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# Interleukin-2 Receptor γ Chain Mutation Results in X-Linked Severe Combined Immunodeficiency in Humans

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## Summary

**The interleukin-2 (IL-2) receptor γ chain (IL-2Rγ) is a component of high and intermediate affinity IL-2 receptors that is required to achieve full ligand binding affinity and internalization. We have localized the IL-2Rγ gene to human chromosome Xq13. Genetic linkage analysis indicates that the IL-2Rγ gene and the locus for X-linked severe combined immunodeficiency (XSCID) appear to be at the same position. Moreover, we demonstrate that each of three unrelated patients with XSCID has a different mutation in his IL-2Rγ gene resulting in a different premature stop codon and predicted C-terminal truncation. These data establish that XSCID is associated with mutations of the IL-2Rγ gene product. Since XSCID is characterized by absent or markedly reduced numbers of T cells, our findings imply that IL-2Rγ plays a vital role in thymic maturation of T cells. These results also have important implications for prenatal and postnatal diagnosis, carrier female detection, and gene therapy for XSCID.**

## Introduction

Interleukin-2 (IL-2) and IL-2 receptors critically regulate the magnitude and duration of the T cell immune response following antigen activation (reviewed by Leonard, 1992; Smith, 1989; Waldmann, 1989). Roles for this cytokine receptor system have also been suggested in thymic maturation (Raulet, 1985; Jenkinson et al., 1987; Ceredig et al., 1985; Tentori et al., 1988) and B cell responses (Waldmann et al., 1984; Mingari et al., 1984; Loughnan and Nossal, 1989). Different combinations of the IL-2 receptor α (Leonard et al., 1984; Nikaido et al., 1984), β (Sharon et al., 1986; Tsudo et al., 1986; Teshigawara et al., 1987; Dukovich, 1987; Sharon et al., 1988; Siegel et al., 1987; Hatakeyama et al., 1989), and γ (Takeshita et al., 1990, 1992a, 1992b; Voss et al., 1992; Arima et al, 1992) chains (IL-2Rα, IL-2Rβ, and IL-2Rγ, respectively) result in the formation of three different classes of IL-2 receptors. Low affinity receptors contain IL-2Rα, but not IL-2Rβ or IL-2Rγ; intermediate affinity IL-2 receptors contain IL-2Rβ and IL-2Rγ, but not IL-2Rα; and high affinity receptors contain all three chains. The high and intermediate affinity receptors are important for IL-2 signaling.

IL-2Rα is not expressed on resting cells but is strongly induced following T cell activation (Leonard et al., 1984; Nikaido et al., 1984). Its gene spans more than 35 kb on chromosome 10p14–15 (Leonard et al., 1985a, 1985b; Ishida et al., 1985) and is organized in eight exons and seven introns. IL-2Rβ is constitutively expressed but is induced 5- to 10-fold following T cell activation (Siegel et al., 1987). Its gene spans 24 kb on chromosome 22q11.2–12 and is organized in ten exons and nine introns (Gnarra et al., 1990; Shibuya et al., 1990). IL-2Rγ is constitutively expressed. Its gene spans 4.2 kb and is organized in eight exons and seven introns (Noguchi et al., submitted). We now report that the IL-2Rγ gene and the X-linked severe combined immunodeficiency (XSCID) locus are both present at the same location on chromosome Xq13. By direct sequencing of DNA derived from XSCID patients, we demonstrate that mutation of the IL-2Rγ gene is the defect in XSCID, a disease characterized by absent or greatly reduced T cells and severely diminished cell-mediated and humoral immunity (Cooper and Butler, 1989; Gelfand and Dosch, 1983; Conley, 1992).

## Results

### IL-2Rγ Is Located on Chromosome Xq13

The chromosomal location of the IL-2Rγ gene was determined using a full-length IL-2Rγ cDNA probe and Southern blot analysis of EcoRI-digested DNAs from a panel of human–rodent somatic cell hybrids (see Experimental Procedures). An examination of the entire panel of hybrids revealed a perfect correlation between the presence of the IL-2Rγ gene and the presence of the human X chromosome (Table 1). The IL-2Rγ gene could therefore be unambiguously assigned to the human X chromosome, and it segregated discordantly (≥37%) with all other human chromosomes (Table 1).

Analysis of hybrids containing specific translocations and breaks involving the X chromosome permitted re-

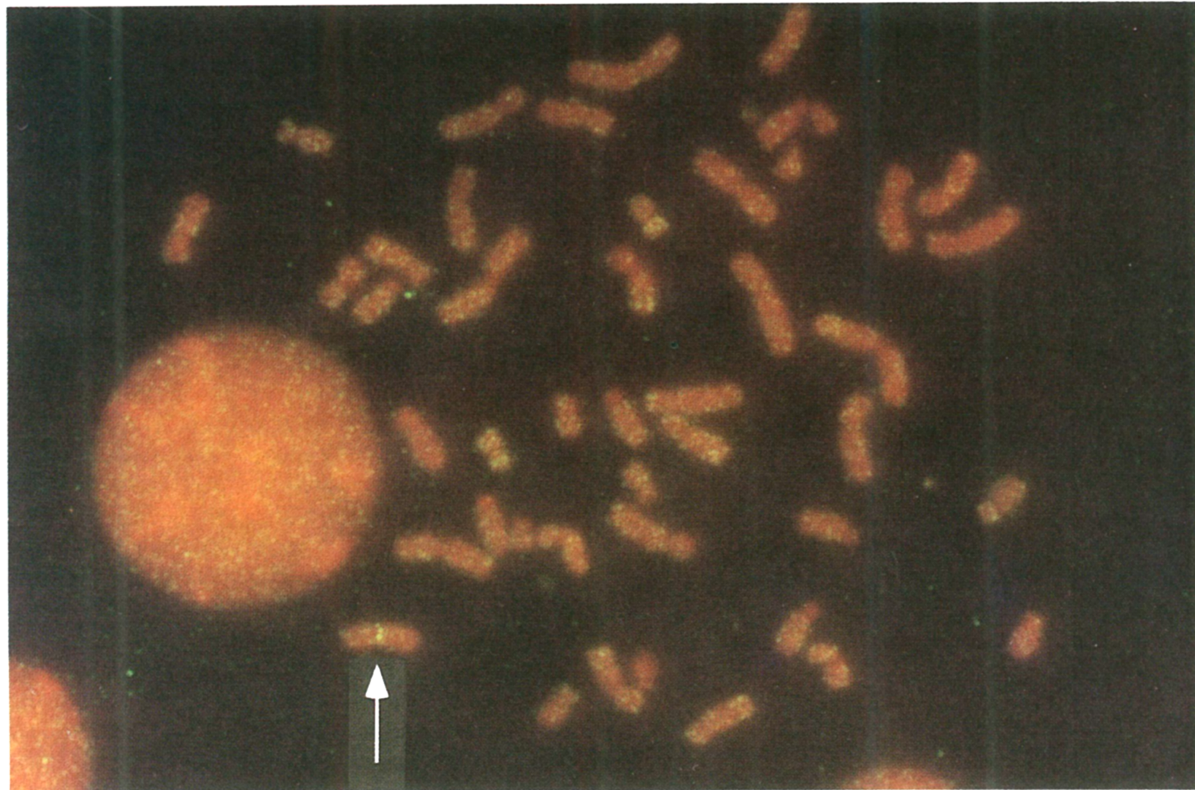

Figure 1. Localization of IL-2Rγ to Xq13 by In Situ Hybridization
Assignment to Xq13 was made by W. S. M. without knowledge of the results of the somatic cell hybrid mapping experiments. Arrow, hybridization of probe on both chromatids at Xq13.

