T-Lymphocyte Differentiation and Proliferation in the Absence of the Cytoplasmic Tail of the Common Cytokine Receptor γc Chain in a Severe Combined Immune Deficiency X1 Patient

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Mutation of the γc chain common to interleukin-2 (IL-2), IL-4, IL-7, IL-9, and IL-15 receptors has been shown to be responsible for the X chromosome-linked severe combined immune deficiency (SCIDX1). Human SCIDX1 patients are characterized by an absence of T and natural killer cell differentiation. We report the case of a SCIDX1 patient who first had few detectable peripheral T cells, then developed, after haploidentical T-depleted bone marrow transplantation (BMT), up to 2,000/μL autologous T cells. These T cells have persisted over 8 years after BMT and were able to proliferate in the presence of mitogens and of some antigens, although to a lesser extent than control T cells. A stop mutation was identified which predicts that the major part of the cytoplasmic tail of γc is truncated. This mutation does not affect high-affinity IL-2 binding, but it partly decreases IL-2 endocytosis and prevents the downmodulation of the IL-2-receptor β chain and the tyrosine phosphorylation of Jak 3 protein in response to IL-2. This report raises questions concerning the role of the γc chain in IL-2 receptor endocytosis and in T-cell development and differentiation. © 1996 by The American Society of Hematology.

X CHROMOSOME-linked severe combined immunodeficiency disease (SCIDX1) is a recessive hereditary disorder characterized by a complete absence of immature and mature T cells and natural killer (NK) cells, whereas B cells are present in normal or elevated numbers. SCIDX1 infants display severe and persistent infections, resulting in failure to thrive and early death if curative bone marrow transplantation (BMT) is not performed. Lymphocytes of patients with SCIDX1 are characterized by an absence of proliferative responses to mitogens and common antigens and failure to produce specific antibodies following immunization. Obligatory female carriers of SCIDX1 are immunologically normal but display a nonrandom pattern of X inactivation in their T-, B-, and NK-cell populations, which suggested a role for the SCIDX1 gene product in the development of these lymphoid lineages.

The gene defective in SCIDX1 has been identified as the IL-2 receptor (IL-2R) γc chain. The IL-2R is composed of three chains (IL-2Rα, β, and γc), which associate noncovalently in different combinations to form high- (αβγc), intermediate- (βγc), or low-affinity (α) IL-2Rs. Low-affinity receptors (kd = 10 to 100 pmol/L) contain the IL-2Rα chain, whereas intermediate (kd = 700 pmol/L-1 nmol/L) and high-affinity (kd = 10 to 100 pmol/L) receptors contain IL-2Rβγc or IL-2Rαβ, and IL-2Rαβγc, respectively. The severity of SCIDX1 results from the participation of the γc chain not only in the IL-2R complex but in multiple cytokine receptors including IL-4R, IL-7R, IL-9R, and IL-15R.

IL-2 signaling requires heterodimerization of IL-2Rβ and γc induced by IL-2 binding. This is followed by the phosphorylation and activation of multiple cellular substrates, principally of Jak1 and Jak 3, associated to IL-2Rβ and IL-2Rγc, respectively, members of the Janus family of kinases. Another early event is the rapid internalization of the multimolecular IL-2R in endocytic compartments. The specific chains responsible for endocytosis of high-affinity IL-2R have not been fully elucidated, but IL-2Rβ has been shown to be constitutively internalized, independently of IL-2Rγc.

Mutations resulting in the SCIDX1 phenotype are heterogeneous. Most of them were identified in the extracellular domain of IL-2Rγc, and result in the loss of high-affinity IL-2R binding. A few mutations were identified within the cytoplasmic tail of IL-2Rγc, and these alter the ability of IL-2R to associate with the tyrosine kinase Jak 3, thus interfering with signal transduction.

