Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 8464–8468, September 1993
Immunology

Characterization of cDNAs encoding the murine interleukin 2 receptor (IL-2R) γ chain: Chromosomal mapping and tissue specificity of IL-2R γ chain expression

XIQING CAO*, CHRISTINE A. KOZAK†, YUE-JIANG LIU*, MASAYUKI NOGUCHI*, ERRIN O'CONNELL‡, AND WARREN J. LEONARD*

*Section on Pulmonary and Molecular Immunology, Office of the Director, Intramural Research Program, National Heart, Lung, and Blood Institute, Bethesda, MD 20892; and †Laboratory of Molecular Microbiology and ‡Laboratory of Cellular and Molecular Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892

Communicated by Ray D. Owen, June 8, 1993

ABSTRACT **The IL-2R γ chain (IL-2Rγ) is an essential component of high- and intermediate-affinity IL-2Rs, playing critical roles for ligand binding and internalization. Recently, our laboratory has demonstrated that IL-2Rγ mutation results in X chromosome-linked severe combined immunodeficiency in humans, suggesting that IL-2Rγ plays a vital role in thymic maturation of human T cells. We now report the isolation and characterization of cDNAs encoding murine IL-2Rγ. The open reading frame encodes 369 aa, identical in length to that encoded by the human IL-2Rγ cDNA. Murine IL-2Rγ and human IL-2Rγ have 69% and 70% identity at the nucleotide and amino acid levels, respectively. As expected, the murine IL-2Rγ retains the WSXWS motif and four cysteine residues characteristic of cytokine receptor superfamily members. IL-2Rγ mRNA distribution shows significant tissue specificity, with particularly high-level expression in spleen and thymus, and higher expression in single-positive ($CD4^+8^-$ or $CD4^-8^+$)-enriched thymocytes than in double-negative ($CD4^-8^-$) thymocytes. Finally, we have localized the murine IL-2Rγ gene, *Il2rg*, to the X chromosome between *Rsvp* and *Plp* and demonstrated that a defect in IL-2Rγ is not responsible for the X chromosome-linked *xid* mutation, which maps to this same region. The cloning of the murine IL-2Rγ cDNA will facilitate the investigation of the role of this protein in lymphocyte function and thymic development.**

Interleukin 2 (IL-2) and IL-2Rs critically regulate the magnitude and duration of the T-cell immune response after antigen activation (for review, see refs. 1–3). Three classes of IL-2Rs exist. Resting lymphocytes express intermediate-affinity IL-2Rs, whereas activated lymphocytes express high- and low-affinity IL-2Rs (1–3). The high- and intermediate-affinity IL-2Rs can transduce IL-2 signals, but the low-affinity IL-2Rs cannot (1–3). Different combinations of three chains, denoted the α (4, 5), β (6–12), and γ (13–17) chains, form these three classes of IL-2Rs. Low-affinity IL-2Rs contain IL-2Rα, but not IL-2Rβ or IL-2Rγ; intermediate-affinity IL-2Rs contain IL-2Rβ and IL-2Rγ chains, but not IL-2Rα; and high-affinity IL-2Rs contain all three chains. Thus, IL-2Rβ and IL-2Rγ are components of high and intermediate IL-2Rs, the receptors capable of transducing IL-2-mediated proliferative and cytolytic signals. IL-2Rβ has been extensively characterized, but studies of IL-2Rγ are more limited.

Human IL-2Rα (4, 5), IL-2Rβ (12), and IL-2Rγ (15) cDNAs have been isolated, but murine cDNAs have only been identified for IL-2Rα (18, 19) and IL-2Rβ (20). We now report the cloning of the murine IL-2Rγ cDNA, its chromosomal mapping, and characterization of IL-2Rγ mRNA expression in different tissues.§ This represents an important step to facilitating the study of the functional and developmental roles of IL-2Rγ. Such studies are particularly important in view of recent data indicating that mutations of IL-2Rγ in humans result in X chromosome-linked severe combined immunodeficiency (XSCID) (21), a disease characterized by absent or profoundly diminished T cells and severely depressed cell-mediated and humoral immunity (for review, see refs. 22 and 23).

MATERIAL AND METHODS

Oligonucleotides and DNA Sequence Analysis. Oligonucleotides were synthesized on an Applied Biosystems model 392 DNA/RNA synthesizer and are summarized in Table 1. All DNA sequencing was performed by the dideoxynucleotide chain-termination method, using Sequenase Version 2.0 (United States Biochemical).

Hybridization Probe and PCR Conditions. Primers γ3 and γ10 corresponding to the human IL-2Rγ sequence (see Table 1) were used to amplify ≈2 μg of DNA derived from a cDNA library prepared from murine 32D myeloid progenitor cells (24). These cells are capable of responding to IL-2 after transfection of IL-2Rβ (25) and, therefore, were assumed to express endogenous murine IL-2Rγ. PCR amplification was performed with 30 cycles of 94°C, 50°C, and 72°C, each for 1 min. The resulting 660-bp fragment was isolated and designated as the γ3-10 PCR probe.