gional localization of the gene (data not shown). One human–mouse hybrid containing the human IL-2Rγ gene retained only the long arm of the human X chromosome translocated to a mouse chromosome, indicating that the gene must be located on the long arm of X. Hybrids isolated after fusing human fibroblasts (GM0073) containing a well-characterized X;14 reciprocal chromosome translocation with Chinese hamster cells (McBride et al., 1982a) provided additional information. Nine independent hybrids retained the Xq13–qter translocation chromosome; the reciprocal translocation chromosome, the normal human X chromosome, and the human IL-2Rγ gene were all absent from each of these hybrids. In contrast, the human IL-2Rγ gene was present in each of 4 independent hybrids that retained only the Xpter–q13 translocation chromosome. These results permit localization of the gene to Xcen–q13. The results with several other hybrids containing spontaneous breaks involving human X supported this interpretation.

To confirm this assignment, we performed in situ chromosomal hybridization utilizing plasmids containing the 7.9 and 4 kb genomic EcoRI IL-2Rγ gene fragments (Noguchi et al., submitted) as biotinylated probes. A total of 50 metaphase spreads from one male were examined. Twenty-four of the cells, one of which is shown in Figure 1, revealed hybridization of the IL-2Rγ probe at one or both chromatids at Xq13 (arrow). Hybridization was not consistently observed at any other site.

## The IL-2Rγ Gene Is Tightly Linked to the Locus for XSCID

Since IL-2Rγ is a critical T cell signaling molecule, it was striking that this locus was in the general region previously determined to be the locus for XSCID (de Saint Basile et al., 1987; Puck et al., 1989). Since the XSCID locus had been mapped by linkage analysis, we used genetic linkage analysis to further localize the IL-2Rγ gene. Polymorphisms at the IL-2Rγ locus were sought for this purpose. No restriction fragment length polymorphisms were detected on hybridization of the IL-2Rγ cDNA to DNAs from ten unrelated individuals digested with 12 different restriction endonucleases (data not shown). We examined the IL-2Rγ gene intron sequences for di- or trinucleotide repeats that would facilitate the detection of microsatellite length polymorphisms, but none were found. However, examination of DNAs from parents in the Centre d'Etude du Polymorphisme Humain (CEPH) pedigrees (Dausset et al., 1990) demonstrated the presence of single-strand conformational polymorphisms (SSCPs) (Orita et al., 1989) within both introns 1 and 2 of the IL-2Rγ gene.

A representative SSCP analysis of intron 2 using CEPH family 1331 is shown in Figure 2A. The segregation of band patterns within members of this family follows typical Mendelian genetics, with bands 2 and 4 representing allele 1 and band 5 representing allele 2. Bands 1 and 3 were present in all of the DNAs. The parents from each of the 40 CEPH families were examined, and all members of

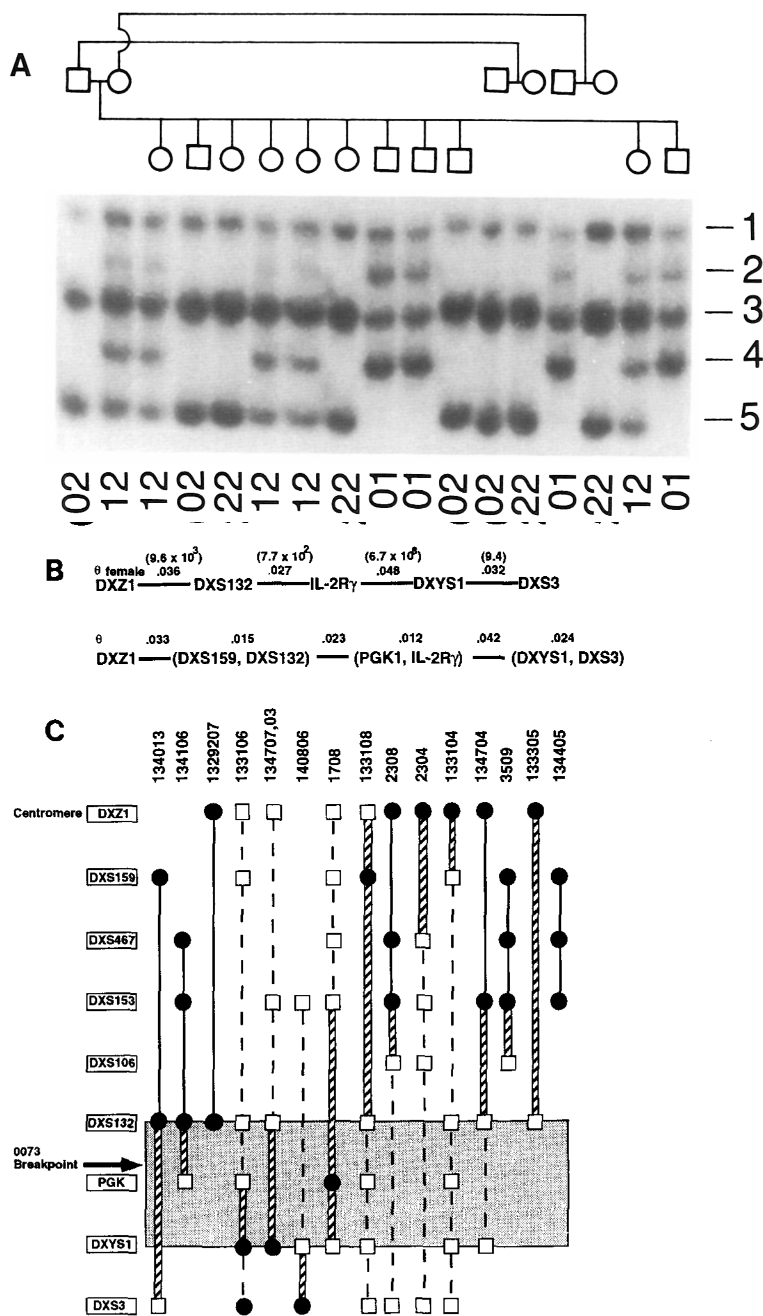


Figure 2. The IL-2Rγ Gene Is Linked to the XSCID Locus

(A) Mendelian inheritance of the IL-2Rγ gene in the three generation CEPH family 1331. The pedigree of the family is shown (squares, males; circles, females) above the lanes, and the alleles in each lane are indicated below the lanes. SSCP analysis of this family was performed as described in Experimental Procedures, using PCR primers corresponding to intron 2 (see Table 3A). Bands 1 and 3 were present in all DNAs in this family. Bands 2 and 4 represent allele 1, and band 5 represents allele 2. In genotypes, the designation 0 indicates hemizygosity, as inferred from the male sex and X chromosomal location of the gene.

