Interleukin-2 Receptor β Chain Gene: Generation of Three Receptor Forms by Cloned Human α and β Chain cDNA's

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Interleukin-2 (IL-2) binds to two distinct receptor molecules, the IL-2 receptor α (IL-2Rα, p55) chain and the newly identified IL-2 receptor β (IL-2Rβ, p70–75) chain. The cDNA encoding the human IL-2Rβ chain has now been isolated. The overall primary structure of the IL-2Rβ chain shows no apparent homology to other known receptors. Unlike the IL-2Rα chain, the IL-2Rβ chain has a large cytoplasmic region in which a functional domain (or domains) mediating an intracellular signal transduction pathway (or pathways) may be embodied. The cDNA-encoded β chain binds and internalizes IL-2 when expressed on T lymphoid cells but not fibroblast cells. Furthermore, the cDNA gives rise to the generation of high-affinity IL-2 receptor when co-expressed with the IL-2Rα chain cDNA.

CYTOKINES, A CLASS OF SOLUBLE MEDIATORS INVOLVED IN cell-to-cell communications, are essential in the regulation of the immune system. Cytokines induce proliferation, differentiation, and activation of target cells through interaction with specific cell surface receptors. Interleukin-2 (IL-2), previously defined as T cell growth factor (*1*), is one of the best characterized cytokines, and it has a pivotal role in the antigen-specific clonal proliferation of T lymphocytes (T cells) (*2*). IL-2 also appears to act on other cells of the immune system such as immature thymocytes (*3*), B lymphocytes (B cells) (*4*), macrophages (*5*), natural killer cells (NK cells) (*6*), and lymphokine-activated killer cells (LAK cells) (*7*). These multifunctional properties of IL-2 have opened new possibilities in the formulation of immunotherapies such as adoptive immunotherapy (*8*). Furthermore, IL-2 has been shown to function also on neural cells such as oligodendrocytes (*9*), suggesting a possible involvement of this cytokine in the central nervous system. Despite extensive studies on the IL-2 system, information on the molecular mechanisms underlying the IL-2–mediated signal transduction (*10*) is limited.

The IL-2 receptor (IL-2R) is present in three forms: high-, intermediate-, and low-affinity forms with respect to binding ability to IL-2, and respective dissociation constants (K_d's) of $10^{-11}M$, $10^{-9}M$, and $10^{-8}M$ (*11, 12*). After the IL-2Rα chain (Tac antigen, p55) was characterized (*13*), it became evident that the α chain constitutes the low-affinity form and is not functional in IL-2 internalization and signal transduction unless it is associated with another specific membrane component (or components) of lymphoid cells (*14, 15*). Subsequently, the lymphoid membrane component was identified as a novel receptor chain, termed β chain (or p70–75) (*12, 16, 17*). In fact, experimental evidence has suggested that the IL-2Rβ chain by itself constitutes the intermediate-affinity form (*12*). In addition, its association with the IL-2Rα chain seems to result in the high-affinity form of the receptor (*12, 16, 17*). Expression studies with wild-type and mutated IL-2Rα chain cDNA's support the possibility that the IL-2Rβ chain but not the IL-2Rα chain has one or more domains responsible in driving the intracellular signal transduction pathways (*18*). Thus, elucidation of the structure and function of the IL-2Rβ chain should provide further insight on the molecular basis of the high-affinity IL-2R as well as on the mechanism of signal transduction operating in IL-2 responsive cells. We now report the isolation, structural analysis, and expression of the cDNA's encoding the human IL-2Rβ chain. We present data showing the reconstitution of three forms of IL-2R in Jurkat cells, an IL-2R–negative human T cell line, and discuss the IL-2–mediated events in reconstituted systems.

