Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *The Year in Immunology*

Severe combined immunodeficiency—an update

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Severe combined immunodeficiencies (SCIDs) are a group of inherited disorders responsible for severe dysfunctions of the immune system. These diseases are life-threatening when the diagnosis is made too late; they are the most severe forms of primary immunodeficiency. SCID patients often die during the first two years of life if appropriate treatments to reconstitute their immune system are not undertaken. Conventionally, SCIDs are classified according either to the main pathway affected by the molecular defect or on the basis of the specific immunologic phenotype that reflects the stage where the blockage occurs during the differentiation process. However, during the last few years many new causative gene alterations have been associated with unusual clinical and immunological phenotypes. Many of these novel forms of SCID also show extra-hematopoietic alterations, leading to complex phenotypes characterized by a functional impairment of several organs, which may lead to a considerable delay in the diagnosis. Here we review the biological and clinical features of SCIDs paying particular attention to the most recently identified forms and to their unusual or extra-immunological clinical features.

Keywords: severe combined immunodeficiency; SCID; primary immunodeficiency; nude/SCID; DiGeorge syndrome; cytokine; thymus

Introduction

Severe combined immunodeficiencies (SCIDs) are a group of inherited disorders responsible for severe dysfunctions of the immune system that lead to the absence or dysfunction of the T and B cells derived from the thymus gland and bone marrow, thus affecting both cellular and humoral adaptive immunity. Recently, Kwan *et al.*, on the basis of data obtained from 11 U.S. newborn screening programs in the general population, reported an incidence of SCID of 1 in 58,000 live-births, an incidence much higher than the previous estimate of one in 100,000 based on retrospective clinical diagnosis of SCID.[1] This group of diseases belongs to the most severe forms of primary immunodeficiency (PID), which are often fatal when the diagnosis is made too late.[2] Even though children with SCID appear healthy at birth, they are predisposed to severe bacterial, viral, and fungal infections as the maternal transferred antibodies decline. During the first year of life, failure to thrive, diarrhea, and oral candidiasis are common findings; *Pneumocystis jiroveci* may frequently cause a severe interstitial pneumopathy; and maternal engraftment of lymphocytes can cause graft-versus-host disease (GVHD).[3] SCID patients often die during the first two years of life if appropriate treatments to reconstitute their immune system are not undertaken.[4] For most patients, the only curative treatment is the allogeneic hematopoietic stem cell transplantation (HSCT).[5] Gene therapy offers a cure for two specific forms of SCID and, although other SCID forms may become amenable to this treatment in the future, it is likely that HSCT will continue to be used for the majority of SCID patients.[6]

Conventionally, SCIDs can be classified according either to the main pathways affected by the molecular defect or on the basis of the specific immunologic phenotype related to that genetic defect, as T cell–deficient but normal B cell (T^-B^+) SCID and both T cell– and B cell–deficient (T^-B^-) SCID, with a further subdivision depending on the presence or

doi: 10.1111/nyas.12849

absence of NK cells (NK^+ or NK^-, respectively).[2] This classification, traditionally considered as representative of the stage where the blockage occurs during the differentiation process, was, until a few years ago, very useful in directing molecular studies toward a certain genetic alteration. However, during the last years many new causative gene alterations have been identified with peculiar clinical and immunological phenotypes. In a few cases, the genetic alteration allows for a normal T cell differentiation program but compromises T cell functionality by affecting the initial or final phase of intracellular signaling. These functional T cell disorders are characterized by immune dysregulation and cancer predisposition, as well as infections. In addition, hypomorphic mutations in some SCIDs genes make possible the development of nonfunctional oligoclonal T cells that are responsible for a complex of clinical conditions that may include hyperinflammation or autoimmunity. Many of the novel forms of SCID also show extra-hematopoietic alterations, leading to complex phenotypes characterized by functional impairment of organs different from primary lymphoid organs, which can make the diagnostic process very complex by standard methods. Taking this into account, the traditional international classification of SCIDs based on immunophenotype may no longer be optimal for clinical and research purposes[7,8]—diagnostic criteria have to be continuously updated to take into account these unusual phenotypic presentations. In his work of 2014, Shearer emphasizes that currently there is no consensus among clinical immunologists on how best to diagnose and treat these rare disorders. It is not surprising that an important clinical dilemma concerns the distinction of SCIDs from other diseases such as combined immunodeficiencies (CIDs). Recently, it was proposed that patients who exhibit an absence or a severe reduction of T cells ($CD3^+ < 300/\mu L$), absence or severe reduction (<10% of the lower limit) of a proliferative response to phytohemagglutinin, or a maternal lymphocyte engraftment should be defined as having typical SCID.[5] Moreover, the European Society for Immunodeficiency suggested as criteria for the diagnosis of CID the presence of one of the following parameters: one severe infection, an immunodysregulation disorder, cancer, familial CID associated with moderate age-related reduction of $CD3^+$, $CD4^+$, $CD8^+$ T cells or of naive T cells. However, a cutoff to distinguish SCID from CID has not yet been well defined.

A main aim of this review is to report on the biological and clinical features of SCID, paying attention to the most recently identified forms and to the unusual or extra-immunological clinical features (Table 2). An attempt to relate together pathogenetic mechanisms to specific clinical features is proposed (Table 1).

SCID due to defective survival of hematopoietic lineage precursors

Reticular dysgenesis (RD) is an autosomal recessive form of SCID characterized by both early myeloid lineage differentiation arrest and impaired lymphoid development.[9] It is considered the most severe form of SCID, accounting for less than 2%. A peculiarity of this disorder is the presence of sensorineural deafness. RD is caused by biallelic mutations in the adenylate kinase 2 gene (*AK2*), which cause the absence or the strong reduction of the expression of AK2 protein.[9,10] The syndrome is characterized by the absence of granulocytes and lymphocytes in peripheral blood. Compared to all the other forms of SCID, RD-associated neutropenia, which is unresponsive to granulocyte-colony stimulating factor (G-CSF), predisposes the patients to severe infections.[11] The only available treatment for RD is allogeneic HSCT, which indicates that the inherited defect is cellular and not linked to the micro-environment, as previously thought. Neutrophil differentiation abnormalities of RD patients are corrected by the restoration of AK2 expression in the bone marrow, thus confirming the specific role of AK2 in the development of the myeloid lineage.[12] Moreover, AK2 is specifically expressed in the stria vascularis region of the inner ear, which explains the sensorineural deafness observed in these individuals.[10] AK2 is localized in the mitochondrial intermembrane space where it regulates adenine nucleotide interconversion within the intermembrane space;[13] a very similar function is mediated by the cytoplasmatic enzyme AK1. The function of AK1/2 is classically described to be the maintenance of a constant concentration of adenine nucleotides and the monitoring of mitochondrial energy state through a fine mechanism of nucleotide sensing and signaling. The molecule also plays a central role in the control of apoptosis through the Fas-associated protein with death domain (FADD)