(B) Multipoint linkage analysis of IL-2Rγ with other loci spanning Xcen–q22. The five loci (upper part of panel) were ordered using the CILINK program, and all possible orders were considered. The most likely recombination fractions between loci and the odds against reversing the order of adjacent loci (in parentheses) are shown. Although DXS3 is telomeric of DXYS1, in the linkage analysis the odds (9.4) for ordering DXYS1 and DXS3 are not significant, and the odds against reversing DXS132 and IL-2Rγ are only $7.7 \times 10^2$; the odds against all other orders exceed $3 \times 10^3$. Below, two more loci (DXS159 and *PGK1*) were then added to this cluster, and the order and most likely recombination fractions were determined by sequential use of the programs CMAP and CILINK, considering all eight possible orders; all other orders were excluded by CMAP with odds $>10^3$. The three pairs of loci within parentheses could not be ordered with odds of even $10^2$. The most likely recombination fractions between adjacent loci are shown, including those between loci in parentheses. The total interval spanned is approximately 15 cM.

(C) Recombinants between the IL-2Rγ gene and other loci within the region Xcen–q22. Only meioses that are informative for at least two loci in addition to IL-2Rγ are shown. Circles indicate informative loci that are recombinant with IL-2Rγ in a meiosis, and these circles are joined by solid lines. Squares indicate loci that are nonrecombinant with IL-2Rγ in a meiosis, and these squares are joined by broken lines. Hatched lines join adjacent circles to squares and indicate the regions containing a recombination breakpoint in each meiosis. The absence of circles or squares at loci indicates maternal homozygosity at those loci. The order of loci is according to previous linkage (de Saint Basile et al., 1987; Puck et al., 1989, 1991; Mahtani et al., 1991) and physical mapping (Cremers et al., 1988; Lafreniere et al., 1991) studies, but the distances between these loci are not proportional to genetic distance. The gene must be distal to DXS132, based upon individuals 134013, 134106, and 1329207. Recombinants of DXYS1 and/or DXS3 in individuals 133106, 134703, 134707, and 140806 indicate that the gene must be proximal to these loci. Thus, the shaded area between DXS132 and DXYS1 indicates the region containing the IL-2Rγ gene. Double recombinants in proximal Xq were found in individuals 1708 and 133108.

The region delimiting the IL-2Rγ gene can be substantially further reduced by combining the results of physical mapping studies with those of the genetic linkage studies. Lafreniere et al. (1991) demonstrated that the X chromosome translocation breakpoint in parental cell line GM0073 is at the boundary of q13.1 and q13.2; *PGK1*, DXS72, DXYS1, and DXS3 are all telomeric to this breakpoint, whereas DXS159–DXS467, DXS153, DXS106, and DXS132 are all centromeric to the breakpoint (indicated by the arrow in [C]). The locus for IL-2Rγ lies between DXS132 and the 0073 breakpoint. We confirmed these results by Southern blot analysis of our somatic cell hybrid DNAs with probes pHPGK-7e, identifying the *PGK1* gene and *PGK1P1* pseudogene loci, cpX289 identifying the DXS159 locus, and pX65H7 identifying the DXS72 locus.

the 10 families informative for the SSCP in intron 2 were subsequently analyzed. Nineteen mothers in the CEPH pedigrees were heterozygous for the SSCP involving intron 1, and 24 of the 40 CEPH families were informative for SSCPs involving one or both of these introns. All 24 families were examined at one or both of the SSCP loci, and these results were combined as a haplotype. The data were then analyzed versus all relevant published loci in

Table 1. Segregation of the IL-2Rγ Gene with the Human X Chromosome

| Human Chromosome | Gene/Chromosome | | | | Discordancy |
|---|---|---|---|---|---|
| | +/+ | +/− | −/+ | −/− | (%) |
| 1 | 18 | 27 | 16 | 34 | 45 |
| 2 | 13 | 32 | 14 | 36 | 48 |
| 3 | 21 | 24 | 14 | 36 | 40 |
| 4 | 31 | 14 | 29 | 21 | 45 |
| 5 | 16 | 29 | 9 | 41 | 40 |
| 6 | 25 | 20 | 23 | 27 | 45 |
| 7 | 18 | 27 | 21 | 29 | 51 |
| 8 | 13 | 32 | 13 | 37 | 47 |
| 9 | 16 | 29 | 16 | 34 | 47 |
| 10 | 12 | 33 | 7 | 43 | 42 |
| 11 | 17 | 28 | 15 | 35 | 45 |
| 12 | 20 | 25 | 17 | 33 | 44 |
| 13 | 17 | 28 | 17 | 33 | 47 |
| 14 | 24 | 21 | 18 | 32 | 41 |
| 15 | 23 | 22 | 24 | 26 | 48 |
| 16 | 19 | 26 | 18 | 32 | 46 |
| 17 | 27 | 18 | 29 | 21 | 49 |
| 18 | 29 | 16 | 20 | 30 | 38 |
| 19 | 15 | 30 | 14 | 36 | 46 |
| 20 | 21 | 24 | 19 | 31 | 45 |
| 21 | 36 | 9 | 26 | 24 | 37 |
| 22 | 16 | 29 | 12 | 38 | 43 |
| X | 45 | 0 | 0 | 50 | 0 |

Discordancy analysis of somatic cell hybrids. Discordancy is the presence of the gene in the absence of the chromosome (+/−) or the absence of the gene in the presence of the chromosome (−/+), and the sum of these numbers divided by the total number of hybrids examined (×100) is percent discordancy.

CEPH data base version 5 by two point and multipoint linkage analysis, using LINKAGE version 5.10 for PC compatibles (Lathrop et al., 1984). Two point linkage analysis with other loci in the region from the centromere to band q22 on human X (Table 2) shows close linkage of IL-2Rγ to all these loci, with highly significant LOD scores (LOD(θ) = log[odds for linkage at θ / odds for nonlinkage]; maximum LOD scores ≥ 3). The IL-2Rγ gene is most closely linked to *PGK1*, DXS106, and DXS132 in the Xq12–q13 region. Multipoint linkage analysis (Figure 2B) was used to order the IL-2Rγ gene with respect to other loci in this region. IL-2Rγ is located very near the *PGK1* gene, and it is distal to the DXS159 and DXS132 loci but proximal to DXYS1 and DXS3. This represents the same region to which XSCID has previously been mapped by linkage analysis in families segregating this locus (de Saint Basile et al., 1987; Puck et al., 1989, 1991).

