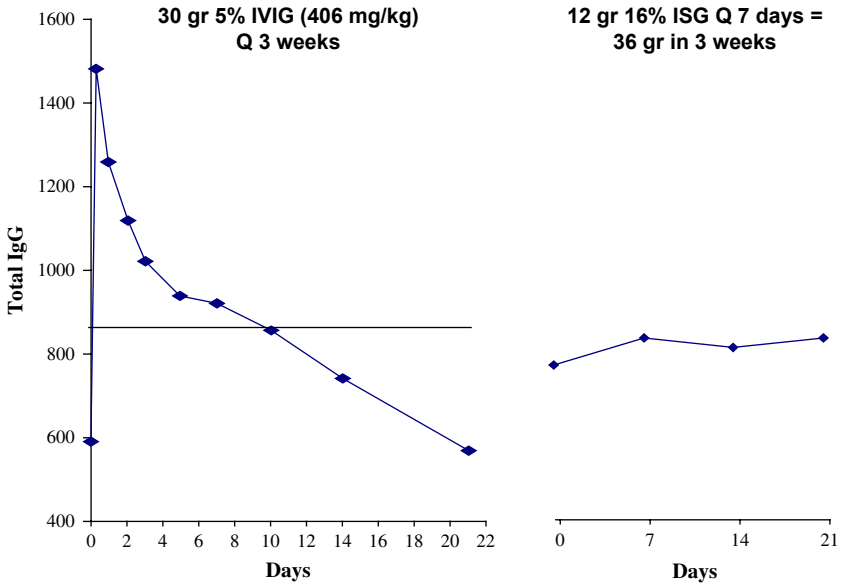

Fig. 1. Serum IgG concentrations in a 34-year-old man who has X-linked agammaglobulinemia. (*Left panel*) The diamonds and blue line indicate serum IgG concentrations at various times after a single intravenous infusion of 30 g (406 mg/kg). The black line indicates the average daily IgG level calculated by interpolation from points indicated by diamonds. Mean ± SEM = 850.6 ± 43 mg/dL. (*Right panel*) Serum IgG concentrations in the same patient receiving 12 g 16% IgG every 7 days. Mean ± SEM = 816 ± 16 mg/dL. (*From* Berger M. Subcutaneous immunoglobulin replacement in primary immunodeficiencies. Clin Immunol 2004;112:1–7; with permission.)

reactions tend to lessen with time, but the reasons for this change are not known. Although many patients have used the subcutaneous route for more than 25 years, chronic changes, fibrosis, or lipodystrophy at the infusion sites have not been problematic. It is rare to be able to identify the site at which a subcutaneous IgG infusion was given more than 24 hours after the infusion has been completed. Because it is easier for the patients to adjust their infusion schedules to their school or work schedule, rather than vice versa, and because patients no longer have to travel to infusion centers or the hospital for their treatments, many patients find that home IGSC regimens increase their sense of independence and autonomy. In turn, this response results in improved quality of life experienced by many, but not all, patients [37–40].

At present, only one ISG preparation marketed in the United States is licensed for subcutaneous administration. Small series and anecdotal reports, however, suggest that most preparations licensed for administration by other routes are well tolerated when administered by the subcutaneous route. Several preparations are marketed for subcutaneous use in Europe, and it is likely that additional products with concentrations as high as

16% or even 20% will be available in the United States in the next few years. A comparison of the volumes required to give comparable amounts of IgG by the subcutaneous and intravenous routes using currently available preparations is given in Box 3, along with some sample subcutaneous regimens.

Box 3. Comparison of intravenous and subcutaneous dosing

1. For a 20-kg 6-year-old receiving 500 mg/kg per month = 10 g IgG
   = 200 mL of 5% intravenous solution
   = 100 mL of 10% intravenous solution
   = 62.5 mL of 16% subcutaneous solution
2. In subcutaneous treatment regimens using the unit dose approach to deliver approximately 10 g IgG per month to a 20-kg 6-year-old child, the volume of 16% solution required = approximately 62.5 mL
   = 16 mL/week (one 10-mL and two 3-mL bottles of 16% solution) administered as one infusion into two or three sites = 2.56 g/week = 10.24 g/month
   = 10 mL (one 10-mL bottle of 16% solution) administered every fifth day (six times/month) into one or two sites = 9.6 g/month
   = 6 mL (two 3-mL bottles of 16% solution) administered every third day as one infusion into one site = 0.96 g/dose = 9.6 g/month
3. In subcutaneous regimens for a 30-kg-child receiving approximately 15 g/month, the volume of 16% solution required = approximately 94 mL
   = 10 mL/week (one 10-mL bottle) administered every third day into one or two sites = 16 g/month
   = 20 mL administered once a week into two or three sites, with one extra infusion per month = 16 g/month
   = 3 mL administered daily into one site over 15 minutes (''push'') = 14.4 g/month
4. In subcutaneous regimens for a 40-kg-child receiving approximately 20 g/month, the volume of 16% solution required = 125 mL
   = 30 mL/week administered into one or two sites once a week = 4.8 g/week = 19.2 g/month
   = 20 mL administered every fifth day (six times per month) into one site = 19.2 g/month
   = 16 mL administered every Saturday and Sunday into one or two sites = 20.5 g/month
   = 10 mL delivered every Monday, Wednesday, and Friday (weekends off) by push or into one site = 19.2 g/month

How much to give

The efficacy of IgG replacement in patients who have PID disease has been clearly demonstrated in studies going back to the 1950s and 1960s. A recent survey documented the decrease in the incidence of pneumonia after patients who had PID disease were put on IGIV treatment [41]. The doses of IgG given in standard replacement regimens has increased over time, as more convenient routes of administration have become practical. In classic studies in the early 1960s, the British Medical Research Council working group on hypogammaglobulinemia reported that 50 mg/kg/week was more effective than 25 mg/kg/week in preventing febrile episodes, otitis media, and pneumonia, but the differences did not seem to be of sufficient clinical importance to warrant widespread use of the higher dosage [3]. Licensing studies of the first generation of intravenous products in the United States used doses in the range of 100 to 200 mg/kg/month [42–44]. Studies by Roifman and his colleagues [45,46] beginning in the mid-1980s showed that patients receiving doses of 200 mg/kg/month did not achieve trough serum IgG levels higher than 500 mg/dL. In contrast, when the patients were crossed over to the arm that received 600 mg/kg/month, they did sustain trough levels above 500 mg/kg. In turn, the incidence of both major and minor infections was reduced greatly when the patients received the higher dose and maintained higher levels. Similar findings were presented by Roifman and colleagues [47] from a more recent study in which patients were maintained on doses of IGIV selected by their physicians: patients receiving higher doses and maintaining higher serum trough IgG levels had fewer infections. A crossover study comparing higher IGIV doses (600 mg/kg/month in adults and 800 mg/kg/month in children) with ''standard'' doses (300 mg/kg/month in adults and 400 mg/kg/month in children) showed statistically significant reductions in the incidence of infection and cumulative days of illness in the higher-dose groups [48]. Licensing studies of the current generation of IGIV products marketed in the United States have used doses in the range of 400 to 500 mg/kg/month, reflecting the dose the patient had been receiving before entering the study [20,24–27]. These regimens all resulted in an annual incidence of serious bacterial infections (meeting FDA definitions) of 0.1 infection/patient/year or less and an overall incidence of infection of two to four infections per patient per year. The Practice Parameters promulgated by the JCAAI call for maintaining trough serum IgG levels of 500 mg/dL as a useful guideline [13]. This level typically requires doses in the range of 300 to 600 mg/kg/month. In patients who have gastrointestinal and/or renal protein loss, higher doses and/or shorter dosing intervals may be necessary to maintain trough levels above 500 mg/dL and keep the patient free from infection. Higher doses—up to 800 mg/kg/month or even higher—are recommended for patients who have chronic lung and/or sinus disease [13,45–48]. There have been several reports of progressive lung disease and dysfunction in patients who seem clinically free from

pneumonia and who report few or no symptoms of chronic bronchitis or bronchiectasis [49,50]. These reports are consistent with the anecdotal experience of most immunologists who have large numbers of antibody-deficient patients in their practices. Close monitoring, which may include annual high-resolution CT scans of the chest, formal pulmonary function testing, and even bronchoscopy in some cases, is recommended for all patients who have these diseases. In the current era, most experts agree that prevention of pneumonia and serious infection is no longer sufficient indication that the patient's management is optimal. Efforts aimed at normalizing pulmonary function tests, maximizing the patients' ability to participate in a full range of activities, and preventing progressive loss of lung function seem warranted [51]. Preventing and/or arresting chronic lung and sinus disease in patients who have PID disease frequently requires IgG replacement doses higher than 750 mg/kg/month, particularly in patients who already have chronic infection and structural damage before therapy is started. Such patients usually benefit by comprehensive approaches, including intense antibiotic therapy, bronchodilators and/or inhaled corticosteroids, mucolytics, and physical therapy, to improve pulmonary toilet and/or sinus surgery. Patients who have selective or lacunar antibody deficiencies and who do not have severe hypogammaglobulinemia per se still require full doses of ISG replacement to maintain adequate titers of the specific antibodies they cannot produce on their own. Similarly, antibody-deficient patients who have high IgG levels caused by polyclonal B-cell activation, as occurs in systemic lupus erythematosus or HIV infection, and patients who have monoclonal gammopathies actually may have elevated serum IgG levels but still need full doses of ISG replacement to provide the spectrum of antibodies necessary for protection against the pathogens to which they may be exposed.

