



CME Review

Primary immunodeficiency disorders: general classification, new molecular insights, and practical approach to diagnosis and treatment



Hans D. Ochs, MD; and David Hagin, MD, PhD

Seattle Children's Research Institute, University of Washington, Seattle, Washington

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At the conclusion of this activity, participants should be able to:

- Describe a classification of PIDDs on the basis of clinical phenotype, immunologic characteristics and molecular defect.
- Discuss recently discovered PIDDs and the complexity of translating a clinical phenotype into a molecular diagnosis.

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Introduction

The field of primary immunodeficiency diseases (PIDDs), formally born in the early 1950s with the discovery of agammaglobulinemia,¹ has progressed exponentially and undergone a revolution with the discovery of new genetic disorders and

Reprints: Hans D. Ochs, MD, Department of Pediatrics, Seattle Children's Research Institute, University of Washington, 1900 Ninth Ave, Seattle, WA 98101; E-mail: allgau@u.washington.edu.

recognition of novel molecular and genetic principles.² This explosive growth was made possible by new technologies for next-generation sequencing and the development of highly specific reagents, allowing flow-based identification of membrane, cytoplasmic, or nuclear proteins and functional assessment of signaling pathways. Perhaps more important is the concept that the term *PIDDs* is no longer reserved to describe severe and frequently lethal genetic diseases characterized by general susceptibility to infections but is extended to include susceptibility to single microorganisms, autoimmunity, immune dysregulation, allergic diseases, autoinflammatory disorders, and malignant tumors.³ Although these advances in diagnosis and treatment directly benefit our patients and provide new insights into how the immune system works, they pose new challenges to the clinician when confronted with atypical clinical phenotypes, new signaling pathways, and complex genetic mechanisms. In this review, we focus on the updated classification of *PIDDs*, discuss recently discovered entities, explore the association of selected *PIDDs* with atopy features or immune dysregulation, and, finally, provide guidelines for how to approach patients suspected of having *PIDDs*.

Classification of *PIDDs*

The International Union of Immunological Societies (IUIS) Expert Committee for Primary Immunodeficiency generates and updates biannually a detailed classification of molecularly defined *PIDDs*.⁴ The IUIS document provides a brief description of the clinical and immunologic features, the genetic defect, and the type of inheritance. The committee has created tables that group entities based on similar clinical phenotypes with the understanding that a specific genotype may lead to phenotypes that fit more than one category. As an example, CD40 ligand (CD40L) deficiency is classified as both antibody deficiency and combined immunodeficiency. Despite these limitations, the classification offers a common language for describing *PIDDs*, which are divided into the following groups: (1) combined immunodeficiencies; (2) combined immunodeficiencies with associated or syndromic features; (3) predominantly antibody deficiencies; (4) diseases of immune dysregulation; (5) congenital defects of phagocyte number, function, or both; (6) defects that involve innate immunity; (7) autoinflammatory disorders; (8) complement deficiencies; and (9) phenocopies of *PIDDs*.

Combined immunodeficiencies include 12 molecularly defined severe combined immunodeficiencies (SCIDs) and combined immunodeficiencies with less profound defects. This group consists of genetic defects that involve signaling pathways, such as mutations in the common γ chain, abnormal DNA repair mechanisms that are crucial for V(D)J recombination, and the development of T and B cells with a sufficiently wide repertoire, exemplified by RAG1/2 deficiencies, or result in a metabolic defect that leads to lymphocyte apoptosis (adenosine deaminase deficiency).

The *PIDDs* included in the combined immunodeficiencies with associated or syndromic features group have other characteristic syndromic features that might bring these patients to medical attention before the manifestation of immune defects. This group includes Wiskott-Aldrich syndrome, which is characterized by small platelets, congenital thrombocytopenia, eczema, and bloody diarrhea; DNA repair defects, such as ataxia telangiectasia and Nijmegen breakage syndrome; thymic defects, such as DiGeorge syndrome; and various hyper-IgE syndromes (HIEs) with characteristic clinical findings and unique gene defects. Most of these disorders have some degree of combined immunodeficiency.

