

15. Primary immunodeficiencies

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In the last years, advances in molecular genetics and immunology have resulted in the identification of a growing number of genes causing primary immunodeficiencies (PIDs) in human subjects and a better understanding of the pathophysiology of these disorders. Characterization of the molecular mechanisms of PIDs has also facilitated the development of novel diagnostic assays based on analysis of the expression of the protein encoded by the PID-specific gene. Pilot newborn screening programs for the identification of infants with severe combined immunodeficiency have been initiated. Finally, significant advances have been made in the treatment of PIDs based on the use of subcutaneous immunoglobulins, hematopoietic cell transplantation from unrelated donors and cord blood, and gene therapy. In this review we will discuss the pathogenesis, diagnosis, and treatment of PIDs, with special attention to recent advances in the field. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Primary immunodeficiency, T-cell immunodeficiency, antibody deficiency, innate immunity defects, immunoglobulin replacement therapy, hematopoietic cell transplantation, gene therapy

Primary immunodeficiencies (PIDs) comprise more than 130 different disorders that affect the development, function, or both of the immune system.¹ In most cases PIDs are monogenic disorders that follow a simple mendelian inheritance; however, some PIDs recognize a more complex polygenic origin. Disease penetrance and expression variability and interactions between genetic and environmental factors can also contribute to the phenotypic diversity of PIDs.

With the exception of IgA deficiency (IgAD), all other forms of PID are rare and have an overall prevalence of approximately 1:10,000 live births; however, a much higher rate is observed among populations with high consanguinity rates or among genetically isolated populations.

PIDs are classified according to the component of the immune system that is primarily involved.¹ Defects in adaptive immune responses include antibody deficiency syndromes and combined immunodeficiencies (CIDs). Defects of innate immunity

comprise disorders of phagocytes, Toll-like receptor (TLR)–mediated signaling, and complement. All of these forms are characterized by increased susceptibility to recurrent infections, severe infections, or both, with distinctive susceptibility to various types of pathogens depending on the nature of the immune defect. In addition, some forms of PIDs present with immune dysregulation, and others (immunodeficiency syndromes) have a more complex phenotype in which immunodeficiency is only one of multiple components of the disease phenotype. For each of the various categories of PIDs, we will review cause and pathogenesis, clinical and laboratory features, prognosis, and treatment.

PATHOGENESIS AND CLINICAL FEATURES **CIDs**

CIDs comprise a heterogeneous group of disorders with impaired development, function, or both of T lymphocytes associated with a defective antibody response.² The latter might result from intrinsic defects in B lymphocytes or might reflect inadequate T_H cell activity. In the most severe forms of CID (also known as severe combined immunodeficiency [SCID]), there is a virtual lack of functional peripheral T cells, whereas residual number, function, or both of T lymphocytes are present in other forms of CID.² Patients with SCID present early in life with infections of bacterial, viral, or fungal origin (*Table I*). Pneumonia caused by *Pneumocystis jiroveci* is common; however, interstitial lung disease might also be due to cytomegalovirus (CMV), adenovirus, respiratory syncytial virus, or parainfluenza virus type 3. Many infants with SCID have chronic diarrhea, leading to failure to thrive. Skin rash might reflect graft-versus-host disease caused by maternal T-cell engraftment in infants with SCID³ or tissue damage caused by infiltration by activated autologous T lymphocytes, as typically seen in Omenn syndrome. In addition, some forms of SCID are associated with distinctive features in other systems.

SCID defects are classified according to the immunologic phenotype and are categorized into (1) SCID with absence of T lymphocytes but presence of B lymphocytes (T[−]B⁺ SCID) or (2) SCID with absence of both T and B lymphocytes (T[−]B[−] SCID). Both main groups of SCID include forms with or without natural killer (NK) lymphocytes. Regardless of the immunologic phenotype (T[−]B[−] or T[−]B⁺), patients with SCID present with similar clinical features, including early-onset severe respiratory tract infections, chronic diarrhea, and failure to thrive. SCIDs have a prevalence of approximately 1:50,000 live births and are more common in male subjects, reflecting the overrepresentation of X-linked SCID (SCIDX1), the most common form of SCID in human subjects.

The pathogenesis of SCID reflects distinct mechanisms that affect various steps in T-cell development (*Fig 1*).² Impaired survival of lymphocyte precursors is observed in reticular dysgenesis (RD) and in adenosine deaminase (ADA) deficiency. Both forms

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Abbreviations used

AD:	Autosomal dominant
ADA:	Adenosine deaminase
AID:	Activation-induced cytidine deaminase
AIRE:	Autoimmune regulator
ALPS:	Autoimmune lymphoproliferative syndrome
ANC:	Absolute neutrophil count
AR:	Autosomal recessive
AT:	Ataxia-telangiectasia
BCR:	B-cell receptor
BTK:	Bruton tyrosine kinase
CD40L:	CD40 ligand
CGD:	Chronic granulomatous disease
CHS:	Chediak-Higashi syndrome
CID:	Combined immunodeficiency
CMV:	Cytomegalovirus
CSR:	Class-switch recombination
CVID:	Common variable immunodeficiency
DC:	Dendritic cell
DGS:	DiGeorge syndrome
DHR-123:	Dihydrorhodamine-123
DP:	Double-positive
FOXP3:	Forkhead box protein 3
HCT:	Hematopoietic cell transplantation
HIES:	Hyper-IgE syndrome
HLH:	Hemophagocytic lymphohistiocytosis
ICOS:	Inducible T-cell costimulator
IgAD:	IgA deficiency
IPEX:	Immune dysregulation–polyendocrinopathy–enteropathy–X-linked
IRAK:	IL-1 receptor–associated kinase
IVIg:	Intravenous immunoglobulin
JAK:	Janus kinase
LAD:	Leukocyte adhesion deficiency
MBL:	Mannose-binding lectin
MMR:	Mismatch repair
MSMD:	Mendelian susceptibility to mycobacterial disease
NADPH:	Reduced nicotinamide adenine dinucleotide phosphate
NHEJ:	Nonhomologous end-joining
NK:	Natural killer
PID:	Primary immunodeficiency
RAG:	Recombinase-activating gene
RD:	Reticular dysgenesis
SBDS:	Schwachman-Bodian-Diamond syndrome
SCID:	Severe combined immunodeficiency
SCIDX1:	X-linked severe combined immunodeficiency
SCIG:	Subcutaneous immunoglobulin
SCN:	Severe congenital neutropenia
SHM:	Somatic hypermutation
STAT:	Signal transducer and activator of transcription
TACI:	Transmembrane activator and calcium modulator and cyclophilin ligand interactor
TAP:	Transporter of antigenic peptide
TCR:	T-cell receptor
THI:	Transient hypogammaglobulinemia of infancy
TLR:	Toll-like receptor
TREC:	T-cell receptor excision circle
Treg:	Regulatory T
UNG:	Uracil N-glycosidase
WAS:	Wiskott-Aldrich syndrome
WASP:	Wiskott-Aldrich syndrome protein
XLA:	X-linked agammaglobulinemia

are inherited as autosomal recessive traits and are characterized by extreme lymphopenia. In addition, patients with RD also have severe neutropenia and sensorineural deafness. RD is a very rare form of SCID and is caused by mutations of the adenylylate kinase 2 gene (*AK2*).^{4,5} This mitochondrial enzyme regulates levels of adenosine diphosphate. Adenylylate kinase 2 deficiency results in increased apoptosis of myeloid and lymphoid precursors. ADA is an enzyme of the purine salvage pathway that mediates conversion of adenosine (and deoxyadenosine) to inosine (and deoxyinosine). In the absence of ADA, high intracellular levels of toxic phosphorylated metabolites of adenosine and deoxyadenosine cause apoptosis of lymphoid precursors in the bone marrow and thymus.^{6,7} ADA deficiency accounts for 10% to 15% of all forms of SCID. Clinical manifestations of ADA deficiency extend beyond the immune system (deafness, behavioral problems, costochondral abnormalities, and liver toxicity), reflecting the fact that ADA is a housekeeping enzyme. Purine nucleoside phosphorylase is another enzyme of the purine salvage pathway. Purine nucleoside phosphorylase deficiency is rare (1% to 2% of all forms of SCID). In this disease immunologic abnormalities become progressively manifest within a few years after birth and are more pronounced in T than in B lymphocytes.⁶ Progressive neurological deterioration and autoimmune hemolytic anemia are typically observed.

