

Reviews in Immunology

Severe Combined Immunodeficiency (SCID) including Omenn Syndrome and Zap-70 Deficiency



Correlagen Diagnostics, Inc.
307 Waverley Oaks Road
Suite 101
Waltham, MA 02452

Tel 866.647.0735
Fax 781.647.0626

www.correlagen.com

Severe Combined Immunodeficiency (SCID) including Omenn Syndrome (SCID/OS) and Zap-70 deficiency

Frequently used abbreviations: **ADA** - adenosine deaminase; **Ado** - adenosine; **D** - diversity; **dAdo** - 2'-deoxyadenosine; **dATP** - deoxyadenosine triphosphate; **GVHD** - graft-versus-host disease; **HSCT** - hematopoietic stem cell transplantation; **IL** - interleukin; **IL-7R** - interleukin-7 receptor; **J** - joining; **Jak** - Janus kinase; **NHEJ** - non-homologous end-joining; **NK** - natural killer cell; **OS** - Omenn Syndrome **RAG1/2** - recombination-activating proteins 1/2; **RSS** - recombination signal sequences; **RS-SCID** - radiation-sensitive SCID; **SCID** - Severe Combined Immunodeficiency; **STAT** - signal transducer and activator of transcription proteins; **TCR** - T-cell receptor; **V** - variable; **XSCID** - X-linked SCID; **Zap-70** - ζ -chain associated protein of 70 kDa

Introduction

Severe Combined Immunodeficiency (SCID) is characterized by severe lymphopenia and lack of adaptive immunity and, if untreated, leads to death through infection. SCID occurs with an estimated incidence of 1 in 75,000 births (1) and is considered a pediatric emergency because of the potentially lethal outcome of recurrent or persistent infections suffered by SCID patients. Several monogenic causes with different modes of inheritance have been identified for SCID (reviewed in 2).

Genetic testing for SCID can allow distinction between the various forms of this syndrome. Knowledge of the defective gene may have implications for treatment and prognosis. This knowledge may also enable more effective genetic counseling, in addition to facilitating identification of asymptomatic carriers and timely initiation of treatment in affected descendants of carriers.

Types and Causes of SCID

Depending on the underlying genetic defect, four different primary phenotypes associated with SCID have been characterized (for comprehensive reviews, refer to references 1-3). Categorization is based on the classes of lymphocytes that are absent or severely reduced. T-cell lymphopenia is generally common to all forms of SCID, but levels of B and natural killer (NK) cells vary depending on the genetic defect. For certain rare subtypes of SCID, T cells may be present, but their function is impaired.

Table 1

| Gene | Gene Product | Lymphocyte Phenotype | Associated Mode of Inheritance |
|----------------|--|---|--------------------------------|
| IL2RG | Common γ chain of IL receptors (γ_c) | T ⁻ B ⁺ NK ⁻ | X-linked recessive |
| JAK3 | Janus kinase 3 (Jak3) | | autosomal recessive |
| ADA | Adenosine deaminase (ADA) | T ⁻ B ⁻ NK ⁻ | autosomal recessive |
| RAG1 | Recombination-activating protein 1 (RAG1) | | autosomal recessive |
| RAG2 | Recombination-activating protein 2 (RAG2) | T ⁻ B ⁻ NK ⁺ | autosomal recessive |
| DCLRE1C | DNA-cross-link repair protein 1C (Artemis) | | autosomal recessive |
| IL7R | IL-7 receptor α chain (IL-R α , CD127) | | autosomal recessive |
| CD3D | CD3 δ chain | T ⁻ B ⁺ NK ⁺ | autosomal recessive |
| CD3E | CD3 ϵ chain | | autosomal recessive |
| ZAP70 | ζ -associated protein of 70 kDa (Zap-70) | T ⁺ (CD4 ⁺ CD8 ⁻)B ⁺ NK ⁺ | autosomal recessive |

T⁻B⁺NK⁻ SCID

T⁻B⁺NK⁻ SCID is the most common type of SCID. It is most often caused by X-linked recessive mutations in *IL2RG* (XSCID), which encodes the γ chain (γ c) common to several cytokine receptors such as IL-2R, IL-4R, IL-7R, IL-9R, IL-15R, and IL-21R (reviewed in 4). T⁻B⁺NK⁻ SCID has also been associated with autosomal recessive mutations in *JAK3*, which codes for the γ c-associated tyrosine kinase known as Janus kinase 3 (Jak3) (reviewed in 5). Mutations in *IL2RG* or *JAK3* lead to defective signaling through the γ c receptors, resulting in an absence of both T cells and NK cells. B cells are present at normal levels, but have impaired function.