Identification of a Full-Length IL-2Rγ cDNA. A cDNA library in λgt10 prepared from mRNA from Con A-activated murine splenocytes (Clontech, 3×10^5 clones) was initially screened with the γ3-10 PCR probe labeled with ^{32}P by nick translation. Duplicate nitrocellulose filters were hybridized in Nitrohyb (Digene Diagnostics, Silver Spring, MD) solution overnight at 42°C and washed under high-stringency conditions (twice in 2× SSPE/0.1% SDS at room temperature for 15 min and once in 0.1× SSPE/0.1% SDS at 56°C for 30 min; 1× SSPE = 0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA). The insert from one clone, denoted 9E, was subcloned into pBluescript II SK(+) and sequenced using Sp6 and T7 primers. The insert was 798 bp long and had 60% sequence identity to the human cDNA. This partial-length cDNA was used to screen another murine cDNA library in the *Bst*XI site of pcDNAI (Invitrogen) prepared from Con A-activated splenocytes from CBA/Ca mice. Five clones were identified, and combination of two of these clones yielded the full-length cDNA sequence. Initial sequencing

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IL-2, interleukin 2; IL-2R, IL-2 receptor; XSCID, X chromosome-linked severe combined immunodeficiency.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L20048).

Table 1. Oligonucleotides

Name	Sequence
γ3	5'-GCACTTGGAACAGCAGCTCTGAG-3'
γ10	5'-GATCCTCTAGGTTCTTCAGGG-3'
	Top-strand sequencing primers
Mγ1	5'-GCACCATGTTGAAACTATTATTG-3'
MG2	5'-GGTATCTGATAATAATACATTC-3'
MG7	5'-TCTGCCACGTCAGCGAGATTC-3'
MG8	5'-GCTAGAGCTGAGATGGAAAAG-3'
MG9	5'-CCTAAGTGACGCTAACCTCCCC-3'
MG10	5'-CATACTGTAGAGGAGAATCCTTCC-3'
MG11	5'-GGCTGGGAGCTCACTATGTAG-3'
	Bottom-strand sequencing primers
X7	5'-CCTCAGCAGGAGCAGCTGAAGG-3'
Int B	5'-TGGGAGCACTGAGGTGTTCAGGGG-3'
Int 1	5'-CAGCCAGAAGTAATCTCTTTGG-3'
Int 2	5'-GTAAACAGCGTTCTTTAATATGTCTG-3'
Int 3	5'-AATCAACCCCATGGTGCCAAC-3'
Int 5	5'-CACTTAGGACTATAGGACTTTGAGG-3'
Int 6	5'-GTAGTCTGGCTGCAGACTCTCAG-3'
Mγ4C	5'-GCAGGGAAAGAGGGCAAGGGAC-3'
MG6C	5'-AGGGAGGGAGTATCCTATAAAAG-3'

was performed with the γ3 and γ10 primers; subsequent primers (Table 1) were synthesized as needed on the basis of newly determined sequence. Both strands were completely sequenced. Analysis of the sequence was performed using DNA STRIDER and GENEWORKS for Macintosh.

Cell Preparation, RNA Extraction, and Northern Blot Analyses. RNA was isolated from spleens of both 7- to 8-week-old male CBA/N mice carrying the *xid* mutation (CBA/CaHN-*xid*/J mice, The Jackson Laboratory) and their normal counterparts (CBA/CaH-T6/J, The Jackson Laboratory). In addition, unfractionated thymocytes, single-positive ($CD4^+$ or $CD8^+$)-enriched thymocytes, and double-negative thymocytes were isolated from 7-week-old C57BL/6 female mice. The single-positive-enriched fraction was prepared by complement-mediated killing with J11d (anti-HSA) monoclonal antibody (mAb) (26) and thus contains a minor population (<5%) of α/β, T-cell-receptor-bearing double-negative cells. The double-negative ($CD4^-/CD8^-$) thymocytes prepared by complement-mediated killing with 3-155 (anti-CD8) (27) and RL172 (anti-CD4) (28) mAbs; this population contains mainly immature double-negative cells but also contains ≈10% mature γ/δ and α/β double-negative cells (29).

RNA was isolated by using guanidine isothiocyanate followed by centrifugation through a cesium chloride gradient. Total RNA (10 μg) was separated on formaldehyde/1% agarose gels and transferred to nitrocellulose membranes (Schleicher & Schuell). Blots were hybridized with ^{32}P-labeled cDNA insert and washed under high-stringency conditions. Levels of RNA loaded on the gel were controlled by hybridization with pHe7, a cDNA whose expression is constant over a wide range of cells and activation states (30).

A mouse multiple tissue Northern blot (Clontech) containing poly(A)$^+$ RNA at 2 μg per lane was also hybridized with the murine IL-2Rγ cDNA probe and washed under high-stringency conditions, as described above.

