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Review

The Molecular Pathology of Primary Immunodeficiencies

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Primary immunodeficiencies are a heterogeneous group of disorders, which affect cellular and humoral immunity or non-specific host defense mechanisms mediated by complement proteins, and cells such as phagocytes and natural killer (NK) cells.[1–3] These disorders of the immune system cause increased susceptibility to infection, autoimmune disease, and malignancy. There are over 80 primary immunodeficiencies, many of which are very rare, and in most cases associated with inherited genetic defects. One in 500 individuals, in the United States, is born with a defect in some component of the immune system.[4] In the majority of cases, the primary immunodeficiencies manifest in the first year of life.[4] They can, however, present at any age, including adulthood.[5] The advent of molecular genetic analyses now allows for the detection and confirmation of immunodeficiencies that were otherwise not severe enough during childhood to have led to a specific diagnosis. In addition, effective treatment for many disorders has led to increased survival of many children with primary immunodeficiency into adult life.

Recent advances in molecular techniques have led to the identification and characterization of more than 25 newly recognized immunological disease genes since 1997.[6,7] The identification of many genes responsible for primary immunodeficiencies has provided insights regarding the spectrum of clinical severity seen in a particular disorder and the phenotypic overlap resulting from mutations of different genes.

This review focuses on the molecular genetic features of primary immunodeficiencies with emphasis on the molecular pathophysiology of the diseases. In large part, the molecular detection of gene mutations leading to these diseases is carried out in research laboratories. Thus, information regarding comparison of analytical methods and prioritization of specific targets for study is largely lacking. Diagnostic perspectives for disease entities for which this information exists are included in their respective sections. We have organized the review into categories of B cell, T cell, severe combined immunodeficiencies, and defects of phagocytes and other miscellaneous immunodeficiencies. The clinico-pathological and immunological aspects are beyond the scope of this review and are available elsewhere. Various websites containing pertinent databases including those organized by the Pan-American Group for Immunodeficiencies (PAGID) http://www.clinimmsoc.org/pagid/, mutation registries, and the large European Society for Immunodeficiencies (ESID) registry which contains clinical data for over 7000 patients from 24 countries are listed in Table 1.[8] In addition, the website for GeneTests-GeneClinics which provide information regarding specific laboratories and the nature of the molecular assays is also shown in Table 1.

Classification

The International Union of Immunological Society's Scientific Committee on Primary Immunodeficiency Diseases/World Health Organization[4] define several major categories of primary immunodeficiencies including: defects in non-specific host defense (phagocytes, natural killer cells, complement); defects of specific humoral immunity (B lymphocytes, antibodies); combined deficiency of cellular (T cell mediated) and humoral immune defense; immune defects associated with other major defects; and immunodeficiencies associated with or secondary to other diseases. Selected primary immune disorders, their presumed pathogenesis, inheritance pattern, and diagnostic tests are summarized in Table 2.

Epidemiology

Many immunodeficiencies are rare, however, the incidence of primary immunodeficiencies has increased by

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Table 1. Immunodeficiency Mutation Databases and Other Related Websites

Database	Immunodeficiency disorder	Internet address of database
ATbase	Ataxia-telangiectasia	http://www.ent.ki.se/ATbase/
ATM	Ataxia-telangiectasia	http://www.vmresearch.org/atm.htm
BLMbase	Bloom syndrome	http://www.uta.fi/imt/bioinfo/BLMbase/
BTKbase	X-linked agammaglobulinemia (XLA)	http://www.uta.fi/imt/bioinfo/BTKbase/
CD3Ebase	CD3epsilon deficiency	http://www.uta.fi/imt/bioinfo/CD3Gbase/
CD40Lbase	X-linked hyper-IgM syndrome (XHIM)	http://www.uta.fi/imt/bioinfo/CD40Lbase/
CYBAbase	Autosomal recessive CGD p22 phox deficiency	http://www.uta.fi/imt/bioinfo/CYBAbase/
CYBBbase	X-linked chronic granulomatous disease (XCGD)	http://www.uta.fi/imt/bioinfo/CYBBbase/
FAA and FAC	Fanconi anemia	http://www.rockefeller.edu/fanconi/mutate/
IL2RGbase	X-linked severe combined immunodeficiency (XSCID)	http://www.nhgri.nih.gov/DIR/LGT/SCID/
JAK3base	Jak3 deficiency	http://www.uta.fi/imt/bioinfo/JAK3base/
NCF1base	Autosomal recessive CGD p47 phox deficiency	http://www.uta.fi/imt/bioinfo/NCF1base/
NCF2base	Autosomal recessive CGD p67 phox deficiency	http://www.uta.fi/imt/bioinfo/NCF2base/
RAG1base	RAG1 deficiency	http://www.uta.fi/imt/bioinfo/RAG1base/
RAG2base	RAG2 deficiency	http://www.uta.fi/imt/bioinfo/RAG2base/
SH2D1Abase	X-linked lymphoproliferative syndrome (XLP)	http://www.uta.fi/imt/bioinfo/SH2D1Abase/
ZAP70base	ZAP70 deficiency	http://www.uta.fi/imt/bioinfo/ZAP70base/
Immune Deficiency Foundation		http://www.primaryimmune.org
National Organization for Rare Disorders		http://www.raredisease.org
Online Mendelian Inheritance in Man		http://www3.ncbi.nim.nih.gov
GeneTests		http://www.genetests.org

10-fold since 1969 to 1 in 10,000.[4] This is partly due to increased identification of affected patients, reduced morbidity and mortality from the introduction of antibiotics, enhanced methods of detection of immunological abnormalities, and the identification of gene mutations responsible for the disorders. Approximately 400 new cases of primary immunodeficiency are diagnosed per year in the U.S. See Table 3 for prevalence of primary immunodeficiencies.

Approach to Diagnosis

The primary immunodeficiencies characteristically present in childhood with infections that persist for long duration with multiple recurrences that are resistant to antibiotics. Failure to thrive and developmental delay are significant clues to the seriousness of their infections. Many immunodeficient children develop other symptoms such as skin rashes, and many have associated developmental anomalies of the face, skeletal system, heart, and pigmentation.

The nature of the pathogens and sites of infections can provide insight as to the underlying immunodeficiency. Defects involving B cell function result in recurrent sinopulmonary infections, often with bacterial septicemia. The lack of antibody production may also increase susceptibility to invasive disease with enteroviruses, resulting in chronic viral meningitis, and giardiasis. T cells are essential for the control of viral and fungal disease, however they also provide helper function to B cells for effective antibody responses. Thus, T cell disorders present as combined T and B cell immunodeficiency with susceptibility to both bacterial and chronic, invasive viral, and fungal pathogens. Patients with disorders of granulocytes are susceptible to staphylococcal diseases and gram-negative infections.

The primary immunodeficiencies are commonly inherited disorders, thus a family history is one of the best diagnostic clues. Unfortunately, because these diseases are rare with low carrier frequencies, a negative family history does not rule out a primary immunodeficiency. Furthermore, occurrence of new mutations, especially for X-linked disorders, is so high that the majority of patients with proven X-linked immunodeficiency mutations have no history of affected male relatives.

Laboratory Assessment

Initial laboratory evaluation for immunodeficiency should include a minimum of tests that can be performed reliably by any laboratory.[9] The initial screening should include a complete blood count and quantitation of serum IgG, IgM, and IgA levels. Laboratories should provide age-matched normal values for cell counts, immunoglobulin measurements and proper controls for functional studies. Other readily available tests are: 1) Quantification of blood mononuclear cell populations: T cells (CD3, CD4, CD8, TCR $\alpha\beta$, TCR $\gamma\delta$); B cells (CD19, CD20, CD21, Ig); NK cells (CD16/CD56); monocytes (CD15); activation markers (HLA-DR, CD25, CD80 for B cells), CD154 for T cells. 2) T cell functional evaluation: delayed hypersensitivity skin tests (PPD, Candida, histoplasmin, and tetanus toxoid); proliferative response to mitogens (anti-CD3 antibody, phytohemagglutinin, concanavalin A) and allogeneic cells (mixed lymphocyte response); cytokine production. 3) B cell functional evaluation: natural or commonly acquired antibodies; response to immunization proteins and carbohydrate antigens; and quantitative

Table 2. Gene Defects, Inheritance, and Diagnostic Tests for Primary Immunodeficiencies

Predominantly antibody deficiencies			
Designation	Pathogenesis/defect	Inheritance	Diagnostic tests
1. X-linked agammaglobulinaemia	Mutations in *btk*	XL	Btk by immunoblotting or FACS analysis, mutation analysis
2. Autosomal recessive agammaglobulinaemia	Mutations in μ or λ5 genes; others	AR	—
3. Ig heavy-chain gene deletions	Chromosomal deletion at 14q32	AR	—
4. κ chain deficiency mutations at AR	Point mutations at chromosome 2p11 in some patients	AR	—
(a) IgG subclass deficiency	Defects of isotype differentiation	Unknown	—
(b) IgA deficiency	Failure of terminal differentiation in IgA = B cells	Variable	Autoimmune and allergic disorders
5. Common variable immunodeficiency	Variable; undetermined	Variable	See text
T cell deficiencies			
1. Purine nucleoside phosphorylase (PNP) deficiency	Deficiency of PNP	AR	Red cell PNP levels and metabolite Mutation analysis
2. CD3 γ or CD3ε deficiency	Defect in TCR signaling	AR	CD3 fluorescence intensity Mutation analysis
3. ZAP-70 deficiency	ZAP70	AR	ZAP70 expression and activation Mutation analysis
4. X-linked lymphoproliferative syndrome	Uncontrolled B cell stimulation SH2DIA(Xq25)	XL	Mutation analysis, SAP expression under development
Combined immunodeficiencies			
1. T-B SCID			
(a) X-linked (γc deficiency)	Mutations in γ chain of IL-2, 4, 7, 9, 15 receptors	XL	CD154 expression on activated T cells by FACS analysis Mutation analysis
(b) Autosomal recessive (Jak3 deficiency)	Mutation in Jak3	AR	JAK3 expression/activation Mutation analysis
2. T-B-SCID			
(a) RAG ½ deficiency	Mutation in RAG 1/2 genes	AR	RAG1 and RAG2 mutation analysis
(b) Adenosine deaminase (ADA) deficiency	T-cell and B-cell defects from toxic metabolites (eg dATP, S-adenosyl homocysteine)	AR	Red cell ADA levels and metabolites Mutation analysis
(c) Other	Defective VDJ recombination	AR	
3. T + B-SCID			
(a) Omenn Syndrome	Missense mutations in RAG 1 and 2 genes	AR	RAG 1 and RAG2 Mutation analysis
(b) IL-2Rα deficiency	Mutations in IL-2Rα gene	AR	IL-2R FACS expression
4. X-linked hyper IgM	Mutations in CD40 ligand gene	XL	CD145 expression on activated T cells by FACS analysis Mutation analysis
5. MHC class II deficiency	Mutation in transcription factors (CIITA bare lymphocyte syndrome, or RFX5, RFXAP, RFXANK genes) for MHC class II molecules	AR	HLA-DR expression Mutation analysis
6. TAP-2 deficiency	Mutations in TAP-2 gene	AR	HLA class I expression
7. CD45 deficiency	PTPRC		Mutation analysis
Congenital defects of phagocytic number and/or function			
	Pathogenesis/gene defect	Inheritance	Diagnostic tests
1. Severe congenital neutropenia	Mutation in *ELA2*	AR	Mutation analysis
2. Cyclic neutropenia	Mutation in *ELA2*	AR	Mutation analysis
3. Leucocyte adhesion defect 1 [deficiency of beta chain (CD18) of LFA-1, Mac 1, p150, 95]	Chemotaxis, adherence, endocytosis, *Integrin B2*	AR	Flow cytometry
4. Leukocyte adhesion defect 2 (failure to convert GDP mannose to fucose)	Chemotaxis, rolling	AR	ND
5. Chediak-Higashi syndrome	Defective cytotoxic T/NK cells, *LYST* gene	AR	Mutation analysis
6. Specific granule deficiency	Chemotaxis/*C/EBP* gene	AR	Mutation analysis
7. Shwachman syndrome	Chemotaxis, mutations in *SBDS* gene	AR	Mutation analysis