The predominantly antibody deficiencies group can be divided into several subcategories. The first category is those with severe reduction in all serum immunoglobulin isotypes associated with a profound decrease in B-cell counts, with the prototype being X-linked agammaglobulinemia due to mutations in the *BTX* gene and

autosomal recessive agammaglobulinemia caused by at least 6 genetic defects. The second type is antibody deficiencies with severe reduction in at least 2 immunoglobulin isotypes, with the most common being common variable immunodeficiency (CVID) due to unknown genetic defect and CVID-like syndromes with a dozen well-defined genetic defects. The third type is antibody deficiencies with impaired class switch recombination due to abnormal signaling (CD40L or CD40 deficiency) or impaired machinery for isotype switching and somatic hypermutation (activation-induced cytidine deaminase and uracil–DNA glycosylase deficiency). CD40L deficiency, characterized by low IgG and IgA with normal or elevated IgM levels, is the most common single-gene defect in this category but in fact is a defect of T-cell activation.

The diseases of immune dysregulation include a wide spectrum of disorders. Familial hemophagocytic lymphohistiocytosis without hypopigmentation may be caused by mutations in perforin, UNC13D, Syntaxin11, and Munc18-2 or familial hemophagocytic lymphohistiocytosis with hypopigmentation (eg, Chediak-Higashi, Griscelli, and Hermansky-Pudlak syndromes), all resulting in decreased natural killer and cytotoxic T-cell activity and leading to enhanced lymphocyte proliferation in response to viral and other triggers. Impaired apoptotic signals lead to accumulation of autoaggressive T and B lymphocytes, resulting in various autoimmune lymphoproliferative syndromes. Genetic defects lead to abnormal regulatory T-cell development and/or function. The prototype disease in this category is immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) with mutations in the *FOXP3* master regulator gene, a transcription factor required for the generation of regulatory T cells. Other genetic defects result in impaired T-regulatory cell function (CD25 or STAT5b deficiency) or impaired ability to respond to regulatory signals, as in the case of interleukin (IL) 10 and IL-10 receptor deficiencies (leading to immune dysregulation with devastating early-onset colitis⁵), and STAT1 gain-of-function mutations, leading to an IPEX-like presentation.⁶

The congenital defects of phagocyte number, function, or both group includes the following entities. The first entity is genetic defects that lead to abnormal myeloid differentiation, causing severe congenital neutropenia (the prototype being Kostmann disease caused by mutations in *HAX1*), or cyclic neutropenia due to mutation in the neutrophil-expressed elastase (*ELANE*), occasionally resulting in overt bone marrow failure. The second entity is defects that lead to abnormal neutrophil motility due to impaired adherence to or abnormal rolling along the vascular endothelium. These leukocyte adhesion defects share common features of leukocytosis, periodontitis, and poor wound healing. This important category includes the third entity, which is defects in the respiratory oxidative burst, leading to X-linked or autosomal recessive chronic granulomatous disease. The fourth entity is defects in macrophages and lymphocyte signaling pathways (interferon γ and IL-12), which are responsible for a unique susceptibility to nontuberculous mycobacteria and salmonella (mendelian susceptibility to mycobacterial diseases).^{7–10}

Unlike the adaptive immune system, innate immune elements do not require genetic recombination events or a developmental phase to mediate function. The innate immune system is hardwired to recognize and respond to danger and serves as the initial immunologic defense against foreign and dangerous material.¹¹ Syndromes included in the category of innate immunity defects are caused by defects in pathways that involve or are downstream of germline encoded receptors (ie, Toll-like receptors [TLRs]). Nevertheless, these defects may affect specific lymphocyte functions but often affect one or more cell types other than lymphocytes. Examples are X-linked anhydrotic ectodermal dysplasia due to mutations in NEMO and other TLR pathway defects, including IRAK4 and MyD88 deficiencies. Although defects in adaptive immunity usually result in susceptibility to a wide range of pathogens,

defects that involve genes required for innate pathways are often restricted to few and sometimes a single microorganism(s).¹² Although mutations in IRAK4 or MyD88 cause invasive pyogenic infections, defects in TLR3 signaling interferes with the generation of interferon α, β and renders the central nervous system susceptible to herpes simplex virus 1. Molecular defects within the IL-17 pathway or in CARD9 lead to chronic mucocutaneous candidiasis (IL-17 pathway) or invasive *Candida* infection (CARD9). A unique susceptibility to human papillomavirus infections is associated with gain-of-function mutation in CXCR4 (WHIM [warts, hypogammaglobulinemia, infections, and myelokathexis] syndrome) and with mutations in *EVER1* or *EVER2* (*Epidermodyplasia verruciformis*).