Isolation and analysis of the cDNA clones. In isolating the cDNA clones, we applied an expression cloning strategy by using two monoclonal antibodies, Mik-β 1 and Mik-β 2 (*19*), to the IL-2Rβ chain found on the human leukemic cell line YT (*20*). The cDNA library was prepared with the polyadenylated (poly(A)$^+$) RNA (30 μg) from YT cells according to standard procedures. In the successful experiment, a pool of recombinant plasmid DNA's representing 5.6×10^6 cDNA clones were transfected into COS-7 cells, and cDNA clones giving rise to the expression of the antigenic epitopes were selected as described (*21*). After four cycles of expression screening, we isolated two independent cDNA clones, pIL-2Rβ 9 and pIL-2Rβ 30; each of the expression products specifically reacted with the antibodies. The two clones contained cDNA inserts of 1.3 kb and 2.3 kb, respectively, and cross-hybridized with each other. Subsequent sequence analysis of the cDNA's revealed that they represent the same mRNA. In fact, RNA blotting analysis revealed that the mRNA is approximately 4 kb (see below). Subsequently, we screened other YT cDNA libraries with the cloned cDNA's as probes, and isolated several independent cDNA clones which together cover the entire mRNA for the IL-2Rβ chain.

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Complete nucleotide sequencing of four of the cloned cDNA's (Fig. 1) revealed a large open reading frame that encodes a protein consisting of 551 amino acids. No significant similarity or identity with other known proteins was found (*22*). Unlike many other cytokine receptors, the IL-2Rα and IL-2Rβ chains do not appear to belong to the immunoglobulin superfamily. From the deduced structure of the protein, the NH_2-terminal 26 amino acids may represent the signal sequence (Figs. 1 and 2) (*23*). Thus, the mature form of the IL-2Rβ chain consists of 525 amino acids with a calculated molecular size of 58,358 daltons. The receptor molecule contains an extracellular region consisting of 214 amino acids (Fig. 1). This region contains eight cysteine residues of which five are found in the NH_2-terminal half and they are interspaced rather periodically by 9 to 12 amino acids. Abundance of cysteine residues seems to be one of the common features of the ligand-binding domain of many receptors (*24*). The predicted number of amino acids (aa) within the extracellular region of the IL-2Rβ chain (214 aa) is almost comparable in number to that of the IL-2Rα chain (219 aa). Such size similarity may be significant in considering the conformation of the heterodimeric receptor complex that is a distinguishing mark for this receptor because both α and β chains individually interact with distinct sites of the same IL-2 molecule (*25*). A hydrophobic stretch of 25 amino acids from residues 215 to 239 appears to constitute the membrane spanning region of the receptor (Figs. 1 and 2).

The cytoplasmic region of the β chain consists of 286 aa and is far larger than that of the α chain, which is only 13 aa long. The consensus sequences of tyrosine kinase (Gly-x-Gly-x-x-Gly) (*26*) are absent in the β chain, but a triplet, Ala-Pro-Glu(293–295), that has been implicated to be the consensus motif for a catalytic domain of some protein kinases (*26*) is present. The possibility that the cytoplasmic region of the β chain may have a protein kinase activity is yet to be tested. The primary structure of this region revealed yet another interesting feature, namely, it is rich in proline (42 of 286)

