Brief General Approach for the Investigations of the Most Common Primary Immunodeficiencies

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Abstract

Primary immunodeficiencies are congenital disorders caused by defects in different elements of the immune system. Laboratory evaluation of primary immunodeficiencies (PIDs) is a complex process requiring a multi-tiered approach to accurately diagnose these heterogeneous disorders. Initial assessment typically involves a thorough clinical history and physical examination, focusing on recurrent infections, autoimmunity, and other associated symptoms. This is followed by a comprehensive laboratory workup designed to evaluate different components of the immune system. The first line of investigation often includes basic immunological tests, such as complete blood counts (CBC) with differential, assessing lymphocyte subsets (CD4+, CD8+, B cells, NK cells), and immunoglobulin levels (IgG, IgA, IgM). Significant deviations from established reference ranges may indicate specific immune deficiencies. For example, low IgG levels suggest hypogammaglobulinemia, while decreased lymphocyte counts point towards lymphopenia. Further evaluation might include assessment of antibody function through specific antibody titers against common pathogens (e.g., pneumococcus, tetanus, diphtheria). Functional assays constitute a crucial second tier of investigation, providing a deeper understanding of immune cell activity. These can encompass flow cytometry analysis to assess lymphocyte activation, proliferation, and surface marker expression; assessment of phagocytic function (e.g., neutrophil oxidative burst test); and in vitro assays to evaluate complement function. These tests help identify defects in cellular immunity, humoral immunity, or complement pathways. Genetic testing is increasingly important, offering molecular confirmation of suspected diagnoses. Next-generation sequencing (NGS) panels targeting a wide range of PID-associated genes are becoming the standard of care, allowing for rapid and comprehensive genetic analysis. Interpreting the results requires expertise and integration of clinical information with laboratory findings. A normal result for a single test does not exclude PID, as the defects can be subtle or affect specific immune components. Therefore, the diagnostic process is often iterative, with additional testing guided by initial results and evolving clinical presentations. Advancements in technology, including high-throughput screening and sophisticated molecular techniques, are constantly improving the accuracy and efficiency of PID diagnosis, ultimately leading to

earlier intervention and improved patient outcomes. However, a strong clinical suspicion remains essential for initiating appropriate investigations.

Keywords: primary immunodeficiencies, investigations

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Introduction

In the last 20 years, not only has the clinical spectrum of known primary immunodeficiencies (PIDs) grown substantially, but the genetic foundation for most PIDs has also been found. Patients with PIDs must have an accurate diagnosis in order to receive the right treatment, and it also gives doctors a chance to counsel the family on genetic matters. Early identification and treatment offer the best chance to avoid substantial disease-associated morbidity, because infection susceptibility is a hallmark of almost all clinical symptoms. For the purpose of identifying the immunologic problem, the laboratory data is crucial in this context.

Recurrent sinusitis, pneumonia, otitis media, and sinusitis are the most common bacterial infections of the sinopulmonary tract in patients with primary antibody deficits. (Table I).[1,2] Although Streptococcus pneumoniae is the most often isolated organism, other species such as Haemophilus influenzae (which is often not typeable), Staphylococcus, and Pseudomonas are also detected. Infection with Giardia lamblia is a common cause of diarrhea in up to 25% of these patients. It is also possible to find infections with Shigella, Campylobacter, enterovirus, and rotavirus.1Furthermore, autoimmune hemolytic anemia and autoimmune thrombocytopenia are the most often detected autoimmune symptoms, and they can be observed in as many as 25% of these patients. Lastly, there is granulomatous disease, which can affect multiple organs but primarily the lungs; this process can cause serious illness in certain patients.[1]

Table I
Common pathogens and infection sites according to the underlying immune defect

Affected immunity arm	Typical site of infection	Common pathogens
B cells	Sinopulmonary tract, GI tract, joints, CNS	Pyogenic bacteria: streptococci, staphylococci, *Haemophilus influenzae*
		Enteroviruses: ECHO, polio
		Mycoplasma species
T cells	Sepsis, lung, GI tract, skin	Viruses: CMV,
		adenovirus, measles, molluscum
		Fungi: *Candida* and *Aspergillus* species, *Pneumocystis jiroveci*
		Pyogenic bacteria
		Protozoa: *Cryptosporidium* species
Phagocytes	Skin infections, lymphadenitis, liver, lung, bone, GI tract, gingivitis/periodontitis	Bacteria: staphylococci, *Serratia marcescens, Burkholderia cepacia, Klebsiella* species, *Escherichia coli, Salmonella* species, *Proteus* species
		Fungi: *Candida, Aspergillus,* and *Nocardia* species
Complement	Systemic infections, meningitis	Pyogenic bacteria: streptococci, *Haemophilus influenzae, Neisseria* species

GI, gastrointestinal; *CNS,* central nervous system; *ECHO,* echovirus; *CMV,* cytomegalovirus.

Table II shows that certain inherited immune deficiencies, such as selective IgA deficiency, common variable immunodeficiency, and congenital agammaglobulinemias, can cause hypogammaglobulinemia to varying degrees. Some less common causes include X-linked lymphoproliferative syndrome (XLP) and agammaglobulinemia with thymoma (Good syndrome).If a man has recurrent otitis or even just one incident of pneumonia, regardless of whether there is a negative family history, X-linked agammaglobulinemia should be considered a possible diagnosis. Pseudomonas or Staphylococcus infection can also cause neutropenia and sepsis, which are symptoms of this illness.[3] Before overt neurologic symptoms appear, ataxia-telangiectasia syndrome can occasionally present with upper respiratory tract symptoms related to an IgA deficit and recurrent infections.[4] It is reasonable to suspect a cellular deficiency that impacts antibody production, such as impairments in nuclear factor κB essential modulator (NEMO; also termed IKK-γ) or CD40 ligand (CD154), if low pathogenic mycobacteria and opportunistic infections occur simultaneously.[5,6] Potentially investigateable conditions that share symptoms with antibody deficit include phagocytic abnormalities and selected complement deficiency. (Table II).