Autoinflammatory disorders are associated with recurrent fevers and can be categorized into defects that affect the inflammasome and non-inflammasome-related conditions. Defects that affect the inflammasome lead to dysregulated maturation and overproduction of IL-1 β , which activates inflammatory processes; examples are familial Mediterranean fever, hyper-IgD syndrome, familial cold autoinflammatory syndrome, Muckle-Wells syndrome, and neonatal onset multisystem inflammatory disease. Non-inflammasome-related conditions consist of a heterogeneous group of diseases that share a common mechanism of cytokine hypersecretion or unopposed inflammatory cytokine production; examples are deficiency of IL-1 receptor antagonist and tumor necrosis factor receptor-associated periodic syndrome.

Complement deficiencies include defects within the classic, alternative, and complement regulatory pathways. The resulting clinical phenotypes are broad and include susceptibility to neisserial and other encapsulated bacteria (early and late complement components, properdin, and factors H and I of the alternative pathway), autoimmune diseases (early complement components), hereditary angioedema (C1 esterase inhibitor), and atypical hemolytic uremic syndrome (factors B, F, and I).

Phenocopies of PIDDs is a new category that was first presented in the IUIS 2014 update. This category includes conditions that resemble PIDDs and are not due to germline mutations. A typical example would be autoantibodies against IL-17 or IL-22 in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome, which is similar to IL-17 pathway defects and leads to chronic mucocutaneous candidiasis.¹³ Another example is somatic mutations in *FAS*, leading to an autoimmune lymphoproliferative syndrome-like syndrome.

Although the IUIS classification lists more than 250 known PIDDs, it is less useful for practitioners treating a patient suspected of having a PIDD. To correct this handicap, the IUIS committee has recently compiled a phenotypic approach to help non-PIDD specialists navigate their way through this complex classification and to offer a more user-friendly flowchart guide, which is designed to provide a roadmap for initial workup and targeted investigation.¹⁴

New PIDDs

With increased interest in the field, advances in next-generation sequencing, and improved functional assays, new genes associated with PIDDs are discovered at increasing velocity. A recent publication by Parvaneh et al¹⁵ summarizes 19 novel single-gene defects that were described within 1 year of the last IUIS report published in 2011. The following selection of newly described PIDDs was made to provide new concepts and ideas.

The *CARD11* gene mutation results in agammaglobulinemia and a profound defective T-cell function despite normal T- and B-cell counts. *CARD11* is a scaffold protein required for T-cell receptor (TCR)- and B-cell receptor (BCR)-induced canonical nuclear factor- κ B (NF- κ B) activation, resulting in lymphocyte activation and proliferation.¹⁶ Because *CARD11* is specifically expressed in

hematopoietic cells, defects in *CARD11* are expected to result in profound immunologic defects. A heterozygous gain-of-function mutation in *CARD11* was described in 2012 in patients with polyclonal B-cell lymphocytosis and abnormal responses to polysaccharide-based vaccines.¹⁷ One year later, a profound combined immune defect was observed in 2 unrelated patients born to consanguineous parents of Palestinian and Central European ancestry; both had homozygous mutations in *CARD11* (a nonsense mutation in one and a deletion in the other), disrupting the canonical NF- κ B pathway. The index cases of both families presented at the age of 6 months with *Pneumocystis jirovecii* pneumonia and panhypogammaglobulinemia in the presence of normal B-cell counts, although most B cells were naive or transitional with absent mature B cells. One patient demonstrated a near-normal T-cell compartment, whereas the other patient had abnormal proliferative responses to mitogens and a predominantly naive T-cell phenotype despite normal absolute T-cell counts.^{18,19} Differences in presentation and immune status of the 2 *CARD11* mutations highlight the complexity in recognizing new PIDDs because different mutations in the same gene may result in completely different clinical and immune phenotypes.