Analysis of recombination breakpoints in individual meioses (Figure 2C) supports IL-2Rγ gene localization between DXS132 and DXYS1 (stippled region). Details of this analysis are in the legend. In fact, physical mapping allowed more refined mapping to the region between DXS132 and the X chromosome breakpoint location in GM0073 cells (Figure 2C). The close linkage of the XSCID and IL-2Rγ loci suggests that IL-2Rγ is the XSCID gene; however, linkage analysis cannot prove their identity. Furthermore, it should be noted that the mappings of XSCID and IL-2Rγ were performed in different sets of families.

**XSCID Patients Have Mutant IL-2Rγ Genes**

We next sought to demonstrate directly that IL-2Rγ is the XSCID gene. We isolated DNA from Epstein–Barr virus (EBV) lines derived from B cells from three XSCID patients. The pedigrees for these families are shown in Figure 3A, and the details of each patient's history are summarized in Experimental Procedures. All were typical of XSCID both in their clinical presentations and in their family histories. To determine whether these individuals had large deletions or insertions in their IL-2Rγ genes, we performed genomic Southern blot analysis with multiple enzymes. All patients had normal sized fragments for each enzyme (data not shown). We next amplified all eight exons for each patient by polymerase chain reaction (PCR) and found that each was normal in size by agarose gel analysis (data not shown). Having found no gross abnormality in the gene, we proceeded to sequence each exon and exon–intron splice junction for each patient (Figure 3B). All three patients had different point mutations resulting in premature stop codons. For each patient, the relevant area of sequencing gel containing the mutation is shown adjacent to the same area from a normal individual. In each case the normal and mutated nucleotides are identified by arrowheads; adjacent to the gel for each patient, the relevant stop codon, shown on the noncoding strand, is boxed. These occurred at Lys-97, Arg-267, and Ser-286

Table 2. Two Point LOD Scores between IL-2Rγ and Other X Chromosomal Loci[a]

| Locus[b] | $Z_{max}$ | $\theta_{max}$[c] | Region[d] |
|---|---|---|---|
| DXZ1 | 13.3 | 0.075 (0.028–0.154) | Xcen |
| DXS159 | 16.8 | 0.048 (0.014–0.115) | Xq11.2–q12 |
| DXS467 | 8.9 | 0.075 (0.023–0.180) | Xq11.2–q12 |
| DXS153 | 13.2 | 0.068 (0.023–0.150) | Xq11.2–q12 |
| DXS106 | 18.1 | 0.00 (0.00–0.036) | Xq11.2–q12 |
| DXS132 | 15.1 | 0.043 (0.010–0.115) | Xq11.2–q12 |
| *PGK1* | 13.8 | 0.018 (0.001–0.087) | Xq13.2–q13.3 |
| DXYS1X | 14.2 | 0.074 (0.028–0.250) | Xq21.3 |
| DXS3 | 9.8 | 0.060 (0.025–0.158) | Xq21.3–q22 |

[a] Most likely recombination fractions ($\theta_{max}$) and LOD scores ($Z_{max}$) between IL-2Rγ and other loci. LOD (Z) is log(odds for linkage at some value θ / odds for nonlinkage [i.e., θ = 0.5]). A value of ≥3 (i.e., 1000-fold odds) for Z is considered significant evidence for linkage; all LOD scores in this table are highly significant.

[b] Probe–enzyme combinations at loci are as follows: DXZ1 is pBamX-E-XbaI; DXS159 is cpX289–PstI; DXS467 is cpX12–RsaI; DXS153 is cX37.1–BstEII; DXS106 is cpX203–BglII; DXS132 is cpX23–DraI; *PGK1* is pSPT-PGK–BglI; DXYS1X is pDP34–TaqI; DXS3 is p19-2–TaqI and p19-2–MspI combined as a haplotype. Only the IL-2Rγ data were generated in this study. Data (from CEPH data base version 5) for DXZ1, DXS159, DXS467, DXS153, DXS106, DXS132, and *PGK1* were from the lab of Dr. H. F. Willard (Mahtani et al., 1991); for DXS3 from the lab of J. Mandel (de Saint Basile et al., 1987), and for DXYS1X and DXS3 from Donis-Keller et al. (1987).

[c] Confidence interval for $\theta_{max}$ over a 10-fold range of likelihood is shown in parentheses. These values were computed from LODSCORE tables using the MLINK option of the LINKAGE program (Lathrop et al., 1984).

[d] Physical locations of loci on the X chromosome. Loci are listed in most probable order based upon published results (de Saint Basile et al., 1987; Puck et al., 1989; Puck et al., 1991; Mahtani et al., 1991). DXS159, DXS467, and DXS153 cannot be ordered (Mahtani et al., 1991); DXS159 and DXS467 are separated by ≤6.5 kb (Mahtani et al., 1991).