We report clinical, immunological, and genetic findings pertaining to a patient affected by a typical form of SCIDX1, in whom a subnormal autologous T-cell population developed a few months after an allogenic BMT and persisted thereafter. Analysis of the functional consequences of the common γc chain mutation on high-affinity IL-2 binding, endocytosis and downmodulation of the IL-2R may contribute to a better understanding of the precise role of the γc chain in cytokine receptor function.

PATIENT

The patient was the second child of a couple of Italian origin. His parents and older sister were healthy, and there was no history of disease in the family. Infections began at 1 month of age, when he developed protracted diarrhea with failure to thrive and repeated bronchopneumonitis. At diagnosis at 4 months of age, immunological investigations showed a T-cell lymphocytopenia (300 CD3+ T cells/μL), a high B-cell count (2,370 CD19+ B cells/μL), and hypogammaglobulinemia. Adenosine deaminase activity was normal. There was no proliferation to phytohemagglutinin, nor was...
T-cell differentiation with truncated γc chain

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MATERIALS AND METHODS

Immunological Investigations

T-cell numeration was performed on whole blood using phycoerythrin (PE) conjugated anti-CD3 monoclonal antibody (MoAb) (Leu 4-Becton Dickinson, Mountain View, CA). Proliferation assays were performed on peripheral blood mononuclear cells (PBMC) stimulated either with the mitogen phytohemagglutinin (Difco, Detroit, MI) (final dilution 1/1000 for 3 days, or for 6 days with either candida antigen (2 µg/mL; Institut Pasteur, Paris, France) or tetanus toxoid (final dilution 1/250; Institut Merieux, Lyon, France). In some experiments, recombinant IL-2 (Genzyme, Cambridge MA; 5 ng/mL) and anti-CD25 MoAb (BB10 Biotech, Germany; 1 µg/mL) were used. Proliferation was measured by 3H-Thymidine uptake.

DNA Analysis

Sequence analysis of the gene encoding the γc chain. Full length IL-2Rγ, chain transcripts were polymerase chain reaction (PCR)-amplified from a B-lymphoblastoid cell line (B-LCL) using reverse transcriptase (RT)-nested PCR and directly sequenced with 32P-end-labeled internal primers using a thermal cycler sequencing kit (New England Biolabs, Beverly, MA). Sequences of the primers were previously reported. Exon 7 of the IL-2Rγ, gene were also amplified from genomic DNA of ST B-LCL and T cells by using flanking intronic primers and directly sequenced by the same method.

Search for chimerism, in the patient following BMT from the mother was performed on DNA isolated from patient E-rosette-positive (E+) PB cells, E-rosette-negative (E-) PB cells and polymorphonuclear cells (PMN). PCR was performed on 101 pg of the DNA preparation using the primers ATT and AAT which detect a triplet sequence polymorphism at the DXS 101 locus, as previously described. One tenth of each reaction mixture was analyzed on a 5% polyacrylamide 8 mol/L urea sequencing gel. In these conditions the sensitivity of chimerism detection was 0.1%.

Cell lines. IARC 301.5 is a subclone from a cell line derived from a human T lymphoma. It expresses high- and low-affinity IL-2R,24,25 YT 12881, a subclone from the NK cell line YT, was obtained from Dr K. Smith (Dartmouth Medical School, New Hampshire). ST is an Epstein-Barr virus (EBV)-transformed B–lymphoblastoid cell line derived from the SCIDX1 patient studied here. The γc-B–cell line is an EBV-transformed B–lymphoblastoid cell line derived from SCIDX1 patient with a genomic deletion of the IL-2Rγ, gene. All cells were grown in RPMI 1640, 10% decomplemented fetal calf serum (FCS), 10 mMol/L HEPES, pH 7.2, supplemented with 2 mMol/L L-glutamine.

