

Brief Report

**ATYPICAL X-LINKED SEVERE
COMBINED IMMUNODEFICIENCY DUE
TO POSSIBLE SPONTANEOUS
REVERSION OF THE GENETIC DEFECT
IN T CELLS**

VOLKER STEPHAN, M.D., VOLKER WAHN, M.D.,
FRANÇOISE LE DEIST, M.D., UTA DIRKSEN, M.D.,
BARBARA BRÖKER, PH.D., INGRID MÜLLER-FLECKENSTEIN,
GERD HORNEFF, M.D., HORST SCHROTEN, M.D.,
ALAIN FISCHER, M.D., PH.D.,
AND GENEVIÈVE DE SAINT BASILE, M.D., PH.D.

X-LINKED severe combined immunodeficiency is a recessive hereditary disease characterized by severe and persistent infections starting in the first months of life and associated with diarrhea and failure to thrive.¹ Affected infants almost invariably present with an absence of T cells and natural killer cells, normal or elevated B-cell counts, and hypogammaglobulinemia. This disease is rapidly fatal without bone marrow transplantation.²

The disease locus has been mapped to Xq12-13,³ and the genetic defect identified as a mutation of the γ chain of the interleukin-2 receptor,⁴ which has been cloned and was recently renamed the common γ (γ c) chain because of its association with cytokine receptors for interleukin-4, 7, 9, and 15.⁵⁻¹² Thus, the early lymphoid progenitor cells in patients with X-linked combined immunodeficiency are unable to respond to the cytokine signals that are crucial for the normal development of T cells and late-stage B cells.

A number of different point mutations and deletions have been described in patients with typical X-linked severe combined immunodeficiency. An attenuated phenotype was observed in a patient with a splice-site mutation resulting in diminished expression of the γ c chain and in another patient with a

point mutation in the intracytoplasmic domain of the γ c gene.^{13,14}

We describe a boy in whom X-linked severe combined immunodeficiency was diagnosed at one year of age on the basis of family history, clinical symptoms, and evidence of a genetic defect in the γ c gene in B-cell lines derived from his peripheral blood. The unusual finding of low-to-normal numbers of T cells, attenuated proliferative responses to antigens and mitogens, and a positive skin test for purified protein derivative led to further genetic analysis that showed normal expression of the γ c chain and an absence of the γ c gene mutation in the patient's T cells. Reversion of the mutation in early T-cell precursors in this patient resulted in subnormal development of peripheral T cells. Partial reversion of the mutation may thus occur in lymphocyte progenitors and produce an atypical severe combined immunodeficiency with partially functional lymphocyte clones.

CASE REPORT

A male infant was born to a 32-year-old woman after an uncomplicated pregnancy and normal vaginal delivery. There was no history of consanguinity in the family, and his two older sisters were healthy. One maternal uncle and a maternal granduncle had died of pneumonia at the respective ages of four months and six months. Vaccination with bacille Calmette-Guérin was performed at two weeks of age. At six months of age the patient was hospitalized for severe interstitial pneumonia. At one year of age he was referred to our hospital for suspected immunodeficiency. Physical examination revealed no signs of graft-versus-host disease and no abnormalities except for a large abscess in the left lumbar region. Examination of the drainage fluid revealed acid-fast bacilli with genetic evidence of bacille Calmette-Guérin.

At diagnosis at 12 months of age, immunologic investigations showed a normal number of T cells (1200 per cubic millimeter), a high B-cell count (2400 per cubic millimeter), and hypogammaglobulinemia (IgG, 1.9 g per liter; IgM, 0.6 g per liter; and undetectable levels of IgA) with no detectable specific antibody responses. The T-cell responses *in vitro* are shown in Table 1. The patient's reaction to purified protein derivative was strongly positive. HLA typing and karyotyping of peripheral-blood mononuclear cells showed no evidence of maternal engraftment.

The abscess was drained and successfully treated by a regimen of three antituberculosis drugs. Over the following two years the patient had no further infectious complications. As of this writing, he has been living at home in good health for 12 months. He continues to receive isoniazid and trimethoprim-sulfamethoxazole (co-trimoxazole) prophylaxis as well as monthly infusions of immune globulin.