Ligand binding to cytokine receptors containing the γ c subunit results in activation of Jak3. Activated Jak3 phosphorylates the cytoplasmic domain of the cytokine receptor, creating a binding site for the signal transducer and activator of transcription (STAT) proteins. Upon binding, STAT proteins are also phosphorylated by Jak3, resulting in their dimerization and translocation to the nucleus where they activate transcription of various genes necessary for the growth and differentiation of lymphocytes (reviewed in 4, 6). Mutations in either γ c or Jak3 disrupt signaling of all γ c-dependent cytokines. Defective IL-7 signaling is thought to be primarily responsible for the T-cell deficiency observed in patients with T⁻B⁺NK⁻ SCID (7), while failed IL-15 signaling leads to the NK-cell deficiency (8, 9). Although B cells are present at normal or elevated levels, B-cell activation, maturation, and class switch recombination are impaired, probably due to the combined effects of defective IL-4 and IL-21 signaling as well as failure of T-cell help (10). The T-cell lymphopenia associated with T⁻B⁺NK⁻ SCID allows infection by opportunistic organisms, while the lack of NK cells causes susceptibility to pathogens such as viruses. The defect in B-cell function further contributes to susceptibility to recurrent infections.

T⁻B⁻NK⁻ SCID

T⁻B⁻NK⁻ SCID results from a purine metabolism defect due to autosomal recessive mutations in *ADA*, the gene encoding adenosine deaminase. Defects in adenosine deaminase allow accumulation of cytotoxic adenosine derivatives in lymphoid organs, resulting in the death of lymphocyte precursors and a lack of T cells, B cells, and NK cells (reviewed in 11).

Adenosine deaminase (ADA) catabolizes adenosine (Ado) and 2'-deoxyadenosine (dAdo) generated by apoptotic cells (reviewed in 11, 12). The large number of apoptotic cells in the lymph nodes, thymus, and bone marrow continually generate high concentrations of Ado and dAdo. Breakdown of dAdo, in particular, is important because its accumulation is highly toxic to lymphoid cells. Excess dAdo causes an increase in levels of deoxyadenosine triphosphate (dATP). Increased dATP levels block DNA synthesis by inhibiting ribonucleotide reductase (13) and trigger lymphocyte apoptosis by facilitating apoptosome formation (14, 15). It is thought that the pro-apoptotic effects of elevated levels of dAdo and dATP are the primary cause of T-, B-, and NK-cell deficiencies observed in *ADA*-related SCID patients. The resulting combined B-cell, T-cell, and NK-cell lymphopenia allows severe, persistent, or recurrent infections with all types of pathogens, including pyogenic and opportunistic bacteria, as well as viruses.

T⁻B⁻NK⁺ SCID

T⁻B⁻NK⁺ SCID is caused by autosomal recessive mutations in at least three genes necessary for antigen receptor rearrangement, *RAG1*, *RAG2*, and *DCLRE1C* (*ARTEMIS*). Defects in these genes lead to impaired development of both B and T cells, while NK-cell development is normal (reviewed in 1). Notably, mutations in *RAG1*, *RAG2*, or *DCLRE1C* allowing limited production of B and T cells result in a condition that is clinically distinct from SCID, commonly known as Omenn Syndrome (OS) (16, 17) (see below).