Some evidence suggests that the bioavailability of IgG is decreased is when it is given subcutaneously instead of intravenously [52]. The tendency toward higher trough levels with weekly subcutaneous treatment may counterbalance any decreased efficacy caused by tissue degradation of the IgG administered by the subcutaneous route, however. Data that would allow determination of the effects of maintaining higher trough levels by fractionating the cumulative dose that otherwise would be given by a single monthly intravenous bolus are sparse. In particular, the relative importance of the high peaks achieved with the intermittent boluses is unclear. Two major efficacy studies of the single ISG product currently licensed for subcutaneous administration in the United States have been performed. In the United States pivotal trial, a dose increase of 37% above the previous intravenous dose was used to meet the FDA requirement that the area under the curve of serum IgG concentration versus time be the same for both routes of therapy [52]. In contrast, a study performed contemporaneously in Europe and Brazil used one fourth of the previous monthly intravenous dose as the weekly subcutaneous dose. In both studies, equal rates of infection were

obtained: 0.04 serious bacterial infections per patient per year, and 4.4 infections per patient per year overall. The range of subcutaneous doses spanned 34 to 352 mg/kg/week in the United States study and 51 to 147 mg/kg/week in the European/Brazil study [53]. These dose ranges and results are quite consistent with those used in licensing trials of intravenous products in the United States in the past few years.

Monitoring therapy

Most patients who have PID disease require IgG replacement therapy for life. It therefore is extremely important to monitor them closely to be sure that (1) the treatment itself is associated with as few adverse events and as little interference with normal activity as possible; (2) the treatment regimen is adequate and maintains control of acute infections as well as chronic complications of the underlying disease; (3) patients do not acquire any bloodborne infections or other long-term complications of their therapy.

Monitoring during infusions

Before beginning any infusion, particularly an intravenous infusion, the patient should be reassessed. It is important to note any changes in medications and signs/symptoms of chronic or acute infection. Adverse effects occurring for up to 72 hours after the previous infusion should be noted. Changes in risk factors for adverse effects also should be noted. For example, starting oral contraceptives or increased cigarette smoking may increase the risk of thrombotic complications of IGIV infusions. Many patients who have PID disease and chronic bronchitis/bronchiectasis have some degree of reversible airway obstruction. If increased secretions and/or bronchospasm are present, bronchodilators may be helpful before or during the infusion. The author and colleagues find it useful to measure and record expiratory flow rates with an office spirometer or even a simple peak flow meter to help with this assessment and to document a patient's status over time. Having the airways as open as possible at the beginning of the infusion may help prevent serious problems if the patient experiences bronchospasm/chest tightness during the infusion. If patients are or recently have been febrile, it usually is helpful to pretreat with antipyretics. If there are signs/symptoms of acute bacterial infection, it may be desirable to defer the infusion for a few days while initiating antibiotic therapy, to avoid shaking chills and severe myalgias/headache during the infusion. The IgG may be needed to help resolve the infection, however, so this delay must be done with caution. Patients who have intercurrent acute gastroenteritis may benefit from antispasmodic or antiemetic treatment before or during the infusion. Some patients may require antipyretics, antiemetics, and/or antimigraine premedication on a routine basis, and some also may require corticosteroids. The author and colleagues often have the latter type of patient take

a dose of steroids orally several hours before the infusion is initiated. The patient's hydration status should be assessed carefully, and it usually is a good idea to record the patient's weight at the time of each infusion to allow comparisons. It is possible that dehydration may increase the risk of renal complications, hyperviscosity, and thromboses. It may be prudent to give patients intravenous fluids before actually starting the IGIV to minimize these risks. Conversely, patients who have or are at risk for congestive heart failure may require deferring the fluid/salt/protein load of an infusion if they have recently gained weight, have increased dyspnea and/or rales on chest examination, and/or have increased peripheral edema. Patients who have congestive heart failure and/or fluid retention from other causes may benefit from the administration of diuretics before, during, or following their infusions. Obviously, monitoring such patients closely during the infusion for signs of dyspnea/fluid retention is important.

Before the infusion begins, vital signs should be recorded. It may be important to be sure that the patient has time to relax and adjusted to the ambient conditions before recording the "baseline" vital signs to avoid the phenomenon of "white coat hypertension" that then might lead to a misinterpretation of decreased blood pressure as the patient relaxes once the intravenous has been placed and the infusion is running. If the patient is comfortable or sleeping, and particularly if the pulse has decreased rather than increased, blood pressure drops of 10% or 20% or even more can be expected and do not necessarily mean that shock is imminent, especially if the patient is not flushed or having dyspnea. Intravenous infusions usually are started at 0.01 mL/kg/minute, and the rates are increased or doubled at 15- to 30-minute intervals to a maximum of 0.08 or 1.0 mL/kg/minute in most cases. Stepwise increases in rates should be made only if the patient is tolerating the infusion well. Therefore vital signs and the patient's condition should be recorded before and 5 to 10 minutes after each rate change. The maximum rate tolerated by the patient at previous infusions should be exceeded only with caution, and it is important to realize that stepwise increases in rates and the maximum infusion rate may vary when the same patient is given different products. Therefore, when any patient must switch products, close monitoring of the patient's condition and vital signs is necessary, because the infusion rates and/or intervals between rate increases may require adjustment.

Subcutaneous infusions of ISG very rarely are associated with systemic adverse effects or significant changes in vital signs. Inadvertent intravascular administration should be avoided by checking to be sure there is no blood return from the needle before actually starting the ISG infusion. Because with most subcutaneous regimens the total monthly dose is fractionated into four or more individual doses, because the IgG is given more slowly, and because adsorption from the subcutaneous site into the circulation is slower than with intravenous infusions, this route may be preferred in patients at risk from cardiovascular, thrombotic and/or renal complications.

Many patients who complain of severe headaches during or following intravenous ISG infusions have less severe problems after subcutaneous infusions, but migraines still may occur, and medication may be required for up to 48 hours after the infusion has been completed. Vital signs usually are not monitored repeatedly during subcutaneous infusions, but the infusion site should be observed by the patient a few times during the infusion. Most subcutaneous infusions are accompanied by swelling with or without redness, and many patients report local pruritus or a burning sensation. These symptoms may be obviated by treatment with antihistamines before or during the infusion. Pretreatment with corticosteroids or antiemetics is rarely needed. One area of special concern with subcutaneous infusions is the risk of cellulitis or other local infection at the infusion sites. Because most patients have swelling and redness, and the injected fluid may lead to a feeling of fluctuance, distinguishing this manifestation from infection sometimes can be difficult. Application of warm compresses or gentle massage may increase local circulation and help dissipate the infused product. In general, most local reactions to IGSC infusions subside within hours after the infusion is completed. Any site at which redness, swelling, or warmth is increasing with time after the infusion should be considered potentially infected and observed very closely or examined by a professional. Patients who self-infuse at home always should be able to contact a physician or nurse on-call. In some cases it may be helpful to ask the patient to mark the size of any local reaction with a pen, so that potential enlargement can be tracked objectively. Although most home-care services and physicians in the United States prescribe preloaded epinephrine injectors to be kept at home, that practice is no longer followed in the United Kingdom [53].

Monitoring adequacy of dose and control of disease

Selection of the appropriate dose has been discussed in a previous section. Selection of the initial dose and monitoring its adequacy over time should be individualized. Certainly freedom from acute bacterial infections would be a readily agreed-upon goal, but many patients who have chronic bronchitis or bronchiectasis and some who seem to have reactive airways actually may be experiencing progressive subclinical lung disease. Similarly, patients may have progressive erosive sinus disease but may have learned to "live with" the symptoms. Monthly measurements of the white blood cell count, sedimentation rate, and C-reactive protein often are used to assess the presence of subclinical infection. In turn, these data are used to adjust ISG and antibiotic therapy. Frequent sputum cultures and physical examinations also may be useful. Although the practice parameters of the JCAAI suggest that 500 mg/dL is a suitable target serum IgG level, patients should be treated more according to their clinical condition than to achieve any designated level. Nevertheless, monitoring serum IgG levels at routine intervals is important for several reasons. They may serve as markers for adequacy of

therapy and enable comparison of one regimen with another. Thus, in patients who experience exacerbations of underlying infection and/or chronic nonspecific symptoms when the IgG trough level falls below a certain value during treatment with intermittent IGIV infusions, that value may serve a target for subcutaneous therapy. Monitoring the serum IgG level also helps assess whether a patient is having increased gastrointestinal or renal protein loss and requires a higher dose or shorter dosing interval to maintain protection against infections. In patients who do not have severe hypogammaglobulinemia per se and/or who actually have elevated serum IgG levels because of monoclonal gammopathy or nonspecific polyclonal B-cell activation, monitoring the trough levels of specific antibody titers (eg, against pneumococcal polysaccharides) may be preferable to using the total IgG level for monitoring the adequacy of therapy.

Besides control of infection, it is important to remember that patients who have common variable immunodeficiency and some other PID diseases are at greater risk for developing malignancies and autoimmunity. Thus, part of the routine monitoring of all such patients, even if they are free from infection, should include careful review of the interval history and physical examination. In addition, selected patients may require radiographic and/or radionuclide studies at regular intervals. In many cases, the immunologist sees the patient much more frequently than any other physician. Sometimes the immunologist, by default, becomes the principal caregiver for the patient and must be diligent to be sure that routine health screening/maintenance is not neglected by the patient's primary care provider, if there is one. Clear communication is required to make sure that developmental and lead screenings are being performed on young children and that monitoring of serum lipids and other risk factors for cardiovascular disease, common malignancies (ie, mammograms, stool guaiac testing, prostate examinations, among others), and bone density is being performed as appropriate in adult patients. Bone density monitoring may be particularly important in postmenopausal women and even in some men who chronically require oral or high-dose inhaled corticosteroids.