(*Table continues*)

Table 2. *(Continued)*

Designation	Pathogenesis/defect	Inheritance	Diagnostic tests
Predominantly antibody deficiencies			
8. Chronic granulomatous disease			
(a) X-linked CGD (deficiency of 91 kDa chain of cytochrome b)	Killing (faulty production of superoxide metabolites), *CYBB*	AR	Mutation analysis
(b) Autosomal recessive-CGD (deficiencies of 22 kDa chain of cytochrome b or of p47 or p67 cytosol factors)	Killing-as above	AR or XL	Mutation analysis
1. p22 phox	Mutations in *LYBA*	XL	Mutation analysis
2. p47 phox	Mutations in *NCF1*	AR	Mutation analysis
3. p67 phox	Mutations in *NCF2*	XL	Mutation analysis
9. Myeloperoxidase deficiency	Killing, mutations in *MPO* gene	AR	Mutation analysis
10. Leukocyte mycobactericidal defect	Killing, mutations	AR	
(a) TNF-γ receptor 1 deficiency	Killing defect	AR or AD	Mutation analysis
(b) TNF-γ receptor 2 deficiency	Killing defect	AR or AD	Mutation analysis
(c) Interleukin-12 receptor deficiency	Killing defect	AR or AD	Mutation analysis
(d) Interleukin-12 deficiency	Killing defect	AR or AD	Mutation analysis
Other well-defined immunodeficiency syndromes			
1. Wiskott-Aldrich syndrome	Mutations in WAS gene; cytoskeletal defect affecting haematopoietic stem cell derivatives	XL	Mutation analysis Detection of WASP mRNA or protein
2. Ataxia-telangiectasia	Mutation in A-T gene (*ATM*); disorder of cell cycle checkpoint pathway leading to chromosomal instability	AR	Mutation analysis DNA radiation sensitivity
3. Nijmegen breakage syndrome	Defect in NBS1 (*Nibrin*); disorder of cell cycle check point and DNA double strand break repair	AR	Mutation analysis DNA radiation sensitivity
4. DiGeorge anomaly	Contiguous gene defect in 90% affecting thymic development	De novo defect or AD	FISH, mutation analysis
5. Immunodeficiency with albinism			Mutation analysis
(a) Chediak-Higashi syndrome	Defect in *Lyst*	AR	Mutation analysis
(b) Griscelli syndrome	Defect in *myosin 5a*	AR	Mutation analysis Defect in *RAB27A*
6. X-linked lymphoproliferative syndrome	Defect in *SAP*	XL	Mutation analysis
7. Familial hemophagocytic lymphohistiocytosis	*Perforin* mutation (10q21–22)	AR	Mutation analysis
8. ICF Syndrome	*DNMT3B* mutation (20q11.2)	AR	Mutation analysis

IgG subclass determination. 4). Phagocyte function: reduction of nitroblue tetrazolium; chemotaxis assays, and bactericidal activity.

Diagnostic Criteria

The PAGID and ESID[10] have established diagnostic criteria for immunodeficiencies which are divided into three categories: definitive, probable, and possible. These criteria provide objective guidelines that ensure that the same definitions are being used universally for diagnosis and clinical research studies. Patients with a definitive diagnosis are assumed to have a greater than 98% probability that in 20 years they would still be given the same diagnosis. Detection of a gene mutation is the most reliable method of making a diagnosis. In some disorders, the absence of the specific transcript or protein is diagnostic, although in others this may be hampered by transient or low levels of expression. The clinical and laboratory findings in several of the X-linked immunodeficiencies are sufficiently distinctive that if coupled with a family history that is specific to X-linked inheritance, a definitive diagnosis can frequently be made. In families with a known mutation in a particular gene, pre- or peri-natal testing can be used to establish a definitive diagnosis in a newborn or fetus. Table 4 highlights the major genetic abnormalities required for definitive diagnosis of primary immunodeficiency disorders.

Patients with a probable diagnosis are those with all of the clinical and laboratory characteristics of a particular disorder but do not have a documented abnormality in the gene, mRNA, or protein that is known to be abnormal in the given disorder. They are assumed to have a greater than 85% probability that in 20 years they will be given the same diagnosis. Patients with a possible diagnosis are those that have some but not all of the characteristic clinical or laboratory findings of a particular disorder.

Table 3. Prevalence of Primary Immunodeficiencies

Disease	Prevalence
IgA deficiency	1/600
X-linked agammaglobulinemia (XLA)	1/200,000
X-linked lymphoproliferative syndrome	<1/1,000,000 live births in males
X-linked immunodeficiency with hyper-IgM	<1/1,000,000 live births in males
X-linked severe combined immunodeficiency	1/50,000 to 1/100,000
JAK3-deficient severe combined immunodeficiency (autosomal recessive T-B+ SCID)	<1/500,000 live births
Adenosine deaminase deficiency	½-1/100 births
B-cell negative SCID, Omenn syndrome	~1/100,000
Ataxia-telangiectasia	~1/100,000
Leukocyte adhesion deficiency type I	1/100,000
X-linked chronic granulomatous disease (X-CGD)	1/250,000
Autosomal CGD – p22phox deficiency	<1/2,000,000
Autosomal CGD – p47phox deficiency	~1/500,000
Autosomal CGD – p67phox deficiency	<1/2,000,000

Since early diagnosis can prevent serious consequences, timely diagnosis of an index case can provide opportunity for genetic counseling, carrier detection, and prenatal diagnosis. Figure 1 outlines the algorithm for evaluation and diagnosis of patients with suspected severe combined immunodeficiency. As defined above, the identification of a gene mutation involving any of the cytokine common gamma chain ($\gamma\chi$), JAK3, RAG1, RAG2, IL-7Rα, or CD45 would lead to a definitive diagnosis of SCIDs. Those without evidence of gene mutation but the clinical and laboratory features of SCIDs will have a "probable" diagnosis of the disease.

Table 4. Genetic Abnormalities Required for Definitive Diagnosis of Primary Immunodeficiency Disorders

Disease	Gene defect
SCID	
	Mutation in the cytokine gamma chain (γ)
	Mutation in JAK3
	Mutation in RAG1 or RAG2
	Mutation in IL-7Rα
	Mutations in both alleles of adenosine deaminase
DiGeorge syndrome	
	Deletion of chromosome 22q11.2
MHC-class II deficiency	
	Mutation in one of the following genes: CIITA, RFX-B, RFX-5, RFX-AP
Leukocyte adhesion defect	
	Mutation in the β_2 integrin gene
	Absence of the β_2 integrin mRNA in leukocytes
Chronic granulomatous disease	
	Mutation in gp91, p22, p47 or p67 phox
	Absent mRNA for one of the above genes by Northern blot analysis
X-linked SCID	
	Mutation in the cytokine common gamma chain (γ)
	Absent γc mRNA on Northern blot analysis of lymphocytes
X-linked Agammaglobulinemia	
	Mutation in Btk
	Absent Btk mRNA on Northern blot analysis of neutrophils or monocytes
	Absent Btk protein in monocytes or platelets
X-linked hyper IgM	
	Mutation in the CD40L gene
Ataxia telangiectasia	
	Mutations in both alleles of ATM
Wiskott-Aldrich syndrome	
	Mutation in WASP
	Absent WASP mRNA on Northern blot analysis of lymphocytes
	Absent WASP protein in lymphocytes
X-linked lymphoproliferative syndrome	
	Mutation SH2D1 A/SAP/DSHP

Modified from[10].

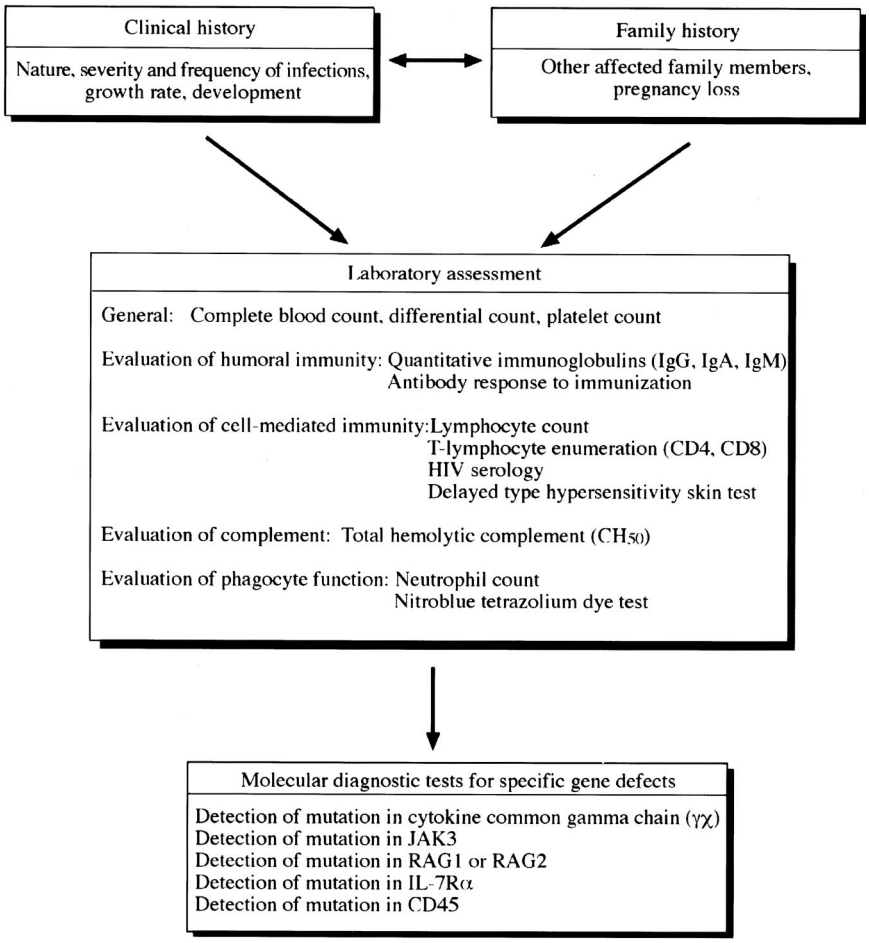

Figure 1. Algorithm for evaluation and diagnosis of a patient with suspected severe combined immunodeficiency.

