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Immunology

Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor γ chain

(homologous recombination/cytokine/severe combined immunodeficiency X1)

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ABSTRACT **The interleukin 2 receptor γ chain (IL-2Rγ) is a component of the receptors for IL-2, IL-4, IL-7, and IL-15. Mutations in IL-2Rγ in man appear responsible for the X chromosome-linked immunodeficiency SCIDX1, characterized by a defect in T-cell and natural killer (NK)-cell differentiation with the presence of poorly functioning B cells. To explore at which level IL-2Rγ affects lymphoid development *in vivo*, we have analyzed mice derived from embryonic stem (ES) cells with mutant IL-2Rγ loci generated by Cre/*loxP*-mediated recombination. In the peripheral blood of chimeric animals, lymphoid cells derived from IL-2Rγ$^-$ ES cells were not detected, although control ES cells carrying an IL-2Rγ gene with embedded *loxP* sites gave rise to T-, B-, and NK-cell lineages. Germline IL-2Rγ-deficient male animals, however, developed some mature splenic B and T cells, although the absolute number of lymphocytes was almost 10-fold reduced. In contrast, there was a complete disappearance of NK cells (over 350-fold reduction). Development of gut-associated intraepithelial lymphocytes was also severely diminished, and Peyer's patches were not detected. *In vitro* mitogenic responses of thymocytes, IL-4-directed immunoglobulin class switch of splenocytes, and NK activity were defective. Thus, IL-2Rγ facilitates mainstream B- and T-cell generation and function and also appears to be essential for NK-cell development.**

Lymphoid development results from the expansion and differentiation of committed precursor cells under the influence of the bone marrow, thymic, and gut microenvironments, which requires both stem cell–stromal cell contact and interactions between soluble cytokines and their receptors (1, 2). The interleukin 2 receptor γ chain (IL-2Rγ), initially identified as an effector component of the IL-2R (3), figures prominantly in lymphopoiesis, through its participation in the receptors for IL-2, IL-4, IL-7, and IL-15 (4–8). Severe combined immunodeficiency X1 (SCIDX1), an X chromosome-linked immunodeficiency characterized by a severe block in T-cell and NK-cell differentiation with the presence of normal or elevated numbers of poorly functioning B cells (9, 10), is associated with mutations in IL-2Rγ (11). Still, the level at which IL-2Rγ mutations disrupt normal cytokine/receptor function and cause SCIDX1 is not known.

A variety of lymphokines play a role in the early stages of lymphoid development. In mice, *in vivo* blockade of the IL-7/IL-7R system with antibodies results in a complete block in B-cell generation and a substantial decrease in T lymphopoiesis (12, 13). In contrast, mice deficient for IL-2, IL-4, or both lymphokines have normal numbers of mature B and T cells (14–16). Therefore the phenotype in human SCIDX1 (near absence of T cells, elevated proportions of B cells) cannot be easily explained by defects in the IL-2, IL-4, or IL-7 receptor systems, without a species-specific difference in receptor function. In addition, the use of IL-2Rγ in additional cytokine receptors (such as IL-15R and perhaps others) and the role of these systems in lymphoid development remain unknown. To further analyze the role of this molecule *in vivo* and to establish a mouse model for SCIDX1, we have generated IL-2Rγ-deficient mice.

MATERIALS AND METHODS

Gene Targeting. The Cre/*loxP* system (17, 18) was used to produce (*i*) a defined deletion of the mouse (m)IL-2Rγ locus (γ$^-$) and (*ii*) an IL-2Rγ locus with embedded *loxP* sites (γ^{flox}) (Fig. 1*a*). The targeting plasmid contained (5′ to 3′): (*i*) a 6.0-kb fragment starting with 5′ upstream promoter region and including exons 1–6, having a single *loxP* site inserted into an *Eco*RI site in the first intron; (*ii*) a *loxP*-flanked neomycin-resistance cassette derived from pL2neo (18); and (*iii*) a 0.8-kb fragment comprising exons 7 and 8. mIL-2Rγ genomic DNAs were of 129/Ola origin as described (19). The targeting construct (80 μg) was electroporated into 4 × 10^7 E14.1 embryonic stem (ES) cells with selection in medium containing the neomycin analogue G418 (15). Clones were screened by PCR using primers 5′-TAGCCGAATAGCCTCTCCAC-CCAAGC-3′ and 5′-GCAGGGAAAGAGGGCAAGGGA-CACT-3′. Conditions were 35 cycles of 1 min of denaturation at 94°C followed by 2 min of annealing and polymerization at 72°C. Seven of 600 clones were positive by PCR, and 2 clones were verified by Southern analysis to harbor only the desired recombination event (γ^T, Fig. 1*a*). After transient transfection with pIC-Cre (18), the subsequent deletion events were characterized by PCR using primers specific for exons 1 and 7 (5′-GATCCTTCTTAGTCCTTCAGC-3′ and 5′-CGAAAA-GTTCCCTTGGTA-3′) followed by Southern analysis. Genomic DNA was digested with *Bam*HI, *Eco*RI, or *Hin*dIII and hybridized with mIL-2Rγ probes: a 0.6-kb PCR fragment containing the promoter region, a 0.6-kb cDNA fragment containing exons 2–6, or a 0.4-kb cDNA fragment hybridizing 3′ of the targeting construct (Fig. 1*a*; ref. 19). Frequencies of Cre-mediated deletions yielding γ$^-$ and γ^{flox} loci were 4% and 2%, respectively.

