

Advances in basic and clinical immunology in 2016

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Advances in basic immunology in 2016 included studies that further characterized the role of different proteins in the differentiation of effector T and B cells, including cytokines and proteins involved in the actin cytoskeleton. Regulation of granule formation and secretion in cytotoxic cells was also further described by examining patients with familial hemophagocytic lymphohistiocytosis. The role of prenylation in patients with mevalonate kinase deficiency leading to inflammation has been established. We reviewed advances in clinical immunology, as well as new approaches of whole-genome sequencing and genes newly reported to be associated with immunodeficiency, such as linker of activation of T cells (*LAT*); B-cell CLL/lymphoma 11B (*BCL11B*); RGD, leucine-rich repeat, tropomodulin domain, and proline-rich domain-containing protein (*RLTPR*); moesin; and Janus kinase 1 (*JAK1*). Trials of hematopoietic stem cell transplantation and gene therapy for primary immunodeficiency have had relative success; the use of autologous virus-specific cytotoxic T cells has proved effective as well. New medications are being explored, such as pioglitazone, which is under study for its role in enhancing the oxidative burst in patients with chronic granulomatous disease. Development of vaccines for HIV infection continues to provide insight into the immune response against a virus with an extraordinary mutation rate. (*J Allergy Clin Immunol* 2017;■■■:■■■-■■■.)

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New research developments in basic and clinical immunology continue to expand the number of immune mechanisms and explain the clinical manifestations resulting from impairment of the immune response. We present selected articles describing progress in basic and clinical immunology that were published in

the scientific literature in 2016, with emphasis on themes related to immunodeficiency.

ADVANCES IN BASIC IMMUNOLOGY T cells

T cells play a central role in cell-mediated immunity. Development of T cells in the thymus, their differentiation into different subpopulations, interaction with other cells involved in an immune response, and signaling downstream of surface receptors are all key aspects of understanding T-cell function in health and disease. Wiekmeijer et al¹ studied thymic T-cell development using a xenograft mouse model in which NOD-*Scid-Il2rg*^{-/-} mice underwent transplantation with CD34⁺ stem cells isolated from patients with severe combined immunodeficiency (SCID) caused by different gene defects. The authors showed that common γ chain cytokine signaling is required almost immediately after T-cell progenitors seed the thymus because deleterious mutations in *IL2RG*, encoding IL-2 receptor γ , and *IL7RA*, encoding IL-7 receptor α , block T-cell development at the CD4⁺CD8⁻CD7⁻CD5⁻ and CD4⁺CD8⁻CD7⁺CD5⁻ double-negative (DN) stages, respectively (Table I).^{1,2} Artemis-deficient cells stop developing at the CD4⁺CD8⁻CD7⁺CD5⁺ DN stage, demonstrating the importance of T-cell receptor (TCR) rearrangements at this stage.

An alternative approach for studying T-cell development using samples from patients with SCID was reported by Brauer et al.² They generated induced pluripotent stem cells (iPSCs) using dermal fibroblasts from patients with recombination-activating gene 1 (*RAG1*) mutations differentiated *in vitro* into CD34⁺ cells and then into T cells. The differentiated cells had a block at the CD4⁺CD8⁻CD7⁺CD5⁺CD38⁻CD31^{-/lo}CD45RA⁺ stage, even those obtained from patients with Omenn syndrome (OS), who had residual RAG1 recombination activity. This was attributed to accumulation of single-strand DNA breaks in cells from patients with OS, resulting in impaired cell survival and differentiation. CD4⁺ single-positive and CD4⁺CD8⁺ double-positive T-cell precursors generated *in vitro* from iPSCs from patients with OS and those with SCID had comparable severe restriction in repertoire diversity.

Resop et al³ demonstrated that expression of sphingosine-1-phosphate (S1P) receptor 1 on mature thymocytes is essential for their egress from the thymus. Using *in vitro* transwell migration assays, the authors showed that mature CD3^{hi}CD27⁺CD45RA⁺CD62L⁺CD69⁻ thymocytes had the greatest migration toward S1P and that thymocytes downregulate S1P receptor 1 on interaction with S1P. The investigators also suggested a role for S1P receptor 2 in thymocyte retention in the thymus. Modulation of the interaction between S1P and its receptor was proposed as a potential strategy for increasing T-cell counts in primary immunodeficiencies (PIDs) characterized by

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Abbreviations used

AIP1:	Actin-interacting protein 1
APDS:	Activated PI3K δ syndrome
BCL11B:	B-cell CLL/lymphoma 11B
BCR:	B-cell receptor
CARD9:	Caspase recruitment domain-containing protein 9
CC:	Coiled-coil leucine zipper
CD40L:	CD40 ligand
CE:	C-terminal extension
CGD:	Chronic granulomatous disease
CNV:	Copy number variant
COPA:	Coatomer protein complex subunit α
CTLA-4:	Cytotoxic T-lymphocyte antigen 4
CVID:	Common variable immunodeficiency
DN:	Double negative
DOCK8:	Dedicator of cytokinesis 8
FHL:	Familial hemophagocytic lymphohistiocytosis
GEF:	Guanine exchange factor
HIES:	Hyper-IgE syndrome
HLH:	Hemophagocytic lymphohistiocytosis
HPS2:	Hermansky-Pudlak syndrome type 2
HSCT:	Hematopoietic stem cell transplantation
ILC:	Innate lymphoid cell
iPSC:	Induced pluripotent stem cell
JAK1:	Janus kinase 1
KIR:	Killer cell immunoglobulin-like receptor
LAT:	Linker for activation of T cells
LFA-1:	Leukocyte function-associated antigen 1
LRBA:	LPS-responsive beige-like anchor protein
LYST:	Lysosomal trafficking regulator
mTOR:	Mammalian target of rapamycin
MVK:	Mevalonate kinase
nAIGA:	Neutralizing anti-IFN- γ autoantibody
NF- κ B:	Nuclear factor κ B
NGS:	Next-generation sequencing
NK:	Natural killer
OS:	Overall survival
OTULIN:	OTU deubiquitinase with linear linkage specificity
PID:	Primary immunodeficiency
PI3K:	Phosphoinositide 3-kinase
POLE2:	DNA polymerase ϵ subunit 2
PRF1:	Perforin 1
PTEN:	Phosphatase and tensin homolog
RAG1:	Recombination-activating gene 1
RLPR:	RGD, leucine-rich repeat, tropomodulin domain, and proline-rich domain-containing protein
ROS:	Reactive oxygen species
RV:	Rubella virus
SCID:	Severe combined immunodeficiency
S1P:	Sphingosine-1-phosphate
STAT:	Signal transducer and activator of transcription
STING:	Stimulator of interferon genes
TAP1:	Transporter, ATP-binding cassette, major histocompatibility complex 1
TCR:	T-cell receptor
T _{FH} :	Follicular helper T
TFRC:	Transferrin receptor
THI:	Transient hypogammaglobulinemia of infancy
TLR:	Toll-like receptor
TMEM:	Transmembrane protein
UNG:	Uracil N-glycosylase
VST:	Virus-specific T cell
WAS:	Wiskott-Aldrich syndrome
X-HIGM:	X-linked hyper-IgM syndrome

impaired T-cell egress. On thymic egress, T cells undergo a short period of postthymic maturation in the periphery, which has now been shown to be important for tolerance induction to tissue-restricted antigens.⁴ The outcome of this T-cell interaction with tissue-restricted antigens is determined by the context of the interaction: in the absence of inflammation, recent thymic emigrants are tolerized; in the presence of inflammation, recent thymic emigrants are directed toward effector cell differentiation in which mature T cells contribute to autoimmune disease.

The ability of naive CD4⁺ T cells to differentiate into effector populations *in vitro* was studied in patients with different PIDs.⁵ IL-12 receptor β 1/TYK2 and IFN- γ receptor/signal transducer and activator of transcription (STAT) 1 signaling were demonstrated to be important for induction of T_H1 cells, and IL-21/IL-21 receptor/STAT3 signaling was demonstrated to be important for induction of T_H17, follicular helper T (T_{FH}), and IL-10-secreting cells. Signaling by IL-12 receptor β 1/TYK2 and nuclear factor κ B (NF- κ B) essential modulator were also proved to be necessary for T_H17 induction. The authors showed that hyperactivation of STAT1 inhibits STAT3 signaling by showing that gain-of-function STAT1 and dominant-negative STAT3 mutations had a similar effect on T_{FH} and T_H17 cells. Noster et al⁶ demonstrated that T_H17 cells assume an anti-inflammatory function and produce IL-10 in the absence of IL-1 β , thereby suppressing T_H cell proliferation and downregulating cytokines secreted by monocytes. However, exposure to IL-1 β impairs IL-10 secretion by T_H17 cells, which is seen in patients with Schnitzler syndrome, a disease characterized by spontaneous IL-1 β secretion. IL-1 β inhibition in patients with Schnitzler syndrome rescued IL-10 production by T_H17 cells.

Yee et al⁷ identified the functions of the coiled-coil leucine zipper (CC) and C-terminal extension (CE) domains in the filamentous actin-binding protein coronin 1A through the study of patients expressing a truncated coronin 1A mutant lacking the CC and CE domains. The CC and CE domains were shown to be important for coronin 1A oligomerization and subcellular localization, regulation of filamentous actin content, *in vivo* T-cell survival, and host defense against viruses but were not essential for calcium flux or cell cytotoxicity.