METHODS

Flow Cytometry

Flow cytometry was performed according to standard protocols with a FACScan flow cytometer (Becton Dickinson, San Diego, Calif.). The following monoclonal antibodies were used: anti-CD2, anti-CD3, anti-CD4, anti-CD8, anti-CD16, anti-HLA-DR, anti-CD20, anti-CD56, and anti-CD14 (all from Becton Dickinson) and anti-V β 2, anti-V β 3, anti-V β 8, anti-V β 13.6, anti-V β 17, and anti-V β 21 (all from Immunotech, Marseille, France). The rat monoclonal antibody TuGh4 (kindly provided by Dr. K. Sugamura, Tohoku University, Sendai, Japan) is directed against the γ chain of the interleukin-2 receptor.

From Universitätskinderklinik, Heinrich-Heine Universität, Düsseldorf, Germany (V.S., V.W., U.D., G.H., H.S.); INSERM Unité 429, Hôpital Necker-Enfants Malades, Paris (F.L.D., A.F., G.S.B.); Bernhard Nocht-Institut, Hamburg, Germany (B.B.); and Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg, Nuremberg, Germany (I.M.-F.). Address reprint requests to Dr. Wahn at Heinrich-Heine University Düsseldorf, Department of Pediatrics, Moorenstr. 5, 40225 Düsseldorf, Germany.

©1996, Massachusetts Medical Society.

Lymphocyte Proliferation

Proliferation assays were performed as described previously.¹⁵ Recombinant human interleukin-2 (generously provided by Eurocetus, Amsterdam) was used at concentrations of up to 30 IU per milliliter.

Establishment of B-Cell and T-Cell Lines

B-lymphoblastoid cell lines were obtained from the patient according to standard protocols for Epstein-Barr virus (EBV) infection.¹⁶ For the generation of permanent, growing T-cell lines the patient's T cells were stimulated with phytohemagglutinin and interleukin-2 in the presence of irradiated allogeneic peripheral-blood mononuclear cells from a healthy donor. T-cell blasts were then infected with herpesvirus saimiri C488 and allowed to proliferate in the presence of interleukin-2 without further stimulation with mitogens or accessory cells.¹⁷

DNA Analysis

For the sequence analysis of the gene encoding the γ chain, full-length transcripts of the γ chain of the interleukin-2 receptor from a B-lymphoblastoid cell line were amplified with an assay involving reverse transcription and a nested polymerase chain reaction (PCR), and the mutant portion was then sequenced directly. We searched for the mutation in other cell populations from the patient by sequencing exon 3 of the γ chain of the interleukin-2 receptor from genomic DNA isolated from sorted B cells (CD19+), T cells (CD3+), monocytes (CD14+), and polymorphonuclear cells as previously described.^{18,19} The fragments were directly sequenced with a thermal cycler sequencing kit (Amersham, Paris).

We searched for X-chromosome mosaicism in the patient by microsatellite typing of DNA isolated from his B-cell line, the T-cell line infected with herpesvirus saimiri, and sorted CD3+ cells from the patient's and his mother's peripheral-blood cells. PCR was performed with 1 μ g of the DNA preparation with the specific primers at the DXS106 and DXS441 loci as previously described.²⁰

TABLE 1. IMMUNOLOGIC ANALYSIS OF THE PATIENT'S PERIPHERAL-BLOOD LEUKOCYTES.

TYPE OF CELL	PATIENT	NORMAL RANGE OR CONTROL VALUE
		cells/mm ³
T cells		
CD3+	815-2050	1200-2500
CD4+	250-828	720-2000
CD8+	360-1860	240-1000
B cells		
CD20+	930-2400	100-600
Natural killer cells		
CD56+	Undetectable	100-500
	T-cell proliferation (cpm $\times 10^{-3}$)	
Stimulus*		
None, day 4†	0.5 \pm 0.1	0.4 \pm 0.2
Phytohemagglutinin	3.3 \pm 1.3	34.0 \pm 15.0
OKT3	5.7 \pm 3.0	31.5 \pm 13.2
None, day 6‡	1.0 \pm 0.2	0.3 \pm 0.1
Tetanus toxoid	4.6 \pm 1.2	23.2 \pm 9.7
Purified protein derivative	7.1 \pm 2.3	24.0 \pm 8.1
Allogeneic cells	12.3 \pm 3.9	34.9 \pm 11.1

*Plus-minus values are means \pm SD.