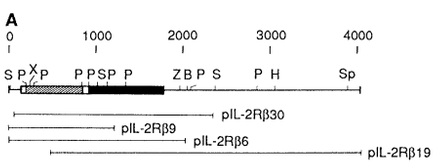

Fig. 1. Structure of the human IL-2Rβ chain cDNA. (**A**) Representation of the mRNA as deduced from the cloned cDNA's. Dotted, hatched, open, and closed rectangles represent, respectively, the signal sequence, the extracellular, the transmembrane, and the cytoplasmic regions. Different cDNA clones encoding for specific overlapping regions of the mRNA are shown below. S, Sac I; P, Pst I; X, Xho I; Z, Sma I; B, Bam HI; H, Sph I; Sp, Ssp I. (**B**) Nucleotide and amino acid sequences of the human IL-2Rβ chain cDNA. The sequence was deduced after complete DNA sequence analysis of the above described cDNA clones. Nucleotides are numbered on the right margin and amino acids are numbered on the left margin. Clones pIL-2Rβ 19 and pIL-2Rβ 6 contained the G→A mutation at nucleotide residues 425 and 1531, respectively. Thus pIL-2Rβ 6 cDNA acquired a TAG triplet in the cytoplasmic region. It is thought to be an error in reverse transcription, since all other clones, pIL-2Rβ 30, pIL-2Rβ 19, and pIL-2Rβ 16 (*32*), have a TGG triplet at that position. The first underlined 26 amino acid residues represent the signal sequence as predicted by the consensus sequence (*23*). The 25 transmembrane amino acid residues are marked by underlining. The cysteine residues are boxed. The potential N-glycosylation sites are underlined twice. The possible polyadenylation signals are shown by open rectangle. RNA was prepared from the NK-like human lymphoid cell line YT, and cDNA libraries were prepared with CDM8 vector as described (*21*), except using either random primers (Amersham) (for pIL-2Rβ 6, 9, and 30), or oligo(dT) primer (for pIL-2Rβ 19). Briefly, 30 μg of poly(A)$^+$ RNA was used for each cDNA library. The cDNA was synthesized by modified procedures of Gubler and Hoffman as described earlier and cloned into CDM8 (*21*). The cDNA libraries were screened with a mixture of monoclonal antibodies to IL-2Rβ, Mik-β1 and Mik-β2, as described (*21*). The panning procedures were repeated four times. Nucleotide sequences were determined by dideoxy chain termination and chemical cleavage methods.

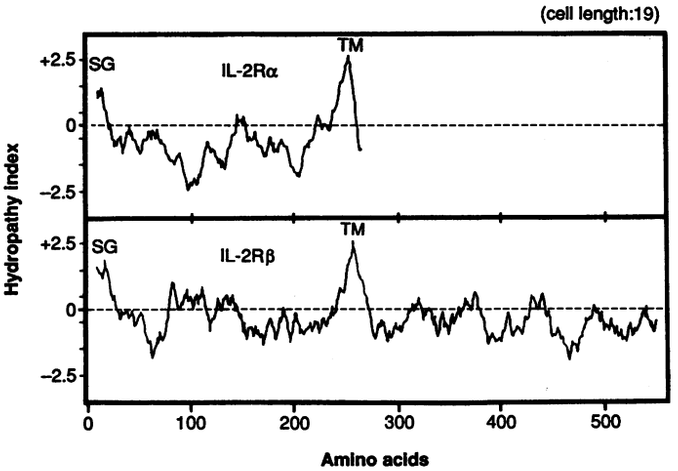

Fig. 2. Hydropathy plot analysis of deduced human IL-2Rα and IL-2Rβ chain precursor structures. The analysis was carried out according to Kyte and Doolittle (*38*). SG and TM each represents signal sequence and transmembrane sequence, respectively.

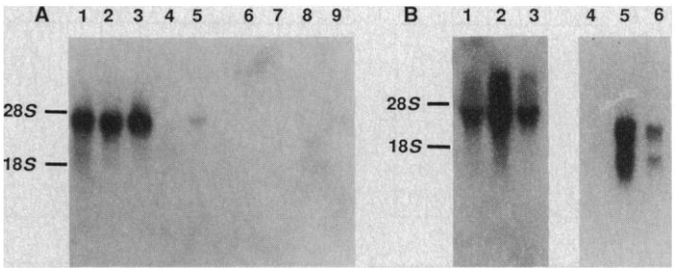

Fig. 3. Expression of human IL-2Rβ chain mRNA. (**A**) Poly(A)$^+$ RNA (2 μg per lane) from different cell sources was prepared and subjected to RNA blotting analysis with the Xho I–digested human IL-2Rβ chain cDNA fragment derived from pIL-2Rβ 30 as a probe (*14, 18, 28*). Lane 1, YT; lane 2, Hut102 (HTLV-1–transformed human T cell line); lane 3, MT-2 (HTLV-1–transformed human T cell line); lane 4, ARH-77 (multiple myeloma line); lane 5, SKW6.4 (Epstein-Barr virus–transformed human B lymphoblastoid line); lane 6, U937 (histiocytic leukemia line); lane 7, MT-1 (HTLV-1–transformed human T cell line); lane 8, Jurkat (human T leukemic line); lane 9, HeLa (human cervical carcinoma cell line). (**B**) Expression of IL-2Rβ and IL-2Rα mRNA's in human PBL's. Total RNA (15 μg per lane) was placed on each lane. Lanes 1 and 4, unstimulated human PBL's; lanes 2 and 5, PBL's stimulated with phytohemagglutinin (PHA) at 5 μg/ml for 24 hours; lanes 3 and 6, PBL's stimulated with PHA at 5 μg/ml for 72 hours. The RNA-blotted filter was hybridized with the IL-2Rβ probe (lanes 1 to 3). After dehybridization of the IL-2Rβ probe, the same filter was hybridized with the IL-2Rα probe [Xba I–Bcl I fragment derived from pSVIL2R-3 (*14*)] (lanes 4 to 6).