Chromosomal Mapping. DNA from the progeny of two multilocus crosses were analyzed for restriction endonuclease polymorphisms of IL-2Rγ. For the first cross, NFS/N or C58/J females were mated with *Mus mus musculus* males and the F_1 progeny were mated with *M. m. musculus* (31). For the second cross, NFS/N females were mated with *Mus spretus* males and the F_1 female progeny were mated with *M. spretus* or C58/J (32). DNA (10 μg) was digested with restriction enzymes, separated on 0.4% agarose gels, transferred to Hybond-N$^+$ nylon membranes (Amersham), and hybridized with a ^{32}P-labeled murine IL-2Rγ probe. Restriction fragment length polymorphisms were detected using *Sst* I in the *M. spretus* cross; 13 restriction enzymes failed to identify polymorphic fragments in the *M. m. musculus* cross.

The progeny of the *M. spretus* cross were also typed for the markers *Plp* (proteolipid protein), *Zfx* (zinc-finger protein, X chromosome-linked), and *Rsvp* (red-sensitive visual protein). *Plp* and *Rsvp* were typed as described (33). *Zfx* was typed using a probe obtained from ATCC after digestion with *Eco*RI. Recombination between loci was calculated as r/n and standard error was calculated as $[(1 - r/n)/n]^{1/2}$, as described (34), where r is the number of recombinants and n is the total number of progeny.

RESULTS

Isolation of Murine IL-2Rγ cDNA Clones and Comparison of Murine and Human IL-2Rγ Sequences. We screened two libraries to obtain a full-length murine IL-2Rγ cDNA. The DNA and deduced amino acid sequences for the murine IL-2Rγ chain are shown in Fig. 1*A*; a schematic of the cDNA is shown in Fig. 1*B*. The full-length murine cDNA sequence was 1608 bp (Fig. 1*A*). The 5' untranslated region is 24 bp; the 3' untranslated region is 474 bp (not including the stop codon) and contains a canonical polyadenylylation signal (underlined). A comparison of the murine and human amino acid sequences is shown in Fig. 2. The mouse and human IL-2Rγ cDNAs are 69% identical at the DNA level (80% identical in the coding region) (data not shown) and 70% identical at the amino acid level (71% if one excludes the signal peptide) (Fig. 2). Both are encoded as 369-aa proproteins and have N-terminal hydrophobic signal peptides. The murine IL-2Rγ signal-peptide cleavage site is not certain but, perhaps, is most likely to occur between Ala-21 and Gly-22, based on the weight matrix algorithm of von Heijne (35). In the sequences from five clones, we have found an apparent nucleotide polymorphism, C or T at nt 183; in either case a proline is still encoded.

Analogous to all cytokine receptor superfamily members (36) including IL-2Rγ, the murine IL-2Rγ protein conserves four cysteines and a WSXWS motif (Fig. 1). Six N-linked glycosylation sites (Asn-Xaa-Ser/Thr) are found in the extracellular domain. Although the overall sequence is well conserved, it is most divergent at the N terminus. Of the first 31 aa of the predicted mature protein (i.e., after signal peptide cleavage), only 10 of 31 aa are identical. In contrast, the cytoplasmic tail is the most highly conserved region (82% amino acid identity). Interestingly, however, although the human IL-2Rγ cytoplasmic domain has a region of limited homology to the fourth and fifth Src homology region 2 (SH2) subdomains (15), this region is less well conserved in mouse (in particular, Arg-289 and Thr-292 in human IL-2Rγ are conserved in Lck, Hck, Lyn, and Blk, but not in murine IL-2Rγ). A "leucine-zipper"-like motif was noted to exist in the human IL-2Rγ sequence (formed by Leu-165, -172, -179, and -186), although no clear functional role for this was demonstrated (15). In the murine sequence, Leu-165 and -172 are conserved, Leu-179 is replaced by Ile, and the final Leu is at position 187 instead of 186. The lack of rigorous conservation in the murine sequence of the limited SH2 subdomain homology and leucine-zipper-like regions tends to lessen the probability that they play critical roles; however, direct experiments are required to clarify this issue.

Genomic Southern Blot Analysis and Chromosomal Mapping. We have recently characterized the human IL-2Rγ gene and found that it consists of eight exons and seven introns, spanning ≈4.2 kb (37). Consistent with this relatively small size, it is noteworthy that the entire murine IL-2Rγ gene appears to be contained on a single 4.0-kb *Eco*RI fragment (Fig. 3*A*). This simple pattern is most consistent with its being encoded by a single-copy gene. We have confirmed this by