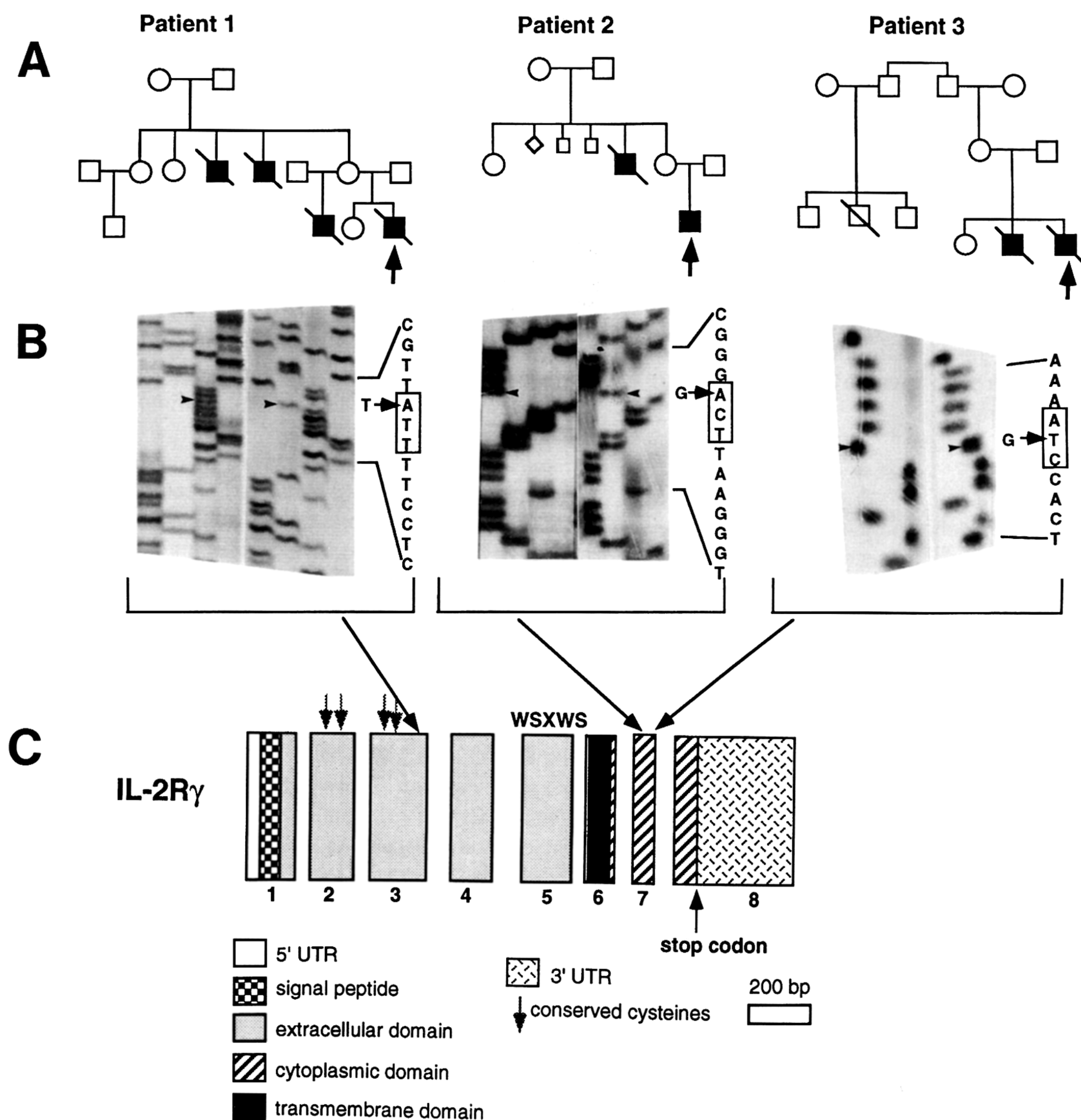


Figure 3. XSCID Patients Have Mutations in the IL-2Rγ Gene

(A) Pedigrees and histories of the XSCID patients studied (see Experimental Procedures). Circles, female; squares, male; closed squares, males with SCID; squares with slashes, deceased males. Small squares and diamond in the pedigree of patient 2 indicate miscarriages (male and of undetermined sex, respectively).

(B) Sequencing of XSCID IL-2Rγ gene sequences. Shown is the sequence of DNA from a normal donor (left panel in each pair) and of DNAs from patients 1, 2, and 3 (right panel in each pair). Patient 1 has an AAA (Lys) to TAA (stop codon) transversion in exon 3, resulting in a truncation of the carboxy-terminal 251 amino acids; patient 2 has a CGA (Arg) to TGA (stop codon) transition in exon 7, resulting in the truncation of 81 amino acids; and patient 3 has a TCG (Ser) to TAG (stop codon) transversion in exon 7, resulting in the truncation of 62 amino acids. The location of each mutation is indicated in the sequence to the right of each set of panels. The sequence shown is complementary to the coding strand. The boxed nucleotides for patients 1, 2, and 3 are complementary to TAA, TGA, and TAG stop codons, respectively.

(C) Schematic showing locations of the artificial stop codons (diagonal arrows) present in the XSCID patients. Patient 1 has a premature stop codon in exon 3, and patients 2 and 3 have premature stop codons in exon 7. UTR, untranslated region.

in patients 1, 2, and 3, respectively, with predicted truncations of 251, 81, and 62 amino acids. The diagonal arrows (connecting Figures 3B and 3C) show the positions of the predicted stop codons on a map of the exons of the IL-2Rγ gene.

Based on the positions of the stop codons, patients 2 and 3 might be expected to express a cell surface form of IL-2Rγ; however, the sequences predict proteins lacking the majority of the cytoplasmic domain, including substantial parts of the Src homology 2 (SH2) subdomain regions contained in IL-2Rγ (Takeshita et al., 1992b). In patient 1, 251 amino acids are deleted, including the entire cytoplasmic domain and transmembrane region and much of the extracellular domain. No mutations were found in the normal individual. Moreover, the three patients showed no abnormalities except for their respective premature stop codons. The only variation between their sequences was that Leu-33 was encoded either by CTA or CTG, a variation

noted previously in IL-2Rγ cDNA and genomic clones, which presumably represents a polymorphism (Noguchi et al., submitted). These data establish that XSCID is associated with mutations of the IL-2Rγ gene product. It is noteworthy that the same results were obtained for each patient when DNA was independently amplified by PCR and again sequenced. Compared with the published IL-2Rγ sequence (Takeshita et al., 1992b), genomic DNA from a normal donor contained no mutations, nor did two IL-2Rγ cDNA clones (Noguchi et al., submitted) or one IL-2Rγ genomic clone (Noguchi et al., submitted) derived from non-XSCID sources. These data indicate that only XSCID-derived DNA has been found to have mutations in the IL-2Rγ gene.

## Discussion

Severe combined immunodeficiency diseases (SCIDs) are characterized by severe and persistent infections from early life, which are due to profound impairment of both cellular and humoral immune function (reviewed by Cooper and Butler, 1989; Gelfand and Dosch, 1983; Conley, 1992). One in every $10^5$–$10^6$ live births is affected by these diseases. Classical SCID (Swiss-type agammaglobulinemia) is characterized by the absence of both T and B cells, presumably related to a defect affecting the lymphocytic stem cell. Autosomal recessive forms of SCID may also result from deficiencies of adenosine deaminase or purine nucleoside phosphorylase, be associated with major histocompatibility complex class II deficiency (bare lymphocyte syndrome) or IL-2 deficiency, or have no known defect (reviewed by Cooper and Butler, 1989; Gelfand and Dosch, 1983; Conley, 1992).