Defects of cytokine-mediated signaling are responsible for the majority of SCID in human subjects. SCIDX1 accounts for 40% of all cases of SCID and is caused by mutations of the IL-2 receptor γ gene (*IL2RG*), which encodes for the common γ chain (γ_c) shared by cytokine receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. In particular, IL-7 mediates expansion of early thymocyte progenitors, whereas IL-15 plays a role in NK cell development.⁸ Accordingly, patients with SCIDX1 lack both T and NK cells, whereas they have a normal number of circulating B lymphocytes.⁹ The γ_c is physically and functionally coupled to the intracellular tyrosine kinase Janus kinase (JAK) 3, which delivers γ_c -mediated intracellular signaling. Hence defects of JAK3 result in an autosomal recessive form of SCID with an immunologic phenotype undistinguishable from that of SCIDX1.¹⁰ Mutations of the *IL7R* gene (encoding for the α chain of the IL-7 receptor) abrogate T-lymphocyte development but leave B-cell and NK cell development intact.¹¹

Expression of the pre-T-cell receptor (TCR) is a critical landmark during thymocyte development. Similar molecular mechanisms govern expression of the pre-TCR and of the pre-B-cell receptor (BCR) in developing T and B lymphocytes, respectively.¹² In particular, recombinase-activating gene 1 (RAG1) and RAG2 proteins mediate DNA cleavage at the TCR and immunoglobulin heavy and light chain loci, thus initiating V(D)J recombination. Several DNA repair proteins complete this process, allowing assembly of variable (V), diversity (D), and joining (J) elements. Defects of pre-TCR and pre-BCR expression account for a significant fraction of autosomal recessive T[−]B[−] SCID in human subjects and might reflect mutations in the *RAG1* and *RAG2* genes (which account for 4% to 20% of all cases of SCID) or in genes that encode proteins involved in nonhomologous end-joining (NHEJ) and DNA repair, in particular Artemis, DNA protein-kinase catalytic subunit, Cernunnos/XLF, and DNA ligase IV.^{2,12–15} In all of these diseases, development of NK cells proceeds normally, so that NK lymphocytes represent almost all of the circulating lymphocytes, but generation of T and B lymphocytes is severely compromised. However, a leaky phenotype, with residual

TABLE I. Type of infections associated with major categories of PIDs

Organism	Antibody deficiencies	CIDs	Phagocytic defects	Complement deficiencies
Viruses	Enteroviruses	All, especially: CMV, respiratory syncytial virus, EBV, parainfluenza type 3	No	No
Bacteria	<i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Moraxella catarrhalis</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Neisseria meningitidis</i> , <i>Mycoplasma pneumoniae</i>	As for antibody deficiencies, also: <i>Salmonella typhi</i> , <i>Listeria</i> <i>monocytogenes</i> , enteric flora	<i>S aureus</i> , <i>P aeruginosa</i> , <i>Nocardia asteroides</i> , <i>S typhi</i>	As for antibody deficiencies: especially <i>N meningitidis</i> in deficiency of late components
Mycobacteria	No	Nontuberculous, including BCG	Nontuberculous, including BCG	No
Fungi	No	<i>Candida</i> species, <i>Aspergillus</i> species, <i>Cryptococcus neoformans</i> , <i>Histoplasmosis</i> <i>capsulatum</i>	<i>Candida</i> species, <i>Aspergillus</i> species	No
Protozoa	<i>Giardia lamblia</i>	<i>Pneumocystis jiroveci</i> , <i>Toxoplasma gondii</i> , <i>Cryptosporidium parvum</i>	No	No

development of T and B lymphocytes, is typically seen in patients with Cernunnos/XLF deficiency.¹⁶ Genetic defects that affect the NHEJ pathway are characterized also by increased cellular radiosensitivity with extramural manifestations (microcephaly, facial dysmorphisms, and defective tooth development).¹⁷

Mutations of the CD3 δ , CD3 ϵ , and CD3 ζ components of the CD3 complex affect signaling through the pre-TCR and hence cause SCID.¹⁸ Few cases of mutations of the tyrosine phosphatase CD45, which mediates signaling in both T and B lymphocytes, have been also reported.^{19,20}

Defects in T-cell development that occur beyond the CD4⁺CD8⁺ double-positive cell stage result in CID with residual numbers of T lymphocytes. Mutations of the ζ chain-associated protein of 70 kDa (ZAP-70) tyrosine kinase in human subjects compromise positive selection of CD8⁺ thymocytes, thus causing severe deficiency of circulating CD8⁺ T lymphocytes. CD4⁺ T cells are generated and exported to the periphery; however, their proliferative response to stimulation through the TCR is drastically impaired.²¹

Positive selection of CD8⁺ thymocytes is dependent on low-affinity recognition of self-peptides in the context of class I MHC molecules. The transporters of antigenic peptides 1 and 2 (TAP1/2) and tapasin shuttle newly synthesized peptides across the endoplasmic reticulum, where they are loaded onto HLA class I molecules and are then directed to the cell membrane. Mutations in the *TAP1*, *TAP2*, or tapasin genes cause selective CD8⁺ cell deficiency.²² However, this defect is rarely severe, reflecting residual MHC class I expression. The clinical phenotype of MHC class I deficiency is often marked by midline granulomatous lesions and vasculitis.

Defective expression of MHC class II molecules impairs positive selection of CD4⁺ lymphocytes. This disease can be caused by mutations in 4 genes that encode for transcriptional activators of MHC class II genes. The disease is more common in certain geographic regions (North Africa). It is characterized by CD4⁺ cell lymphopenia and has a progressive and severe clinical course.²³

Recently, novel genetic defects have been identified in patients with other rare forms of CID. Calcium flux is essential to mediate the response of various cell types, including mature lymphocytes, to activatory stimuli. Genetic defects of *STIM1* (a sensor of calcium release from the endoplasmic reticulum) and *ORAI1* (a component of the calcium-release activated channels) cause inability of T lymphocytes to respond to TCR-mediated activation.^{24,25} Muscular cells are also affected, causing myopathy.

The signal transducer and activator of transcription (STAT) 5b is a transcription factor that is activated in response to IL-2 and other cytokines and growth factors, including growth hormone. Mutations of *STAT5B* result in a rare form of immunodeficiency with short stature. Because IL-2 plays a critical role in immune homeostasis, STAT5b deficiency is often associated with autoimmune manifestations.²⁶

Hypomorphic mutations in genes that are typically associated with SCID can allow residual T-cell development. In these cases impaired cross-talk between thymocytes and thymic epithelial cells might compromise mechanisms of central tolerance, with failure to delete autoreactive T cells and impaired generation of regulatory T (Treg) cells.^{27,28} Accordingly, autoimmune or dysreactive manifestations are common, with infiltration of target tissues by activated and oligoclonal T lymphocytes. Omenn syndrome, which is caused by mutations in *RAG1/2* or other genes, is the prototype of these conditions and is characterized by erythroderma, lymphadenopathy, and inflammatory gut disease. Hypomorphic *RAG* mutations have been also associated with a novel phenotype characterized by granuloma formation, EBV-related lymphoma, and survival into late childhood.²⁹

Idiopathic CD4 lymphopenia is defined based on a persistently low CD4⁺ T-cell count ($<0.3 \times 10^9/L$ in adults and $<1.0 \times 10^9/L$ in childhood). It is a diagnosis of exclusion: infections sustained by HIV or other T-cell lymphotropic viruses, immunosuppressive treatment, or underlying autoimmune disease must be ruled out. Most patients with idiopathic CD4 lymphopenia are adults. Clinical features include opportunistic (caused by *P jiroveci*, *Cryptococcus neoformans*, *Candida* species, and mycobacteria) and