B Cell Immunodeficiencies-Predominantly Antibody Defects

X-Linked Agammaglobulinemia

X-linked agammaglobulinemia (XLA) is a typical antibody deficiency in which production of antibodies is prevented due to a block in B cell maturation. Serum concentrations of IgG, IgA, and IgM are markedly reduced. Levels of circulating B cells are significantly decreased and plasma cells are absent from lymph nodes and bone marrow, although the number of T cells is normal or even increased. The clinical phenotype may be variable and even members of the same family can have different symptoms. The majority of affected boys present with recurrent bacterial infections from the age of 4 to 12 months following the disappearance of maternal immunoglobulin.[11] Infections caused by pyogenic bacteria are the most common clinical manifestations. Rare cases of XLA have been described in adults.[12]

Molecular and Cellular Defects

X-linked agammaglobulinemia was the first immunodeficiency to be characterized at the genetic level.[13] XLA is caused by a block in B cell differentiation due to mutations involving the Bruton kinase gene, *Btk*, which encodes a tyrosine kinase that regulates the activity of signaling pathways by phosphorylation.[14,15] BTK is activated by immunoglobulins and other membrane receptors that in turn activate phospholipase Cγ and calcium influx. Although the specific defect in the signaling pathway that impairs B-cell development is unknown, it is proposed that BTK is important in mediation of survival signals.[16] The phenotypic heterogeneity seen in patients does not reflect the consequences of different mutations as most of the mutations identified thus far lead to complete absence of BTK protein. Modifier genes and environmental factors have been postulated to play a role in influencing the expression pattern of the B cell deficiency. Flow cytometric methods to detect expression of BTK protein expression[17] and molecular diagnostic tests allow the detection of the carrier state and enable prenatal diagnosis of the disorder.[18] However, since approximately one third to one half of XLA cases are sporadic, alternative mechanisms of diagnosis are necessary. Furthermore, deleterious mutations of the μ heavy chain gene,[19] lambda 5/14–1 surrogate light chain gene,[20] *Blnk* gene which encodes an adaptor protein that has a critical role in pro-B to pre-B cell progression,[21] and the *CD79a* gene[22] have been identified in agammaglobulinemia patients.

Hyper-IgM Syndrome

Hyper-IgM syndrome (HIM) represents a group of distinct entities characterized by defective normal or elevated IgM in the presence of diminished IgG and IgA levels.[23] Seventy per cent of the cases are X-linked in inheritance,[24] and others are autosomal recessive.[25] Male patients with X-linked hyper-IgM have a history of recurrent pyogenic infections, and are particularly susceptible to *Pneumocystis carinii*. They are also prone to profound neutropenia, autoimmune hemolytic anemia, and thrombocytopenic purpura. Liver disease including sclerosing cholangitis, viral hepatitis as well as hepatic lymphoma are common and their frequencies increase with age.[26] The long-term survival rate for patients with XHIM is poor despite regular use of intravenous immunoglobulins. Less than 30% of the patients are alive at 25 years of age. Major causes of death include *Pneumocystis carinii* pneumonia early in life, liver disease, and malignancies in later life.[27] Allogeneic bone marrow transplantation[28] or nonmyeloablative bone marrow transplantation from matched, unrelated donors[29] have been successful in the treatment of hyper-IgM syndrome.

Molecular and Cellular Defects

The genetic anomaly in X-linked hyper-IgM syndrome has been mapped to Xq26, and resides in mutations of the *CD40 ligand* gene now known as CD154.[30] CD40L is a member of the tumor necrosis factor family that binds to its receptor (CD40) expressed in B cells. Interactions between the CD40 ligand present on activated T cells and CD40 on B cells is required for productive isotype switching in B cells.[31] The defect in X-linked hyper-IgM syndrome is a failure of isotype switch. Failure of this switch results in defective formation of germinal centers and immunoglobulin switching. Studies of CD40L (CD154) knockout mice that are also susuceptible to *Pneumocystis carinii* infections show that the defect of CD40L expression prevents CD40-mediated up-regulation of CD80/CD86 expression in B cells and other antigen presenting cells, ultimately resulting in poor T cell priming and defective type 1 immune response.[32]

Another form of X-linked hyper-IgM is associated with ectodermal dysplasia (XHM-ED). Interestingly, the expression of CD40 and CD40L are normal but a specific missense mutation in the putative zinc-finger domain of the gene that encodes *nuclear factor* κB (*NF-*κB) essential modulator (*NEMO*, also known as IKKγ) have been described in these patients.[33] The mutations of *NEMO* prevent CD40L-mediated degradation of inhibitor of NF-κB (IκB-α) resulting in defects in immunoglobulin class-switching, inability of antigen-presenting cells to synthesize the NF-κB-regulated cytokines such as IL-12 and TNF-α when stimulated with CD40L.[33]

A minority of patients show an autosomal recessive inheritance pattern named hyper IgM-2. A mutation of the activation-induced cytidine deaminase (*AID*) gene, the product of which is selectively expressed in B cells from germinal centers[34] has been reported. There is a similar defect in immunoglobulin switching, however significant differences exist in that they have lymph nodes with hyperplastic germinal centers and patients do not suffer from opportunistic infections. The B cells characteristically exhibit few somatic mutations of the variable part of the rearranged immunoglobulin genes. The exact mechanism of how a member of a RNA editing enzyme family leads to the defect is unknown.

More recently, homozygous mutations of the *CD40* gene leading to lack of surface expression of CD40 have been reported in another form of autosomal recessive hyper IgM. The clinical and immunological features are indistinguishable from those of the X-linked form.[35]

Common Variable Immunodeficiency

Common variable immunodeficiency (CVID) refers to a heterogeneous group of disorders which are characterized by defective antibody formation.[4] CVID is the most frequent of the primary immunodeficiency diseases among populations of European descent and affects both sexes equally.[36] The feature common to all patients is hypogammaglobulinemia, generally affecting all antibody classes but sometimes only IgG. Several modes of inheritance (autosomal recessive, autosomal dominant, and X-linked) have been reported, however sporadic cases are most common. The usual age at presentation is in the second or third decade of life. CVID is the most common clinically significant primary immunodeficiency disease that can present initially in adult life.[5]

The clinical manifestations of CVID include manifestations of antibody deficiency, ie, recurrent pyogenic sinopulmonary infections. Recurrent attacks of herpes simplex are common, and herpes zoster develops in about 20% of patients.[3] Some patients may have unusual enteroviral infections with a chronic meningoencephalitis and a dermatomyositis-like illness. In addition, CVID patients are also prone to persistent episodes of diarrhea caused by *Giardia lamblia*. A high frequency of autoimmune diseases including rheumatoid arthritis, pernicious anemia, hemolytic anemia, thrombocytopenia, and neutropenia, is also seen.[3] A syndrome resembling sarcoidosis can also affect some patients with CVID. The granulomas tend to involve the lung, liver, spleen, and conjunctivae. They are also at risk for inflammatory bowel diseases such as Crohn's disease, celiac disease, and nodular lymphoid hyperplasia. A few patients present with opportunistic infections such as *Pneumocystis carinii*, mycobacteria, or fungal infections.

Molecular and Cellular Defects

The primary cause of CVID is not known. In part because CVID comprises a heterogeneous group of disorders, non-random recurrent cytogenetic abnormalities unique to CVID have not been identified. Current evidence suggests that the humoral defect in CVID patients is as a result of insufficient *in vivo* stimulus for B cell activation rather than an intrinsic inability of the B lymphocytes to undergo terminal differentiation into plasma cells.[37] Furthermore, circulating B cells from CVID pa-

tients failed to undergo somatic hypermutation in immunoglobulin-variable region genes, similar to cord blood B cells. They were also unable to produce IgA on engagement of the Ig receptor suggesting the presence of severe deficiency of switched memory B cells (CD27+ IgM−IgD−) in CVID patients.[38,39]

Abnormalities in T cell signaling and thus defective T cell to B cell interactions, are thought to underlie the diminished *in vivo* stimulation of B cell activation and differentiation into immunoglobulin secreting plasma cells.[3,7] Experimental evidence indicates that the molecular basis of abnormal B cell differentiation is varied. Some CVID patients have mutations interfering with the regulation of the expression of immunoglobulin genes.[40,41] Others have functional abnormalities of $CD4^+$ (helper) cells or CD8+ (suppressor) T cells, as well as defective B cells[42] and apoptosis.[43] An aberrantly spliced *lck* transcript, a key molecule in T cell receptor-mediated signaling, lacking the entire exon 7 associated with decreased expression of LCK protein has been described in a patient with CVID.[44] Low levels of serum interleukin 2 (IL-2) and interferon gamma (IFN-γ)[45] due to defects in these cells can contribute to hypogammaglobulinemia.

Recent studies have also implicated genes within the HLA complex predisposing to CVID; many patients have deletions of the *C4A* gene or possess rare alleles of the *C2* gene. Both CVID and isolated IgA deficiency may affect different individuals of the same family, suggesting that they may be related disorders with a common genetic defect.[41] A subset of male patients with CVID have mutations in the X-linked lymphoproliferative disease gene *SH2D1A/SAP*[46] suggesting a link between defects in the *SAP* gene to abnormal B cell development. The diagnosis is based on the exclusion of other known causes of humoral immune defects.

IgA Deficiency

Selective IgA deficiency is the most common form of immunodeficiency in the Western world, affecting approximately 1 in 600 individuals.[47] Only about one third of the patients are particularly prone to infections. Most patients have IgA levels below 5 mg/dl. The serum concentrations of the other immunoglobulins are usually normal, but patients have a high incidence of autoantibodies, many with allergies including food reactions, allergic conjunctivitis, rhinitis, urticaria, atopic eczema, and bronchial asthma.[48] In about two thirds of the cases, the deficiency does not lead to an increased occurrence of infections, whereas the remaining patients suffer from bacterial infections in both the upper and lower respiratory tract.

