An Interleukin-2 Receptor γ Chain Mutation with Normal Thymus Morphology

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Abstract

One of the most common human immunodeficiencies is an X-linked condition arising from mutations of the γ subunit of the interleukin-2 receptor (IL-2Rγ). The IL-2Rγ protein is one chain of the heterotrimeric (α, β, γ) IL-2 receptor, but also participates in the formation of the IL-4, 7, 9, and 15 receptor complexes. The diagnosis of X-linked SCID is usually relatively simple due to the distinctive immunological presentation; IL-2Rγ–deficient patients typically lacking mature T lymphocytes (T^-B^+). However, it is becoming clear that this merely represents one extreme of a potential range of clinical presentations. We describe here a novel mutation of the human IL-2Rγ chain (R222C) resulting in an unusual immunological phenotype. Although clinically immunodeficient, this patient has normal numbers of peripheral T and B cells, responds normally to mitogenic stimuli, and unusually, has a normal thymus gland. This IL-2Rγ mutation is distinctive in that the protein is sufficiently stable to be expressed at the cell surface. While the T cell receptor repertoire appears complete, suggesting normal T cell differentiation occurs, patient T cells demonstrate a reduced ability to bind IL-2 and this appears sufficient to cause a deficiency in their ability to participate in antigenic responses. Early clinical recognition of this phenotype is critical as a delay in diagnosis may result in a fatal infection. (*J. Clin. Invest.* 1997. 100:3036–3043.) Key words: human • T cell • signaling • X-linked • immunodeficiency

Introduction

The interleukin-2 cytokine receptor is essential to the normal function of the human immune system and alterations to any of its components can severely compromise this function (1). Indeed, one of the most commonly recognized human immunodeficiencies is a recessive hereditary condition arising from mutations of the γ subunit of the interleukin-2 receptor (IL-2Rγ)[1] (2–4). The IL-2Rγ protein is one chain of the heterotrimeric (α, β, γ) IL-2 receptor (1–5), but also participates in the formation of the IL-4, 7, 9, and 15 receptor complexes (6–10); and as such has been termed the γ common chain. In these complexes the γ chain contributes to both ligand binding affinity and to initiation of signal transduction pathways, through activation of the Jak-family protein tyrosine kinases (11–14). In both the IL-2R and IL-7R complexes a functional γ chain is also required for efficient receptor complex internalization after ligand binding (8).

Potentially, mutation of any component of the IL-2R complex could result in immunodeficiency. However, the most commonly observed form of human severe combined immunodeficiency (SCID) results from mutations of the IL-2Rγ chain. As this gene maps to Xq12–13 (15, 16), an X-chromosome linked condition results from its mutation. Due to the multiple roles played by the IL-2Rγ in B and T cell development (as part of the IL-2, IL-4, IL-7, and IL-15 receptors) the phenotype of γ-deficient patients is extremely severe. IL-2Rγ–deficient patients typically have a reduction in, or absence of, peripheral T lymphocytes, but normal or elevated levels of circulating B cells (17), although these appear to be functionally compromised. This results in a critically compromised immune defense, manifesting in the early development of serious infections, for which currently the only treatment is reconstitution of the immune system by transplantation. Failure to treat early is inevitably fatal.

The diagnosis of X-linked SCID is usually relatively simple due to the distinctive immunological presentation. However, slowly it is becoming clear that these classical cases merely represent one extreme of a potential range of clinical presentations, all resulting from defects of the X-linked IL-2Rγ gene. We describe here a novel mutation of the IL-2Rγ chain resulting in an immunological phenotype quite distinct from the clinical presentation of the classical X-linked SCID and from atypical cases with a few T cells. Although clinically immunodeficient, normal numbers of T and B cells are observed and a normal thymus gland is present. While the T cell receptor (TCR) repertoire appears to be complete (suggesting that events regulated by interleukin-7 proceed normally during T cell differentiation, references 18–22) patient T cells are unable to respond efficiently to interleukin-2, demonstrating a reduced ability to bind this cytokine.

Methods

Peripheral lymphocyte function assays. Cell surface markers of peripheral blood cells were determined by immunofluorescent staining and flow cytometry (Epics V; Coulter Electronics, Hialeah, FL) with antibodies purchased from Coulter Diagnostics. To assay lymphocyte proliferation, peripheral blood mononuclear cells (isolated by Ficoll-Hypaque gradient centrifugation) were incubated at 37°C (5% CO_2) in complete culture medium RPMI-1640 supplemented with 10% (vol/

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1. *Abbreviations used in this paper:* IL-2Rγ, γ subunit of the IL-2 receptor; PHA, phytohemagglutinin; RT-PCR, reverse transcriptase PCR; SCID, severe combined immunodeficiency; TCR, T cell receptor.

vol) fetal calf serum (Cansera, Rexdale, Canada), 2 mM glutamine (Gibco/BRL, Gaithersburg, MD). Cells were incubated in round bottom tissue culture plates with or without phytohemagglutinin (PHA) (Difco Laboratories Inc., Detroit, MI), SPA 25 μg/ml (Pharmacia Fine Chemicals, Piscataway, NJ), anti-CD3 (Ab-1 antibody, Oncogene Science Inc., Mineola, NY), formalin treated SAC (Calbiochem Corp., La Jolla, CA), or recombinant hIL-2 (100 U/ml) (Genzyme Corp., Boston, MA). 4 h before termination of the culture, 1 μCi [^{3}H]thymidine (6.7 Ci/mmol) was added to each well. The cells were then harvested and samples counted in a liquid scintillation counter.