Surface expression of IL-2Rβ and IL-2Rγ, and downregulation. For surface expression analysis, indirect immunofluorescence using the murine MoAb 341 directed against the IL-2Rβ chain,27 a kind gift of Dr Robb (Dupont Merck Pharmaceutical Co, Delaware), or the rat MoAb TuGb4 directed against the IL-2Rγ, chain,28 a kind gift of Dr Sugamura (Tokohu University School of Medicine, Sendai, Japan), was performed as described. Isotype-matched control mouse IgG1 (Pharmingen, San Diego, CA) and Rat IgG2b (Pharmingen) were respectively used as controls. The second antibodies used with MoAb 341 were PE-conjugated anti-IgG(ab)'; (Immunotech, Marseille, France); and with TuGb4, a PE-conjugated antirat Ig (Jackson, West Grove, PA).

To study IL-2Rβ downregulation, exponentially growing cells were incubated at 2.106 cells/mL at 37°C for the indicated times with IL-2 at specified concentrations. After two washes in cold phosphate-buffered saline (PBS), the cells were stained with anti-IL-2Rβ antibodies for 60 minutes at 4°C. After one wash in cold PBS, they were labeled with the second antibody for 45 minutes at 4°C, washed once, and analyzed by cytofluorimetry.

Scatchard analysis and 125I-IL–2 endocytosis. Pure recombinant IL-2 was obtained from SANOFI (Labege, France) and Roussel-Uclaf (Romainville, France). IL-2 was radiolabeled with 125I by the chloramine T method to a specific activity of 30 to 100 × 106 cpm/µg. Nonspecific binding of radiolabeled IL-2 was measured in the presence of a 500-fold molar excess of cold IL-2 and was subtracted. Scatchard analysis studies using 125I-labeled IL-2 were performed as described, and the data were fit by computer analysis.

For endocytosis studies, B-cell lines were incubated in 100 µL RPMI-HEPES, pH 7.2, 10% FCS, with 150 µmol/L,125I-labeled IL-2 for 1 hour at 4°C. At the end of the incubation, the cells were washed twice at 4°C to remove unbound ligand. The cells were then resuspended in the same medium at 37°C. Aliquots were removed at different times and rapidly cooled down to 4°C by adding 1 mL cold medium. Cell surface associated radioactive ligand was then removed by two successive acid pH treatments (2 minutes at pH 2 at 4°C) as previously described. Nonspecific binding, measured for each ligand by adding a 500-fold excess of the same unlabeled ligand was subtracted. The efficiency of removal of cell-surface associated ligands by acid pH washes was measured for each ligand and was greater than 99%. The data presented here show specific binding and internalization.

Tyrosine kinase phosphorylation study. B-LCLs were procured, washed in RPMI 1640, and resuspended at a density of 2.106/mL in the same medium. They were then incubated at 37°C for 10 minutes with 250 U/mL IL-2 (Genzyme, Cambridge, MA). Cell activation was stopped by incubation at 4°C and centrifugation. The cells were lysed for 30 minutes on ice in lysis buffer (20 mMol/L Tris, pH 7.5; 140 mMol/L NaCl; 2 mMol/L EDTA; 50 mMol/L NaF; 1 mMol/L Sodium orthovanadate; 1% [vol/vol] Nonidet P-40) to which the protease inhibitors antipain, pepstatin, leupeptin (each at 2 µg/mL), aprotinin (10 µg/mL), and phenyl methylsulfonyl fluoride (1 mMol/L) were freshly added. Immunoprecipitations of precleared lysates were performed at 4°C overnight with the polyclonal antibody directed against the Jak 3 protein (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoprecipitated proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membrane. Western blots were performed with antibodies against phosphotyrosine 4G10 (Upstate Biotechnological, Lake Placid, NY) and polyclonal antibodies against Jak 3 protein. Immunoreactive bands were visualized with the chemiluminescence Western blotting system (Amer sham, Arlington Heights, IL).

