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Disabled Receptor Signaling and New Primary Immunodeficiency Disorders

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Related articles, pages 1901, 1913

During the past decade, we have witnessed major advances in the identification of mutations that cause primary immunodeficiency disorders—exciting results of the successful blending of fundamental and clinical research. Among the disturbances that disable the immune system is severe combined immunodeficiency (SCID), which occurs at a rate of 1 per 75,000 births. Babies born with SCID have greatly increased susceptibility to many environmental pathogens, and without treatment, these babies die of infection within 6 to 12 months. The curative treatment is allogeneic hematopoietic stem-cell transplantation, which provides

the T-cell progenitors that are missing in patients with SCID.

The genetic defects that cause SCID have been identified in more than 95 percent of cases. This information permits precise diagnoses and should also permit the development of therapeutic agents that are tailor-made for each SCID variant. An example is the historic advance made by Alain Fischer and colleagues at the Hôpital Necker-Enfants Malades in Paris, who used gene transfer to repair a particular genetic defect in a group of patients with SCID.¹ Some 10 different molecular defects are now known to underlie the various forms of SCID, with each defect corresponding

to a key step in the differentiation of T cells.

The process of T-cell differentiation begins with the migration of a progenitor stem cell from the bone marrow to the thymus. Within the thymus, this progenitor converts to a thymocyte with a pre-T-cell-receptor complex (see Figure 1A). This complex consists of a pre-T-cell-receptor dimer and four polypeptide chains: CD3 γ , CD3 δ , CD3 ϵ , and ζ -chain dimer (ζ - ζ). These chains facilitate expression of the complex on the cell surface and transmit the intracellular signals that are needed for the progression of the cell to a thymocyte expressing a mature T-cell-receptor complex with the coreceptors

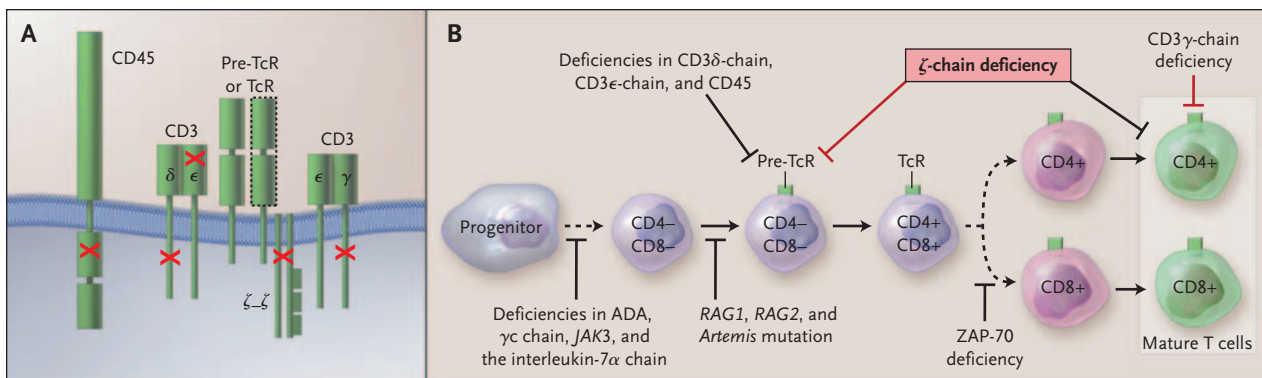


Figure 1. Mutations in Multiple Proteins, Including the CD3 and ζ Chains, That Cause T-Cell Immunodeficiencies.

The pre-T-cell-receptor (pre-TcR) and mature TcR complexes consist of a receptor dimer associated with CD3 chains γ , δ , and ϵ and a ζ -chain dimer (Panel A). The pre-TcR complex differs from the mature complex owing to the presence of a surrogate chain (indicated by a dotted line) in the pre-TcR dimer. The CD3 and ζ chains facilitate the expression of the complex on the cell surface and send intracellular signals. Mutations in the receptor complexes that have been linked to T-cell immunodeficiencies are indicated with a red X. T-cell differentiation (Panel B) entails the progression from a progenitor cell to a CD4-CD8- thymocyte that expresses a pre-TcR, followed by differentiation into a CD4+CD8+ thymocyte expressing the mature TcR. This cell develops into a single CD4+CD8- or CD4-CD8+ thymocyte and, finally, into a CD4+CD8- or CD4-CD8+ mature T cell. Dashed lines indicate that intervening steps occur that are not shown. The stages of differentiation affected by mutations and deficiencies of different proteins are shown by T bars. Red bars indicate a partial effect. ADA denotes adenosine deaminase, JAK3 Janus kinase 3, RAG recombination-activating gene, and ZAP-70 zeta-chain-associated protein of 70 kD.

CD4 and CD8 (see Figure 1B). At this stage of differentiation, self-reactive thymocytes are deleted from the T-cell repertoire, whereas others become single CD4+ or CD8+ thymocytes and, eventually, mature T cells.