Table II
Differential diagnosis of antibody deficiencies and associated laboratory findings

Primary B-cell disorders
Common variable immunodeficiency: low IgG and IgA levels, variable IgM levels, usually normal B-cell numbers
Selective IgA deficiency: low IgA levels, normal IgG and IgM levels, normal B-cell numbers
Congenital agammaglobulinemia: low IgG, IgA, and IgM levels; undetectable or very low B-cell numbers (<2%)
Specific antibody deficiency: normal IgG, IgA, and IgM levels; normal B-cell numbers; defective antibody response to vaccination
Agammaglobulinemia with thymoma (Good syndrome): low IgG and IgA levels, variable IgM levels, low B-cell numbers
Combined cellular and humoral disorders
Hyper-IgM syndromes: low IgG and IgA levels, normal, low or high IgM levels, normal B-cell numbers
Ectodermal dysplasia with immunodeficiency syndrome (NEMO/IκBα deficiency): variable immunoglobulin levels, normal B-cell numbers
XLP: low IgG and IgA levels, variable IgM levels, typically normal B-cell numbers
Ataxia-telangiectasia syndrome: low IgA levels
Other causes to consider
Drug-induced hypogammaglobulinemia; sickle cell disease with secondary hyposplenism; primary asplenia; immunodeficiency, centromeric instability, facial anomalies syndrome; cystic fibrosis; complement component deficiency; myelodysplasia; chronic lymphocytic leukemia; multiple myeloma; dysmotile cilia syndrome; warts, hypogammaglobulinemia, immunodeficiency and myelokathexis (WHIM) syndrome

Table III
Evaluation of suspected antibody deficiency

Screening tests
Quantitative immunoglobulins
Specific antibody levels
Circulating specific antibody levels to prior vaccines and blood group antigens (isohemagglutinins)
Pre/postimmunization antibody levels
Protein antigens
Carbohydrate antigens
IgG subclasses
Secondary tests
B-cell immunophenotyping
In vitro functional studies
Tests to exclude rare and secondary causes
Thoracic computed tomography to exclude thymoma (particularly useful if patient is >50 years old with low B-cell numbers)
Intracellular flow cytometry or genetic evaluation for BTK (XLA) or SAP/XIAP (XLP)
Genetic evaluation of NEMO to rule out anhydrotic ectodermal dysplasia with immune deficiency
Fecal α_1-antitrypsin, urinary protein, serum albumin, absolute lymphocyte count to exclude gastrointestinal or urinary protein loss or lymphatic loss
HIV testing to exclude AIDS
Complement function (CH50, AP50) to exclude complement deficiency
Karyotype to exclude immunodeficiency, centromeric instability, facial anomalies syndrome
Sweat chloride or genetic evaluation to exclude cystic fibrosis

BTK, Bruton tyrosine kinase; *XLA,* X-linked agammaglobulinemia; *SAP/XIAP,* SLAM-associated protein/X-linked inhibitor of apoptosis.

Laboratory evaluation

By monitoring the concentrations of the four main immunoglobulin classes—IgG, IgA, IgM, and IgE—clinical laboratories can perform the first screening of antibody-mediated immune activity. (Table III). It is necessary to compare the outcomes with reference intervals (normal ranges) that are matched by age and usually given as 95% CIs. Although there are no hard and fast rules for diagnosing immunoglobulin deficiency, additional testing should be initiated if an IgG level is below 3 g/L (300 mg/dL) in adults or adolescents and when a child's value is obviously lower than the age-appropriate reference (95% confidence interval). Level quantification of IgG subclasses is another accessible and supplementary assay. An IgA-deficient patient with severe recurring bacterial infections is the ideal candidate for this test. The test's limited value is due to the fact that, in most contexts, detecting an IgG subclass shortage still requires documentation of an anomaly in specific antibody production before therapy may be initiated. When total immunoglobulin levels are only slightly lower (or even normal) in the context of recurrent bacterial infection, measuring particular antibody responses is crucial for verifying faulty antibody synthesis. Testing for antibodies that have developed on their own (such as isohemagglutinins, which are antibodies to blood groups) or that have been introduced by past illnesses or vaccines is the quickest and easiest way. One of the most reliable ways to measure in

vivo antibody generation is to immunize a patient with protein and polysaccharide antigens, such as Pneumovax (Merck & Co, Inc., Whitehouse Station, NJ) and then measure their antibody levels before and three to four weeks after the injection. Typically, according to the testing laboratory's guidelines for normal responses, there should be a minimum of a fourfold increase in antibody levels and/or protective antibody levels following immunization. Patients now receiving immunoglobulin replacement therapy have an additional option for accessing the humoral immune response: vaccination with a neoantigen, like the bacteriophage Phi X174. However, this is only offered at a select number of specialized centers.[7]

Table IV
Most common T-cell and combined immunodeficiencies and distinctive features

SCID: failure to thrive, chronic diarrhea, oral thrush, recurrent or severe bacterial, viral and/or fungal infections
CD40 and CD40 ligand deficiency: recurrent sinopulmonary and opportunistic infections with low IgG and IgA levels and variable IgM levels
Wiskott-Aldrich syndrome: easy bruising, eczema, recurrent otitis media, diarrhea, thrombocytopenia with small platelets
DiGeorge syndrome: hypoparathyroidism, cardiac malformations, dysmorphic features, variable T- and B-cell defects
Anhydrotic/hypohidrotic ectodermal dysplasia with immunodeficiency (NEMO or IκBα deficiency): recurrent mycobacterial or pyogenic infections, with or without skin, hair, and nail abnormalities; poor fever responses
XLP: hypogammaglobulinemia, persistent or fatal EBV infection
Chronic mucocutaneous candidiasis: recurrent oroesophageal and skin *Candida* species infection

Flow cytometry is used to confirm or rule out the presence of B cells in further testing. Since the underlying abnormalities in this group of disorders usually cause circulating B-cell counts to be either nonexistent or drastically reduced, this is especially helpful as a diagnostic for congenital forms of agammaglobulinemia.[2] Patients with common variable immunodeficiency have recently been characterized by the use of B-cell subset analysis, with a focus on memory and immature B cells.[8] In most cases, only specialized research facilities conduct studies that examine immunoglobulin production and in vitro B-cell signaling.

Evaluating Suspected T-Cell or Combined T- and B-Cell Immunodeficiency Disorders

Patients with primary disorders characterized by abnormal T-cell function, such as severe combined immunodeficiency (SCID), often experience recurrent infections with opportunistic pathogens, such as Candida albicans (thrush), Pneumocystis jiroveci, or cytomegalovirus, and often fail to thrive during early childhood (Table I).[9] Persistent infections despite sufficient conventional treatment, recurring bacterial infections impacting various areas, and prolonged diarrhea are other prevalent symptoms. The clinical outcome of SCID can be greatly improved with an early diagnosis, making it a pediatric emergency. Omenn and Wiskott-Aldrich syndromes are two examples of the many T-cell illnesses that can cause skin rashes [10] Other severe cellular or combined defects present with varied clinical symptoms, as listed briefly in Table IV.

Laboratory evaluation

Evaluation of individuals suspected of cellular immunodeficiency diseases requires meticulous review of white blood cell count and differential. To properly understand the absolute lymphocyte count, it is necessary to compare it with age-matched control ranges. If a repeat test confirms severe lymphopenia in a newborn (<3,000/mm3), it is an urgent result that should trigger immunologic assessment immediately. One catch is that it wouldn't pick up on children with Omenn syndrome if low T-cell numbers in infancy were used as a screening for T-cell development problems. Despite severe cellular immunodeficiency due to an oligoclonal proliferation of T cells, this illness is characterized by normal or increasing numbers of T cells [10] Another possible outcome of maternal T-cell engraftment is the presence of circulating T cells in patients with a severe cellular immunological deficiency. Memory CD45RO+ cells, rather than the naive CD45RA+ cells seen in healthy infants, will make up the majority of the mother's T cells. These cells do not offer host protection. Furthermore, a significant cellular immunological deficiency may also be accompanied by the presence of circulating T cells in cases of maternal T-cell engraftment. In contrast to healthy newborns' naive CD45RA+T cells, which do not offer host protection, the majority of a mother's T cells will be memory CD45RO+ cells.[11] One last point: if you have a significant cellular immune deficiency and get a transfusion of nonirradiated blood products, it could lead to graft-versus-host disease, which is a potentially deadly condition. In this case, it is very important to irradiate any blood product that is given to a baby who may have a T-cell deficit.