RESULTS

Immunological time course study and chimerism after BMT. The patient first presented a typical SCIDX1 phenotype with an immunodeficiency characterized by panhypogammaglobulinemia, lymphocytopenia with a very low T-cell count and an elevated B-cell count, and the absence of proliferation to mitogens and antigens. At 11 months of age, he received a depleted haploidentical BMT from his mother. At 14 months of age, a rise in the CD3+ lymphocyte count was detected, which subsequently persisted during the following years (900 to 2,800/µL) (Fig 1). This population contained mature-phenotype CD4+ or CD8+ T cells. How-
trifugation. Fluorescence staining was performed on PBMC using anti-CD3 antibodies followed by a FITC-conjugated goat antimouse Ig. The number of CD3+ cells was then determined by flow cytometry.

Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density centrifugation isolated from freshly drawn heparinized blood by means of Ficoll Hypaque. The cell count occurred from 32 months of age (700/µL) until now represents the date of BMT.

The cell count decreased from 580/mL before BMT to 40/mL years later (not shown). Mitogen-induced T-cell proliferation, initially negative at 4 and 9 months of age, became positive 5 months after BMT, and remained positive during the seven following years (Table 1). Similarly, T cells were able to proliferate in response to tetanus toxoid and candida antigens after BMT, although the responses rarely reached normal values (Table 1). To study the role of the IL-R complex in the proliferation of the patient’s T cells in response to antigens, either soluble IL-2 or anti-IL-2Ra antibody was added to the culture. As shown in Table 2, antigen mediated response of patient’s and control’s cells was enhanced when IL-2 was added. Also, the anti-IL-2Ra antibody BB10 inhibited the proliferation in response to antigens of both patient’s and control cells.

Chimerism was repeatedly analyzed from 3 months to 7 years after BMT by using different methods. HLA typing of recipient T and B cells was performed by serotyping 3 months after BMT and by single strand conformational polymorphism (SSCP) allele-specific PCR on E+ and E− cells, 7 years later. The sensitivity of the latter method is sufficient to detect between 1/5,000 and 1/10,000 cells. Both methods showed that these cells had exclusively the patient HLA phenotype (data not shown). Karyotype analysis on E-rosette+ PB cells, performed 9 and 16 months after BMT, showed that 100% of the cells studied were XY (data not shown). DNA haplotype analysis using the polymorphic trinucleotide repeat DXS101 X chromosome marker was performed on distinct circulating hematopoietic cell populations of the patient and of his mother, 21 months following BMT. As shown in Fig 2, a unique allele is detected at DXS101 locus in the patient’s cell populations, including the T-cell subset, whereas two alleles are detected in his mother’s cell populations. These results show the presence of an autologous T-cell population in the patient following allogenic BMT, and the absence of detectable peripheral cells from maternal origin.

In conclusion, donor cells were not detectable after BMT, indicating that the mother’s BM did not engraft and that the patient had autologous BM reconstitution with development of partially functional T cells.

IL-2Rγc gene mutation in the patient. Because of the typical XSCID phenotype presented by the patient before BMT, we analyzed the IL-2Rγc transcript of the patient’s B-LCL by direct sequencing of the PCR amplified product. A single point mutation was detected in the patient’s IL-2Rγc gene consisting of a C to T base transition at position 865 in exon 7, corresponding to amino acid 289 and replacing the normal arginine-encoding codon by a premature stop codon (not shown). The mutation was also detected by sequencing genomic DNA from B-LCL and T cells (Fig 3). This transition results in a predicted protein lacking 81 amino acids at the carboxy terminus (Fig 3). We have immunoprecipitated IL-2Rγc from control B-LCL, the patient B-LCL, IL-2Rγc-B-LCL, and from YT cells. By western blotting with an antiserum directed against a peptide corresponding to amino-acid 342-361 mapping at the carboxy terminus of IL-2Rγc, the presence of IL-2Rγc was clearly detected in YT cells and control B-LCL, whereas no IL-2Rγc could be observed with ST cells or IL-2Rγc−cells, in agreement with the predicted truncation shown by the DNA analysis.