Monitoring for complications of therapy with blood products

Because all ISG products are prepared from large pools of plasma, and there are always threats of emerging diseases and of lapses in standard safety procedures, patients should be monitored for any sign of development of a potential bloodborne disease, including spongiform encephalopathies. Although there have been reports of chronic, slowly progressive neurodegenerative disease in patients receiving IGIV therapy for PID disease, it is not clear whether these conditions might represent chronic viral infections of the central nervous system or other complications of the PID disease per se [54]. Transient Coombs' positivity has been reported with certain IGIV preparations, but clinically significant hemolytic anemia is rare as

a complication of IGIV therapy [22]. Patients who have PID disease, especially those who have common variable immunodeficiency and hyper-IgM syndromes, also may develop hematologic and/or hepatic abnormalities resulting from their underlying disease or treatment with other medications. Obviously it is important to follow renal function in patients receiving repeated therapeutic infusions of fluid and protein. Careful initial characterization of the patient's baseline, including documentation of hematologic, hepatic, and renal function, and developmental screening thus are essential. In patients who do have some antibody production, tests for exposure to Epstein-Barr virus and cytomegalovirus, as well as HIV and viral hepatidies should be documented before therapy is begun because the presence of passively acquired antibody may make this documentation more difficult. Documentation of a patient's baseline virologic status may have important clinical and medicolegal implications in the future. To monitor for complications of therapy, the complete blood cell count, hepatic and renal function tests, and urinalysis should be repeated every 6 to 12 months. To monitor for bloodborne and other infectious diseases, nucleic acid tests such as polymerase chain reaction or reverse transcription polymerase chain reaction, when available, are preferred to serologic screening tests, because patients who have PID disease generally are antibody deficient and may not produce antibody as expected after exposure to a pathogen. These tests probably should be repeated once a year.

Hyperimmune and other specific immune globulins

Besides standard preparations of polyclonal ISG, several special hyperimmune globulins are available. These are listed and described in detail elsewhere [55]. In general, hyperimmune globulins are prepared from the plasma of individuals who have been exposed accidentally (eg, to snake or other venoms), are convalescing from specific infections (eg, chickenpox), or whose plasma has been found on testing to contain high titers of certain desirable antibodies. For a few antigens, plasma is drawn from persons specifically immunized against unusual but potentially important pathogens such as vaccinia or from healthy normal donors repeatedly immunized with common vaccines such as tetanus toxoid. Most pediatricians are familiar with Varicella zoster immune globulin and with the use of anti-Rh(D) to prevent alloimmunization of mothers. Guidelines for plasma donations from which these products are produced and the steps used to assure that they are free from bloodborne pathogens are the same as for standard polyclonal ISG products, as discussed previously.

Unanswered questions

Although great progress has been made since the painful intramuscular injections of ISG in the 1950s and 1960s, many questions about the

characteristics and uses of ISG remain unanswered. Important issues about the mechanisms of action of polyclonal IgG in inflammatory and autoimmune diseases remain, and it is not clear to what extent specifically modified or monoclonal preparations might obviate the use of polyclonal IgG for those conditions. Reports describing the spectrum of antibodies and the appropriate uses of ISG for prevention and/or management of influenza are scarce, and it is even less clear what will be done if new pandemic strains emerge. As the population of plasma donors shifts from those who have recovered from natural infection with measles, mumps, and similar viruses in childhood to those who were immunized and never had the wild-type disease, antibody levels in ISG preparations are changing. Concerns have been raised about how well the protection of antibody-deficient individuals will be maintained. On the other hand, a steadily increasing number of new vaccines has been introduced in recent years, now including meningococcal conjugate vaccine and human papilloma virus vaccine. Studies of the antibody content and efficacy of standard ISG preparations in preventing those infections are not available. In addition, with the experience of rapid, worldwide transmission of severe acute respiratory syndrome and West Nile virus infection in recent years, the threat of emerging diseases is no longer an exotic science fiction scenario. Thus, production of ISG and other blood-derived products and optimal care of patients who have PID disease will continue to evolve.

Summary

Advances in the large-scale production of polyclonal ISG preparations during the last 2 decades have greatly improved the management of patients who have PID disease. The continued development of products with improved safety and tolerability profiles has allowed treatment to focus on quality of life and long-term freedom from the complications of PID disease rather than just on freedom from severe acute infections and survival. Currently available ISG preparations allow routine therapy by a variety of routes and regimens that can be tailored to suit any individual patient. Continued vigilance is required, however, because problems with emerging diseases and the costs and availability of ISG are likely to present continuing challenges.

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Advances in Hematopoietic Stem Cell Transplantation for Primary Immunodeficiency

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Primary immunodeficiencies (PIDs) are a genetically heterogeneous group of diseases affecting distinct components of innate and acquired immunity including the development and function of complement proteins, phagocytes, dendritic cells, natural killer cells, and T and B lymphocytes. More than 120 gene defects have been described [1], giving rise to more than 150 disease phenotypes. The most severe defects causing absent or disordered function of T lymphocytes (and sometimes B lymphocytes and natural killer cells) are known collectively as "severe combined immunodeficiency" (SCID); without treatment patients usually die by 12 to 18 months of age. Other T-lymphocyte immunodeficiencies may present later. Defects in innate immunity may present in early infancy, but prophylaxis has meant that many patients survive until early adulthood. Although patients may do well on prophylactic medication, it is becoming clear that the long-term outlook for such patients is poor, with many patients dying from complications caused by infectious or inflammatory sequelae or malignancy in early adulthood. Since the first bone marrow transplant for SCID in 1968, the number of patients for whom hematopoietic stem cell transplantation (HSCT) is considered has grown significantly, and the range of indications for HSCT also has widened. This article considers how more precise diagnosis as a result of increasing knowledge of the molecular causes of PID, together with registry data on the natural history of the diseases, in conjunction

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with more successful HSCT techniques, has meant that HSCT now is a successful curative treatment option for a large number of disorders hitherto amenable only to supportive care.

Natural history of primary immunodeficiencies

As genetic testing becomes more widely available, more patients are being diagnosed with PID. Increasingly, disease caused by hypomorphic genetic mutations presenting atypically with less profound immune defects are being recognized [2,3]. It is important to try to identify an underlying genetic defect, because this identification may help ascertain future prognosis and therefore which treatment to offer. In conjunction with this identification, registry data from large cohorts of patients give more precise information for the long-term outlook of patients with a given disease. Important registries that have been published include those for patients who have X-linked lymphoproliferative disease [4], chronic granulomatous disease (CGD) [5], CD40 ligand deficiency [6], Wiskott-Aldrich syndrome (WAS) [7], and severe congenital neutropenia [8]. Such registries also can give information about phenotype–genotype correlations, if any, that also can guide treatment.

Hematopoietic stem cell transplantation

The first HSCT for SCID was performed in 1968. Subsequently, many more patients have undergone transplantation for SCID and for an expanding number of other PIDs. HSCT still is often viewed as a risky procedure, with a significant morbidity and mortality, and historically, in the context of hematologic malignancy, it has been used as a treatment of last resort. For SCID, in which there are no other effective treatments, HSCT is the conventional treatment, although gene therapy may have a role in some SCID genotypes, albeit with potential adverse events [9,10]. When prophylactic treatments are available, however, the risk of HSCT for patients who have other PIDs, such as CGD, previously was thought to be too high to recommend routinely [11]. For PID, successful HSCT usually brings cure; for most patients, regular, long-term prophylaxis can be discontinued, and life quality improves dramatically. Combined multicenter European results showed an overall 5-year survival rate of 56% for patients who had undergone transplantation before 1995 [12]. Since 2000 overall survival for patients undergoing HSCT for SCID is 71% (P. Landais, personal communication, 2007). For patients who have other immunodeficiencies, survival after transplantation before 1995 was 55%; survival rates now have risen to 74% overall, with even better results (90% survival) with well-matched donors for defined conditions such as CGD. In the light of this experience, should HSCT now be considered the treatment of choice for the majority of PIDs? If so, what evidence supports this assertion? To answer these

questions, it is necessary to look at the advances there have been in transplantation techniques that give such good survival rates (Box 1).

Factors contributing to improved survival after hematopoietic stem cell transplantation

Refined HLA tissue typing

During the last 20 years tissue typing has become more refined. Increasingly patients now are matched at an allelic level using molecular DNA techniques rather than less accurate serologic techniques. Patients who received hematopoietic stem cells (HSCs) from a serologically matched unrelated donor actually can have a significant degree of mismatch when examined at the molecular level, because the anti-HLA antibody used in serologic typing fails to distinguish differences between some peptide groups (Fig. 1). This refinement probably accounts for the recent improvement in matched unrelated-donor transplant results for patients undergoing transplantation for both SCID and other PIDs [12,13]. Indeed HSCT with a molecularly matched or nearly matched donor may give results as good as those using an HLA-identical sibling [14].

Reduced-intensity chemotherapy conditioning

Prior to infusion of HSC for the treatment of PID, many centers give chemotherapy (so-called ''conditioning'') to create marrow space and destroy host cells that otherwise might reject an incoming graft. For many years myeloablative therapy with busulfan and cyclophosphamide was used. Significant toxicities are seen after giving these drugs, including

Box 1. Factors contributing to improved survival after hematopoietic stem cell transplantation for primary immunodeficiencies

Molecular HLA tissue typing
Reduced-intensity chemotherapy conditioning
Improved viral detection and treatment
Improved fungal detection and treatment
New treatments for graft-versus-host disease
New treatments for veno-occlusive disease
Increased use of cord blood as a stem cell source
Graft engineering
- Positive hematopoietic stem cell selection
- Allo-depleted T-cell infusions
- Viral antigen–specific cytotoxic T-cell infusions

A. Patient:	**A2**	**A3**	**B62**	**B60**			**DR9**	**DR11**		
Donor:	**A2**	**A3**	**B62**	**B60**			**DR9**	**DR11**		
B. Patient:	**A02**	**A03**	**B1505**	**B4023**	**Cw7**	**Cw10**	**DRB1 0901**	**DRB1 1101**	**DQB 0301**	**DQB 0302**
Donor:	**A02**	**A03**	**B1501**	**B4001**	**Cw1**	**Cw10**	**DRB1 0901**	**DRB1 1101**	**DQB 01**	**DQB 0303**

Fig. 1. Serologic and molecular DNA HLA tissue typing of the same donor and recipient pair. (*A*) Serologic typing showing HLA matching of donor and recipient. (*B*) High-resolution molecular typing of HLA class 1 molecules showing allelic mismatching (*double underlining*) at both HLA-B loci and antigen mismatching (*single underlining*) at one HLA-C locus and high-resolution molecular typing of HLA class 2 molecules showing an antigen mismatch (*single underlining*) at one HLA-DQ locus and an allelic mismatch (*double underlining*) at the other HLA-DQ locus. (*Courtesy of* The Paediatric Immunology Unit, Newcastle General Hospital, Newcastle upon Tyne, UK; with permission.)

mucositis, hepatic veno-occlusive disease, and pneumonitis in the immediate post-HSCT period and markedly decreased fertility and a risk of lung dysfunction in the long term. Although most patients undergoing transplantation for PID do not undergo radiotherapy before transplantation, significant morbidity may be seen from the chemotherapy used in transplantation. The use of reduced-intensity chemotherapy (RIC) conditioning regimens, using agents such as fludarabine, melphalan, and treosulphan as well as anti-CD45 monoclonal antibodies, has meant that patients who have significant pre-existing end-organ damage to liver and lungs can undergo HSCT successfully with good engraftment and relatively few complications. Overall survival is much improved, with 93% in one series versus 60% for those undergoing myeloablative conditioning [15]. Donor engraftment may be lost gradually over time, however [16]. This loss may be reversed by a reduction in immunosuppression or by a boost infusion of HSCs, although this procedure usually is more successful if performed shortly after the original transplantation procedure [17,18]. With these maneuvers, the results are very promising.