Activated PI3K- δ syndrome includes respiratory infections, lymphadenopathy, infections caused by herpes group viruses, and variable antibody deficiency with abnormal B- and T-cell phenotyping. One of the most interesting with the potential to be one of the more common PIDDs is the recently described gain-of-function mutation in the catalytic p110 δ subunit of phosphoinositide 3-kinase δ (PI3K δ), a heterodimer lipid kinase that phosphorylates phosphatidylinositol 4,5-bisphosphate to generate phosphatidylinositol 3,4,5-triphosphate, an important second messenger molecule. PI3K δ is expressed predominantly in hematopoietic lineage cells and receives signals via TCR, BCR, TLRs, costimulatory molecules, and cytokine receptors in T, B, and myeloid cells.²⁰ Therefore, PI3K δ is at the checkpoint of many immune signaling pathways. Two groups simultaneously described a gain-of-function mutation, each in a relatively large cohort of patients from multiple unrelated families. In the first study,²¹ whole exome sequencing performed in 35 patients with a positive family history of susceptibility to infections (CVID-like) identified 4 patients from 2 families with the same heterozygous mutation resulting in amino acid substitution (E1021K) in the PI3K δ . Subsequent Sanger sequencing found 4 additional affected family members. DNA samples from a second cohort of 134 PIDD patients were studied similarly, identifying 5 more patients from 3 unrelated families carrying the same heterozygous *PIK3CD* mutation. Because one of these patients was previously diagnosed as having hyper-IgM syndrome (HIES), 15 patients with hyper-IgM syndrome from 13 families without a molecular diagnosis were evaluated, and 3 patients from 2 unrelated families were also found to have the same mutation, suggesting a high incidence of this mutation among PIDD patients. The second study performed a more targeted screening of patients with early-onset sinopulmonary infections, bronchiectasis, lymphoproliferation, cytomegalovirus or Epstein-Barr virus viremia, and nodular lymphoid hyperplasia of mucosal surfaces.²² Fourteen patients from 7 unrelated families were found to have heterozygous gain-of-function mutations in the *PIK3CD* gene. Although there was a significant variability in the severity of disease, even among patients who shared the same mutation, several common features were observed: recurrent sinopulmonary infections and bronchiectasis; lymphadenopathy and splenomegaly; variable antibody levels, often elevated IgM and low IgG2 levels and abnormal responses to polysaccharide vaccines; abnormal B-cell phenotyping with decreased memory B cells and increased transitional B cells; and abnormal T-cell phenotyping with decreased CD4 cell counts and increased terminally differentiated CD8 T-cell counts. These features often present in a subset of CVID patients. The authors of

both articles suggest the use of either specific p110 δ inhibitors or mTOR inhibitors because at least some of the observed effects were demonstrated to be secondary to highly activated PI3K δ and to uncontrolled cell activation and activation-induced cell death.

IL-21 receptor deficiency leads to susceptibility to *Cryptosporidium* infection and chronic cholangitis. Activation of IL-21 receptor induces signaling mediated by phosphorylation of the transcription factors STAT1, STAT3, and STAT5. Two distinct homozygous loss-of-function mutations in the *IL-21R* gene were found in 2 sets of siblings born to 2 unrelated families of consanguineous parents. All patients presented with cholangitis and liver disease associated with chronic cryptosporidial infection and a history of recurrent respiratory infections. T and B cells were variably dysfunctional, and patient cells had decreased STAT1, STAT3, and STAT5 phosphorylation in response to IL-21 stimulation with normal response to other cytokines.²³

Genetic defects that interfere with normal expression of and signaling via the pre-BCR typically result in the near absence of circulating mature B cells, agammaglobulinemia, and accumulation of pre-B cells in the bone marrow.²⁴ Recently, a new genetic defect associated with agammaglobulinemia was discovered in 4 unrelated patients with a family history negative for immunodeficiency. All 4 patients shared a similar phenotype that was different from that usually observed in agammaglobulinemia, with increased CD19 expression and absence of BCR in the few detectable circulating B cells. Bone marrow analysis revealed a differentiation block at the common lymphoid precursor stage, which is earlier than that seen in other known agammaglobulinemia syndromes.²⁵ Interestingly, all 4 patients had the same autosomal dominant de novo germline mutation with dominant negative effect in the *TCF3* gene, resulting in altered ability of the transcription factor E47 to bind to target DNA sites. It is assumed that E47 plays a critical role in normal B-cell development and blocks the development of pro-B cells that lack a pre-BCR. In the absence of E47, nonfunctional BCR-negative B cells develop.²⁶