Thymus biopsy analysis. 4-μm serial sections of frozen thymus tissue were mounted on glass slides, air-dried, and stained with hematoxylin and eosin.

Western blotting. 2×10^6 cells were pelleted and lysed in 50 μl lysis buffer (20 mM Tris pH 7.4, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 2 mM Na_3VO_4, 1 mM PMSF), incubated on ice for 15 min followed by centrifugation for 10 min at 12,000 *g.* The supernatant was collected, 2 × SDS-gel sample buffer was added, and the samples separated on an 8% SDS-polyacrylamide gel. Proteins were electrophoretically transferred to nitrocellulose membrane (Hybond-C; Amersham Corp., Arlington Heights, IL), and blocked overnight with 5% milk in 10 mM Tris, 150 mM NaCl pH 8.0 (TBS). The blots were incubated with the primary antibody in TBS-T (0.05% Tween-20) + 1% milk for 2 h at room temperature, washed, and incubated with secondary dectecting antibodies directly conjugated to horseradish peroxidase (donkey-anti–rabbit-horseradish peroxidase [HRP] or sheep-anti–mouse-HRP; Amersham Corp.). The filters were then developed using enhanced chemilumuinescence reagents from Amersham Corp., as per manufacturer's instructions and exposed to film. Antibodies to the IL-2Rγ chain and Jak3 were purchased from Santa-Cruz Biotechnology (Santa Cruz, CA).

Immunoprecipitation. 1×10^6 transfected COS-7 cells per sample were stimulated with rIL-2 (Genzyme Corp.) and 1 ml 1% Triton lysates prepared. Anti-Jak3 and Protein-A were added to the lysates, after clarification by centrifugation, and incubated overnight at 4°C with agitation. The immunoprecipitates were washed three times in lysis buffer, SDS-sample buffer added, and electrophoresed as above.

RNA and DNA preparation. DNA and RNA were isolated from peripheral blood mononuclear cells after Ficoll-Hypaque gradient centrifugation. To isolate RNA, cells were resuspended in Trizol reagent (Gibco/BRL) and total RNA isolated per the manufacturer's instructions. DNA was isolated by proteinase K digestion of cells in lysis buffer (100 mM Tris pH 8.0, 5 mM EDTA, 0.2% SDS, 200 mM NaCl) at 37°C for 4 h. DNA was purified by two phenol/chloroform extractions followed by ethanol precipitation.

PCR of the IL-2Rγ transcript and genomic sequence. Oligo-dT primed first strand cDNA synthesis was performed on total RNA. Aliquots were used in PCR reactions designed to isolate the entire coding region of the IL-2Rγ chain message.

The primers ATGTTGAAGCCATCAT and GGTTCAGGTTTCAGGCTT were used, corresponding to the 5′ and 3′ ends of the IL-2Rγ message, respectively. PCR cycle conditions 94°C (30 s), 45°C (30 s), 72°C (90 s) were used for five cycles, followed by 30 cycles with an annealing temperature of 60°C. The resultant PCR products were cloned and sequenced. For PCR analysis of the genomic sequence the exon 5 primers AGACATAAGTTCTCCTTGCCTAG and CCAGTGGATTGGGTGGCTCC were used with the cycle conditions 94°C (30 s), 60°C (30 s), 72°C (60 s) for five cycles, followed by 30 cycles with an annealing temperature of 65°C.

Reverse transcriptase PCR (RT-PCR) of TCR vβ transcripts. Total RNA was obtained from peripheral blood mononuclear cells as described above. RT-PCR was performed as previously described using a specific TCR constant region primer for the reverse transcription reaction (23). For analysis of the TCRvβ repertoire, 20 PCR amplifications were performed using the same constant region primer, together with one of 20 vβ family-specific primers. ^{32}P-dATP was included in PCR reactions to permit visualization of PCR products by autoradiography.

Analysis of IL-2 binding to patient EBV-transformed B cells. Patient and normal control EBV B cell lines were incubated at 4°C in PBS containing 50 nM, 5 nM, or 500 pM phycoerythrin labeled interleukin-2 (R&D Systems, Minneapolis, MN). The level of nonspecific binding was determined by incubation with streptavadin-PE as per the manufacturer's instructions. After 1 h, cells were washed four times in PBS and IL-2 binding analyzed by flow cytometry. The number of cells demonstrating positive staining at each concentration of the fluorescent cytokine was determined.