Viral and fungal detection and treatment

Although infection remains a significant risk following HSCT, it now rarely causes death thanks to prophylactic and pre-emptive antibiotic and antifungal therapy and nursing in highly specialized isolation units. Human herpes viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpes virus 6 (HHV6), as well as adenovirus, sometimes can be a problem, however, particularly when transplanting patients who have PID who, before HSCT, may have chronic infection with these pathogens as a complication of the underlying condition. Additionally, a consequence of RIC is prolonged immunosuppression leading to viral infection with EBV, CMV, or adenovirus [19]. New molecular methods of polymerase chain reaction and viral and fungal antigen detection in the blood and other tissues make it possible to detect infectious organisms before there is

evidence of disease, allowing pre-emptive therapy with newly developed antiviral agents such as ganciclovir, foscarnet, and cidofovir and with new antifungal agents including caspofungin, voriconazole, and posaconazole [20]. EBV infection can be treated with rituximab, which removes EBV-infected B lymphocytes as well reducing immunosuppression to permit some T-lymphocyte function. Treatment with EBV antigen–specific cytotoxic T lymphocytes has been very effective [21]. Clinical trials in patients who have adenovirus and CMV infections are planned [22].

Treatment of graft-versus-host disease

Graft-versus-host disease (GvHD), the most frequent complication after HSCT, depends on various factors including histoincompatibility between donor and host, older age at transplantation, greater intensity of conditioning regimen leading to tissue damage and inflammation, and pre-existing tissue inflammation. Unlike the hematologic setting, in which GvHD may be encouraged for the graft-versus-leukemia effect, GvHD usually is not encouraged following HSCT for PID. Donor chimerism, however, is more likely to be complete in the presence of GvHD, which may be important following treatment with RIC. Depletion of T lymphocytes will prevent most GvHD, an important consideration in a severe HLA-mismatched setting, but immune reconstitution is slower, with a greater risk to the patient of severe viral infection. GvHD is easier to prevent than to treat once established, and most patients receiving a T-lymphocyte–replete stem cell source receive prophylactic treatment with a calcineurin inhibitor such as cyclosporine. Acute GvHD generally is treated with a tapered course of methylprednisolone. Secondary treatment for GvHD is much more successful now thanks to an increasing array of agents, including mycophenolate mofetil, monoclonal antibodies including anti-CD 25, anti-CD3, and anti-CD52, and anti-tumor necrosis factor-α antibodies as steroid-sparing agents [23]. For refractory GvHD, extracorporeal phototherapy, pentostatin (a purine antimetabolite), and antithymocyte globulin may be useful [24,25].

Treatment of veno-occlusive disease

Veno-occlusive disease or sinusoid obstructive syndrome is a potentially fatal complication of HSCT particularly associated with busulphan or pre-existing hepatic damage. The introduction of novel busulphan dosing regimens has decreased the incidence of hepatic toxicity [26,27]. Other, less toxic conditioning regimens also are associated with a reduced incidence of veno-occlusive disease.

Defibrotide, a single-stranded oligonucleotide polydisperse mixture with antithrombotic and fibrinolytic effects on the microvascular endothelium, is used to treat severe veno-occlusive disease and seems to be well tolerated and effective, with a low risk of adverse effects, particularly hemorrhage [28].

In high-risk patients who have pre-existing liver disease or a propensity for developing veno-occlusive disease, prophylactic defibrotide also seems to be effective [29,30].

Cord blood

Umbilical cord blood contains large numbers of HSCs and is used as an alternative source of these cells. Cord blood has a number of advantages over other sources of HSCs (Box 2). Survival following cord-blood transplantation in children who have PID is as good as with transplantation using other stem cell sources, even for conditions such as Omenn syndrome or reticular dysgenesis for which HSCT results generally are poorer [31]. The available data suggest immune reconstitution is as complete as when other stem cell sources are used, and B-cell function seems better than after haploidentical HSCT [32].

Particular advantages of cord blood include the use of infant HSCs which, when used in infants, may have a longer life than stem cells from older donors. Recent data indicate that transplanted cord-blood mononuclear cells have significantly longer telomere length than transplanted peripheral blood HSCs from adult donors [33]. Cord-blood transplantation also can be arranged very quickly. Another advantage is the ability to transplant across HLA types. Most cord-blood collections have been matched at only the class 1 HLA-A and -B and class 2 HLA-DR levels, historically using serologic methods and more recently using low-resolution molecular DNA typing. Despite less precise matching, results have been good, and many transplantations with class I HLA-C or class 2 HLA-DQ and -DP mismatches are successful. Indeed, in the authors' experience, cord-blood transplantation using units matched at five of six HLA antigens have been as successful as bone marrow transplantation using units matched at six of six HLA antigens. Despite earlier claims to the contrary, however, significant GvHD is likely if a cord blood unit matched at less than five of six HLA antigens is used.

Box 2. Advantages of umbilical cord blood as a source of stem cells for transplantation

Rapid access to the donor unit
Ease of arranging date for transplantation
No medical risk to the donor
Lower risk of latent viral transmission
Lower risk of graft-versus-host disease
Higher frequency of rare HLA haplotypes compared with bone marrow registries
Stem cell nascence

Graft manipulation

New methods of graft engineering have enabled specific cell populations to be negatively or positively selected, to enhance a particular characteristic of the graft. Since the late 1990s, European centers have used positive selection of CD34+ hematopoietic cells for mismatched-donor transplantations. Patients receiving T-lymphocyte–depleted HSCT can have antiviral immunity boosted by infusing donor T lymphocytes that have been allo-depleted of the T lymphocytes likely to cause GvHD but retaining those with antiviral specificity [34].

Outcome of hematopoietic stem cell transplantation for primary immunodeficiencies

Many molecularly defined PIDs now have been treated successfully by HSCT (Box 3).

Overall survival is improving markedly, with advances in donor and recipient HLA matching by high-resolution molecular typing leading to similar results for transplants from HLA-matched sibling donors and HLA-matched unrelated donors [14]. Outcome data from many European centers for transplantation of patients who had PIDs have been collected in Paris, France for many years in the Stem Cell Transplantation for Immunodeficiencies (SCETIDE) registry. This enormously valuable collaborative project has enabled the gathering of data on large numbers of patients who have the same disease. Regular working party meetings of members have ensured that common transplantation guidelines have been adopted, thus providing valuable information on relatively large cohorts of patients undergoing similar treatment. The last data analysis was published in 2003 [12], and the latest data currently are being analyzed and prepared for publication. Currently, 716 patients who have SCID and 806 patients who have other PIDs have been entered in the registry. Survival generally seems to be good, and patients who survive beyond the first 12 to 24 months generally have good long-term outcome because, unlike patients who have hematologic malignancy, relapse does not occur. Furthermore, unlike the situation following solid-organ transplantation, where lack of immune tolerance necessitates lifelong immunosuppression, HSCT recipients rapidly develop tolerance, and most patients can stop immunosuppressive treatment within 6 months after HSCT.

Severe combined immunodeficiency

In the European series, the overall probability of 5-year posttransplantation survival for patients who underwent transplantation for SCID before 1995 was 56%, rising to 71% for patients who underwent transplantation between 2000 and 2005. The survival rate now is 90% after transplantation from a matched sibling donor for SCID and is 69% when a matched

Box 3. Immunodeficiencies that have been successfully treated by hematopoietic stem cell transplantation

Lymphocyte immunodeficiencies
Common gamma chain ($C\gamma C$) deficiency
Janus-associated kinase 3 (JAK3) deficiency
Interleuken-7 receptor α (IL7Rα) deficiency
Recombinase-activating gene (*RAG*) 1/2 deficiency
Adenosine deaminase (ADA) deficiency
CD45 deficiency
CD3δ/CD3ϵ/CD3ξ deficiency
CD3γ deficiency
Reticular dysgenesis
Artemis deficiency
DNA ligase IV
Cernunnos/X-linked lymphoproliferative disease (XLF) deficiency
Purine nucleoside phosphorylase (PNP) deficiency
Major histocompatibility class II (MHC II) deficiency
Zeta-chain-associated protein-70 (ZAP-70) deficiency
CD8 deficiency
Winged helix deficiency
Omenn syndrome
Ca^{++} channel deficiency
DiGeorge syndrome
Coloboma, heart anomalies, choanal atresia, retardation of growth and development, and genital and ear anomalies (CHARGE syndrome)
Wiskott-Aldrich syndrome
CD40 ligand deficiency
X-linked lymphoproliferative disease
1. XLP1 (*SH2D1A*)
2. XLP2 (*XIAP*)

Phagocytic deficiencies
Chronic granulomatous disease
Severe congenital neutropenias
Leukocyte adhesion deficiency (1)
Schwachman-Diamond syndrome
Chediak-Higashi syndrome
Griscelli syndrome, type 2
Familial hemophagocytic lymphocytosis (FHL)
1. Perforin deficiency
2. MUNC13-4 deficiency

3. Syntaxin 11 deficiency
Interferon-γ receptor (IFN- γR) deficiencies

Other immunodeficiencies
Cartilage hair hypoplasia
Hyper IgD syndrome
Autoimmune lymphoproliferative syndrome (ALPS) (Fas)
Hyper-IgE syndrome
Immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome
CD25 deficiency
Nuclear factor-κB (NF-κB) essential modulator (*NEMO*) deficiency
NF-κB inhibitor, alpha (IκBα) deficiency
Immunodeficiency, centromeric instability, facial dysmorphism (ICF) syndrome
Nijmegen breakage syndrome

unrelated donor is used (P Landais, personal communication, 2007). Results published by other groups highlight the similar outcomes of matched sibling-donor and matched unrelated-donor transplantations for SCID [13,35]. The major risk factors for complications and death are pre-existing infection and lung and liver disease.