Table 1. New clinical phenotypes associated with old forms of nonsyndromic SCID/CID and new genetic defects

Gene defect	Old phenotype	New phenotype	Pathogenetic mechanism	Reference
AK2	Absence of granulocytes, severe lymphopenia sensorineural deafness	OS	Peripheral expansion of oligoclonal T lymphocytes	15
IL2RG (γc)	$T^-B^+NK^-$ SCID, leaky $T^+B^+NK^-$ SCID, immune-dysregulation and autoimmunity	Hodgkin like features, invagination and HLH	Not clear; maternal GVHD	55,56
JAK3		Selective $CD4^+$ T lymphopenia	Hypomorphic mutation associated with somatic chimerism	51
RAG	Severe hypogammaglobulinemia, marked reduction of T and B cells, OS, incomplete OS	Granulomatous lesions, EBV-related lymphoma, Idiopatic $CD4^+$ T lymphopenia with extensive chickenpox	Hypomorphic mutations	70
CORO1A	$T^-B^-NK^+$ SCID, severe postvaccination chickenpox, language delay, behavioral and cognitive impairment	EBV B cell lymphoproliferation	Not clear; null and hypomorphic mutations of *Coro1A* in mice are associated with defects in T cell survival and migration	79
FOXN1	Human nude/SCID	Eczematous rash, erythroderma, severe diarrhea and alopecia	Residual T cell development sustained by rudimentary thymus or extrathymic lymphoid sites	80
IL21R	NA	Cryptosporidiosis, chronic cholangitis and liver disease, abnormal IL-21 induced proliferation, defect of immunoglobulin class-switching, and NK cell cytotoxicity	Abrogation of IL-21 ligand binding, defective cytokine secretion	99
ZAP70	Selective $CD8^+$ lymphopenia and normal/elevated numbers of not functional $CD4^+$ T cells	Late onset disease, cutaneous, erythematous lesions, immune dysregulation erythroderma	Possible role of hypomorphic mutations on T lymphocytes effector and suppressive function	113
MALT1	NA	CID	Abnormal IL-12 production, failure of IκBα degradation	114
BCL10	NA	Profound T and B memory cell deficiency, severe hypogammaglobulinemia	Impairment of NF-κB pathways	115
CARD11	NA	CID	Abnormal IL-12 production, T_{reg} cells deficiency	101
TTC7A	NA	CID-MIA	Defective thymopoiesis	116
LCK, UNC119	NA	$CD4^+$ lymphopenia, restricted T cell repertoire, immune dysregulation	Impaired TCR signaling	122
IKBK2	NA	Mycobacterium avium and tuberculosis infections, neurological impairment, hypogammaglobulinemia, normal T cells count with absence of T_{reg} and γ/δ T cells	Impairment of IKK2–NF-κB signaling	124

Note: OS, Omenn syndrome; HLA, hemophagocytic lymphohistiocytosis, GVHD, graft versus host disease; MIA, multiple intestinal atresia; NA, not applicable.

Table 2. Pathogenetic mechanisms of SCID

Pathogenetic mechanism	Defect	Phenotype	Inheritance
Defective survival of haematopoietic precursors	AK2	$T^-B^-NK^-$	AR
Toxic metabolite accumulation	ADA	$T^-B^-NK^-$	AR
	PNP	$T^-B^+NK^-$	AR
Cytokine signaling anomalies	IL-2RG	$T^-B^+NK^-$	XL
	JAK3	$T^-B^+NK^-$	AR
	IL-7RA	$T^-B^+NK^+$	AR
V(D)J recombination and TCR abnormalities	RAG1/RAG2, Artemis, DNA-PKcs, Cernunnos, LIG4	$T^-B^-NK^+$	AR
TCR abnormalities	CD45	$T^-B^+NK^+$	AR
	CD3ε, δ, ζ	$T^-B^+NK^+$	AR
	CORO1A	$T^-B^-NK^+$	AR
Thymic abnormalities	FOXN1	$T^{-/low}B^+NK^+$	AR
	DiGeorge sindrome	$T^-B^+NK^+$	De novo or AD

and caspase 10 pathways.[14] Omenn syndrome (OS), resulting from residual development and peripheral expansion of oligoclonal T lymphocytes, has recently been described in a patient with RD due to missense mutation in *AK2*.[15] OS is a clinical condition characterized by generalized skin rash, hepatomegaly, splenomegaly, lymphadenopathy (similar to that which occurs in SCID patients with detectable $CD3^+$ T cells), absent or low T cell proliferation to common antigens, and no maternal engraftment. Increased IgE serum levels and eosinophil count are also common features. In rare patients with RD, no mutations in *AK2* have been found, suggesting a potential role for other molecules involved in this pathway. For instance, a similar phenotype has been described in a murine models either deficient for growth factor independence-1 (Gfi-1) or transgenic for expression of Gfi-1b nucleoproteins, suggesting a role for these two factors in the pathogenesis of RD.[16]