Two studies have further delineated the role of the guanine exchange factor (GEF) dedicator of cytokinesis 8 (DOCK8) in T-cell function. Janssen et al⁸ showed that DOCK8 interacts directly with Wiskott-Aldrich syndrome (WAS)-interacting protein, which bridges DOCK8 to WAS protein and actin in T cells. The GEF activity of DOCK8 is essential for TCR-driven WAS protein activation, actin cytoskeleton reorganization, and subcortical actin cytoskeletal integrity, which in turn are important for filamentous actin assembly, immune synapse formation, actin foci formation, mechanotransduction, T-cell transendothelial migration, and homing of T cells to the lymph nodes. Hyper-IgE syndrome (HIES) caused by autosomal dominant mutations in *STAT3* or autosomal recessive mutations in *DOCK8* have multiple overlapping features, including T_H17 dysfunction.

Keles et al⁹ demonstrated that patients with mutations in *DOCK8* have a block in T_H17 differentiation similar to that previously reported in patients with autosomal dominant *STAT3* mutations. The authors showed that DOCK8 constitutively associates with STAT3 and regulates its phosphorylation through DOCK8 GEF activity. DOCK8 also promotes STAT3

TABLE I. Arrest in human T-cell development caused by SCID gene defects using 2 different experimental models

Gene	T-cell developmental arrest	TCRD locus rearrangement	Method
<i>ADA</i>	Normal development	Normal	Xenograft mouse model
<i>Artemis</i>	CD4 ⁺ CD8 ⁺ CD7 ⁺ CD5 ⁺ DN stage	Broad repertoire TCRD δ 2-D δ 3	Xenograft mouse model
		No rearrangement TCRG, TCHRB	
<i>IL7RA</i>	CD4 ⁺ CD8 ⁺ CD7 ⁺ CD5 ⁺ DN stage	No rearrangement	Xenograft mouse model
<i>IL2RG</i>	CD4 ⁺ CD8 ⁺ CD7 ⁺ CD5 ⁺ DN stage	No rearrangement	Xenograft mouse model
<i>IL2RG</i> hypomorphic	Normal development, decreased T-cell numbers	Not done	Xenograft mouse model
<i>RAG1</i>	CD4 ⁺ CD8 ⁺ CD7 ⁺ CD5 ⁺ CD38 ⁺ CD31 ^{+/lo} CD45RA ⁺ DN stage	No rearrangement	iPSC

Normal development of adenosine deaminase-deficient T cells results from exogenous mouse adenosine deaminase (Wiekmeijer et al¹ and Brauer et al²).

TABLE II. Selected key advances in B-cell immunology

	References
● Decreased somatic hypermutation causes impaired peripheral B-cell tolerance in patients with <i>AICDA</i> mutations.	14
● Kabuki syndrome caused by <i>KMT2D</i> mutation leads to humoral immune deficiency with impaired terminal B-cell differentiation.	15
● B cell-intrinsic defective STAT3 signaling results in impaired specific antibody production and increased IgE levels.	16
● Overexpression of IL-10 in plasmacytosis inhibits neutrophil migration and results in increased susceptibility to bacterial infection.	17
● CD24 ^{high} CD38 ^{high} transitional B cells can be distinguished into subsets with distinct regulatory function profiles.	18
● CD19 mediates TLR9-induced B-cell activation, integrating TLR and BCR signaling.	19
● Autoreactive naive IgD ⁺ IgM ⁺ B cells are rescued from anergy by T _H 2 signals through upregulation of CD45 activity.	20
● The integrin LFA-1 is required for T _{FH} survival and differentiation in the germinal center.	21

translocation to the nucleus and consequently induces STAT3-dependent T_H17 differentiation.

Infection-triggered induction of hemophagocytic lymphohistiocytosis (HLH) in perforin-deficient mice, as well as in patients with familial HLH, leads to decreased regulatory T-cell numbers.¹⁰ This was attributed to changes in the homeostatic regulation of IL-2, specifically decreased IL-2 secretion, increased IL-2 consumption by activated CD8⁺ T cells, and increased secretion of soluble CD25 (IL-2 receptor α), which competes for IL-2. Reduction of levels of circulating IL-2, which is essential for the maintenance of regulatory T cells, thereby contributes to the persistence of inflammation in these patients.

Several reports have expanded our understanding of T-cell function in patients with viral infections. IL-6 is known to induce IL-21 production by CD4⁺ T cells and is required for the development of T_{FH} cells, which in turn promote production of antibodies by means of interaction with B cells through the production of IL-21. Yang et al¹¹ showed that IL-6 also induces CD8⁺ T cells to differentiate into cells that produce low levels of IFN- γ and high levels of IL-21, which facilitates isotype switching in B cells. This is particularly relevant for the secretion of virus-specific IgG during an influenza virus infection, thus identifying an additional contribution of CD8⁺ T cells in host immunity against viruses. Persistent TCR activation and signaling, as seen in patients with chronic viral infections, leads to T-cell exhaustion, a state characterized by compromised effector T-cell function. Termination of TCR signaling is achieved through multivesicular body-mediated lysosomal degradation of the TCR.

Hu et al¹² showed that deficiency of transmembrane protein 16F (TMEM16F), the dominant lipid scramblase in T lymphocytes that transports phospholipids across membranes, amplifies T-cell activation. Chronic infection of TMEM16F-deficient

mice with lymphocytic choriomeningitis virus resulted in increased T-cell activation in the early stages of infection and T-cell exhaustion at later stages. Mechanistically, the authors demonstrated that TMEM16F is located in late endosomes and is important for the formation of the multivesicular body, TCR degradation, and thus signal termination. Targeting of TMEM16F to enhance its function is a potential strategy for avoiding T-cell exhaustion in patients with chronic viral infections or cancer.

Autoimmune lymphoproliferative syndrome is characterized by chronic lymphoproliferation and accumulation of TCR $\alpha\beta$ ⁺CD4⁺CD8⁺ DN T cells. Völkl et al¹³ showed that DN T cells and their CD4⁺ or CD8⁺ precursors have hyperactive mammalian target of rapamycin (mTOR) signaling, which can be ameliorated by rapamycin.

B cells

Disturbances in humoral immunity manifest clinically as immune deficiency, autoimmunity, or allergy. Multiple studies have addressed the production and regulation of B cells and antibodies (Table II).¹⁴⁻²¹ Cantaert et al¹⁴ studied the mechanisms by which mutations in *AICDA*, which encodes activation-induced cytidine deaminase, impair peripheral B-cell tolerance. They found that B cells from patients with *AICDA* mutations produced increased autoreactive antibodies compared with those of control subjects and patients with uracil N-glycosylase (UNG) deficiency. UNG deficiency causes defective class-switch recombination, as seen in patients with activation-induced cytidine deaminase deficiency, but is distinguished by intact somatic hypermutation. Patients with *AICDA* mutations, but not UNG-deficient patients, had altered germinal center reactions, defective regulatory T-cell suppressive function, increased T_{FH} cell production, and enhanced cytokine production. The authors postulated

that somatic hypermutation regulates peripheral B-cell tolerance through production of highly mutated antibodies that eliminate antigens and prevent alterations in T-cell composition and function that favor autoimmunity.

Lindsley et al¹⁵ studied the extent of humoral immunity impairment in patients with Kabuki syndrome caused by autosomal dominant mutations in lysine methyltransferase 2D (*KMT2D*). Affected patients have variable hypogammaglobulinemia, decreased numbers of total and class-switched memory B cells, and reduced rates of somatic hypermutation in IgG. Autoimmunity was found in a subset of patients with a specific missense mutation. The authors emphasized the importance of serial immune follow-up for patients with Kabuki syndrome.

Using a mouse model of B cell lineage-specific inactivation of *Stat3*, Kane et al¹⁶ observed impaired expansion of antigen-specific B cells, reduced serum antigen-specific IgM and IgG levels, significantly increased serum IgE levels, and impaired affinity maturation after immunization with a T cell-dependent antigen. The results highlight the role of defective B cell-intrinsic *Stat3* signaling in causing the humoral immune defects observed in autosomal dominant HIES in human subjects.

Kulkarni et al¹⁷ examined the effect of IL-10 on neutrophil function in a murine myeloma model. IL-10 derived from normal and neoplastic cells inhibited neutrophil migration toward the anaphylatoxin C5a and suppressed neutrophil-dependent inflammation. Interestingly, the authors demonstrated increased susceptibility to streptococcal pneumonia in this mouse model that could be reversed with IL-10 receptor blockade.

Simon et al¹⁸ characterized CD24^{high}CD38^{high} transitional B cells in human subjects and reported 4 subsets of transitional B cells based on distinct phenotypic and regulatory function properties. In addition to transitional type 1 and type 2 B cells, the authors described anergic type 3 B cells and IL-10-producing CD27⁺ transitional B cells. These subsets were differentially represented in patients with specific autoimmune diseases, suggesting potential clinical utility for this classification.