XSCID accounts for approximately half of all cases of SCID. This disease is characterized by an absence or diminished number of T cells and histologic evidence of hypoplastic and abnormal differentiation of the thymic epithelium (Nezelof, 1986). Levels of B cells can be normal or even elevated, however; thus, the patients are only mildly lymphopenic (Cooper and Butler, 1989; Gelfand and Dosch, 1983; Conley, 1992). The B cells are not functional; therefore, these males are hypo- or agammaglobulinemic. In some cases, however, B cells are capable of synthesizing and secreting immunoglobulin if cultured with normal T cells in vitro. This suggests that a lack of T cell help could be the dominant problem, rather than an intrinsic B cell defect (Cooper and Butler, 1989; Gelfand and Dosch, 1983; Conley, 1992). Following successful bone marrow transplantation, the donor T cells engraft and develop normally, suggesting that the gene defect primarily affects the bone marrow–derived T cell precursors and that the thymic epithelial abnormalities are secondary. The macrophages and B cells remain those of the host. The development of posttransplantation antibody responsiveness usually takes more than 1 year, suggesting that a subtle B cell defect could contribute to the long delay in establishing donor T and host B cell collaboration (Cooper and Butler, 1989; Gelfand and Dosch, 1983; Conley, 1992). X chromosome inactivation patterns from mothers of children with XSCID provide further support for an intrinsic B cell defect. These XSCID carrier females are immunologically normal. They exhibit nonrandom X chromosome inactivation in T cells (i.e., they inactivate the mutant X chromosome; Puck et al., 1987; Conley et al., 1990). Whereas random X chromosome inactivation is observed in B cell hybrids derived from less mature surface immunoglobulin M (IgM)–positive B cells, nonrandom X chromosome inactivation is seen in surface IgM–negative B cells that have undergone further replication and differentiation (Conley et al., 1988). These data suggest that the XSCID gene product is required during terminal B cell differentiation.

We have now demonstrated that three out of three XSCID patients studied have mutations that truncate IL-2Rγ. This striking result has a number of important implications.

First, it suggests that IL-2Rγ plays a critical role in thymic maturation of bone marrow–derived precursor human T cells. A number of reports have discussed the potential role of IL-2 receptors in thymocytes. Immature (L3T4$^-$, Lyt2$^-$) murine thymocytes express receptors for IL-2 and can proliferate vigorously in response to IL-2 if provided with a costimulatory mitogen; more mature thymocytes express far lower levels of IL-2Rα (Raulet, 1985; von Boehmer et al., 1985). In fact, the initiation of CD3, CD4, and CD8 acquisition parallels down-regulation of IL-2Rα (Petrie et al., 1990). The levels of IL-2Rβ and IL-2Rγ have not been examined. These data regarding murine thymocytes are consistent with a role for IL-2 receptors in early thymic maturation, as suggested by Tentori et al. (1988). Interestingly, however, mice made deficient of IL-2 by gene targeting are grossly normal in terms of thymocyte and peripheral T cell subset composition (Schorle et al., 1991), even though they have dysregulated in vitro T cell responses and altered serum levels of immunoglobulin isotypes. It is possible that IL-2 and IL-2Rγ deficiencies result in different phenotypes (i.e., IL-2Rγ mutation appears to result in a more profound loss of T cells), perhaps suggesting that IL-2Rγ may have an additional role beyond the IL-2 system (see below). Alternatively, it is possible that thymic maturation in humans and mice may differ in the degree of dependence on the IL-2–IL-2 receptor system.

Second, the critical role of IL-2Rγ in XSCID suggests that mutation of other components of the IL-2–IL-2 receptor system could be responsible for certain autosomal recessive forms of SCID or less severe forms of immunodeficiency. In this regard, it is noteworthy that SCID has been associated with defective IL-2 production (Pahwa et al., 1989; Weinberg and Parkman, 1990), which in one case resulted from defective production of NF-AT, a nuclear factor required for IL-2 gene transcription (Chatila et al., 1990). However, the patient's cells were capable of producing low levels of IL-2 mRNA (Chatila et al., 1990), perhaps explaining why this SCID patient had T cells, and consistent with the possibility that the amount of IL-2 produced was sufficient for a degree of thymic maturation but not for normal mature T cell immune function in the periphery.

Weinberg and Parkman (1990) described a phenotypically similar patient; however, in this case no IL-2 mRNA

was detectable in peripheral blood T cells activated with mitogens. Although peripheral T cells were present, some circulating cells appeared to be thymocytes, consistent with a thymic maturation defect. The basis for the lack of IL-2 mRNA production in this patient is unknown, particularly since genomic Southern blot analysis revealed that the IL-2 gene was grossly intact. It is therefore unknown whether some IL-2 could have been produced within the thymus, allowing the degree of maturation seen. Nevertheless, these two cases of IL-2-deficient SCID suggest that in humans early T cell maturation might proceed in the absence of IL-2. The apparent differences in phenotypes of SCID with deficiencies in IL-2 versus IL-2Rγ are consistent with IL-2Rγ being a critical component of more than one cytokine receptor. Although there is no evidence yet in support of this possibility, such a system would be analogous to the IL-3, IL-5, and granulocyte–macrophage colony-stimulating factor receptors, which share a common β chain (Kitamura et al., 1991; Tavernier et al., 1991), and to the IL-6, leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor receptors, which all used gp130 as a signaling molecule (Gearing et al., 1992; Taga et al., 1992). Such a model could explain why defects in IL-2Rγ, as compared with defects in IL-2, appear to have a more profound impact on the degree of T cell maturation.

Third, because XSCID individuals can recover B cell function following T cell engraftment, our data suggest that the absence of IL-2Rγ expression in B cells can be significantly compensated for by the presence of normal T cells during terminal B cell activation and differentiation.

Fourth, it is noteworthy that three distinct mutations were found in examining three patients. This may suggest that there is no single dominant mutation, as is found, for example, in cystic fibrosis (reviewed by Fanen et al., 1992), although larger numbers of XSCID patients need to be examined to determine the prevalence of each mutation. In fact, in a severe X-linked disease, assuming constant prevalence of the disease, new mutations must constantly arise to replace the mutant X chromosomes (in males) that are not passed on to the next generation, so that a variety of mutations would be expected. All three mutations are predicted to result in truncations of IL-2Rγ protein. Deletion of as few as 62 amino acids from the cytoplasmic domain appears to be sufficient to abrogate IL-2Rγ function. Other types of point mutations, such as those that affect residues required for IL-2 binding, would also be expected to be found if DNAs from enough XSCID patients were sequenced. It is reasonable to speculate that less severe phenotypes will also be found, resulting from mutations that diminish but do not abrogate IL-2Rγ chain function.

Finally, these findings have important implications for prenatal and post-natal diagnosis, carrier female identification, and gene therapy of XSCID.

## Experimental Procedures

### Oligonucleotides

All oligonucleotides were made using an Applied Biosystems model 392 DNA/RNA synthesizer.

### Somatic Cell Hybrids

The human and rodent parental cells, fusion procedure, and isolation and characterization of hybrids that were used have been described previously (McBride et al., 1982a, 1982b). Most hybrid cells were analyzed for the presence of all human chromosomes except Y by standard isoenzyme analyses, by Southern blot analysis with probes from previously localized genes, and, in many cases, by cytogenetic analysis. DNA (10 μg) was digested with EcoRI, separated by 0.7% agarose gel electrophoresis, transferred to a nylon membrane, and hybridized with a full-length IL-2Rγ cDNA insert derived from pIL-2Rγ7 (Noguchi et al., submitted). The hybridizations and washings were performed at high stringency (Gnarra et al., 1990), intended to allow less than 10% sequence divergence. The IL-2Rγ gene was detected as 4.0 and 7.9 kb hybridizing EcoRI bands. Cross-hybridizing 5.9 and 4.2 kb bands were detected in Chinese hamster and mouse fibroblast DNAs, respectively. The 42 human–hamster hybrids consisted of 29 primary hybrids and 13 subclones, and the 53 human-mouse hybrids consisted of 19 primary hybrids and 34 subclones. Twenty of the human–hamster and 25 of the human–mouse hybrids contained the human IL-2Rγ gene. Detection of the gene was correlated with the presence or absence of each human chromosome in the group of somatic cell hybrids (Table 1).