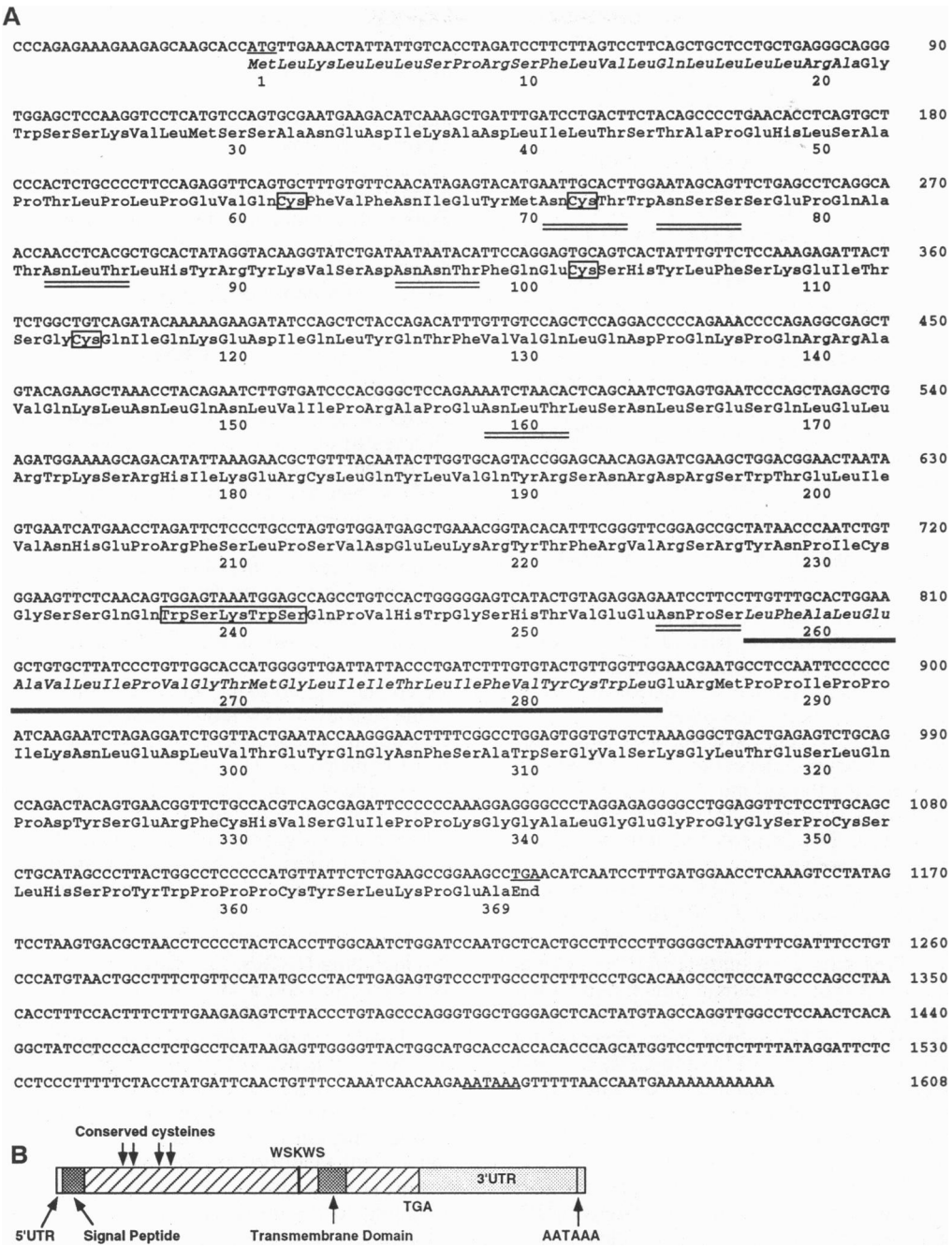

FIG. 1. Sequence of the murine IL-2Rγ cDNA. (*A*) DNA and deduced amino acid sequence. The putative signal peptide and transmembrane domain are in italics; the transmembrane domain is additionally underscored with a heavy bar. The four conserved cysteines and the WSXWS motif are boxed. The ATG start codon, TGA stop codon, and AATAAA polyadenylylation signal are underlined. Each N-linked glycosylation consensus motif (Asn-Xaa-Ser/Thr) is double underlined. (*B*) Schematic representation of domains of the murine IL-2Rγ cDNA.

chromosomal mapping studies using Southern blot analysis of DNAs derived from progeny of two multilocus crosses. *Sst* I digestion identified IL-2Rγ-reactive fragments of 3.0 kb in *M. spretus* and 4.2 kb in NFS/N (Fig. 3*B*). Inheritance of these fragments was consistent with X chromosome linkage, and analysis of other X chromosome-linked markers in this same cross positioned the gene for IL-2Rγ, *Il2rg*, ≈3.3 centimorgans distal to *Zfx* with gene order *Rsvp–Zfx–Il2rg–Plp* (Table 2). This assignment is consistent with the human IL-2Rγ map location at Xq13 (21).