The best outcomes following HSCT for SCID are in patients diagnosed at birth because of family history and undergoing transplantation in the neonatal period before they develop infection and end-organ damage [32,36]. In the authors' updated series, 18 of 20 patients undergoing transplantation in the neonatal period have survived.

Results of transplantation for cohorts of patients who have specific diseases now are being published; this information, in conjunction with registry data of nontransplanted patients, also can help inform best treatment options.

CD40 ligand deficiency

Until 1993, CD40 ligand deficiency was considered a B-lymphocyte disorder, and patients were treated with immunoglobulin replacement. The discovery that the defect was on the T lymphocyte [37], together with the finding that cryptosporidial, *Pneumocystis*, and viral infection caused significant morbidity and mortality, led to a reappraisal of the best way to treat this condition [6]. Registry data showed that although 95% of patients were alive at 10 years, only 20% were alive at 25 years. Furthermore, ongoing liver disease caused by cryptosporidial infection causes major morbidity as well as mortality in older patients. HSCT now is the treatment of choice. Initial results were not good, but in the European series, 58% of patients

who had CD40 ligand deficiency were cured following transplantation. Of those who underwent transplantation by 5 years of age, 86% survived, compared with 58% of those underwent transplantation after 5 years of age. Adverse risk factors included pre-existing infection and pre-existing liver and lung disease [38]. Results have improved more recently: in the authors' own, updated series three of seven boys (43%) who underwent transplantation before 2000 survived, compared with five of five (100%) who underwent transplantation since 2000 [39].

Wiskott-Aldrich syndrome

The natural history of patients who have mutations in the *WAS* gene is debated. Some believe there is a better outcome for patients who have mutations resulting in the "more mild" X-linked thrombocytopenia phenotype [40]. More recently, the presence or absence of WAS protein (WASP) was shown to correlate better with outcome: patients who have no expressed protein have a worse outcome than those who have expressed protein, regardless of genotype [7]. There were no WASP-negative patients alive by 15 years, compared with 96% of WASP-positive patients. Although 96% of WASP-positive patients were alive at 30 years, however, the intracranial hemorrhage–free survival was only 37%. The incidence of autoimmune complications was the same in WASP-positive and WASP-negative patients (24%). The efficacy of HSCT for patients who have WAS is long established, with good outcomes following matched sibling-donor and matched unrelated-donor HSCT (100% and 76.9%, respectively, in one series Ref. [41]). Mortality is associated with mismatched donors but also with pre-existing organ damage and infection [41–44]. A recent study has emphasized the importance of gaining full donor chimerism following transplantation, because autoimmune disease is significantly more likely in survivors who have partial chimerism [45]. These studies, and similar ones, are of immense importance, because they allow transplantation protocols to be tailored with specific features to ensure the optimal outcome for patients who have WAS, including appropriate stem cell doses and careful monitoring of post-HSCT donor chimerism with early reduction of post-HSCT immunosuppression.

Phagocyte disorders

Neutrophil disorders also are amenable to transplantation. Early attempts at HSCT for CGD were poor. Subsequent attempts at transplanting patients who have CGD using non-myeloablative preparatory regimens and T-lymphocyte–depleted allografts were disappointing, with 2 of 10 patients rejecting the graft and an overall mortality of 30% [46]. More recent series indicate good outcomes following fully myeloablative conditioning and T-lymphocyte–replete stem cell sources, with 85% survival and 82% cure overall. Deaths in this study were confined to those who had active fungal

disease at the time of transplantation [47]. Modification of the preparatory chemotherapy regimen has enabled successful transplantation of adults who have significant pre-existing organ damage [48]. At the authors' center, 18 of 20 HSCTs for CGD were successful, although unrelated donors were used in 10 cases. The two deaths were associated with active fungal infection at the time of transplantation. There was resolution of inflammatory lung and bowel lesions, and boys who had experienced growth failure demonstrated catch-up growth after HSCT (Fig. 2). Most of these patients, more than 2 years after HSCT, no longer require prophylactic medication, lead completely normal lives, and come to the hospital only for an annual examination, an outcome that is markedly better than that seen in nontransplanted patients receiving antibiotic and antifungal prophylaxis.

Data on the long-term outcome of patients who have severe congenital neutropenia (Kostmann syndrome) and Schwachman-Diamond syndrome are becoming available [49,50]. The significant mortality risks are from major sepsis or malignant transformation. The cumulative risk of death from sepsis while receiving treatment with granulocyte colony-stimulating factor is 8% over 10 years, with a mortality rate from sepsis of 0.9% per year [50]. Although the debate about the contribution of granulocyte colony-stimulating factor to malignant transformation continues, recent data suggest that the risk is not dependant on the underlying gene mutation, with a cumulative incidence of malignant transformation of 36% by 15 years [51]. Once these patients have developed malignancy, the outcome of HSCT is poor [52]. It could be argued that, as in patients who have CGD and combined immunodeficiencies, patients who have severe congenital neutropenia should undergo transplantation when they are in the optimum condition to tolerate this procedure, before they develop significant organ damage from infection or from treatment of malignancy.

Long-term outcomes

As the survival of patients undergoing HSCT for PID improves, the long-term outcome of these patients is important. Several studies now have been published that analyze long-term immunoreconstitution in patients who underwent transplantation for PID and survived more than 10 years [53–57]. It is increasingly clear that long-term T-lymphocyte function requires generation of new T lymphocytes from the recipient's thymus. Although this result may be achieved in common gamma chain or Janus-associated kinase 3 SCID without engraftment of other cell lineages, myeloid chimerism generally is required for stable long-term T-lymphocyte reconstitution. This goal is most likely to be achieved if preparative chemotherapy is given. Other quality-of-life indicators also need to be considered. Autoimmunity occurs in about 10% of patients after HSCT for PID [56–58] and is seen particularly in patients undergoing transplantation for WAS who achieve only partial donor chimerism [45]. Other long-term issues to be addressed include fertility after

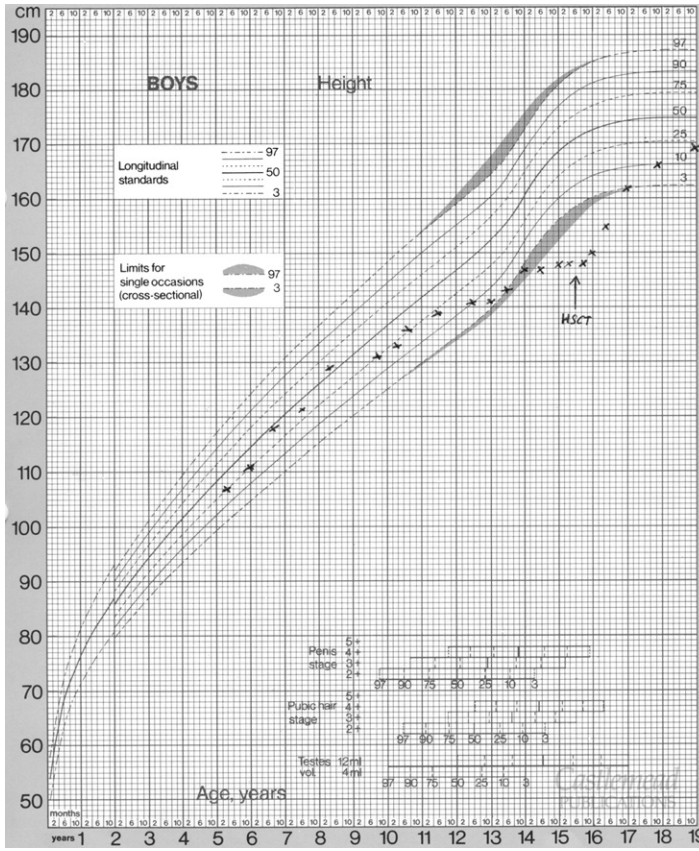

Fig. 2. Growth chart of a boy who has X-linked chronic granulomatous disease, demonstrating growth failure coincident with the onset of colitis and catch-up growth following successful hematopoietic stem cell transplantation from an unrelated donor. (*Courtesy of* The Paediatric Immunology Unit, Newcastle General Hospital, Newcastle upon Tyne, UK; with permission.)

chemotherapy [59,60] and long-term lung function [61]. These long-term complications, however, should be considered in the context of complications occurring in patients who are receiving prophylactic treatment for PID and suffer from recurrent infection, hospitalization, and lung and bowel disease with a continued risk of developing malignancy and of dying prematurely.

Immunoregulatory disorders

As the understanding of the functions of the immune system expand, it is clear that immune system failure leads both to recurrent or persistent infection and to lymphoid malignancy resulting from the failure of immune surveillance or aberrant lymphocyte receptor formation [62], autoimmunity caused by the absence of immunoregulatory cells [63], lymphoproliferation

caused by the failure of normal regulatory lymphocyte apoptosis [64], and autoinflammatory syndromes (Fig. 3) [65]. Current conventional treatments for many of these disorders are at best palliative, perhaps alleviating symptoms but not abolishing the underlying problem or altering the long-term outcome. HSCT has a role in the treatment for at least some of these patients [66–69]. Given the good outcome of HSCT for the disorders discussed in previous sections, perhaps HSCT should be considered more favorably for the ''new'' immunodeficiencies.