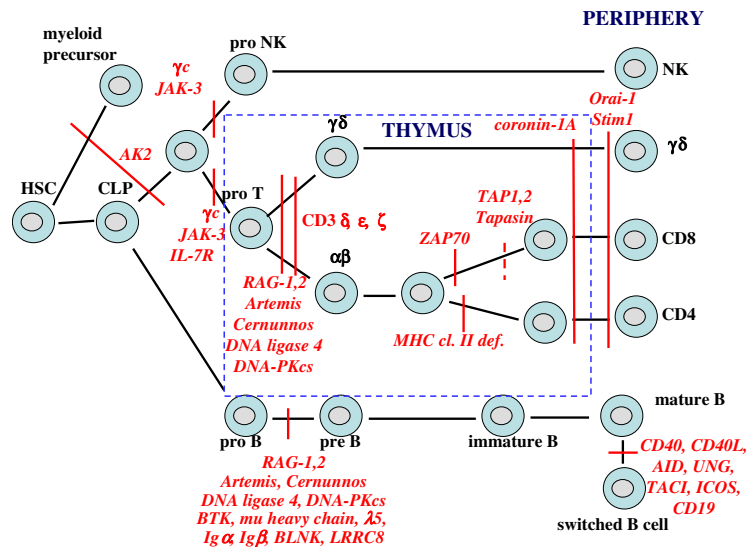


FIG 1. Blocks in T- and B-cell development associated with PIDs.

viral infections.³⁰ Naive CD4⁺ T cells are affected more than memory CD4⁺ lymphocytes. Some degree of hypogammaglobulinemia is common.

CD40 ligand (CD40L) deficiency is inherited as an X-linked trait. CD40L is predominantly expressed by activated CD4⁺ T lymphocytes and interacts with CD40, which is expressed by B lymphocytes, monocytes, dendritic cells (DCs), and other cell types. CD40L-CD40 interaction is a key signal in driving B-cell activation and, combined with interleukin-mediated signaling, promotes class-switch recombination (CSR). Accordingly, male subjects with CD40L deficiency have a severe defect of all immunoglobulin isotypes other than IgM. In addition, CD40L-CD40 interaction also promotes DC maturation and IL-12 secretion, favoring T-cell priming and production of IFN- γ , a key molecule in the defense against intracellular pathogens. Therefore patients with CD40L deficiency are also prone to opportunistic infections (*P. jiroveci* and *Cryptosporidium parvum*), making CD40L deficiency a form of CID.³¹ Neutropenia, which is usually associated with a block at the promyelocyte-myelocyte stage of differentiation in the bone marrow, is found in 65% of the patients with CD40L deficiency. A similar phenotype has been reported in patients with CD40 deficiency, a rare PID with autosomal recessive inheritance.³²

T-cell immunodeficiencies caused by thymic defects

DiGeorge syndrome (DGS) is a developmental defect of the third and fourth pharyngeal pouches and arches, resulting in impaired development of the thymus and parathyroid glands, conotruncal heart abnormalities, facial dysmorphisms, feeding difficulties, and increased frequency of psychiatric disorders in childhood and adulthood.³³ In different series hemizygous deletion of chromosome 22q11 has been observed in 35% to 90% of patients; a minority of patients show deletion of 10p13-14. In most cases (referred to as "partial DGS"), there is mild-to-moderate T-cell deficiency, reflecting residual thymic development. Complete DGS with athymia is rare (1% of all cases) and presents with SCID-like features. Atypical complete DGS includes presentation with development of oligoclonal T cells that

undergo extensive *in vivo* activation and infiltrate target organs, mimicking that observed in Omenn syndrome.

Forkhead box N1 (FOXN1) is a transcription factor required for thymic epithelial cells development. Autosomal recessive *FOXN1* deficiency has been reported in a few patients and is characterized by SCID associated with alopecia and nail dystrophy.³⁴

Impaired egress of mature thymocytes has been reported in a single patient with mutations in coronin-1A, a regulator of actin cytoskeleton.³⁵

Finally, Good syndrome is characterized by the association of hypogammaglobulinemia with thymoma. Opportunistic infections (candidiasis, CMV, and recurrent herpes simplex virus infections) and autoimmune cytopenia (especially red cell aplasia, neutropenia, or both) are common.³⁶

Antibody deficiencies

Defective antibody production causes increased susceptibility, mostly to bacterial infections (Table I) that typically involve the upper and lower respiratory tract (otitis, sinusitis, and pneumonia) but might also cause abscesses in the skin or other organs, meningitis, urinary tract infections, and arthritis. Recurrent viral infections are also common. Intestinal *Giardia* species infection can cause protracted diarrhea. Antibody deficiencies might depend on a variety of defects that interfere with B-cell development, maturation, and/or function (Fig 1).³¹

Signaling through the pre-BCR is an essential step in B-cell development. The pre-BCR is composed of immunoglobulin heavy μ chains, surrogate light chains (V-preB and $\lambda 5$), and the signal-transducing subunits Ig α (CD79a) and Ig β (CD79b). This complex recruits a number of intracytoplasmic proteins, among which are the adaptor molecule B cell linker protein (BLNK) and Bruton tyrosine kinase (BTK). Defects in BTK account for X-linked agammaglobulinemia (XLA), the most common form (85%) of early-onset agammaglobulinemia in human subjects. Mutations in the immunoglobulin heavy μ chain gene (*IGHM*) are the second most common cause (5%), whereas only a few patients have been identified with defects in $\lambda 5$, Ig α , Ig β , and BLNK.^{31,37} In all of these cases, there is a block at the pro-B to

pre-B stage of differentiation in the bone marrow, resulting in virtual absence (<1%) of circulating B lymphocytes. However, the defect is often incomplete in patients with XLA, and few B cells might be identified in peripheral blood. A chromosomal translocation involving the leucine-rich repeat-containing protein 8 gene (*LRRC8*) has been reported in 1 patient in whom congenital agammaglobulinemia with developmental arrest at the pro-B-cell stage was associated with facial dysmorphisms.³⁸ In addition to bacterial infections, patients with agammaglobulinemia are uniquely susceptible to enteroviral infections (which might cause meningoencephalitis or severe dermatomyositis) and mycoplasma (arthritis). Fortunately, both these complications are rare in patients treated appropriately with replacement immunoglobulins.

Maturation of the antibody response is marked by 2 key processes: CSR and somatic hypermutation (SHM).³⁹ During CSR, the μ chain is replaced by other immunoglobulin heavy chains, resulting in the production of IgG, IgA, and IgE, which have distinct physicochemical and biologic properties. SHM is the process by which point mutations are introduced in the variable region of the immunoglobulin genes, leading to increased binding affinity for antigen (affinity maturation). Although CSR and SHM are independent and distinct processes, they both occur in the germinal centers and are triggered by similar signals, such as CD40L-CD40 interaction and TLR-mediated signaling. CSR involves active transcription through the heavy chain constant-regions loci, with formation of DNA/RNA hybrids that leave one strand of DNA accessible to changes and cleavage. Induction of CSR promotes transcription of the activation-induced cytidine deaminase gene (*AICDA*), which encodes a DNA-editing enzyme (also known as activation-induced cytidine deaminase [AID]) that replaces deoxycytidine residues with deoxyuracil. The resulting mismatch in the DNA is recognized by the enzyme uracil N-glycosylase (UNG), which removes the deoxyuracil residues, leaving abasic sites that are resolved by means of DNA repair mechanisms. Similar events occur during SHM; however, the mechanisms of DNA repair between these processes are distinct.³⁹ In particular, DNA repair during CSR involves proteins of the NHEJ pathway, as well as the Ataxia-Telangiectasia Mutated (ATM), Meiotic Recombination 11 (MRE11) and Nijmegen Breakage Syndrome 1 (NBS1) proteins. Furthermore, the DNA mismatch repair (MMR) system also participates in CSR. In contrast, SHM involves error-prone DNA polymerases and the MMR pathway. Mutations in the *AICDA* and *UNG* genes account for B-cell intrinsic defects of CSR, resulting in absent or very low levels of serum IgG, IgA, and IgE, whereas IgM levels are often increased (thus resulting in a hyper-IgM phenotype).³⁹ The vast majority of AID and all of the few cases of UNG deficiency reported thus far are inherited as autosomal recessive traits; however, few cases of mutations in the C-terminal region of AID have autosomal dominant inheritance.^{31,39} SHM is differently affected by AID versus UNG deficiency; in particular, SHM is abolished in the former (with the exception of mutations that affect the C-terminus of the AID molecule), whereas it is preserved but biased (with lack of mutations at A:T residues) in UNG deficiency.³⁹ Impaired CSR with reduced levels of IgG, IgA, and IgE is also observed in patients with ataxia-telangiectasia (caused by mutations of the *ATM* gene), ataxia-telangiectasia-like syndrome (*MRE11* mutations), Nijmegen breakage syndrome (*NBS1* mutation), and ligase IV syndrome in keeping with the role that these proteins play in CSR.^{39,40} Also, mutations of *Post-Meiotic*