The Murine X Chromosome-Linked Immunodeficiency *xid* Is Not Due to a Mutation in IL-2Rγ. Since IL-2Rγ mutations in humans result in XSCID, it was noteworthy that the genetic locus for a murine immunodeficiency, *xid*, has been mapped to the same general region as *Il2rg*. However, *xid* is not the result of an IL-2Rγ mutation based on three lines of evidence. (*i*) No differences were found by genomic Southern blot analysis with multiple enzymes of DNAs derived from CBA/CaH-T6/J vs. CBA/CaHN-*xid*/J mice (Fig. 4*A*). (*ii*) Both of these strains of mice have identically sized and similar levels of IL-2Rγ mRNA (Fig. 4*B*). (*iii*) When IL-2Rγ mRNA was amplified by reverse transcriptase-mediated PCR and then subjected to DNA sequencing, no differences were found in the *xid* IL-2Rγ sequence (data not shown).

IL-2Rγ mRNA Is Expressed Preferentially in Lymphoid Tissue. We next investigated the tissue distribution of IL-2Rγ mRNA expression (Fig. 5). Of the tissues examined, highest levels of IL-2Rγ mRNA expression were found in spleen

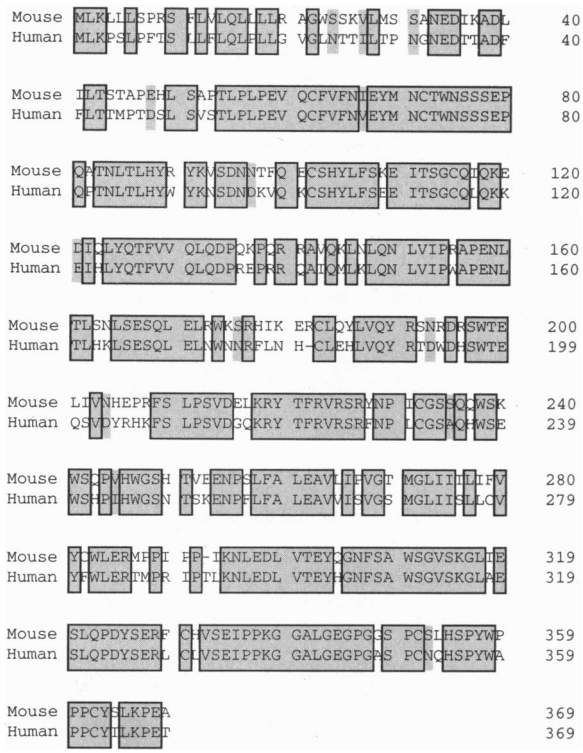

FIG. 2. Alignment of the mouse and human IL-2Rγ amino acid sequences. Identical residues are boxed and shaded. Residues with similar but not identical properties are shaded. Dashes represent inserted gaps to maximize the alignment.

(Fig. 5*A*). Modest IL-2Rγ hybridization was found using mRNA derived from lung, and very low levels were found in heart, skeletal muscle, and kidney; we cannot exclude the possibility that these weaker signals in part resulted from the presence of blood cells within these organs. In addition, we examined IL-2Rγ mRNA expression in thymocytes. IL-2Rγ was expressed in double-negative thymocytes, single-positive-enriched thymocytes, and unfractionated thymocytes (Fig. 5*B*), with highest expression in the single-positive-enriched population. Although the single-positive population

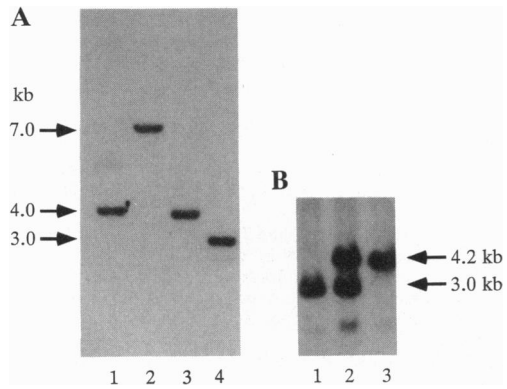

FIG. 3. IL-2Rγ is encoded by a single-copy gene. (*A*) Southern blot hybridization of mouse genomic DNA. DNA was isolated from splenocytes from CBA/CaH-T6/J mice, digested with *Eco*RI (lane 1), *Hin*dIII (lane 2), *Bam*HI (lane 3), and *Sst* I (lane 4), and then electrophoresed in a 1% agarose gel. The DNA was transferred to nitrocellulose membranes and hybridized with the radiolabeled IL-2Rγ cDNA. Size markers are indicated on the left. (*B*) Southern blot analysis of *Sst* I-digested DNAs extracted from the progeny of the cross (NFS/N × *M. spretus*) × C58/J and hybridized to a 798-bp IL-2Rγ probe. Lanes: 1 and 3, male progeny; 2, female. The 4.2- and 3.0-kb bands are indicated.