Immunofluorescence and Histological Analysis. Flow cytometric analysis on cell populations isolated from bone marrow, thymus, and spleen was performed as described (20). Rat anti-mIL-2Rγ monoclonal antibody (mAb) TUGm2 (4), rat anti-mIL-7Rα mAb (13), and NK-specific mAb DX5 were gifts of K. Sugamura (Tohoku University, Sendai, Japan), S. Nishikawa (Kumamoto University, Kyoto, Japan), and L. Lanier (DNAX, Palo Alto, CA), respectively. For histology, tissues were fixed in Carnoy's solution and embedded in paraffin. Sections were stained with methyl green/pyronin or by the

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Abbreviations: IL-*n*, interleukin *n*; IL-*n*R, IL-*n* receptor; IL-2Rγ, IL-2R γ chain; mIL-2Rγ, mouse IL-2Rγ; ES, embryonic stem; LPS, lipopolysaccharide; mAb, monoclonal antibody; NK, natural killer; SCID, severe combined immunodeficiency; TCR, T-cell receptor.

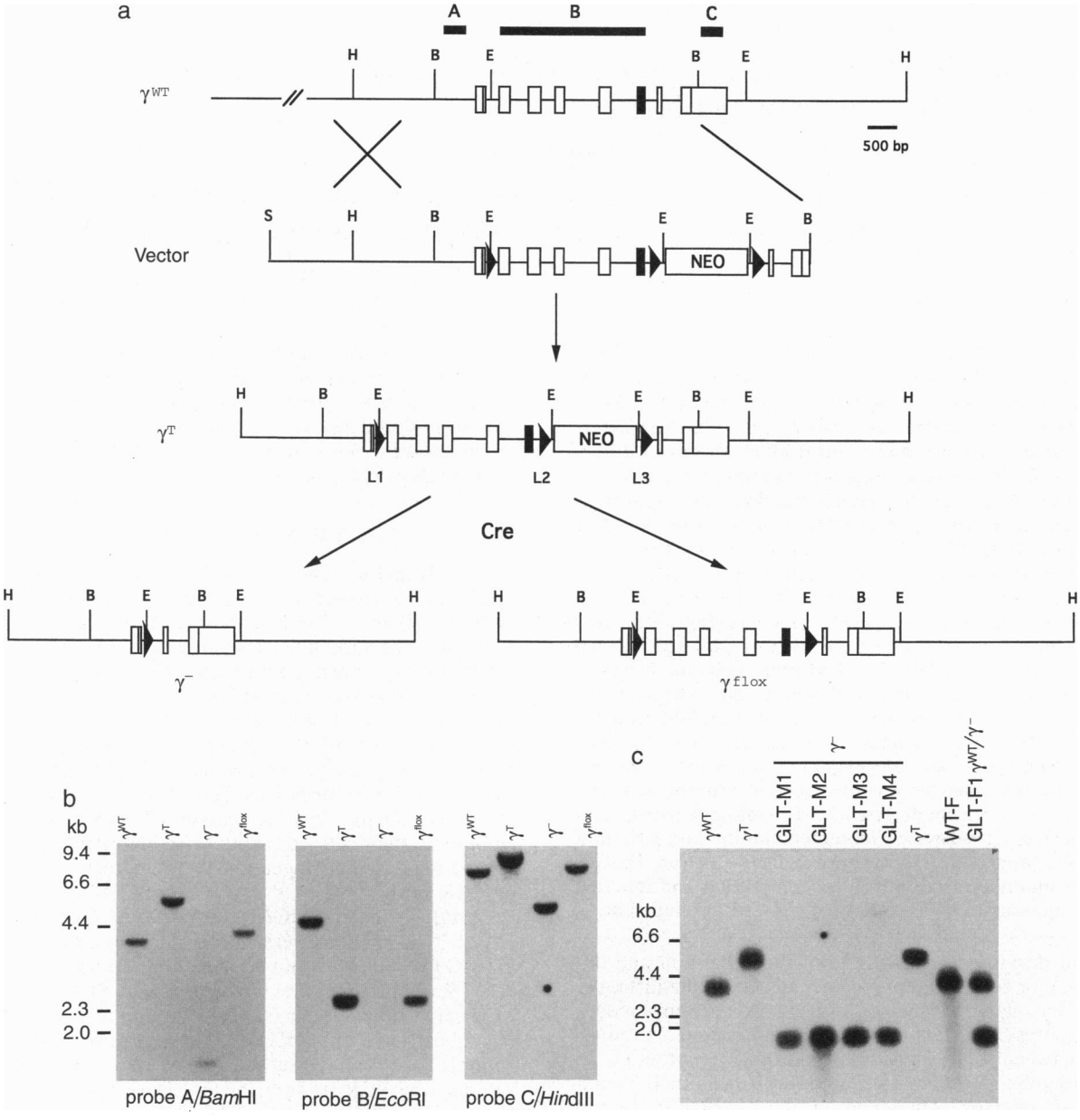

FIG. 1. Targeting of the IL-2Rγ gene. (*a*) Normal, wild-type IL-2Rγ locus (γ^{WT}) is shown with restriction enzyme sites: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sac* I. Exons are indicated by boxes with the transmembrane exon in black. A–C are probes used in Southern analysis. Vector contains *loxP* sites (L1–L3, black triangles) and the neomycin-resistance gene (NEO). γ^{T} is the resultant homologous recombinant. Cre-mediated deletion between L1 and L3 generates the γ^{-} locus, and between L2 and L3, the γ^{flox} locus. (*b*) Southern blot analysis of parental and modified ES clones. (*c*) Southern blot analysis of offspring derived from γ^{-} female chimeras. Germline-transmitted male (GLT-M) and female (GLT-F) as well as control wild-type female (WT-F) DNAs are shown. DNA was digested with *Bam*HI and hybridized with probe A.

periodic acid–Schiff reaction. Intraepithelial lymphocytes were enumerated and expressed as a percentage of epithelial cells (21).

Assays of Lymphocyte Function. Isolated splenocytes and thymocytes were prepared aseptically and depleted of erythrocytes. For thymocyte stimulation, 10^5 cells per well were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum in 96-well plates with or without Con A (2.5 μg/ml) and cytokines (IL-2, IL-4, and IL-7 at 20 ng/ml) for 72 hr. Cells were incubated with 0.5 μCi of [*methyl*-^{3}H]thymidine during the final 6 hr (1 μCi = 37 kBq). Splenic NK activity on ^{51}Cr-labeled YAC target cells was assessed after induction (40 hr) with poly(I-C) (0.2 mg per mouse, i.p.). IL-4-directed isotype switch and measurements of serum immunoglobulin were as described (15, 22).

RESULTS