An essential role of CD19 in mediating Toll-like receptor (TLR) 9-induced human B-cell activation was reported by Morbach et al.¹⁹ The authors found that CD19 deficiency not only interfered with B-cell receptor (BCR)-mediated B-cell activation but also led to impaired activation responses after TLR9 stimulation. The authors demonstrated that B-cell signaling downstream of TLR9 activation through the molecules phosphoinositide 3-kinase (PI3K), Bruton tyrosine kinase, and AKT was dependent on CD19.

The subset of autoreactive B cells that undergo functional anergy and thus do not result in autoimmunity have been characterized as naive IgD⁺IgM⁻ B cells. Szodoray et al²⁰ demonstrated that BCR signaling can be restored in naive IgD⁺IgM⁻ B cells and they can be rescued from anergy on receiving T_H2 signals (IL-4/CD40 ligand [CD40L]). T_H2 signals increase the activity of the protein tyrosine phosphatase CD45, allowing activation of Lyn kinase and increasing BCR signaling.

Meli et al²¹ showed that a highly active form of the integrin leukocyte function-associated antigen 1 (LFA-1) is required for T_{FH} cell survival within the germinal center and promotes the expression of BCL-6, which is critical for T_{FH} cell differentiation. This paves the way for a potential therapeutic target for regulating the humoral immune response.

Natural killer cells

Natural killer (NK) cells are known to produce a cytotoxic effect on their target cells by releasing granules containing lethal proteins. While doing so, many bystander cells can also die as a consequence if there is no control of this function. Convergence is an intracellular process induced by dynein and through an integrin signal that orients the polarization of the lethal granules toward the target cell. Hsu et al²² designed experiments that demonstrated that convergence improved killing efficiency and reduced the bystander effect. NK cells stimulated with LFA-1 and CD16 signals produced a greater concentration of lytic granules in the immunologic synapse than CD16 signal alone. LFA-1 signal by itself does not induce degranulation, and without this signal, killing was reduced.

Killer cell immunoglobulin-like receptors (KIRs) are expressed on NK cells and T-cell subsets. Wang et al²³ examined KIR and HLA allele frequencies in a cohort of patients with common variable immunodeficiency (CVID) and in healthy control subjects. They identified an association of KIR genes and KIR/HLA genotype combinations with CVID. The study encourages further work to explore the potential role of NK cell activation in the pathogenesis of CVID variants.

Patients with Chediak-Higashi syndrome present with mutations on the lysosomal trafficking regulator (LYST). Gil-Krzawska et al²⁴ showed that NK cells from these patients had cytotoxic granules of larger size and were able to converge toward target cells; however, these are not secreted or fuse with the plasma membrane, suggesting an essential role of the LYST protein in cytotoxic granule exosome and secretion. Of note, cytokine secretion was not affected.

Lougaris et al²⁵ investigated NK cell function in patients with a gain-of-function mutation in p85α (*PI3KR1*). They were able to show impairment of cytotoxic activity and reduction of INF-γ secretion on IL-12 stimulation, which might contribute to the increased susceptibility of these patients to viral infections.

Innate lymphoid cells

The role of innate lymphoid cells (ILCs) in the immune response continues to be revealed. ILCs are of lymphoid lineage, characteristically do not express antigen-specific receptors, and play a role in inflammation and in the immune response against pathogens. They have been classified as 3 types (type 1, type 2, and type 3) based on their cytokine secretion pattern. Antignano et al²⁶ investigated pathways of ILC differentiation based on previous studies on T_H2 differentiation and reported that type 2 ILC differentiation also required expression of the G9a protein, a lysine methyl transferase that activates histone H3. Of note, absence of G9a expression resulted in expansion of type 3 ILCs.

By measuring ILCs in patients with SCID before and after hematopoietic stem cell transplantation (HSCT), Vély et al²⁷ found that the presence or absence of ILCs in these patients does not affect the frequency of infections, strongly suggesting a redundant role in this setting. When HSCT was performed with myeloablation, ILCs were detected in peripheral blood, although without discernible benefit in the risk of infection.

In contrast to redundancy in the control of infection, Cols et al²⁸ reported that patients with CVID might have an expanded population of ILCs from both peripheral blood and mucosal tissues expressing INF-γ and suggested that ILCs might have a role in inflammatory conditions observed in patients with CVID.

Monocytes/macrophages and inflammation

Monocytes in peripheral blood and the more differentiated macrophages and dendritic cells in tissues have a variety of functions: antigen presentation to lymphocytes, cytotoxicity, antibody-mediated phagocytosis, and cytokine secretion. These cells have an essential role in regulation of immune and inflammatory responses. Prandini et al²⁹ studied the *in vitro* differentiation of monocytes into dendritic cells of patients with Hermansky-Pudlak syndrome type 2 (HPS2). Patients with HPS2 present with deficiency of the adaptor protein 3 complex, which is involved in protein transport to lysosomes and associated with impairment of cytotoxic activity. Consistent with the role of the adaptor protein 3 complex in TLR7 and TLR9 signaling, monocytes from patients with HPS2 demonstrated delayed maturation into dendritic cells; impaired cytokine secretion after stimulation with CpG, LPS, and herpes simplex virus 1; and defective ability to induce T-cell proliferation.

Mechanisms of inflammation in periodic fever caused by mevalonate kinase (MVK) deficiency have not been clearly defined. Jurczyk et al³⁰ studied the prenylation of Rab small GTPases, which, when absent, might cause increased inflammation activity and release of IL-1 β , resulting in systemic symptoms of inflammation, such as fever and rash. By using lymphoblastoid cell lines derived from MVK-deficient patients, heat stress induced by increasing incubation temperature to 40°C resulted in decrease of the mutated MVK activity and subsequent detection of unprenylated Rab proteins. These data suggest a possible mechanism for periodic fever in MVK deficiency. Akula et al³¹ showed that MVK deficiency is associated with decreased protein geranylgeranylation, which is necessary for activation of PI3K mediated by GTPase Kras. Decreased PIK3 activity leads to spontaneous and TLR-induced activation of the inflammasome and increased expression of proinflammatory cytokines.

ADVANCES IN CLINICAL IMMUNOLOGY

Diagnosis of PIDs

The diagnostic workup of patients with suspected PIDs involves taking a detailed personal and family history coupled with laboratory assays that are guided by knowledge of the molecular pathways. Classically, the next step would involve Sanger sequencing of multiple candidate genes in search of a reasonable genotype-phenotype correlation. More recently, whole-exome sequencing has been used to provide rapid diagnosis. However, whole-exome sequencing is still relatively expensive and provides a sizeable amount of information that requires time and highly trained investigators for analysis. Novel approaches in next-generation sequencing (NGS) for the detection of specific PID-causing genes have enabled targeted NGS panels as a rapid and cost-effective alternative to whole-exome sequencing (Fig 1).

Yu et al³² established a targeted gene capture/NGS panel that included 46 genes associated with SCID and other severe PIDs. This was coupled with the simultaneous detection of copy number variants (CNVs) and Sanger sequencing for exons with insufficient coverage. Of 20 patients with abnormal T-cell receptor excision circle newborn screening test results or a positive family history for PIDs, the assay identified mutations in disease-causing genes in 14 (70%) patients. The authors highlight the utility of targeted sequencing platforms in the setting of a positive

family history because newborn screening results might be non-informative for severe PIDs with normal T-cell numbers.

Kojima et al³³ studied 97 patients using a broader NGS panel, including 286 genes associated with PIDs, 42 genetic causes of congenital bone marrow failure syndromes, and the 47 genes within the 22q11.2 region coupled with CNV detection. The cost of this panel was \$500 per sample. In this cohort, 38 patients had known PID-causing mutations, and 59 lacked a genetic diagnosis. The system detected all the known mutations; of the 59 patients without a diagnosis, 9 diagnostic gene variants in 8 patients were identified.

Al-Mousa et al³⁴ developed an NGS sequencing panel of 162 genes with simultaneous CNV detection. The study included 122 samples with known causative PID mutations: mutations in 117 samples were detected, but the remainder missed the rest because of low sequencing coverage. When tested on samples from patients without a known diagnosis, the assay detected mutations in 35 (25%) of 139 tested samples. Because atypical presentations of PIDs are increasingly frequent, the authors propose that a comprehensive panel including all genes associated with PIDs is more reliable than panels limited to specific phenotypes.

Transient hypogammaglobulinemia of infancy (THI) is defined as a primary antibody deficiency characterized by delayed immunoglobulin production that spontaneously recovers before 4 years of age. Unclassified hypogammaglobulinemia is a term that might be used for patients with low IgG values in the first 3 years of life; those with unclassified hypogammaglobulinemia who eventually normalize their IgG levels by 4 years of age receive a diagnosis of THI. Moschese et al³⁵ enrolled 21 patients with a history of recurrent infections with low IgG levels in the first years of life and measured IgM, IgA, and IgG levels, as well as specific antibody levels, after anti-pneumococcal vaccine was given. The investigators found that children with THI had significantly decreased responses to pneumococcal immunization compared with healthy control subjects. Responses to *Haemophilus influenzae* type B and tetanus vaccines were not different from those of healthy control subjects.