†The experiments with phytohemagglutinin and OKT3 ended on day 4.

‡The experiments with antigens and allogeneic cells ended on day 6.

TABLE 2. EXPRESSION OF DIFFERENT T-CELL-RECEPTOR $V\beta$ FAMILIES BY THE PATIENT'S CD4+ AND CD8+ CELLS, AS DETECTED BY MONOCLONAL ANTIBODIES.*

$V\beta$	PATIENT		AGE-MATCHED NORMAL CONTROLS	
	CD4+	CD8+	CD4+	CD8+
	percentage of cells expressing TCR $V\beta$ chains†			
$V\beta 2$	2	5	10.5 \pm 1.7	4.1 \pm 1.6
$V\beta 3$	3	0.2	3.6 \pm 2.3	5.8 \pm 3.0
$V\beta 8$	3	0.4	4.6 \pm 0.9	4.9 \pm 1.8
$V\beta 13.6$	3	0.2	2.2 \pm 0.4	2.0 \pm 1.7
$V\beta 17$	5	0.6	5.1 \pm 1.6	5.0 \pm 1.9
$V\beta 21$	2	0.1	2.9 \pm 0.9	2.3 \pm 1.2

*Plus-minus values are means \pm SD.

†TCR denotes T-cell receptor.

RESULTS

Immunologic Studies

The absolute number of circulating lymphocytes ranged from 2200 to 4600 per cubic millimeter. At least 20 percent of the lymphocyte population stained with antibodies directed against CD2 or CD3 (Table 1). This T-cell population had a mature phenotype of CD4+ or CD8+. However, a severely diminished number of CD4+ T cells and an increased number of CD8+ T cells and CD20+ B cells were repeatedly detected. There was normal expression of HLA class I and class II antigens and adhesion molecules CD11 and CD18 (data not shown). The expression of the $V\beta$ family of monoclonal antibodies by CD4+ cells was normal, whereas their expression by CD8+ cells was substantially lower than that in age-matched controls (Table 2). The absence of γ/δ + cells in the CD8+ population may provide some evidence that the altered pattern of use of clonal $V\beta$ was constitutive rather than due to mycobacterial infection (data not shown). When stimulated, the T-cell proliferative responses to mitogens, tetanus toxoid, and purified protein derivative were repeatedly low but detectable (Table 1). The patient's T-cell responses to allogeneic cells were low but clearly positive (Table 1). Immunophenotype and proliferative responses did not change over a period of 18 months.

Studies of the γ Chain

Because the patient's pedigree was suggestive of an inherited X-linked immune deficiency, we measured the expression of the γ chain on the surface of the patient's EBV-transformed B-cell lines. The expression of the β chain of the interleukin-2 receptor was normal in both the patient's and the control B-cell lines (data not shown), whereas the expression

of the γ c chain was detectable only in the control B-cell lines (Fig. 1A). Direct genomic sequence analysis of the patient's B-cell lines detected a single point mutation in the γ c gene consisting of a change from T to C at position 343 in exon 3, corresponding to amino acid 115, which replaced the normal cysteine-encoding codon with one coding for arginine (Fig. 1B). DNA analysis revealed that the patient's mother was heterozygous for this mutation (data not shown).

Because of the unexpected presence of circulating mature T cells, we sorted and analyzed the patient's CD3+ T cells for expression of the γ c chain and to determine whether the mutation was present. Surprisingly, the expression of the γ c chain by T cells with either the CD4+ or CD8+ phenotype was normal (data not shown), and sequencing of the γ c gene

revealed the wild-type sequence at position 343 (Fig. 2) (and data not shown). In contrast, the sorted CD19+ B cells, the sorted CD14+ monocytes, and the polymorphonuclear-cell population had no detectable expression of the γ c chain on their surface and contained the Cys→Arg mutation at position 115.