Generation of ES Cells with a Defined Deletion of the IL-2Rγ Locus. Homologous recombination was used in conjunction with Cre/*loxP* recombination (17, 18) to modify the X chromosome-encoded IL-2Rγ locus. A single *loxP* site (L1) was inserted into intron 1 and a *loxP*-flanked neomycin-resistance cassette (L2*neo*L3) was placed into intron 6 of the IL-2Rγ gene (Fig. 1*a*). After homologous recombination and Cre-mediated deletion, two types of ES subclones were derived. In one type, a deletion between L1 and L3 removed the *neo* gene and the region encompassing exons 2–6 (encoding the extracellular and transmembrane domains; ref. 19) of the IL-2Rγ gene, thus creating a nonfunctional locus (γ^{-}). Southern hybridization confirmed the deletion event (Fig. 1*b*). In addition, an ES cell subclone with a deletion removing only *neo*

and leaving single *loxP* sites in introns 1 and 6 was isolated (Fig. 1 *a* and *b*). This *loxP*-flanked IL-2Rγ locus (γ^{flox}) should not, in principle, inactivate the gene, as the *loxP* sites are intronic. γ^{flox} ES cells serve as an internal control for unanticipated ES cell mutations and also allow for future experiments involving conditional IL-2Rγ gene inactivation (see *Discussion* and ref. 17).

Analysis of Chimeric Animals. Both γ^- and γ^{flox} ES cell clones were injected into CB20 blastocysts to generate chimeric animals. Because E14.1 ES cells differ from CB20 with respect to major histocompatibility complex haplotype and only one X chromosome needs to be inactivated in a male ES cell line, the phenotype caused by the mutation could be directly analyzed in the chimeric mice. No T, B, or NK cells expressing H-2^b (ES-derived) could be detected in the peripheral blood of chimeric animals (n = 8) derived from two different γ^- ES cell subclones (data not shown). Hematopoietic chimerism was documented, since H-2^b-expressing granulocytes were found in the γ^- chimeras. In contrast, chimeras derived from γ^{flox} ES cells generated T, B, and NK cells bearing H-2^b (data not shown). In chimeric animals, the developmental potential of γ^- lymphoid cells might not have been appreciated, however, if γ^+ cells (blastocyst-derived) had a growth advantage. Lymphoid competition has been previously demonstrated in female carriers of SCIDX1, where nonrandom X chromosome inactivation patterns are found with preferential expansion of cells bearing the normal X chromosome (9). Indeed, some degree of IL-2Rγ$^-$ B-cell development had occurred in the chimeric animals, because serum IgM^a (ES-derived), although low, was clearly detectable (γ^- chimeras, average of 12 μg of IgM^a per ml with 644 μg of total IgM per ml; γ^{flox} chimeras, average of 257 μg of IgM^a per ml with 673 μg of total IgM per ml).