Deficiency of protein kinase C δ (PRKCD) associated with a CVID-like phenotype and severe autoimmunity. CVID, a heterogeneous group of disorders with various clinical presentations and biomarkers, have been classified into 4 phenotypes: uncomplicated CVID (infections only), CVID associated with cytopenias, CVID complicated by polyclonal lymphoproliferation, and CVID with severe enteropathy.²⁷ Although several genetic defects have been described (including those with mutations in ICOS, CD19, CD81, CD20, CD21, TACI, BAFF-R, and more), most cases have no known genetic defect. It is likely, however, that new genetic defects will be discovered in cases in which CVID is associated with a unique phenotype. PRKCD, a member of the protein kinase C family, regulates cell survival, proliferation, and apoptosis and was found to play a unique negative regulatory role in B cells. Interestingly, *Prkcd*^{-/-} mice develop B-cell proliferation, lymphadenopathy, and autoantibodies.²⁸ This regulatory effect is assumed to be mediated by suppression of IL-6 production. The group of Boztug reported a homozygous *PRKCD* mutation in a patient born to consanguineous parents of Turkish origin who presented with B-cell deficiency and severe autoimmunity.²⁹ The patient had recurrent infections that started during the first year of life and developed early-onset immune dysregulation with membranous glomerulonephritis, lymphadenopathy and splenomegaly, relapsing polychondritis, and antiphospholipid syndrome. Immune workup revealed low IgG levels with elevated IgA and IgM. B-cell phenotyping revealed a progressive decrease in CD19⁺ B cells, decreased memory B cells, and increased number of CD21^{low} B cells known to be associated with autoimmunity. The heterozygous father was diagnosed as having Behçet disease and autoimmune thyroiditis. Interestingly, the patient and his father were found to have a heterozygous variant in *CTLA4* reported to be associated with autoimmune

thyroiditis. This genetic defect offers new insight into B-cell homeostasis and autoimmunity. The discovery of a second variant in a different gene contributes to the complexity of interpreting the results of whole-exome sequencing as a diagnostic approach.

Mutations in the *NFKB2* gene of the noncanonical NF- κ B pathway can present as CVID, autoimmunity and adrenal insufficiency. Although the canonical NF- κ B pathway uses numerous ligands and is involved in a broad range of immune functions, the noncanonical (NF- κ B2) pathway is more narrow and activated specifically by lymphotoxin β , BAFF, CD40L, and RANKL. Defects that affect the BAFF receptor and the CD40L-CD40 axis are well known to cause B-cell abnormalities, making the downstream transcription factor, NF- κ B2, a candidate gene for antibody deficiency, even though NF- κ B2 expression is not limited to B lymphocytes. Whole genome sequencing of DNA from members of a family with an autosomal dominant form of early-onset CVID, features of autoimmunity and immune dysregulation (alopecia totalis, autoantibodies, and asthma), and central adrenal insufficiency revealed that all affected individuals were heterozygous for a frameshift deletion in *NFKB2*. Following this finding, an additional 33 unrelated CVID patients were tested for variants in the *NFKB2* gene. One person with a heterozygous nonsense mutation was identified, who also had early-onset infections, autoimmunity, and central adrenal insufficiency.³⁰ This comprehensive study demonstrates how a detailed history and specific attention to additional nonimmune conditions make it possible to cluster patients with similar phenotypes and to identify new molecularly defined PIDDs.