and serine (30 of 286) residues. The "proline-rich" structure has also been demonstrated in the cytoplasmic region of CD2, a T cell membrane antigen involved in the activation pathway of T cells (*27*). In addition, the cytoplasmic region contains 40 negatively charged amino acids (glutamic and aspartic acids), whereas only 18 amino acids account for the positively charged residues (lysine and arginine). Such a distribution is notable in the middle portion (aa 345–390) of the cytoplasmic region. Thus, the cytoplasmic region of the β chain may be quite acidic. Taken together, some if not all of these characteristic features may contribute to driving further the downstream signal transduction pathways. The receptor protein contains five potential sites for N-linked glycosylation (Fig. 1), in which four are extracellular. Such a posttranslational modification may account for the difference between the size of the estimated mature (70 to 75 kD) and the calculated (58 kD) protein molecules. Hydropathy plot analysis of the α and β chains revealed hydrophilic regions just adjacent to the cell membrane in both chains (Fig. 2). These regions may participate in the noncovalent intermolecular association between the two chains.

Expression of IL-2Rβ chain mRNA. Expression of the IL-2Rβ mRNA was examined with the cDNA insert from pIL-2Rβ 30 as the probe. The RNA blot analysis revealed the presence of a 4-kb mRNA (Fig. 3A), the expression of which is restricted to lymphoid cells previously identified to bear IL-2Rβ chain (that is, YT, MT-2, Hut102, SKW6.4) (*12, 16, 17*). In contrast, the mRNA expression was not detected in cells such as Jurkat, MT-1, U937, ARH-77, and HeLa. Essentially, the amount of mRNA expression correlated with that of the IL-2Rβ chain. We next examined the mRNA expression in normal peripheral blood lymphocytes (PBL's). The IL-2Rβ mRNA was detectable in unstimulated PBL's and its expression increased transiently only 2.5 times after mitogen stimulation (Fig. 3B). Previous data derived from flow cytometric analysis (*19*) make it likely that the mRNA induction patterns differ between the different lymphocyte populations. This expression pattern is quite different from that of the IL-2Rα chain whose expression strictly requires mitogenic stimulation of the cells (Fig. 3B), suggesting the presence of distinct mechanisms of gene expression between the two genes.

IL-2 binding properties of the cDNA-encoded IL-2Rβ chain. We next performed cDNA expression studies in order to determine whether the cDNA product binds IL-2 and indeed manifests the properties of the IL-2Rβ chain that have been demonstrated or suggested (or both) in previous studies. Two cDNA expression plasmids were constructed in which expression of the cDNA spanning the entire coding region was directed by either the mouse *lck* gene (*28*) promoter (pLCKRβ) or Moloney leukemia virus long terminal repeat (*29*) (pMLVRβ) (*30*). The plasmid pLCKRβ was introduced together with neomycin resistance gene into the mouse T lymphoma EL-4 and the human T cell leukemia Jurkat lines (*31*), both of which are devoid of surface molecules that bind human IL-2. Stable transformant clones expressing the cDNA product were obtained for both the EL-4 (ELβ-13) and Jurkat (Jβ-8) cells as judged by FACS analysis (Fig. 4A). In addition, we also introduced the same gene into the Jurkat transformant clone, Jα-5, which expresses the transfected, human IL-2Rα chain cDNA. Two of the resulting transformants, Jαβ-2 and Jαβ-10, expressed both α and β chains (Fig. 4A, d and e). As expected, RNA blotting analyses of the mRNA expressed in those Jurkat transformants revealed that the α and β chain–specific mRNA's are derived from the transfected cDNA's but not from the endogenous genes as judged by size differences between cDNA-derived and endogenous receptor mRNA's (*32*). Furthermore, in order to examine the property of the cDNA product in nonlymphoid cells, the plasmid pMLVRβ was introduced into an NIH 3T3 cell–derived cell line ψ2 (*31*), and the resulting transformant expressing the cDNA, Fβ-3, was obtained (Fig. 4A, f).