IL-2Rγ$^-$ Mice. To fully explore the phenotype associated with the absence of IL-2Rγ, animals carrying the γ^- mutation in the germline were generated from γ^- female chimeras, identified by coat color, and confirmed through Southern analysis (Fig. 1*c*). IL-2Rγ$^-$ males appeared normal at birth and developed as well as their control littermates. For the experiments described below, (129 × CB20)F_1 mice which had been housed under conventional conditions were studied at 3–4 weeks of age.

B-cell generation in the bone marrow follows an ordered developmental pattern and can be partitioned into distinct fractions (A–F) by using cell surface markers CD43, CD45R (B220), heat-stable antigen, BP-1, and surface (s) IgM (23). The pre-B-cell compartment in the bone marrow of IL-2Rγ$^-$ mice (Fig. 2*a*) showed a 20-fold decrease in CD45R(B220)$^+$CD43$^-$ cells (fraction D). This fraction derives from progenitor cells whose growth is dependent on IL-7 (24, 25). Mature, sIgM$^+$sIgD$^+$ B cells were also detected, although in reduced numbers (Fig. 2*a*). Thus, a defect in the expansion of pre-B cells in the bone marrow of γ^- males is present, although this block appears to be incomplete.

The thymi of γ^- mice were also markedly diminished in size and cellularity (22-fold reduction) relative to controls (Table 1). Histologically, both cortical and medullary zones could be demarcated (data not shown). Thymocyte differentiation did not appear to be arrested, as the cells exhibited normal patterns of expression for CD4 and CD8 (Fig. 2*b*) and for CD3 and the T-cell receptor (TCR)αβ heterodimer (data not shown). As expected, γ^- thymocytes did not express cell surface IL-2Rγ molecules (Fig. 2*b*), which can be specifically recognized by the TUGm2 antibody (4). As IL-2Rγ functions in IL-7 signaling, we examined the expression of the IL-7Rα molecule, which can be expressed as a single chain. Interestingly, IL-7Rα expression on Thy-1^{lo} cells in γ^- thymi appeared to be increased (Fig. 2*b*).

The spleens of γ^- males were small and the absolute lymphoid cell number was 8-fold reduced relative to controls (Table 1). Histologically, the clusters of white pulp were diminished in number and size but contained T-cell areas and primary follicles without germinal centers (data not shown). The number of mature, sIgM$^+$sIgD$^+$ B cells was 12-fold