The possibility that the patient's circulating T-cell population was derived from the engraftment of T cells from the mother in utero was excluded by T-cell karyotyping and HLA typing (data not shown). In addition, the X chromosome present in the patient's T-cell and B-cell populations was assessed by study of two microsatellites flanking the severe combined immunodeficiency locus on the X chromosome. These cell populations had only one X chromosome derived from the mother, with

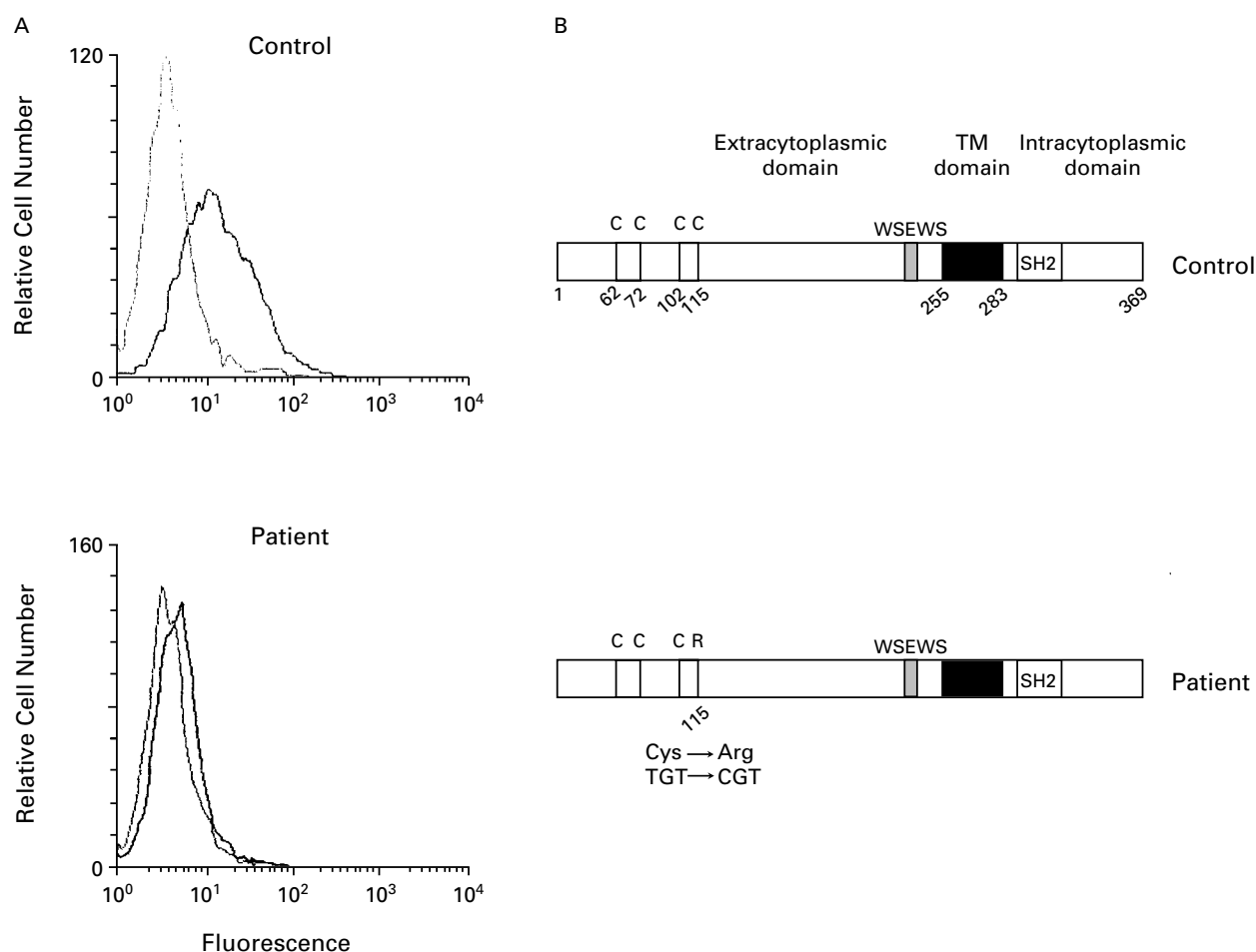


Figure 1. Study of the γ c Chain in B-Cell Lines from the Patient and a Normal Control.