We have checked that no secondary mutation was present on the patient’s T cells. This study was performed twice at 5 years interval by SSCP analysis and by direct sequencing.

| Table 1. Time Course of Mitogen and Antigen-Induced Proliferation of the Patient’s T Cells |
|---------------------------------|---|---|---|---|---|---|---|---|
| Time after BMT (mo) | 9 | 16 | 20 | 24 | 34 | 44 | 78 | 95 |
| PHA | 0.5 (0.4) | 0.1 (0.3) | 0.2 (0.2) | 1.4 (1.7) | 1.1 (2.5) | 0.1 (0.2) | 0.9 (2) | Positive Threshold |
| No stimulation, day 3 | 6 (89) | 14 (47) | 18 (107) | 26 (146) | 22 (85) | 13 (75) | 16 (106) | ≥15 |
| Candida antigen | 0.5 (0.5) | 0.8 (1) | 1.4 (1.6) | 3.4 (2.5) | 0.3 (0.1) | 0.5 (1.7) | | |
| Tetanus toxoid antigen | 6 (44) | 4 (20) | 9 (12) | 28 (62) | 8 (81) | 6.5 (90) | | |
| Positive Threshold | | | | | | | | |

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of DNA isolated from the patient’s E-rosette+ PB cells (containing more than 95% T lymphocytes). The DNA sequence obtained from the patient’s T cells was exactly the same, in the 2 samples taken at 5 years interval, as the DNA sequence obtained from his B-LCL (Fig 3). This rules out the possibility that there may exist a second corrective genetic change in the T-cell population altering the SCIDX1 phenotype. Sequence analysis of the same region IL-2Rγc transcript of the patient’s mother did not detect any mutations, indicating that the mutation occurred de novo.

Surface expression of IL-2Rβ and IL-2Rγc. We next determined whether the IL-2Rβ and IL-2Rγc chains were expressed on the cell surface of the patient’s lymphoid cells. We studied the expression of the IL-2Rβ and IL-2Rγc chains on the patient’s EBV-transformed B-LCLs. This EBV-transformed cell line will be referred to as ST from now on.

Previous studies have shown that EBV-transformed human B cells express both high- and low-affinity IL-2R, indicating the presence of IL-2Rα, β, and γc chains. Surface expression of IL-2Rβ and IL-2Rγc was determined by immunofluorescence and fluorescence-activated cell sorter analysis on ST cells and control B-LCLS. ST cells expressed both IL-2Rβ and IL-2Rγc. The level of expression of IL-2Rγc and IL-2Rβ was similar in ST and in control cells. In contrast, no γc chain expression was detectable in the B-cell line of a SCIDX1 patient in whom a genomic deletion of the γc gene has been identified (Fig 4).

ST cells express high-affinity IL-2R. Because IL-2Rβ and γc were expressed on the cell surface of ST cells, we next determined if the truncated form of IL-2Rγc expressed in this patient was capable of associating with the other IL-2R components to form high-affinity receptors. Three different affinity IL-2Rs can be formed by association of the IL-2R subunits. By Scatchard analysis, ~140 high-affinity receptors with a kd ~ 70 pmol/L were detected on the patient’s B-LCLS, as compared to ~120 receptors with the same kd on control B-LCLS and (Fig 5). In each experiment, Scatchard analysis was performed at the same time on the IARC 301.5 T-cell line, which expresses well-defined high-affinity IL-2R, with a kd ~ 60 to 80 pmol/L. Similar [125I]-IL-2 binding experiments with B-LCLS derived from SCIDX1 patients which do not express IL-2Rγc and whose receptors are only composed of IL-2Rα and IL-2Rβ, did not allow us to detect any pseudo high-affinity receptors (αβ).