Segregation increased 2 (*PMS2*), which is involved in MMR, cause impaired CSR, which is associated with high susceptibility to malignancies and café au lait spots.⁴¹ In spite of these advances, a significant proportion (about 15%) of CSR defects currently grouped under the definition of hyper-IgM syndrome remain genetically undefined.³⁹

Common variable immunodeficiency disorders (CVIDs) are the most common form of clinically significant PIDs and present mainly in adults, although they can also be observed in children. CVIDs are defined by reduced levels of 1 or more isotypes and impaired antibody production in response to immunization antigens or natural infections.⁴² CVIDs are a diagnosis of exclusion of all known causes of poor antibody production or low serum immunoglobulin levels. In addition to recurrent infections of the respiratory tract (sinusitis, otitis, bronchitis, and pneumonia) caused by common bacteria (eg, nontypeable *Haemophilus influenzae* and *Streptococcus pneumoniae*), some patients with a CVID are highly prone to autoimmune manifestations (cytopenias and inflammatory bowel disease), granulomatous lesions, lymphoid hyperplasia, and tumors (especially lymphomas).⁴²⁻⁴⁴ There is now clear evidence that CVIDs include a group of clinically and genetically heterogeneous conditions. CVIDs are mostly sporadic; however, autosomal dominant and autosomal recessive forms are also possible. Although most patients with a CVID have a normal number of B lymphocytes, some (12%) will turn out to have differentiation defects. Among those who do have B cells, some have reduced switched memory (CD27⁺IgD⁻) B lymphocytes and a low rate of SHM⁴⁵; this might turn out to be due to a T- or B-cell failure. Most cases of CVIDs remain genetically undefined and might well turn out to be polygenic. Several studies of familial cases of CVIDs have shown association with the MHC region; however, the underlying gene defect has not been clearly identified. Mutations of the Tumor Necrosis Factor Receptor Soluble Factor 13B (*TNFRSF13B*) gene, which encodes the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), a member of the TNF receptor family, have been identified in 15% of the patients, most often in heterozygosity,^{31,42,46} and have been found to be present in the general population as well. TACI is expressed by B lymphocytes and interacts with 2 ligands: the B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL). In particular, APRIL/TACI interaction promotes B-cell activation and CSR. Disruption of the *tnfrsf13b* gene in mice leads to lymphoid proliferation, and lymphoid hyperplasia and granulomatous lesions are often seen in patients with a CVID. Nine patients have been identified with mutations in the inducible T-cell costimulator gene (*ICOS*).⁴⁷ ICOS is expressed by activated T cells and interacts with ICOS ligand expressed by B lymphocytes. This interaction promotes B-cell activation and antibody production. Finally, a few cases of CVIDs are due to mutations of the *CD19* gene.⁴⁸ The CD19 protein forms a complex with CD21, CD81, and CD225 and decreases the threshold of BCR-mediated activation. CD19 deficiency does not affect B-cell development, as shown by a normal number of circulating CD20⁺ lymphocytes in CD19-deficient patients.

IgAD is the most common PID, with approximately 1:700 affected individuals worldwide. Both partial and complete forms of IgAD are known. The pathophysiology of IgAD remains poorly understood, although association with MHC alleles and higher frequency within families with a CVID have been reported. Approximately two thirds of adults with IgAD are

asymptomatic, but the remaining might experience recurrent infections, autoimmunity, or allergy.⁴⁹ Adult patients with IgAD and a history of infections often have associated defects of IgG subclasses, especially IgG2. However, children are more likely to have delayed maturation of immunoglobulin synthesis and will not progress to significant immunodeficiency. IgG subclass deficiency might also occur without abnormalities in other isotypes. The pathophysiology of IgG subclass deficiency remains unknown in most cases; interstitial deletions on both alleles of the immunoglobulin heavy chain constant region have been reported in a minority of patients, most of whom have been infection free.

Specific antibody deficiency is characterized by impaired antibody production (especially to carbohydrate antigens) without abnormalities in total immunoglobulin levels or in B-cell numbers. The pathophysiology of this disorder, which is associated with recurrent upper and lower respiratory tract infections,⁵⁰ remains unclear.

Patients with transient hypogammaglobulinemia of infancy (THI) have low immunoglobulin levels that spontaneously return to normal, usually within 2 years of age, although this is very variable.^{51,52} Although many subjects with THI remain asymptomatic, this condition is associated with a higher rate of recurrent infections, especially upper respiratory tract infections of viral origin. The pathophysiology of THI is unknown. In a prospective study of infants who presented with hypogammaglobulinemia, a low number of memory B cells and inability to produce IgG *in vitro* were associated with persistence of hypogammaglobulinemia and increased risk of infection beyond 2 years of age.⁵¹

Immunodeficiency with immune dysregulation

Some forms of immunodeficiency are characterized by significant autoimmune manifestations, reflecting disturbance in immune homeostasis.^{53,54} Central immune tolerance is achieved through deletion of autoreactive T-cell clones in the thymus. The autoimmune regulator (AIRE) protein is a transcription factor expressed by mature medullary thymic epithelial cells. AIRE drives expression of tissue-restricted antigens that are presented by medullary thymic epithelial cells and thymic DCs to nascent T lymphocytes, thereby permitting deletion of T-cell clones that recognize self-antigens with high affinity. Mutations of the *AIRE* gene disrupt this protein, causing autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy syndrome.⁵⁵ Hypoparathyroidism and adrenal insufficiency are prominent autoimmune manifestations of this autosomal recessive disorder. Treg cells mediate suppression of immune responses to self-antigens in the periphery. Generation of Treg cells in the thymus is controlled by the transcription factor forkhead box protein 3 (FOXP3). Mutations of the *FOXP3* gene cause immune dysregulation–polyendocrinopathy–enteropathy–X-linked (IPEX) syndrome, with severe and early-onset autoimmune enteropathy, insulin-dependent diabetes, and eczema.⁵⁶ In typical cases the disease evolves rapidly, unless treated with hematopoietic cell transplantation (HCT). IL-2 plays an important role in immune homeostasis and upregulates expression of FOXP3 by CD4⁺CD25^{hi} Treg cells. Mutations of the *IL2RA* gene, which encodes for the α chain of the IL-2 receptor, cause immunodeficiency with IPEX-like features.⁵⁷ Peripheral immune homeostasis is also based on apoptosis of autoreactive

lymphocytes in the periphery. Interaction between Fas ligand, expressed by activated lymphocytes and Fas (CD95) triggers intracellular signaling pathways that ultimately result in activation of caspases and cell death. Mutations of Fas are the predominant cause of autoimmune lymphoproliferative syndrome (ALPS), with lymphadenopathy, hepatosplenomegaly, and autoimmune cytopenia.⁵⁸ There is an increased risk of malignancies (especially B-cell lymphomas), which occur in 10% of the patients with Fas mutations.⁵⁹ ALPS is most often inherited as an autosomal dominant trait and is caused by dominant-negative mutations that interfere with the signal-transducing activity of Fas trimeric complexes. Somatic mutations of the Fas gene have been reported in a few cases. A rare variant of Fas is caused by Fas ligand mutations. In a few patients, mutations of caspase-8 and caspase-10 have been identified also.^{60,61} IL-2 starvation induces apoptosis through a mechanism that depends on the proto-oncogene Neuroblastoma RAS viral oncogene homolog (NRAS). Mutations of this gene have been identified in a single family with ALPS.⁶² A significant fraction of patients with ALPS remain genetically undefined.