SCID due to accumulation of toxic metabolites

Adenosine deaminase (ADA) deficiency and purine nucleoside phosphorylase (PNP) deficiency are inherited disorders of the purine metabolism characterized by abnormal accumulation of toxic nucleoside products.[17] ADA deficiency is responsible for a T cell–, B cell–, and NK cell–deficient ($T^-B^-NK^-$) form of SCID associated with thymic hypoplasia and absence of lymphocyte proliferative response. Before the introduction of newborn screening, the incidence of this autosomal recessive disorder was estimated to be between 1:375,000 and 1:660,000 live births.[18] However, a recent trial on a population-based neonatal screening revealed that the incidence of ADA-SCID is much higher, and closer to 1:50,000.[19] The ADA gene of 12 exons is located in a 32 kb region on chromosome 20q13.11. Several genetic alterations, with more than seventy mutations, have been identified in ADA-SCID patients.[20] The product of *ADA* is an ubiquitous enzyme that catalyzes the irreversible deamination of adenosine (Ado) and deoxyadenosine (dAdo) to inosine and deoxyinosine, respectively. Despite ADA protein being present in virtually every cell of the human body, it is particularly expressed in the lymphoid system, especially in the thymus, where it plays a key role in its differentiation and maturation. The absence of ADA activity is responsible for a massive accumulation of Ado and dAdo, in particular in thymocytes, lymphocytes, and erythrocytes.[17,21] dAdo phosphorylation by nucleoside kinases leads to the production of deoxynucleotide triphosphates (dATP) whose accumulation, altering lymphocyte signaling pathways and serving as a danger signal, may cause the severe lymphopenia observed in ADA deficiency. Another alternative pathogenic mechanism proposed is the inhibition of *S*-adenosylmethionine–mediated transmethylation reactions required for

cell viability and normal differentiation.[22] By the first 6 months of age up to 80% of patients show multiple recurrent opportunistic infections that rapidly may become fatal and hypoplasia or apparent absence of lymphoid tissue. However, in the remaining patients, a late-onset phenotype, presenting at two or three years of life, or even later,[23] has been reported. These patients may also present with autoimmune diseases and usually exhibit a milder T cell immunodeficiency, which gradually progresses. Owing to its ubiquitous expression normally, ADA deficiency can affect several organs, leading to the development of skeletal alterations, such as anterior rib cupping, scapular spurring, and pelvic dysplasia, which can be reversible with appropriate therapy. In addition, pulmonary alveolar proteinosis, probably caused by a surfactant metabolism defect, and hepatic, gastrointestinal, and neurological disorders, mainly due to Purkinje cell damage, may be found. Bone marrow hypocellularity and myeloid dysplasia also have been observed in some ADA-deficient patients; in others, renal impairment.[24,25] A genotype–phenotype correlation has been documented and, in particular, severity of disease seems to correlate with residual ADA activity and the types of substrates that accumulate.[26] The therapeutic approach currently available for this particular form of SCID includes three options: enzyme replacement therapy with polyethylene glycol-modified bovine adenosine deaminase, HSCT, or gene therapy.[27–29] The use of dried blood spot samples tested by tandem mass spectrometry has been recently proposed as part of a neonatal program of screening in several countries.

Purine nucleoside phosphorylase gene (*PNP*) mutations result in an extremely rare autosomal recessive disorder accounting for 4% of all form of SCIDs.[30] Autoimmunity, recurrent infections, failure to thrive, and neurologic dysfunction are some of the main features of PNP deficiency. *PNP* maps to chromosome 14q13 and encodes a protein that catalyzes the phosphorolysis of guanosine, deoxyguanosine, inosine, and deoxyinosine, to their respective purine bases.[17,31,32] Mutations in the PNP pathways result in elevated deoxyguanosine triphosphate storage and in T cell toxicity due to the inhibition of the mechanisms of DNA synthesis and repair, resulting in an increased sensitivity to DNA damage and apoptosis, especially in T lymphocytes during selection within the thymus.[33] T cell defects typically become evident by the first year of life, with a milder phenotype than what is normally seen in ADA deficiency. PNP deficiency can be suspected when lymphopenia is associated with reduced PNP enzymatic activity in red blood cells in a patient with recurrent respiratory infections and other typical manifestations.[34] Low serum uric acid (hypouricemia) is usually found, although PNP deficiency should not be ruled out if patients do not exhibit it. The immunodeficiency in these patients is progressive, since the severe T cell deficiency usually appears after the second year of life and is characterized by a normal B cell compartment. Among the neurological disorders associated with PNP deficiency, ataxia, developmental delay, and spasticity have been described. Autoimmune diseases observed include hemolytic anemia and sclerosing cholangitis,[35] and in some patients megaloblastic or dysplastic bone marrow has been described.[33]

SCID due to cytokine signaling anomalies

Cytokines are soluble regulators of immune system homeostasis. Alterations of their signaling are implicated in the pathogenesis of the major SCIDs. In particular, SCIDs caused by defects of the common gamma chain (γc), Janus kinase 3 (JAK3), or the IL-7 receptor α chain (IL-7Rα) are prototypic cytokine-associated disorders, accounting for 67–74% of all cases of SCIDs.[36,37]

Mutations of γc gene cause X-linked SCID (X-SCID), one of the most common forms of SCID, accounting for 50% of all cases. The γc gene (*IL2RG*) maps to chromosome Xq13.1 and encodes a transmembrane protein that is a component of several cytokine receptors, including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, all critical for lymphocyte development and function.[38] The γc interacts with the intracellular tyrosine kinase JAK3, which acts as a transducing element[39] indispensable for cell growth and control of hematopoietic cell development. Evidence indicates that γc is widely expressed in non-hematopoietic cells as well, even though its function in these cells has not yet been clearly elucidated. It has been reported that γc is implicated in the growth hormone receptor signaling, suggesting the existence of a subtle interaction between endocrine and immune systems.[40–44]

JAK3, mainly expressed in lymphoid and myeloid cells, is essential for the differentiation of

hematopoietic precursors;[45–47] its deficiency is responsible for an autosomal recessive SCID. Molecular alteration of JAK3 may affect any of its functional domains and results in a $T^-B^+NK^-$ form of SCID, with a clinical phenotype similar to that observed in γc deficiency.[48] The immunological phenotype is due to the key role of γc/JAK3 signaling in both early T and NK cell, but not B cell, differentiation programs. However, B cell–intrinsic abnormalities, such as impaired class switch recombination and defective antibody production, have been documented. The identification of IL-7R–deficient SCID patients with a selective T cell defect[37] implies that the T cell defect observed in SCID due to mutations of γc/JAK3 results from defective IL-7 signaling. The ability of IL-15 to drive NK cell development[49] explains the lack of NK cells in γc/JAK3-deficient patients as a consequence of defective IL-15 signaling.[50] The molecular basis of the B cell functional abnormalities in patients with γc/JAK3 deficiency is probably linked to a defect in IL-21 secretion, a cytokine involved in proliferation, Ig isotype switching, plasma cell generation, and antibody secretion through activation of the JAK/STAT pathway.