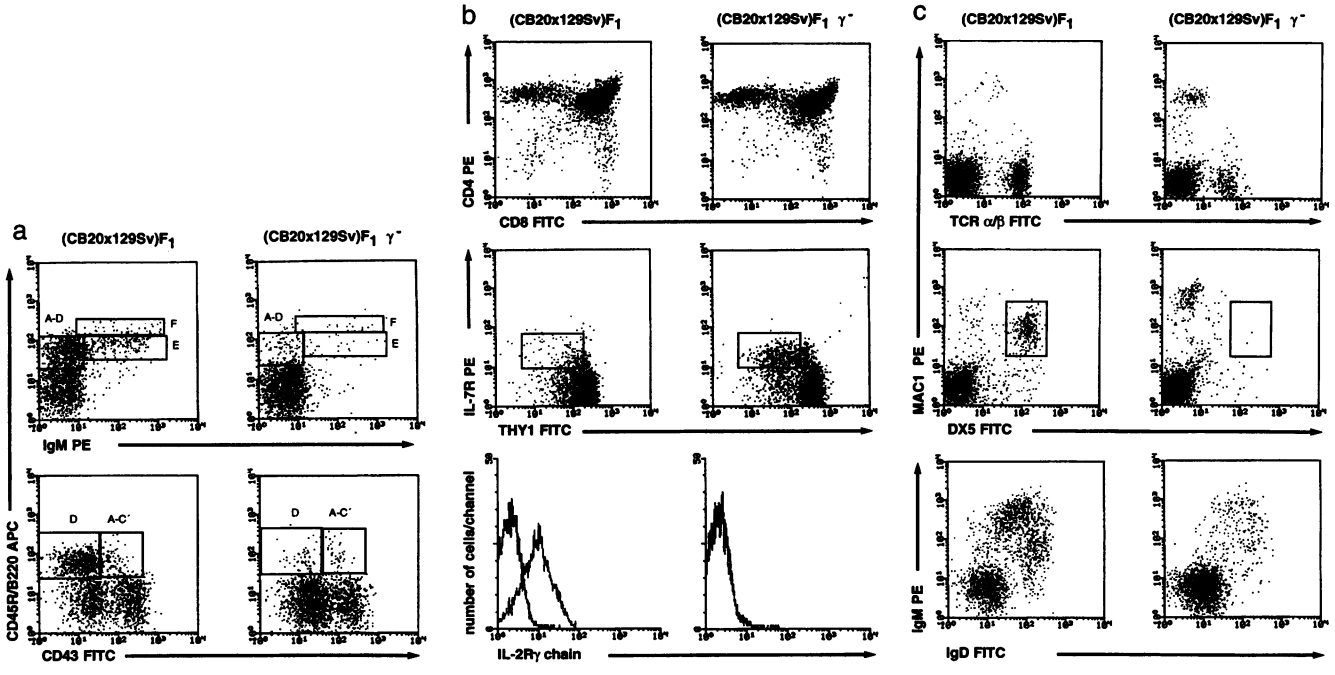

FIG. 2. Flow cytometric analyses of bone marrow, thymus, and spleen from 4-week-old control (*Left*) and IL-2Rγ$^-$ (*Right*) mice. (*a*) Bone marrow stained with phycoerythrin (PE)-anti-IgM and allophycocyanin (APC)-anti-B220 gated on lymphocytes (*Upper*) or with fluorescein isothiocyanate (FITC)-anti-CD43 and APC-anti-B220 gated on IgM$^-$ lymphocytes (*Lower*). (*b*) Thymocytes stained with FITC-anti-CD8 and PE-anti-CD4 (*Upper*), FITC-anti-Thy1 and PE-anti-IL7Rα (*Middle*), or anti-IL-2Rγ followed by biotinylated-anti-rat immunoglobulin and streptavidin-PE (*Lower*). (*c*) Splenocytes stained with PE-anti-Mac1 and either FITC-anti-TCRαβ (*Upper*), FITC-anti-NK (*Middle*), or FITC-anti-IgM and PE-anti-IgD (*Lower*). Boxed areas indicate populations referred to in the text. The Mac1$^+$ cells appear increased due to the decreased lymphoid component in the spleens of IL-2Rγ$^-$ mice.

Table 1. Quantitation of lymphoid cells in IL-2Rγ⁻ and control (wild-type, WT) mice

Mouse strain	Bone marrow cells* Lympho-cytes, %	No. × 10^{-5} A–C′	D	E	F	Thymus cells,† no. × 10^{-6}	Gut IELs,‡ %	Spleen cells,† no. × 10^{-6} Total	Lymphocytes	B	T	NK
IL-2Rγ⁻	44	2.7	2.2	1.2	0.5	11.7 ± 6.6	0.35 ± 0.05	19.2 ± 15.2	8.2 ± 3.2	3.9 ± 1.9	5.1 ± 4.6	0.009 ± 0.001
WT	68	4.8	42	14	3.7	247 ± 33.8	12.8 ± 4.5	84.0 ± 13.8	66.5 ± 10.9	47.3 ± 12.9	17.0 ± 6.3	2.8 ± 0.8

*Fractions A–C′ are CD43⁺B220⁺IgM⁻, D is CD43⁻B220⁺IgM⁻, E is IgM⁺IgD⁻, and F is IgM⁺IgD⁺ cells. Two mice of each strain were analyzed with similar results. For one IL-2Rγ⁻ mouse, fractions A–C′ were found to contain 50% of cells in fraction A and 50% in fractions B and C′, similar to the proportions found in normal mice (23).

†Six mice from each strain were analyzed. B cells are B220⁺, T cells are Thy-1⁺ or TCRαβ⁺, and NK cells are DX5⁺.

‡Intraepithelial lymphocytes are expressed as a percentage of epithelial cells. Results are from four mice of each strain.

reduced (Fig. 2*c*). We noted a decrease in B-cell numbers in the spleen of older (>6 weeks old) IL-2Rγ⁻ mice (unpublished observations). Interestingly, mature T cells bearing TCRαβ were also detected in the spleen of γ⁻ mice, although 3-fold reduced relative to in controls (Fig. 2*c*; Table 1). NK cells, identified by the DX5 mAb, were ≈350-fold reduced. Small lymph nodes were detectable in γ⁻ mice but contained 10-fold fewer lymphoid cells than controls (data not shown).

We also examined the gut-associated lymphoid tissue. Peyer's patches were not detected in IL-2Rγ⁻ mice. In conventional mice, the gut epithelium contains CD3/TCR-bearing lymphocytes of thymus-dependent and -independent origins (26). These populations were 40-fold decreased in γ⁻ mice (Table 1). A lymphoid origin for the few cells detected between the epithelial crypts has not been ascertained. Lymphocytes in the lamina propria showed a similar reduction, and plasma cells were exceptionally rare (Fig. 3).