Instead of serologic testing for anti-HIV antibodies, testing for the presence of the virus (i.e., an HIV viral load assay) is usually necessary to rule out HIV infection in all individuals exhibiting symptoms of cellular immunodeficiency.(Table V).

Table V
Evaluation of suspected T-cell and combined immunodeficiency

Screening tests
HIV testing
Lymphocyte immunophenotyping
Delayed-type hypersensitivity skin testing
Secondary tests
T-cell proliferation (mitogens, alloantigens, recall antigens)
T-cell cytokine production
Flow cytometric evaluation of surface or intracellular proteins, such as CD40 ligand (CD154 on activated T cells), IL-2 receptor γ chain (CD132), MHC class I and II, IL-7 receptor α chain (CD127), CD3 chains, WASP
Enzyme assays: adenosine deaminase, PNP
FISH for 22q11 deletion
TREC numbers
TCR repertoire analysis
Mutation analysis

WASP, Wiskott-Aldrich syndrome protein; *PNP*, purine nucleoside phosphorylase; *FISH*, Fluorescence *in situ* hybridization; *TREC*, T-cell receptor excision circle.

After T-cell screening tests, the next step would be a directed assessment of cellular immunity (Table V). This includes immunophenotyping of T cells by means of flow cytometry together with *in vitro* functional testing (eg, proliferation and cytokine production assays).[12] The immunophenotyping for a patient suspected of having SCID not only helps to establish the diagnosis, but it can also point to the potential underlying genetic defect (Table VI).[12] It is important to carefully review the percentage and absolute numbers for all lymphocyte subsets, comparing them with age-appropriate reference ranges. Typically, defects in cytokine signaling molecules result in a $T^-B^+NK^-$ phenotype, whereas mutations in DNA-editing proteins required for T- and B-cell receptor expression are associated with a $T^-B^-NK^+$ phenotype; severe metabolic defects usually are toxic for all lymphocyte types, resulting in a $T^-B^-NK^-$ phenotype (Table VI).

Table VI
Immunophenotypic findings and associated genetic defects in patients with SCID

Phenotype	Pathway affected and genetic defect(s)
$T^-B^+NK^-$	Cytokine signaling: IL-2 receptor γ, JAK3
$T^-B^-NK^+$	DNA editing: RAG1/2, Artemis, ligase 4, Cernunnos
$T^-B^-NK^-$	Metabolic defects: adenosine deaminase, AK2
$T^-B^+NK^+$	Cytokine signaling: IL-7 receptor α chain
$CD8^+CD4^-B^+NK^+$	Positive selection/signaling: MHC class II, p56lck
$CD4^+CD8^-B^+NK^+$	Signaling: ZAP70

JAK3, Janus kinase 3; *RAG*, recombination-activating gene; *AK2*, adenylate kinase 2; *ZAP70*, zeta-chain associated protein kinase, 70 kD.

Other useful tests in special circumstances include fluorescence *in situ* hybridization for the 22q11 microdeletion found in the majority of patients with DiGeorge syndrome and specific enzyme assays to evaluate for adenosine deaminase and purine nucleoside phosphorylase (PNP) deficiencies.[13] Evaluation for intracellular Wiskott-Aldrich syndrome protein expression by means of flow cytometry can be performed in selected centers to screen for possible Wiskott-Aldrich syndrome.[14] Direct evaluation of T-cell function, as assessed by the proliferative response to mitogens, recall antigens, and/or alloantigens, is an important part of evaluating cellular immunity. The same sort of culture conditions can also be used to evaluate for cytokine production using the culture supernatant (alternatively, one can evaluate cytoplasmic cytokine expression using flow cytometry).[15]

Quantification of T-cell receptor excision circles (TRECs) and evaluation of the T-cell repertoire can be used for additional evaluation of immune status. TRECs are formed during the normal editing of

the T-cell receptor (TCR) genes during T-cell differentiation and maturation within the thymus and persist within the cell as extragenomic circular pieces of DNA. TREC copies are diluted over time as the T cells proliferate after antigen encounter. Therefore naive T cells that have recently emigrated from the thymus will present relatively high TREC levels compared with those of aged, antigen-experienced T cells.[16] TREC evaluation (also $CD4^+CD45RA^+CD31^+$ T cells by flow cytometry) can be used as a diagnostic confirmation of low thymic output that would be found in DiGeorge syndrome or to monitor immune reconstitution after bone marrow transplantation. More recently, the quantification of TRECs on blood derived from the Guthrie card obtained from infants after delivery has been initiated as a neonatal screening tool for SCID (and other significant T-cell defects) in both Wisconsin and Massachusetts.[17] The finding of low TREC levels in neonates should prompt immediate follow-up with immunophenotyping by means of flow cytometry. A recent report from Wisconsin suggests that this test has a very low rate of false-positive or inconclusive results (approximately 0.00009% and 0.0017%, respectively).[18]

Analysis of the T-cell repertoire can be useful in specific clinical situations. The T-cell repertoire in circulating T cells from healthy subjects includes expression of the majority of the 24 TCR Vβ chain families, which can be promptly assessed by flow cytometry.[19] Alternatively, evaluation of TCR Vβ CDR3 region diversity can be performed by PCR and is commonly referred to as spectratyping. The PCR-amplified product from each of these Vβ families normally demonstrates a Gaussian distribution of variously sized PCR products, each differing by 3 nucleotides. In settings in which there is an oligoclonal T-cell population, such as is found in patients with Omenn and atypical DiGeorge syndromes, a very limited number of Vβ families will be represented, with each demonstrating a very distorted (non-Gaussian) distribution.[19]

Evaluating Suspected Phagocyte Dysfunction Syndromes

The clinical features of neutrophil dysfunction (including neutropenia) usually include recurrent bacterial and fungal infections of the skin, lymph nodes, lung, liver, bone, and, in some cases, the periodontal tissue (Table I).[20] The clinical pattern of infection often can help to discriminate the underlying problem. Common phagocyte defects and accompanying laboratory findings are presented in Table VII. Patients with neutropenia and those with leukocyte adhesion deficiency (LAD) tend to have recurrent cellulitis, periodontal disease, otitis media, pneumonia, and rectal or gastrointestinal infections with diminished inflammation and lack of pus formation.[20] Although LAD is accompanied by a persistent granulocytosis, there is effectively a tissue neutropenia caused by the underlying adhesion defect that prevents the directed movement of these phagocytic cells to sites of infection. Delayed umbilical cord separation is commonly seen in patients with LAD; however, LAD is very rare, and most infants whose cords persist for up to 1 month are actually healthy. In patients with cyclic neutropenia, there are short periods of fever, mouth ulcers, and infections recurring at intervals of 18 to 21 days in concert with the decreased neutrophil count. Other more common instances of neutropenia include drug-induced and immune-mediated neutropenia.

