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CLONING OF THE MOUSE INTERLEUKIN 2 RECEPTOR γ CHAIN: DEMONSTRATION OF FUNCTIONAL DIFFERENCES BETWEEN THE MOUSE AND HUMAN RECEPTORS+

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We isolated a cDNA clone for the γ chain of the mouse interleukin 2 receptor. Introduction of the mouse γ chain cDNA clone into a mouse fibroblast cell line, L929, expressing the mouse αβ heterodimer IL-2 receptor converted pseudohigh affinity of the IL-2 receptor into functional high, resulting in internalization of IL-2 and induction of the c-myc, c-fos and c-jun genes. The mouse βγ heterodimer, however, failed to bind IL-2 unlike the human βγ heterodimer intermediate-affinity receptor. These results indicate that the mouse functional IL-2 receptor is a complex comprising three distinct subunits, α, β and γ chains, but the βγ heterodimer is not functional and different from the human heterodimer. © 1993 Academic Press, Inc.

Interleukin 2 (IL-2) is a member of the family of cytokines, and is known to affect T cells, B cells, natural killer cells, macrophages and glioma cells through its receptor (1). In addition to the α and β subunits, we recently cloned the human gene for the γ chain that is shown to participate in formation of the human IL-2 receptor complexes with high affinity (dissociation constant, Kd=10^{-11} M) and intermediate affinity (Kd=10^{-9} M) (1-3). Interestingly, a mouse T cell line, EL-4, failed to bind IL-2 with its endogenous mouse β chain (4), although introduction of the human α or β chain led the cells to bind IL-2 with high or intermediate affinity, respectively (5, 6). Thus we assumed that the mouse IL-2/IL-2 receptor system may be different from the human. In the present study, we first report isolation of a cDNA clone for the mouse γ chain and demonstrate that only the mouse αβγ heterotrimer with high affinity to IL-2 is functional for signal transduction, and that the mouse βγ heterodimer is not capable of binding IL-2 unlike the human heterodimer.

+Sequence data from this article have been deposited with the DDBJ/EMBL/GenBank Data Libraries under Accession No. D13565.

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Abbreviations: IL-2, interleukin 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SH2, *Src* homology region 2.

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MATERIALS AND METHODS

Cell lines: Cell lines used were a mouse IL-2-dependent T cell line, CTLL-2, and a mouse fibroblast cell line, L929.

cDNA cloning: Poly A-enriched RNA from CTLL-2 cells was used to construct a cDNA library with λZAPII phage (Stratagene), yielding a cDNA library containing 4.3×10^5 independent clones. The cDNA library was screened by plaque hybridization using a 0.9 kb XbaI-NcoI fragment of human IL-2 receptor γ chain cDNA (3) as a probe. The mouse IL-2 receptor β chain cDNA was also screened from the same CTLL-2 library with a mouse β chain probe which was synthesized according to the published sequence (corresponding 51 nucleotides from the ATG starting site of the open reading frame) (4).

Transfection of mouse β and γ chain cDNAs: Fragments encompassing the whole mouse β and γ chain coding regions were isolated from cDNA clones and inserted into expression vector pcDSRα (7) by using an EcoRI linker to yield pSRmB3 and pSRmG1, respectively. L929 cells were transfected with pSRmB3 along with pSV2neo carrying the neomycine-resistance gene by electroporation, establishing Lmβ-1. Lmβ-1 was then co-transfected with pSRmG1 and pcDSα-mouse IL-2Rα (8) carrying the mouse IL-2 receptor α chain gene along with the hygromycin-resistance gene, pHygJM109, yielding Lmβmγ-3 and Lmαmβ-7, respectively. Lmαmβ-7 was further transfected with pSRmG1 along with pSV2bsr (9) carrying the blasticidin S-resistance gene (Kaken), establishing Lmαmβmγ-1. The transfectants were selected at each step of transfection in the medium containing either G418 (GIBCO), hygromycin B (Sigma), or blasticidin S (Kaken) at final concentrations of 500 μg/ml, 350 μg/ml and 2.5 μg/ml, respectively. Clones were isolated by limiting dilution.