In ST cells, Scatchard analysis showed that IL-2 binding was due to high-affinity IL-2R composed of the three chains. Therefore, the IL-2Rγc mutation of ST does not affect the formation and ligand binding properties of high-affinity IL-2R.

IL-2 endocytosis by ST cells. One of the early events following IL-2 binding to high-affinity receptors is ligand and receptor endocytosis. Because the cytoplasmic tail of IL-2Rγc is mostly deleted in ST cells, we asked how this intracellular deletion affects IL-2 endocytosis. ST cells having bound [125I]-IL-2 at 4°C were washed and transferred to 37°C, and the kinetics of IL-2 internalization were analyzed. [125I]-IL-2 was internalized in ST cells (Fig 6). However, it was internalized less efficiently than in control cells because a 50% reduction was observed. Therefore, although most of the intracellular IL-2Rγc region is absent, IL-2 can still be internalized, but not as well as when IL-2Rγc is intact.

Downmodulation of IL-2R in ST cells. We have previously shown that IL-2 high-affinity receptors are constitutively internalized in the absence of ligand and that IL-2 increases their endocytosis rate by a factor of two. Thus, IL-2 induces high-affinity receptor downregulation. Because high-affinity receptors are composed of three noncovalently linked chains, downregulation of only one chain should be sufficient to cause downregulation of the whole receptor.

### Table 2. Effect of IL-2 and Anti-IL-2Ra Antibodies on Antigen-Induced Proliferation of the Patient’s T Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Candida Antigen</th>
<th>Tetanus Toxoid Antigen</th>
<th>No Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2R (5 ng/mL)</td>
<td>+Anti-IL-2Rα Antibody</td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.7</td>
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Each value is the mean of duplicates. Results are representative of two similar experiments.

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**Fig 2.** Analysis of the polymorphic pattern at the DXS 101 locus (X chromosome) on DNA isolated from blood polymorphonuclear (PN), E+, and E- cells of the ST patient 21 months after BMT compared to the maternal donor haplotype. The CA repeat polymorphism at the DXS 101 locus was analyzed by PCR using a single end-labeled primer. Only one allele is detected in the various hematopoietic cells of ST patient, whereas two alleles are present in the mother cells at this locus.
Fig 3. Characterization of the mutation in the $\gamma_\varepsilon$ chain of the ST patient. The pictures show the C to T base transition at position 865 in exon 7 of the IL-2R$\gamma_\varepsilon$ gene in genomic DNA extracted from the patient's B-LCL and T cells. A schematic representation of the normal and truncated ST $\gamma_\varepsilon$ chain is also shown.

Fig 4. Surface expression of IL-2R$\beta$ and IL2R$\gamma_\varepsilon$ on ST, control B-cell lines and control $\gamma_\varepsilon$-B-cell line. Cells from the ST B-cell line, from a control B-cell line or from a SCIDX1 patient B-cell line with genomic deletion of the IL-2R$\gamma_\varepsilon$ gene were stained for IL-2R$\beta$ (left panel) and IL-2R$\gamma_\varepsilon$ (right panel) and analyzed by flow cytofluorimetry (thick-line histograms), as described in Materials and Methods. In each panel, thin-line histograms represent the fluorescent profiles of cells stained with an isotype-matched irrelevant antibody followed by second antibodies.
T-CELL DIFFERENTIATION WITH TRUNCATED \( \gamma_c \) CHAIN

know that IL-2R\( \beta \) is constantly internalized without ligand in T, B, and NK cell lines.\(^{18}\) Therefore, we analyzed the effect of IL-2 on IL-2R\( \beta \) surface expression both in T and B cell lines expressing high-affinity receptors (IL-2R\( \alpha \), \( \beta \), and \( \gamma_c \)). As seen in Fig 7B and C, IL-2 induces IL-2R\( \beta \) downregulation in both T- and B-cell lines expressing high-affinity receptors (IL-2R\( \alpha \), \( \beta \), and \( \gamma_c \)). Downregulation of IL-2R\( \beta \) is also observed in the NK YT cell line expressing only intermediate affinity receptors (IL-2R\( \beta \) and \( \gamma_c \)) (Fig 7D).