Recently, hypomorphic mutations in JAK3 associated with somatic chimerism have been reported in a patient with predominant $CD4^+$ lymphopenia.[51] This observation suggests that hypomorphic mutations and/or somatic chimerism in other genes, which usually cause a SCID phenotype, eventually could be implicated in selective $CD4^+$ lymphopenia. Individuals with mutations that result in the production of a small amount of gene product or a protein with residual activity are less frequently seen. These individuals may have an atypical "leaky" disease characterized by $T^+B^+NK^-$ phenotype that is associated with immune dysregulation and autoimmunity, rashes, splenomegaly, gastrointestinal malabsorption, and/or short stature;[52,53] a few patients have presented with an OS phenotype,[54] which is characterized by elevated IgE, erythroderma, and an expansion of cells with a lymphocyte profile.

A peculiar extranodal lymphoproliferative disorder characterized by a polymorphous $CD20^+$ B lymphocyte infiltrate, resembling Hodgkin Reed-Sternberg cells, has also been observed in two patients affected with X-SCID.[55] Recently, a novel mutation in exon 5 of the γc gene has been reported that causes a classical severe immunological phenotype associated with invagination and hemophagocytic lymphohistiocytosis (HLH).[56] The HLH phenotype, previously described in two other cases with γc gene mutations,[57] is probably explained by maternal GVHD, and highlights the need for a fine-grained evaluation of the immunological phenotype and associated genotypes in patients with HLH.[58] As for the mechanism by which maternal engrafted T cells may be responsible for HLH in such cases, it is reasonable to hypothesize that unchecked T cell dysregulation of $CD8^+$ cells, activated by alloantigens, may result in cytokine hypersecretion and massive macrophage activation, eventually leading to hemophagocytosis.

The mutations of IL-7Rα gene (*IL7R*) cause a $T^-B^+NK^+$ SCID with an autosomal recessive transmission that is responsible for 10% of all SCIDs. The human *IL7R* maps to chromosome 5p13.2 and encodes for a protein[59] that is a component of two cytokine receptors, namely IL-7R and thymic stromal lymphopoietin receptor (TSLPR). Following the binding of IL-7 to IL-7R, JAK1 (coupled to IL-7Rα) and JAK3 are activated, which induces the phosphorylation of IL-7Rα, the recruitment of STAT5, and phosphatidylinositol 3-kinase (PI3K) at the receptor signaling apparatus. STAT5 molecules dimerize and translocate to the nucleus, leading to the transcription of IL-7–dependent genes. PI3K induces Akt activation, which prevents cell death through inhibition of Bad and regulates the kinase activity of Tor, eventually leading to the induction of several nuclear targets, including nuclear factor of activated T cells (NF-AT), NF-κB, and cyclin D1. Finally, activation of the Ras/MAPK/ERK pathway results in the induction of other nuclear targets, such as c-Myc, STAT1/3, and the Ets transcription factors. IL-7R is almost exclusively expressed by cells of the lymphoid lineage and is involved in thymocyte survival and maturation, particularly during $CD8^+$ positive selection.[60]

TSLPR, expressed mainly on monocytes, dendritic cells (DCs), and some types of T lymphocytes, is able to activate JAK2/STAT5 pathway, although this does not lead to cell proliferation. Human TSLP acts primarily on DCs, promoting DC-mediated expansion of $CD4^+$ T lymphocytes that acquire a memory T cell phenotype. The clinical phenotype of this form of SCID is quite heterogeneous and includes peculiar features such as OS,[61] cytopenia,[62]

severe and unresponsive cytomegalovirus (CMV) infection, or diarrhea of probable viral origin.[61]

SCID due to V(D)J recombination and TCR abnormalities

V(D)J recombination is a complex process that occurs in early B and T cell development. It is responsible of the introduction of site-specific DNA double strand breaks (DSBs) by the recombination activating genes (RAG) 1 and 2.[63,64] The cleavage of the hairpin and the joining of these segments requires the DNA nonhomologous end-joining (NHEJ) DNA repair factors, which generate the diversity through recombination of the V, D, and J segments and junction.

NHEJ also plays a role in preserving the genomic stability of cells exposed to X-ray DNA damage. Consistent with these functions, it is not surprising that mice lacking NHEJ components exhibit a SCID phenotype and radiosensitivity (RS), a phenotype referred to as RS-SCID. In humans, several mutations in NHEJ genes have been identified, including mutations in genes for DNA ligase IV (*LIG4*), XLF/Cernunnos (*NHEJ1*), DNA-PKcs (*PRKDC*), and Artemis (*DCLREIC*), that are associated with SCID.[64–66] Of note, the increased radiosensitivity peculiar to these forms of SCID can be used as a diagnostic tool.[67,68]

Owing to the essential role of RAG1/RAG2 genes in V(D)J recombination, mutations of *RAG1* and/or *RAG2*, associated with partial protein expression and limited production of T and B cells, have been associated with a $T^-B^-NK^+$ SCID, OS, and autoimmunity.[69] Hypomorphic RAG gene mutations have also been described in patients with granuloma formation[70] and EBV-related lymphoma.[71] Since different clinical phenotypes have been associated with similar RAG mutations resulting in the same biological effect, a complex pathogenetic mechanism, based not only on the residual recombinase activity but also on the type and the moment of antigenic pressure has been postulated.

Artemis deficiency causes T cell maturation and B cell differentiation arrest at the pre-B cell checkpoint, resulting in a $T^-B^-NK^+$ SCID.[68] DNA-PKcs is involved in Artemis regulation and activation by both phosphorylation and complex formation, thus regulating enzymatic activities critical for V(D)J recombination.[64,72] Deficiency of DNA-PKcs causes a phenotype similar to Artemis deficiency.