Cetica et al³⁶ reported the gene defects found in a cohort of 500 patients with HLH. Homozygous mutations causing familial hemophagocytic lymphohistiocytosis (FHL) were found in 34% of the patients. Mutations in perforin 1 (*PRF1*) and Unc 13 homolog D (*UNC13D*) accounted for 70% of cases of FHL. Absent perforin protein expression was always indicative of a homozygous *PRF1* mutation. A complete degranulation defect was also found to be predictive of homozygous mutations in degranulation-related genes, including *UNC13D*, syntaxin binding protein 2 (*STXBP2*), *LYST*, and *RAB27A*. Notably, of 281 patients with sporadic HLH, 43 harbored heterozygous mutations in one of the FHL-causing genes. On testing 28 of the 43 patients, 11 had a partial degranulation defect. Thirty-two percent of patients with reduced (but not absent) perforin expression had monoallelic mutations in *PRF1*, suggesting an association between monoallelic mutations in genes associated with FHL and sporadic HLH.

Lévy et al³⁷ reported 21 patients presenting with chronic mucocutaneous candidiasis (CMC) before 6 months of age. Fourteen patients also had early-onset recurrent staphylococcal skin infections. Twelve different mutations in *IL17RA* were identified, the majority of which abrogated protein expression; 1 missense mutation preserved protein expression. All reported mutations abolished cellular responses to IL-17A and IL-17F in fibroblasts and IL-17E/IL-25 in leukocytes.

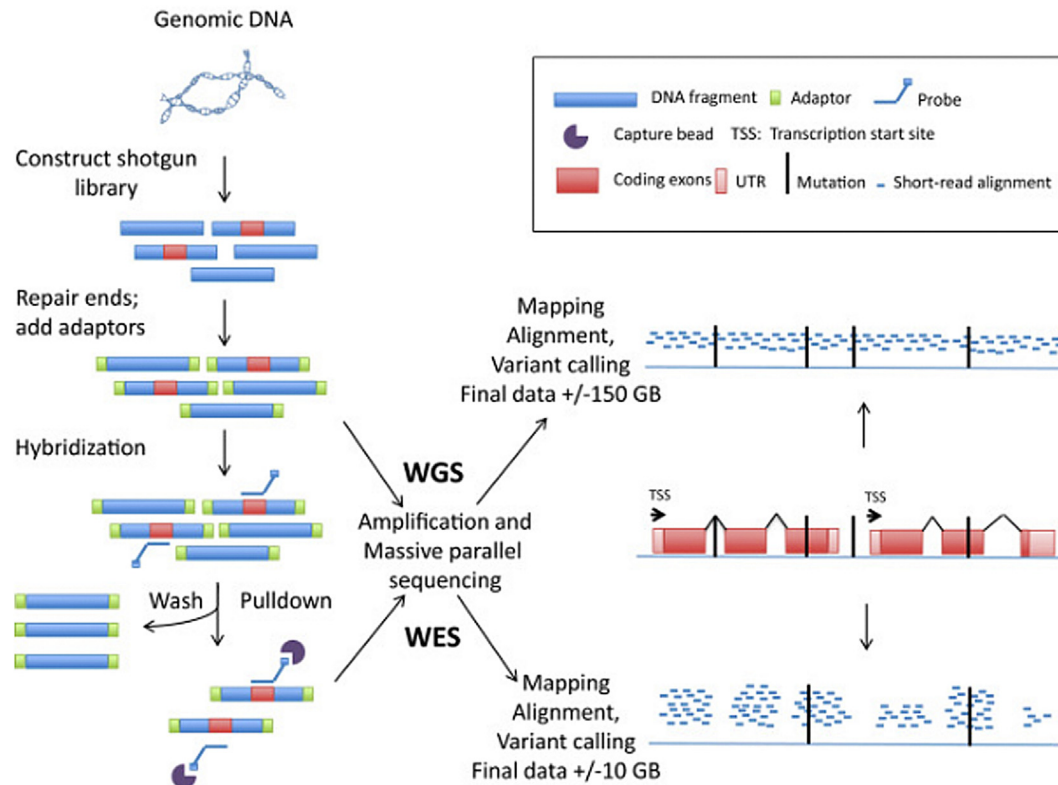


FIG 1. Schematic representation of whole-exome sequencing (WES) and whole-genome sequencing (WGS). Genomic DNA is fragmented and amplified, and then a “library” is made of these DNA fragments and fused with adaptors to facilitate sequencing. In WES the coding exome is enriched. Differences in the patient’s DNA sequence and the reference genome are identified and analyzed for possible clinical significance. TSS, Transcription start site; UTR, untranslated region. Reproduced with permission from Meyts I, Bosch B, Bolze A, Boisson B, Itan Y, Belkadi A, et al. Exome and genome sequencing for inborn errors of immunity. *J Allergy Clin Immunol* 2016;138:957-69.

Two studies demonstrated the effects of viral infections on influencing the clinical phenotype of PIDs. Perelygina et al³⁸ found that rubella virus (RV) vaccine strain granulomas were most common in patients with ataxia telangiectasia but were also found in patients with combined immunodeficiencies (CIDs) and cartilage-hair hypoplasia syndrome. The authors demonstrated that M2 macrophages and epidermal keratinocytes harbored the RV antigens and hypothesized that the attenuated RV strain persists in the setting of a nonprotective immune response, resulting in M2-type granuloma formation.

Walter et al³⁹ showed that patients with hypomorphic mutations in *RAG1* or *RAG2*, particularly those with delayed-onset combined immunodeficiency and granulomatous autoimmune manifestations, were found to produce a broad spectrum of autoantibodies that correlate with their clinical phenotype. For example, patients with neutralizing antibodies to IFN- α or IFN- ω had a history of severe viral infections. Mice with leaky SCID caused by a *Rag1* mutation were injected with agonists for TLR3, TLR7/8, and TLR9 and were found to have significantly increased autoantibody production after the TLR challenge, demonstrating that recurrent or chronic viral infections can trigger immune dysregulation.

Pelham et al⁴⁰ described the genotype-phenotype correlation in patients with autosomal dominant HIES and heterozygous *STAT3* mutations in the DNA binding domain, the SH2 domain, or the transactivation domain. The authors profiled the effect of the

mutations on STAT3 phosphorylation, STAT3 dimerization, nuclear translocation, and DNA-binding capacity. All mutations had a similarly damaging effect on generation of T_H17, T_{FH}, and memory B cells.

Several studies have elucidated mechanisms driving the immune response to fungal infections. Mutations in caspase recruitment domain-containing protein 9 (*CARD9*) are associated with increased susceptibility to fungal infections. *Candida albicans* is known to robustly induce GM-CSF from monocytes. Gavino et al⁴¹ showed that a hypomorphic mutation in *CARD9* (p.Y91H) in 4 patients with spontaneous central nervous system candidiasis resulted in an impaired interaction between *CARD9* and the Ras protein-specific guanine nucleotide-releasing factor 1 (RASGRF1), leading to reduced signaling and impaired production of GM-CSF after fungal stimulation. Gazendam et al⁴² studied monocytes and neutrophils from patients with chronic granulomatous disease (CGD) to determine the role of NADPH oxidase activity in the production of cytokines against bacteria and fungi. Bacterial stimulation of monocytes from patients with CGD resulted in impaired secretion of IL-6 and IL-1 β , whereas fungal stimulation resulted in an enhanced proinflammatory cytokine response. These findings indicate that intact generation of reactive oxygen species (ROS) negatively regulates the production of proinflammatory cytokines by monocytes and neutrophils in response to fungal infection. In patients with defective NADPH oxidase function, impaired ROS production might result

in a persistent and overactive innate immune response after certain infections.

Although bacterial and fungal infections are the most common infections in the setting of CGD, mycobacterial infections have also been reported in some patients. Conti et al⁴³ retrospectively analyzed 71 patients with CGD and mycobacterial disease. Forty-four percent of the patients had tuberculosis, 75% presented with an adverse reaction to BCG vaccination (local BCG-itis, regional BCG-itis or BCG-osis), and 18% had both tuberculosis and BCG infections. Twenty-four percent of those patients presented only with mycobacterial disease in the absence of other infections, whereas 60% of the patients had mycobacterial disease as the first clinical manifestation of CGD. The authors highlight the importance of the oxidative burst in host immunity to mycobacteria and suggest mycobacterial infections as a clinical indicator of CGD. Furthermore, BCG vaccination should be contraindicated in patients suspected of having or given a diagnosis of CGD and their newborn siblings.

Neutralizing anti-IFN- γ autoantibodies (nAIGAs) constitute another cause of disseminated nontuberculous mycobacteria, which are found predominantly in patients from Southeast Asia. Ku et al⁴⁴ demonstrated that HLA-DR*15:02/16:02 and HLA-DQ*05:01/05:02 are strongly associated with the presence of serum nAIGAs in different populations across Southeast Asia, suggesting that HLA class II alleles might confer a risk of nAIGA-associated immunodeficiency.

Homozygous mutations in complement component C3 result in a syndrome characterized by susceptibility to recurrent bacterial infections caused by *Streptococcus pneumoniae* and *Neisseria meningitidis*, as well as immune complex-mediated diseases, such as systemic lupus erythematosus-like illness or renal diseases. Okura et al⁴⁵ analyzed 37 cases of 29 families with mutations in C3 and found that mutations affecting the N-terminal portion of the protein upstream of the thioester-containing domain were associated with susceptibility to severe infections. In contrast, mutations in the C-terminus of the protein, within or downstream of the thioester-containing domain, were associated with immune complex-mediated diseases.