Conclusions and future perspectives

Treatment of PID by HSCT has become safer, and more patients are surviving. New developments in the field of HSCT, often developed by physicians treating adult patients who have malignancy, have meant that treatments enabling the sickest patients to survive transplantation are available and have been adopted by physicians treating PID. The best outcomes, however, are still achieved in patients who do not have concurrent or preexisting respiratory or hepatic end-organ damage or active viral or fungal infection. The compilation of disease-specific registries has enabled the long-term outcome of large cohorts of patients, often receiving prophylactic therapy, to be assessed. It is becoming clear that the natural life span of most of these patients is foreshortened despite prophylactic treatment, usually with considerable comorbidities and a poor quality of life. Thus, there is an increasing argument for performing HSCT in the first decade of life. Long-term analysis of the outcome of patients who have PID treated by HSCT demonstrates cure and, in many cases, a normal life quality. Many issues remain to be resolved, including developing less toxic but effective chemotherapy. Exciting potential therapies include the use of mesenchymal stem cells for the treatment of GvHD and enhancement of engraftment

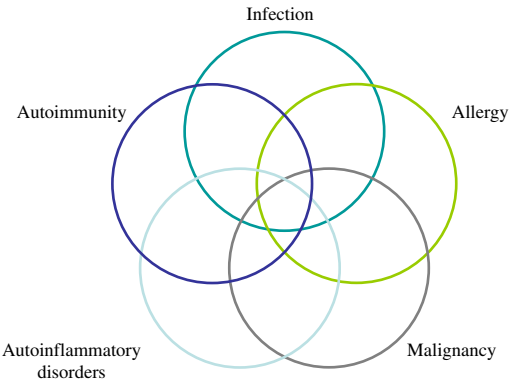

Fig. 3. Immune system failure. (*Courtesy of* The Paediatric Immunology Unit, Newcastle General Hospital, Newcastle upon Tyne, UK; with permission.)

[70–72] and the use of growth factors in encouraging immune reconstitution and shortening the time between transplantation and the emergence of newly formed T lymphocytes from the thymus of the recipient [73].

The development of collaborative transplant protocols, the pooling of transplantation results, and the publishing of large registries will encourage accurate assessment of outcomes and development of future treatments. The role of organizations such as the European Group for Bone Marrow Transportation/European Society for Immunodeficiencies and the International Bone Marrow Transplant Registry/National Marrow Donor Program in developing these collaborations is to be applauded.

Finally, for many PIDs that potentially are amenable to treatment with HSCT, perhaps the question now to be asked for any given patient should not be ''What are the indications?'' but rather ''What are the contraindications to transplantation?''

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Gene Therapy for Primary Immunodeficiencies

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Human primary immunodeficiencies (PID) are a heterogeneous group of disorders in which inherited genetic defects compromise host immunity [1]. During recent years, mutations in more than 130 genes have been identified that result in a broad range of defects in innate and adaptive immune mechanisms [2]. The most severe forms of PID are known as severe combined immunodeficiency (SCID), in which T lymphocyte development is invariably compromised and associated with diverse disorders of development and functionality of B lymphocytes and natural killer (NK) cells [3,4]. Although invariably fatal without treatment, hematopoietic stem cell (HSC) transplantation (HSCT) is usually highly successful if a genotypically matched family donor or unrelated donor is available [5,6]. However, for most individuals, this is not the case, and survival from mismatched family (usually parental haploidentical donor) transplants is substantially lower (approximately 52% 10-year survival for all forms of SCID, SCETIDE European registry) and associated with predictable toxicity arising from the administration of chemotherapeutic agents to ensure adequate HSC engraftment. SCID and some forms of PID are particularly attractive initial targets for gene therapy because a profound growth and survival advantage is conferred to corrected cells (although this may be variable among different molecular types). In other words, because of the huge proliferative capacity of the hematopoietic system (and particularly the lymphoid compartment), effective gene transfer to a small proportion of bone marrow

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precursor cells can result in substantial correction of the immunologic deficit, most clearly exemplified by the accumulation of normally functioning lymphocytes in patients with rare somatic gene reversion events [7]. Other PIDs predominantly affecting B lymphocytes or myeloid cells may be less severe immediately but are often associated with significant accumulative morbidity and mortality. Mismatched HSCT is also particularly problematic in this group. For these disorders, a combination of effective gene transfer to HSC and the use of myelosuppressive conditioning regimens is almost certainly necessary to achieve effective engraftment of transduced cells.

HSCs are important targets for the development of effective gene therapy because of their self-renewal capacity, pluripotentiality, and ability to repopulate myeloablated recipients. Gene transfer to HSC and their progeny requires stability, which currently can only be achieved efficiently using integrating vectors, most commonly based on mammalian retroviruses, which, although effective, may be associated with variegation of transgene expression (dependent on the local chromatin environment) and the potential for harmful mutagenesis. Most clinical studies conducted to date have used murine gammaretroviruses, which depend on active proliferation of the target cell population because the nuclear membrane must be disrupted for entry of the preintegration complex. Because HSCs are mostly quiescent or only slowly dividing, extensive studies have been dedicated to the identification of optimal ex vivo culture conditions that stimulate HSC proliferation, without inducing differentiation and loss of long-term repopulating ability. Vectors based on lentiviruses and foamy viruses are under investigation as alternatives to murine gammaretroviruses because they are less dependent on cell division for effective gene transfer and are highly efficient [8–12]. Clinical trials using HIV-1–based lentiviral vectors for transduction of HSC have recently been initiated.

Gene therapy applications in primary immunodeficiencies

X-linked severe combined immunodeficiency

X-linked SCID (SCID-X1) accounts for approximately 40% to 50% of all SCIDs, and is caused by mutations in the gene encoding the interleukin (IL)-2 receptor gamma chain (*IL2RG*) [13,14]. This molecule is now known to be a key subunit of the cytokine receptor complex for IL-2, -4, -7, -9, -15, and -21, and is designated the common cytokine receptor gamma chain (γc). In the absence of γc signaling, many aspects of immune cell development and function are compromised, leading to absence of T and NK cells, and persistence of dysfunctional B cells (T-B+NK- SCID), although atypical individuals with partial T-cell development have also been identified. If a genotypically matched donor is available, allogeneic bone marrow transplantation is a highly successful procedure with a long-term survival rate of more than 90%. Survival rates are high partly because efficient

engraftment occurs in the absence of myelosuppressive conditioning. In contrast, survival in a large cohort of patients treated by mismatched HSCT has been shown to be significantly lower (72% 10-year survival for patients treated after 1995, SCETIDE European registry), and is often associated with poor long-term immunologic recovery with minimal HSC engraftment [15]. Furthermore, chemotherapeutic conditioning is often used to facilitate HSC engraftment in this setting, and may itself be associated with late toxic effects.

Many incremental advances in gene transfer technology have contributed to the successful application of gene therapy for PID (including the optimization of cell culture and gene transfer conditions ex vivo), which complement any intrinsic profound selective growth advantage imparted to successfully transduced cells (Table 1). Two completed trials in 19 infants have demonstrated highly effective immunologic reconstitution in patients with SCID-X1 [16–18]. Gammaretroviral vectors encoding a *IL2RG* cDNA (regulated by flanking Moloney murine leukemia virus long terminal repeat (LTR) sequences in both trials), were used to transduce autologous $CD34^+$ cells ex vivo, which were reinfused into the patients in the absence of preconditioning. In nearly all patients, NK cells appeared between 2 and 4 weeks after infusion, followed by new thymic T lymphocyte emigrants at 8 to 12 weeks. With some variation, the number and distribution of these T cells normalized rapidly, within 6 months (apparently more rapidly than observed following haploidentical transplantation). They also performed normally in terms of proliferative response to mitogens, T-cell receptor (TCR), and specific antigen stimulation, and had a normally complex phenotypic and molecular diversity of TCR. Functionality of the humoral system was also restored, maybe not quite as effectively, but to a degree sufficient that discontinuation of immunoglobulin therapy was possible in most patients. Persistent long-term marking in peripheral myeloid cells (between 0.1% and 1%) suggests that long-lived stem or progenitor cells have also been successfully transduced, albeit at a low level. This suggestion is supported by the detection of common vector integration sites between myeloid and lymphoid cells, and the presence of transgene in nonobese-diabetic/SCID mouse repopulating cells obtained from 1 patient 1 year after gene therapy [25]. However, the contribution to the initial burst of thymopoiesis from relatively late T-cell precursors in the original transduced $CD34^+$ cell population, versus that from cells earlier in the hematopoietic differentiation hierarchy that have engrafted in the bone marrow, has not yet been determined, which may have important implications for the durability of immunologic reconstitution and for sustained production of new T cells. Ultimately, the longevity of functional reconstitution can only be determined by clinical monitoring, but it may also be feasible to repeat gene therapy on multiple occasions. Definition of the window within which gene therapy will be effective is therefore vitally important, as suggested for other more conventional therapeutic modalities [26]. This

Table 1
Clinical trials of gene therapy for primary immunodeficiencies

Disease	Gene	Retroviral vector	Envelope	Cell type treated	Patients treated (to end 2007)
ADA-D	ADA	GIADA1 [19]	A	BM CD34^{+a}	13
ADA-D	ADA	GCsap-M-ADA MND-ADA [20]	A	BM CD34^{+a}	6
ADA-D	ADA	SFFV-ADA-WPRE [21]	G	BM CD34+^{a}	5
ADA-D	ADA	GCsap-M-ADA [22]	A	BM CD34+	2
SCID-X1	γc	MFG [17]	A	BM CD34^{+}	12
SCID-X1	γc	MGF [16]	G	BM CD34^{+}	11
SCID-X1	γc	MFG [23]	G	PB CD34^{+}	3
AR-SCID	JAK-3	MSCV (Sorrentino, unpublished data)	G	BM CD34^{+}	1
X-CGD	Gp91phox	MFG-gp91phox (Thrasher, unpublished data)	A	BM CD34+^{a}	1
X-CGD	Gp91phox	SF71gp91phox [24] (Thrasher, unpublished data)	G	PB CD34+^{a}	8
X-CGD	Gp91phox	MFG-gp91phox (Malech, unpublished data)	A	PB CD34+^{a}	2
WAS	WASp	MFG-WASp (Klein, unpublished data)	G	PB CD34+^{a}	2

Summary of clinical trials for PID using gammaretroviral vectors from 2000–2007.