There are four categories of SCID, each of which results in a differentiation defect and a failure to produce T cells (see Figure 1B). The first involves deficiencies in adenosine deaminase (ADA) that lead to the death of progenitor cells and accounts for one fifth of cases. The second category involves mutations in the γ c subunit of cytokine receptors (for interleukins 2, 4, 7, 9, 15, and 21) that lead to X-linked SCID (SCID-X1). These mutations are responsible for about 50 percent of cases. In the same category, mutations in the interleukin-7 receptor α chain and the signaling protein Janus kinase 3 (JAK3) produce the same phenotype (lack of T cells and natural killer cells

but detectable B cells) and account for 5 to 10 percent of cases of SCID. The third category consists of mutations in the recombination-activating gene 1 or 2 (*RAG1* or *RAG2*) or the *Artemis* gene; these genes are required for somatic V, D, and J rearrangements that encode the antigen-binding site of the pre-T-cell receptor and the mature T-cell receptor. They account for 20 percent of cases. The fourth category involves mutations in the genes for the CD3 δ and CD3 ϵ chains that cause a block at the pre-T-cell-receptor stage of development and account for less than 1 percent of cases (see Figure 1B). Mutations in the gene for CD45 — a phosphatase that modulates intracellular signaling — can cause a similar immunodeficiency and also account for less than 1 percent of cases. Mutations in other genes, such as those for the CD3 γ chain and the signaling kinase zeta-chain-associated protein of 70 kD (*ZAP-70*),

cause milder immunodeficiencies. Although these mutations fail to block differentiation completely, the resultant T cells are impaired in their responses to antigen. *ZAP-70* mutations can cause the selective loss of CD8+ T cells and nonfunctional CD4+ cells.

With this progress, a key remaining question is this: Have we seen the last of mutations that cause SCID and other, less severe immunodeficiencies? In this issue of the *Journal*, Rieux-Laucat et al. (pages 1913–1921) describe a four-month-old boy with recurrent pulmonary infections and mutations (termed Q70X) at position 70 in both alleles of the gene for the ζ chain of the pre-T-cell-receptor complex. This mutation resulted in reduced numbers of T cells and reduced expression of the T-cell receptor. Furthermore, the mutation led to the deletion of the signaling portion of the molecule, resulting in impairment of the intracellular signaling need-

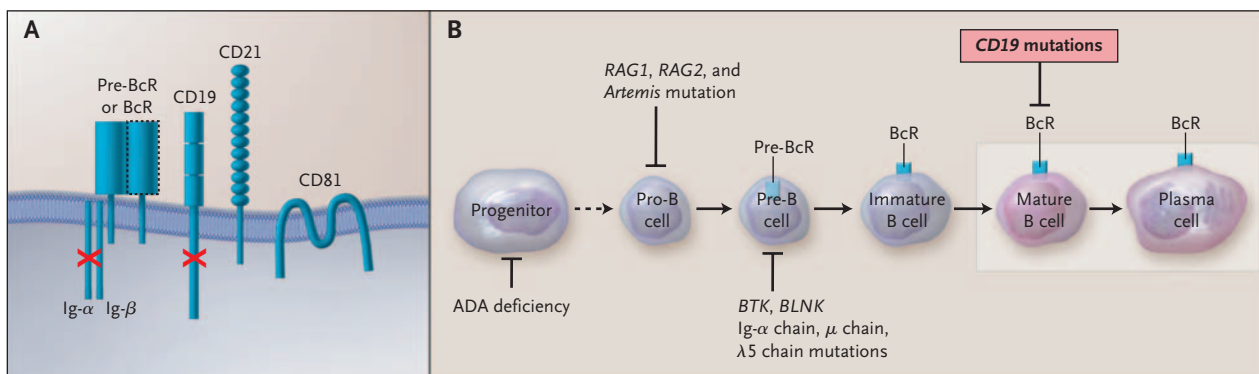


Figure 2. Mutations in Multiple Proteins, Including the Pre-B-Cell Receptor (Pre-BcR) and CD19, That Cause B-Cell Immunodeficiencies.

The pre-BcR and mature BcR complexes consist of an immunoglobulin dimer associated with the Ig- α and Ig- β subunits that generate intracellular signals (Panel A). The pre-BcR differs from the mature complex owing to the presence of a surrogate light chain (indicated by a dotted line) in the pre-BcR dimer. Further associated with the BcR are CD19, CD21, CD81 (TAPA-1), and CD225 (Leu-13, not shown), which act as coreceptors to modulate the threshold of signaling. Mutations in the receptor complexes that have been linked to B-cell immunodeficiencies are indicated with a red X. B-cell differentiation (Panel B) entails a progression from a progenitor stem cell to a pro-B cell to a pre-B cell to an immature B cell and, finally, to a mature B cell. The dashed line indicates that intervening steps occur that are not shown. The pre-BcR provides signals for pre-B-cell differentiation. The stages of B-cell differentiation affected by mutations and deficiencies of different proteins are shown by T bars. ADA denotes adenosine deaminase, RAG recombination-activating gene, BTK Bruton's tyrosine kinase, and BLNK mutated B-cell-linked protein.