Molecular and Cellular Defects

The major role of IgA is to facilitate presentation of antigen to mucosal T cells. The pathogenesis of IgA deficiency involves a block in B cell differentiation that occurs due to defective interaction between T and B cells. This is demonstrated by the observation that IL-12 treatment can overcome IgA deficiency by providing adequate T cell priming in mice.[49] The pathogenesis of IgA deficiency is associated with genes within the major histocompatability complex such as *HLA-B8, SC01*, and *DR3*.[50] Other studies have implicated genomic polymorphisms in the *tumor necrosis factor* gene[51] as a protective factor in IgA deficiency. The defect is manifested at the stem cell level as transfer of bone marrow from an IgA-deficient donor to a normal recipient results in IgA deficiency in the recipient.[52]

Selective IgG Subclass Deficiencies

Selective deficiencies of IgG subclasses, with or without IgA deficiency, are caused by defects in several genes. IgG2 deficiency is most common in children, whereas adults more often have low levels of IgG3.[4] Hyper-IgE syndrome is a rare immunodeficiency state characterized by recurrent skin and pulmonary abscesses and extremely elevated serum IgE levels. It is a single-locus disease with an autosomal dominant pattern of transmission with variable expressivity.[53] Genotypic analysis of 19 kindreds with multiple cases of hyper-IgE syndrome with polymorphic markers in a candidate region on human chromosome 4 suggests the proximal 4q region as a candidate locus for the disease.[54] Single-strand conformation polymorphism analysis followed by DNA sequencing revealed mutations in the α subunit of the interleukin-4 receptor α[55] in 3 of 3 patients with hyper-IgE. The R576 is a novel IL-4 receptor α allele causing a change from glutamine to arginine at position 576 (R576) in the cytoplasmic domain of the IL-4 receptor α protein. However, this allele is also commonly found in those patients with atopic dermatitis suggesting that the mutation may predispose persons to allergic disease rather than a primary genetic cause of the disease.

T Cell Immunodeficiencies

Purine Nucleoside Phosphorylase Deficiency

The ubiquitous purine nucleoside phosphorylase (PNP) plays a key role in the purine salvage pathway.[56] PNP deficiency is a lethal, autosomal recessive disease that causes profound T cell deficiency with variable deficiencies in the humoral system. Patients with PNP deficiency experience recurrent bacterial, viral, and fungal infections usually beginning early in life. Immunodeficiency is accompanied by a neurological disorders and developmental retardation.[57]

Molecular and Cellular Defects

The PNP protein reversibly catalyzes the degradation of the purine nucleosides inosine and deoxyinosine to hypoxanthine and that of guanosine and deoxyguanosine to guanine. Deficiency of nucleoside phosphorylase leads to a dysfunction of the purine salvage pathway and

accumulation of deoxyguanosine triphosphate (dGTP) which is preferentially toxic to T cells compared to B cells.[58] Mutations result in truncated proteins leading to variable levels of enzymatic activity.[59] Recent mouse models of purine nucleoside phosphorylase deficiency suggest that accumulation of dGTP in the mitochondria result in impaired mitochondrial DNA repair with enhanced sensitivity of T cells to spontaneous DNA damage leading to T cell apoptosis.[60]

Zap-70 Deficiency

ZAP-70 deficiency is inherited in an autosomal recessive manner. Recurrent and opportunistic infections occur within the first year of life.[4] There is lymphopenia involving CD8+ T cells, with normal numbers of non-functional $CD4^+$ T cells and severe combined immunodeficiency.[61] Zap-70 (ζ-associated polypeptide of 70 kd) is a tyrosine kinase that binds to the TCR's phosphorylated immunoreceptor tyrosine-based activation motif (ITAM) sequences. Recruitment of ZAP-70 to the TCR and its subsequent phosphorylation and activation, largely by Lck, is essential for downstream signaling events.[62] In Zap-70 deficiency, a mutation within the kinase domain of ZAP-70 results in abolished protein expression. Signaling through TCR is defective, influencing T cell development with a selective block in positive selection of CD8+ cells. There is failure of peripheral $CD4^+$ T cell proliferative response to mitogens or anti-CD3 antibody. By contrast, the activity of natural killer cells, B cells, and serum immunoglobulin levels are normal. The severe combined immunodeficiency associated with ZAP-70 deficiency is fatal unless treated by allogeneic bone marrow transplantation.

CD3ε and CD3γ Deficiencies

Rare congenital immunodeficiencies are caused by mutations in the γ[63] and ε[64] subunits of *CD3*. They are inherited in an autosomal recessive (11q23) manner and result in moderate to severe immunodeficiency due to decreased circulating CD3+ T cells, and poor responses to T cell mitogens. They show decreased lymphocyte membrane expression of TCR/CD3.

Severe Combined Immunodeficiencies (SCIDs)

The severe combined immunodeficiencies (SCIDs) syndrome is characterized by gross impairment of both the humoral and cell-mediated immunity and by susceptibility to overwhelming fungal, bacterial, and viral infections. The syndrome comprises a heterogeneous group of primary immunodeficiencies associated with various defects of the immune system involving T, B, and sometimes natural killer cells.

T-B-SCID

RAG1 Deficiency, RAG2 Deficiency, and Omenn Syndrome

Two related deficiencies of recombination activating genes, *RAG1* and *RAG2* result in a spectrum of severe combined immunodeficiencies called RAG1/RAG2 deficiency and Omenn syndrome. Both conditions are inherited as autosomal recessive diseases. RAGs are crucial proteins that play a role in activating V(D)J recombination in the B cell and T cell receptor genes required for generation of the diversity of the recognition sites. Absence or defective V(D)J recombination results in the arrest of B and T cell development such that most of the circulating lymphocytes in affected patients are natural killer cells. Mutations that lead to total absence of *RAG1* or *RAG2* gene product (null mutations) are known to lead to severe combined immune deficiency without mature lymphoid cells,[65] whereas mutations that result in partial V(D)J recombinase activity due to missense mutation on at least one allele lead to Omenn syndrome.[66] A clinical phenotype similar to Omenn syndrome has been also seen as a result of engraftment of maternal T cells as a complication of a transplacental transfusion.[67] Thus, analysis of the *RAG* genes by direct sequencing may be an effective way to provide accurate diagnosis of RAG-deficient as opposed to RAG-independent V(D)J recombination defects.

Omenn syndrome is an immunodeficiency disease with autoimmune features resembling graft-*versus*-host disease.[68] It is characterized by the absence of circulating B cells and infiltration of many organs by activated oligoclonal T cells. Most present with infantile diffuse erythrodermia, alopecia, protracted diarrhea, lymphadenopathy, hepatosplenomegaly, fever, hypereosinophilia, and elevated serum IgE levels leading to failure to thrive and ultimately death. Protein loss due to diarrhea and exudative erythrodermia often leads to generalized edema. The condition is fatal unless it is corrected by bone marrow transplantation.

Molecular and Cellular Defects

Molecular studies of patients with Omenn syndrome[66] have revealed missense mutations of the *RAG1* and *RAG2* genes which result in impaired but not absent rearrangement of the B cell receptor and T cell receptor genes. These findings indicated that the immunodeficiency manifested in patients with Omenn syndrome arises from mutations of RAG1 and RAG2 genes that decrease the efficiency of V(D)J recombination. More recent studies have shown that some cases of Omenn syndrome are characterized by mutations identical to those seen in T-B-SCID patients suggesting the role of additional factors that may play a part in the development of Omenn syndrome.[69] An analysis of TCR repertoire demonstrates exquisite restriction of TCR clonotypes indicating antigen-driven proliferation of T cells. The TCR from some patients lacked N- or P-nucleotide insertions and used proximal variable and joining gene segments,

suggesting abnormal intrathymic T cell development. Abnormal assembly of gene segments and truncated rearrangements within non-productive alleles also suggest abnormalities in the TCR rearrangement mechanism.[70]

Adenosine Deaminase Deficiency

Adenosine deaminase (ADA) deficiency accounts for about half of the autosomal recessive forms of SCIDs. It is one of the most severe immunodeficiencies and is associated with severe depletion of B cells, T cells, and NK cells. It is the second-most prevalent form of SCID accounting for approximately 20% of the group. Affected individuals die from overwhelming opportunistic infections within the first few months of life if untreated. ADA follows purine nucleoside phosphorylase in purine nucleoside catabolism, but deficiency in this enzyme causes even more severe symptoms than PNP deficiency that is largely limited to T cells. In addition to immunological defect, most patients with ADA deficiency also have skeletal abnormalities.

Molecular and Cellular Defects

ADA, an enzyme of the purine salvage pathway, catalyzes the conversion of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively. ADA deficiency results in accumulation of the toxic metabolites, adenosine and deoxyadenosine, which accumulate in the cells of affected patients. Deficiency of adenosine deaminase results in a profound decrease in the maturation of lymphocyte precursors.[71] Defective enzymatic activity in lymphocyte precursors results in selective accumulation of dGTP that inhibits cellular division. Typical patients are diagnosed by age 6 months and rarely survive beyond 1 to 2 years unless immune function is restored by stem cell transplation or enzyme replacement therapy. Partial ADA deficiency has been found in less severely affected patients with delayed or late/adult onset of immune deficiency[72,73] and autoimmunity.[71] About 70 known mutations, the majority missense, span the 32-kb, 12 exon *ADA* gene on chromosome 20q.[74] Expression studies demonstrate that missense mutations appear more deleterious yielding 0.005% to 0.6% of the ADA activity from wild-type cDNA whereas splicing mutations are associated with mild severity.[74] Studies of *ADA*-deficient mice show multiple defects of T cells including increased thymic apoptosis as well as defective T cell receptor signaling.[75] Treatment of ADA deficiency by gene transfer has also been attempted.[76] Flow cytometric analysis of ADA expression can be used to assess the expression of ADA in follow-up of patients treated in clinical gene transfer protocols.[77]

T-B+ SCID

SCIDs with lack of circulating T cells but a normal number of B cells accounts for 30 to 50% of all cases of human SCIDs. The X-linked form is the most common form that provides rationale for the prevalence of SCID being three times as common in boys as in girls. The second most common variant is autosomal recessive and due to mutations of the *JAK3* gene.

X-Linked SCID

This group of immunodeficiency diseases appears to be as a result of several genetic causes. Affected infants are susceptible to recurrent severe infections caused by a wide range of pathogens including, *Candida albicans, Pneumocystis carinii, Pseudomonas*, cytomegalovirus, and varicella. Death from varicella, herpes, adenovirus, or cytomegalovirus may occur very soon after infection. Infants with severe combined imunodeficiency invariably have profound lymphopenia.[3] The number of natural killer cells may be normal or high. In contrast to the autosomal recessive forms of SCID in which both T and B cells are profoundly deficient, the X-linked form of SCID is characterized by the presence of normal number of peripheral blood B-cells.