Abbreviations: A, amphotropic envelope; ADA, adenosine deaminase; ADA-D, adenosine deaminase deficiency; AR-SCID, autosomal recessive; BM, bone marrow; G, gibbon ape leukemia virus envelope; PB, peripheral blood; WAS, Wiskott-Aldrich Syndrome; WASp, WAS protein; X-CGD, X-linked chronic granulomatous disease; γc, common cytokine receptor gamma chain.

[a] Denotes the use of preconditioning.

necessity has been clearly demonstrated by the failure of immunologic reconstitution in several older patients following effective gene transfer to bone marrow or peripheral blood CD34+ cells [23,27]. At least for SCID, it is likely that host-related restrictions to efficacy exist, for example because of the inability to initiate or reinitiate an exhausted or failed program of thymopoiesis.

Adenosine deaminase deficiency

Adenosine deaminase (ADA) catalyzes the deamination of deoxyadenosine and adenosine to deoxyinosine and inosine, respectively. Deficiency of this enzyme results in the accumulation of metabolites, which have detrimental effects on lymphocyte development and function through a number of cellular mechanisms [28]. The severity of the condition varies, but most ADA patients have low numbers of T, B, and NK lymphocytes. As for all forms of SCID, allogeneic HSCT is highly successful in the human

leukocyte antigen (HLA) genotypically matched setting, but mismatched transplants for this particular disease have a poor survival outcome, perhaps because of the systemic nature of the metabolic abnormalities [29]. An alternative modality of treatment for the ADA-deficient (ADA-D) form of SCID is exogenous enzyme replacement with polyethylene glycol-conjugated bovine ADA (PEG-ADA). Regular intramuscular injections of PEG-ADA result in rapid systemic detoxification, with reduction of dAdo and dATP to near-normal levels within a few weeks of starting therapy. Approximately 70% of children show an improvement in lymphocyte counts to near-normal levels, and of these, 50% remain on immunoglobulin replacement because of continuing impairment of humoral immunity. The long-term prognosis for children on PEG-ADA without any corrective procedure being undertaken is unclear, although evidence is emerging of declining effectiveness over time [30]. For this reason, it appears sensible to implement definitive therapy as soon as possible, and while an opportunity exists to restore effective thymopoiesis.

Early gene therapy studies using gammaretroviral vectors conducted on patients with ADA-D were largely unsuccessful in terms of immunologic correction [31–34], at least in part because of the continued use of PEG-ADA enzyme replacement therapy, which may have abrogated the survival advantage of gene-modified cells. However, a detailed analysis of two patients treated by repeated ADA gene transfer into autologous peripheral blood lymphocytes demonstrated that transduced human T cells (or their progeny) are able to survive in the peripheral circulation for more than 10 years and that transgenes regulated by gammaretroviral sequences continue to be expressed in peripheral T cells in vivo over long periods of time in the absence of significant silencing [35]. In another study, umbilical cord blood CD34+ cells were transduced and reinfused in the first week of life in three affected infants [34]. Although no significant clinical benefit was observed, clear evidence existed of the selective accumulation of transduced T lymphocytes over a subsequent 4-year period (in contrast to other lymphoid and myeloid lineages), and also for the persistence of these cells following withdrawal of PEG-ADA in one patient. Similarly, withdrawal of PEG-ADA from a patient who had received multiple infusions of autologous transduced peripheral blood lymphocytes as part of another study led to an increase in the proportion of transduced T cells from less than 10% to nearly 100% [36]. It is therefore apparent that successful gene therapy for ADA-D depends on the discontinuation of enzyme replacement; other clinical trials in which it has been maintained at the time of engraftment of transduced lymphocytes or bone marrow progenitor cells have failed to show significant clinical benefit, even with improved vectors and cell transduction protocols.

It remains undetermined whether optimal correction by gene therapy can be achieved by withdrawal of PEG-ADA alone or whether additional strategies are necessary to facilitate engraftment of transduced cells. Given that

ADA is a metabolic enzyme expressed in all cells, and that systemic detoxification may be beneficial for normal functioning of other tissues such as the brain, it appears reasonable to attempt high levels of engraftment of all hematopoietic lineages. With this theory in mind, two studies have now used low-intensity bone marrow conditioning regimens before infusion of transduced cells. In the first of these, bone marrow CD34+ cells were transduced with an amphotropic gammaretroviral vector [19]. Patients received a myelosuppressive dose of busulfan on 2 successive days before return of gene-modified cells. Thirteen patients have been treated in this study to date, which has demonstrated impressive reconstitution of peripheral T, B, and NK cells and substantial reduction of blood metabolite levels (Alessandro Aiuti, personal communication, 2008). As for patients with SCID-X1, the TCR repertoire was highly diverse. Lineage-specific transgene analysis demonstrated high level marking in T, NK, and B cells and persistence of gene-modified granulocytes, monocytes, and megakaryocytes at levels of up to 10% to 20%, indicating that multipotent progenitors had engrafted efficiently. In a second study using a gibbon ape leukemia virus pseudotyped gammaretroviral vector, patients have been treated with similar good clinical effect, although in this case, myelosuppression was achieved using a single dose of melphalan, and PEG-ADA was discontinued 1 month before gene therapy [21]. Levels of long-term myeloid marking were also substantial (and in one patient, the mean levels of red cell ADA were normalized), which may well also reflect the potent activity of the spleen focus-forming virus (SFFV) LTR in this cell lineage.

Two patients have been treated in Japan with a protocol involving the cessation of PEG-ADA but without cytoreductive conditioning [22]. Both patients were in good health after treatment, with no need for enzyme replacement, although the number of circulating lymphocytes remained low. The question of whether a conditioning regimen is absolutely required for effective therapy therefore remains unresolved, although for maximal systemic detoxification, high-level engraftment of HSC would appear to be desirable. One other patient suffered prolonged marrow aplasia after busulfan treatment in association with pre-existing trisomy 8 mosaicism in bone marrow cells [37]. The unsuccessful outcome of gene therapy in this particular case suggests that cytogenetic screening should be performed to test the eligibility of patients, particularly if cell counts are abnormal before treatment. It also highlights the potential for toxicity arising from conditioning of patients.

Other primary immunodeficiencies as targets for gene therapy

Mutations of the receptor tyrosine kinase gene *JAK-3* result in an autosomal recessive form of SCID that is immunophenotypically identical to SCID-X1, and the rationale for gene therapy is therefore similar [38]. Correction of a murine model of JAK-3-deficient SCID has been achieved using myelosuppressive and, more relevant to clinical studies, conditioning-free

protocols [39,40]. Attempts to rescue one patient with this strategy failed, although this may be related to the failure of previous allogeneic stem cell transplantation. Patients with mutations of the recombinase activating genes *RAG-1* and *RAG-2* characteristically present with absence of B and T cells. Moloney-based gammaretroviral vectors have been shown to reconstitute RAG-2-deficient mice effectively in the absence of detectable toxicity, even though gene expression was not tightly regulated [41]. Similar efforts to correct RAG-1 deficiency were also successful but required high vector copy numbers for efficacy [42]. Other lymphohematopoietic disorders, including Artemis deficiency and ZAP-70 deficiency, have been shown to be amenable to correction by gene transfer, and clinical studies in these are likely to commence in the near future [43,44].

Chronic granulomatous disease (CGD) is caused by mutations in genes encoding components of the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase complex, which is responsible for mediating efficient killing and digestion of many bacteria and fungi. In many ways, this disorder has become a model for testing HSC gene therapy strategies, because corrected cells have no growth or survival advantage [45–47]. Gene expression is also primarily important in relatively short-lived, terminally differentiated effector cells such as neutrophils and macrophages, meaning that long-term efficacy is entirely dependent on efficient stable transduction and engraftment of HSCs. In addition to data from murine reconstitution models, important information can be obtained from the study of variant patients who retain partial NADPH-oxidase activity, and carriers of the X-linked form of the disease. From this finding, it can be predicted that more than 10% correction in terms of cell numbers will be therapeutically effective, but that levels of correction per cell (in other words, the levels of enzyme activity) probably needs to be more than 30%. Therefore, the challenges are to achieve sufficient engraftment of transduced HSC and efficient gene expression in terminally-differentiated cells. Several clinical studies have been performed using standard gammaretroviral vectors but, in the absence of bone marrow preconditioning, only transient, low-level correction has been achieved [48]. More recently, studies have incorporated low-intensity conditioning (busulfan or melphalan, as for ADA gene therapy studies) to create space for incoming transduced HSC. These studies have provided good evidence for functional correction associated with substantial therapeutic effect and clearance of infections refractory to conventional treatment [24]. However, extended follow-up has revealed unexpected clonal selection through mutagenesis, which, although contributing to therapeutic efficacy in the short term, was eventually associated with silencing of the therapeutic gene and myelodysplastic changes in the bone marrow of two patients [49]. One of these patients died from overwhelming sepsis. Despite these unexpected events, it is clear that gene transfer has the potential for therapeutic efficacy in CGD as a transient rescue procedure in untreatable patients and, in the longer term, as a permanent cure.