The deficiency of XLF/Cernunnos causes a $T^-B^-NK^+$ SCID phenotype associated with microcephaly.[73] In particular, the phenotype is characterized by a progressive decrease of B cells and the presence of only memory T cells. Crystallography studies showed that XLF/Cernunnos is a component of the LIG4/XRCC4 complex, which exerts a role in aligning the two DNA ends in the DNA repair complex machinery. Deficiency of LIG4 is responsible for facial dysmorphisms, microcephaly, and variable forms of PID, ranging from SCID/OS to hypogammaglobulinemia or moderate defects in T and B cell functions.[74]

Gene mutations that abrogate early TCR signaling are associated with profound abnormalities of T lymphocyte development and function. CD45 (leukocyte common antigen) is a transmembrane tyrosine phosphatase involved in both TCR signaling and T cell development within the thymus and B cell development and maturation. CD45 deficiency is responsible for a very rare form of $T^-B^+NK^+$ SCID in which lymph nodes lack germinal centers.[75] Despite a normal monocyte numbers, T lymphocyte numbers are considerably decreased, with normal expression of TCR $\gamma\delta$ chains but a reduction of TCR$\alpha\beta^+$ cells. B cells, even though nonfunctional, are increased in number.

CD3 is a multimeric complex involved in TCR signaling and required for T cell differentiation. Defects of the complex can involve all the chains, resulting in a $T^-B^+NK^+$ phenotype. Alterations of the subunits epsilon (CD3ε), delta (CD3δ), and zeta (CD3ζ), have been reported in patients with severe forms of SCID, while alterations of the CD3γ have been associated with a more benign course. These disorders are rare and inherited as autosomal recessive SCIDs. Some mutations can allow residual T cell maturation, even though the cross-talk between thymocytes and thymic epithelial cells may be impaired, thus compromising central tolerance and regulatory T cell (T_{reg}) development. Autoimmune manifestations, including autoimmune hemolytic anemia, vitiligo, Hashimoto's thyroiditis, autoimmune enteropathy, Evans syndrome, autoimmune hepatitis, and nephrotic syndrome are frequently observed in such patients.[76]

Coronin-1A is important for regulation of actin polymerization of cytoskeleton and essential for T cell migration from the thymus to the secondary lymphoid organs.[77] The human coronin-1A gene

(*CORO1A*) maps to chromosome 16p11.2 and encodes a highly conserved 57-kDa actin-binding protein expressed in both hematopoietic and immune cells. Coronin 1A–deficient neutrophils of mice have a normal adherence, membrane dynamics, migration, phagocytosis, and oxidative burst; dendritic cells are similarly not impaired. However, coronin 1A–deficient mice exhibit T cell lymphocytopenia and a normal number of B and NK cells, thus confirming its prominent role in T cell homeostasis and TCR signaling. In humans, deficiency of coronin 1A is associated with the absence of peripheral T cells.[78] However, different from other SCIDs due to other genetic alterations, a normal size thymus has been observed in the context of coronin 1A deficiency.[79] Hypomorphic *CORO1A* mutations have been associated with aggressive Epstein Barr virus–associated B cell lymphoproliferation, occurring at an early age.[79]

SCID due to thymic abnormalities: from DiGeorge syndrome to nude/SCID

The prototype of athymic disorders caused by abnormalities of the stromal component of the thymus—the primary lymphoid organ for T cell differentiation—is the nude/SCID syndrome, described in humans in 1996.[80] This form of SCID is the only one not primarily related to an intrinsic abnormality of the hematopoietic cell, but rather to a defect in hematopoietic cell–supporting thymic epithelial cells.[81–83] This human SCID is the equivalent of the murine nude/SCID phenotype described in 1966, although in humans the phenotype is more severe. It is one of the rarest forms of SCID, and only three mutations have been associated thus far with nude/SCID.[84] The gene responsible for the disease in humans is *FOXN1*, located on chromosome 17,[85] which encodes a member of the forkhead/winged helix class proteins; this same gene is mutated in the same type of SCID in mice and rats. Forkhead/winged helix proteins is a large family of transcriptional factors implicated in several biological processes governing development, metabolism, cancer, and aging. FOXN1 is mainly expressed in the epithelial cells of the skin and thymus, where it plays a role in maintaining the balance between growth and differentiation. Thymic epithelial cell precursors require FOXN1 for full differentiation into cortical and medullary thymic epithelial cells capable of supporting T cell development. In epithelial cells, FOXN1 contributes to keratinocyte proliferation and differentiation in hair follicles, and to the development of the choroid plexus epithelium; this could explain the major features that characterize patients with nude/SCID, namely the absence of the thymus, with a severe T cell defect (though normal B and NK cells) and abnormal skin development, including congenital alopecia and nail dystrophy. The syndrome belongs to the $T^-B^+NK^+$ subgroup of SCIDs.[81] Usually, there is a significant reduction of $CD3^+CD4^+$ T helper lymphocytes, while the number of $CD3^+CD8^+$ T cells is less reduced. Functionally, there is a severe impairment of the proliferative response to mitogens, as found in the other forms of SCIDs.

The mutations described in nude/SCID cause a complete absence of functional FOXN1 protein. The first known mutation identified in humans, R255X, truncates the protein before the start of the forkhead domain, while a second mutation, R320W, leads to a substitution in the protein's DNA binding domain. A third mutation, c.562delA, results in a frameshift and premature truncation of the protein (p.S188fs) after the first 24 amino acids of the forkhead domain. The disease is inherited as an autosomal recessive trait. Heterozygous patients show minor ectodermal anomalies, such as nail dystrophy and, in particular, leukonychia or koilonychia (spoon nail).[86,87] Recent studies support a role for FOXN1 as cofactor in the development and differentiation of the central nervous system.[88]

Bone marrow transplantation (BMT) to treat this nude/SCID, despite the favorable clinical course, often results in a progressive decline of the $CD4^+$ T cell compartment[89] owing to the fact that a normal thymus is necessary for the generation of the $CD4^+$ naive subset. Conversely, the production of $CD8^+$ naive lymphocytes after BMT is less thymus dependent and even occurs in nude/SCID patients. In addition, a recent study showed the presence of T lymphocytes in a $FOXN1^{-/-}$ human fetus, suggesting partial T cell ontogeny in a thymus- and FOXN1-independent process.[90] Thymus transplantation has been shown to lead to immune reconstitution in two nude/SCID patients affected with disseminated Bacillus Calmette-Guérin infection and cytopenia.[91]