IL-2 binding assay and IL-2 internalization: Human recombinant IL-2 labeled with [^{125}I]Na (Amersham) by the chloramine T method was used for IL-2 binding assay and IL-2 internalization by the transfectants as described previously (3).

[^{125}I]IL-2 affinity labeling: Cells (5×10^6) were incubated with 1 nM [^{125}I]IL-2 for 1 h at 4 °C. The cells were then washed twice with ice cold phosphate-buffered saline, and treated with a chemical cross-linker, disuccinimidyl suberate (Pierce) for 20 min at 4 °C. The cross-linked cells were solubilized with 1 % Nonidet P-40. Subsequently, the cell lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 7.5 % gel. Cells preincubated with 200 nM unlabeled IL-2 for 1 h at 4 °C prior to treatment with [^{125}I]IL-2 were used as controls.

RNA blot analysis: Total cellular RNAs were isolated from cell lines and mouse tissues by extraction with guanidinium thiocyanate. Northern blot hybridization was performed as described previously (10). The probe for the mouse IL-2 receptor γ chain was a 0.4 kb EcoRV fragment from pSRmG1. The probes for the c-myc, c-fos, c-jun and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were labeled with [α-^{32}P]dCTP by the random primer method as described previously (10).

RESULTS

Characterization of the mouse γ chain. To isolate a cDNA clone for the mouse γ chain, a cDNA library from CTLL-2 was screened by cross-hybridization using the human γ chain cDNA as a probe. Four positive clones were obtained. Sequence analyses of the isolated clones revealed that two of them contained an open reading frame that encodes a protein consisting of 369 amino acid residues. Hydrophobicity analysis predicted a putative signal sequence of the N-terminal 22 amino acid residues and a putative transmembrane domain of 29 amino acid residues (Fig. 1A). The predicted figure of the mouse γ chain is identical to that of the human γ chain except for one amino acid in each extracellular and cytoplasmic regions in number; the mouse γ chain extracellular region is one amino acid longer and the cytoplasmic region is one amino acid shorter than the human counterpart. The homology between the two species is 70 % at the amino acid level.

The extracellular domain of the mouse γ chain, having 66 % amino acid homology with the human γ chain, included the consensus sequences of the cytokine receptor superfamily;