Progress in gene transfer strategies for CGD will likely be directly translated to similar approaches for myeloid diseases such as leukocyte adhesion deficiency type 1, an inherited disorder of leukocyte function caused by defective expression of the common β-integrin subunit (CD18). As in CGD, no survival advantage is expected in gene-corrected cells and the critical cell population to be targeted is terminally differentiated neutrophils. One previous attempt at treating this disease, by gammaretrovirus vector-mediated transfer into HSC and engraftment into nonmyelosuppressed recipient patients, resulted in only minimal and transient correction of myeloid cells [45,50]. Recent studies in a canine model have demonstrated effective therapy using foamy virus-based vectors and represent the first successful use of this vector type to correct a genetic disease [51].

The Wiskott-Aldrich syndrome (WAS) is a PID characterized by lymphocytic and myeloid abnormalities, although an additional invariable feature is nonimmune microthrombocytopenia [52]. The WAS protein (WASp) is expressed in all hematopoietic cell types, and is responsible for regulated organization of the actin cytoskeleton. Considerable evidence exists from WAS patients who have somatic reversions, of a potent growth and survival advantage for lymphocytes expressing WASp [53]. Furthermore, patients with attenuated WAS (in whom some residual WASp activity is present), usually have very mild immunodeficiency with microthrombocytopenia, although the latter can be largely corrected by splenectomy. Therefore, partial restoration of function is likely to be successful therapeutically. Several preclinical studies have demonstrated that many of the cellular defects, including T-cell proliferation and aberrant cell motility in multiple lineages, can be corrected using gammaretroviral and lentiviral vectors [54–62]. One clinical protocol using a conventional gammaretroviral configuration has recently been initiated, and another, using a lentiviral vector incorporating endogenous *WAS* gene promoter sequences, is in preparation.

Risks and side effects of gene therapy

The dependence of retroviruses on chromosomal integration for stability of transduction is an important feature that enables permanent modification of proliferating cell populations but brings with it the risk of insertional mutagenesis. Reproducible leukemogenesis and oncogenesis has now been demonstrated clearly in preclinical models, and may be directly associated with vector dose or copy number and transduction conditions [63]. Co-operating effects from expression of the transgene, or from other elements within the vector backbone, may also be important and are likely to be context dependent [64]. Analysis of transduced human CD34+ progenitor cells using standard culture methods has revealed a preference for gammaretroviral vector integration near proto-oncogenes and in genes that are highly expressed at the time [65,66]. Furthermore, characterization of integration sites in T lymphocytes from patients receiving successful gene therapy for

SCID-X1 has revealed an unexpected skewing after engraftment, suggesting the existence of widespread, subtle, vector-mediated influences on gene expression that alter survival and growth of cells in vivo [66,67]. A more dramatic illustration of the potential for vector sequences to disturb normal gene function inadvertently has been observed in SCID-X1 trials, where five patients have developed acute T lymphoblastic leukemia (T-ALL) between 2 and 6 years after the gene therapy procedure (Ref. [68] and unpublished data). In four of these patients, a single retroviral vector insertion into, or near, the *LMO-2* proto-oncogene resulted in high-level expression of LMO-2, as a result of retroviral enhancer-mediated activation of transcription (in one of these, a second insertion resulted in overexpression of *BMI1*). A fifth patient had a single activating insertion close to *CCND2*, which encodes cyclin D2 [69]. Activation of *LMO-2* is known to participate in human leukemogenesis by chromosomal translocation (probably often through deletion of a negative regulatory region), and results in the development of T-cell lymphoproliferation and leukemia in mice, albeit with a long latency [70,71]. Translocations at this locus have also been found in normal human thymus tissue, although the precise site of translocation may be critical for determining the extent of gene activation [70]. It is therefore likely that other contributing factors are required for these terminal leukemic events to manifest. For example, analysis of leukemic clones from patients has revealed other acquired molecular and cytogenetic defects, including activating mutations in *NOTCH-1* (Ref. [69] and unpublished data).

A matter of some discussion has been the contribution of the *IL2RG* transgene to leukemogenesis, although currently, no evidence exists of dysregulated expression or aberrant downstream signaling in peripheral lymphoid cells or, indeed, leukemic clones from patients (it is not feasible to determine expression profiles in patient thymocytes). In experimental systems, one tumor derived in susceptible mice following infection with replication competent gammaretroviruses has been shown to harbor separate but coincident integrations at the *Il2rg* and *Lmo-2* gene loci, suggesting the possibility of a significant interaction [72]. Another study reported a significant incidence of leukemia in a mouse model of SCID-X1 gene therapy using high doses of a lentiviral vector encoding a murine *Il2rg* transgene, although the contribution from mutagenesis was not evaluated [73]. In contrast, other studies using conventional gene transfer methodologies, transgenic animals, or fetal thymic organ cultures of transduced human CD34+ cells have failed to support a potent direct influence over and above a permissive effect through restoration of thymocyte development [74,75]. Reconstitution experiments in tumor-susceptible mice (Arf−/−) have also shown a dependence on mutagenesis but suggest a unique contribution from the SCID-X1 genetic background, possibly associated with an expanded population of lymphocyte progenitors that are blocked in their normal differentiation pathway [76]. It is conceivable that SCID, per se, is a risk factor irrespective of the genetic lesion, as a result of dysregulated early thymopoiesis or

diminished immunologic surveillance. Whether ADA-D confers a similar risk is unknown, although no patients have developed lymphoproliferative complications, and the slower kinetics of T-cell reconstitution suggest possible additional metabolic influences. Further evidence of the mutagenic potential of gammaretroviral vectors in humans has been obtained from a CGD study in which the transgene itself is clearly neutral [24]. Part of the therapeutic efficacy in two reported patients almost certainly resulted from the preferential expansion of functional myeloid clones caused by activation of the zinc finger transcription factor homologs *MDS1-EV11, PRDM16*, and *SETBP*. Unfortunately, acquired secondary mutations in these expanded clones (including monosomy 7) led to preleukemic myelodysplasia in both patients [49]. This process was associated with the coincident epigenetic silencing of the vector promoter region through methylation, and decline in transgene expression (and therefore loss of functional correction), whereas enhancer-mediated activation of the growth-promoting genes was selectively retained (Manuel Grez, personal communication, 2008).

Future prospects

Overall, it appears that the prominent events resulting in abnormal clonal growth and frank leukemogenesis are the inadvertent insertional activation of proto-oncogenes or cell growth-promoting genes by vector regulatory sequences. Although it is not clear why only some patients develop these complications, it cannot be assumed that neutral transgenes in the context of integrating vectors are necessarily safe. Efforts to eliminate vector mutagenesis, and to prevent epigenetic silencing, are therefore of paramount importance in achieving reduction in toxicity and maintaining efficacy. Even so, the realization that somatic gene therapy produces such remarkable therapeutic effects in SCID and CGD offers considerable hope for the treatment of many inherited immunologic and hematologic disorders, and is therefore likely to spawn rapid progress in terms of technologic development and clinical efficacy. The applicability of any novel therapy, including gene therapy, ultimately depends on the balance of risks against those of alternative treatments. Of the five patients who developed gene therapy–related lymphoproliferation (out of a total of more than 30 children with SCID-X1 and ADA-D now treated worldwide), one has died, but four are in complete remission following chemotherapy. These figures have to be balanced against the established risks and side effects of human leukocyte antigen–mismatched allogeneic HSCT, which are considerable.

Much can be done to improve the efficiency and safety of current protocols. The design of vectors used for gene delivery is clearly important and modifications may be possible that will limit the risks of mutagenesis, for example by incorporation of DNA insulator sequences in integrating vectors (to suppress influence on/by the surrounding chromatin environment), by

the use of self-inactivating vectors (in which the powerful viral LTR enhancer sequences are deleted), or by targeting safe regions in the genome. Self-inactivating vectors, in particular, allow the incorporation of more physiologic or less potent gene regulatory sequences and can substantially reduce mutagenic potential [77,78]. Clinical studies using these vectors are about to commence for SCID-X1 and WAS [60,79]. Culture conditions required to achieve effective gene transfer have been optimized for high efficiency, particularly for gammaretroviruses, but may also influence the pattern of vector integration and the susceptibility of cells to additional mutagenic events. Shortened protocols (which are more feasible using lentiviral or foamy viral vectors) may therefore be beneficial, although these also will have to be optimized with respect to cytokines and other agents that promote successful transduction. An equally important consideration is the preparation of patients before engraftment of transduced cells. For some diseases such as SCID-X1, this preparation may be minimal, but for others, where corrected cells have little survival or growth advantage, substantial myelosuppression (probably even fully myeloablative regimens) will be required for engraftment to therapeutic levels, which will increase the potential for toxicity, even if vector-related toxicity is eliminated. Clearly, the development of alternative methodologies to create space for incoming cells will be useful (eg, using antibody-mediated selective clearance of HSC niches) [80].

In the longer term, development of homologous recombination or gene repair to correct mutations, or the construction of mitotically stable extrachromosomal vectors, would obviate many problems, but until recently, applicable technologies have been inefficient. The use of zinc finger technology for enhancing homologous recombination at specific target sites is a promising development (as recently demonstrated for a human γc mutation in vitro) and, if specificity and efficacy in primary hematopoietic progenitor cells or HSC can be demonstrated, may become an important strategy [81]. Alternative sources of HSC may facilitate this type of technology. RAG2−/− mutant murine embryonic stem cells, repaired by standard homologous recombination technology, have be grown in vitro to provide sufficient hematopoietic progenitors for engraftment and correction of RAG-2 mutant mice [82]. More recently, it has been shown that pluripotent stem cells can be derived from reprogrammed somatic cells, and that these also provide a good opportunity for individualized genetic correction of a wide range of diseases [83,84].

PID has been at the forefront of the development of allogeneic HSCT strategies for the last 40 years, and has recently provided the first evidence for successful human gene therapy using an ex vivo approach. In the future, it is equally likely that novel developments and technologic refinements applied to this group of patients, perhaps eventually using vectors to modify target hematopoietic cells directly in vivo, will lead to the successful curative therapy of many other genetic diseases.

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