However, IL-2R\( \beta \) downregulation could not be observed in ST cells expressing the truncated IL-2R\( \gamma_c \) (Fig 7A). We have shown that IL-2 induced receptor downregulation is caused by an increase in the net rate of receptor internalization.\(^{24}\) Therefore, sequences missing in this patient’s IL-2R\( \gamma_c \) are necessary for IL-2R\( \beta \) downmodulation.

Absence of Jak 3 tyrosine phosphorylation in ST cells. Tyrosine phosphorylation of the Janus Kinase proteins is one of the early biochemical events which follows IL-2 stimulation. The protein kinase Jak 3 is phosphorylated in response to cytokines whose receptors contain the \( \gamma_c \) chain (IL-2, IL-4, IL-7, and IL-9).\(^{13}\) Furthermore, Jak 3 is physically associated with the cytoplasmic domain of the \( \gamma_c \) chain. Based on transfection studies in COS cells, it has been reported that this association requires the presence of the 48 COOH terminal aminoacids.\(^{13}\) Jak 3 has been recently shown to play a key role in T-cell differentiation and activation in humans.\(^{11,12}\) Given the phenotype of the patient, we examined whether Jak 3 might be tyrosine phosphorylated on IL-2 stimulation of the endogenous IL-2R in ST cells. As shown in Fig 8 immunoprecipitation with antibody to Jak 3 yielded a tyrosine phosphorylated band in response to IL-2 in control B-LCL, but no band was detected in ST cells. When the filter was stripped and reprobed with anti-Jak 3 antibody, equivalent amounts of Jak 3 were precipitated in each case. Therefore, as expected, Jak 3 was not phosphorylated in ST patient in response to IL-2.

DISCUSSION

We report the case of an atypical SCIDX1 patient who had a mature peripheral T-cell population even though the cells expressed a \( \gamma_c \) chain missing most of the cytosolic tail.
Because of this unexpected finding, structural and functional studies of the IL-2R of the cells from this patient (ST cells) were undertaken. The ST B-cell line displayed high-affinity IL-2R similar in number and affinity to those of control B cell lines. Following binding to high-affinity IL-2R, IL-2 was internalized, implying that most of the cytosolic part of IL-2Rγc is not required for IL-2 internalization, as already observed for shorter deletions of the cytosolic region. The fact that IL-2 is endocytosed in ST cells also agrees with previous observations made in normal T, B, or NK cells showing that IL-2Rβ carries the necessary molecular signal for endocytosis but is internalized twofold less efficiently in the absence of IL2Rγc (and unpublished results).

An important functional consequence of endocytosis is receptor downmodulation. Here we show that in normal B, T, and NK cell lines, IL-2 induces the downmodulation of IL-2Rβ, whereas in ST cells, IL-2Rβ downmodulation is not observed. The impairment of IL-2R endocytosis can account for the lack of downmodulation. These data suggest that endocytosis of high-affinity receptors in ST cells is governed by IL-2Rβ. In normal high-affinity receptors, the role...
of the intracellular tail of IL-2Rγc would thus be to accelerate receptor entry. Two mechanisms can be proposed to explain the role of IL-2Rγc. The first is that each of the chains, IL-2Rβ and γc, carries its own endocytic signal: when the heterodimer is formed, both signals function in an additive way, increasing the rate of entry. Such a mechanism has been described for the transferrin receptor when two endocytic signals were introduced in its cytoplasmic tail. Phosphorylation may modify the rate of IL-2R endocytosis, as described for EGF receptors.