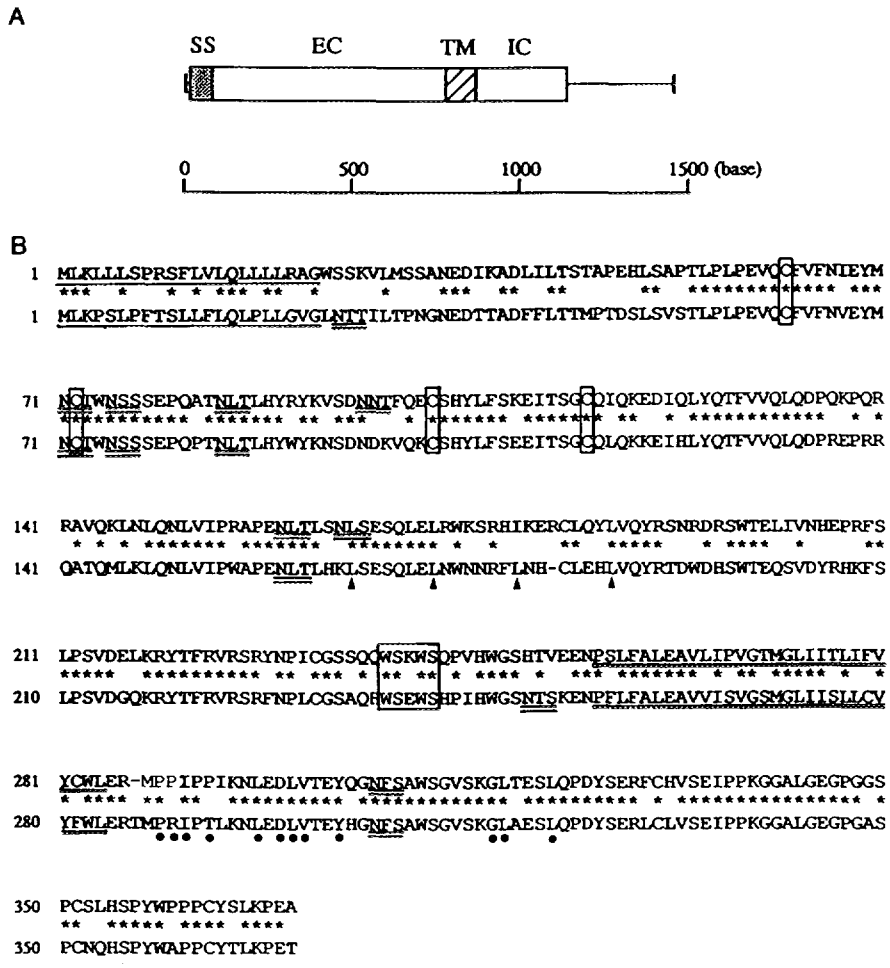

Figure 1. The mouse IL-2 receptor γ chain cDNA. (A) Schematic representation of the mouse γ chain cDNA. The coding region is boxed. SS, signal sequence, EC, extracellular region, TM, transmembrane region, IC, intracellular region. (B) Amino acid sequence of the mouse γ chain. Upper and lower lines are the amino acid sequences of the mouse and human γ chains, respectively. Asterisks represent identical amino acids between the two species. Gaps (-) are introduced to maximize homology. Thin underlines indicate the putative signal sequence. The predicted transmembrane region is indicated by thick underlines. The conserved four cysteine residues and the WS motif are boxed. Four leucine residues similar to the leucine zipper structure and the SH2 subdomains of the human γ chain are indicated by closed triangles and circles below the human γ chain amino acid sequence, respectively. Seven potential N-glycosylation sites are doubly underlined.

characteristic four conserved cysteine residues near the N-terminus and the unique WS motif (Trp-Ser-X-Trp-Ser) located at the proximal end of the transmembrane region (11, 12). The mouse γ chain also has amino acid sequences (positions 165 to 187) similar to the putative leucine zipper between the conserved cysteine region and the WS motif, like the human γ chain (3), with one substitution of the third leucine to isoleucine residue and with one amino acid insertion between the third and fourth leucine residues. The cytoplasmic domain, showing 83 % amino acid homology with the human γ chain, included the highly conserved *Src* homology region 2 (SH2) subdomains (13), which is also seen in the human γ chain (3) (Fig. 1B). No consensus sequence for the tyrosine kinase domain was identified.

Functional expression of the mouse IL-2 receptor γ chain. The function of the mouse γ chain was examined with a mouse fibroblast cell line, L929, in which the mouse IL-2 receptor was reconstituted by transfection. No expression of the mouse α, β and γ chains in parental L cells was confirmed by flow cytometric analysis and mRNA blot (Fig. 4B and data not shown).

The IL-2-binding properties of the transfectants were analyzed by Scatchard plots. L cell sublines transfected with cDNA clones for either the mouse β or γ chain could not bind IL-2 like with the human β or γ chain (data not shown). Surprisingly, as seen with Lmβmγ-3 cells, simultaneous expression of the mouse β and γ chains did not show any appreciable binding to both human and mouse IL-2 under physiological IL-2 concentrations (Fig. 2A). This contrasts well with the results from the L929 subline, Lβγ-9, expressing the human β and γ chains with intermediate affinity to IL-2 (Kd=2.4 nM, 5400 sites/cell) (3). Introduction of the mouse γ chain cDNA into Lmαmβ-7, which has IL-2 receptors with pseudohigh affinity (Kd=254 pM, 2325 sites/cell) and low affinity (Kd=9.3 nM, 43975 sites/cell), altered the IL-2 binding affinity from pseudohigh to high (Kd=60 pM, 1735 sites/cell) without affecting the low-affinity receptor (Kd=9.3 nM, 48011 sites/cell) (Fig. 2A). These results clearly demonstrate that the mouse high-affinity IL-2 receptor comprises the α, β and γ subunits.