Table 2. Segregation of *Il2rg* with *Zfx*, *Rsvp*, and *Plp* in the cross (NFS/N × *M. spretus*) × *M. spretus* or C58/J

Mice	Inheritance of parental alleles				Mice, no.
	Rsvp	*Zfx*	*Il2rg*	*Plp*	
Nonrecombinant	+	+	+	+	36
	−	−	−	−	40
Single recombinant	+	+	+	−	5
	−	−	−	+	4
	+	+	−	−	0
	−	−	+	+	3
	+	−	−	−	1
	−	+	+	+	3

Mice were scored as heterozygous (+) or homozygous (−). Recombination frequencies and standard errors in 92 mice are as follows: *Rsvp* – 4.3 ± 2.1% – *Zfx* – 3.3 ± 1.9% – *Il2rg* – 9.8 ± 3.1% – *Plp*.

is contaminated by some (<5%) double-negative thymocytes, in comparison to the double-negative population (lane 1), we can conclude the single-positive cells on average express more IL-2Rγ than the double-negative cells. By densitometric analysis, normalizing RNA loading to the intensity of the pHe7 hybridization signals, the relative ratios of IL-2Rγ mRNA are 1.0, 4.6, and 1.9, respectively, for the double-negative, single-positive, and unfractionated thymocyte populations shown in Fig. 5*B*.

DISCUSSION

The IL-2R is known to consist of three proteins that contribute to ligand binding. IL-2Rα is different from all other cytokine receptor proteins, whereas both IL-2Rβ and IL-2Rγ are cytokine receptor superfamily members. IL-2Rα cannot transduce IL-2 signals but augments ligand binding affinity. Both IL-2Rβ and IL-2Rγ must be present to allow transduction of IL-2 signals. With this manuscript, all three principal IL-2R chains have now been cloned in both humans and mice. Comparing murine and human sequences at the amino acid levels, the percent identities for IL-2Rα, IL-2Rβ, and IL-2Rγ are 62, 58, and 70%, respectively, making IL-2Rγ the most conserved of the three chains. The region of highest conservation between human and mouse IL-2Rγ proteins is the cytoplasmic domain, suggesting the important role this region plays in IL-2Rγ function and consistent with the observation that deletion of as few as 62 aa of the human IL-2Rγ cytoplasmic domain was sufficient to result in XSCID (21).

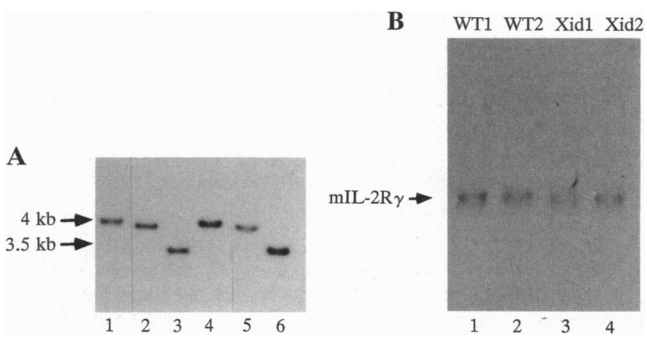

FIG. 4. *xid* mice have normal *Il2rg* loci. (*A*) Genomic Southern blot using an IL-2Rγ cDNA probe and DNA from CBA/CaH-T6/J mice (lanes 1–3) or CBA/CaHN-xid/J mice (lanes 4–6) digested with *Eco*RI (lanes 1 and 4), *Bam*HI (lanes 2 and 5), and *Sst* I (lanes 3 and 6). (*B*) Northern blot using an IL-2Rγ cDNA probe and 10 μg of RNA derived from CBA/CaH-T6/J mice (lanes 1 and 2) or CBA/CaHN-*xid*/J mice (lanes 3 and 4). mIL-2Rγ, murine IL-2Rγ.

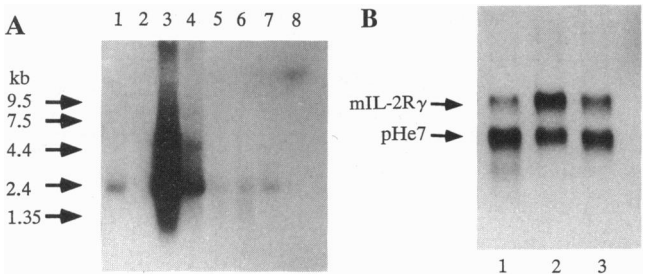

FIG. 5. IL-2Rγ mRNA expression. (*A*) Multiple tissue Northern blot. RNA size markers are shown to the left and RNA is from murine heart (lane 1), brain (lane 2), spleen (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7), and testis (lane 8). (*B*) IL-2Rγ mRNA expression in double-negative thymocytes (lane 1), single-positive-enriched thymocytes (lane 2), and unfractionated thymocytes (lane 3). The blot was rehybridized with a radiolabeled pHe7 cDNA probe to control for uniformity of loading of the gel. Positions of murine IL-2Rγ (mIL-2Rγ) and pHe7 mRNAs are indicated. Similar results were obtained when RNAs from independently isolated thymocyte populations were examined by Northern blot analysis (data not shown).