Mutation in *VPS45* results in congenital neutropenia and neutrophil defect syndrome. The ability to fuse membrane compartments is required for normal neutrophil functions, including secretion of proteins stored in vesicles and fusion of phagosomes and lysosomes to form phagolysosomes. More than 40 *VPS* genes have been identified so far and are assumed to play a role in vesicle-mediated protein trafficking. Two recent reports identified homozygous mutations in one member of this group of proteins (*VPS45*) in 10 children from 7 unrelated families. Affected patients presented with congenital neutropenia, neutrophil dysfunction and accelerated apoptosis, lack of response to granulocyte colony-stimulating factor, life-threatening bacterial and fungal infections, myelofibrosis, and nephromegaly secondary to extramedullary hematopoiesis.^{31,32} Two children died of infection after hematopoietic stem cell graft failure.³²

Interferons, many interleukins and colony-stimulating factors that represent innate immunity use a common mechanism for signal transduction: the JAK-STAT pathway. Four JAKs (JAK1, JAK2, JAK3, and TYK2) selectively associate with the cytoplasmic domains of various cytokine receptors. Cytokine-binding activates JAKs, which in turn phosphorylate cytokine receptors. This process allows the selective binding of 1 of 7 members of the STAT family.³³ The antiviral α/β -interferons induce activation of STAT1 and STAT2. Two siblings with severe viral infections were found to have homozygous mutation in the *STAT2* gene. The first had developed disseminated vaccine strain measles, and the second died after 2 days of a febrile illness assumed to have been due to an unknown viral infection. Interestingly, the same homozygous mutation was found in 3 more family members, one with a history of prolonged febrile encephalitis after measles-mumps-rubella vaccine and another one having been hospitalized for a viral illness.³⁴

PIDDs Associated With an Atopic Phenotype

A number of PIDDs develop an atopic phenotype that may include eczema, elevated eosinophil counts, and increased IgE levels. Examples include Wiskott-Aldrich syndrome; autosomal dominant HIES (AD-HIES; also known as Job syndrome) due to heterozygous mutation in *STAT3*; autosomal recessive HIES (AR-

Table 1

PIDDs for which HSCT is indicated, warning signs other than infections, and initial supportive treatment until transplantation

Disease	Warning signs	Major Precautions and Tx awaiting transplant
Severe combined immunodeficiency	Abnormal newborn screen Lymphopenia Growth failure or diarrhea Eczema or rash	Emergency referral to (PIDD) transplant center and protective isolation Immunoglobulin replacement therapy PJP prophylaxis Consider antifungal prophylaxis Hold breastfeeding based on maternal CMV status Use irradiated leukodepleted CMV [−] blood products if needed Avoid live vaccines
Chronic granulomatous disease	Growth failure Diarrhea or irritable bowel disease Abnormal wound healing Hepatosplenomegaly or lymphadenitis Infections with typical unusual organisms	Antibacterial (trimethoprim-sulfamethoxazole) Antifungal (itraconazole) Interferon γ Consider low-dose prednisone with infections and abscesses Aggressive treatment to prevent infectious complications
Wiskott-Aldrich syndrome	Congenital thrombocytopenia Bloody diarrhea Eczematous rash	Immunoglobulin replacement therapy PJP prophylaxis Avoid live vaccines but give all killed vaccines
IPEX	Severe watery diarrhea due to enteropathy Type 1 diabetes mellitus Immune-mediated cytopenias Eczema Severe food allergies	Immune suppression (steroids and steroid-sparing agents) Avoid all vaccination to prevent severe disease flares Feed breast milk or hydrolyzed formula to reduce exposure to food allergens
Hyper-IgM syndrome	Diarrhea or growth failure Liver disease or sclerosing cholangitis Typical opportunistic infection (<i>Pneumocystis</i> , <i>Cryptosporidium</i> , <i>Histoplasma</i>)	Immunoglobulin replacement therapy PJP prophylaxis Suspect <i>Cryptosporidium</i> infection with diarrhea and gastrointestinal symptoms
Hemophagocytic lymphohistiocytosis	Fever Hepatosplenomegaly or lymphadenopathy Cytopenias Abnormal liver function test results Elevated ferritin levels and elevated soluble CD25 Hypertriglyceridemia or hypofibrinogenemia Low or absent NK cell activity Evidence of hemophagocytosis on biopsy	Urgent referral to hematology or oncology department to initiate intensive chemotherapy or HSCT

Abbreviations: CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplantation; NK, natural killer; PIDDs, primary immunodeficiency diseases; PJP, *Pneumocystis jirovecii* pneumonia.