We further investigated the involvement of the γ chain in the IL-2 binding by chemical cross-linking after treatment of cells with human [^{125}I]IL-2. [^{125}I]IL-2 cross-linked proteins

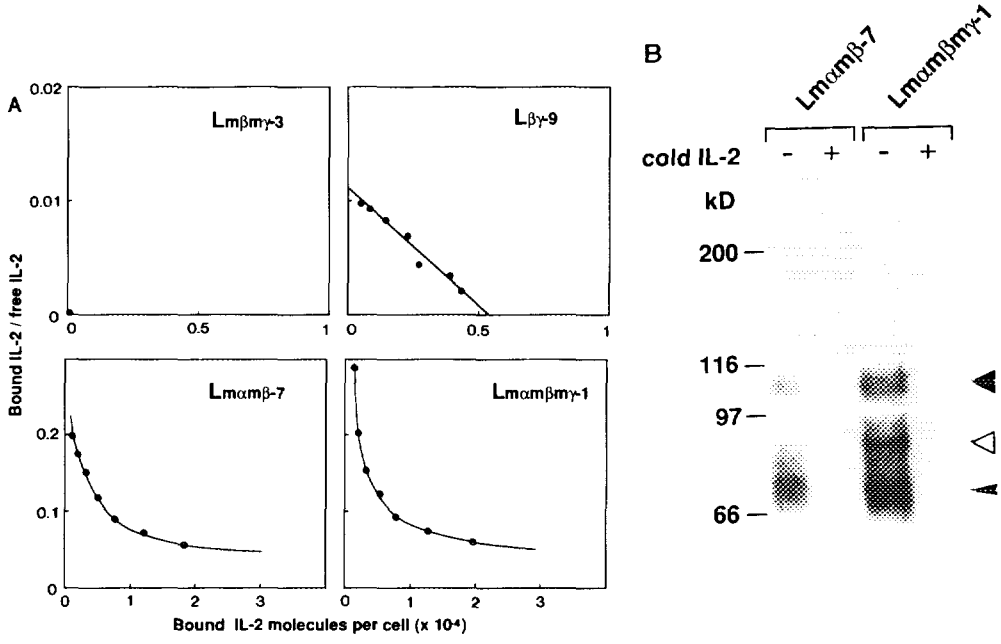

Figure 2. IL-2 binding properties of the L transfectant cells. (A) Scatchard plot analysis of human [^{125}I]IL-2 binding to the transfectants. (B) Affinity labeling of the transfectants with human [^{125}I]IL-2. Cells were incubated for 1 h in the presence (+) or absence (-) of 200 fold excess cold IL-2 prior to addition of [^{125}I]IL-2, and then treated with a chemical cross-linker. Complexes of the mouse IL-2 receptor α (◀), β (◀) and γ chains (◁) with [^{125}I]IL-2 are indicated. Molecular sizes are marked at the left margin.

with molecular masses of 105, 85 and 70 kDa were detected with Lmαmβmγ-1 cells, while Lmαmβ-7 cells showed only 105 and 70 kDa bands (Fig. 2B); obviously the detection of the 85 kDa protein is γ chain dependent and presumably it is a complex of the γ chain with human IL-2. Molecular masses (90 and 55 kDa) estimated from apparent molecular masses of 105 kDa and 70 kDa by subtraction of the molecular weight of human IL-2 (15 kDa) are consistent with those of the mouse IL-2 receptor α and β chains, respectively (14, 15). Similarly a molecular mass of the putative mouse IL-2 receptor γ chain protein is calculated to be 70 kDa. The mature form of the mouse γ chain peptide backbone consists of 347 amino acid residues with a calculated molecular weight of 39,762. The difference between molecular masses from the deduced amino acid sequence and from the cross-linked protein is probably due to glycosylation, as the six potential N-linked glycosylation sites are in the extracellular region of the mouse γ chain (Fig. 1B).