RAG1 and *RAG2* encode the recombination-activating proteins RAG1 and RAG2, respectively. These proteins play a fundamental role in the rearrangement of the antigen-binding domain of B- and T-cell receptors by initiating V(D)J recombination (18, 19). Sites of recombination are specified by recombination signal sequences (RSS) that flank each variable (V), diversity (D), and joining (J) gene segment. RAG1 recognizes and binds to the RSS, and then recruits RAG2 to form a stable complex on the DNA (20, 21). Within this complex, the RAG proteins introduce a DNA double-strand break at the junction of the RSS and the coding sequence. DNA cleavage by the Rag1/Rag2 complex generates covalently sealed hairpins at the ends of the V, D, and J coding sequences that require further processing. The *DCLRE1C* gene product, DNA cross-link repair protein 1C (commonly known as Artemis), is recruited to the DNA hairpin structures by components of the non-homologous end joining (NHEJ) DNA repair machinery. Upon joining the NHEJ complex, Artemis cleaves the DNA hairpin structures (22), allowing other NHEJ components to repair the damaged DNA, thus completing the recombination process. Defects in either RAG1, RAG2, or Artemis prevent productive rearrangement of both the B- and T-cell receptors, which is a prerequisite for B and T cell maturation, thereby blocking development of B and T cells at very early stages (23, 24). The resulting T- and B-cell lymphopenia leads to increased susceptibility to a wide range of infections, including opportunistic pathogens. NK-cell levels are normal or elevated. Defects in Artemis also cause cellular sensitivity to ionizing radiation, likely due to a role for Artemis in the general DNA double-strand break repair pathway (25). *DCLRE1C*-related SCID is therefore also known as radiation-sensitive SCID (RS-SCID).

T⁻B⁺NK⁺ SCID

T⁻B⁺NK⁺ SCID is often associated with autosomal recessive loss-of-function mutations in *IL7R*, which encodes the α subunit of the IL-7 receptor (reviewed in 6). Additional causes of this type of SCID include autosomal recessive loss-of-function mutations in *CD3D* and *CD3E*, which code for proteins necessary for signaling through the pre-T-cell receptor (pre-TCR) or the TCR (reviewed in 1). Autosomal recessive loss-of-function mutations in *ZAP70*, which encodes a signaling protein that associates with the CD3 complex, give rise to a rare subtype of SCID characterized by the selective absence of CD8⁺ T cells (reviewed in 26, 27).

IL-7R α , the *IL7R* gene product, is a component of the interleukin-7 receptor (IL-7R) (reviewed in 28, 29). Interaction of IL-7 with IL-7R leads to recruitment of the intracellular signaling molecules Jak1 and Jak3. Phosphorylation of IL-7R α by Jak proteins activates multiple downstream signaling pathways which are important for transcriptional activation of genes involved in T-cell differentiation (30), T-cell survival and maturation (31), and TCR rearrangement (32). Disruption of IL-7 signaling arrests T-cell development at the double negative (CD4⁻CD8⁻) stage, preventing productive TCR rearrangement (33) and leading to T-cell lymphopenia.

The *CD3D* and *CD3E* gene products, CD3 δ and CD3 ϵ , respectively, are components of the invariant CD3 protein complex that pairs with the variable antigen-recognition subunits to form both the pre-TCR and the TCR. The CD3 complex is comprised of the CD3 γ , δ , ϵ , and ζ transmembrane protein subunits; each subunit contains at least one immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic tail (reviewed in 34). Upon ligand binding to the pre-TCR or the TCR, these ITAMs are phosphorylated, initiating a signaling cascade that results in transcriptional activation of genes involved in T-cell differentiation and proliferation. CD3 association with the pre-TCR is required for the transition from double negative to double positive T cells during thymocyte maturation and for rearrangement of the TCR α chain (35). Surface expression of and signaling by the α/β TCR/CD3 complex is necessary for thymocyte maturation from double positive to single positive CD4 or CD8 T cells (reviewed in 36). Defects in any of the CD3 subunits can result in impaired TCR surface expression and/or signaling, thereby hindering thymocyte maturation. Defects in CD3 δ or CD3 ϵ lead to an arrest of thymocyte development at the double negative (CD4⁻CD8⁻) stage, causing a severe deficiency of both α/β and γ/δ T cells (36, 37).

Loss-of-function mutations in *IL7R*, *CD3D*, or *CD3E* result in severe T-cell lymphopenia. NK and B cells are present at normal levels, but B-cell activation and class-switch recombination are impaired, probably due to the failure of T-cell help. The T-cell lymphopenia associated with *IL7R*-, *CD3D*-, and *CD3E*-related SCID leads to recurrent opportunistic infections, while the defect in B-cell function further contributes to susceptibility to severe or recurrent infections.