Molecular and Cellular Defects

Approximately 50% of patients with SCID have an X-linked recessive pattern of inheritance. It has been mapped to Xq13.[78] These patients have a mutation in the common cytokine receptor gamma chain gene that encodes a shared, essential component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15.[79] The mutation results in the expression of the gamma chain which exhibits reduced binding to JAK3.[80] Thus, the early lymphoid progenitor cells lacking intact interleukin receptors, fail to be stimulated by these growth factors that are vital to the normal development of T and B cells. Affected newborns are profoundly deficient in thymic size and there is progressive T cell deficiency resulting from ineffective rescue from apoptosis or replication senescence.[81] Prenatal mutational screening by single-strand conformational polymorphism, heteroduplex analysis and dideoxy fingerprinting (ddF) followed by direct sequencing for mutations in the gamma chain reveal that ddF is the most sensitive method for detection of heterozygous mutations.[82] Allogeneic stem cell transplantation even *in utero*, as well as *ex vivo* gene therapy can correct the immunodeficiency in these patients.[83]

JAK3 Deficiency

Mutations of the *JAK3* gene lead to a form of non-X-linked autosomal recessive form of SCID. Clinically, they resemble infants with X-linked SCID with elevated levels of B cells and very low levels of T cells and natural killer cells in the blood.[79] The *JAK3* gene maps to chromosome 19p12–13.1 and encodes the JAK3 protein, an intracellular tyrosine kinase, which is crucial for signal-transmission of cytokine receptors to signal transducers and activators of transcription (STATs). Once recruited into the cytokine receptor complex, STATs are phosphorylated and then translocated into the nucleus to regulate tran-

scription at multiple sites. As a result, there is almost complete absence of JAK3 kinase activity with impairment of IL-2 and IL-4 signaling.[84,85] Due to the multiple cytokines using this signaling pathway, an early and severe block in T and natural killer cell development combined with impaired B cell function is observed. The identification of the genomic organization of the human *JAK3* gene into 23 exons[86] has made rapid mutation detection and prenatal diagnosis feasible.[87] Twenty-seven unique mutations have been identified that affect all seven structural Jak homology (JH) functional domains. The ability to test the function of a specific and expressed mutant in a phosphorylation assay using physiological substrates (JAK3 and STAT5) has enabled the verification of the consequences of the observed mutations. While some mutations result in absence of protein activity, others such as the C759R substitution results in constitutive phosphorylation of JAK3 which cannot be up-regulated by cytokine stimulation and thus block signal transduction.[88] Thus, demonstration of JAK3 protein expression by Western blot does not rule out functional JAK3 deficiency until cytokine-induced phosphorylation of JAK3 itself and/or STAT5 is excluded. JAK3 deficiency is a candidate for gene therapy. *In vitro* biochemical correction of JAK3-deficient human B-cell derived cell lines has been accomplished.[89]

Other SCIDs

Bare Lymphocyte Syndrome Type I (TAP1 and TAP2 Deficiency)

Bare lymphocyte syndrome is characterized by a severe decrease of HLA class I and/or class II molecules. Patients show reduced numbers of CD8+ T cells and lack natural killer cell activity. HLA class I expression depends on the formation of a peptide-loading complex composed of class I heavy chain, β_2-microglobulin, the transporter associated with antigen processing (TAP) and tapasin which links TAP to the heavy chain. Type I bare lymphocyte syndrome is caused by a deficiency in the TAP proteins encoded by genes within the major histocompatibility complex and plays a role in presentation of antigenic peptides to T cells. TAP transports peptides from the cytoplasm into the inner lumen of the endoplasmic reticulum and thus defects in TAP induce poor peptide loading on class I heavy chains. The TAP complex is a composed of TAP1 and TAP2 which, via the ATP-binding cassette transporter, translocates peptides from the cytosol to the waiting MHC class I molecules in the endoplasmic reticulum. TAP1 and TAP2 deficiency have identical clinical presentations that manifest within the first 6 years of life with recurrent bacterial infections of the upper respiratory tract.

Molecular and Cellular Defects

Mutations of both *TAP1* and *TAP2* genes result in deficient expression of class I HLA proteins on the cell surface with defects in natural killer cell cytotoxicity.[90,91] A novel genetic cause of type I bare lymphocyte syndrome has been identified within the *Tapasin* gene resulting from a deletion of 4 exons by Alu-mediated recombination.[92]

Bare Lymphocyte Syndrome Type II (MHC Class II Deficiency)

The cell surface glycoproteins of the MHC class II are crucial players in the immune response. Defective expression of major histocompatibility complex class II molecules account for 5% of SCID.[93] Children with autosomal recessive form of hereditary MHC class II deficiency, or bare lymphocyte syndrome type II are extremely susceptible to bacterial, viral, and fungal infections, beginning in the first year of life. Mortality rate is high, with most children dying from overwhelming infections by the age of 4 years.[94]

Molecular and Cellular Defects

The genetic lesions responsible for this syndrome do not lie within the MHC-II locus itself, but reside instead in genes encoding transcription factors controlling MHC-II expression. Different subtypes of human MHC II molecules are transcribed from TATA-less promoters that contain conserved S, X, and Y boxes. Protein complexes that bind to these proximal promoter elements attract the class II transactivator (CIITA) by an unknown mechanism. S and X boxes bind a tripartite regulatory factor X (RFX) complex, while the Y box binds the nuclear factor Y (NFY) complex. While the genes for MHC-II determinants remain intact, different mutations have been found in four *trans*-acting factors, RFX5, RFXAP, RFXANK(B), and CIITA.[95] MHC II molecules expressed on the surface of B cells present processed peptide fragments to the TCR of CD4 + T helper cells, triggering the antigen-specific T cell response. The regulatory factor (RF) X, a complex binding to the X-box of MHC-II promoters in the nucleus, is mutated in one type.

CD4 +T cells are decreased in all forms, although circulating lymphocyte numbers are normal and immunoglobulin numbers can also be decreased. Mutant forms of CIITA and all of the known subunits of RFX have been found among patients with the bare lymphocyte syndrome because all mutations cause absolute deficiency of MHC class II proteins.[96,97]

CD45 Deficiency

A molecular defect of CD45 is a rare cause of severe combined immunodeficiency with an autosomal recessive inheritance.[98,99] CD45 is an abundant transmembrane tyrosine phosphatase expressed on all leukocytes and is required for efficient lymphocyte signaling, integrin-mediated adhesion, and migration of immune cells. Mutations leading to loss of a component of the extracellular domain of CD45 result in very low circulating T cells but a normal number of B cells. The T cells are unrespon-

sive to mitogens and serum immunoglobulin levels usually decrease with age. A homozygous 6-bp deletion in the *CD45* gene resulting in a loss of glutamic acid 339 and tyrosine 340 in the first fibronectin type II module of the extracellular domain of CD45 has been reported.[98] A male patient with a deficiency in CD45 due to a large deletion at one allele and a point mutation at the other resulting in aberrant splice site[99] has also been reported.

Treatment of SCIDs

SCIDs have been among the first diseases to be cured by bone marrow stem cell transplantation, and there is no need for pretransplant immunosuppression. Preliminary studies show that they are also good candidates for gene therapy. Retroviral reconstitution of the gene defect into autologous marrow hematopoeitic cells have led to full correction of X-linked SCIDs.[100]

Defects of Phagocytic Cells

Most congenital phagocytic disorders are diagnosed in the first year of life, but leukocyte adhesion deficiencies and chronic granulomatous disease may not be diagnosed until adulthood. Chronic granulomatous disease tends to exhibit increased susceptibility to catalase-positive organisms, whereas defects of the interferon-γ-interleukin 12-pathway are characteristically associated with mycobacteria and other intracellular pathogens. Myeloperoxidase deficient individuals will generally not be susceptible to infections except for patients with diabetes who develop severe candidiasis.

Cyclic Neutropenia

Cyclic neutropenia occurs sporadically and by an autosomal-dominant inheritance pattern. The typical presentation is that of recurrent, severe neutropenia (an absolute neutrophil count of less than 200 cells per cubic millimeter) lasting 3 to 6 days of every 21-day period. Diagnosis depends on serial measurements of absolute neutrophil counts over a period of several weeks.[101] Patients are usually asymptomatic, however, during periods of severe neutropenia, they develop recurring episodes of fever, aphthous ulcers, gingivitis, and cellulitis. Deep tissue infections and bacteremia from *Clostridium* species are the most serious complications.

Molecular and Cellular Defects

Although neutropenia in these disorders has been attributed to impaired or ineffective neutrophil production, the molecular and cellular basis for these diseases has remained largely unknown. Positional cloning studies have led to the mapping of candidate genes to chromosome 19p13.3, a region containing the genes for three neutrophil proteases: azurcidin, proteinase 3, and neutrophil elastase. Sequence analysis of the PCR-amplified genomic DNA revealed that all affected members in 13 families and one sporadic case of cyclic neutropenia harbored a mutation within the neurophil elastase gene (*ELA2*), with most of the mutations occurring at the junction of exons 4 and 5.[102] Neutrophil elastase is a serine protease chiefly synthesized by promyelocytes. The mutations affect the catalytic site of the enzyme, resulting in inactive elastase.[103] The link between inactive elastase and the cyclic changes in the level of neutrophils remains to be determined. However, defects in the granulocyte colony-stimulating factor signaling pathway is thought to destabilize normal steady-state conditions and increase the number of circulating lymphocytes, reticulocytes, and platelets during neutropenia.[104]

Subsequent studies also showed that 90% of patients with classic congenital neutropenia also had mutations in *ELA2* gene, however a greater diversity of mutations was found.[105] Those mutations associated with cyclic neutropenia occur in proximity to the active site and the binding pocket for the enzyme's substrate whereas the mutations responsible for congenital neutropenia would be predicted to change the molecular folding of the protein possibly affecting the storage of the enzyme in the primary granules.[106]

Severe Congenital Neutropenia

Severe congenital neutropenia is a heterogeneous disorder related to cyclic neutropenia. There is severe neutropenia with an absolute neutrophil count of less than 500 cells per cubic milliter, recurrent bacterial infections, and absence of myeloid maturation with arrest at the promyelocyte stage.[107] The disease manifests in the first months of life with infectious complications from *Staphylococcus aureus* and *Burkholderia aeruginosa* resulting in cellulitis, perirectal abscess, meningitis, and stomatitis.[108] The disease is more severe than cyclic neutropenia and is three to four times more common.[109] The disease was initially described as an autosomal recessive disease, however it can occur sporadically and as an autosomal dominant disorder.