Table VII
Differential diagnosis of phagocyte defects and associated laboratory findings

Chronic granulomatous disease: defective oxidative burst by means of DHR assay or NBT
Leukocyte adhesion defects
LAD1: low/absent CD18 and CD11 expression by means of flow cytometry; persistent leukocytosis
LAD2: Bombay phenotype; absent CD15 (Sialyl-Lewis X) expression
LAD3: mutation analysis only
Chediak-Higashi syndrome: giant lysosomal inclusion bodies observed on morphologic review of granulocytes (with partial albinism)
Griscelli syndrome type 2: neutropenia without inclusion bodies (with partial albinism)
Severe congenital neutropenia: persistent neutropenia; maturation arrest on bone marrow studies
Cyclic neutropenia: intermittent neutropenia requiring serial measurements
X-linked neutropenia: altered WASP expression by means of flow or mutation analysis
G6PD and MPO deficiency: abnormal functional enzymatic assay
Hyper-IgE syndrome: IgE level >2,000 IU/mL; low T_H17 cell numbers
Other disorders to be considered
Drug-induced neutropenia; autoimmune/alloimmune neutropenia; hypersplenism; chronic mucocutaneous candidiasis; TCII deficiency; hyper-IgM syndrome, XLA; Schwachman-Bodian-Diamond syndrome; warts, hypogammaglobulinemia, immunodeficiency and myelokathexis (WHIM) syndrome

NBT, Nitroblue tetrazolium; *WASP*, Wiskott-Aldrich syndrome protein; *G6PD*, glucose-6-phosphate dehydrogenase; *MPO*, myeloperoxidase; *XLA*, X-linked agammaglobulinemia.

In contrast, patients with chronic granulomatous disease have significant problems with liver and bone abscesses, as well as pneumonias with selected organisms, including *Staphylococcus aureus*, and *Nocardia* and *Aspergillus* species.[21] Furthermore, they tend to have a lower frequency of *Escherichia coli* and streptococcal species infections compared with patients with neutropenia or LAD.

Finally, patients with hyper-IgE syndrome present with recurrent skin abscesses and cavitary pneumonias caused by *S aureus* and other pyogenic bacteria and demonstrate chronic mucocutaneous candidiasis.[22] In addition, they typically demonstrate specific nonimmunologic findings, such as coarse facial features, scoliosis, hyperextensible joints, increased risk for bone fractures, and delayed or failed shedding of primary dentition.[23]

Laboratory evaluation

Screening studies directed at the evaluation of neutrophil function should start with a leukocyte count, differential, and morphologic review (Table VIII). The diagnosis of cyclic neutropenia requires multiple absolute neutrophil counts 2 to 3 times a week for at least 4 to 6 weeks.[24] A diagnosis of severe congenital neutropenia (Kostmann syndrome) is made with neutrophil counts of less than 0.5×10^9/L on several occasions.[24] Bone marrow analysis is useful to exclude insufficient production because of neoplasia or other causes and to document other abnormalities, such as the maturation arrest typical of Kostmann syndrome

If neutropenia and morphologic abnormalities are ruled out, the evaluation should be directed at assays that provide functional information about neutrophils. LAD workup involves flow cytometric assessment of the neutrophil adhesion molecules CD11 and CD18, the expression of which is absent or decreased on neutrophils (and other leukocytes) from patients with LAD1.[25] CD15 (Sialyl-Lewis X) expression is absent on neutrophils from patients with LAD2.[26]

Table VIII
Evaluation of suspected phagocyte defects

Absolute neutrophil count and morphologic analysis: congenital neutropenia syndromes and Chediak-Higashi syndrome
Oxidative burst by means of DHR or NBT assays: chronic granulomatous disease; rarely complete G6PD or MPO deficiency
CD18 (also CD11a, CD11b, and CD11c) expression by means of flow cytometry: LAD1
CD15 expression by means of flow cytometry: LAD2
Bombay phenotype: LAD2
Anti-neutrophil antibodies: autoimmune neutropenia
Bone marrow biopsy: exclude defective myeloid production in neutropenia syndromes
Chemotaxis/phagocytosis assays: limited utility

NBT, Nitroblue tetrazolium; *G6PD*, glucose-6-phosphate dehydrogenase; *MPO*, myeloperoxidase.

The neutrophil oxidative burst pathway can be screened with either the nitroblue tetrazolium tests or a flow cytometric assay (dihydrorhodamine 123 [DHR]), the results of both of which are abnormal in patients with chronic granulomatous disease, but the latter is a more sensitive test.[27]

The diagnosis of autosomal dominant and sporadic hyper-IgE syndrome has been associated with heterozygous pathogenic mutations in the gene encoding signal transducer and activator of transcription (STAT) 3.[28,29] A consistent feature in this disorder is a very increased IgElevel(>2,000IU/mL),andmore recently, low to absent IL-17–producing T cells (T_H17) have been demonstrated.[30]

Finally, evaluation of neutrophil-directed movement (chemotaxis) can be performed *in vivo* by using the Rebuck skin window technique, as well as *in vitro* with a Boyden chamber or a soft agar system. Abnormalities of chemotaxis have been observed after use of certain pharmacologic agents, as well as in patients with LAD, Chediak-Higashi syndrome, Pelger-Huet anomaly, and juvenile periodontitis. However, chemotactic tests are difficult to perform, very hard to standardize, and available in only a limited number of laboratories.

Evaluating Suspected Natural Killer and Cytotoxic T-Cell Defects

Deficiency in natural killer (NK) cell function has been described in a limited number of patients with recurrent herpes virus family infections. Another category of NK and cytotoxic T-lymphocyte defects

results in an uncontrolled inflammatory response initiated in association with certain specific infections that produces multiple organ damage (hemophagocytic lymphohistiocytosis [HLH]). One of these disorders is XLP, which is usually asymptomatic until the patient has an EBV infection and then leads to an uncontrolled lymphoproliferative disorder that is often fatal without aggressive treatment.[31] Importantly, approximately 30% of patients with XLP present with hypogammaglobulinemia without other symptoms. Bone marrow transplantation is the only long-term cure for XLP.[31]

The clinical manifestations of familial HLH are rather nonspecific, requiring a high suspicion index for early diagnosis.[32] They include persistent fever, hepatosplenomegaly, neurological symptoms (ataxia and seizures), lymphadenopathy, and skin rashes. Diagnosis mandates an immediate therapeutic response and prompt referral for bone marrow transplantation because this is currently the only curative approach. Disorders caused by defective intracellular vesicle trafficking, such as Chediak-Higashi syndrome and Griscelli syndrome type 2, also commonly manifest with a secondary lymphohistiocytic syndrome.[32]