Before the identification of human nude/SCID, the DiGeorge syndrome (DGS) was long considered the model of a severe T cell differentiation defect. DGS is a complex disorder that typically

comprises T cell deficiency due to thymic hypo/aplasia, hypoparathyroidism, conotruncal cardiac defects, facial abnormalities, cognitive defects, speech delay, other birth defects, and gastrointestinal disorders.[92] Deletion of 22q11.2 is the most frequent chromosomal change associated with DGS,[93] with an incidence of one in 4000–5000 live births. The alteration is inherited in a familial autosomal dominant pattern in 8–28% of the cases.[94] Most patients have a deletion of 3 Mb that includes about 30 genes, while in 8% of the cases a smaller deletion of 1.5 Mb containing 24 genes is detected. No specific genotype–phenotype relationship has been documented. Both deletions include the gene T-box transcription factor 1 (*TBX1*), which seems to be necessary for normal development of the thymus and parathyroid, the large arteries of heart, and the muscles and bones of face and neck. Thymic hypoplasia, responsible for the thymic dysfunction, is observed in more than 80% of patients. The syndrome may be associated with variable T cell deficiencies, ranging from close to normal T cell numbers and functions, to complete DGS with a $T^-B^+NK^+$ SCID-like phenotype accounting for less than 1% of DGS.[95] Recently, a phenotype characterized by a $T^-B^-NK^+$ SCID has been described in two DGS patients with a concomitant Artemis deficiency.[96] Patients with complete DGS, like other infants with SCID, suffer from severe opportunistic infections and exhibit a high risk of acquired GVHD if transfused. Furthermore, a few patients affected with an atypical complete DGS have mature T cells derived from maternal engraftment or oligoclonal expansion of memory T cells responsible for a severe inflammation. These patients may develop an OS, characterized by erythrodermia, enteropathy, and lymphadenopathy. On the other hand, there are also subjects carrying the deletion who only have a mild phenotype. Some patients diagnosed as 22q11.2DS in early childhood remain clinically asymptomatic and exhibit only minimal immune alterations. Increased prevalence of atopic and autoimmune diseases has been reported in patients with partial deletion syndrome.[97] While normal B, NK, and T cell numbers are frequently observed in 22q11.2DS individuals, sometimes, a decrease of $CD4^+$ and $CD8^+$ T lymphocytes may be found[97] due to lower thymic output of the naive T cell subset, oligoclonal T lymphocyte expansion,[98] or altered T cell differentiation. These observations can be explained by the dysregulation of peripheral T cell homeostasis due to a defect in IL-7 signaling, crucial for T lymphocyte survival and expansion and for homeostasis of the naive $CD4^+$ T cell pool. Indeed, subjects with 22q11.2DS show a significant decrease of $CD3^+$ T lymphocytes expressing IL-7Ra; adults have accelerated conversion of naive to memory cells, shorter telomeres, and a defect in the variability of the TCR repertoire.[98]

A DGS phenotype has been described in patients carrying a 10p deletion, the clinical features being almost undistinguishable from 22q11.2DS. Even though low numbers of T cells, reduced immunoglobulin,[80] and thymus hypoplasia have been observed in 28% of such patients, none have been affected with a severe SCID-like phenotype.[95]

SCID/CIDs associated with syndromic features

According to the International Union of Immunological Societies (IUIS), there are forms of PID associated with highly pleomorphic extra-immunological features responsible for complex syndromes with a genetic basis. Typical features of these syndromes comprise peculiar facial dysmorphism, growth delay, microcephaly, and ectodermal abnormalities. While an increased susceptibility to autoimmunity and (occasionally) cancer associated with the depletion of other blood cell lines is frequently reported, an increased susceptibility to infections is usually less frequent, and its clinical relevance is lower than in other PIDs. The pathogenetic mechanism resides in the involvement of several genes expressed in multiple cell lines, genes responsible for both ontogenesis and maturation of the immune system, as well as morphogenesis and organogenesis of other organs. Some of these conditions may be associated with a SCID/CID phenotype. Several syndromes are included in this group (Table 3), such DGS and CHARGE syndrome. Patients with CHARGE syndrome exhibit variable grades of immune defects, ranging from severe to mild T cell lymphopenia and abnormal T cell functionality, sometimes associated with hypogammaglobulinemia.[102] The incidence of SCID in patients with CHARGE is unknown, even though it may be, as in DGS, rare.[103] These patients, whose clinical phenotype is characterized by coloboma, heart defect, atresia choanae, retarded growth and development, genital hypoplasia, and

Table 3. Peculiar clinical and laboratory findings in the main genetic syndromes which in a few cases may be associated with a SCID/CID phenotype

Disorder	Genetic defect	Clinical phenotype	Immunological features
CHARGE syndrome	*CHD7*	Coloboma, hearth defect, atresia choanae, retarded growth and development	$T^-B^+NK^+$ SCID, OS, T cell lymphopenia, hypogammaglobulinemia
Cartilage–hair hypoplasia (CHH)	*RMRP*	Short limb with metaphyseal dysostosis, sparse hair, neural dysplasia of intestine	T cell lymphopenia, hypogammaglobulinemia, antibody deficiency
Schimke immuno-osseous dysplasia	*SMARCAL1*	Short stature, IUGR, spondiloepiphyseal dysplasia	T cell lymphopenia, bone marrow failure
Hyper IgE syndrome	*PGM3*	Short stature, brachydactyly, facial dysmorphism, intellectual disability	Congenital leucopenia, neutropenia, B and T cell lymphopenia
Hoyeraal-Hreidarsson syndrome (HHS)	*DKC1*	Microcephaly, cerebellar hypoplasia, IUGR	Bone marrow failure, CID or $T^+B^-NK^-$ SCID
Folate and cobalamin metabolism defect	*PCFT, TCN2, MTHFD1*	Failure to thrive, weakness, mental retardation, megaloblastic anemia, neurological disease	Pancytopenia, SCID-like phenotype, hypogammaglobulinemia
Anhydrotic ectodermic dysplasia with immunodeficiency	*NEMO*	Hypohidrosis, hypodontia, conical teeth, facial dysmorphism	SCID/CID-like phenotype

IUGR, intrauterine growth restriction.

ear anomalies/deafness, may suffer from a T^-B^+ NK^+ SCID and, in some cases, OS.[103] The disorder is caused by mutations in the chromodomain helicase DNA binding protein 7 gene (*CHD7*), a member of the chromo domain helicase DNA binding domain family of adenosine-5′-triphosphate dependent chromatin remodeling enzymes. CHD7 is expressed throughout the neural crest containing mesenchyme of the pharyngeal arches, suggesting a pathogenetic overlap between CHARGE and DGS.