An international consensus document was issued on CVID that discusses the definition of CVID, epidemiology, natural history, genetics, diagnosis, management, prognosis, and areas of potential further study.⁴⁶

New gene defects associated with immune dysfunction

Investigating patients with abnormalities in their immune responses continued to reveal novel gene defects as causes of PIDs. In 2016, defects in regulators of known immune pathways, scaffolding proteins of immune receptors, transcription factors, and genes involved in DNA replication and repair have all been shown to affect the immune system (Table III).⁴⁷⁻⁶⁷ Bacchelli et al⁴⁷ reported a family with T⁻B⁺NK⁺ SCID caused by homozygous mutations in linker for activation of T cells (*LAT*) that abrogated protein expression. *LAT*-deficient cell lines reconstituted with the patients' mutant copy of *LAT* lacked TCR signaling and did not induce Ca²⁺ flux after OKT3 stimulation.

Keller et al⁴⁸ reported a hypomorphic mutation in *LAT* in a kindred with early-onset recurrent infections, progressive lymphopenia and hypogammaglobulinemia, lymphoproliferative disease, and life-threatening autoimmunity. Residual T cells were

predominantly $\gamma\delta$ T cells. The patients' cells expressed a truncated form of LAT, resulting in abolished extracellular signal-regulated kinase phosphorylation and upregulation of CD69 after anti-CD3 stimulation. However, Ca²⁺ flux was normal, suggesting residual TCR signal and providing an explanation for the different phenotype.

Punwani et al⁴⁹ described a heterozygous missense *de novo* mutation in the zinc finger DNA-binding domain of the transcription factor B-cell CLL/lymphoma 11B (*BCL11B*) as a cause of T⁻B⁺NK⁺ SCID associated with multisystemic anomalies, including absence of the corpus callosum and craniofacial abnormalities. The mutant protein product heterodimerizes with wild-type BCL11B and exerts a dominant negative effect by impairing binding to promoter targets, leading to decreased activation of target genes, such as *IL2*. BCL11B was shown to regulate the expression of CCR7 and CCR9, which are necessary for the migration of CD34⁺ hematopoietic progenitor cells from the bone marrow to the thymus.

Biallelic mutations in RGD, leucine-rich repeat, tropomodulin domain, and proline-rich domain-containing protein (*RLTPR*), which encodes a scaffolding protein that links CD28 to the CARD11/CARMA1 adaptor and the NF- κ B signaling pathway, were reported⁵⁰ to cause a combined immunodeficiency characterized by allergic disease and an increased susceptibility to molluscum contagiosum, mucocutaneous candidiasis, tuberculosis, and bacterial infections.^{51,52} *RLTPR*-deficient patients have low percentages of regulatory T cells and memory CD4⁺ T cells. Decreased *RLTPR* expression resulted in impaired activation of NF- κ B in CD4⁺ T cells downstream of anti-CD3/anti-CD28 stimulation, as well as failure of CD4⁺ T cells to differentiate into T_H1 and T_H17 under polarizing conditions. The patients have few memory B cells and impaired antibody responses, which was attributed to defective NF- κ B activation downstream BCR activation.

Salzer et al⁵³ described a patient with a homozygous mutation in RAS guanyl nucleotide-releasing protein 1 (*RASGRP1*), which encodes a nucleotide exchange factor critical for activating of the mitogen-activated protein kinase cascade. This patient presented with recurrent bacterial and viral infections and progressive CD4⁺ T- and B-cell lymphopenia. T and B cells had abnormal extracellular signal-regulated kinase phosphorylation after stimulation, as well as abnormal proliferation and activation to anti-CD3/anti-CD28 and anti-CD40L/IL-4, respectively. NK cells from the patient exhibited impaired cytotoxicity associated with defective granule convergence and actin accumulation. This was attributed to the interaction of RASGRP1 with the dynein light chain, thus enabling regulation of cytoskeletal dynamics. RASGRP1-deficient leukocytes had impaired migration caused by impaired signaling of the GTPase RhoA, which was corrected by the RhoA activator lenalidomide.

A homozygous mutation in DNA polymerase ϵ subunit 2 (*POLE2*), encoding a subunit of the DNA polymerase ϵ complex, was described in a patient with combined immunodeficiency, facial dysmorphic features, and autoimmunity.⁵⁴ The patient's cells had impaired cell-cycle progression because of the protein's role in DNA replication. The *POLE2* mutation leads to a more severe clinical evolution and a more profound block in B-cell development than that seen in patients with the previously reported *POLE1* mutation, which might be due to differences in the patients' genetic backgrounds or differences in the function of the POLE subunits.

The first human immunodeficiency caused by defective iron transport was reported by Jabara et al,⁵⁵ who identified a

TABLE III. New PID genes reported in 2016

Gene symbol	Gene name	Immunodeficiency	References
<i>LAT</i>	Linker for activation of T cells	● T ⁺ B ⁺ NK ⁺ SCID	47,48
<i>BCL11B</i>	B-cell CLL/lymphoma 11B	● Combined immunodeficiency	49
<i>RLTPR</i>	RGD, leucine-rich repeat, tropomodulin domain, and proline-rich domain-containing protein	● T ⁺ B ⁺ NK ⁺ SCID, absent corpus callosum	50-52
<i>RASGRP1</i>	RAS guanyl nucleotide-releasing protein 1	● Combined immunodeficiency	53
<i>POLE2</i>	DNA polymerase ϵ , subunit 2	● Combined immunodeficiency, facial dysmorphism, autoimmunity	54
<i>TFRC</i>	Transferrin receptor	● Combined immunodeficiency	55
<i>PTEN</i>	Phosphatase and tensin homolog	● Activated PI3K δ syndrome (APDS)	56,57
<i>IKZF1</i>	IKAROS, transcription factor	● CVID	58
<i>IRF2BP2</i>	Interferon regulatory factor 2-binding protein 2	● CVID	59
<i>JAK1</i>	Janus kinase 1	● Mycobacterial disease and early-onset bladder carcinoma	60
<i>MSN</i>	Moesin	● X-linked PID, increased bacterial and viral infections	61
<i>WDR1</i>	WD repeat-containing protein 1	● Poor neutrophil function	62
		● Neutropenia	
<i>NEIL3</i>	nei-like DNA glycosylase 3	● Autoimmunity	63
<i>C9ORF72</i>	Chromosome 9 open reading frame 72	● Autoimmunity, splenomegaly	64
<i>TNFAIP3</i>	TNF- α -induced protein 3	● Behçet syndrome	65
<i>OTULIN</i>	OTU deubiquitinase with linear linkage specificity	● Neonatal-onset inflammatory syndrome	66,67

homozygous mutation in transferrin receptor (*TFRC*) in patients with mild anemia, intermittent thrombocytopenia, and a combined immunodeficiency characterized by hypogammaglobulinemia and impaired T- and B-cell function, recapitulating the phenotype in a mouse model for *TFRC* deficiency. The mutation disrupts receptor-mediated iron internalization, which is necessary for T- and B-cell proliferation, as well as antibody class-switching. Despite the known importance of *TFRC* in erythrocyte development, the patients did not have severe anemia. This was attributed to the expression of six-transmembrane epithelial antigen of prostate 3 (*STEAP3*), a metalloredutase that associates with *TFRC* and facilitates its cell internalization, in erythroid precursors but not in lymphocytes.

Heterozygous mutations in phosphatase and tensin homolog (*PTEN*) were identified in 2 patients presenting with activated PI3K δ syndrome (APDS).⁵⁶ Both patients had recurrent lower respiratory tract infections, hepatosplenomegaly, and lymphadenopathy. *PTEN* negatively regulates the AKT/mTOR/S6 pathway. Thus defects in *PTEN* result in increased phosphorylation of AKT, mTOR, and S6 in activated T cells. Mutations in *PTEN* have also been previously identified in patients with *PTEN* hamartoma tumor syndrome, which can be associated with a CVID-like phenotype. Driessen et al⁵⁷ demonstrated that dysregulation in PI3K/Akt signaling in B cells from these patients results in impaired class-switch recombination and defective somatic hypermutation.

Kuehn et al⁵⁸ identified heterozygous mutations in *IKZF1*, the gene encoding the transcription factor IKAROS, in patients with CVID. Haploinsufficiency of *IKZF1* was associated with an inverted CD4/CD8 ratio, very low numbers of peripheral blood B cells, residual CD27⁺ memory B cells, and normal CD138⁺ bone marrow plasma cells. Some patients remain asymptomatic despite abnormalities in their immunologic laboratory profile, demonstrating the variable clinical penetrance of this disease. Another cause of autosomal dominant CVID was identified to be a novel heterozygous mutation in interferon regulatory factor 2 binding protein 2 (*IRF2BP2*), a transcriptional corepressor of the nuclear factor of activated T cells family.⁵⁹ Patients' B cells

did not form B-cell plasmablasts after *in vitro* stimulation with CpG, suggesting a defect in B-cell differentiation.

Eletto et al⁶⁰ identified homozygous mutations in Janus kinase 1 (*JAK1*) as a cause of Mendelian susceptibility to mycobacterial disease associated with early-onset metastatic bladder carcinoma. The mutation impaired phosphorylation of *JAK1* after stimulation with IFN- α and IFN- γ , leading to defective *JAK1*-mediated phosphorylation of STAT1/2 and STAT1, respectively, and decreased expression of multiple interferon-regulated genes. The authors highlighted the overlap in the clinical manifestations seen in patients undergoing treatment with the *JAK1/3* inhibitor tofacitinib and the genetic deficiency of *JAK1*.