Zap-70 is a Syk family protein tyrosine kinase expressed exclusively in T cells and NK cells. Zap-70 is recruited to the ζ chain of the invariant CD3 protein complex of the TCR in response to TCR engagement (38, 39). Once associated with the TCR/CD3 complex, Zap-70 is phosphorylated and activated. Activation of Zap-70 leads to recruitment of additional downstream signaling molecules, inducing a signaling cascade that results in transcriptional activation of genes required for T-cell activation, differentiation, proliferation, and function (reviewed in 40, 41). Loss-of-function mutations in *ZAP-70* disrupt T-cell development at the transition from double positive (CD4⁺CD8⁺) to single positive T cells, leading to a selective absence of peripheral CD8⁺ T cells, likely due to a defect in the positive selection of these cells (42). CD4⁺ T cells are present at normal or elevated levels, but do not function properly, indicating that Zap-70 is not essential for CD4⁺ T-cell development (43), but required for their activation and proliferation. As in T⁻B⁺NK⁺ SCID, NK and B cells are present at normal levels, but in most cases, B-cell function is impaired (26).

Omenn Syndrome

Mutations in *RAG1* or *RAG2* that allow residual enzyme activity (so called hypomorphic mutations) result in impaired V(D)J recombination and limited TCR diversity and can give rise to a distinct SCID phenotype commonly known as Omenn Syndrome (OS), which is distinguished from classic SCID by the presence of oligoclonal T cells, elevated levels of serum IgE, and eosinophilia (reviewed in 17).

Although hypomorphic mutations in *RAG1* or *RAG2* are the best characterized cause of OS, they do not account for all cases of OS (44). Hypomorphic mutations in *IL7R* (45) and *DCLRE1C* (*ARTEMIS*) (16) have also been implicated in OS, and the identification of additional causes is anticipated.

Clinical Presentation of SCID

Symptoms of SCID usually appear within the first few months after birth, following a brief asymptomatic period due to the presence of maternal Igs. Infants with SCID typically suffer from severe and persistent infections, intractable diarrhea, and failure to thrive (2). Infections are characteristically caused by opportunistic organisms such as

Pseudomonas, *Salmonella*, *Candida albicans*, or *Pneumocystis carinii* or viruses such as respiratory syncytial virus, adenovirus, or cytomegalovirus. Lymph nodes and tonsils are absent. X-ray imaging will usually reveal a small thymus lacking thymocytes; however, in rare cases, a nearly normal-sized thymus may also be observed (36). SCID-associated deficiency in endogenous T cells can be initially masked by the temporary presence of maternal lymphocytes that have crossed the placenta during gestation (46). Engraftment of such maternal T cells or T cells received through postnatal blood transfusions can lead to graft-versus-host disease (GVHD) (47). The defect in B-cell function results in hypogammaglobulinemia and failure to mount a specific antibody response to immunization (48).

ADA-related SCID ($T^+B^-NK^-$ SCID) is further characterized by the presence of skeletal and neurological abnormalities and/or excretion of large amounts of dAdo in the urine (12). The level of clinical severity and immune dysfunction associated with mutations in *ADA* varies substantially, with mutations that allow some ADA activity giving rise to milder disease or later onset of symptoms (12).

DCLRE1C-related SCID ($T^+B^-NK^+$ SCID or RS-SCID) is also associated with increased sensitivity of both bone marrow and skin fibroblasts to ionizing radiation (49, 50), B-cell lymphomas (51), and oral and genital ulcers (52).

Zap-70 deficiency is clinically similar to classic SCID, but is distinguished by the selective absence of $CD8^+$ T cells in the presence of normal or elevated $CD4^+$ T cells levels. Although $CD4^+$ T cells are present, proliferative responses to antigens are severely reduced. In most cases of Zap-70 deficiency, lymph nodes and tonsils are palpable and X-ray imaging will show a normal-sized thymus (26). Thymic biopsy will reveal the presence of double positive ($CD4^+CD8^+$) thymocytes in the cortex, while $CD4^+$, but not $CD8^+$ single positive cells are present in the medulla (42).

Omenn Syndrome can be distinguished from SCID by the presence normal or elevated T-cell levels with a restricted, oligoclonal T-cell repertoire, elevated levels of serum IgE, and eosinophilia. In rare cases, B cells are also present; however both B cells and T cells are oligoclonal and non-functional (53). Lymph nodes, liver, and spleen are enlarged (17). Patients with OS also suffer from exudative erythrodermatitis and chronic diarrhea due to infiltration of the skin and intestines by activated T cells, resulting in symptoms that resemble graft-versus-host disease (GVHD) (25).