Probes cpX289 (DXS159), pX65H7 (DXS72), and pHPGK-7e (*PGK1*) were obtained from the American Type Culture Collection and used for Southern blot analysis of these somatic cell hybrid DNAs to permit the localization of the X chromosomal translocation breakpoint in the parental cell line GM0073 to Xq13.1–q13.2. The pHPGK-7e probe also identified a processed pseudogene locus (*PGK1P1*) for *PGK1* at Xq12 (Lafreniere et al., 1991; Michelson et al., 1985).

### In Situ Hybridization Experiments

These were performed using peripheral blood lymphocytes from a normal male (46; XY), as previously reported (Tory et al., 1992). Briefly, biotinylated probe (20 ng/ml) was hybridized overnight at 37°C in 2× SSC containing 50% formamide and 10% dextran sulfate. Washes were performed in 2× SSC–50% formamide and then in 2× SSC, both at 40°C. Detection was carried out using avidin–fluorescein isothiocyanate. Chromosome identification was carried out by Q-banding (Tory et al., 1992).

### PCR and SSCP Analyses

SSCP analyses were performed essentially as described by Orita et al. (1989). PCR was performed in a total volume of 15 μl, containing 200 ng of genomic DNA, 1× PCR buffer (Promega), primers (1 μM each), dATP, dGTP, and dTTP (200 μM each), 25 μM dCTP with 1 μCi of [$^{32}$P]dCTP, and 0.45 U of Taq polymerase (Promega). Initial denaturation was for 5 min at 94°C, followed by 30 cycles of 1 min each at 94°C, 55°C, and 72°C. The final extension was for 7 min at 72°C. The oligonucleotide primers used for amplification of each intron are shown in Table 3A. A 2.5 μl aliquot of the $^{32}$P-labeled PCR product was diluted with 10 μl of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol, denatured 10 min at 95°C, and quenched in an ice bath. Aliquots (4 μl) of the $^{32}$P-labeled single-stranded DNA were applied to the wells of a 30 cm × 40 cm × 0.4 mm gel containing 5% polyacrylamide gel in 1× TBE buffer (pH 8.0). Electrophoresis was performed at constant power (25 W) at room temperature, with cooling from a fan blowing over a tray of ice in front of the gel, until the bands had migrated to within approximately 10 cm from the bottom of the gel. Gels were dried, and band locations were determined by autoradiography; 30 min exposures were generally sufficient.

### Description of XSCID Patients 1, 2, and 3

Patient 1 was diagnosed at 11 weeks of age when he presented with failure to thrive, treatment-resistant oral candidiasis, and disseminated adenoviral infection. Recurrent pneumonias began at 4 months of age. At diagnosis, his IgG level was 18 mg/dl (normal range, 294–2069), IgM level was 14 mg/dl (normal range, 41–149), and IgA and IgE were undetectable. Peripheral absolute lymphocyte counts ranged from 100 to 1000 per mm$^3$, with 65%–83% CD19$^+$ B cells and consistently <10% CD3$^+$ T cells. There was no proliferation to phytohemagglutinin, nor was there detectable T cell or natural killer cell cytotoxicity. No maternal engraftment was detected by restriction fragment length polymor-

Table 3A. Oligonucleotides: PCR Primers for SSCP

| Region | Type | Sequence |
|---|---|---|
| Intron 1 | Sense | GAATGAAGACACCACAGCTG |
| | Antisense | GGGCATAGTGGTCAGGAAG |
| Intron 2 | Sense | GATGAAGAAGAGGTGGGTTG |
| | Antisense | GTTGGAAGGAAGAGGAACAG |
| Intron 3 | Sense | AGGGATACTGTGGGACATTG |
| | Antisense | AGTCAGGATCCTGAAGGTAG |
| Intron 4 | Sense | AGTGACTAGGGACGTGAATG |
| | Antisense | GTGGGTGTCTATGAGAGAAG |
| | Sense | GACCTAATATCAAGCTCCTG |
| | Antisense | GTCTATCTGGTATCAGGAAG |
| Intron 5 | Sense | TGTCTTTTGCTCCACTTCCC |
| | Antisense | AGGCAGGAACAATTACCTCC |
| Intron 6 | Sense | AGCTGACAATAGCAGAGGAG |
| | Antisense | TCACCCTTCTCCAGTTGTC |
| Intron 7 | Sense | GCAGTCTATCAACTGCCAAC |
| | Antisense | GACAGATATGTCCAGGTCAG |

PCR primers for SSCP. Sense and antisense PCR primers were prepared to analyze each intron for SSCPs; two different pairs of oligonucleotides were used for intron 4. Polymorphisms were found within introns 1 and 2 but not within introns 3, 4, 5, 6, or 7.