Mutations in genes encoding regulators of cytoskeletal dynamics were shown to cause PIDs. Hemizygous mutations in moesin (*MSN*) were shown to result in X-linked immunodeficiency characterized by early-onset recurrent bacterial infections, as well as severe varicella virus-related infections.⁶¹ These patients have severe lymphopenia, hypogammaglobulinemia, neutropenia, and a poor immune response to vaccine antigens. Moesin is a member of the ezrin-radixin-moesin family of proteins that link cortical actin filaments to the plasma membrane. T cells from the patients had abnormalities in migration and adhesion in response to activation by chemokines and integrins.

Kuhns et al⁶² showed that patients with biallelic mutations in WD repeat-containing protein 1 (*WDR1*), the gene encoding actin-interacting protein 1 (*AIP1*), have recurrent infections, mild neutropenia, impaired wound healing, severe stomatitis, and premature death. *AIP1* promotes cofilin-mediated actin depolymerization. Loss-of-function mutations in *AIP1* results in the accumulation of polymerized actin, causing abnormal neutrophil morphology and migration.

Two new genes have been identified as important regulators of immune tolerance. Massaad et al⁶³ identified a mutation in the base excision repair enzyme nei-like DNA glycosylase 3 (*NEIL3*) in patients with autoimmunity, autoantibodies, and defective B-cell tolerance, a finding that was recapitulated in *Neil3*^{-/-} mice. The mice demonstrated increased apoptosis of splenic T and B cells and germinal center B cells, resulting in

TABLE IV. HSCT for PIDs: Outcomes reported in 2016

Genetic defect	No. of patients who underwent HSCT	HSCT outcomes	References
<i>CTPS1</i>	1	This patient was alive and well after matched unrelated donor HSCT with peripheral blood stem cells.	68
<i>LRBA</i>	4	- Two of 4 patients were alive and well. - Better outcome was seen with transplantations at an early age before patients had disease-related complications.	69
<i>STAT1</i> GoF	5	- Two of 5 patients were alive without serious complications: 1 received cord blood transplantation from a mismatched donor, and the other received phenotypically HLA-identical HSCT. - One patient died from persistent HLH, 1 from interstitial lung disease, and 1 from disseminated CMV infection.	71
<i>DOCK8</i>	11	- Ten of 11 patients were alive and well. - Patients had significant improvements in eczema and frequency and severity of infections. - Food allergies resolved in 8 of 10 patients.	73
<i>CTLA4</i>	8	- Six of 8 patients were alive and well. - One patient died of transplant-related causes, and 1 died of diabetic ketoacidosis. - Four patients had GVHD. - Seven patients had complete resolution of severe enteropathy and cytopenias.	74
<i>TTC7A</i>	4	- Diabetes persisted. - Reduced- or minimal-toxicity conditioning was used. - All patients were alive. - Normal immune reconstitution was achieved in all patients. - Intestinal disease persisted.	75
<i>FOXP3</i>	7	- Six of 7 patients had mixed chimerism despite initial full donor engraftment. - Four of 7 patients were alive, achieving eventual stable donor engraftment. - Three of the survivors needed cellular intervention by either peripheral blood stem cell boost or donor lymphocyte infusion.	76
<i>TAP1</i>	1	This patient was alive, with clinical manifestations improved after a partially matched HSCT.	77
CGD-causing mutations (83% X-linked CGD)	70	- Treosulfan-based conditioning was used. - There were 57 HLA-matched donors and 12 HLA-mismatched donors. - At 34 months, there was a 91.4% survival rate and 81.4% event-free survival. - Fifty-one percent of patients had acute GVHD, 12% of which were grade III to IV.	78

GoF, Gain of function; GVHD, graft-versus-host disease.

systemic release of self-antigens and predisposition to autoimmunity. Burberry et al⁶⁴ showed that mice with a loss-of-function mutation in the murine ortholog of chromosome 9 open reading frame 72 (*C9ORF72*) have splenomegaly, neutrophilia, thrombocytopenia, severe autoimmunity, and increased systemic inflammatory cytokines. *C9ORF72* is the most commonly mutated gene in patients with sporadic amyotrophic lateral sclerosis and frontotemporal dementia. *C9ORF72* also has a role in promoting immune tolerance within the hematopoietic system. *C9orf72* mutant bone marrow transferred an autoimmune phenotype when transplanted into wild-type recipients and wild-type recipients suppressed some of the immune dysregulation when transplanted into mutant animals.

In the field of autoinflammation, Zhou et al⁶⁵ identified heterozygous loss-of-function mutations in TNF- α -induced protein 3 (*TNFAIP3*) as a cause of a systemic inflammatory disease resembling Behçet syndrome. *TNFAIP3* encodes the deubiquitinase A20, an inhibitor of the NF- κ B pathway. Haploinsufficiency of A20 leads to increased NF- κ B signaling and overexpression of

proinflammatory cytokines, including IL-1 β , TNF- α , and IL-6. Patients with homozygous mutations in OTU deubiquitinase with linear linkage specificity (*OTULIN*), which also encodes a deubiquitinase in the NF- κ B pathway, were also reported to have severe systemic inflammation characterized by neonatal-onset fever, neutrophilic dermatitis, arthritis, and failure to thrive.⁶⁶ Similar to patients with A20 haploinsufficiency, *OTULIN*-deficient cells have NF- κ B overactivation and overexpression of proinflammatory cytokines. *OTULIN* deficiency in mice leads to embryonic lethality. Chimeric mice lacking *OTULIN* in their hematopoietic cells only experience severe systemic inflammation. Treatment with TNF inhibitors ameliorates the systemic inflammation in *OTULIN*-deficient human subjects and mice.⁶⁷

Extended phenotypes of previously reported PIDs

It is increasingly recognized that mutations in 1 gene might result in more than 1 clinical presentation. This characteristic of

PIDs and other genetic conditions has been termed phenotypic heterogeneity. Multiple articles published in 2016 aimed to describe clinical presentations for known gene defects that were distinct from the original description.

CTP synthase 1 (CTP1) deficiency has been known to result in early-onset severe chronic viral infections, recurrent encapsulated bacterial infections, and EBV-related B-cell non-Hodgkin lymphoma. Trück et al⁶⁸ have expanded this phenotype in their report of 2 siblings with *CTP1* mutations resulting in recurrent respiratory tract infections, chronic diarrhea, and eczema. Only 1 sibling had evidence of EBV infection. Both patients had increased percentages of transitional B cells and naive CD8⁺ T cells, with reduced percentages of naive B cells. IFN- γ production in response to T-cell agonists was significantly reduced. One sibling successfully underwent HSCT without complications.

The extended phenotype of LPS-responsive beige-like anchor protein (LRBA) deficiency was shown in a cohort of 22 LRBA-deficient patients reported by Gamez-Diaz et al.⁶⁹ Seventeen novel mutations were identified. Although the majority of the reported mutations abrogated protein expression, 2 mutations permitted residual expression. Twenty of the reported patients presented at an age of less than 12 years. Clinical manifestations included immune dysregulation (95%), organomegaly (86%), recurrent infections (71%), and hypogammaglobulinemia (57%). The most common abnormalities in immunophenotype were decreased numbers of regulatory T cells (73% of patients), low percentages of switched memory B cells (80% of patients), and low percentages of plasmablasts (90% of patients). The majority of the living patients are managed with immunoglobulin replacement or immunomodulatory drugs, including systemic steroids, abatacept, cyclosporine, mycophenolate mofetil, sirolimus, and rituximab. Four patients were treated with HSCT, but only 2 survived, demonstrating the high risk inherent in HSCT for these patients.

Autosomal dominant mutations in *PIK3R1*, the gene encoding the regulatory subunit (p85 α) of class 1A PI3Ks, cause APDS2. Elkaim et al⁷⁰ reviewed 36 patients with APDS2 caused by *PIK3R1* mutations in the donor and acceptor splice sites of exon 11. Recurrent upper respiratory tract infections, pneumonitis, and chronic lymphoproliferation were the most common features. Growth retardation was noticed in 45% of patients. Persistent EBV and/or CMV viremia was detected in several patients. Malignancy, particularly B-cell lymphoma, developed in 28% of patients. The majority of patients had decreased levels of IgG and IgA, B-cell lymphopenia, increased percentages of transitional B cells, and a decreased number of naive CD4⁺ and CD8⁺ cells. For 2 patients, rapamycin treatment was beneficial, but the majority of patients are managed with immunoglobulin replacement.

Toubiana et al⁷¹ enrolled 274 patients from 40 countries to describe the clinical spectrum associated with *STAT1* gain-of-function mutations. Predominant clinical features included chronic mucocutaneous candidiasis (98% of patients), recurrent skin and pulmonary bacterial infections (74% of patients), and viral infections (38% of patients). Autoimmune manifestations, particularly hypothyroidism, were seen in 37% of patients. Invasive infections, cerebral aneurysms, and cancers were the cause of premature death in 12% of patients. Circulating IL-17A-producing T-cell counts were low in the majority (82%) of the tested patients. The first line of management in patients is long-term antifungal therapy, but CMC persisted in 39% of patients

receiving antifungal treatment. Five patients with severe CMC and recurrent infections underwent HSCT, but only 2 are currently alive. Second-line treatments, including GM-CSF, granulocyte colony-stimulating factor, and inhibitors of the JAK-STAT pathway have been tried, but more data are needed to quantify the benefit.