Diagnosis of SCID

Patients with SCID and related SCID subtypes are usually diagnosed during the first few months after birth. SCID is suspected in infants presenting with recurrent, severe, or unusual infections and lymphopenia. Diagnosis currently relies on detection of a reduction in total lymphocyte counts, detection of diminished T-cell levels by flow cytometry, and lymphocyte functional tests, and is supported by a family history of death in infancy due to infection or a family history of SCID. Biochemical testing may be considered to identify ADA deficiency (12).

Diagnosis of SCID and its subtypes can also be achieved through genetic testing. Importantly, genetic testing allows detection of carriers, allowing more effective genetic counseling. Genetic testing of newborns known to have a genetic predisposition for SCID can allow diagnosis and treatment of SCID before any life-threatening infections develop. Such early treatment has been correlated to increased survival (47).

Treatment of SCID

Hematopoietic stem cell transfer (HSCT) has proven successful in controlling the defect in T-cell mediated immunity (2), although continued intravenous immunoglobulin therapy is often necessary if B cells do not engraft. HSCT is also the recommended treatment for OS (17) and Zap-70 deficiency (26). When performed before serious infection develops, HSCT can result in a survival rate as high as 97% (3), making early diagnosis critical.

ADA-related SCID patients who are unable to receive HSCT may benefit from enzyme replacement with polyethylene glycol-conjugated ADA (PEG-ADA), which has been shown to increase T-lymphocyte levels and improve cellular immune function.

Recent clinical trials suggest that gene therapy will be another promising treatment option for both ADA-related SCID and *IL2RG*-related XSCID; however, clinical trials are currently on hold due to development of a form of leukemia in two *IL2RG* gene-therapy recipients (2, 47). Research into the development of gene therapy-based replacement of both Jak3 (5) and Zap-70 (54, 55) is also ongoing, but has not yet yielded viable treatment options.

Genetics of SCID

IL2RG-related XSCID is an X-linked recessive disorder, exclusively affecting males. Of note, mutations in *IL2RG* resulting in a milder form of XSCID, distinguished by the presence of circulating T cells and delayed onset of infections, have also been characterized (56-59).

Loss-of-function mutations in ***CD3E*** that allow residual expression of CD3 ϵ are associated with milder immunodeficiency characterized by recurrent bacterial and viral infections (36, 60).

Except for *IL2RG*-related XSCID, all other known types of SCID are inherited in an autosomal recessive manner, affecting both males and females.

Although very rare in the general population, ***DCLRE1C***-related SCID occurs with an incidence of 1 in 2000 live births within the Athabascan-speaking Navajo and Apache Native American population (often referred to as Athabascan SCID or SCIDA) due to a founder mutation in *DCLRE1C* (52).

Table 2

| Lymphocyte Phenotype | Affected Gene | Relative Frequency (3) | Affects |
|--|-----------------------|------------------------|-------------------|
| T ⁻ B ⁺ NK ⁻ | <i>IL2RG</i> | 46% | males only |
| | <i>JAK3</i> | 6.9% | males and females |
| T ⁻ B ⁻ NK ⁻ | <i>ADA</i> | 16.1% | males and females |
| T ⁻ B ⁻ NK ⁺ | <i>RAG1</i> | 3.4% | males and females |
| | <i>RAG2</i> | | |
| | <i>DCLRE1C</i> | 1.1% | |
| T ⁻ B ⁺ NK ⁺ | <i>IL7R</i> | 10.3% | males and females |
| | <i>CD3D</i> | 0.6% | |
| | <i>CD3E</i> | 0.6% | |
| T ⁺ (CD4 ⁺ CD8 ⁻) B ⁺ NK ⁺ | <i>ZAP70</i> | n/a | males and females |
| | <i>unknown</i> | ~15% | |

Omenn Syndrome

Loss-of-function mutations in *RAG1*, *RAG2*, *DCLRE1C*, or *IL7R* can result in either SCID or OS (16, 17, 45). Both SCID and OS are inherited in an autosomal recessive manner, that is, both copies of the affected gene must harbor a loss-of-function mutation for a disease phenotype to be expressed. If both gene copies contain a mutation that leads to complete loss of enzyme function, the resulting phenotype is SCID. If at least one copy of the gene contains a mutation that allows residual enzyme activity, the phenotype will be OS. Thus, a patient affected by OS could also be a carrier of a SCID-associated mutation. With only one exception (a frameshift mutation in *RAG1*), all known OS-associated mutations in *RAG1* and *RAG2* are missense mutations (reviewed in 17, 53).