Transfection of COS cells. COS-7 cells were maintained in DME with 10% FCS. The eucaryotic expression vector pCMV (Invitrogen Corp., San Diego, CA) was used to express complete cDNAs for IL-2Rβ (a gift from Dr. G. Mills, M.D. Anderson Cancer Centre, Houston, TX), IL-2Rγ and the mutated IL-2Rγ isolated from the patient. Human Jak3 was expressed in pcDNA3 (Invitrogen Corp.). COS cells were transfected using the cationic lipid reagent lipofectamine (Gibco/BRL) and 6 μg of construct or empty vector. After 36 h at 37°C in DME, 10% FCS the cells were assayed for IL-2 binding or IL-2–induced Jak3 activation, as described above.

Model of the interleukin-2 receptor gamma chain structure. In the absence of crystallography data for any of the interleukin receptor subunits, a model of the interleukin-2 receptor γ chain has been proposed (24) based upon x-ray crystallography coordinates derived from the related growth hormone receptor (25) and prolactin receptor chains (26). The coordinates for the IL-2Rγ models were obtained from the Brookhaven Protein Databank (Upton; NY). The interactive Rasmol molecular visualization program (R. Sayle; Glaxo R&D, Greenford, Middlesex, United Kingdom) was used to view these structures.

Results

Clinical presentation. The patient (P1) was a one-yr-old only son of healthy Caucasian parents. He grew and developed normally and had no diarrhea or oral thrush, remaining healthy until the age of 9 mo when he developed progressive respiratory symptoms caused by pneumocystic carinii. He was treated successfully with trimethoprim-sulfamethazole and referred to our center for diagnosis and treatment. His physical examination was completely normal and included the presence of normal size cervical lymph nodes. Chest radiography and ultrasound revealed a normal size thymus gland. No hematologic abnormalities were detected and his adenosine deaminase and purine nucleoside phosphorylase enzyme activities were normal. Evidence of maternal T cell engraftment could not be detected by either HLA typing or karyotyping. The patient's serum tested negative for both HIV antigens and antibodies. After failing to reject an allogeneic skin graft, the patient received a matched unrelated bone marrow transplant. He is currently alive and well and fully engrafted with donor cells.

A maternal male cousin (P2) had been previously diagnosed at another center with an unusual combined immunodeficiency. This presentation included normal numbers of both B and T peripheral lymphocytes, accompanied by normal levels of immunoglobulins and specific antibodies. The patient died at the age of 2 yr after a mismatched bone marrow transplant following lectin T cell depletion.

Immunological studies. The thymus of a severe combined immunodeficiency patient typically appears small and dysplastic, with a complete disruption of thymic architecture, depletion of thymocytes, and an absence of Hassall's corpuscles. However, uncharacteristically, the patient's thymus gland was readily detectable by radiography or ultrasound. Histological analysis of a thymic biopsy revealed a heavily populated thymus gland,