The IL-2 binding studies were performed with ^{125}I-labeled recombinant human IL-2 (*11, 12, 14*). The following binding profiles were obtained by Scatchard plot analyses (Fig. 4B). Actually, the EL-4–derived clone (ELβ-13) and the Jurkat-derived clone (Jβ-8), both expressing the β chain cDNA, displayed intermediate-affinity to IL-2 with estimated K_d values of 4.0 n*M* and 2.7 n*M*, respectively. The IL-2 binding to those cells was completely abolished by the Mik-β1 antibody (Fig. 4B, a and b). The Jurkat-derived Jαβ-2 and Jαβ-10 clones expressing both the human IL-2Rα and IL-2Rβ cDNA displayed both high- and low-affinity receptors with estimated K_d values of 22 p*M* and 15 n*M* for Jαβ-2 and 19 p*M* and 33 n*M* for Jαβ-10, respectively (Fig. 4B, d and e). In contrast, the parental, Jurkat-derived Jα-5 cells expressing the α chain cDNA

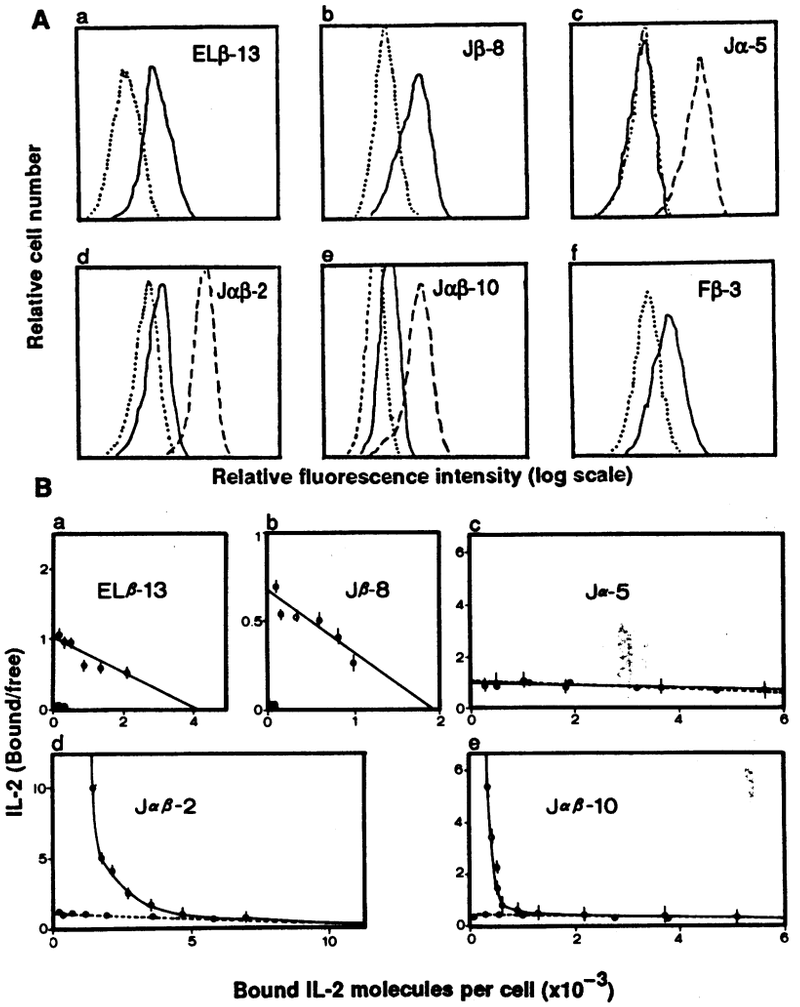

Fig. 4. Expression of human IL-2Rα or IL-2Rβ chain cDNA's. (**A**) Cell surface staining patterns of human IL-2Rα or IL-2Rβ cDNA (or both) transformants. Parental cells and various transformant cells were separately stained with either a monoclonal antibody to human IL-2Rα, anti-Tac (– – –), or monoclonal antibody to human IL-2Rβ, Mik-β1 (——). Dotted line (. . . . .) is a fluorescence profile of the cells stained with fluorescein-conjugated goat-anti-mouse immunoglobulin G alone. Cells used were (a) ELβ-13 (an EL-4–derived clone transfected with pLCKRβ), (b) Jβ-8 (a Jurkat-derived clone transfected with pLCKRβ), (c) Jα-5 (a Jurkat-derived clone transfected with pSVIL2Rneo), (d) Jαβ-2 (a Jα-5–derived clone transfected with pLCKRβ), (e) Jαβ-10 (a Jα-5–derived clone transfected with pLCKRβ), and (f) Fβ-3 (a NIH 3T3–derived line transfected with pMLVRβ). (**B**) Scatchard plot analysis of ^{125}I-labeled IL-2 binding to the transfectants expressing the cloned cDNA's. Scatchard plot of the IL-2 binding data in the absence (○—○) or presence (●—●) of 1:100 diluted ascites of Mik-β1. Binding of ^{125}I-labeled IL-2 to ELβ-13 or Jβ-8 was completely abolished by Mik-β1. Each cell line was examined twice and the findings were reproducible. The nonspecific IL-2 binding was less than 10 percent of the total bound IL-2. No specific IL-2 binding was observed when parental Jurkat or EL-4 cells were examined. The number of IL-2 binding sites per cell and the receptor affinity K_d were determined by computer-assisted analysis of the IL-2 binding data. (a) ELβ-13, 4100 sites per cell, K_d, 4.0 n*M*; (b) Jβ-8, 1900 sites per cell, K_d, 2.7 n*M*; (c) Jα-5, 18,300 sites per cell, K_d, 19.5 n*M*; (d) Jαβ-2, 1250 sites per cell, K_d, 22 p*M*; 13,500 sites per cell; Kd, 15 n*M*; (e) Jαβ-10, 400 sites per cell, K_d, 19 p*M*; 12,500 sites per cell, K_d, 33 n*M*.