Functional Analysis of IL-2Rγ⁻ Lymphocytes. The function of cytokine receptors normally containing IL-2Rγ was tested in T and B cells of the mutant mice. Wild-type thymocytes proliferate when cultured *in vitro* with mitogens, and this response can be augmented by the addition of exogenous lymphokines. IL-2Rγ⁻ thymocytes failed to respond to Con A, and this defect could not be overcome by the addition of IL-2, IL-4, or IL-7 (Table 2). Splenic T cells were also not responsive to Con A with or without lymphokines (data not shown). B-cell immunoglobulin isotype switch *in vitro* was tested after exposure to bacterial lipopolysaccharide (LPS) with or without IL-4. IgG1⁺ and IgG3⁺ cells were present in LPS-treated cultures from IL-2Rγ⁻ splenocytes, at levels similar to controls. However, addition of IL-4 failed to increase the percentage of IgG1⁺ cells (with a decrease in IgG3⁺ cells), whereas control cultures responded appropriately (Table 3). Sera from IL-2Rγ⁻ mice contain IgM (γ⁻ mice, 153 μg of IgM per ml; control mice, 483 μg of IgM per ml) but not IgE (γ⁻ mice, <0.05 μg of IgE per ml; control mice, 4 μg of IgE per ml). A complete analysis of immunoglobulin isotypes in γ⁻ mice was not performed, because of the presence of maternal antibody in the animals. In the case of IgG1, small amounts of IgG1^{a} (endogenous) could occasionally be detected in mutant mice older than 4 weeks. Activity against the NK-sensitive YAC target cell line could not be detected in IL-2Rγ⁻ mice [γ⁻ mice, background level of specific lysis (3%) at an effector/target cell ratio of 100:1; control mice, 65% specific lysis].

DISCUSSION

IL-2Rγ is a component of the receptors for IL-2, IL-4, IL-7, and IL-15 (3–8), and its absence would be expected to perturb lymphoid development at multiple stages. Indeed, we find that overall lymphopoiesis in IL-2Rγ⁻ mice is reduced. However, small numbers of mature peripheral B and T cells can be detected. The role of cytokines in the development of these cells is not known. IL-2 and IL-15 receptors have an absolute requirement for IL-2Rγ for ligand binding and signaling (3, 8). Although IL-4Rα or IL-7Rα can bind cytokine, it is unclear whether these receptors can signal in the absence of IL-2Rγ *in vivo*. The failure to detect IgE in γ⁻ mice strongly suggests that IL-2Rγ is required for proper IL-4R function, despite the residual IL-4-binding capability of IL-4Rα. It is possible that IL-7Rα may signal, albeit inefficiently, without IL-2Rγ. The up-regulation of this chain on γ⁻ thymocytes could allow for signal transduction. Alternatively, activation via other cytokine receptors (not containing IL-2Rγ) or other membrane molecules may allow for the partial lymphopoiesis observed.

IL-2Rγ⁻ mice do not possess NK cells and exhibit a substantial decrease in gut-associated lymphoid cells. Gut lymphocytes are the progeny of clones emerging from the Peyer's patches (thymus-dependent lineage) or may directly arise in the gut microenvironment (thymus-independent lineage) (26, 27). The more severe reduction in gut-associated lymphocytes and NK cells compared with splenic T and B cells in IL-2Rγ⁻ mice may reflect differential cytokine requirements of these lymphoid subpopulations. Our results suggest that the gener-