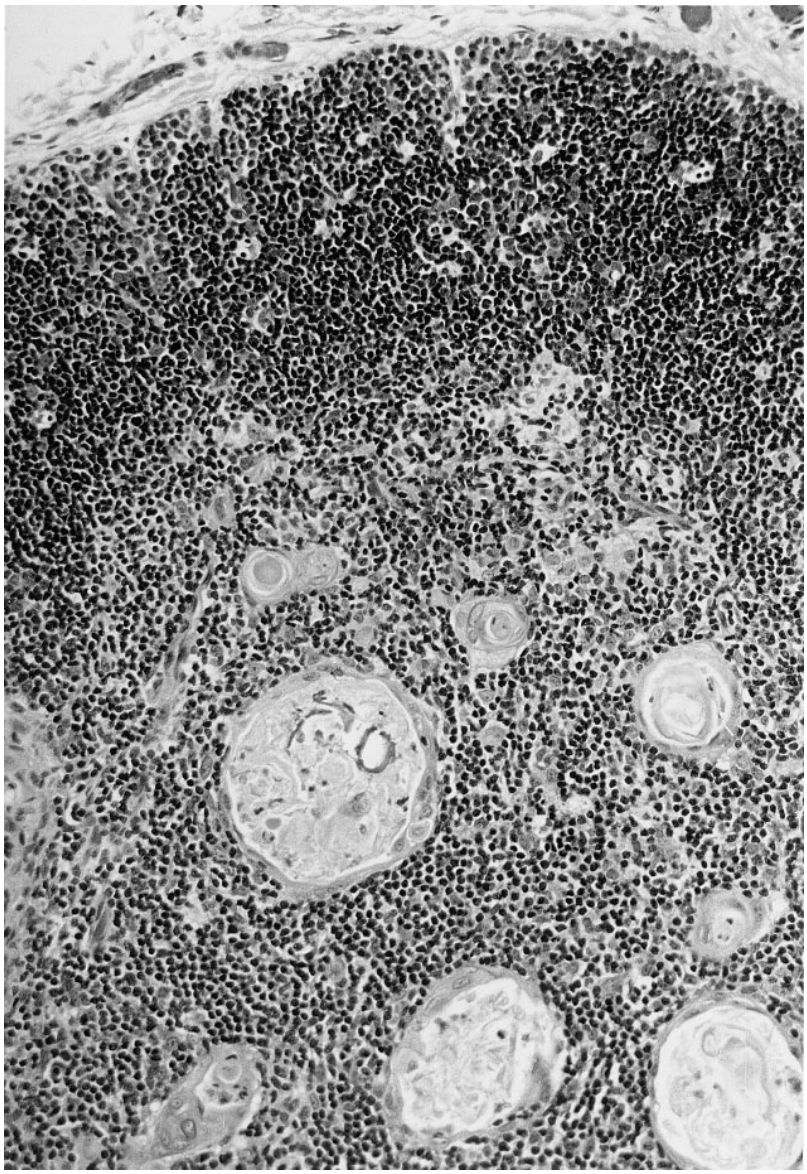


Figure 1. Patient thymus biopsy. Hematoxylin and eosin staining revealed a normal thymus with fibrous septa separating lobules of lymphoid tissue, distinct demarcation between the cortex and medulla, and normal numbers and development of Hassall's corpuscles.

which had clear cortico-medullary demarcation and abundant Hassall's corpuscles (Fig. 1), basically indistinguishable from a normal thymus. Similarly, immunohistochemical stainings with antibodies against CD2, CD3, CD4, CD8, HLA class I and class II were completely normal (not shown). These observations suggested that maturation of thymocytes proceeded normally in this patient. Indeed, analysis of the peripheral T cell repertoire, by examination of TCR V_β families using PCR, revealed that all 20 V_β families were represented (Fig. 2), suggesting that thymocyte selection was unaltered.

In addition to an apparently normal thymus, the patient had normal numbers of both T and B lymphocytes and natural killer cells. Closer examination revealed that both the $CD3^+4^+$ and $CD3^+8^+$ T cell subsets were within normal ranges. However, humoral immunity was abnormal, as although IgG, IgM, and IgA levels were consistently within the normal range (Table I), specific serum antibodies to tetanus and polio were absent despite immunization. T cell mitogenic responses to anti-CD3 were consistently comparable to, and at times better than, control samples. Similarly, PHA responses were considered within normal range, (75% of simultaneously studied normal control sample responses). However, unlike control samples, addition of exogenous IL-2 (100 U/ml) to PHA- or CD3-stimulated patient cells did not result in increased proliferation (Table I), as measured by thymidine incorporation.

The inability of the patient's T cells to respond to exogenous interleukin-2 was most unusual for a combined immunodeficiency, where some degree of response is usually observed. This suggested that one of the IL-2 receptor chains could be mutated, resulting in either reduced receptor expression or perhaps a more subtle change affecting IL-2 binding affinity. When expression of the IL-2R α, β, and γ chains was analyzed by flow cytometry, all three were found to be present and normally expressed. We therefore examined the possibility that a point mutation might have resulted in modification of the receptor binding site for interleukin-2.

IL-2Rγ gene sequences in the patient and family. An alteration to the IL-2Rγ chain appeared to be the most likely

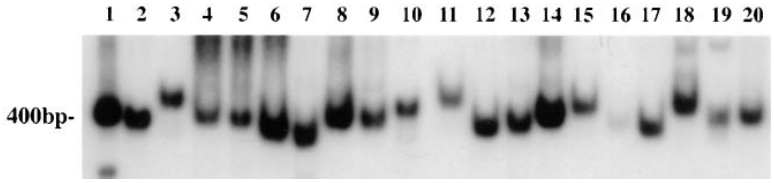


Figure 2. Expression of the Vβ repertoire was examined by reverse transcriptase-PCR upon RNA obtained from patient peripheral blood lymphocytes as described in the Methods. An aliquot of ^{32}P-dCTP was included in PCR reactions and after gel electrophoresis products were detected by autoradiography. All 20 (1–20) Vβ families were found to be present.

source of mutation; as a maternal male cousin had died with a similar disorder, suggesting an X-linked condition. The message for IL-2Rγ was therefore isolated by RT-PCR, cloned and sequenced. In accordance with observation of normal IL-2Rγ protein expression in patient cells (Fig. 3), no major changes were observed. The cDNA sequence of patient IL-2Rγ was found to be normal except for a single base pair change C664 > T (numbered from the initiating ATG), with a resultant change in coding from arginine to cysteine at residue 222 (Fig. 4). This change occurs within the extracellular region of the protein, close to the transmembrane domain.