Immunodeficiency with impaired cell-mediated cytotoxicity

The cytotoxic activity of T and NK lymphocytes depends on the expression of cytolytic proteins that are assembled into granules and transported through microtubules to the lytic synapse that is formed on contact with target cells. Some forms of immunodeficiency are characterized by impairment of the mechanisms of transport, docking, or release of the lytic granules. These disorders are frequently associated with defective intracellular transport of melanin, resulting in immunodeficiency with pigmentary dilution disorders.⁶³ The Chediak-Higashi syndrome (CHS) is an autosomal recessive disease caused by mutations of the *Lysosomal Trafficking regulator (LYST)* gene. In addition to impaired cytotoxicity, the phenotype of CHS includes the presence of giant lysosomes in leukocytes, gray-silvery hair, and peripheral neuropathy that reflects primary involvement of the nervous system.⁶⁴ Cytotoxicity defects characterize also the autosomal recessive forms of hemophagocytic lymphohistiocytosis (HLH) because of deficiency of perforin (which forms the cytolytic pores on contact with target cells), Munc13-4 (involved in intracellular transport of lytic granules), and syntaxin 11 (presumably involved in permitting fusion of the cell membrane between cytotoxic lymphocytes and target cells). In these diseases the inability to extinguish inflammatory reactions results in sustained and excessive production of T_H1 cytokines and IFN- γ in particular. This inflammatory reaction characterizes the life-threatening “accelerated phase” of the disease with hemophagocytosis. A similar phenotype has been occasionally observed also in Hermansky-Pudlak syndrome type 2, a condition with oculocutaneous albinism, severe neutropenia, and tendency to bleeding, which is caused by mutations of the β component of the adaptor-related protein complex 3 (AP3) involved in sorting of granules to the endosomal pathway.⁶⁵

The X-linked lymphoproliferative disease, mainly caused by EBV infection, might be due to mutation of the *SH2D1A* gene, which encodes an adaptor protein involved in intracellular signaling in T and NK lymphocytes,⁶⁶ or of the *BIRC4* gene, which encodes for the X-linked inhibitor of apoptosis.⁶⁷ More recently, an autosomal recessive form of the disease has been identified that

is caused by mutations of *ITK*, an intracellular tyrosine kinase expressed in T lymphocytes.⁶⁸ All of these 3 forms of lymphoproliferative disease share a lack of NK T cells, suggesting a possible role of these rare populations of lymphocytes in controlling EBV infection.

Defects of innate immunity: Phagocytes, TLRs, leukocyte signaling pathways, and complement

Phagocytic cell defects. Phagocytes play a key role in the defense against bacteria and fungi; accordingly, patients with defects of phagocytic cell number, function, or both experience recurrent and severe infections of fungal (especially *Candida* and *Aspergillus* species) and bacterial origin. Respiratory tract and cutaneous infections predominate, but deep-seeded abscesses are also common. Recurrent oral stomatitis is present in most cases.

Severe congenital neutropenia (SCN) is defined as a neutrophil count that is persistently less than 0.5×10^9 cells/L. A variety of genetic defects might cause SCN in human subjects.⁶⁹ The most common form of SCN is due to mutation of the *ELA2* gene, which encodes neutrophil elastase. This disease might be sporadic or autosomal dominant and is associated with a block at the promyelocyte-myelocyte stage in the bone marrow. Some *ELA2* mutations cause cyclic neutropenia, with oscillations in neutrophil count, which reach a nadir approximately every 21 days, resulting in periodicity of the infections. *ELA2* mutations carry an increased risk of myelodysplasia and myeloid leukemia associated with somatic mutations in the granulocyte colony-stimulating factor receptor (*GCSFR*) gene. Among autosomal recessive forms of SCN, *HAX1* deficiency (identified also in the original SCN pedigree reported by Kostmann et al) causes increased apoptosis of myeloid cells. Some *HAX1* mutations cause also increased neuronal cell death, leading to a severe neurological phenotype.⁷⁰ Increased apoptosis caused by intracellular unbalance of glucose levels is also observed in myeloid cells from patients with mutations in the glucose-6-phosphatase catalytic subunit 3 gene (*G6PCS3*) or in patients with glycogenosis 1b caused by defects of the *G6PT1* gene, which encodes a G6P transporter. Heart and urogenital defects and prominence of superficial veins are part of the *G6PCS3* deficiency phenotype.⁷¹ More rarely, autosomal recessive SCN is due to p14 deficiency; this form is associated with partial oculocutaneous albinism and short stature. Mutation of the Growth Factor Independent 1 (*GFI1*) gene, encoding a myeloid transcription factor, causes a rare form of autosomal dominant SCN; T and B lymphopenia is also observed. A rare X-linked recessive form of SCN with increased risk of myelodysplasia is caused by activating mutations of the Wiskott-Aldrich syndrome protein gene (*WASP*). Finally, X-linked neutropenia, heart defects, and growth retardation are features of Barth syndrome, which is caused by mutation of the Tafazzin gene (*TAF*) with mitochondrial defects.

Chronic granulomatous disease (CGD) represents the prototype of defects of phagocyte function and is caused by defects in the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex.⁷² In 75% of cases, CGD is inherited as an X-linked trait caused by mutations in the X-linked *CYBB* gene, which encodes the gp91^{phox} component of NADPH oxidase. Autosomal recessive forms caused by defects of the p22^{phox}, p47^{phox}, and p67^{phox} components are also known. The gp91^{phox} and p22^{phox} proteins are located in the phagosome membrane,

where cytosolic p47^{phox} and p67^{phox} are translocated after phagocytic cell activation. Assembly of the NADPH complex is regulated by 2 glutathione triphosphatases, Rac2 and Rap1, and defects of Rac2 have been identified in 1 patient with CGD-like disease. After phagocytosis, induction of the NADPH oxidase complex results in production of microbicidal compounds (superoxide radicals and hydrogen peroxide) and activation of lytic enzymes (cathepsin G, elastase, and myeloperoxidase), resulting in intracellular killing of bacteria and fungi. Patients with CGD experience recurrent and often severe infections (skin, liver and perirectal abscesses, pneumonia, and lymphadenitis). *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia*, and *Nocardia* species are responsible for most of the bacterial infections, whereas *Candida* and *Aspergillus* species cause most of the fungal infections. Patients with CGD are also at higher risk for mycobacterial infections. The sustained inflammatory response observed in patients with CGD is also responsible for the granulomatous manifestations of the disease that recur, especially in hollow organs, even in the absence of infections, causing gastric outlet obstruction, noninfective colitis, and hydronephrosis.⁷²

Leukocyte adhesion deficiency (LAD) includes a series of syndromes characterized by impaired trafficking of leukocytes.⁷³ LAD1 is due to mutations of the *ITGB2* gene, which encodes the β_2 integrin component (also known as CD18) shared by the lymphocyte function-associated antigen 1, complement receptor 3, and complement receptor 4 molecules. These β_2 integrins mediate stable adhesion between leukocytes and endothelial cells, permitting transendothelial passage of leukocytes that can thus reach the sites of infection/inflammation. LAD2 is caused by mutations in a GDP-fucose transporter. In this disease defective fucosylation of proteins results in a lack of expression of Sialyl-Lewis-X, the ligand for E-selectin. This defect causes impaired rolling of leukocytes along the endothelium.⁷³ Finally, LAD3 is caused by mutations of kindlin-3, which is involved in inside-outside integrin signaling.⁷⁴ All 3 forms of LAD are inherited as an autosomal recessive trait, and they all present early in life with very severe infections and lack of pus formation. Impaired wound healing (even after minor trauma) and severe periodontitis are typically present. In addition, patients with LAD2 have short stature, facial dysmorphisms, mental retardation, and Bombay blood-type phenotype, reflecting the generalized defect of fucose metabolism. LAD3 is associated with increased bleeding caused by platelet functional defects.