Of note, hypomorphic mutations in both copies of *DCLRE1C* can give rise to milder disease characterized by residual V(D)J recombination activity in vitro and partial T- and B- lymphocyte deficiency. These patients also exhibit increased frequency of B-cell lymphomas (51).

OS and SCID have been reported within the same family (49), implying that the identical genetic defect can cause both phenotypes. However, GVHD in SCID patients may also lead to OS-like symptoms (17).

Testing for SCID

Genetic testing can confirm a diagnosis of SCID or establish a diagnosis before infections develop. Such early diagnosis is important, since timely treatment has been shown to increase survival. Genetic testing can also distinguish between the different molecular causes of SCID, with important implications for genetic counseling. Importantly, genetic testing allows detection of asymptomatic carriers of SCID-associated mutations. This is critical in cases of X-linked diseases such as XSCID, since female carriers are asymptomatic, while their sons are at a 50% risk of being affected.

How is Genetic Testing for SCID Performed?

DNA for sequencing is obtained from leukocytes present in a small blood sample. The coding sequences of the genes in question are amplified in a highly specific manner through a polymerase chain reaction (PCR), and all PCR products are fully sequenced. Sequencing results are interpreted, and a detailed result report is sent to the patient's physician.

References

1. Fischer A, Le Deist F, Hacein-Bey-Abina S, Andre-Schmutz I, et al. (2005) Severe combined immunodeficiency. A model disease for molecular immunology and therapy. *Immunol Rev* 203:98-109.
2. Buckley RH (2004) Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. *Annu Rev Immunol* 22:625-55.
3. Buckley RH (2004) The multiple causes of human SCID. *J Clin Invest* 114:1409-11.
4. Leonard WJ (2001) Cytokines and immunodeficiency diseases. *Nat Rev Immunol* 1:200-8.
5. Pesu M, Candotti F, Husa M, Hofmann SR, et al. (2005) Jak3, severe combined immunodeficiency, and a new class of immunosuppressive drugs. *Immunol Rev* 203:127-42.
6. Kovanen PE, Leonard WJ (2004) Cytokines and immunodeficiency diseases: critical roles of the gamma(c)-dependent cytokines interleukins 2, 4, 7, 9, 15, and 21, and their signaling pathways. *Immunol Rev* 202:67-83.
7. Puel A, Ziegler SF, Buckley RH, Leonard WJ (1998) Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. *Nat Genet* 20:394-7.
8. Kennedy MK, Glaccum M, Brown SN, Butz EA, et al. (2000) Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med* 191:771-80.
9. Lodolce JP, Boone DL, Chai S, Swain RE, et al. (1998) IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* 9:669-76.
10. Ozaki K, Spolski R, Feng CG, Qi CF, et al. (2002) A critical role for IL-21 in regulating immunoglobulin production. *Science* 298:1630-4.
11. Hershfield MS (2005) New insights into adenosine-receptor-mediated immunosuppression and the role of adenosine in causing the immunodeficiency associated with adenosine deaminase deficiency. *Eur J Immunol* 35:25-30.
12. Hershfield MS (2003) Genotype is an important determinant of phenotype in adenosine deaminase deficiency. *Curr Opin Immunol* 15:571-7.
13. Benveniste P, Cohen A (1995) p53 expression is required for thymocyte apoptosis induced by adenosine deaminase deficiency. *Proc Natl Acad Sci U S A* 92:8373-7.
14. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, et al. (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91:479-89.
15. Liu X, Kim CN, Yang J, Jemmerson R, et al. (1996) Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* 86:147-57.
16. Ege M, Ma Y, Manfras B, Kalwak K, et al. (2005) Omenn syndrome due to ARTEMIS mutations. *Blood* 105:4179-86.
17. Santagata S, Villa A, Sobacchi C, Cortes P, et al. (2000) The genetic and biochemical basis of Omenn syndrome. *Immunol Rev* 178:64-74.
18. Oettinger MA, Schatz DG, Gorka C, Baltimore D (1990) RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science* 248:1517-23.
19. Schatz DG, Oettinger MA, Baltimore D (1989) The V(D)J recombination activating gene, RAG-1. *Cell* 59:1035-48.
20. Difilippantonio MJ, McMahan CJ, Eastman QM, Spanopoulou E, et al. (1996) RAG1 mediates signal sequence recognition and recruitment of RAG2 in V(D)J recombination. *Cell* 87:253-62.