An analysis of maternal IL-2Rγ message by RT-PCR revealed a pattern of nonrandom X-chromosome inactivation, with the majority of the clones sequenced representing normal sequences and only a few percent containing the mutation. Examination of the genomic IL-2Rγ sequence of the patient confirmed the presence of the single base change, while both the normal and mutated residues were found in the mother's genomic sequences. The presence of apparently nonrandom X chromosome inactivation in the patient's mother strongly suggested that this single base change, and consequent amino acid change, was deleterious. In addition, a similar pattern of nonrandom inactivation was observed in IL-2Rγ cDNA from the maternal grandmother. DNA was also obtained from cells of the maternal male cousin (P2) who had died at 2.5 yr with a similar condition to the patient and when the IL-2Rγ gene was examined, the same mutation was observed.

Table I. Phenotype and Functional Analysis of Patient's Peripheral Mononuclear Cells

	Patient (4,000–6,000)*	Control (4,000–6,000)
Percentage of positive staining cells (%)		
CD2	84±5	75–95
CD3	68±3	60–85
CD4	40±2	30–60
CD8	25±6	15–35
CD19	20±1	5–20
CD56	10±3	5–20
Lymphocyte proliferation (cpm 10^{-3})		
PHA	86±19	120±32
CD3	110±30	105±28
CD3 + IL-2	93±8	126±25
IL-2	0.9±0.3	3±0.7
Immunoglobulin and antibody levels		
IgG	2.72 gram/liter	2.3–14.1 gram/liter
IgM	1.14 gram/liter	0.0–1.4 gram/liter
IgA	0.29 gram/liter	0.0–0.8 gram/liter
Tetanus	< 0.01 IU	> 0.01 IU
Polio	titer < 1–8	titer >1–8

*Lymphocytes/μl. Phenotype and functional analysis of patient's peripheral blood mononuclear cells. Patient lymphocyte counts were consistently within normal ranges. Flow cytometry analysis of peripheral lymphocytes with antibodies to CD2, CD3, CD4, CD8, CD19, and CD56 revealed normal B and T cell subset numbers. Proliferative responses were performed as described in the Methods and are presented as thymidine incorporation after 3-day culture. While serum IgG, IgA, and IgM levels were all within normal age range, specific antibodies to tetanus and polio were absent despite immunization. Polio titers were determined by complement fixation.

Altered ligand binding to patient IL-2 receptors. On the basis of the evidence described, it was strongly felt that the R222C mutation of the IL-2Rγ chain was responsible for the unusual immunodeficiency observed in both P1 and P2. As the mutation did not appear to affect IL-2Rγ expression, we examined whether the ability to bind interleukin-2 was altered, using an EBV-transformed B cell line derived from the peripheral B lymphocytes of P1. In comparison to normal controls, binding of an IL-2 fluorokine to the patient EBV cell line was greatly reduced. Binding at three concentrations of the fluorokine was examined, 50 nM, 5 nM, and 500 pM; a range designed to examine binding to both high and low affinity IL-2 receptors. While binding to both patient and control cells was observed with 50 nM IL-2, only control cells demonstrated detectable binding at the lower concentrations (Fig. 5*A*). In addition, transient transfection of cDNA for a normal IL-2Rγ chain, in the eucaryotic expression vector pCMV (Invitrogen Corp.), into the patient EBV cell line resulted in a detectable increase in IL-2 binding (Fig. 5 *B*), strongly suggesting that the patient IL-2 receptor had a lower than normal affinity for the cytokine.

Alternatively, these results could be explained by a lower level of receptor expression on patient cells, by variations in IL-2Rα chain expression between EBV cell lines, or by the creation of additional receptors using previously unutilized α and β chains upon transfection with the normal IL-2Rγ. However, transient transfection of β and γ chains into COS cells further suggested that an alteration in affinity had occurred. cDNA for a normal IL-2Rβ chain was cotransfected into COS cells with either a normal IL-2Rγ cDNA or the mutated ver-

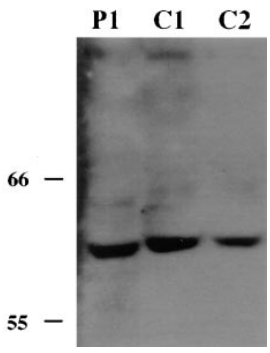
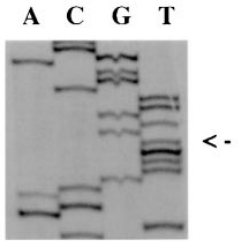


Figure 3. Western blot analysis of IL-2Rγ protein expression in EBV-transformed cell lines derived from mature circulating B cells. IL-2Rγ protein expression in an EBV B cell line from the patient (*P1*) was clearly detectable and comparable to that in two control EBV B cell lines (*C1*, *C2*).