Vece et al⁷² reviewed the knowledge on coatamer protein complex subunit α (COPA) syndrome caused by mutations in the *COPA* gene, encoding the COP α protein, which is essential for Golgi-endoplasmic reticulum transport. COPA syndrome is an autosomal dominant disease with variable penetrance. Defects in COP α lead to increased levels of endoplasmic reticulum stress and aberrant cellular autophagy. Seventy-six percent of reported patients present at less than 5 years of age with a combination of arthritis, interstitial lung disease with pulmonary hemorrhage, and autoantibodies. The immune profile of the patients shows increased T_H17 cells and proinflammatory cytokine expression, including IL-1 β and IL-6. Acute exacerbations are usually managed with cyclophosphamide, rituximab, or systemic corticosteroids.

Progress in PID treatment

Allogeneic HSCT remains the main curative therapy for the most severe PIDs. Several studies reported on the outcomes of patients with PIDs undergoing HSCT and advanced our understanding of disease-specific considerations for HSCT. Additionally, multiple articles discussed targeted therapies that affect the involved pathway in patients with different disorders (Table IV).^{68,69,71,73-78}

Al-Herz et al⁷³ performed a retrospective review of HSCT outcomes for 11 patients with DOCK8 deficiency, a combined immunodeficiency with a high morbidity and mortality rate but without immune reconstitution. The authors reported a survival rate of 91% and significant improvements in the frequency and severity of infections. Although full donor chimerism was achieved in the 8 patients who received a matched related or unrelated donor transplant, the 3 recipients of a mismatched unrelated donor transplant had mixed chimerism. Importantly, the patients with mixed chimerism had high levels of donor chimerism in T cells and switched memory B cells, which is consistent with a high selective advantage of these cell populations. The study supports the efficacy of HSCT in the treatment of DOCK8 deficiency and advocates for early diagnosis and aggressive management.

Slatter et al⁷⁴ reported the experience of HSCT in 8 patients with cytotoxic T-lymphocyte antigen 4 (CTLA-4) deficiency, 7 of which underwent transplantation before diagnosis for manifestations of life-threatening, treatment-resistant immune dysregulation. The majority of patients had 100% donor chimerism, and 7 of 8 patients had complete resolution of severe enteropathy and cytopenias after transplantation. Of note, 4 of 8 patients had graft-versus-host disease, despite having well-matched donors. The authors suggested that pretransplantation and/or posttransplantation management might need to be adjusted, accounting for the patient's high level of inflammation entering HSCT that could promote the development of alloreactivity.

Immunodeficiency associated with a severe form of very early-onset inflammatory bowel disease and multiple intestinal atresias is caused by mutations in the tetratricopeptide repeat domain 7A (*TTC7A*) gene. Published median survival for this disease is less than 12 months of age. Kammermeier et al⁷⁵ reported long-term

follow-up after HSCT of 4 TTC7A-deficient patients. All patients underwent multiple surgeries in the neonatal period to address intestinal strictures and required parenteral nutrition. HSCT was done with reduced-toxicity conditioning or serotherapy alone and resulted in normal immune reconstitution in all cases, whereas intestinal inflammation and an abnormal epithelial phenotype caused by the defect in nonhematopoietic cells persisted.

Kucuk et al⁷⁶ published a single-center 10-year experience of HSCT for 7 patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome caused by mutations in the forkhead box P3 (*FOXP3*) gene. Five of 7 patients received a matched unrelated donor HSCT, 1 received a matched sibling donor HSCT, and 1 received an umbilical cord blood HSCT. All but 1 patient had mixed chimerism, despite initial full donor engraftment. Four of 7 patients survived and achieved eventual stable donor engraftment. However, 3 of the survivors needed cellular intervention by either peripheral blood stem cell boost or donor lymphocyte infusion.

Gao et al⁷⁷ described successful immune reconstitution by means of HSCT in a 13-year-old girl with MHC class I deficiency caused by a homozygous deletion from exon 3 of transporter, ATP-binding cassette, major histocompatibility complex 1 (*TAP1*) through exon 11 of *TAP2*. Posttransplantation *TAP1* and *TAP2* protein expression was restored, MHC class I expression returned to normal, and clinical manifestations improved.

A long-term multicenter experience of 70 patients with CGD undergoing HSCT using treosulfan-based conditioning was reported by Morillo-Gutierrez et al.⁷⁸ Treosulfan is considered to have a low toxicity profile, but long-term effects are not yet well documented given its only relatively recent use in some centers. The large majority of patients had pre-HSCT high-risk factors, including infection, autoimmunity, or both. At a median follow-up of 34 months, treatment was well tolerated, with an overall survival (OS) of 91.4% and event-free survival of 81.4%.

Hartz et al⁷⁹ analyzed the relationship of donor chimerism and occurrence of HLH reactivation after HSCT for FHL in a retrospective international multicenter study. The authors found that an approximate chimerism threshold of more than 20% to 30% after the immediate posttransplantation period (>180 days after transplantation) conferred a decreased risk of late HLH reactivation, whereas lower chimerism levels did not inevitably result in disease recurrence. In the most vulnerable period of immune suppression during the first 6 months after HSCT, low chimerism levels likely also contributed to HLH reactivation, but a definitive protective threshold could not be determined. The study highlights the importance of optimizing HSCT protocols and carefully considering cellular intervention in patients with decreasing or long-term low chimerism status.

Two studies evaluated the safety and efficacy of adoptive transfer of virus-specific T cells (VSTs) in patients with PIDs.^{80,81} VSTs are derived from an HSCT donor or third-party donor or from cord blood and generated by means of different methods, including *ex vivo* culture and expansion or direct isolation from peripheral blood. In a multicenter retrospective study, Naik et al⁸⁰ described 36 patients with CMV, EBV, adenovirus, or human herpesvirus 6 infections receiving VSTs before or after HSCT. A partial or complete response against targeted viruses was observed in 81% of patients. Treatment was overall well tolerated. Graft-versus-host disease occurred in 4 patients and

was thought to be likely related to transplantation rather than VST infusions.

Credy et al⁸¹ reported use of VSTs in 15 cases of post-HSCT viral reactivation with CMV, adenovirus, or both and found a complete antiviral response in one third of the patients. The high morbidity and mortality in this patient population make it challenging to accurately identify VST-related adverse events. Clinical efficacy and advances in production technology are promising and might result in greater availability and use of this therapeutic approach in the future.

Multiple studies reported on the progress in determining the efficacy and safety of gene therapy in the treatment of PIDs. De Ravin et al⁸² described successful gene therapy using a self-inactivating lentiviral vector with low-dose busulfan conditioning in 5 older patients with SCID-X1 in whom previous haploidentical HSCT failed. In patients with a greater than 2-year follow-up, selective expansion of gene-marked T, NK, and B cells was achieved, corresponding to improvement in humoral immune function.

Cicalese et al⁸³ reported on the long-term outcome of 18 patients with adenosine deaminase-deficient SCID for whom an HLA-matched related donor was not available and who underwent gene therapy with a retroviral vector and low-dose busulfan preconditioning. After a median follow-up of 6.9 years, all patients survived and had persistent T-cell reconstitution, stable lymphocyte adenosine deaminase activity levels, and no evidence of leukemic transformation. Although B-cell counts remained low after gene therapy, significant improvements in B-cell function were shown, resulting in less intravenous immunoglobulin dependence.

Hubbard et al⁸⁴ investigated the use of targeted gene editing for correcting X-linked hyper-IgM syndrome (X-HIGM). Effective restoration of CD40L protein expression and function in primary human T cells was demonstrated by using homology-directed repair through engineered nucleases. The edited X-HIGM CD4 T cells were shown to induce naive B cells to class-switch *in vitro*, confirming restored function. In contrast to gene transfer using viral vectors, this methodology preserves endogenous regulation of transgene expression. This might provide a significant advantage in correcting defects like X-HIGM, in which unregulated overexpression of CD40L has led to lymphoproliferative disease in a mouse model of gene therapy.

To study the safety of gene therapy in the treatment of radiosensitive SCID caused by Artemis deficiency, Rivera-Munoz et al⁸⁵ developed a transgenic mouse model highly overexpressing human Artemis (up to 40 times) protein. Transgenic mice were found to have normal T- and B-cell development and function without evidence of increased lymphoid malignancies. Results support the safety of gene therapy in Artemis-deficient patients.

The feasibility of gene transfer for correcting the cytotoxic defect in patients with FHL was tested by Soheili et al,⁸⁶ who transferred gene-corrected mature CD8⁺ T cells derived from patients with FHL3 caused by Munc13-4 deficiency into immunodeficient mice bearing EBV-induced B-cell lymphoma. The gene-corrected T cells were shown to have restored Munc13-4 protein expression and intact granule release capacity. Adoptive transfer of Munc13-4-corrected EBV-specific T cells led to tumor regression, demonstrating restored *in vivo* T-cell cytotoxic function and a potential role of this therapy for treating FLH.