We have demonstrated that IL-2Rγ is strongly expressed in spleen and thymus and absent or expressed at lower levels in other tissues. Thus, IL-2Rγ is expressed in a tissue-type-specific fashion. Furthermore, among thymocytes, our data suggest that the more mature single-positive thymocytes express higher levels than double-negative thymocytes.

Although mutation of human IL-2Rγ results in XSCID in humans (21), it is unclear that a murine analog for XSCID exists. Based on available mapping data and linkage of homologous genes in humans and mice, it has been suggested that the XSCID locus in humans and the locus for X chromosome-linked immunodeficiency (*xid*) in mice could be related, even though the xid phenotype is more similar to human X chromosome-linked agammaglobulinemia (XLA) than to XSCID (38). In this regard, the recently cloned tyrosine kinase whose function is abrogated in XLA (39, 40) has been reported to have apparently normal enzymatic activity in *xid* mice (40), suggesting that XLA and *xid* may not be related. Interestingly, our genetic mapping analysis indicates that *Il2rg* is in the same region of the X chromosome as *xid*. However, our results from Southern and Northern blot analyses and DNA sequence analysis revealed no abnormalities in the murine IL-2Rγ gene in *xid* mice. These data indicate that *Il2rg* is not the defective gene in *xid* and, therefore, that *xid* is not an analog of human XSCID. Since we are not aware of a naturally occurring IL-2Rγ-deficient mouse strain, the cloning of the murine IL-2Rγ cDNA represents an initial step toward the goal of developing such mice by homologous recombination to study the role of IL-2Rγ in a murine model *in vivo*. In addition, the availability of the murine IL-2Rγ cDNA sequence should facilitate the preparation of anti-murine IL-2Rγ antibodies, reagents that do not currently exist.

Note Added in Proof. Since submission of this manuscript, two groups have reported that *xid* is associated with mutation of the *btk* gene tyrosine kinase (41, 42).

We thank Dr. B. J. Fowlkes (National Institute of Allergy and Infectious Disease) for valuable discussions, Dr. Louis Matis (National Cancer Institute, Frederick Cancer Research and Development Center) for providing the murine cDNA library prepared from Con A-activated splenocytes from CBA/Ca mice, and Dr. Atsushi Miyajima (DNAX) in whose laboratory W.J.L. prepared the 32D cDNA library from which DNA was isolated for the initial PCR amplification with γ3 and γ10. We thank Dr. Arnold W. Strauss for advice on potential signal peptide cleavage sites, Dr. Jian-Xin Lin (National Heart, Lung, and Blood Institute) for critical comments, and M. Charlene Adamson for technical assistance.