In Panel A, cells from the patient's B-cell line and from a control B-cell line were stained for the γ c chain and analyzed by flow cytometry (thick lines), as described in the Methods section. In each panel, the thin lines represent the fluorescence profiles of cells stained first with an isotype-matched irrelevant antibody and then with a second antibody. Panel B shows the normal sequence of the γ c chain and the missense mutation identified in the γ c chain of the DNA isolated from the patient's B-cell line. TM denotes transmembrane, and WSEWS denotes the following amino-acid sequence: tryptophan-serine-unconserved amino acid-tryptophan-serine. SH2 denotes Src homology region 2.

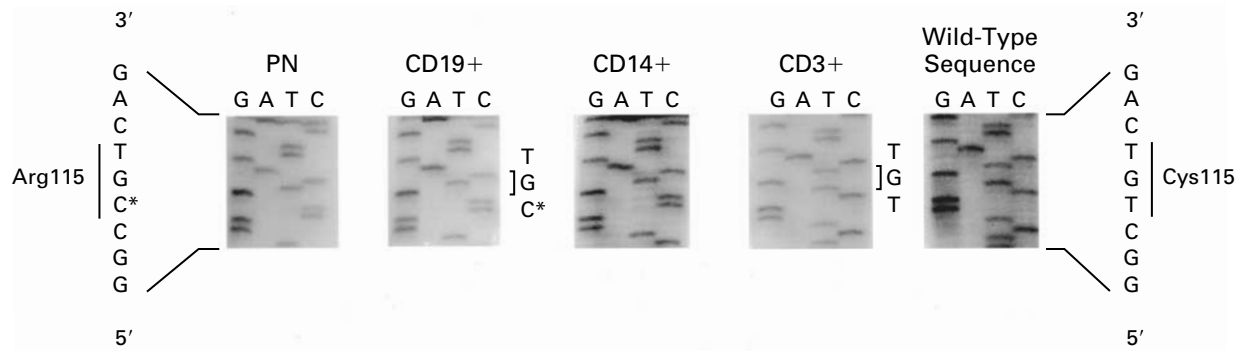


Figure 2. Direct Sequencing of the γc Chain in Polymorphonuclear Cells (PN), CD19+ Cells, CD14+ Cells, and CD3+ Cells from the Patient.

The asterisk denotes the single-nucleotide difference causing the substitution of arginine for cysteine at position 115 in each case. The wild-type sequence is shown on the right.

the same X chromosome present in both T-cell and B-cell populations (data not shown).

DISCUSSION

We describe a boy with an attenuated form of severe combined immunodeficiency resulting from a point mutation in the γc gene in B cells. There was, however, normal expression of the γc chain by T cells and some proliferative responses of T cells. Genetic analysis of the boy's B cells revealed a point mutation leading to the substitution of arginine for the cysteine residue at position 115, thereby confirming the initial clinical diagnosis of severe combined immunodeficiency. The cysteine replaced belongs to the consensus residues common to all members of the cytokine I receptor family.²¹ A similar molecular defect affecting the same amino acid was previously identified in two unrelated patients with typical severe combined immunodeficiency²² (and unpublished data). Therefore, this amino acid residue seems critical for the expression of the γc chain and subsequent normal T-cell development. In contrast to the typical patient with severe combined immunodeficiency, our patient had mature T cells and detectable but diminished mitogen and antigen-specific responses. Subsequent genetic analysis showed an absence of the genetic defect in T cells from his peripheral blood. HLA typing and cytogenetic studies ruled out the possibility of maternal engraftment.

The mosaicism detected in the patient could be due to a postzygotic somatic mutation of the normal maternal X chromosome in some progenitor cells or to a reversion of the mutation, as shown for severe combined immunodeficiency due to adenosine deaminase deficiency.²³⁻²⁵ However, the presence of the identical mutation of the γc gene in the patient's mother and the presence of the same X-chromosome region encompassing the severe combined immunodeficiency locus in the patient's T and B cells argue

for a reversion event causing a correction of the inherited molecular defect. Although a C→T reversion of a specific deleterious point mutation is statistically highly unlikely, *in vivo* selection could allow such a rare event. In patients with severe combined immunodeficiency, a reversion of the γc mutation in T cells would confer a distinct advantage over cells without functional γc chains in terms of growth and differentiation.