In other syndromes, several peculiar skeletric abnormalities are the main feature, which lead the patient to the medical attention, as observed in patients with cartilage–hair hypoplasia (CHH), characterized by severe disproportionate short stature due to short limb with metaphyseal dysostosis, sparse hair and neural dysplasia of the intestine,[104] or in Schimke immuno-osseous dysplasia, which sometimes may show a CID phenotype.

In humans, defects in gene involved in telomere maintenance (*TERT*, *TERC*, *DKC1*, *WRAP53*/*TCAB1*, *NOP10*, *NHP2*, and *TINF2*) are responsible for the dyskeratosis congenita (DC), a rare congenital disorder characterized by progressive bone marrow failure, premature aging, mucocutaneous abnormalities, and cancer predisposition.[106] The most sever infantile variant of X-linked DC is the Hoyeraal-Hreidarsson syndrome (HHS), whose main clinical features are microcephaly, cerebellar hypoplasia, and intrauterine growth retardation. The early-onset bone marrow failure usually leads to either a combined immunodeficiency or a $T^+B^-NK^-$ SCID, which may require HSCT.[107]

Recently, several inborn errors in folate and cobalamin metabolism have been described as having a profound impact on many systems, including hematopoiesis and neuronal function. Immunodeficiency of variable degrees has been associated with defects in these pathways. A CID phenotype

characterized by lymphopenia, responsiveness to folate replacement therapy, and severe bacterial and viral infections has been described in patients with functional methionine synthase deficiency caused by hereditary folate malabsorption due to deficiency in the proton coupled folate transporter (PCFT) and in transcobalamin II (TCN2); this CID usually presents in early infancy in untreated patients as failure to thrive, weakness, pancytopenia, and intellectual disability. Recently, exomic sequencing demonstrated that heterozygous mutations in the trifunctional protein MTHFD1 is responsible for a SCID-like phenotype characterized by $T^-B^-NK^-$ lymphopenia, marked hypogammaglobulinemia, megaloblastic anemia, and neurologic disease.[108] A partial immune reconstitution after vitamin B12 and folate replacement therapy has been documented.

In summary, it must be noted that several syndromes, together with the more typical severe manifestations, can share clinical and immunological signs of SCID/CID, as for example patients affected by NEMO deficiency.

Recently identified combined immunodeficiencies

Combined immunodeficiency (CID) is a group of genetic heterogeneous disorders characterized by severe recurrent infections, moderate reduction of T and B lymphocytes, and impaired cellular and humoral functionality that may reflect late defects in T cell development and function.[109,110] In most cases, it is not always easy to distinguish between patients affected with more severe forms and those with CID. Furthermore, a greater difficulty in making a clear classification is due to the fact that many inborn defects, which underlie these immune disorders, have recently been associated with both SCID and CID, in particular hypomorphic mutations. Several genetic defects responsible for a wide number of clinical conditions are comprised in this group (Table 2).[111] Besides the well-known genetic defects responsible for MHC class I (*TAP1*, *TAP2*, *TAPBP*) or class II deficiency (*CIITA*, *RRFX5*, *RFXAP*, *RFXANK*) associated with a predominant $CD8^+$ or $CD4^+$ selective deficiency respectively, the very rare *CD8A* defects and many others (see the new International Union of Immunological Societies classification, Ref. 111) have been identified recently. Since the number of these conditions is large, we have chosen to discuss only the most common form associated with new phenotypes and novel ones reported over the past 3 to 4 years.

ZAP70-related immunodeficiency is inherited in an autosomal recessive manner. It is caused by abnormal TCR signaling, which leads to a selective absence of $CD8^+$ T cells and normal or elevated numbers of non-functional $CD4^+$ T cells. ZAP70 has a key role in both mature T cell signaling and differentiation of thymic precursors. Finally, in some patients peculiar phenotypes have been observed. In particular, some patients exhibit an attenuated phenotype with a late onset disease and preserved production of $CD4^+$ T follicular helper (T_{FH}), T helper type I (T_H1), T_H17, and T_{reg} cells. Immune dysregulation and severe erythroderma resembling OS have also been described, characterized by skin infiltrative lesions with activated $CD4^+$ T cells in the peripheral blood.[113]

Thanks to next generation sequencing technologies, which have provided a powerful tool to identify the molecular cause of PIDs of unknown genetic origin, new defects have been detected, even though in most cases the genetic cause still remains unknown.

Whole-exome sequencing recently demonstrated the presence of deleterious mutations in the phosphoglucomutase 3 gene (*PGM3*) in three unrelated subjects with recurrent infections, congenital leukopenia, neutropenia, B and T cell lymphopenia, and progression to bone marrow failure due to a congenital disorder of glycosylation (CDG). Two of the three children also had skeletal anomalies characterized by short stature, brachydactyly, dysmorphic facial features, and intellectual disability.[105] Thanks to this technology, Kotlarz *et al.* identified in 2013 two distinct homozygous loss of functions mutations in the interleukin-21 receptor gene (*IL21R*) in two unrelated children affected with cryptosporidiosis, chronic cholangitis and liver disease, recurrent upper and lower airway infections, and failure to thrive.[99] IL-21R binds to common γc and signals via JAK/STAT pathways.[100,101] The authors observed that the mutation was responsible for the aberrant trafficking of the IL-21R to the plasma membrane and for the abrogation of IL-21 ligand binding. These molecular alterations lead to defective phosphorylation of STAT1, STAT3, and STAT5. The immunophenotype of these patients was normal, but abnormal proliferation induced by

IL-21 and defects in immunoglobulin class-switching in B cells and NK cell cytotoxicity were documented. A defect in T cell secretion of several cytokines, including T_H17-associated cytokines IL-17F and IL-22, was reported, thus putatively explaining the increased susceptibility to cryptosporidial infection in these patients.