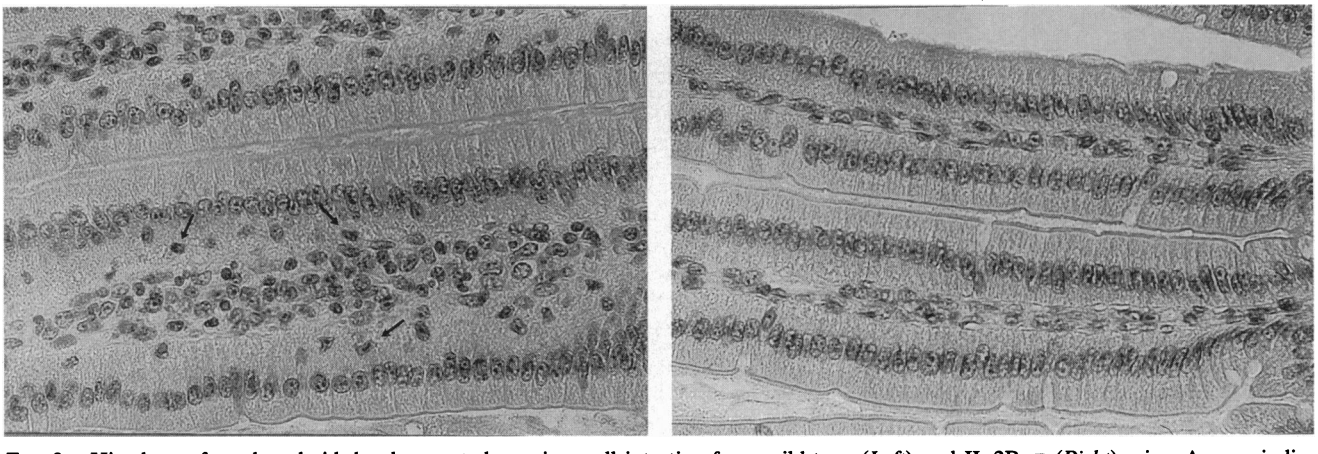

FIG. 3. Histology of gut lymphoid development shown in small intestine from wild-type (*Left*) and IL-2Rγ⁻ (*Right*) mice. Arrows indicate intraepithelial lymphocytes in the control mouse. Note the overall decrease in cellularity of the lamina propria in the IL-2Rγ⁻ mouse. (×150.)

Table 2. Con A-stimulated proliferation of thymocytes

Mouse strain	[^{3}H]Thymidine incorporation, cpm × 10^{-3}			
	Medium	+ IL-2	+ IL-4	+ IL-7
Wild type	62.0	260.0	210.0	190.0
IL-2Rγ$^-$ no. 1	0.40	0.95	0.88	1.60
IL-2Rγ$^-$ no. 2	0.75	0.51	0.68	0.33

Data from a representative experiment are shown as cpm of triplicate wells. Background incorporation in the absence of Con A was <200 cpm for all mice tested. Similar results were obtained with two additional mice from each strain.

ation and/or maintenance of gut lymphoid and NK cells depends on cytokines which require IL-2Rγ for binding (such as IL-2 and IL-15 in combination).

The phenotypes observed in IL-2Rγ$^-$ mice and humans differ in interesting ways. The peripheral blood of SCIDX1 patients contains B cells which produce IgM but fail to undergo isotype switching (9, 10). Peripheral T cells are absent or markedly decreased, and those present are nonfunctional (9). NK cells are less well characterized in SCIDX1 patients, although some patients have been reported to lack these cells (10). Previous histological studies of thymi from a heterogeneous group of SCID patients demonstrated a spectrum of abnormalities ranging from thymic dysplasia to lymphoid atrophy (28). Additional analyses of thymic and splenic specimens and NK-cell activity from IL-2Rγ$^-$ patients are required. Like SCIDX1 patients, IL-2Rγ$^-$ mice have reduced numbers of lymphoid cells in the peripheral blood. In contrast, small numbers of nonfunctional T cells are present in the thymus and spleen. NK cells and activity are completely absent. B cells from IL-2Rγ$^-$ mice make IgM but fail to produce IgE *in vivo*. However, both IgG1$^+$ and IgG3$^+$ cells are found in *in vitro* cultures of IL-2Rγ$^-$ splenocytes, suggesting that immunoglobulin isotype switch is not entirely defective. The ability of IL-2Rγ$^-$ mice to generate immunoglobulin subclasses after *in vivo* antigen exposure awaits further studies. Thus, absence of IL-2Rγ appears to block T-cell development to a greater extent in humans than in mice. This may reflect a species-specific difference in the function of particular cytokine receptors (partial function of the murine receptor versus no function for the human equivalent). In contrast, B cells are decreased in IL-2Rγ$^-$ mice but apparently not in humans, although B-cell function is defective in both species.

γ$^-$ and γ^{flox} mouse strains provide a starting point for additional studies. Complementation analysis of γ$^-$ mice with mutant IL-2Rγ transgenes provides a means to dissect the structure/function of this molecule *in vivo*. A potential therapy for SCIDX1 involves gene transfer of the IL-2Rγ gene into hematopoietic progenitors. The feasibility, efficacy, and safety of this approach can be validated with IL-2Rγ$^-$ mice. γ^{flox} mice will allow for *in vivo* assessment of IL-2Rγ function in particular cell lineages, through matings with transgenic mouse strains which express Cre in a cell type-specific fashion (17). This approach, using promoters specific for gut lymphoid or NK cells, will provide a system to determine the role of these cells in immune responses.

Table 3. Isotype switch by splenocytes

Mouse strain	IgG3$^+$ cells, %		IgG1$^+$ cells, %	
	LPS alone	LPS + IL-4	LPS alone	LPS + IL-4
Wild type	31 ± 2.8	3.6 ± 1.6	6.2 ± 1.8	48 ± 16
IL-2Rγ$^-$	34 ± 1.4	33 ± 2.0	9.7 ± 4.3	8.8 ± 3.4

Four mice from each strain were analyzed. Percentage of IgG3$^+$ or IgG1$^+$ (switched) cells was calculated as number of switched cells divided by the sum of switched cells plus IgM$^+$ cells in each culture.