Laboratory evaluation

Testing of NK cell function includes immunophenotyping NK cells by means of flow cytometry and assaying cytotoxicity with standard *in vitro* assays. Patients with XLP1 will demonstrate absent invariant-chain NK T cells in peripheral blood, as measured by $CD3^{+}V\alpha24^{+}V\beta11^{+}$ staining.[31] Additionally, intracellular flow cytometry can be used to evaluate for expression of SAP (SLAM-associated protein) and XIAP (X-linked inhibitor of apoptosis), the proteins defective in XLP1 and XLP2, respectively.[33,34] Absent protein would indicate disease, whereas normal expression could be the result of an abnormal protein that is not distinguished from the normal protein by means of antibody staining. Therefore this screening test would require further investigation directed at cell function when the protein is detected in a patient suspected of having XLP. HLH is commonly associated with cytopenias, including anemia and thrombocytopenia; increased liver function test results; hypofibrinogenemia; and hypertriglyceridemia.[32] High ferritin and circulating soluble CD25 levels are also typical and represent laboratory findings used to establish the diagnosis of HLH.[32] Low intracellular perforin expression, as determined by flow cytometry, can be used to diagnose HLH2, and decreased surface expression of CD107a (LAMP1, lysosomal-associated membrane protein 1) on NK cells after activation can predict the presence of mutations in MUNC13-4 and syntaxin 11.[35,36]

Evaluating Suspected Defects Involving The Adaptive-Innate Immunity Interface

IL-12/23–IFN-γ pathways

An emerging concept in the field of PIDs is that monogenic disorders can cause recurrent severe infections involving 1 or a very restricted range of pathogens.[37] Recently, patients with severe invasive infections caused by low virulence or environmental *Mycobacteria* and *Salmonella* species have been

found to harbor defects in genes encoding different components of the IL-12/23–IFN-γ pathway: the IFN-γ receptor 1 gene (*IFNGR1*), the IFN-γ receptor 2 gene (*IFNGR2*), the IL-12 receptor β1 gene (*IL12RB1*), *IL12B*, and *STAT1*.[38] The 2 most prevalent genetic defects among this group involve *IL12RB1* and *IFNGR1*, typically resulting in absent cell-surface protein expression.[39] This can be readily assessed by using flow cytometry with monoclonal reagents specific for these 2 proteins.[25] In addition, there is an autosomal dominant defect affecting *IFNGR1* that results in overexpression of the protein, and this also can be detected with flow cytometry.[40] Screening for other defects in IFN-γ signaling (abnormalities in *IFNGR2* or *STAT1*) can be done by evaluating monocyte STAT1 phosphorylation (by means of flow cytometry or Western blotting) *ex vivo* in response to IFN-γ.[41] Defects in IL-12 production can be tested by evaluating IL-12 production in response to *ex vivo* stimulation of mononuclear cells with LPS and IFN-γ.

Toll-like receptor and NF-κB signaling defects

Recently, recurrent infections involving *S pneumoniae* and *Staphylococcus* species have been associated with defects involving molecules of the Toll-like receptor (TLR) pathway, including IL-1 receptor–associated kinase 4 (IRAK4), MYD88 (myeloid differentiation primary response gene 88), and NEMO.[42-44] One of the distinctive features of patients with IRAK4 and MYD88 mutations is the markedly diminished inflammatory response to infection with little or no fever and acute-phase reactants observed.[45] NEMO deficiency is a more complex X-linked recessive disorder with a wide-ranging clinical phenotype and varied degree of immunologic abnormalities.[5] Finally, susceptibility to herpes simplex encephalitis has been linked to mutations in the genes encoding the receptor, TLR3, and an accessory protein of the TLR pathway, unc-93 homolog (UNC-93B).[46,47] Additional defects in TLR function associated with specific clinical phenotypes are likely to be identified and represent an evolving field in clinical immunology. Currently, the evaluation of TLR function is confined to a limited number of centers that usually screen response by stimulating mononuclear cells with various TLR-specific ligands and measuring cytokine production. This can then be followed by direct sequencing of the suspected mutant gene or genes involved in the specific TLR signaling process. Recently, von Bernuth et al[48] described a simplified assay for the screening of TLR function that is reported to detect functional defects in the signaling process by using whole blood samples. This assay involves stimulation of leukocytes with a series of specific TLR ligands and then evaluating for CD62L shedding from granulocytes by using flow cytometry. In cells with intact TLR signaling pathways, CD62L is promptly shed from the cell surface in contrast to the failure of CD62L shedding in cells from patients with IRAK4 or UNC-93B deficiency. One caveat is that the sample has to be analyzed shortly after obtaining the blood sample to prevent interpretation problems resulting from spontaneous CD62L shedding.

The identification of this new class of defects has also opened up potentially new therapeutic approaches, including the use of IFN-γ to augment antibiotics in selected patients with recurrent mycobacterial disease. In the case of herpes simplex encephalitis, the findings that patients with UNC-

93B and TLR-3 defects have diminished virally induced type 1 interferon production suggests that supplementation of conventional antiviral therapy with IFN-α could be beneficial in terms of decreasing morbidity, but this study has yet to be undertaken.[49]

Evaluating Suspected Complement Disorders

The clinical setting in which complement defects should be suspected depends on the site of the defect. Abnormalities in the early components of the classical complement pathway (C1, C4, and C2) typically manifest as systemic lupus erythematosus–like autoimmunity, but recurrent sinopulmonary infections are also seen, especially in C2 deficiency.[50] Defects in C3 produce a clinical phenotype that is indistinguishable from an antibody defect, although this complement deficiency is markedly less frequent than humoral immunodeficiencies.[51] Defects in the late components of complement producing defects in the generation of the membrane attack complex (C5-C9) present with increased susceptibility to infections with *Neisseria* species that might not manifest until adolescence or young adulthood.[51] Clinically, these patients manifest neisserial meningitis, sepsis, or gonococcal arthritis. Alternative complement pathway defects, including properdin, factor B and factor D deficiencies also present with severe neisserial and other bacterial infections. Factor H deficiency is associated with atypical (not associated with diarrhea) hemolytic uremic syndrome or glomerulonephritis and also with secondary C3 deficiency that can result in recurrent pyogenic infections.[51] Finally, C1 esterase inhibitor deficiency causes hereditary angioedema, whereas DAF (decay-accelerating factor) and CD59 defects are seen in patients with paroxysmal nocturnal hemoglobinuria.[51]

Laboratory evaluation

The best screening test for defects in the classical complement pathway is the total hemolytic complement activity (CH50) assay, whereas the AH50 assay screens for defects in the alternative complement pathway. Assuming correct handling of the serum sample (complement components are very labile), a classical complement component deficiency will result in virtual absence of hemolysis on CH50 testing in contrast to the markedly decreased but not absent results seen in diseases like systemic lupus erythematosus. A decreased AH50 test result suggests a deficiency in factor B, factor D, or properdin. A decrease in both CH50 and AH50 test results suggests deficiency in a shared complement component (from C3 to C9).

Selected component immunoassays are available in larger laboratories, whereas specific component functional testing is typically only available in a very limited number of specialized complement laboratories.