21. Spanopoulou E, Zaitseva F, Wang FH, Santagata S, et al. (1996) The homeodomain region of Rag-1 reveals the parallel mechanisms of bacterial and V(D)J recombination. *Cell* 87:263-76.
22. Ma Y, Pannicke U, Schwarz K, Lieber MR (2002) Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell* 108:781-94.
23. de Villartay JP (2002) V(D)J recombination and DNA repair: lessons from human immune deficiencies and other animal models. *Curr Opin Allergy Clin Immunol* 2:473-9.
24. Schwarz K, Gauss GH, Ludwig L, Pannicke U, et al. (1996) RAG mutations in human B cell-negative SCID. *Science* 274:97-9.
25. Le Deist F, Poinignon C, Moshous D, Fischer A, et al. (2004) Artemis sheds new light on V(D)J recombination. *Immunol Rev* 200:142-55.
26. Elder ME (1998) ZAP-70 and defects of T-cell receptor signaling. *Semin Hematol* 35:310-20.
27. Hivroz C, Fischer A (1994) Immunodeficiency diseases. Multiple roles for ZAP-70. *Curr Biol* 4:731-3.
28. Fry TJ, Mackall CL (2002) Interleukin-7: from bench to clinic. *Blood* 99:3892-904.
29. Giliani S, Mori L, de Saint Basile G, Le Deist F, et al. (2005) Interleukin-7 receptor alpha (IL-7Ralpha) deficiency: cellular and molecular bases. Analysis of clinical, immunological, and molecular features in 16 novel patients. *Immunol Rev* 203:110-26.
30. Trigueros C, Hozumi K, Silva-Santos B, Bruno L, et al. (2003) Pre-TCR signaling regulates IL-7 receptor alpha expression promoting thymocyte survival at the transition from the double-negative to double-positive stage. *Eur J Immunol* 33:1968-77.
31. Yu Q, Erman B, Bhandoola A, Sharrow SO, et al. (2003) In vitro evidence that cytokine receptor signals are required for differentiation of double positive thymocytes into functionally mature CD8+ T cells. *J Exp Med* 197:475-87.
32. Smart FM, Venkitaraman AR (2000) Inhibition of interleukin 7 receptor signaling by antigen receptor assembly. *J Exp Med* 191:737-42.
33. Perumal NB, Kenniston TW, Jr., Tweardy DJ, Dyer KF, et al. (1997) TCR-gamma genes are rearranged but not transcribed in IL-7R alpha-deficient mice. *J Immunol* 158:5744-50.
34. Clevers H, Alarcon B, Wileman T, Terhorst C (1988) The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu Rev Immunol* 6:629-62.
35. von Boehmer H, Fehling HJ (1997) Structure and function of the pre-T cell receptor. *Annu Rev Immunol* 15:433-52.
36. Roifman CM (2004) CD3 delta immunodeficiency. *Curr Opin Allergy Clin Immunol* 4:479-84.
37. de Saint Basile G, Geissmann F, Flori E, Uring-Lambert B, et al. (2004) Severe combined immunodeficiency caused by deficiency in either the delta or the epsilon subunit of CD3. *J Clin Invest* 114:1512-7.
38. Chan AC, Iwashima M, Turck CW, Weiss A (1992) ZAP-70: a 70 kd protein-tyrosine kinase that associates with the TCR zeta chain. *Cell* 71:649-62.
39. Chan AC, van Oers NS, Tran A, Turka L, et al. (1994) Differential expression of ZAP-70 and Syk protein tyrosine kinases, and the role of this family of protein tyrosine kinases in TCR signaling. *J Immunol* 152:4758-66.
40. Qian D, Weiss A (1997) T cell antigen receptor signal transduction. *Curr Opin Cell Biol* 9:205-12.
41. van Leeuwen JE, Samelson LE (1999) T cell antigen-receptor signal transduction. *Curr Opin Immunol* 11:242-8.
42. Roifman CM (1995) Selection transduction defect (STD) due to Zap-70 kinase deficiency. *Immunodeficiency* 5:193-211.
43. Gelfand EW, Weinberg K, Mazer BD, Kadlec TA, et al. (1995) Absence of ZAP-70 prevents signaling through the antigen receptor on peripheral blood T cells but not on thymocytes. *J Exp Med* 182:1057-65.
44. Gennery AR, Hodges E, Williams AP, Harris S, et al. (2005) Omenn's syndrome occurring in patients without mutations in recombination activating genes. *Clin Immunol* 116:246-56.
45. Giliani S, Bonfim C, de Saint Basile G, Lanzi G, et al. (2006) Omenn syndrome in an infant with IL7RA gene mutation. *J Pediatr* 148:272-74.
46. Buckley RH, Schiff RI, Schiff SE, Markert ML, et al. (1997) Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. *J Pediatr* 130:378-87.
47. Kalman L, Lindegren ML, Kobrynski L, Vogt R, et al. (2004) Mutations in genes required for T-cell development: IL7R, CD45, IL2RG, JAK3, RAG1, RAG2, ARTEMIS, and ADA and severe combined immunodeficiency: HuGE review. *Genet Med* 6:16-26.
48. O'Shea JJ, Husa M, Li D, Hofmann SR, et al. (2004) Jak3 and the pathogenesis of severe combined immunodeficiency. *Mol Immunol* 41:727-37.
49. Cavazzana-Calvo M, Le Deist F, De Saint Basile G, Papadopoulo D, et al. (1993) Increased radiosensitivity of granulocyte macrophage colony-forming units and skin fibroblasts in human autosomal recessive severe combined immunodeficiency. *J Clin Invest* 91:1214-8.