Molecular and Cellular Defects

The underlying genetic abnormality in severe congenital neutropenia is largely unknown. In about 10% of the patients, a heterozygous mutation of the granulocyte colony-stimulating factor receptor is identified. Germline mutations of the gene encoding neutrophil elastase (ELA2) have been observed in a large percentage of (22 of 25) patients studied according to a recent report.[105] This gene mutation has been found to play a role in other neutropenic disorders including cyclic neutropenia, but not in Shwachman-Diamond syndrome patients. Accelerated apoptosis of neutrophil precursors is a common feature of both cyclic and severe congenital neutropenia. Nevertheless, the mechanism by which mutations of the *ELA2* gene cause the premature death of these cells is unclear.[110] The enzyme neutrophil elastase is synthesized in neutrophil precursors early in the process of primary granule formation. It is currently presumed that

the mutant elastase functions aberrantly within the cells to accelerate apoptosis of the precursors resulting in ineffective and oscillatory production. Although mutations in *ELA2* may be necessary for the phenotype of congenital neutropenia, it may not be sufficient.[111] In most patients, treatment with G-CSF diminishes the number of infections.[107,112] *ELA2* mutations may provide a background for the G-CSF receptor mutations that occur in the transformation to acute myeloid leukemia. More recently, a novel mutation affecting the the GTPase binding domain of the Wiskott-Aldrich syndrome protein (WASP) has been described in an X-linked form of severe congenital neutropenia.[113]

Shwachman-Diamond Syndrome

Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive disorder characterized by exocrine pancreatic insufficiency, skeletal abnormalities, bone marrow dysfunction, and recurrent infections.[108] Neutropenia, either cyclic or intermittent, with or without pancytopenia can occur.[114] Recurrent infections involving sinuses, lungs, bones, skin, and urinary tract begin during the first year of life.[108] Patients have increased risk of bone marrow aplasia, myelodysplasia, and myeloid leukemia.[115]

A variety of immune defects have been observed including decreased *in vitro* B lymphocyte proliferation, lack of specific antibody production, low numbers of $CD4^+$ T cells and decreased numbers of natural killer cells.[116] Additionally, there is hyperactivation of the Fas-mediated apoptotic pathway resulting in higher tendency of bone marrow mononuclear cells to undergo apoptosis.[117] The significant immune dysfunction seen in these patients suggests that Shwachman-Diamond syndrome may involve a marrow defect accounting for aberrant function of hematopoietic and lymphopoietic lineages. The mapping of the *SDS* locus to the centromeric region of chomosome 7 (7p10–7q11)[118] is interesting as both myelodysplastic syndrome and AML frequently exhibit monosomy 7, isochromosome 7, and deletion of 7q.[119]

More recently, mutations involving a previously uncharacterized gene, *SBDS*, have been identified in patients with Shwachman-Diamond syndrome.[120] The mutations are located in the interval of 1.9 cM at 7q11 resulting from gene conversion involving exon 2 in 89% of unrelated individuals with Shwachman-Diamond syndrome (141 of 158 families) with 60% (95 of 158 families) carrying two converted alleles. *SDBS* encodes a predicted protein of 250 amino acids and thought to be involved in RNA processing. The complex and pleiotropic phenotype associated with this syndrome suggests however, the presence of several mutations responsible for the disease.

Leukocyte Adhesion Defect

Leukocyte adhesion deficiency is a rare, autosomal recessive genetic disorder in which neutrophils fail to mobilize and migrate to sites of injury. There is delayed separation of the umbilical cord in infancy, followed by severe, scarring skin infections, gingivitis, and systemic bacterial infections. Because of abnormal aggregation and migration, even when there is no infection, the patients have twice the normal number of neutrophils in the peripheral blood.[121]

Molecular and Cellular Defects

The gene defects associated with this disorder involve CD18 and CD11c, both of which are components of surface integrin complexes that are essential for neutrophil aggregation and attachment to endothelial surfaces.[122] Leukocyte adhesion deficiency type 1 is an autosomal recessive disorder resulting from a lack of β_2 integrin adhesion molecules on neutrophils.[123]

The second type of leukocyte adhesion deficiency is a defect of carbohydrate fucosylation and is associated with growth retardation, dysmorphic features, and neurological deficits.[124] These patients lack CD15s, sialyl-$Lewis^X$, a ligand for the selectin family. In these patients, there is no fucosylation of other glycoconjugates that are required for interactions with P-selectins and E-selectins on endothelial cells.[125] The genetic defect has not been determined, however treatment with oral fucose has reduced the frequency of infections and fevers.[125]

Rac2 Deficiency

Dominant-negative mutations resulting in deficiency of ras-related C3 botulinum toxin substrate (*Rac2*), the predominant hematopoeitic-specific Rho GTPase in neutrophils also leads to leukocyte adhesion deficiency.[126] The Rho family of GTPases plays key roles in regulating cytoskeletal reorganization, integrin complex formation, and cell adhesion. The neutrophils from this disorder exhibit abnormal chemotaxis and secretion of primary granules and defective generation of superoxide.[127] Rac2 is integral to the function of the actin cytoskeleton and its deficiency results in the inability of neutrophils to migrate normally in response to bacterial peptides.[128] Furthermore, biochemical and biological characterization of the human Rac2 GTPase dominant-negative mutant suggests that neutrophils with the mutant Rac2 GTPase exhibit increased apoptosis as well as defective neutrophil function.[129]

Defects of Interferon-γ-Interleukin-12 Axis

The interferon-γ-interleukin-12 axis is critical for defense against intracellular microbes such as mycobacteria, salmonella, and listeria. Defects in the ligand binding and signaling chain of the interferon-γ receptor, the interleukin-12 receptor or interleukin-12 itself result in profound deficiency of T cells and susceptibility to mycobacterial infection and severe combined immunodeficiency.[130] Interferon-γ is important for activation of macrophages and neutrophils with subsequent secretion of tumor necrosis factor α and activation of NADPH oxidase. IL-12 is stimulated when pathogens activate dendritic cells and macrophages which in turn leads to secretion of interferon-

γ.[131] Mutations have been identified in the genes for both chains of the interferon-γ receptor with autosomal recessive and autosomal dominant inheritance.[132] Children with a mutation that cause a complete loss of the ligand-binding chain have severe disease that begins in early infancy. The main features are disseminated atypical mycobacterial disease or fatal Bacilli Calmette-Guerin infection after vaccination and inability to form granulomas.[132]

Specific Granule Deficiency

Neutrophil-specific granule deficiency (SGD) is a rare disorder characterized by a lack of specific or secondary granules containing lactoferrin, transcobalamin, gelatinase B, and collagenase in developing and mature neutrophils.[133] Due to the absence of proteins necessary for the normal respiratory burst, the neutrophils show defects in bactericidal activity as well as abnormal migration and disaggregation.[134] The expression of the primary granules, myeloperoxidase and lysozyme, is unaffected. Patients present with frequent bacterial infections with *S. aureus*, S. epidermidis, and enteric bacteria primarily of the skin and lungs. The neutrophils display atypical bilobed nuclei, and lack expression of primary, secondary and tertiary granule proteins. Defects in chemotaxis, disaggregation, receptor up-regulation, and bactericidal activity are also characteristic features.

Molecular and Cellular Defects

The functional loss of the myeloid-specific transcription factor CCAAT/enhancer binding protein (C/EBPvarepsilon) due to frame-shift mutations leads to deficiencies in mulple myeloid lineages and the development of specific granule deficiency.[135,136]

Chronic Granulomatous Disease (CGD)

CGD is a rare inherited immunodeficiency that is caused by a functional defect of the various components of NADPH oxidase of phagocytes. Patients with CGD present with typical responses to infection such as fever, leukocytosis, and localized inflammation. As a result, many present with lymphadenopathy, hepatosplenomegaly, and recurrent infections. Due to defective microbial killing however, there is incomplete clearing of the infections, with subsequent formation of gramulomatous lesions. The disease is inherited in either an X-linked form, which accounts for two-thirds of cases and an autosomal recessive form.[137]

Molecular and Cellular Defects

The basic cellular defect of CGD is a deficiency of the respiratory burst.[138] Structural components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system is required for the generation of superoxide and other reactive species that are important in the killing of ingested microorganisms. The NADPH oxidase is composed of four polypeptide subunits and mutations in the corresponding genes are responsible for the four different genetic subgroups of CGD. Approximately 2 of 3 of CGD cases arise from mutations involving the X-linked gene encoding the $gp91^{phox}$ subunit. The *CYBA* gene encoding $p22^{phox}$ is localized on chromosome 16q24.[139] Mutations in *CYBA*, a subunit of the cytochrome b558, the redox element of NADPH oxidase, result in a rare autosomal recessive form of the disorder. Twenty-six different mutations in the *CYBA* gene have been described, including deletions, insertions, and substitutions leading to missense, nonsense, frame-shift, and splice-site mutations with no "hot-spots."[139–142] About one quarter of the CGD patients have mutations in the *NCF1* gene encoding $p47^{phox}$. It involves a GT deletion at the beginning of exon 2 of *NCF1*, most likely caused by recombination events between the *NCF1* and one of its pseudogenes.[143] Gene-scan methods have been used to detect one *NCF1* gene (carriers) from controls and from *NCF1*-deficient patients.[144] Prenatal diagnosis of p47phox-mutant CGD has been reported.[145] It is the most common autosomal form of CGD. The remaining cases of autosomal recessive CGD involve defects in $p47^{phox}$ or $c^{x139,146,147}$ with those involving $p67^{phox}$ being the rarest. As a group, those with X-linked form, $p22^{phox}$, $p67^{phox}$ CGD tend to have a worse clinical course compared to those with $p47^{phox}$ CGD.

Myeloperoxidase Deficiency

Hereditary myeloperoxidase (MPO) deficiency is the most common biochemical defect of neutrophils. An estimated prevalence of 1 of 2000 individuals has been reported in the United States.[4] The clinical outcome of hereditary MPO deficiency is much less severe than other genetic diseases of neutrophils such as chronic granulomatous disease (CGD) or Chediak-Higashi syndrome. Approximately half of those affected have a complete deficiency of myeloperoxidase while the rest have structural or functional defects in the enzyme.[148] Myeloperoxidase is a principal component of the azurophilic (primary) granules and an increased susceptibility to infections (particularly *Staphylococcus aureus* and *Candida albicans*) has been reported for some MPO-deficient patients.

Molecular and Cellular Defects

The mutations in the gene encoding myeloperoxidase are heterogeneous and result in transcriptional or post-transcriptional defects.[149] More recent studies demonstrate that hereditary MPO deficiency is not a simple autosomal recessive trait but a result of compound heterozygosity with respect to the R569W missense mutations of the *MPO* gene.[148]

Other Immunodeficiencies

Wiskott-Aldrich Syndrome

Wiskott-Aldrich syndrome (WAS) is inherited as an X-linked recessive disease and characterized by immune dysregulation and microthrombocytopenia. The estimated incidence in the U.S. is 4.0 per million male live births, accounting for six new cases per year.[4] Affected males have eczema, recurrent bacterial infections, and profound thrombocytopenia with platelets of reduced size and function.[150] Serum IgM concentrations are reduced, whereas IgA and IgE concentrations are elevated, and IgG is normal. Antibody formation, especially to bacteria with a polysaccharide capsule, is defective. In addition, affected patients have a poor response to protein antigens. The number of B cells progressively increases with time, while the number of T cells progressively decreases.[151] Further evidence of T cell dysfunction is demonstrated by poor *in vitro* T cell response to the mitogenic effects of anti-CD3.[152] Immune dysregulation may also manifest as food allergy, autoimmune disease including hemolytic anemia, vasculitides, and inflammatory bowel disease. In its less severe form known as X-linked thrombocytopenia, mutations in the same gene produce characteristic platelet abnormalities, but minimal immunological disturbances. The immune defects appear later and are variable and progressive.[153,154] In the past, WAS patients generally died within the first decade of life from infection, hemorrhage, or malignancy. However, the recent improved management modalities including intravenous immune globulin therapy and splenectomy, have improved the life expectancy of WAS patients with median survival of 15 years.