Defects of TLRs. TLRs are a series of molecules that are expressed at the cell surface or endosomal membrane and mediate recognition of pathogen-associated molecular patterns, such as LPS, glycolipids, and single- or double-stranded RNA. The classical pathway of TLR activation involves the adaptor molecules MyD88 and the intracellular kinases IL-1 receptor-associated kinase (IRAK) 4 and IRAK-1 and ultimately results in the induction of nuclear factor κ B and production of inflammatory cytokines (IL-1, IL-6, TNF- α , and IL-12). TLR-3, TLR-7, TLR-8, and TLR-9 can activate an alternative pathway that involves the adaptor molecules TRIF and the UNC-93B protein, resulting in the induction of type 1 interferons (IFN- α/β). Surprisingly, mutations in genes involved in TLR-mediated signaling result in a selective susceptibility to pathogens in human subjects. In particular, *IRAK4* and *MYD88* gene mutations (both inherited as autosomal recessive traits) have a similar phenotype, with severe and invasive pyogenic infections early in life, often

without significant inflammatory response.^{75,76} Infections tend to become less frequent later in life. Heterozygous mutations in *TLR3* and biallelic mutations in *UNC93B* have been identified in patients with selective susceptibility to herpes simplex encephalitis, with reduced production of type 1 IFN.^{77,78}

Defects of the IL-12/IFN- γ signaling pathway. The immune response against mycobacteria is based on secretion of IL-12 by macrophages. IL-12 binds to a specific receptor expressed by T and NK lymphocytes and induces secretion of IFN- γ that triggers macrophage microbicide on binding to the IFN- γ receptor. Defects of the IL-12/IFN- γ axis account for mendelian susceptibility to mycobacterial disease (MSMD).⁷⁹ Mutations might affect the IL-12 p40 subunit, the IL-12 receptor β 1 chain, both chains of the IFN- γ receptor (encoded by the *IFNGR1* and *IFNGR2* genes), and the *STAT1* gene, which encodes a transcription factor downstream of the IFN- γ receptor. Different mutations in these genes account for autosomal dominant and autosomal recessive variants of MSMD. In addition, mutations in the *IKBKG* gene, encoding the IKK- γ (also known as NF- κ B essential modulator, NEMO) regulatory component of the nuclear factor κ B signaling pathway, also cause increased susceptibility to mycobacterial disease associated with CID, impaired CSR, and ectodermal dystrophy.⁸⁰ NEMO deficiency is inherited as an X-linked trait. All forms of MSMD are characterized by increased susceptibility to environmental mycobacteria (*Mycobacterium avium*, *Mycobacterium kansasii*, and *Mycobacterium fortuitum*) and to BCG vaccine strain. *Salmonella*, *Listeria*, and *Histoplasma* species infections can also be observed, especially in patients with *IL12RB1* mutations. Complete STAT1 deficiency causes susceptibility to severe viral infections in addition to MSMD.⁸¹

Complement defects. A variety of inherited defects of complement components have been reported.⁸² Deficiency of the early components of the classical pathway of complement (C1q, C1r, C1s, C4, C2, and C3) causes autoimmune manifestations resembling systemic lupus erythematosus. C2 and C3 deficiencies also lead to increased risk of infections caused by capsulated bacteria. Defects of late components (C5-C9) are associated with recurrent and invasive neisserial infections. A similar phenotype is observed in patients with defects of factor D or of properdin, 2 components of the alternative pathway of complement activation. Deficiencies of the regulatory components Factor H and Factor I cause membranoproliferative glomerulonephritis and recurrent atypical hemolytic-uremic syndrome. The latter can also be caused by deficiency of membrane cofactor protein, a C3b/C4b-binding molecule of the complement system with cofactor activity for the Factor I-dependent cleavage of C3b and C4b. Deficiency of the manose-binding lectin (MBL), a component of the MBL-dependent pathway of complement, has been associated with increased risk of recurrent bacterial infections, especially during the first years of life. However, it is more plausible that MBL deficiency plays a contributory role in patients who have additional risk factors. Mutations of the MBL-associated serine-protease-2 gene (*MASP2*) have been also linked to increased occurrence of infections. Finally, deficiency of the C1 esterase inhibitor, a regulatory component of the classical pathway of complement activation, does not cause immunodeficiency but hereditary angioedema, with recurrent episodes of edema that might involve the mucosa of the larynx and the gut, as well as the face and the extremities. These manifestations reflect uncontrolled release of

bradykinin caused by lack of inhibition by C1 esterase inhibitor of the kallikrein-kinin system.

Immunodeficiency syndromes

The term immunodeficiency syndromes applies to several disorders in which other clinical features are present in addition to immunodeficiency. The Wiskott-Aldrich syndrome (WAS) is an X-linked disease characterized by eczema, immunodeficiency (with increased susceptibility to infections, autoimmunity, and lymphoid malignancies), and congenital small-sized thrombocytopenia.⁸³ However, only one third of the patients present all of the elements of this triad. WAS is caused by mutations in the *WASP* gene, which encodes a regulator of actin cytoskeleton the expression of which is restricted to hematopoietic cells. Hypomorphic mutations of the *WASP* gene, especially in exons 1 and 2, are often associated with a milder variant of the disease isolated X-linked thrombocytopenia.⁸³ In contrast, transactivating mutations of *WASP* cause X-linked neutropenia and myelodysplasia (see above).

Several immunodeficiencies are caused by defects in mechanisms that sense, repair, or both DNA breaks. Some of these defects are associated with SCID (see above). Ataxia-telangiectasia is an autosomal recessive disease caused by mutations of the ataxia-telangiectasia mutated gene (*ATM*).⁸⁴ Patients with ataxia-telangiectasia have ataxia, ocular telangiectasia, increased risk of infections, and tumors. The immunodeficiency is marked by a progressive decrease of T-lymphocyte counts and function and hypogammaglobulinemia. Typically, levels of α -fetoprotein are increased. A similar phenotype, although without the increase in α -fetoprotein levels, is observed in patients with mutations in the *MRE11* gene, which is involved in DNA repair. The Nijmegen syndrome associates immunologic findings with microcephaly, "bird-like" facies, short stature, and increased occurrence of malignancies and is due to mutations in the Nibrin gene (*NBS1*).⁸⁵ Ligase IV (*LIG4*) syndrome is due to defects of the *LIG4* gene, which encodes a factor involved in the DNA repair phase of the NHEJ process. Patients with *LIG4* syndrome present with microcephaly, facial dysmorphism and increased susceptibility to tumors, and a variable degree of immunodeficiency that ranges from SCID/Omenn syndrome to hypogammaglobulinemia with impaired CSR to moderate or even very modest defects of T- and B-cell immunity.⁸⁶ Immunodeficiency-centromeric instability-facial anomalies syndrome is most often due to mutations of the DNA methyltransferase 3B gene (*DNMT3B*). From an immunologic standpoint, immunodeficiency-centromeric instability-facial anomalies syndrome is characterized by recurrent bacterial and opportunistic infections, hypogammaglobulinemia, and a reduced number of T and B lymphocytes.⁸⁷

The hyper-IgE syndrome (HIES) is characterized by eczema, increased occurrence of cutaneous and pulmonary infections sustained by *S aureus* (with formation of pneumatoceles) and *Candida* species, and markedly increased IgE levels.⁸⁸ Life-threatening superinfection of pneumatoceles by *Aspergillus* and *Scedosporium* species is common. Sporadic autosomal dominant and autosomal recessive forms are known. The autosomal dominant HIES associates defective shedding of primary teeth, scoliosis, higher risk of bone fractures, joint hyperextensibility, characteristic facial appearance, and vascular abnormalities with aneurysms and is due to dominant-negative heterozygous mutations of the *STAT3* gene.⁸⁹ STAT3 is a transcription factor

that is activated in response to activation of the JAK-STAT signaling pathway through cytokine and growth factor receptors that contain the gp130 protein. Biologic responses to IL-6 and IL-10 are decreased, and development of T_H17 cells is impaired, resulting in poor secretion of IL-17, IL-21, and IL-22. The phenotype of autosomal recessive HIES is different. These patients might also have viral disease but do not present with skeletal or dental abnormalities. Vasculitis and autoimmunity are common in autosomal recessive HIES. In one case of autosomal recessive HIES, mutations of the tyrosine kinase 2 gene (*TYK2*), encoding one of the members of the JAK family upstream of STAT3, were demonstrated.⁹⁰ However, most cases of autosomal recessive HIES remain genetically undefined.