Evaluating Suspected Immune Dysregulation Disorders

Under this category are included monogenic autoimmune disorders, such as the autoimmune lymphoproliferative syndrome (ALPS); the immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX); and the autoimmune, polyendocrinopathy, candidiasis, ectodermal

dystrophy syndrome (APECED; Table IX). Patients with ALPS present early in life with persistent nonmalignant lymphadenopathy and splenomegaly commonly accompanied by immune thrombocytopenia, hemolytic anemia, or both.[52] Organ-specific or vasculitic-type autoimmunity is rarely seen in patients with ALPS. IPEX is an immunologic emergency and typically presents in the neonatal period with severe watery or bloody diarrhea, skin eczema, and type 1 diabetes.[53] An immediate diagnosis is mandatory because these children require aggressive immunosuppression to control the acute symptoms, and bone marrow transplantation is currently the only curative therapy that should be undertaken before islet cells are destroyed, if at all possible. Finally, APECED is characterized by endocrine organ–directed autoimmunity (adrenal insufficiency and hypothyroidism) and chronic mucocutaneous candidiasis.[54] Patients might also have type 1 diabetes, gonadal failure, pernicious anemia, autoimmune hepatitis, and cutaneous manifestations. This is usually not a life-threatening condition, and immunosuppression is usually not required, with specific therapy directed at the endocrine abnormalities.

Table IX
Main clinical and laboratory findings of immune dysregulation syndromes and causative genes

Disorder	Distinctive clinical findings	Key laboratory findings	Gene(s) involved
ALPS	Lymphadenopathy, splenomegaly, autoimmune hemolytic anemia and/or thrombocytopenia, high risk for lymphomas	↑ $CD3^+$αβ-TCR-$αβ^+$ $CD4^-CD8^-$ cells, hypergammaglobulinemia, Coomb positive, ↑ plasma IL-10 levels, ↑ serum vitamin B12 levels, ↑ soluble Fas ligand levels	*FAS*, *FASL*, *CASP8*, *CASP10*, *NRAS*
IPEX	Early-life enteritis, dermatitis, autoimmune endocrinopathy (usually type 1 diabetes)	↑ IgE levels, diminished $FoxP3^+$ CD4 T-cell subpopulation	*FOXP3*
APECED	Adrenal insufficiency, hypothyroidism, chronic mucocutaneous candidiasis	Organ-specific autoantibodies	*AIRE*

FASL, Fas ligand; *CASP8*, caspase 8; *CASP10*, caspase-10, *NRAS*, neuroblastoma *RAS* viral oncogene homolog; *FOXP3*, forkhead box protein 3; *AIRE*, autoimmune regulator.

Laboratory evaluation

The diagnosis of ALPS currently requires the presence of compatible clinical symptoms and the presence of a characteristic T-cell population on immunophenotyping that expresses CD3 and αβ-TCR but does not express CD4 or CD8 markers, which are referred to as double-negative T cells (Table IX). Because the majority of double-negative T cells in normal samples are γδ-TCR+ and do not contribute to the diagnosis of ALPS, antibodies to αβ-TCR are necessary for determining this subgroup of T cells. Every lab needs to set their own normal ranges for αβ double-negative T cells. An aberrant adult lymphocyte population is defined as having more than one percent of the total population, according to the National Institutes of Health. Hypergammaglobulinemia, elevated levels of IL-10 in the blood, elevated vitamin B12 levels, and soluble Fas ligand levels are further aspects that support this (J.B.O. and T.A.F., unpublished observations).55Further evidence of faulty lymphocyte

apoptosis in vitro or a mutation on FAS, FASL, CASP8, CASP10, or NRAS is required for a definitive diagnosis.[56-61]

Conclusion

If a patient has a history of uncommon illnesses, frequent infections, or both, the most important way to evaluate their immune function is through laboratory testing. Not only does well-directed immune function testing aid in diagnosis, but it also guides choices about the best course of treatment. The latter is of paramount importance in reducing illness-related mortality. Instead of using a scattershot approach, lab testing for immune system disorders should be targeted and organized according to the patient's medical history.

References:

1.Yong PF, Chee R, Grimbacher B. Hypogammaglobulinaemia. Immunol Allergy Clin North Am. 2008;28:691–713. doi: 10.1016/j.iac.2008.06.003. [DOI] [PubMed] [Google Scholar]
2.Conley ME, Dobbs AK, Farmer DM, Kilic S, Paris K, Grigoriadou S, et al. Primary B cell immunodeficiencies: comparisons and contrasts. Annu Rev Immunol. 2009;27:199–227. doi: 10.1146/annurev.immunol.021908.132649. [DOI] [PubMed] [Google Scholar]
3.Conley ME, Howard V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. J Pediatr. 2002;141:566–71. doi: 10.1067/mpd.2002.127711. [DOI] [PubMed] [Google Scholar]
4.Ersoy F, Berkel AI, Sanal O, Oktay H. Twenty-year follow-up of 160 patients with ataxia-telangiectasia. Turk J Pediatr. 1991;33:205–15. [PubMed] [Google Scholar]
5.Hanson EP, Monaco-Shawver L, Solt LA, Madge LA, Banerjee PP, May MJ, et al. Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. J Allergy Clin Immunol. 2008;122:1116–77. e1116. doi: 10.1016/j.jaci.2008.08.018. [DOI] [PMC free article] [PubMed] [Google Scholar]
6.Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science. 1993;259:990–3. doi: 10.1126/science.7679801. [DOI] [PubMed] [Google Scholar]
7.Ochs HD, Buckley RH, Kobayashi RH, Kobayashi AL, Sorensen RU, Douglas SD, et al. Antibody responses to bacteriophage phi X174 in patients with adenosine deaminase deficiency. Blood. 1992;80:1163–71. [PubMed] [Google Scholar]
8.Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008;111:77–85. doi: 10.1182/blood-2007-06-091744. [DOI] [PubMed] [Google Scholar]
9.Notarangelo LD, Giliani S, Mazza C, Mella P, Savoldi G, Rodriguez-Perez C, et al. Of genes and phenotypes: the immunological and molecular spectrum of combined immune deficiency. Defects of the gamma(c)-JAK3 signaling pathway as a model. Immunol Rev. 2000;178:39–48. doi: 10.1034/j.1600-065x.2000.17812.x. [DOI] [PubMed] [Google Scholar]
10.Villa A, Notarangelo LD, Roifman CM. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. J Allergy Clin Immunol. 2008;122:1082–6. doi: 10.1016/j.jaci.2008.09.037. [DOI] [PubMed] [Google Scholar]