Table 3B. Primers for Nested PCR Amplification of Exons and Genomic Sequencing

| Region | Type | Sequence |
|---|---|---|
| Exon1 | Sense | AAGGTTCTTTCCACCGGAAG |
| | Antisense | TGATTGTAATTGGCCAGTGGC |
| | NSense | Biotin-ATGACAGAGGAAACGTGTGG |
| | NAntisense | CAGGCACCAGATCTCTGTAG |
| | Seq1 | ACAGCCACCCTTCTCACCAG |
| | Seq2 | GCAGCTGCAGGAATAAGAGG |
| Exon2 | Sense | ATTACAGACATGAGCCACCG |
| | Antisense | CCCTCTCTCTTGGTCTCTG |
| | NSense | Biotin-CTCCTTCTCACCATCATTTC |
| | NAntisense | CTTGGTCTCTGATCCAACCC |
| | Seq1 | TCTGATCCAACCCACCTCTTC |
| | Seq2 | CAAAACACTGAACCTCTGGG |
| Exon 3 | Sense | TAGGCTCTGGATATCTGCAG |
| | Antisense | CTCTGTAGACTCCAATGTCC |
| | NSense | Biotin-CTGTTCCTCTTCCTTCCAAC |
| | NAntisense | GACTCCAATGTCCCACAG |
| | Seq1 | AATGTCCCACAGTATCCCTG |
| | Seq2 | TTTGGTAGAGGTGGATCTC |
| Exon 4 | Sense | ATTAGGGGCACTACCTTCAG |
| | Antisense | AGGTCCTTCTATCTGTCTGG |
| | NSense | Biotin-ACCTTCAGGATCCTGACTTG |
| | NAntisense | GTTGAATCCTTTAGCCCTAC |
| | Seq1 | ACTTTCTTGGCCTTAGCTGC |
| | Seq2 | AGAATCTGTTGTTCCAGTTC |
| Exon5 | Sense | CAGTAGCAGAGATGACACTG |
| | Antisense | TAGACAGTGTGGAGAGATGG |
| | NSense | Biotin-CTTCTCTCATAGACACCCAC |
| | NAntisense | AGGGAAGTGGAGCAAAAGAC |
| | Seq1 | AGCAAAAGACAGTGGTGTTAG |
| | Seq2 | TCCGAACACGAAACGTGTAG |
| Exon 6 | Sense | TGGAGGTAATTGTTCCTGCC |
| | Antisense | CCAAACCCTCTTGGGTAATC |
| | NSense | Biotin-TCACAGGAGCTGTTGTGAGG |
| | NAntisense | TACTGTCTCATCCTTTACTCC |
| | Seq1 | CTATTGTCAGCTACCGTTCC |
| | Seq2 | GCTGATAATCAATCCCATGG |
| Exon 7 | Sense | TATGGACAACTGGAGAAGGG |
| | Antisense | ACACTCTGTCTGTCTTGCTG |
| | NSense | Biotin-TAACCTATGTGCTCCTGCTC |
| | NAntisense | TCTTGCTGGCAGGCAGTTG |
| | Seq1 | GTTGGCAGTTGATAGACTGC |
| Exon 8 | Sense | TACTCCTTTGGACAGAGCTC |
| | Antisense | TTCCAAATCAGCCACAGTGG |
| | NSense | Biotin-CTGACCTGGACATATCTGTC |
| | NAntisense | TATGAGACGCAGGTGGGTTG |
| | Seq1 | GGAAAGTTAGTACCACTTAGGG |
| | Seq2 | AATCTCACTGACGAGGCAG |

Primers for nested PCR amplification of exons and genomic sequencing. See Experimental Procedures for details of sequencing and nomenclature of oligonucleotides.

phism analysis. Patient 1 died, 43 days after a T-depleted bone marrow transplant from his mother, of progressive adenoviral infection and scopulariopsis pneumonia and fungemia.

Patient 2 was diagnosed with Pneumocystis carinii pneumonitis at 3 months of age. At 5 months of age, his IgM level was 6 mg/dl (normal range, 33–108), IgA and IgE were undetectable, and IgG was 162 mg/dl (normal range, 172–814), while receiving intravenous IgG. Absolute lymphocyte counts varied from 900 to 1600 per mm$^3$, with 62%–78% IgM$^+$ B cells expressing either κ or λ and 23–28% CD3$^+$ T cells, approximately half of which expressed α/β T cell receptors. Engraftment of maternal T cells was confirmed by restriction fragment length polymorphism analysis. Proliferation to phytohemagglutinin was 4% of control. The patient's cells did not generate T cell cytotoxicity in vitro, and natural killer cell cytotoxicity, while measurable, was below the normal range. Patient 2 underwent a T-depleted haploidentical transplant from his father. Two months posttransplant, he developed disseminated EBV lymphoproliferative syndrome (with markedly elevated oligoclonal IgG and IgM). This condition was successfully treated with α-interferon. One year posttransplant, he shows evidence of donor T cells and has normal serum levels of IgA, IgM, and IgE. The patient's mother had one brother who died of SCID. The small squares and diamond in Figure 3A indicate miscarriages (two male and one of undetermined sex).

Patient 3's early clinical course and immune function have been reported (South et al., 1977; Shearer et al., 1985). Diagnosis was made based on clinical course and family history of a male sibling with SCID. A male cousin who died had normal immune function and did not have SCID. Patient 3's immunodeficiency was characterized by panhypogammaglobulinemia, lymphopenia with diminished T cells (10%–40%, varying over 12 years), elevated B cells (30%–80%), and essentially absent proliferation to mitogens or antigens. Following T-depleted haploidentical bone marrow transplantation from his sister, there was no improvement in immune function. He died 124 days posttransplant from an EBV-associated lymphoproliferative syndrome.

**Sequencing of DNA from Cells from Patients with XSCID**

B lymphocytes from patients 1 and 2 were immortalized by EBV as described previously (Tosato, 1991). For patient 3, an autologous B cell line, DV-1, was established from bone marrow obtained postmortem (Rosenblatt et al., 1987). DNA was isolated from approximately $10^6$ cells using the Oncor genomic DNA isolation kit. To obtain genomic sequences, two-step PCR reactions were performed using a DNA thermal cycler (Perkin-Elmer Cetus 9600) and oligonucleotides shown in Table 3B. For each exon, a first PCR was performed with the appropriate sense and antisense oligonucleotides. The second nested PCR was performed with NSense (nested sense) and NAntisense (nested antisense) oligonucleotides. Each NSense oligonucleotide was biotinylated at its 5′ end. PCRs (100 μl) contained 350 ng of template genomic DNA in 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, oligonucleotide primers (1 μM each), dNTPs (200 μM

each), and 2.5 U of Pfu DNA polymerase (Stratagene). Samples were denatured for 4 min at 94°C and then amplified for 35 cycles of 1 min each at 94°C, 55°C or 62°C, and 72°C. The final extension was performed for 10 min at 72°C. First PCR products were purified by gel filtration, and 5 μl of purified PCR product was used as a template for a second, nested, PCR reaction. Second PCR reactions were performed using 4 min of initial denaturation, then 30 cycles of 30 s each at 94°C, 55°C, and 72°C, and final extension for 10 min. PCR products were purifed by gel filtration, and the biotinylated strand was isolated using Dynabeads M280 streptavidin (DYNAL) and sequenced on the beads by the dideoxy method using Sequenase 2.0 (US Biochemical). For each exon, genomic sequencing was performed with oligonucleotide primers Seq1 (each Seq1 annealed in its respective intron near the end of the nested PCR fragment) and Seq2 (located approximately in the middle of the exon, except in the case of exon 7, where a second sequencing oligonucleotide was not needed). The sense, antisense, NSense, NAntisense, and Seq1 oligonucleotide sequences are located in noncoding regions. The organization of the IL-2Rγ gene with full map and sequences of exons, exon–intron splice junctions, and partial intron sequences will be published elsewhere (Noguchi et al., submitted). In an unsuccessful effort to identify dinucleotide or trinucleotide repeats to facilitate the detection of polymorphisms (see text), we examined intron sequences and visually scanned regions of sequencing gels encompassing regions of introns that could not be read with perfect accuracy.

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**Note Added in Proof**

The data referred to throughout as Noguchi et al., submitted, are now in press: Noguchi, M., Adelstein, S., Cao, X., and Leonard, W. J. (1993). Characterization of the human interleukin-2 receptor γ gene. J. Biol. Chem. *268*, in press.