Y	T	F	R	V	R	S	R	
TAC	ACG	TTT	CGT	GTT	CGG	AGC	CGC	normal
			+					
TAC	ACG	TTT	TGT	GTT	CGG	AGC	CGC	mutation
Y	T	F	C	V	R	S	R	

Figure 4. Mutation of the patient IL-2Rγ sequence. The mutation detected in the patient IL-2Rγ gene is shown and compared to the normal sequence. The C to T transition at base 664 results in an alteration in coding from arginine to cysteine at residue 222. An identical mutation was found in the mother, grandmother, and a similarly affected male cousin. Both the mother and grandmother demonstrated nonrandom X-chromosome inactivation.

sion derived from the patient. A clear difference between the contribution of the patient and normal IL-2Rγ chains was evident (Fig. 5 *C*). Although appreciably greater IL-2 binding was observed to the combined β and γ (R222C) chains, than to β alone, it was significantly lower than IL-2 binding to the normal βγ complex. In summary, these results indicate that although receptors containing the mutated gamma chain are capable of binding IL-2, their affinity is reduced.

Reduced Jak3 activation by patient βγ receptors. As the R222C mutation of the γ chain did not completely abrogate IL-2 binding, it was expected that signaling through the receptor complex would proceed normally, but to a lesser degree than normal at low IL-2 concentrations. IL-2–induced Jak3 phosphorylation could be detected in the patient EBV B cell line (not shown); but, it was not possible to quantify the degree of Jak3 activation relative to normal EBV B cell lines, due to the variations in Jak3 activation levels encountered in the normal controls. This variation in normal controls reduces the usefulness of assessing Jak3 activation as a clinical marker in identifying other atypical X-linked SCIDS.

In an attempt to quantify the relative abilities to activate Jak3, expression vectors for a normal β + γ, or β + γ_{R222C}, and Jak3 were cotransfected into COS cells and the level of Jak3 phosphorylation induced by low levels of IL-2 examined. γ_{R222C} was clearly less effective in modulating Jak3 activation (Fig. 6). Thus, at limiting concentrations of IL-2, patient T cell activation may be compromised, by a reduced level of signaling.

Discussion

Patients with X chromosome–linked severe combined immunodeficiency typically present with recurrent and severe infections and failure to thrive at 5–6 mo of age. This immunological profile characteristically includes decreased or absent T cell and natural killer cell development, elevated B cell numbers, and panhypogammaglobulinemia. However, it is becoming clear that less definitive immunological phenotypes can occur as the result of mutations in the X-linked IL-2Rγ gene, as recently a case with a near normal T cell phenotype but abnormal cellular and antibody responses to antigens was identified (27). A definitive diagnosis of SCID in our patient was greatly delayed because of the unusual presentation; with normal growth and development, a low number of infections and nor-

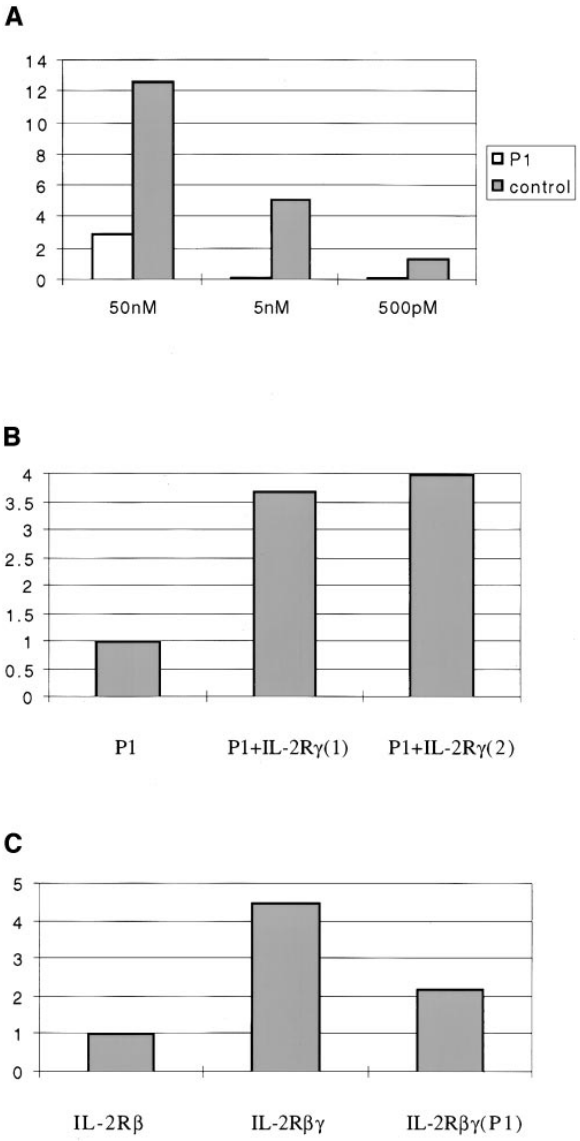


Figure 5. Reduced IL-2 binding to patient IL-2 receptors. (*A*) The binding of an IL-2 fluorokine to patient and control EBV cell lines was compared at three concentrations: 50 nM, 5 nM, and 500 pM, and the percentage of positively staining cells was measured. (*B*) cDNA coding for a normal IL-2Rγ was transfected into the patient EBV B cell line and IL-2 binding analyzed relative to the untransfected EBV line. The results of two independent experiments are shown. In both cases transfection resulted in an approximately fourfold increase in IL-2 binding. Binding is presented relative to the untransfected EBV cells (= 1.0). (*C*) COS-7 cells were transfected with cDNA for an IL-2Rβ chain in combination with either a normal IL-2Rγ cDNA or cDNA for the patient IL-2Rγ and binding of the IL-2 fluorokine analyzed. IL-2 binding is presented relative to the control of IL-2Rβ alone (= 1.0).