Internalization of human [^{125}I]IL-2 bound to the transfectant cells was also examined. Lmαmβmγ-1 cells internalized IL-2 significantly, whereas little, if any, internalization was observed with Lmαmβ-7 cells (Fig. 3A), although numbers of (pseudo)high- and low-affinity IL-2 receptors in both transfectants are comparable, demonstrating that the γ chain is responsible for internalization.

Effects of the mouse γ chain on induction of these proto-oncogenes was examined. Lmαmβmγ-1 cells were able to induce c-myc, c-fos and c-jun expression in response to IL-2, but the Lmαmβ-7 cells were not (Fig. 3B). Taken together, the γ chain in the L transfectants is functional not only in IL-2 binding and internalization of IL-2 but also in induction of the proto-oncogenes.

Cell specific expression of the mouse γ chain. The γ chain mRNA was constitutively expressed in mouse T cell lines, CTLL-2 and EL-4, a pro-B cell line, BAF/B03, and a natural killer cell line, NK3, whereas mouse fibroblast cell lines, Balb/3T3, NIH/3T3

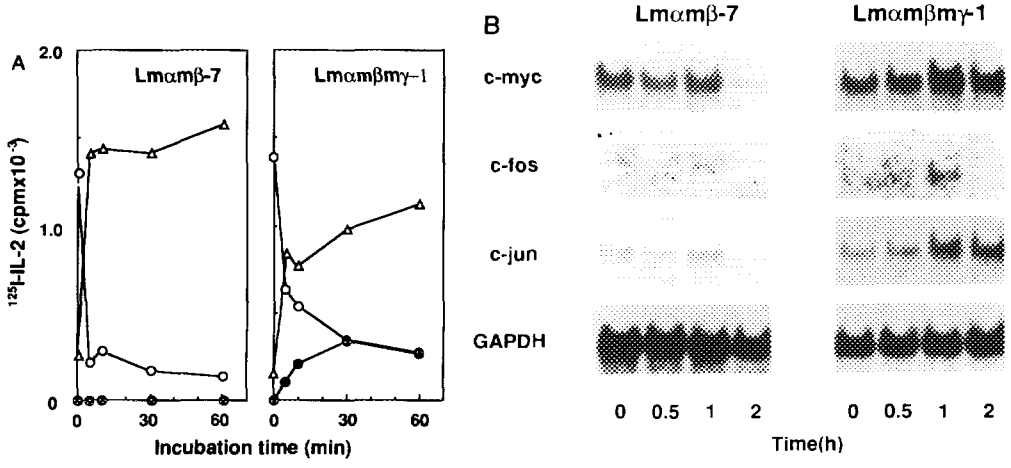

Figure 3. (A) Internalization of IL-2 in the transfectants. Radioactivities of unbound IL-2 (Δ), cell surface-bound IL-2(○), and internalized IL-2 (●) were separately determined. (B) IL-2-induced mRNA expression of nuclear proto-oncogenes, c-myc, c-fos and c-jun, in the transfectants. Cells were stimulated with 10 nM IL-2 for indicated times. The relative amount of GAPDH mRNA was monitored to check the amount of total RNA applied to each lane.