11.Muller SM, Ege M, Pottharst A, Schulz AS, Schwarz K, Friedrich W. Transplacentally acquired maternal T lymphocytes in severe combined immunodeficiency: a study of 121 patients. Blood. 2001;98:1847–51. doi: 10.1182/blood.v98.6.1847. [DOI] [PubMed] [Google Scholar]
12.Fleisher TA, Oliveira JB. Functional and molecular evaluation of lymphocytes. J Allergy Clin Immunol. 2004;114:227–35. doi: 10.1016/j.jaci.2004.06.001. [DOI] [PubMed] [Google Scholar]
13.Sullivan KE. Chromosome 22q11.2 deletion syndrome: DiGeorge syndrome/velocardiofacial syndrome. Immunol Allergy Clin North Am. 2008;28:353–66. doi: 10.1016/j.iac.2008.01.003. [DOI] [PubMed] [Google Scholar]
14.Kawai S, Minegishi M, Ohashi Y, Sasahara Y, Kumaki S, Konno T, et al. Flow cytometric determination of intracytoplasmic Wiskott-Aldrich syndrome protein in peripheral blood lymphocyte subpopulations. J Immunol Methods. 2002;260:195–205. doi: 10.1016/s0022-1759(01)00549-x. [DOI] [PubMed] [Google Scholar]
15.Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. J Immunol Methods. 1995;188:117–28. doi: 10.1016/0022-1759(95)00209-x. [DOI] [PubMed] [Google Scholar]
16.Douek, et al. Changes in thymic function with age and during the treament of HIV infection. Nature. 1998;396:690–5. doi: 10.1038/25374. [DOI] [PubMed] [Google Scholar]
17.Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. J Allergy Clin Immunol. 2005;115:391–8. doi: 10.1016/j.jaci.2004.10.012. [DOI] [PubMed] [Google Scholar]
18.Routes JM, Grossman WJ, Kumar A, Verbsky J, Brokopp C, Laessig RH, et al. Update of statewide newborn screening program for severe combined immunodeficiency (SCID) by T-cell receptor excision circles (TRECs) J Allergy Clin Immunol. 2009;123(suppl):S68. [Google Scholar]
19.Pilch H, Hohn H, Freitag K, Neukirch C, Necker A, Haddad P, et al. Improved assessment of T-cell receptor (TCR) VB repertoire in clinical specimens: combination of TCR-CDR3 spectratyping with flow cytometry-based TCR VB frequency analysis. Clin Diagn Lab Immunol. 2002;9:257–66. doi: 10.1128/CDLI.9.2.257-266.2002. [DOI] [PMC free article] [PubMed] [Google Scholar]
20.Rosenzweig SD, Holland SM. Phagocyte immunodeficiencies and their infections. J Allergy Clin Immunol. 2004;113:620–6. doi: 10.1016/j.jaci.2004.02.001. [DOI] [PubMed] [Google Scholar]
21.Martire B, Rondelli R, Soresina A, Pignata C, Broccoletti T, Finocchi A, et al. Clinical features, long-term follow-up and outcome of a large cohort of patients with chronic granulomatous disease: an Italian multicenter study. Clin Immunol. 2008;126:155–64. doi: 10.1016/j.clim.2007.09.008. [DOI] [PubMed] [Google Scholar]
22.Ozcan E, Notarangelo LD, Geha RS. Primary immune deficiencies with aberrant IgE production. J Allergy Clin Immunol. 2008;122:1054–63. doi: 10.1016/j.jaci.2008.10.023. [DOI] [PubMed] [Google Scholar]
23.Grimbacher B, Holland SM, Puck JM. Hyper-IgE syndromes. Immunol Rev. 2005;203:244–50. doi: 10.1111/j.0105-2896.2005.00228.x. [DOI] [PubMed] [Google Scholar]
24.Boztug K, Welte K, Zeidler C, Klein C. Congenital neutropenia syndromes. Immunol Allergy Clin North Am. 2008;28:259–75. vii–viii. doi: 10.1016/j.iac.2008.01.007. [DOI] [PubMed] [Google Scholar]
25.Oliveira JB, Notarangelo LD, Fleisher TA. Applications offlowcytometry for the study of primary immune deficiencies. Curr Opin Allergy Clin Immunol. 2008;8:499–509. doi: 10.1097/ACI.0b013e328312c790. [DOI] [PubMed] [Google Scholar]
26.Etzioni A. Leukocyte adhesion deficiencies: molecular basis, clinical findings, and therapeutic options. Adv Exp Med Biol. 2007;601:51–60. doi: 10.1007/978-0-387-72005-0_5. [DOI] [PubMed] [Google Scholar]
27.Jirapongsananuruk O, Malech HL, Kuhns DB, Niemela JE, Brown MR, Anderson-Cohen M, et al. Diagnostic paradigm for evaluation of male patients with chronic granulomatous disease, based on the

dihydrorhodamine 123 assay. J Allergy Clin Immunol. 2003;111:374–9. doi: 10.1067/mai.2003.58. [DOI] [PubMed] [Google Scholar]
28.Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med. 2007;357:1608–19. doi: 10.1056/NEJMoa073687. [DOI] [PubMed] [Google Scholar]
29.Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature. 2007;448:1058–62. doi: 10.1038/nature06096. [DOI] [PubMed] [Google Scholar]
30.Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, et al. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature. 2008;452:773–6. doi: 10.1038/nature06764. [DOI] [PMC free article] [PubMed] [Google Scholar]
31.Nichols KE, Ma CS, Cannons JL, Schwartzberg PL, Tangye SG. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. Immunol Rev. 2005;203:180–99. doi: 10.1111/j.0105-2896.2005.00230.x. [DOI] [PubMed] [Google Scholar]
32.Filipovich AH. Hemophagocytic lymphohistiocytosis and other hemophagocytic disorders. Immunol Allergy Clin North Am. 2008;28:293–313. viii. doi: 10.1016/j.iac.2008.01.010. [DOI] [PubMed] [Google Scholar]
33.Marsh RA, Villanueva J, Zhang K, Snow AL, Su HC, Madden L, et al. A rapid flow cytometric screening test for X-linked lymphoproliferative disease due to XIAP deficiency. Cytometry B Clin Cytom. 2009;76:334–44. doi: 10.1002/cyto.b.20473. [DOI] [PMC free article] [PubMed] [Google Scholar]
34.Shinozaki K, Kanegane H, Matsukura H, Sumazaki R, Tsuchida M, Makita M, et al. Activation-dependent T cell expression of the X-linked lymphoproliferative disease gene product SLAM-associated protein and its assessment for patient detection. Int Immunol. 2002;14:1215–23. doi: 10.1093/intimm/dxf084. [DOI] [PubMed] [Google Scholar]
35.Marcenaro S, Gallo F, Martini S, Santoro A, Griffiths GM, Arico M, et al. Analysis of natural killer-cell function in familial hemophagocytic lymphohistiocytosis (FHL): defective CD107a surface expression heralds Munc13-4 defect and discriminates between genetic subtypes of the disease. Blood. 2006;108:2316–23. doi: 10.1182/blood-2006-04-015693. [DOI] [PubMed] [Google Scholar]
36.Kogawa K, Lee SM, Villanueva J, Marmer D, Sumegi J, Filipovich AH. Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members. Blood. 2002;99:61–6. doi: 10.1182/blood.v99.1.61. [DOI] [PubMed] [Google Scholar]
37.Casanova JL, Fieschi C, Zhang SY, Abel L. Revisiting human primary immunodeficiencies. J Intern Med. 2008;264:115–27. doi: 10.1111/j.1365-2796.2008.01971.x. [DOI] [PubMed] [Google Scholar]
38.Al-Muhsen S, Casanova JL. The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. J Allergy Clin Immunol. 2008;122:1043–52. doi: 10.1016/j.jaci.2008.10.037. [DOI] [PubMed] [Google Scholar]
39.Filipe-Santos O, Bustamante J, Chapgier A, Vogt G, de Beaucoudrey L, Feinberg J, et al. Inborn errors of IL-12/23- and IFN-gamma-mediated immunity: molecular, cellular, and clinical features. Semin Immunol. 2006;18:347–61. doi: 10.1016/j.smim.2006.07.010. [DOI] [PubMed] [Google Scholar]
40.Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondaneche MC, Dupuis S, et al. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nat Genet. 1999;21:370–8. doi: 10.1038/7701. [DOI] [PubMed] [Google Scholar]
41.Fleisher TA, Dorman SE, Anderson JA, Vail M, Brown MR, Holland SM. Detection of intracellular phosphorylated STAT-1 by flowcytometry. Clin Immunol. 1999;90:425–30. doi: 10.1006/clim.1998.4654. [DOI] [PubMed] [Google Scholar]