50. Nicolas N, Moshous D, Cavazzana-Calvo M, Papadopoulo D, et al. (1998) A human severe combined immunodeficiency (SCID) condition with increased sensitivity to ionizing radiations and impaired V(D)J rearrangements defines a new DNA recombination/repair deficiency. *J Exp Med* 188:627-34.
51. Moshous D, Pannetier C, Chasseval Rd R, Deist FI F, et al. (2003) Partial T and B lymphocyte immunodeficiency and predisposition to lymphoma in patients with hypomorphic mutations in Artemis. *J Clin Invest* 111:381-7.
52. Li L, Moshous D, Zhou Y, Wang J, et al. (2002) A founder mutation in Artemis, an SNM1-like protein, causes SCID in Athabaskan-speaking Native Americans. *J Immunol* 168:6323-9.
53. Villa A, Sobacchi C, Vezzoni P (2001) Recombination activating gene and its defects. *Curr Opin Allergy Clin Immunol* 1:491-5.
54. Adjali O, Marodon G, Steinberg M, Mongellaz C, et al. (2005) In vivo correction of ZAP-70 immunodeficiency by intrathymic gene transfer. *J Clin Invest* 115:2287-95.
55. Steinberg M, Swainson L, Schwarz K, Boyer M, et al. (2000) Retrovirus-mediated transduction of primary ZAP-70-deficient human T cells results in the selective growth advantage of gene-corrected cells: implications for gene therapy. *Gene Ther* 7:1392-400.
56. DiSanto JP, Rieux-Laucat F, Dautry-Varsat A, Fischer A, et al. (1994) Defective human interleukin 2 receptor gamma chain in an atypical X chromosome-linked severe combined immunodeficiency with peripheral T cells. *Proc Natl Acad Sci U S A* 91:9466-70.
57. Ginn SL, Smyth C, Wong M, Bennetts B, et al. (2004) A novel splice-site mutation in the common gamma chain (gammac) gene IL2RG results in X-linked severe combined immunodeficiency with an atypical NK+ phenotype. *Hum Mutat* 23:522-3.
58. Puck JM, Pepper AE, Henthorn PS, Candotti F, et al. (1997) Mutation analysis of IL2RG in human X-linked severe combined immunodeficiency. *Blood* 89:1968-77.
59. Russell SM, Johnston JA, Noguchi M, Kawamura M, et al. (1994) Interaction of IL-2R beta and gamma c chains with Jak1 and Jak3: implications for XSCID and XCID. *Science* 266:1042-5.
60. Fischer A, de Saint Basile G, Le Deist F (2005) CD3 deficiencies. *Curr Opin Allergy Clin Immunol* 5:491-5.