In the last few years mutations in the CARD9–BCL10–MALT1 (CBM) complex involved in NF-κB signaling have been associated with PID. In particular, autosomal recessive mutations in MALT1 gene have been described in patients with CID and severe bacterial, fungal and viral infections.[114] The MALT1-deficient T cells are not able to degrade IκBα or produce IL-2 following T cell activation. BCL10 has a role in several immune pathways critical for the function of the innate and the adaptive immune systems, and for the response to bacterial and fungal infections. Mutations in *BCL10* and other genes encoding for proteins interacting with MALT1, such as *CARD11* and *CARD9*, have also been recently described. Patients with BCL10 deficiency show a profound defect of memory T and B cells and severe hypogammaglobulinemia, with a reduction of CD69 and CD25 percentages and ICOS levels.[115] Even though CARD9 deficiency has been shown to selectively compromise defenses toward a limited number of fungal infections, mutations in CARD11, which plays a crucial role in the differentiation of both neuronal and immunologic tissues as a scaffold protein, are associated with a more profound CID characterized by abnormal T cell proliferation to anti-CD3/CD28 stimulation, expansion of late transitional B cells, mature B cells deficiency, and hypogammaglobulinemia.[101] Furthermore, CARD11-deficient T cells do not produce normal amounts of IL-2 or upregulate the IL-2 receptor α chain (CD25) after TCR stimulation, which contributes to T_{reg} cell deficiency in these patients.

Mutations in tetratricopeptide repeat domain 7A (TTC7A), a member of the large family of proteins containing the tetratricopeptide repeat (TPR) domain, have recently been found in patients affected with CID and multiple intestinal atresia (MIA).[116] MIA is a clinical condition that can be isolated or may occur in association with variable grades of immunodeficiency ranging from SCID to a mild decrease of T cells and partially preserved thymic function. However, in all these genetic forms, profound $CD8^+$ T cell lymphopenia, reflecting the impaired cellular immunity and the defective thymopoiesis, has been observed. Severe hypogammaglobulinemia is also frequent. A higher frequency of bloodstream infections due to intestinal microbes has also been reported.

The clinical and immunological phenotypes of Ras homolog family member H gene (*RHOH*) deficiency is characterized by naive $CD4^+$ T cell deficiency, absence of recent thymic emigrants, increased number of effector memory T cells, restricted T cell repertoire, and reduced *in vitro* proliferation via CD3 stimulation.[117] Expressed mainly in hematopoietic cells, RhoH is a small GTPase that mediates interaction between Zap70 and LCK. RhoH deficiency determines both alterations in pre-TCR–mediated signaling and in positive selection, as observed in Zap70 deficiency. Expansion of memory T cells has also been observed in other CIDs, such as deficiency of DOCK8 or MST1. DOCK8 deficiency is an autosomal recessive form of CID associated with a hyper-IgE phenotype. Viral infections (especially of the skin) and malignancies are very common. Lymphopenia of $CD4^+$ and $CD8^+$ T cells, or predominantly $CD4^+$ lymphocytes, may be found. In addition, DOCK8 deficient patients exhibit defective differentiation of T_H17 cells and a reduction of B lymphocytes.[118]

The lymphocyte specific kinase LCK is involved in the initiation of signaling from the TCR [121] through the adaptor protein unc-119 lipid binding chaperone (UNC119). Recently, mutations in LCK or UNC119, which impairs LCK activation and signaling, have been identified. Main features of this phenotype include $CD4^+$ T cell lymphopenia, a restricted T cell repertoire, and impaired TCR signaling.[122] Patients with LCK deficiency frequently present with immune dysregulation and autoimmunity. Mutations in the magnesium transporter protein1 gene (*MAGT1*) result in a CID phenotype characterized by $CD4^+$ lymphopenia and abnormal T cell proliferation, which are responsible for chronic viral infections and EBV-related lymphoma, respectively.[123] Recently, a CID was observed in four unrelated patients with mutation of inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta (*IKBKB*); the patients had severe bacterial, viral, fungal, mycobacterial infections associated with failure to thrive and neurological impairment. The immunological phenotype was

characterized by a/hypogammaglobulinemia and absence of T_{reg} and γ/δ T cells. Even though T cell counts were normal, all the patients exclusively showed naive T and B lymphocytes.[124]

Newborn screening for SCID

Recently, T cell receptor excision circles (TREC)–based newborn screening has been implemented in several countries. Compared with patients identified by the clinical features, patients identified through newborn screening programs, similar to children identified because of a positive familial history, can receive an early and accurate diagnosis by one month of life and then undergo HSCT or gene therapy by 3 months of age, before the occurrence of severe complications. This results in a significantly improved outcome.[125,126] The TREC assay, based on the detection of intracellular accumulation of products derived from process of T cell receptor gene splicing and rearrangement, is able to detect several defects, which result in either SCID or profound T cell lymphopenia that is also seen in patients affected with 22q11.2DS, CHH, CHARGE, and AT.[127] However, one limitation of the TREC assay is that it is not able to identify all forms of CID or atypical SCID. Some genetic disorders, such as deficiency of ZAP70, late onset ADA, Nijmegen breakage syndrome, MHC class II deficiency, and many others, are likely to be missed because TRECs are usually found at normal levels. The identification of kappa-deleting recombination excision circles (KREC), a sensitive marker of newly formed B cells, increases the possibility of identifying other forms of SCID/CID that are associated with low numbers of B lymphocytes, such as NBS and late onset ADA. Furthermore, it has been reported that tandem mass spectrometry can easily identify abnormal purine metabolites in newborns with typical or late onset ADA and PNP deficiency,[19] thus increasing the spectrum of disorders detectable through newborn screening.

Conclusions

SCIDs are a heterogeneous group of syndromes related to alterations of distinct genes that cause abnormalities in the maturation and/or function of T, B, and/or NK cells. Recently, advances in next generation DNA sequencing have allowed new gene identification through whole exome sequencing or whole genome sequencing of several forms of SCID and CID of unknown cause. The phenotypic and the molecular heterogeneity of SCIDs, as revealed by the expanding phenotypes observed, is making traditional classification of this group of disorders very intricate. Frequently, different mutations in the same gene can lead to different clinical phenotypes, such as OS, leaky SCID, or CID, that may even be inherited with different mechanisms.

In this review we have focused in detail on different forms of SCID and CID, paying attention to the distinctive peculiar clinical and laboratory features, in order to provide information to clinicians for recognizing and carefully managing these novel forms of PIDs.

Conflicts of interest

The authors declare no conflicts of interest.

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