Recent studies based on Jak3, IL-7Rαc, IL-7, and γc knock-out mice and on SCID patients with mutations in Jak3 or in γc show a critical nonredundant role for γc as part of the IL-7R in T-cell development and for the Jak3 signaling pathway. Although ST IL-2Rγc truncation does not affect IL-2 high-affinity binding and partially decreases endocytosis, it dramatically affects the Jak3 signaling pathway. The presence in patient ST of an autologous T-cell population with partially preserved functions suggests that γc-dependent cytokines or other signaling pathways allow some T-cell differentiation and function to occur. The recently identified IL-7-like cytokine termed “thymic stromal-derived lymphopoietin” could account for such a mechanism. In addition, a cytokine combination that excluded IL-7 allowed in vitro T-cell development from pro-T cells. In this regard, one could hypothesize that the haploidentical BM transplant provided a cytokine that could substitute for IL-7 in supporting early T-cell development. However, none of the cell populations tested in the patient were from the donor, and BMT performed in a number of SCIDX1 patients were never reported to induce autologous T-cell reconstitution.

Although the truncated γc molecule in the ST patient have lost the ability to mediate Jak3 phosphorylation, one cannot conclude that ST γc is completely nonfunctional. IL-2R are not downmodulated by IL-2, and their constant expression may favor alternative signaling pathways. Indeed, multiple cellular substrates besides the Janus kinase family were shown to be activated in response to IL-2. The results observed in the ST patient suggest that the functions of the cytoplasmic region of γc, whether in the context of the IL-2R or any of the other receptors sharing the γc chain, may be partially bypassed during the development of a mature T-cell population in humans.

Although most of the mutations described so far in SCIDX1 patients are localized in the extracellular or transmembrane region of the γc chain, few mutations were found in the cytosolic tail. In three previously described patients, the same nonsense mutation as found in the ST patient has been reported, thus identifying a hot-spot for mutations. In two other patients, stop-mutations further downstream were identified. The few clinical studies concerning these patients do not report the precise levels of circulating T cells, but they are reported as typical SCIDX1 patients, and no data from follow-up studies are available.

The relatively late age of the patient at the time of BMT may also be one of the variables that influence the appearance of peripheral T cells. It is worth noting that, in two unrelated families, the SCIDX1 patients previously described with a leaky phenotype displayed a T-cell lymphocytopenia during the first months of life. The peripheral T-cell counts became normal later, although with a restricted repertoire, which may partly explain the impaired T-cell functions observed. A restricted T-cell repertoire cannot be excluded in patient ST.

Different genetic backgrounds may also account for heterogeneous expression of SCIDX1, as already reported within some XLA families, where considerable phenotypic heterogeneity can be observed. Species-species differences in SCIDX1 have been well-documented: the IL-2Rγc-deficient mice and SCIDX1 dogs develop some mature B and T cells, although they are not functional, and considerable variation of T-cell numbers among affected dogs has been noticed. In this respect, they differ from the typical form of SCIDX1 described in humans, but not from the patient reported here or the two other patients with a mild form of SCIDX1 already described. Patient ST differs from the mouse and canine SCICX model, in which the γc gene is either deleted or not expressed on the surface due to a microdeletion very early in the coding region. However, the recent observation that γc mutant mice with a truncated γc cytoplasmic tail display a similar phenotype to that of the null mutant in terms of development of lymphocyte populations suggests that the truncated γc in patient ST might not play a dominant role in the T-cell differentiation observed.

It is likely that the diagnostic criteria of SCIDX1 based on the absence of circulating T cells, may have led to misdiagnosis of a number of SCIDX1 patients with an “atypical” phenotype. Molecular studies of IL-2Rγc should now allow detection of patients having phenotypes closer to the one of the ST patient. Similar to what has been described in animal models, it thus appears that the absence of a functional γc chain in humans does not necessarily lead to a severe block in T-cell differentiation and that, in certain circumstances, alternative pathways may partly overcome the γc chain defect to allow some T-cell differentiation and function, even if a severe immune deficiency persists.

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