ed to mount a response to antigens such as tetanus toxoid. In a fascinating twist, multiple somatic mutations in about 10 percent of the child's T cells had occurred at the same site (Q70) as the germ-line mutations in the ζ -chain gene. These cells expressed normal levels of the T-cell receptor and contributed to the residual response of the patient's immune system. The deficiency of the ζ chain seems to cause a less severe form of immunodeficiency than is caused by mutations in the *CD3 δ* or *CD3 ϵ* gene but a more severe form than is caused by mutations in the *CD3 γ* gene, which result in only a slight reduction in T-cell numbers and mild immunodeficiency (see Figure 1B).

Overall, the work of Rieux-Laucat et al. is important, first because it identifies the first mutation in the ζ -chain gene that is responsible for an immunodeficiency, and second, because it identifies a connection in the signaling property of the chain with the immune defect. Gene-transfer therapy with the ζ -chain gene could be an option in the treatment of patients who have a deficiency of the ζ chain.

A connection between defects in receptor signaling and disorders is not new to the field of B-cell immunodeficiencies. Patients with B-cell-immunodeficiency diseases are especially susceptible to recurrent bacterial infections, because of a lack of immunoglobulins (agammaglobulinemia), an imbalance of isotypes (dysgammaglobulinemia), or the presence of common variable immunodeficiency, a syndrome characterized by hypogammaglobulinemia. In

this case, the pre-B-cell receptor is made up of a μ chain linked to a surrogate light chain and the Ig- α and Ig- β subunits that provide signals for pre-B-cell-receptor differentiation (see Figure 2A). The pre-B-cell receptor eventually is converted to a mature B-cell-receptor complex with rearranged heavy and light chains.

As in T cells, disabling mutations in B cells interfere with the process of differentiation of a progenitor cell to a pro-B cell and, from there, to immature and mature B cells (see Figure 2B). Most cases of agammaglobulinemia are due to mutations in the gene for either the B-cell signaling kinase — Bruton's tyrosine kinase, the deficiency of which causes X-linked agammaglobulinemia — or the B-cell-linked protein, a signaling molecule in pre-B cells. Mutations in the gene for the Ig- α subunit can cause a disorder that is clinically similar to XLA. Mutations in *ADA*, *RAG1*, *RAG2*, and *Artemis* also impair B-cell development at the progenitor, pro-B-cell, and pre-B-cell stages. Mutations in the μ chain and the $\lambda 5$ chain can also interfere with the pre-B-cell-receptor stage (see Figure 2B).

Notably, the pre- and mature B-cell receptors on the cell surface also associate with so-called coreceptors — CD19, CD21, CD81 (TAPA-1), and CD225 (Leu-13) — that modulate the amplitude of the response of the B cell (see Figure 2A). Until now, mutations in coreceptors have not been linked to an immunodeficiency. In this issue of the *Journal*, however, van Zelm et al. (pages 1901–1912) describe four patients with hypogam-

maglobulinemia and germ-line mutations in *CD19*. Unlike patients with XLA, the patients described by van Zelm et al. had normal numbers of B cells. Levels of CD19 were undetectable in one patient and reduced in the other three. As in the ζ -chain mutation in T cells, the *CD19* mutation resulted in the deletion of the signaling portion of the protein. For this reason, the remaining CD19 protein was profoundly defective in generating the signals needed for B-cell activation and the development of immunologic memory. This work not only links a defect in CD19 to an antibody-deficiency syndrome but also pinpoints a defect in CD19-mediated intracellular signaling as the mechanism.

Can mutations in the genes for coreceptors on T cells also cause immunodeficiencies? CD28 and inducible T-cell costimulator (ICOS), which modulate the threshold of T-cell activation, are two such coreceptors. In CD28-deficient mice, responses to most antigens are greatly reduced, and mice deficient in ICOS are especially defective in the formation of germinal centers and the production of immunoglobulin. Mutations in *CD28* would be predicted to affect T-cell immunity, whereas deleterious mutations in ICOS would be expected to cause antibody deficiencies.

Genes with a role in more severe cases of immunodeficiency that might be explored in the future include those encoding the linker for the activation of T cells (LAT) and the SH2 domain-containing leukocyte protein of 76 kD (SLP-76). The lack of these proteins in mice causes a block

in T-cell differentiation similar to that caused by the loss of the CD3 δ or CD3 ϵ chain. Similarly, Grb2-related protein (GADS) bridges LAT with SLP-76. GADS-deficient mice possess normal numbers of T cells that are impaired in their responses to antigen. Research on new candidate genes will extend the field's exciting advances and ultimately lead to increasingly refined diagnoses and treatments for primary immunodeficiency disorders.

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1. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* 2000;288:627-9.