This possibility is in keeping with the finding of a nonrandom pattern of X-chromosome inactivation in T cells and natural killer cells of heterozygous female carriers of X-linked severe combined immunodeficiency, resulting in the survival only of cells with the normal X chromosome.²⁶ Therefore, given the selection advantage of T cells expressing the functional γc chain in the different cytokine receptors, it is conceivable that all T cells in this patient arose from a single revertant T-cell precursor. This possibility will be important to consider in future attempts at gene therapy.

Although a normal γc chain is present in the reverted T cells, the population remains poorly functional. Low antigen-specific proliferative responses can be the result of a restricted repertoire of T-cell-receptor antigens because of a late reversion during thymocyte ontogeny. However, the expression of $V\beta$ by CD8+ cells was substantially less than that in the patient's age-matched normal controls, whereas the expression of $V\beta$ by the responsive CD4+ cells was similar to that in the controls. Alternatively, to be normal, T-cell proliferative responses to mitogens and antigens may need the help of monocytes activated through their γc chain, which was nonfunctional in our patient.²⁷

The presence of normal γc -chain expression by both CD4+ and CD8+ cells, which are partially functional, in addition to the absence of natural killer cells in this patient, suggests that reversion of

the mutation occurred in early T-cell precursors. This event most likely occurred after B-cell and natural-killer-cell progenitors were already committed and before the rearrangement of the β -chain gene of the T-cell receptor, at a stage of differentiation that can be reached in the absence of γ c-chain expression.

If one assumes that there was only a single reversion event and that the single T-cell progenitor gave rise to a number of diversified T-cell clones, then the process of cell proliferation must have been very active before the rearrangements of the β - and α -chain genes of the T-cell receptor. A careful analysis of the T-cell repertoire is under way. The determination of the T-cell-receptor sequence in our patient may provide further insight into the process of clonal expansion following infection or immunization as well as into the risk of progressive exhaustion of the differentiated T-cell clones. Unless the revertant progenitor cell has the capacity for self-renewal — an unlikely possibility — further changes in the T-cell repertoire are to be expected.

Supported by grants from the European Economic Community (BIO2-CT92-0164) and the Association Française contre les Myopathies, le Ministère de la Recherche et de la Technologie (ACC-SV).

We are indebted to N. Lambert, N. Vente, and S. Schumacher for excellent technical assistance; to Dr. J. Peake for revising the manuscript; and to B. Neveu for typing the manuscript.