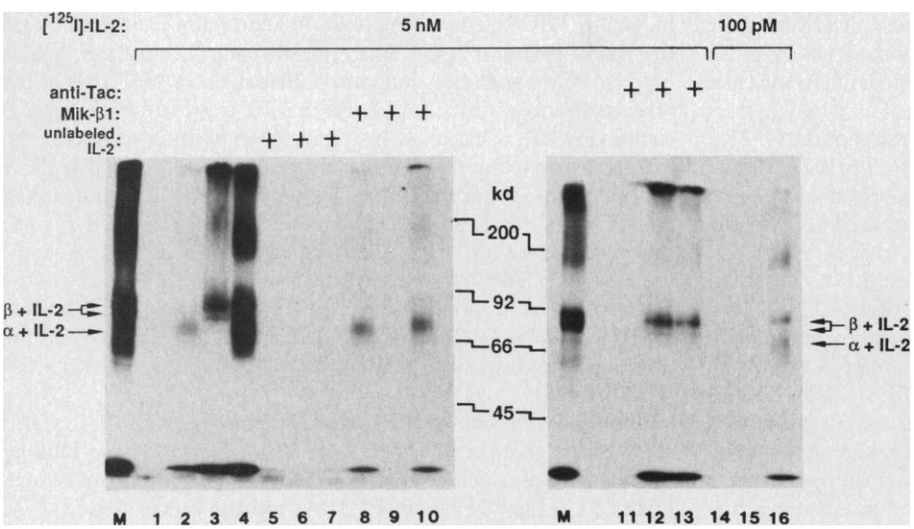

Fig. 5. Affinity cross-linking studies of the IL-2R–positive transformants. Cells were incubated with 5 n*M* (lanes 1 to 13) or 100 p*M* (lanes 14 to 16) ^{125}I-labeled IL-2 in the absence (lanes 1 to 4, 14 to 16) or presence of a molar excess (250-fold) of unlabeled IL-2 (lanes 5 to 7), a molar excess (500-fold) of affinity column–purified Mik-β1 (lanes 8 to 10) or a molar excess (500-fold) of affinity column–purified anti-Tac (lanes 11 to 13). Then cells were chemically cross-linked with dissuccinimidyl suberate (DSS) (*16*). The cells were then solubilized, and the lysates were subjected to 7.5 percent SDS-PAGE. Two independent gels were run simultaneously with lysates from the same cell number (5×10^6). The cross-linking efficiencies and the exposure times (4 days at −70°C) were the same. The cells used were Jurkat (lane 1); Jα-5 (lanes 2, 5, 8, 11, 14); Jβ-8 (lanes 3, 6, 9, 12, 15); Jαβ-10 (lanes 4, 7, 10, 13, 16). YT cells cross-linked with ^{125}I-labeled IL-2 were used as a marker (M).

alone manifested exclusively low affinity (K_d, 19.5 n*M*) to IL-2 (Fig. 4B, c). The number of the high-affinity IL-2R expressed on Jαβ-2 and Jαβ-10 cells was comparable to that of expressed IL-2Rβ molecules.

In addition, treatment of these cells with Mik-β1 antibody completely abolished high-affinity IL-2 binding sites from the cell surface, while retaining the expression of low-affinity IL-2R (Fig. 4B, d and e). These observations demonstrate unequivocally that the cDNA-encoded IL-2Rβ molecule is directly involved in the formation of high-affinity receptor complex in association with the IL-2Rα chain. In contrast to the T cell transformants described above, the Fβ-3 cells did not show any IL-2 binding on the cell surface under the same binding conditions. The same observation was made with monkey COS cells that express the β chain, but failed to bind

IL-2 (*32*). Thus, the results suggest the involvement of either a cell type–specific processing mechanism or an additional cellular component, or both for the functional IL-2Rβ chain expression.

In order to characterize further the molecular structure of reconstituted IL-2R, we performed chemical cross-linking experiments with ^{125}I-labeled IL-2 and the noncleavable chemical cross-linker, dissuccinimidyl suberate (DSS). When cells expressing only IL-2Rβ chain were cross-linked with ^{125}I-labeled IL-2 and analyzed by SDS–polyacrylamide gel electrophoresis (PAGE), a doublet band consisting of 90 kD major and 85 kD minor was detected and its migration profile was indistinguishable from that of YT cells (Fig. 5, arrows) (*16, 17*). The appearance of the doublet was inhibited by an excess of unlabeled IL-2 or by Mik-β1. The doublet formation may be due to degradation of the receptor–IL-2 complex. It is also possible that both protein products are derived by a differential posttranslational modification. Alternatively, one of the doublet may represent a third component of the receptor complex. A broad band migrating around the position of 150 kD was also detected in the transformant (Jαβ-10) as well as YT cells (Fig. 5, lanes M and 4). The appearance of this band is also inhibited by either unlabeled IL-2, anti-Tac, or Mik-β1 (Fig. 5, lanes 7, 10 and 13); it may represent the ternary complex of IL-2, IL-2Rα, and IL-2Rβ molecules. In a series of chemical cross-linking experiments (Fig. 4), the properties of the receptor complex expressed on the surface of Jαβ-10 were indistinguishable from those of high-affinity receptor expressed on cultured T cell lines or PBL's (*12, 16, 17*).

Whether the expression of the α and β chains in nonlymphoid cells results in the formation of high-affinity receptor is not known. However, we have some data indicating that, when the α and β chain cDNA's are coexpressed transiently in COS cells, both chains can cross-link with ^{125}I-labeled IL-2 at the concentration (400 p*M*) where the similarly expressed α chain alone cannot (*32*). The results may suggest the formation of the αβ heterodimeric receptor in this nonlymphoid cell line.