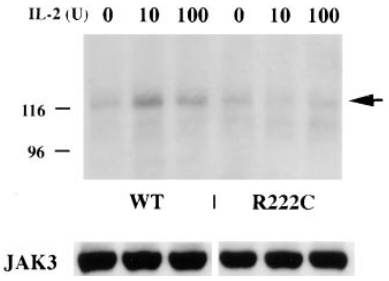


Figure 6. Jak3 activation through the γR222C chain. cDNAs for β + γ, or β + γ_{R222C}, and Jak3 were transfected into COS-7 cells and Jak3 phosphorylation in response to IL-2 determined by anti-phosphotyrosine Western blot of Jak3 immunoprecipitates. Lysate from each transfection was blotted with anti-Jak3 to ensure equal expression.

mally functioning cellular immunity as measured by in vitro assays. Moreover, even a thymus biopsy appeared completely normal. This highlights clearly the variation that can occur in clinical presentation as the result of mutations of the IL-2Rγ gene. In other SCID cases it is noticeable that further deterioration of the immune system, and consequently the development of an even greater susceptibility to infection, occurs with advancing age. Unusually, in this case, no such further deterioration was detected before the unrelated bone marrow transplant was performed at the age of 16 mo. However, the complete inability of the patient to reject an allogeneic skin graft, combined with a family history of a male cousin with a similar condition, who had deteriorated clinically in the second year of life, suggested a grave prognosis and prompted the decision to perform the transplant.

The mutation detected in the IL-2Rγ chain of these two SCID patients was unusual in that the protein is sufficiently stable to be expressed at the cell surface. Most IL-2Rγ mutations result in the complete ablation of protein expression, whether the mutation is a deletion or substitution. Many of these mutations appear to be associated with amino acid substitutions which destabilize protein conformation sufficiently as to prevent expression. In this case, the R222C mutation does not appear to alter expression, but rather the function of the protein would appear to be compromised. However, there are grounds for believing that the mutated protein still retains a significant degree of function. Unlike the cases where IL-2Rγ protein expression is ablated, peripheral T cells are not absent or even significantly decreased. T cell differentiation appears to be unaffected, as mature T cells are present, with the correct ratio of CD4 to CD8 cells observed in the periphery (2:1), and immunostaining of thymus sections for CD3, CD2, CD4, and CD8 is normal. This is of importance because the early stages of T cell differentiation are known to be dependent upon signals from the interleukin-7 receptor. Ablating expression in mice of either IL-7 (28), or its receptor (29), results in a near complete arrest of T cell development; similar to that observed in the absence of IL-2Rγ. This strongly suggests that the IL-2Rγ chain plays its normal role in the IL-7R complex of this patient; contributing to the affinity for IL-7 binding, receptor internalization after ligand induced cross-linking, and in recruiting the cytoplasmic Jak3 tyrosine kinase into the signaling complex. The deficiency in T cell function appears to arise after maturation is completed, in the periphery, where responses to foreign antigen are affected. Interleukin-2 plays a critical role in this process and we have shown that the ability of receptors containing the mutated γ chain to bind IL-2 is reduced.