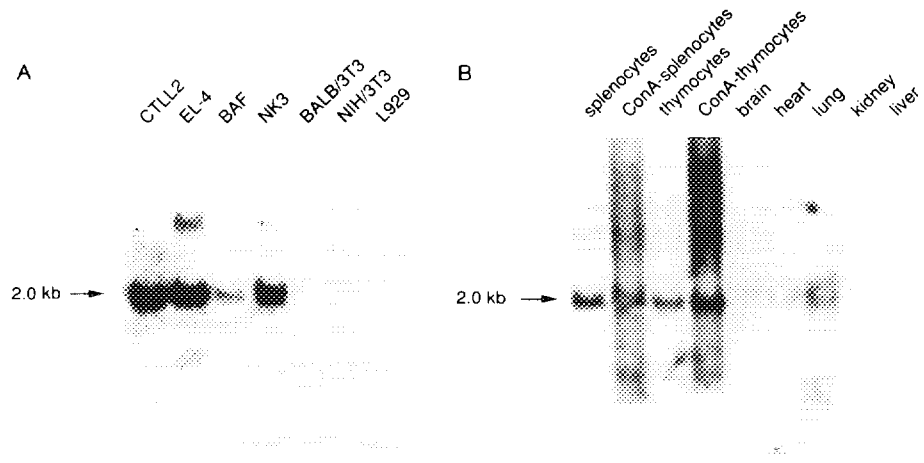

Figure 4. Expression of IL-2 receptor γ chain mRNA in various mouse cell lines and tissues. Total RNAs (20μg) from various mouse cell lines (A) and BALB/c mouse tissues (B) were subjected to Northern blot analysis. Splenocytes and thymocytes were stimulated with 5 μg/ml concanavalin A for 48 h. Molecular size is indicated in kilobase at the left margin.

and L929 did not express appreciable mRNA for the mouse γ chain (Fig. 4A). This limited expression within hematopoietic cell lines, is similar to that of the human γ chain (3).

Expression of the γ chain in tissues was also examined. The mRNA for the mouse γ chain was detected in spleen, thymus and lung, but detectable expression was not observed in brain, heart, kidney and liver. Upon stimulation with concanavalin A, splenocytes and thymocytes increased the mRNA level (Fig. 4B), suggesting that expression of the γ chain may be associated with lymphocyte activation and proliferation. Further study is needed to determine which population in the lung tissue expresses the γ chain.

DISCUSSION

The mouse γ chain is the same as the human counterpart structurally and functionally except for the inability of the mouse βγ heterodimer to bind IL-2, unlike the human βγ heterodimer which binds IL-2 with intermediate affinity (2, 3, 10). The mouse γ chain, when transfected along with both the mouse α and β chains, generates the high-affinity receptor that can mediate the IL-2 internalization and nuclear proto-oncogene induction, thus the mouse γ chain we cloned is functional. Since these results were observed with transfectants of the same parental cell line, L929 cells, the difference in function of the βγ heterodimer between mouse and human is not attributable to cell specificity and presumably reflects the subtle differences between the mouse and human β and/or γ chains.

In this context, it is of interest to learn that EL-4 cells cannot bind either human or mouse IL-2 under the physiological conditions (4, 5, 15), although they express the mouse β and γ chains endogenously. The DNA sequence for the mouse β chain of EL-4 is reported to be indistinguishable from that of spleen cell-derived cDNA (4), moreover, the EL-4 γ chain seems functional, because of acquisition of intermediate IL-2 binding in combination with the human β chain (6). In addition, IL-2 binding with high affinity on CTLL-2 cells was

completely prevented by addition of an anti-IL-2 receptor α chain monoclonal antibody that blocks IL-2 binding to the α chain (16). Furthermore, the mouse βγ heterodimer comprising the transfected β chain and endogenous γ chain on a mouse lymphoma cell line, BW5147, could not bind IL-2 (our unpublished observation). A simple interpretation of these phenomena is that a heterodimer complex between the human β chain and mouse γ chain, as well as the human βγ complex, is able to generate the intermediate-affinity IL-2 receptor, but the cognate complex of the mouse β and γ chains is not functional in IL-2 binding as seen with the L subclone transfected with the mouse β and γ chains. Taken together, the mouse system seems to require the α chain as an element responsible for IL-2 binding, like other cytokine receptors for IL-3, IL-5, IL-6, granulocyte-macrophage colony-stimulating factor, Oncostatin M, leukemia inhibitory factor and ciliary neurotrophic factor (17). On the other hand, the combination of the human β and γ chains is sufficient for generating IL-2 binding with the intermediate affinity without the α chain (3). There is, however, a report that Ba/F3(β^+) cells, expressing exogenously the mouse β chain but not the α chain, are able to transduce growth signals in response to mouse IL-2 through its intermediate-affinity receptor (18). To clarify the discrepancy in IL-2 binding of cell lines expressing both the mouse β and γ chains needs further examination.

Availability of the mouse genes for the IL-2 receptor subunits not only will provide a useful tool for better understanding of functions of the IL-2 receptor on mature functional cells, but also will facilitate study on the functional roles of the subunits in development and differentiation of the hematopoietic cell lineage.

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