Veno-occlusive disease with immunodeficiency syndrome is a CID associated with early-onset severe liver disease and profound hypogammaglobulinemia and is caused by a mutation of the gene encoding the nuclear body protein Sp110.⁹¹

Immuno-osseous dysplasias include cartilage hair hypoplasia, Schimke disease, and Shwachman-Bodian-Diamond syndrome (SBDS). Cartilage hair hypoplasia is caused by mutations of the RNase mitochondrial ribonucleoprotein gene (*RMRP*). It is characterized by short-limbed dwarfism, sparse hair, and frequent occurrence of anemia or other forms of bone marrow failure associated with a variable degree of immunodeficiency, ranging from SCID to virtually normal immune function.⁹² Schimke disease (caused by mutations of *SMARCAL1*)⁹³ associates short stature and progressive renal disease. SBDS is characterized by exocrine pancreatic insufficiency, bone marrow failure, and metaphyseal chondrodysplasia and is caused by a mutation of a gene involved in ribosome biogenesis.⁹⁴ Neutropenia is a prominent feature of SBDS and might cause severe infections.

Warts-hypogammaglobulinemia-infections-myelokathexis syndrome is an autosomal dominant disease caused by heterozygous mutations of the *CXCR4* chemokine receptor, which cause sustained and inappropriate signaling mediated by the ligand CXCL12. CXCL12-CXCR4 interaction is important in the governance of leukocyte trafficking. In patients with Warts-hypogammaglobulinemia-infections-myelokathexis, there is retention of mature neutrophils in the bone marrow (myelokathexis), resulting in severe neutropenia. Trafficking of B lymphocytes is also affected, causing B-cell lymphopenia and a variable degree of hypogammaglobulinemia. Patients are highly prone to papilloma-virus infections (warts).⁹⁵

DIAGNOSTIC APPROACH TO PIDS

The main forms of PID (CIDs, antibody deficiencies, and defects of innate immunity) are characterized by different susceptibilities to pathogens (Table I). Accordingly, medical history (with particular regard to type, location, age at onset, and severity of infections) might provide important insights into the possible underlying mechanisms of immunodeficiency. Additional aspects of past medical history might also help. Certainly a history of HIV infection is very important in the differential diagnosis of SCID. Also, a history of seizures during neonatal age should prompt one to consider DGS.

Family history is also important in the approach to PIDs because of the monogenic nature of most forms of these disorders. However, most patients with PIDs do not have a positive family history because they represent *de novo* mutations or the first occurrence of an autosomal recessive disease.

Physical examination can also provide important hints. Patients with agammaglobulinemia show absence of tonsils and other lymphoid tissues. Partial albinism characterizes pigmentary dilution disorders, ataxia and ocular telangiectasias are observed in ataxia-telangiectasia, microcephaly is common in PIDs associated with defects in DNA repair, petechiae and other bleeding manifestations associated with eczema are highly suggestive of WAS, and patients with immuno-osseous disorders have short stature. Generalized erythroderma is typical of Omenn syndrome but can also occur in IPEX, in SCID with maternal T-cell engraftment, in atypical complete DGS, and occasionally in NEMO deficiency.

Clinical immunologic laboratory tests are very important to validate the suspicion of PID. Lymphopenia, and marked reduction of T-lymphocyte counts in particular, is a hallmark of SCID, but HIV infection must be excluded in all cases. It is very important to compare lymphocyte counts with those of age-matched healthy control subjects. The presence of maternal T-cell engraftment or of residual autologous T cells in patients with CID might result in relatively preserved (and even normal) T-lymphocyte counts and hence confound the picture; however, in these cases most circulating T lymphocytes have an activated/memory (CD45R0⁺) phenotype, and there is a virtual lack of naive (CD45RA⁺) T lymphocytes. T-cell receptor excision circles (TRECs), consisting of circularized signal joints, are a byproduct of V(D)J recombination and are exported to the periphery by newly generated T lymphocytes that leave the thymus. Levels of TRECs in circulating lymphocytes are particularly high in newborns and infants (reflecting active thymic function) and progressively decrease with age. No TRECs are detected in infants with SCID; assessment of TREC levels by means of PCR has been proposed for newborn screening of SCID,⁹⁶ and a pilot study has been recently started in Wisconsin and Massachusetts.⁹⁷ In addition to the severe T-cell lymphopenia, *in vitro* response to mitogens is absent in patients with SCID; however, the number and proliferative responses of circulating T lymphocytes are often variable in patients with other forms of CID.

Evaluation of patients with putative antibody deficiency should include enumeration of B lymphocytes, measurement of total immunoglobulins, and assessment of specific antibodies to both protein and polysaccharide antigens. B lymphocytes (identified based on CD19 or CD20 expression) are absent in patients with congenital agammaglobulinemia, some adult patients with CVIDs, and patients with thymoma. Differential diagnosis includes some forms of SCID and myelodysplasia. Immunoglobulin serum levels should be compared with values of age-matched control subjects. It is important to remember that during the first months of life, IgGs are predominantly of maternal origin. Therefore apparently normal IgG serum levels can be detected during the first 2 to 3 months of life, even in patients with impaired ability to produce antibodies. There is large variability in the ability to produce IgA, and some individual attain normal levels only after the first few years of life. Demonstration of very low levels of serum IgG and IgA, with normal to increased serum IgM levels, is suggestive of CSR defects caused by either intrinsic B-cell problems (AID or UNG deficiency) or impaired cross-talk between T and B lymphocytes (CD40L or CD40 deficiency or NEMO defect). The term hyper-IgM syndrome, which is commonly used to identify these disorders, is in fact misleading because the majority of patients with these disorders have normal IgM serum levels.

Determination of serum levels of IgG subclasses has limited value and should not be used as a screening assay. More important information is provided by the assessment of antibody titers. In particular, antibodies to tetanus toxoid and diphtheria toxoid represent robust assays to measure the antibody response to protein (T-dependent) antigens. It is important to remember that use of conjugated vaccines to pneumococcus, *H influenzae*, or meningococcus elicits T-dependent responses, even if the antibodies are ultimately directed against polysaccharide antigens. Therefore pneumococcal polysaccharide vaccine should be used to test the antibody response to polysaccharide (T-independent) antigens. If basal antibody titers appear nonprotective, a boosting immunization should be performed, followed by repeat measurement of specific antibodies 4 weeks later. A 4-fold increase of specific antibodies is considered indicative of robust antibody production. Isohemagglutinins are antibodies directed against the polysaccharide moieties of ABO blood group antigens and represent "natural" anti-polysaccharide antibodies. However, isoagglutinin titers are often low in the first 2 years of life, thus limiting the value of this test in infancy.

The occurrence of severe bacterial and fungal infections since early life, especially if associated with a history of gingivostomatitis, should prompt consideration of disorders of neutrophil numbers, functions, or both. The absolute neutrophil count (ANC) is markedly reduced in all forms of SCN. If infections recur approximately every 3 weeks, the possibility of cyclic neutropenia should be entertained. In this case ANCs should be evaluated once a week for 6 consecutive weeks to identify possible decreases in the neutrophil count. Diagnosis of CGD is most commonly based on evaluation of dihydrorhodamine-123 (DHR-123) oxidation, as assessed by means of flow cytometry. This assay is quantitative and objective and also permits identification of carriers of X-linked CGD, who have 2 populations of neutrophils, only one of which is capable of mediating DHR-123 oxidation. Patients with autosomal recessive CGD often have very modest but detectable levels of activity, as detected by using this assay. Diagnosis of LAD1 is straightforward and is based on flow cytometric evaluation of CD18 expression on the surface of leukocytes. Partial defects (2% to 10% of normal density of CD18 molecules at the cell surface) are associated with a moderate form of the disease that permits more prolonged survival.

Investigation of patients with putative TLR-signaling defects can be facilitated by using a screening assay to measure IL-6 and TNF- α production on stimulation of whole blood with TLR agonists or by the failure of affected neutrophils to shed CD62 ligand following stimulation *in vitro*.⁹⁸

Measurement of hemolytic activity of the classical (CH50) and alternative (AP50) pathways of complement, as well as of C3 and C4 levels, might guide in the diagnosis of complement deficiencies. However, as for other forms of PIDs, gene mutations might allow production of nonfunctional proteins, so that ultimately the diagnosis of deficiency of single complement components can rely on appropriate functional assays. The importance of measuring MBL levels is less well defined because of the uncertainties on the pathogenic role of this deficiency, if isolated.