Severe hyperinflammation with immunopathologic manifestations in multiple tissues are characteristic of HLH. Hyperactivated CD8⁺ T cells and macrophages that produce excessive amounts of proinflammatory cytokines, such as IFN- γ , TNF- α , IL-6, and IL-18, are responsible for target organ damage. Signaling downstream of IFN- γ , IL-2, and IL-6 involves activation of the JAK-STAT pathway, which activates gene transcription. The therapeutic efficacy of the JAK1/2 inhibitor ruxolitinib was reported in 2 murine studies using models for primary and secondary HLH.^{87,88} Inhibition of JAK1/2 signaling *in vivo* significantly increased the long-term survival rate by improving weight loss, organomegaly, anemia, thrombocytopenia, increased cytokine levels, and lymphocytic infiltration of the spleen, liver, and central nervous system. These studies provide promise for the potential use of JAK inhibition in the treatment of primary and secondary HLH.

In a clinical setting ruxolitinib was used in the treatment of 3 children with gain-of-function mutations in *TMEM173*.⁸⁹ *TMEM173* encodes the stimulator of interferon genes (STING) protein, which senses cytosolic DNA derived from bacteria and viruses and promotes production of type I interferons. Constitutive activation of STING gives rise to the autoinflammatory disease STING-associated vasculopathy with onset in infancy, leading to severe vasculopathy involving multiple organ systems. Fremont et al.⁸⁹ noted a marked amelioration in the clinical phenotype of all 3 children treated with ruxolitinib. This case series supports the role of JAK inhibition in the management of autoinflammatory disorders involving type I interferon activation.

IL-7 is a hematopoietic growth factor that plays a central role in T-cell development, homeostasis, and survival. Sheikh et al.⁹⁰ explored the use of recombinant human IL-7 as a therapy for idiopathic CD4 T-cell lymphopenia. Treatment with recombinant human IL-7 was well tolerated and led to an increase in peripheral blood CD4 T-cell numbers in 8 of 9 study patients and to increased numbers of tissue CD3 T cells in gut mucosa and bone marrow, making it a promising intervention for patients with idiopathic CD4 T-cell lymphopenia.

Migliavacca et al.⁹¹ reported the first case of using pioglitazone in the treatment of patients with CGD. Pioglitazone, a peroxisome proliferator-activated receptor γ agonist, enhances mitochondrial ROS production, partially overcoming defective ROS production seen in patients with CGD. The authors described a case of a child who showed clinical improvement along with enhanced DHR fluorescence as a quantitative measurement of the oxidative burst of the child's granulocytes after treatment with pioglitazone. This intervention led to clinical stabilization that allowed proceeding with a HSCT for definitive treatment.

Lee et al.⁹² investigated using abatacept, a soluble fusion protein composed of the IgG₁ Fc region linked with the extracellular domain of CTLA-4-Ig, for treating a child with refractory multi-system autoimmune disease caused by CTLA-4 haploinsufficiency. Treatment resulted in attenuation of inflammatory cytokine production, including IL-10, IL-17A, and IL-6. Moreover, it led to dramatic clinical improvement of the disease manifestations, controlling the patient's severe autoimmune enteropathy and cytopenias without requiring other immunosuppressants.

Arts et al.⁹³ reported the therapeutic effect of INF- γ in 3 patients with a history of recurrent severe mucosal herpesvirus infection. Their immunologic studies had shown absent INF- γ production by PBMCs stimulated with a TLR3 agonist. Whole-genome

sequencing was not helpful to definitively identify a gene defect. After starting recombinant INF- γ 3 times a week, the number of viral reactivation episodes decreased from once or twice a month to once a year.

Specific control of T_H17 responses might be helpful to control autoimmune conditions in which there is excess inflammation mediated by T_H17 cytokines. de Wit et al.⁹⁴ targeted the T_H17 pathway by using 2 small molecules that were selected to best interfere with the binding of steroid receptor coactivator 1 to the retinoic acid receptor orphan related γ t. The addition of these molecules *in vitro* reduced T_H17 skewing and IL-17A secretion by CD4 T cells from healthy control subjects and patients with inflammatory conditions, such as inflammatory bowel disease and ankylosing spondylitis.

Another example of the use of small molecules targeting the control of inflammatory diseases was illustrated by Borroto et al.⁹⁵ They reported the effects of a molecule that interferes with T-cell activation, effectively preventing development of autoimmune encephalitis and psoriasis in mouse models without increasing their susceptibility to infections. The authors hypothesized that this small molecule might be active only when interacting with low-affinity antigens, although not with high-affinity antigens, which are most characteristic of pathogens.

Possibly the most formidable challenge in the treatment of autoimmune disease is the restoration of tolerance. It has been postulated that treatment with rituximab eliminates autoreactive B cells and might induce cure of autoimmunity. Indeed, patients with autoimmune disorders have shown benefit after receiving rituximab; however, the therapeutic effect is often transient. Chamberlain et al.⁹⁶ studied B-cell self-antigen specificities from patients with type I diabetes mellitus who received rituximab and demonstrated a decrease in the requirement for exogenous insulin. However, the percentage of autoreactive B-cell clones remained similar before and 52 weeks after rituximab treatment.

Neonatal thymectomy

With advances in heart surgery, mortality caused by cardiac malformations has decreased significantly. Heart surgery techniques include removal of the thymus, resulting in mild-to-moderate T-cell lymphopenia, although without a significant increase in susceptibility to infections. This progress has allowed investigations on the role of the thymus after birth. Studying the T-cell compartment in infancy and a decade after neonatal thymectomy, van den Broeck et al.⁹⁷ described that in the first years (1-5 years) after thymectomy, there is a decrease in naive T-cell numbers and a compensatory increase in memory and effector T-cell numbers. In children surviving 10 years after neonatal thymectomy, T-cell phenotyping discriminated 2 groups of patients: those with low CD31⁺ naive T-cell percentages (n = 7), who were also lymphopenic, and those with normal or high CD31⁺ naive T-cell percentages (n = 17), with a lymphocyte phenotype similar to that of healthy donors. A similar study in Sweden⁹⁸ analyzing lymphocyte phenotypes of children who had heart surgery 18 years earlier showed a similar decrease of T-cell numbers and reduction of naive T-cell numbers. In addition, this study reported oligoclonality of CD8 T cells and shortened telomere in T cells, whereas B-cell telomere size was conserved. These studies continue to uncover long-term immunologic alterations caused by absence of thymus, although there is not a clear significant clinical consequence. However, researchers

are calling for conservation of thymus tissue during heart surgery when possible.

HIV

Research in HIV continues to contribute to our understanding of basic and clinical immunology. HIV targets CD4 T cells and leads to CD4 T-cell depletion and immunodeficiency in patients infected with HIV unless treated with anti-HIV medications. Although a combination of these drugs has proved to be effective in controlling HIV replication, they have significant side effects, and discontinuation of treatment is associated with viral reactivation. One current focus in HIV research is the development of an effective vaccine by using complementary strategies to induce effective specific immune responses. Hu et al⁹⁹ used a rhesus macaque model to demonstrate that targeting conserved epitopes of pGag results in high protective cellular immunity. The use of conserved epitopes produces a broader range of cellular responses, including those to antigen epitopes considered “subdominant.” By amplifying full-length copies of proviral DNA in CD4 T cells, Bruner et al¹⁰⁰ showed that more than 93% of infected T cells from treated HIV-infected patients contain provirus defective with either deleted or hypermutated HIV genes. This is of importance to correctly estimate cure strategies because estimates of reservoir load might include defective proviruses of uncertain significance.

Many efforts are reportedly aimed at the development of broadly neutralizing antibodies directed against epitopes of Env binding to CD4.¹⁰¹⁻¹⁰³ One of them, 3BNC117, was shown to decrease viremia after treatment interruption when it was administered passively to 13 HIV-infected patients who were receiving antiretrovirals. This antibody also appeared to induce an increase in host antiviral antibody levels and to accelerate the clearance of human HIV-infected cells in a mouse model. Eventually, all patients had viral load rebounds, with strains resistant to the neutralization by this antibody, suggesting reactivation of dormant variants.

Mucosal vaccination is desirable because it does not involve injections and addresses mucosal immunity, a port of entry for several common infections, including HIV infection. One difficulty is poor antigen uptake through the mucosa epithelia, although a successful influenza vaccine using this route is available. Rochereau et al¹⁰⁴ reported delivery of HIV p24 linked to secretory IgA intranasally in a mouse model, with detection of specific IgG and IgA levels in serum, saliva, and vaginal lavage fluid, as well as specific anti-HIV cytotoxic responses.

CONCLUSIONS

Research in basic and clinical immunology continues to advance, with the identification of new genes involved in the immune response and in the characterization of their function. Gene-sequencing approaches are described to highlight their potential, as well as their limitations, in the discovery of genetic causes for immune syndromes. Treatment approaches with HSCT and gene therapy for PIDs are expanding to establish effectiveness and indications, and new uses of medications are being explored. Significant research is being focused on the control of immune dysregulation and autoimmunity. The role of the thymus after birth has started to be defined. HIV research is now focused on developing an effective vaccine, a goal that has remained elusive in part because of the extraordinary mutation rate of the virus.

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