Molecular and Cellular Defects

Several nonsense and missense mutations as well as deletions and insertions of the *WAS* gene (Xp11.22)[155] have been identified in WAS patients.[156] The normal *WAS* gene encodes the Wiskott-Aldrich syndrome protein (WASP),[155] that belongs to a member of a unique family of proteins which are responsible for transduction of signals from the cell membrane[157] to the actin cytoskeleton. The interaction between WAS protein, the Rho family GTPase CDC42 and the cytoskeletal organizing complex, Arp2/3 is disturbed and leads to defects in cell signaling, polarization, motility, and phagocytosis.[158] The cell-surface sialoglycoproteins, most notably CD43 (sialophorin/leukosialin), are unstable with decreased expression in the cell membranes of lymphocytes.[155] Although the neutrophils and macrophages in WAS patients have been shown to exhibit impaired chemotactic responses,[159] other functional properties of these cells appear to be unimpaired.[157] Deficiency of WAS protein can be reliably detected in peripheral blood mononuclear cells of affected patients by flow cytometry.[160,161] Prenatal molecular diagnosis of WAS patients can be carried out by SSCP and heteroduplex assays followed by automatic DNA seqencing from chorionic villus samples.[162]

Most patients with X-linked thrombocytopenia have missense mutations within exons 1 and 2 of the *WAS* gene leading to decreased but detectable protein expression whereas a wide spectrum of mutations, most often leading to complete absence of protein have been detected in classical WAS.[163] The observation of missense mutations of the *WAS* gene in two families with an intermittent form of X-linked thrombocytopenia[164] broadens the spectrum of clinical phenotypes associated with defects of the *WAS* gene and indicates the need for molecular analysis in males with reduced platelet volume, regardless of platelet number. The only definitive therapy for the disease at present is allogeneic bone marrow transplatation, however retrovirus-mediated *WAS* gene transfer has been shown to correct the T cell abnormalities.[165]

DiGeorge/Velocardiofacial Syndrome

DiGeorge/velocardiofacial syndrome (DGS) is a congenital immune disorder characterized by lack of embryonic development or underdevelopment of the thymus and surrounding organs. The syndrome is associated with several defects including cardiac outflow tract anomalies, abnormal facies, thymic hypoplasia, cleft palate, and hypocalcinemia.[166] The immune deficit is caused by hypoplasia or aplasia of the thymus. The degree of thymic abnormality varies and only about 20% of the patients have decreased T cell number and function. Autoimmune diseases and recurrent infections are other common features.[167] Although mostly diagnosed in infancy, in some cases hypocalcemic tetany during adulthood have resulted in the diagnosis of DiGeorge syndrome with underlying cardiovascular malformations.[168]

Molecular and Cellular Defects

The DiGeorge anomaly was originally considered as a clinical paradigm for thymus deficiency but has been redefined as a member of a group of disorders that have in common a chromosome deletion involving 22q11.2 (the DiGeorge syndrome chromosome region, or DGCR) that commonly include 24 continguous genes.[166] The embryologic defect is due to inadequate development of facial neural-crest tissues resulting in defective organogenesis of the pharyngeal pouch derivatives. No single gene or combination of genes has been demonstrated to contribute to the complex phenotypic spectrum of DGS. Knockout mice studies have shown that mice heterogyzous for a transcription factor of the T-box gene, *Tbx1,* dsplayed a wide range of developmental anomalies encompassing almost all of the common DGS features[169,170] Other candidate genes that have been studied are *RanBP1*, a GTPase important for nucleocytoplasmic transport,[171] and *CRKL* which encodes an SH2-SH3-SH3 adaptor protein. Mice defective in these candidate genes result in phenotypes with similarities with human DiGeorge syndrome.[172] With the rapid progress in molecular cytogenetics, the investigation of choice is now a standard karyotype to exclude major rearrangements. Polymerase chain reaction and fluorescence *in situ* hybridization using probes from

within the deletion segment are also available for prenatal diagnosis of the 22q11.2 deletion.[173]

Ataxia Telangiectasia

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by various neurological deficits including, cerebellar degeneration with progressive ataxia, oculocutaneous telangiectasia, and immunodeficiency.[4] Most patients develop recurrent bacterial sinopulmonary infections and increased susceptibility to hematological cancer.[174,175] The immunological defects are of variable severity and may affect both T and B cells. Other important features include thymic hypoplasia, growth retardation, hypogonadism, and consistently elevated levels of α-fetoprotein, which may be used to establish the diagnosis.[174] In addition to these findings, 80% of patients are IgA deficient and serum levels of IgG4 and IgG2 may also be decreased. AT patients also exhibit increased susceptibility to the effects of ionizing radiation, due to defective DNA repair and chromosomal instability.[176]

Molecular and Cellular Defects

The *ATM* gene has been mapped to chromosome 11q22–23.[177] The gene designated *ATM* has been found to encode a polypeptide with a phosphatidyl inositol-3′ (PI-3) kinase domain.[178] It has similarities to the catalytic subunit of DNA-dependent protein kinase[179] and can directly phosphorylate and activate many genes involved in cell cycle checkpoint responses including *p53*,[180] *CHK2*,[181] *Nbs1*,[182] and *BRCA1*.[183] *ATM* is involved in mitogenic signal transduction, meiotic recombination, response to DNA damage and control of the cell cycle, and apoptosis.[184] Biallelic loss of the *ATM* gene or its substrates results in a defect in the radiation-activated S phase checkpoint. The *ATM* gene is mutated in AT patients from all four complementation groups, indicating that it is probably the sole gene responsible for the disorder.[178] In addition, in AT patients of any age, approximately 10% of all T lymphocytes show the presence of translocations and inversions, mainly involving chromosomes 7 and 14 at specific breakpoints; the chromosomal location of the T cell antigen receptor genes, as well as the immunoglobulin gene loci.[174] Whereas truncating mutations are the most common type of mutation observed in individuals affected with AT,[185] those that occur in sporadic breast cancer are missense mutations. Furthermore analysis of ATM mutations in sporadic lymphomas and leukemias show that there is a mixture of both missense and truncating alterations distributed across the whole of the ATM coding sequence. Interestingly, mutations in sporadic T-prolymphocytic leukemia were predominantly missense, clustering in the region encoding the PI-3 kinase catalytic domain of the protein, while those seen in mature B cell lymphomas such as chronic lymphocytic leukemias and mantle cell lymphomas were distributed across the whole of the ATM coding sequence.[186] The highest percentage of ATM mutations is found within mantle cell lymphoma.[187] *ATM* heterozygous mice harboring an in-frame deletion corresponding to the human 7636del9 mutation show an increased susceptibility to malignancies. Expression of *ATM* cDNA containing the 7636del9 mutation had a dominant-negative effect inhibiting radiation-induced ATM kinase activity.[188] These findings indicate that different *ATM* mutations might differ in the degree of associated cancer risk. There is emerging data that show that many chromosomal instability disorders share components of converging signaling pathways. For example, phosphorylation of the Fanconi anemia protein FANCD2 by ATM kinase following ionizing radiation is required for activation of the S phase checkpoint.[189]

Immunodeficiency with Albinism

Chediak-Higashi Syndrome

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder characterized by partial skin and ocular albinism, increased susceptibility to infections, and progressive neuropathy.[190] About 85 to 90% of the patients present in early childhood with life-threatening bacterial infections due to neutropenia and lack of natural killer cell function. Most patients with CHS develop lethal complications, called the "accelerated phase" during which T cells and macrophages undergo overwhelming activation involving multiple organs.[191]

Molecular and Cellular Defects

Mutations of the gene encoding a protein named *LYST* in humans (lysosomal trafficking regulator/CHS) or *Beige* in mice, that is a cytoplasmic protein that controls lysosome traffic is thought to cause CHS.[192] The CHS1 protein is predicted to contain three to four defined domains including a weak ARM/HEAT repeat domain, a perilipin domain, a BEACH domain, and a series of WD-40 repeats. These motifs are thought to be important in mediating membrane associations, vesicular transport, and lipid association. The exact biochemical function of *LYST/CHS1* is unknown however, yeast two-hybrid studies demonstrate that LYST interacts with proteins important for vesicular transport and signal transduction including SNAR-complex protein HRS, 14–3-3, and casein kinase II.[193] Many cell types have large intracytoplasmic granulations[194] and deficient cytotoxic lymphocyte activity as a result of defective vesicular trafficking of the endosome/lysosome compartment. Defective T cells and natural killer cell cytotoxic function develop due to defective exocytosis and delivery of lytic proteins.[195] Macrophages and neutrophils show decreased chemotaxis with normal phagocytic function. The defective lysosomal function results in a delay in fusion of phagosomes with lysosomes. Recent data suggest the presence of genotype-phenotypic correlation in childhood and adult forms of CHS. Most of the mutations in patients with severe childhood presentations result in null alleles, frame-shifts, nonsense mutations, or gene deletions that abolish the expression of the full-length polypeptide.[196] About 10 to

15% of patients exhibit a much milder clinical phenotype and survive to adulthood. In these patients, missense mutant alleles that encode peptides with partial function are found[197]

Griscelli Syndrome

Griscelli syndrome is a rare autosomal recessive disorder that results in hypopigmentation of hair and skin, the presence of large clumps of pigment in hair shafts, and an accumulation of melanosomes in melanocytes.[198] Most patients develop an uncontrolled T lymphocyte and macrophage activation syndrome, known as hemophagocytic syndrome, potentially leading to death.[199] Some Griscelli syndrome patients, early in life, show severe neurological impairment without apparent immune abnormalities. Despite an adequate number of T and B lymphocytes, the patients are hypogammaglobulinemic, deficient in antibody production, and incapable of delayed skin hypersensitivity and skin graft rejection. Griscelli syndrome resembles Chediak-Higashi syndrome, but differs in that the polymorphonuclear leukocytes in GS are morphologically normal, and lack the abnormal giant granules that are characteristic of CH syndrome.