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1. Kincade, P. W. (1991) *Semin. Immunol.* **3,** 379–390.
2. Ritter, M. A. & Boyd, R. L. (1993) *Immunol. Today* **14,** 462–469.
3. Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. & Sugamura, K. (1992) *Science* **257,** 379–382.
4. Kondo, K., Takeshita, T., Ishii, N., Nakamura, M., Watanabe, S., Arai, K.-I. & Sugamura, K. (1993) *Science* **262,** 1874–1877.
5. Noguchi, M., Nakamura, Y., Russel, S. M., Ziegler, S. F., Tsang, M., Cao, X. & Leonard, W. J. (1993) *Science* **262,** 1877–1880.
6. Kondo, K., Takeshita, T., Higuchi, M., Nakamura, M., Sudo, T., Nishikawa, S.-I. & Sugamura, K. (1993) *Science* **263,** 1453–1454.
7. Russel, S. M., Keegan, A. D., Harada, N., Nakamura, Y., Noguchi, M., Leland, P., Friedman, M. C., Miyajima, A., Puri, R. K., Paul, W. E. & Leonard, W. J. (1993) *Science* **262,** 1880–1882.
8. Giri, J. G., Ahdieh, M., Eisenman, J., Shanebeck, K., Grabstein, K., Kumaki, S., Namen, A., Park, L. S., Cosman, D. & Anderson, D. (1994) *EMBO J.* **13,** 2822–2830.
9. Conley, M. E. (1991) *Clin. Immunol. Immunopathol.* **61,** 94–99.
10. Gougeon, M.-L., Drean, G., Le Deist, F., Dousseau, M., Fevrier, M., Diu, A., Theze, J., Griscelli, C. & Fischer, A. (1990) *J. Immunol.* **145,** 2873–2879.
11. Noguchi, M., Huafang, Y., Rosenblatt, H. M., Fillpovich, A. H., Adelstein, S., Modl, W. S., McBride, O. W. & Leonard, W. J. (1993) *Cell* **73,** 147–157.
12. Grabstein, K., Waldschmidt, T. J., Finkelman, F. D., Hess, B. W., Alpert, A. R., Boiani, N. E., Namen, A. E. & Morrissey, P. J. (1993) *J. Exp. Med.* **178,** 257–264.
13. Sudo, T., Nishikawa, S., Ohno, N., Akiyama, N., Tamakoshi, M., Yoshida, H. & Nishikawa, S.-I. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9125–9129.
14. Schorle, H., Holtschke, T., Hünig, T., Schimpl, A. & Horak, I. (1991) *Nature (London)* **352,** 621–624.
15. Kühn, R., Rajewsky, K. & Müller, W. (1991) *Science* **254,** 707–710.
16. Sadlack, B., Kühn, R., Schorle, H., Rajewsky, K., Müller, W. & Horak, I. (1994) *Eur. J. Immunol.* **24,** 281–284.
17. Gu, H., Marth, J. D., Orban, P. C., Mossman, H. & Rajewsky, K. (1994) *Science* **265,** 103–106.
18. Gu, H., Zou, Y.-R. & Rajewsky, K. (1993) *Cell* **73,** 1155–1164.
19. DiSanto, J. P., Certain, S., Wilson, A., MacDonald, H. R., Avner, P., Fischer, A. & de Saint Basile, G. (1994) *Eur. J. Immunol.* **24,** 3014–3018.
20. Kühn, R., Löhler, J., Rennick, D., Rajewsky, K. & Müller, W. (1993) *Cell* **75,** 263–274.
21. Rocha, B., Vassalli, P. & Guy-Grand, D. (1994) *J. Exp. Med.* **180,** 681–686.
22. Radbruch, A., Müller, W. & Rajewsky, K. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3954–3957.
23. Löffert, D., Schaal, S., Ehlich, A., Hardy, R. R., Zou, Y.-R., Müller, W. & Rajewsky, K. (1994) *Immunol. Rev.* **137,** 135–153.
24. Era, T., Ogawa, M., Nishikawa, S., Okamoto, M., Honjo, T., Akagi, K., Miyazaki, J. & Yamamura, K. (1991) *EMBO J.* **10,** 337–342.
25. Hardy, R. R., Carmack, C. E., Shinton, S. A., Kemp, J. D. & Hayakawa, K. (1991) *J. Exp. Med.* **173,** 1213–1225.
26. Guy-Grand, D., Cerf-Bensussan, N., Malissen, B., Malassis-Seris, M., Briottet, C. & Vassalli, P. (1991) *J. Exp. Med.* **173,** 471–481.
27. Guy-Grand, D., Malassis-Seris, M., Briottet, C. & Vassalli, P. (1991) *J. Exp. Med.* **173,** 1549–1552.
28. Borzy, M. S., Schulte-Wisserman, H., Gilbert, E., Horowitz, S. D., Pellet, J. & Hong, R. (1979) *Clin. Immunol. Immunopathol.* **12,** 31–51.