REFERENCES

1. Primary immunodeficiency diseases: report of a WHO scientific group. In: Rosen FS, Seligmann M, eds. Immunodeficiencies. Chur, Switzerland: Harwood Academic, 1993:1-29.
2. Stephan JL, Vlekova V, Le Deist F, et al. Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 patients. *J Pediatr* 1993;23:564-72.
3. de Saint Basile G, Arveiler B, Oberle I, et al. Close linkage of the locus for X chromosome-linked severe combined immunodeficiency to polymorphic DNA markers in Xq11-q13. *Proc Natl Acad Sci U S A* 1987;84:7576-9.
4. Noguchi M, Yi H, Rosenblatt HM, et al. Interleukin-2 receptor γ chain mutation results in X-linked severe combined immunodeficiency in humans. *Cell* 1993;73:147-57.
5. Takeshita T, Asao H, Suzuki J, Sugamura K. An associated molecule, p64, with high-affinity interleukin 2 receptor. *Int Immunol* 1990;2:477-80.
6. Kondo M, Takeshita T, Ishii N, et al. Sharing of the interleukin-2 (IL-2) receptor γ chain between receptors for IL-2 and IL-4. *Science* 1993;262:1874-7.
7. Kondo M, Takeshita T, Higuchi M, et al. Functional participation of the IL-2 receptor γ chain in IL-7 receptor complexes. *Science* 1994;263:1453-4.
8. Russell SM, Keegan AD, Harada N, et al. Interleukin-2 receptor γ chain: a functional component of the interleukin-4 receptor. *Science* 1993;262:1880-3.
9. Giri JG, Ahdieh M, Eisenman J, et al. Utilization of the β and γ chains of the IL-2 receptor by the novel cytokine IL-15. *EMBO J* 1994;13:2822-30.
10. Russell SM, Johnston JA, Noguchi M, et al. Interaction of IL-2R β and γ c chains with Jak1 and Jak3: implications for XSCID and XCID. *Science* 1994;266:1042-5.
11. Nakamura Y, Russell SM, Mess SA, et al. Heterodimerization of the IL-2 receptor β - and γ -chain cytoplasmic domains is required for signaling. *Nature* 1994;369:330-3.
12. Minami Y, Kono T, Miyazaki T, Taniguchi T. The IL-2 receptor complex: its structure, function, and target genes. *Annu Rev Immunol* 1993;11:245-68.
13. DiSanto JP, Rieux-Laucat F, Dautry-Varsat A, Fischer A, de Saint Basile G. Defective human interleukin 2 receptor γ chain in an atypical X chromosome-linked severe combined immunodeficiency with peripheral T cells. *Proc Natl Acad Sci U S A* 1994;91:9466-70.
14. Morelon E, Dautry-Varsat A, Hacelion-Bay S, Fischer A, de Saint Basile G. T-lymphocyte differentiation in the absence of the cytoplasmic tail of the common cytokine receptor γ c chain in a severe combined immunodeficiency X1 patient. *Blood* (in press).
15. Wahn V, Yokota S, Meyer KL, et al. Expansion of a maternally derived monoclonal T cell population with CD3⁺/CD8⁺/T cell receptor- γ/δ ⁺ phenotype in a child with severe combined immunodeficiency. *J Immunol* 1991;147:2934-41.
16. Tosato G. Generation of Epstein-Barr virus (EBV) — immortalized B cell lines. In: Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W, eds. Current protocols in immunology. Vol. 3. New York: John Wiley, 1994:7.22.1-7.22.3.
17. Biesinger B, Müller-Fleckenstein I, Simmer B, et al. Stable growth transformation of human T lymphocytes by herpesvirus saimiri. *Proc Natl Acad Sci U S A* 1992;89:3116-9.
18. DiSanto JP, Dautry-Varsat A, Certain S, Fischer A, de Saint Basile G. Interleukin-2 (IL-2) receptor γ chain mutations in X-linked severe combined immunodeficiency disease result in the loss of high-affinity IL-2 receptor binding. *Eur J Immunol* 1994;24:475-9.
19. Noguchi M, Adelstein S, Cao X, Leonard WJ. Characterization of the human interleukin-2 receptor γ chain gene. *J Biol Chem* 1993;268:13601-8.
20. Markiewicz S, DiSanto JP, Chelly J, et al. Fine mapping of the human SCIDX1 locus at Xq12-13.1. *Hum Mol Genet* 1993;2:651-4.
21. Bazan JF. Structural design and molecular evolution of a cytokine receptor superfamily. *Proc Natl Acad Sci U S A* 1990;87:6934-8.
22. Puck JM, Deschènes SM, Porter JC, et al. The interleukin-2 receptor γ chain maps to Xq13.1 and is mutated in X-linked severe combined immunodeficiency, SCIDX1. *Hum Mol Genet* 1993;2:1099-104.
23. Hirschhorn R, Yang DR, Israni A, Huie ML, Ownby DR. Somatic mosaicism for a newly identified splice-site mutation in a patient with adenosine deaminase-deficient immunodeficiency and spontaneous clinical recovery. *Am J Hum Genet* 1994;55:59-68.
24. Youssoufian H. Natural gene therapy and the Darwinian legacy. *Nat Genet* 1996;13:255-6.
25. Hirschhorn R, Yang DR, Puck JM, Huie ML, Jiang CK, Kurlandsky LE. Spontaneous in vivo reversion to normal of an inherited mutation in a patient with adenosine deaminase deficiency. *Nat Genet* 1996;13:290-5.
26. Puck JM, Nussbaum RL, Conley ME. Carrier detection in X-linked severe combined immunodeficiency based on patterns of X chromosome inactivation. *J Clin Invest* 1987;79:1395-400.
27. Bosco MC, Espinoza-Delgado I, Schwabe M, et al. Regulation by interleukin-2 (IL-2) and interferon γ of IL-2 receptor γ chain gene expression in human monocytes. *Blood* 1994;83:2995-3002.