Flow cytometric assays targeted to disease-associated proteins might help define the diagnosis,⁹⁹ as discussed above for assessment of CD18 expression in patients with LAD1. Impaired expression of CD40L is typically observed following *in vitro*

activation of CD4⁺ T cells in patients with CD40L deficiency. Patients with IPEX usually lack circulating Treg cells, which are defined as CD4⁺CD25^{bright} cells that express intracellular FOXP3. However, a minority of patients with IPEX show residual expression of nonfunctional FOXP3 protein. Caution should be used in the enumeration of Treg cells because FOXP3 can also be expressed by activated T lymphocytes. Protein-specific flow cytometric assays can also be used in the diagnosis of WAS (lack of WASP protein), SCIDX1 (absence of γ c), XLA (lack of BTK protein in monocytes), and HLH caused by perforin deficiency. However, interpretation of these assays should take into account that some mutations are permissive for residual protein expression.

Increase of TCR $\alpha\beta$ ⁺CD4⁻CD8⁻ (double-negative) T cells is suggestive of ALPS; increased levels of Fas ligand and IL-10 represent additional biomarkers of this disease.¹⁰⁰

Functional assays are also important. In addition to *in vitro* proliferative response to mitogens and antigens for the diagnosis of CID, specific antibody responses in patients with hypogammaglobulinemia, and DHR-123 oxidation in the diagnosis of CGD, other clinically relevant functional assays include the demonstration of markedly reduced NK cytotoxicity (measured against K562 erythroleukemic target cells) in patients with familial forms of HLH and impaired Fas-mediated apoptosis in patients with ALPS.

In the last years, genetic tests have become more widely available. Identification of specific mutations is important not only to confirm the diagnosis but also to guide genetic counseling and to facilitate carrier detection and prenatal diagnosis. However, it should be noted that not all DNA changes are necessarily disease causing; some might represent polymorphisms, rare variants, or disease-contributing variations. These non-disease-causing DNA changes contribute to the heterogeneity of clinical and immunologic phenotypes associated with PIDs, and overall, they make genotype-phenotype correlation less stringent. Analysis of mRNA and protein expression and functional studies are often warranted to define in better detail the possible pathogenicity of specific gene mutations. However, it is important to recognize that in some cases (eg, patients with SCID), the urgency of the condition might require that patients be treated aggressively, even if definition of the genetic defect is still under way.

TREATMENT

Infants with a suspicion of SCID or other CIDs require prompt intervention with use of cotrimoxazole to prevent *Pneumocystis* infection, prophylactic use of antifungal drugs, immunoglobulin replacement therapy, and aggressive treatment of any infectious episodes. Nutritional support is often necessary. Immune suppression helps in controlling the inflammatory reactions associated with Omenn syndrome. Only irradiated and filtered blood products should be used in patients with SCID because of the high risk of otherwise fatal graft-versus-host disease and of transmitting infections (CMV in particular). Use of live attenuated viral vaccines must be avoided to prevent uncontrolled vaccine-associated infections. Ultimately, permanent cure of SCID depends on HCT. When performed from an HLA-identical sibling, HCT can allow greater than 90% long-term survival and very robust and long-lasting immune reconstitution.¹⁰¹ HCT from HLA-mismatched related donors provides excellent results when performed in the first 3.5 months of life¹⁰²; however, the outcome is less satisfactory in older patients. Encouraging results have

been reported with HCT from matched unrelated donors.¹⁰³ It is important to distinguish typical forms of SCID (T^+B^- or T^+B^+) from the most severe forms of CID. Although HCT is required in both, the presence of residual autologous T cells in patients with CID has important implications, both because it might cause symptoms associated with immune dysregulation and because chemotherapy is usually needed to eliminate autologous T cells before HCT. Nonetheless, some forms of CID are characterized by worse outcome after HCT. In particular, reduced survival and a higher rate of complications after HCT have been reported in patients with radiation-sensitive SCID, Omenn syndrome, MHC class II deficiency, and ADA deficiency who did not have HLA-matched siblings.

Gene therapy has been shown to be effective for patients with ADA deficiency and with SCIDX1, leading to survival with immune reconstitution.^{104,105} However, 5 of the 20 infants with SCIDX1 treated at 2 centers have experienced clonal proliferation caused by insertional mutagenesis, calling for the development of novel and safer vectors. Enzyme replacement therapy with weekly intramuscular injections of pegylated bovine ADA is available for patients with ADA deficiency.¹⁰⁶ Thymic transplantation from unrelated donors has been shown to restore T-lymphocyte development in patients with complete DGS.¹⁰⁷ Alternatively, unmanipulated bone marrow transplantation from HLA-identical siblings can also allow T-cell reconstitution caused by expansion of mature T lymphocytes contained in the graft.¹⁰⁸

Treatment of antibody deficiency is based on immunoglobulin replacement therapy. This can be performed with intravenous or subcutaneous preparations (intravenous immunoglobulin [IVIG] and subcutaneous immunoglobulin [SCIG]). Both are effective in reducing the incidence of infections. The usual dose of IVIG is 400 mg/kg per 21 days, but higher doses might be needed in patients with bronchiectasis or enteroviral meningoencephalitis. SCIG is administered at the dose of 100 mg/kg/wk; potential advantages of SCIG include a lower rate of adverse reactions and more stable IgG trough levels.¹⁰⁹ On the other hand, IVIG might be more useful when there is a need to administer higher doses (as in the treatment of patients with bronchiectasis or with associated autoimmune complications). Although no studies have been performed to support the use of continuous antibiotic prophylaxis in patients with antibody deficiency, they might be beneficial in patients with bronchiectasis and recurrent sinusitis.

Defects of neutrophils require regular antibiotic and antifungal prophylaxis. Cotrimoxazole and itraconazole are efficacious in the treatment of CGD, and injection of IFN- γ might further reduce the incidence of severe infections. Overall survival in patients with CGD is now around 90%.⁷² HCT is the treatment of choice for LAD and can be proposed for patients with CGD who have HLA-matched family donors. Use of HCT from Matched Unrelated Donors or cord blood in patients with CGD remains controversial, although recent reports have shown that it can correct severe inflammatory complications.¹¹⁰ Gene therapy has been attempted in a few patients with CGD, although because of the lack of selective advantage for gene-corrected cells, only a very small fraction of cells in the periphery carry the transgene if no conditioning regimen is used. In a recent trial with nonmyeloablative conditioning, clonal expansion caused by insertional mutagenesis has been reported.¹¹¹ Regular subcutaneous administration of recombinant granulocyte colony-stimulating factor can increase ANC in patients with SCN.

Management of HIES is based on hygiene and regular prophylaxis of staphylococcal and fungal infections.⁸⁸

Patients with WAS should be treated with infusion of immunoglobulins, antibiotic prophylaxis, and appropriate measures to prevent severe bleeding episodes (eg, use of helmets).⁸³ However, the ultimate therapy of WAS is represented by means of HCT. Excellent results have been obtained with HCT from HLA-identical related donors. Results of MUD-HCT are also good, but mixed chimerism is associated with an increased rate of autoimmune complications.¹¹² Demonstration of lack of WASP protein expression might help identify high-risk patients who should be treated with HCT; in contrast, the approach to patients with XLT is more controversial.

HCT should be used without delay in patients with familial forms of HLH and in those with X-linked lymphoproliferative disease. Immunosuppressive treatment is required to treat the accelerated phase of the disease both in patients with X-linked lymphoproliferative disease and in those with PIDs with defective cytotoxicity associated with pigmentary dilution. Although HCT can cure the hematologic and immunologic manifestations of the disease, it does not prevent progressive neurological deterioration in patients with CHS.

Finally, although all patients with immunodeficiency associated with autoimmunity benefit from immunosuppressive treatment, alternative strategies can be considered, depending on the severity of the underlying defect. In particular, HCT is needed in patients with IPEX, whereas the significant splenomegaly of ALPS might require splenectomy.

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