The arginine to cysteine (R222C) substitution occurs in the

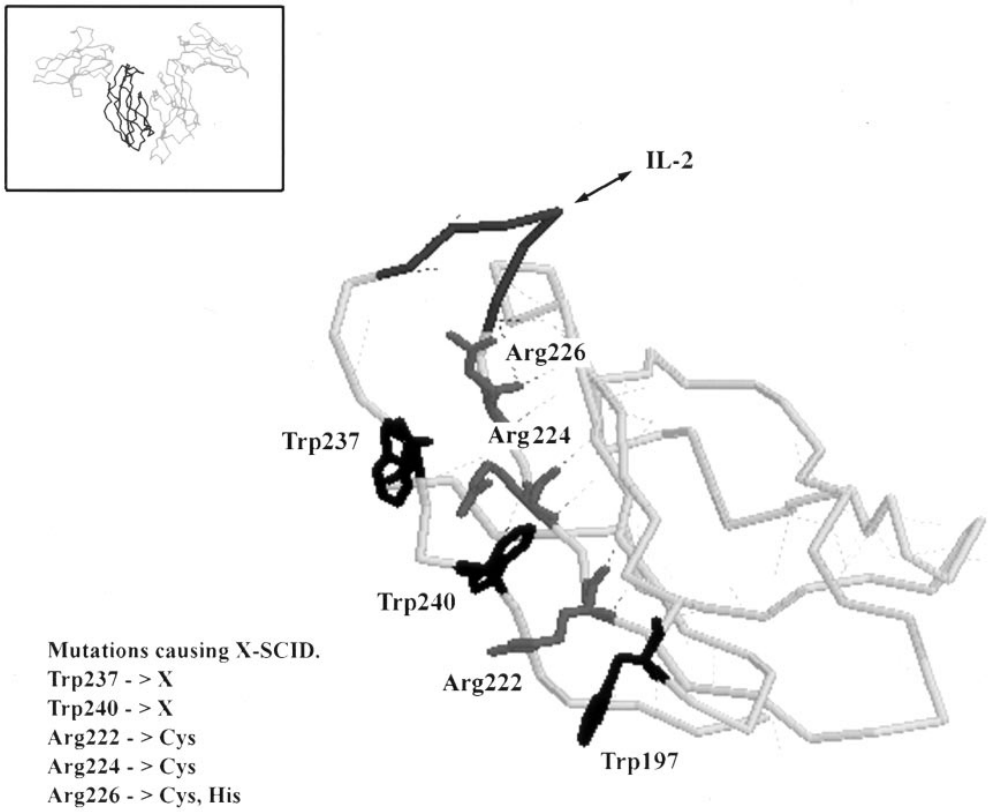


Figure 7. Model of the IL-2Rγ. The carbon backbone model of the membrane proximal portion of the extracellular domain of IL-2Rγ is based upon the structure of the prolactin and growth hormone receptors. A small schematic of the extracellular domains of the IL-2Rβγ complex is presented (*inset*), with the highlighted γ domain enlarged below. The area potentially affected by the R222C mutation is highlighted by inclusion of amino acid functional groups, while the rest of the protein is represented only by the alpha carbon backbone. The tryptophan and arginine residue stack is clearly visible, with only the position of Trp197 demonstrating significant positional variation in the different crystal structures and models. Mutation of either Arg222, 224, or 226 results in X-SCID and mutation of the Trp residues is similarly deleterious.

extracellular domain where several mutations have already been described, including a similar arginine–cysteine substitution at residue 224 (17, 30), many resulting in SCID characterized by an absence of IL-2Rγ expression. In the absence of crystallography data for the IL-2Rγ chain it is difficult to assess the exact contribution of these residues to the structure of the protein. However, IL-2Rγ is a member of a large family of closely related receptor chains, several of which have well described structures based on crystallographic data; primarily the human growth hormone and prolactin receptors (24–26). Models of both the IL-2Rβ and IL-2Rγ chains have been proposed based upon these crystal structures (24). In the original structures, and the models formulated upon them, Arg222 is part of a complex stacked structure. Arginine and lysine residues intercalate between the stacked rings of a series of tryptophan amino acids (Fig. 7) and hydrogen bonding between amino acids appears to stabilize this conformation, forming a distinct structural unit. Most of the residues forming this structure are highly conserved within the receptor family (31).

Mutation of any of the stacked tryptophan residues results in a loss of IL-2Rγ protein expression and development of SCID. The importance of this structure is further emphasized by the R224C mutation (30), where loss of this positively charged residue alone destabilizes structure sufficiently to ablate protein expression. Although the R222C substitution observed in our patient does not appear as deleterious to protein integrity, the loss of the positively charged arginine and substitution of the polar cysteine, will significantly alter the H-bonding pattern of the stack and may alter its precise structure and position. Because the tryptophan–arginine stack is directly connected to a short loop protruding into the putative ligand binding pocket, alterations in the stack are likely to influence loop positioning, potentially altering its orientation or simply the distance it extends into the pocket. This alone may be sufficient to affect ligand binding, while other functions remain unaffected.

The effect of such an alteration on the function of the IL-2, IL-7, and IL-4 (and −9, −15) receptors is likely to differ, as the only constant of these complexes is the IL-2Rγ chain itself, and its contribution is known to vary. The IL-2Rγ chain exerts a much greater influence upon the ligand binding affinity of the IL-2 receptor complex than either of the IL-7 or IL-4 receptor complexes. Inclusion of the γ chain increases the affinity of IL-4 binding two- to threefold, IL-7 binding 7–10-fold, but IL-2 binding 100-fold (8, 9, 17). Therefore, mutations in regions of the IL-2Rγ chain contributing to the affinity of binding are likely to have a far greater effect upon T cell responses to IL-2 than IL-7 (or IL-4).

Thus, the single base substitution observed in the IL-2Rγ gene of this patient and a male cousin appears sufficient to cause immunodeficiency, as the result to T cell dysfunction. T lymphocyte differentation and maturation does not appear to be significantly altered; but the ability to participate in antigenic responses may be severely limited due to the inability of mature T lymphocytes to effectively bind interleukin-2.

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