1. Leonard, W. J. (1992) in *Interleukin-2*, eds. Waxman, J. & Balkwill, F. (Blackwell, Oxford), pp. 29–46.
2. Smith, K. A. (1989) *Annu. Rev. Cell Biol.* **5**, 397–425.
3. Waldmann, T. A. (1989) *Annu. Rev. Biochem.* **58**, 875–911.
4. Leonard, W. J., Depper, J. M., Crabtree, J. R., Rudikoff, S., Pumphrey, J., Robb, R. J., Kronke, M., Svetlik, P. B., Peffer, N. J., Waldmann, T. A. & Greene, W. C. (1984) *Nature (London)* **311**, 625–631.
5. Nikaido, T., Shimizu, A., Ishida, N., Sabe, H., Teshigawara, K., Maeda, M., Uchiyama, T., Yodoi, J. & Honjo, T. (1984) *Nature (London)* **311**, 631–635.
6. Sharon, M., Klausner, R. D., Cullen, B. R., Chizzonite, R. & Leonard, W. J. (1986) *Science* **234**, 859–863.
7. Tsudo, M., Kozak, R. W., Goldman, C. K. & Waldmann, T. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9694–9698.
8. Teshigawara, K., Wang, H. M., Kata, K. & Smith, K. A. (1987) *J. Exp. Med.* **165**, 223–238.
9. Dukovich, M., Wano, Y., Thuy, L.-T. B., Katz, P., Cullen, B. R., Kehrl, J. H. & Greene, W. C. (1987) *Nature (London)* **327**, 518–522.
10. Sharon, M., Siegel, J. P., Tosato, G., Yodoi, J., Gerrard, T. L. & Leonard, W. J. (1988) *J. Exp. Med.* **167**, 1265–1270.
11. Siegel, J. P., Sharon, M., Smith, P. L. & Leonard, W. J. (1987) *Science* **238**, 75–78.
12. Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. & Taniguchi, T. (1989) *Science* **244**, 551–556.
13. Takeshita, T., Asao, H., Suzuki, J. & Sugamura, K. (1990) *Int. Immunol.* **2**, 551–556.
14. Takeshita, T., Ohtani, K., Asao, H., Kumaki, S., Nakamura, M. & Sugamura, K. (1992) *J. Immunol.* **148**, 2154–2158.
15. Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. & Sugamura, K. (1992) *Science* **257**, 379–382.
16. Voss, S. D., Sondel, P. M. & Robb, R. J. (1992) *J. Exp. Med.* **176**, 531–541.
17. Arima, N., Kamio, M., Imada, K., Hori, T., Hattori, T., Tsudo, M., Okuma, M. & Uchiyama, T. (1992) *J. Exp. Med.* **176**, 1265–1272.
18. Miller, J., Malek, T. R., Leonard, W. J., Greene, W. C., Shevach, E. M. & Germain, R. N. (1985) *J. Immunol.* **134**, 4212–4217.
19. Shimuzu, A., Kondo, S., Takeda, S. I., Yodoi, J., Ishida, N., Sabe, H., Osawa, H., Diamantstein, T., Nikaido, T. & Honjo, T. (1985) *Nucleic Acids Res.* **13**, 1505–1516.
20. Kono, T., Doi, T., Yamada, G., Hatakeyama, M., Minamoto, S., Tsudo, M., Miyasaka, M., Miyata, T. & Taniguchi, T. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1806–1810.
21. Noguchi, M., Yi, H., Rosenblatt, H. M., Filipovich, A. H., Adelstein, S., Modi, W. S., McBride, O. W. & Leonard, W. J. (1993) *Cell* **73**, 147–157.
22. Cooper, M. D. & Butler, J. L. (1989) in *Fundamental Immunology*, ed. Paul, W. E. (Raven, New York), pp. 1034–1039.
23. Conley, M. E. (1992) *Annu. Rev. Immunol.* **10**, 215–238.
24. Greenberger, J. S., Sakakeeny, M. A., Humphries, R. K., Eaves, C. J. & Eckner, R. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2931–2935.
25. Otani, H., Siegel, J. P., Erdos, M., Gnarra, J. R., Toledano, M. B., Sharon, M., Mostowski, H., Feinberg, M. B., Pierce, J. H. & Leonard, W. J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2789–2793.
26. Bruce, J. F., Symington, F. W., McKern, T. J. & Sprent, J. (1981) *J. Immunol.* **127**, 2496–2501.
27. Sarmiento, M., Glasebrook, A. L. & Fitch, F. W. (1980) *J. Immunol.* **125**, 2665–2672.
28. Ceredig, R., Lowenthal, J., Nabholz, M. & MacDonald, R. (1985) *Nature (London)* **314**, 98–100.
29. Fowlkes, B. J. & Pardoll, D. M. (1989) *Adv. Immunol.* **44**, 207–264.
30. Kaczmarek, L., Calabretta, B., Kao, H.-T., Heinz, N., Nevins, J. & Baserga, R. (1987) *J. Cell Biol.* **104**, 183–187.
31. Kozak, C. A., Peyser, M., Krall, M., Mariano, T. M., Kumar, C. S., Pestka, S. & Mock, B. A. (1990) *Genomics* **8**, 519–524.
32. Adamson, M. C., Silver, J. & Kozak, C. A. (1991) *Virology* **183**, 778.
33. Mueller, R. E., Baggio, L., Kozak, C. A. & Ball, J. K. (1992) *Virology* **191**, 628–637.
34. Green, M. C. (1989) *Genetics and Probability in Animal Breeding Experiments* (Macmillan, New York), pp. 77–113.
35. von Heijne, G. (1986) *Nucleic Acids Res.* **14**, 4683–4690.
36. Bazan, J. F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6934–6938.
37. Noguchi, M., Adelstein, S., Cao, X. & Leonard, W. J. (1993) *J. Biol. Chem.* **268**, 13601–13608.
38. Puck, J. M., Nussbaum, R. L., Smead, D. L. & Conley, M. E. (1989) *Am. J. Hum. Genet.* **44**, 724–730.
39. Vetrie, D., Vorechovsky, I., Sideras, P., Holland, J., Davies, A., Flinter, F., Hammarstrom, L., Kinnon, C., Levinsky, R., Bobrow, M., Smith, C. I. E. & Bentley, D. R. (1993) *Nature (London)* **361**, 226–233.
40. Tsukada, S., Saffran, D. C., Rawlings, D. J., Parolini, O., Allen, R. C., Klisak, I., Sparkes, R. S., Kubagawa, H., Mohandas, T., Quan, S., Belmont, J. W., Cooper, M. D., Conley, M. E. & Witte, O. N. (1993) *Cell* **72**, 279–290.
41. Thomas, J. D., Sideras, P., Smith, C. I. E., Vorechovsky, I., Chapman, V. & Paul, W. E. (1993) *Science* **261**, 355–358.
42. Rawlings, D. J., Saffran, D. C., Tsukada, S., Largaespada, D. A., Grimaldi, J. C., Cohen, L., Mohr, R. N., Bazan, J. F., Howard, M., Copeland, N. G., Jenkins, N. A. & Witte, O. N. (1993) *Science* **261**, 358–361.