HIES) due to mutations in *DOCK8* or *TYK2*; *STA5b*; IPEX... and Netherton syndrome. A unique entity with atopic features is Omenn syndrome, which is caused by hypomorphic mutations in genes associated with SCID, including *RAG1/2*, *IL-7RA*, *DCLRE1C* (*ARTEMIS*), *ADA*, *LIG4* (DNA ligase IV) and *RMRP* (cartilage hair hypoplasia), and represents deficiencies that lead to a SCID phenotype with other characteristic findings, including erythrodermia, eosinophilia, hepatosplenomegaly, lymphadenopathy, and elevated IgE. CD4⁺ T_H2 cells control immunity to extracellular parasites and all forms of allergic inflammatory responses. Therefore, conditions that favor differentiation of CD4⁺ T cell to T_H2 cell such as activation in the presence of certain cytokines (eg, IL-4, IL-25, and thymic stromal lymphopoietin) will likely result in atopy. However, accumulated evidence suggests that weak TCR stimulation by itself can drive naive CD4 cells to differentiate into T_H2 cells.^{35,36} Weak TCR signaling can be the result of either low-affinity antigen or abnormal TCR signaling as is the case in many combined PIDDs. For example, abnormal TCR rearrangement in Omenn syndrome results in low-affinity limited-repertoire TCRs and therefore skewed T_H2 differentiation that may be responsible for the observed phenotype. In other cases, such as in WAS and *DOCK8* deficiency, abnormal cytoskeleton rearrangement can impair the formation of effective immune synapse and may lead to weak TCR stimulation. High-intensity and prolonged TCR stimulation is also required for normal thymic differentiation of regulatory T cells³⁷; therefore, abnormal TCR signaling can also affect regulatory T-cell generation and further contribute to the development of atopy features. Without underestimating the role of other genetic and environmental factors, one should wonder what role impaired TCR signaling may play in atopic diseases and which of our atopic patients actually has an underlying hypomorphic PIDD.³⁸

Elevated IgE levels are a common finding in allergic patients. When presented together with severe atopic dermatitis and skin infections, there is always concern about whether infections are secondary to abnormal skin barrier or to an underlying HIES. The National Institutes of Health scoring system^{39,40} for the diagnosis of AD-HIES is based on the presence and severity of 21 clinical and laboratory findings (immune and nonimmune) and was recently revalidated to separate HIES from atopic dermatitis⁴¹ and AD-HIES from other AR-HIESs (eg, *DOCK8* and *TYK2*).⁴² AR-HIES due to *DOCK8* deficiency results in combined immunodeficiency, leading to recurrent sinopulmonary infections, viral infections of the skin (including human papillomavirus infections), chronic mucocutaneous candidiasis, and liver disease possibly due to cryptosporidial infections. Other features include allergies, neurologic complications (seizures and hemiparesis), and an increased risk of

Table 2The 10+1 warning signs of PIDDs^a

Four or more new ear infections within 1 year
Two or more serious sinus infections within 1 year
Two or more months taking antibiotics with little effect
Two or more pneumonias within 1 year or 1 pneumonia per year for more than 1 year
Failure of an infant to gain weight or grow normally or chronic diarrhea with weight loss
Recurrent, deep abscesses of the skin or internal organs
Persistent thrush or fungal infection on skin or elsewhere
Need for intravenous antibiotics to clear infections
Two or more deep-seated infections, including septicemia
A family history of primary immunodeficiency
Infection with atypical mycobacteria

^aThe presence of 2 or more warning signs should trigger an evaluation for immunodeficiency. (adapted from the Jeffery Model Foundation: <http://www.info4pi.org>).