42.von Bernuth H, Picard C, Jin Z, Pankla R, Xiao H, Ku CL, et al. Pyogenic bacterial infections in humans with MyD88 deficiency. Science. 2008;321:691–6. doi: 10.1126/science.1158298. [DOI] [PMC free article] [PubMed] [Google Scholar]
43.Picard C, von Bernuth H, Ku CL, Yang K, Puel A, Casanova JL. Inherited human IRAK-4 deficiency: an update. Immunol Res. 2007;38:347–52. doi: 10.1007/s12026-007-0006-2. [DOI] [PubMed] [Google Scholar]
44.Doffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A, et al. Xlinked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired eNF-kappaB signaling. Nat Genet. 2001;27:277–85. doi: 10.1038/85837. [DOI] [PubMed] [Google Scholar]
45.von Bernuth H, Puel A, Ku CL, Yang K, Bustamante J, Chang HH, et al. Septicemia without sepsis: inherited disorders of nuclear factor-kappa B-mediated inflammation. Clin Infect Dis. 2005;41(suppl 7):S436–9. doi: 10.1086/431994. [DOI] [PubMed] [Google Scholar]
46.Zhang SY, Jouanguy E, Ugolini S, Smahi A, Elain G, Romero P, et al. TLR3 deficiency in patients with herpes simplex encephalitis. Science. 2007;317:1522–7. doi: 10.1126/science.1139522. [DOI] [PubMed] [Google Scholar]
47.Casrouge A, Zhang SY, Eidenschenk C, Jouanguy E, Puel A, Yang K, et al. Herpes simplex virus encephalitis in human UNC-93B deficiency. Science. 2006;314:308–12. doi: 10.1126/science.1128346. [DOI] [PubMed] [Google Scholar]
48.von Bernuth H, Ku CL, Rodriguez-Gallego C, Zhang S, Garty BZ, Marodi L, et al. A fast procedure for the detection of defects in Toll-like receptor signaling. Pediatrics. 2006;118:2498–503. doi: 10.1542/peds.2006-1845. [DOI] [PubMed] [Google Scholar]
49.Sancho-Shimizu V, Zhang SY, Abel L, Tardieu M, Rozenberg F, Jouanguy E, et al. Genetic susceptibility to herpes simplex virus 1 encephalitis in mice and humans. Curr Opin Allergy Clin Immunol. 2007;7:495–505. doi: 10.1097/ACI.0b013e3282f151d2. [DOI] [PubMed] [Google Scholar]
50.Truedsson L, Bengtsson AA, Sturfelt G. Complement deficiencies and systemic lupus erythematosus. Autoimmunity. 2007;40:560–6. doi: 10.1080/08916930701510673. [DOI] [PubMed] [Google Scholar]
51.Botto M, Kirschfink M, Macor P, Pickering MC, Wurzner R, Tedesco F. Complement in human diseases: Lessons from complement deficiencies. Mol Immunol. 2009;46:2774–83. doi: 10.1016/j.molimm.2009.04.029. [DOI] [PubMed] [Google Scholar]
52.Oliveira JB, Fleisher T. Autoimmune lymphoproliferative syndrome. Curr Opin Allergy Clin Immunol. 2004;4:497–503. doi: 10.1097/00130832-200412000-00005. [DOI] [PubMed] [Google Scholar]
53.Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: forkhead box protein 3 mutations and lack of regulatory T cells. J Allergy Clin Immunol. 2007;120:744–51. doi: 10.1016/j.jaci.2007.08.044. [DOI] [PubMed] [Google Scholar]
54.Moraes-Vasconcelos D, Costa-Carvalho BT, Torgerson TR, Ochs HD. Primary immune deficiency disorders presenting as autoimmune diseases: IPEX and APECED. J Clin Immunol. 2008;28(suppl 1):S11–9. doi: 10.1007/s10875-008-9176-5. [DOI] [PubMed] [Google Scholar]
55.Magerus-Chatinet A, et al. FAS-L, IL-10, and double-negative CD4-CD8-TCR alpha/beta= T cells are reliable markers of autoimmune lymphoproliferative syndrome (ALPS) associated with FAS loss of function. Blood. 2009;113:3027–30. doi: 10.1182/blood-2008-09-179630. [DOI] [PubMed] [Google Scholar]
56.Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. cell. 1995;81:935–46. doi: 10.1016/0092-8674(95)90013-6. [DOI] [PubMed] [Google Scholar]
57.Bi LL, Pan G, Atkinson TP, Zheng L, Dale JK, Makris C, et al. Dominant inhibition of Fas ligand-mediated apoptosis due to a heterozygous mutation associated with autoimmune lymphoproliferative syndrome (ALPS)

Type Ib. BMC Med Genet. 2007;8:41. doi: 10.1186/1471-2350-8-41. [DOI] [PMC free article] [PubMed] [Google Scholar]
58.Chun HJ, Zheng L, Ahmad M, Wang J, Speirs CK, Siegel RM, et al. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. Nature. 2002;419:395–9. doi: 10.1038/nature01063. [DOI] [PubMed] [Google Scholar]
59.Rieux-Laucat F, Le Deist F, Hivroz C, Roberts IA, Debatin KM, Fischer A, et al. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. Science. 1995;268:1347–9. doi: 10.1126/science.7539157. [DOI] [PubMed] [Google Scholar]
60.Holzelova E, Vonarbourg C, Stolzenberg MC, Arkwright PD, Selz F, Prieur AM, et al. Autoimmune lymphoproliferative syndrome with somatic Fas mutations. N Engl J Med. 2004;351:1409–18. doi: 10.1056/NEJMoa040036. [DOI] [PubMed] [Google Scholar]
61.Wang J, Zheng L, Lobito A, Chan FK, Dale J, Sneller M, et al. Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. Cell. 1999;98:47–58. doi: 10.1016/S0092-8674(00)80605-4. [DOI] [PubMed] [Google Scholar]