Molecular and Cellular Defects

Two closely linked genes located on human 15q21 region have been found to be responsible for the disease. Nucleotide substitutions of myosin 5A (*MYO5A*) gene have been reported[200,201] while others have reported the involvement of *RAB27A*.[199] Myosin 5A, a member of the unconventional myosin family thought to participate in organelle-transport is important for lymphocyte cytotoxic function. *RAB27A* encodes a RAB guanosine triphosphate (GTP)-binding protein required for prenylation and activation of RAB GTPases which are critical regulators of vesicular transport. Studies of the mouse model of *Rab27A* mutant *ashen* homozygotes show that it is important for the regulated secretion of granzyme A and hexosaminidase of cytotxic T cells.[202] Griscelli disease presents with a heterogenous clinical picture that seem to reflect the involved gene defect. The form due to myosin 5A mutation is associated with a primary neurological impairment, and immune features such as susceptibility to infections and occurrence of hemophagocytic syndrome are absent. The form due to *RAB27A* mutations has no neurological features but is associated with an uncontrolled T cell and macrophage activation syndrome, the hemophagocytic syndrome. T cells of *RAB27A*-deficient individuals had normal granule content in perforin and granzymes but showed defective granule release. Indeed, bone marrow transplantation with peripheral blood stem cell transplant in a 6-month-old girl with Griscelli syndrome due to deletion of the *RAB27A* has been beneficial.[203]

Familial Hemophagocytic Lymphohistiocytosis

Familial hemophagocytic lymphohistiocytosis (FHL) is inherited as an autosomal recessive disorder with a rapidly fatal clinical presentation.[204] It is characterized by the overwhelming activation of T cells and macrophages which results in fever, hepatosplenomegaly, pancytopenia, coagulation abnormality, and liver dysfunction. Multiple organs including liver, spleen, bone marrow, and central nervous system become infiltrated by activated T cells and macrophages that become engulfed by phagocytes, a process called hemophagocytosis.[205] In addition, neurological involvement develops during the latter course of the syndrome with manifestations ranging from confusion to severe seizures and neurological deficits. These clinical features are associated with the overproduction by T lymphocytes and macrophages of cytokines including interferon-γ, tumor necrosis factor-α, IL-1, and IL-6.[206] Combination of chemotherapy and bone marrow transplantation can cure many patients.[207]

Molecular and Cellular Defects

Both homozygous and heterozygous mutations[208] involving the perforin gene have been identified in patients with familial hemophagocytic lymphohistiocytosis. Lymphocytes obtained from FHL patients demonstrated significantly reduced CD3-dependent perforin-mediated cytotoxic activity of CD8+ T and natural killer cells than from normal control patients. Clonal expansion of $\alpha\beta$-T cells with restricted Jβ1 usage of T cells was seen in these patients[209] which could be detected at relapse after chemotherapy.[210] The level of perforin-mediated cytotoxic activity is correlated with perforin immunoreactivity. More recently, a comprehensive survey of 34 patients for mutations in the coding region of the perforin gene have been reported.[211] Homozygous and heterozygous mutations of the perforin gene were detected in 20% (7 of 34) of all FHL patients investigated, with a somewhat higher prevalence, approximately 30% (6 of 20), in children whose parents originated from Turkey. Prenatal diagnosis of perforin gene mutations can identify a subset of patients with FHL.[212]

X-Linked Lymphoproliferative Disease

In X-linked lymphoproliferative disease (XLP) or Duncan's disease, patients are exceptionally susceptible to Epstein-Barr virus (EBV)[213,214] leading to uncontrolled expansion of EBV-infected B cells and cytotoxic T cells. The average age of onset is 2.5 years, with 100% mortality by the age of 40 years.[215] Patients are totally asymptomatic before EBV infection however, following infection with EBV, patients mount a vigorous, uncontrolled polyclonal expansion of T and B cells. The subtle, progressive combined variable immunodeficiency disease is characterized by benign or malignant proliferation of lymphocytes that may be polyclonal or overly malignant with monoclonal populations. Proliferation of histiocytes and alterations in concentrations of serum immunoglobulins are also characteristic. In many patients, a differential diagnosis of lymphoma is operative as proliferating lymphocytes infiltrate the lymph nodes, spleen, and liver. Patients' symptoms are fever, pharyngitis, lymphadenopathy, hep-

atosplenomegaly, atypical lymphocytosis, and a spectrum of deficiencies ranging from agammaglobulinemia to polyclonal hypergammaglobulinemia. The primary cause of death is hepatic necrosis and bone marrow failure.

Molecular and Cellular Defects

The genetic abnormality associated with XLP involves a gene designated SH2 domain protein-1A (*SH2D1A*)[214] also called *SAP* (SLAM-associated protein). SLAM (signaling lymphocyte activation molecule), also known as CDw150, appears on the surface of T cells, where it has a crucial function in cell stimulation. SAP is physiologically expressed in T cells and NK cells and is an SH2 domain-containing protein. SAP is associated with the SLAM protein that is expressed on the surface of activated T-cells[216] and 2B4 molecule on NK cells and cytotoxic T cells.[217] It is thought to act as an adaptor molecule involved in transducing activation signals in T cells following T-B cell contact. It is expressed in activated T and NK cells but not in activated B cells.[218] Mutations in *SAP* affect the interaction between T and B cells and leads into uncontrolled B cell proliferation in EBV infection.[213] The identification of the *SAP* gene and its association with XLP has led to molecular diagnoses of related disorders although the mutation detection rate in several series is only 55 to 60%. Disease causing mutations of the *SAP* gene have been reported in male patients initially diagnosed as affected by CVID,[219] suggesting the possibility that a subgroup of patients with CVID may represent phenotypic variants of XLP. As the molecular genetic basis of CVID remains elusive, the diagnosis involves the exclusion of other molecularly defined disorders.

Immunodeficiency, Centromeric Instability, Facial Anomaly Syndrome

Immunodeficiency, centromeric instability, facial anomaly (ICF) syndrome is a rare autosomal recessive disorder with variable immune deficiency. The most common diagnostic feature is the branching of chromosomes due to centromeric instability of chromosomes 1, 9, 16, and rarely, 2, with an increased frequency of somatic recombination of the arms of these chromosomes.[220] The affected children suffer serious variable immunodeficiency, mild developmental delay, and facial abnormalities with hypertelorism, a flat nasal bridge, epicanthal folds, protrusion of the tongue, and mild micrognathia. The severity of the disorder is indicated by the fact that most die during childhood.

Molecular and Cellular Defects

Positional cloning has revealed the *de novo* DNA methyltransferase 3B (*DNMT3B*) as the candidate gene in a large percentage of ICF patients.[221,222] ICF patients show marked hypomethylation of their DNA. Mutations involving the DNA methyltransferase 3B (*DNMT3B*) gene were identified in four patients from three families. There is evidence however for genetic heterogeneity, since two ICF patients from consanguineous families who did not show autozygosity (homozygosity by descent) for the *DNMT3B* locus also did not reveal DNMT3B mutations. Mutations including missense, nonsense, and splice-site involvement[223] have been documented. No homozygous mutations have been found suggesting that the absence of the enzyme is incompatible with life. Genomic methylation is critical for maintenance of structure and expression and undermethylation of certain chromosomal segments is associated with centromere instability and abnormal expression of key immunoregulatory genes. Microarray expression analysis of B cell lymphoblastoid cell lines from ICF patients with diverse *DNMT3B* mutations demonstrated dysregulation of numerous genes associated with lymphocyte signaling, maturation, and migration compared to normal lymphoblastoid cell lines[224]

Nijmegen Breakage Syndrome

Nijmegen breakage syndrome (NBS) is a rare autosomal disorder characterized by microcephaly, stunted growth, mental retardation, cafe-au-lait spots, and immunodeficiency with impaired DNA repair and hypersensitivity to ionizing radiation.[225,226] Most patients suffer from recurrent respiratory tract infections and others have repeated urinary tract infections. There is a high predisposition for malignancy, occuring in 40% of patients before the age of 21. Hypogammaglobulinemia is typically present involving the levels of IgA, IgG2, IgG4, and IgE.[226] NBS patients lack the classical signs and symptoms of AT such as cerebellar ataxia, oculocutaneous telangiectasia, and increased α-fetoprotein (AFP).

Molecular and Cellular Defects

Most patients have a truncating deletion of the *NBS-1* gene, which is located on chromosome 8q21. The *NBS-1* gene encodes a protein nibrin, a member of the hMre11/hRad50 nuclease complex involved in DNA double-strand break repair.[227] The defect in DNA repair mechanism is thought to contribute to decreased immunoglobulins as a result of defective class switching.[228] Such findings as recurrent infections, immunodeficiency, chromosomal instability with multiple 7 and/or 14 rearrangements, cellular and chromosomal hypersensitivity to ionizing radiation and bleomycin, and radioresistance of DNA replication, are common features of both NBS and AT. More recent studies demonstrate a functional link between ataxia-telangiectasia and Nijmegen breakage syndrome gene products. The phosphorylation and function of NBS1 protein induced by ionizing radiation requires the catalytically active kinase function of ATM.[229] These results demonstrate a biochemical link between cell-cycle checkpoints activated by DNA damage and DNA repair in two genetic diseases with overlapping phenotypes.

Job Syndrome

Job syndrome of hyperimmunoglobulinemia E and recurrent infections (hyper-IgE syndrome) was described in 1966.[230] The disorder is characterized by markedly elevated serum IgE levels and recurrent cutaneous and sinopulmonary infections.[230] Many also present with eczematoid rashes, mucocutaneous candidiasis, subcutaneous abscesses, bony abnormalities, and osteoporotic fractures.[231]

Molecular and Cellular Defects

The genetic basis for Job syndrome is not known and the central immunological defect is largely undefined. Reduced neutrophil chemotaxis is often described, and variable T cell defects have been demonstrated in some patients. It has been hypothesized that hyper-IgE is associated with a Th1/Th2 imbalance.[232] It is inherited as a single-locus, autosomal dominant trait with variable expressivity.[54]

Autoimmune Lymphoproliferative Syndrome

Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of defective lymphocyte apoptosis due to genetic aberrations of several members of the Fas-signaling pathway. Patients with ALPS present with peripheral lymphocytosis, diffuse lymphadenopathy, hepatosplenomegaly, hypergammaglobulinemia, autoimmune cytopenias and rarely autoimmune glomerulonephritis and hepatitis.[233] The peripheral blood is characterized by a markedly expanded population of CD5+ B cells, NK cells, and TCR$\alpha\beta$ CD4-CD8- (double-negative) HLA-DR+ naive T cells.[234] Many patients display a dysregulated cytokine pattern with dysfunctional T cells, suggesting that Fas defects may alter pathways of T cell activation[235] and differentiation. In most patients, this population constitutes between 15 to 70% of the peripheral blood T cells. The T cells have impaired proliferative and cytokine reponses to T cell activation.

Molecular and Cellular Defects

ALPS result from mutations in several genes critical in the apoptosis pathway, including *Fas*,[236] *Fas ligand*, *Caspase 8*, and *10*.[237] The genetics and pathogenesis of ALPS is complex. The disease can be inherited as an autosomal dominant trait, but development of full phenotype depends on additional factors suggesting that other host factors or genes play a role in modulating the disease phenotype. Moreover, the observation of ALPS in individuals without mutations of the above mentioned genes suggest a role for other apoptosis pathway genes in the predisposition to this disorder.

Conclusions

Recent developments in molecular diagnostics and gene-based therapies have led to fundamental shifts in the diagnosis, treatment, and management of families affected by primary immunodeficiencies. The discovery of specific gene abnormalities important in lymphocyte biology and their link to these rare diseases has led to significant insights into the complexity of the immune system and provided new insights regarding the spectrum of clinical severity seen in a particular disorder and the phenotypic overlap resulting from mutations of different genes.

Immunophenotypic and molecular genetic studies are very useful in the evaluation of these processes. However, the results should be interpreted with caution and correlation with clinical features is imperative. Despite the increased susceptibility of primary immunodeficiency patients to the development of lymphoma, the most frequent cause of mortality in these patients continues to be infection. Further delineation of the molecular and cellular defects in these diseases may provide the framework for the development of therapies targeted at the specific defects which may prevent the occurrence of devastating secondary complications such as infection and malignancy.

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