Table 3Initial screening for evaluating the different components of the immune system^a

System	Quantitative evaluation	Qualitative evaluation
Adaptive Immunity		
B cells and antibody production	Flow cytometry to enumerate and characterize B cells Serum immunoglobulin isotype levels (IgG, IgA, IgM, IgE) Specific antibody levels (to vaccines)	B-cell flow–based immunophenotyping to evaluate B-cell development and maturation Antibody responses to booster immunization (polysaccharide and protein antigens) Antibody responses to neoantigen
T cells	Flow cytometry to enumerate and characterize T-cell subsets	T-cell flow–based immunophenotyping to evaluate T-cell development and maturation Mitogen and antigen proliferation assays Anti-CD3 proliferation assay
Innate Immunity		
Complement system	Levels of individual complement component (usually ordered after abnormal pathway activity)	CH ₅₀ (classic pathway activity) AH ₅₀ (alternative pathway activity) Lectin pathway activity
Phagocytic system	Blood cell count and differential Examination of stained blood smear Flow cytometry for adhesion molecules Bone marrow biopsy	Function of individual component Measurement of oxidase function (nitroblue tetrazolium test or DHR dye reduction)
NK cells	Flow cytometry to enumerate NK cells	Target cell lysis Expression of degranulation surface marker
TLRs		Cytokine production in response to different TLR agonists

Abbreviations: AH₅₀, complement, alternate pathway; CH₅₀, total hemolytic complement assay; NK, natural killer, TLR, Toll-like receptor.^aAdapted from Bonilla FA et al.⁴⁷

hematologic and skin malignant tumors.⁴³ Because of the poor prognosis, early hematopoietic stem cell transplantation (HSCT) is indicated. More recently, another clue to help separate AD-HIES from atopy was described. The observation that AD-HIES patients rarely have allergic diseases (probably because STAT3 signaling plays a role in mast cell activation⁴⁴) further differentiates AD-HIES from other forms of HIESs.

When to Suspect and How to Approach a Candidate PIDD Patient

Early diagnosis before permanent organ damage or death has occurred is important, especially in those PIDDs in which HSCT offers a curative treatment (Table 1). Although frequent, difficult-to-treat infections, sometimes with unusual organisms, are the most common presentation, other clinical and laboratory findings are equally informative. In an attempt to serve the general population, the Jeffrey Model Foundation has published 10 warning signs for PIDDs (Table 2). These signs are a starting point to suspect immunodeficiency and to initiate a diagnostic workup.

The first steps include a thorough medical history in search for anamnestic clues such as the time of cord separation, possible bleeding during circumcision, early-onset eczema, oral thrush, infections, and response to antimicrobial treatment. We perform careful and complete physical examination with special attention to any dysmorphic features, evaluation of lymphoid tissue (either absent or enlarged), oral hygiene and periodontitis, rashes, warts, onychomycosis, thrush, and so on. History and physical examination should further direct the initial workup, aiming to screen the different component of the immune system. Quantitative and qualitative evaluation of relevant components of the immune system is required (Table 3). If the result of the initial evaluation is positive, further testing should be performed with the aim of eventually reaching a molecular diagnosis.

With the understanding that PIDD includes more than just recurrent infections, the search for immune dysfunction should be expanded to include other cases of unusual presentations, such as early-onset autoimmunity, strong family history of recurrent infections or other immune phenomena, and additional nonimmune features that are shared by other family members. An example is the recently described PLCγ2 deletions observed in 3 families.

Initially presented with cold-induced urticaria, they were found to have antibody deficiency, susceptibility to infections, and autoimmunity.⁴⁵

Conclusion

Establishing and identifying the underlying condition that causes a primary immunodeficiency is challenging and may require a high index of suspicion. With the introduction of newborn screening for SCID, it is possible that immunodeficiencies that present later in life will have different features and genetic etiologies. Therefore, recognizing which part of the immune system is primarily affected, even in the absence of a definitive diagnosis, is important for selecting initial treatment. Except for patients with SCID or severe life-threatening immune dysregulation, most cases of PIDD can be initially treated with immunoglobulin replacement therapy, prophylactic antibiotics, and/or immunosuppressive drugs. Eventually, the clinical phenotype will declare itself and a molecular diagnosis will be achieved, allowing disease-specific therapy, anticipation of complications and outcomes, and effective genetic counseling.

Much of the progress achieved in recent years can be attributed to scientific curiosity and physician dedication to improve patient care. Although immune deficiencies are rare, they teach us how the immune system works. Yet with a risk of serious complications, such as leukemia,⁴⁶ as has been described by Alain Fischer group, monogenetic diseases can be corrected by gene replacement and, in the future, by gene correction.

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