IL-2 internalization by reconstituted receptors. Intermediate- and high-affinity IL-2 receptors can both internalize IL-2 (*33–35*). Ligand internalization is usually accompanied with the IL-2 signal transduction, suggesting that this process is essential. In experiments on internalization of IL-2 by reconstituted receptors (Fig. 6), the cells expressing IL-2Rβ chain alone, or both α and β chains were found capable in internalizing IL-2 as judged by a kinetic pattern similar to that of the native receptor. In contrast, the Jurkat cells expressing only IL-2Rα failed to internalize IL-2, similar to previously reported observations (*33, 34*). We have some results indicating that the growth of the cells expressing the intermediate- or high-affinity receptors is selectively inhibited by IL-2 (*14, 36*). We also have some results that the β chain expressed in another host cell line functions in stimulating the cell growth in response to IL-2 (*32*).

Signal transduction in IL-2 system. The availability of the gene encoding the IL-2Rβ chain makes it possible to explore novel approaches for the functional studies of the IL-2 system. As revealed from our study as well as previous reports, the receptor structure operating in the IL-2 system is unique in that two structurally distinct membrane molecules, the IL-2Rα and IL-2Rβ chains, both bind IL-2 independently. The series of cDNA expression studies in this report substantiate further the previous notion that the α and β chains constitute the high-affinity IL-2R complex via a noncovalent association of the molecules (*18, 37*). Thus the peculiarity of this system is the involvement of three intermolecular interactions between one ligand and two distinct receptor components. The availability of both receptor cDNA's (α and β) will make it possible to elucidate functional domains of this cytokine receptor system. Mutational analyses of the cloned β chain cDNA may provide clues as to the identification of respective domains involved in ligand

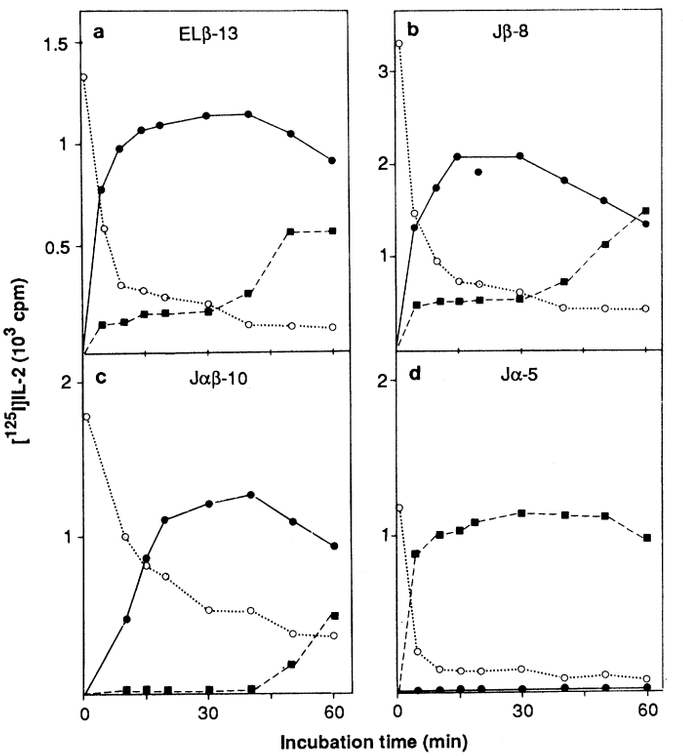

Fig. 6. IL-2 internalization via the reconstituted receptors. IL-2 internalization was examined as described (*35*). Briefly, cells (5×10^7) were treated with ^{125}I-labeled IL-2 at a final concentration of 200 p*M* (Jαβ-10) or 5 n*M* (Jα-5, Jβ-8, and ELβ-13) at 0°C for 30 minutes. The cells were suspended in warm culture medium (37°C), and the kinetic pattern of IL-2 internalization was examined (*35*). (**a**) ELβ-13, (**b**) Jβ-8, (**c**) Jαβ-10, (**d**) Jα-5. (-●-●-●-), internalized IL-2; (··○··○··○··), cell-surface bound IL-2; (-■--■--■-), free IL-2.

binding and association with the α chain. To date, little is known about the cascade of biochemical events triggered by cytokines interacting with their homologous receptors. Our findings on the structure of the IL-2Rβ chain demonstrate the presence of a large cytoplasmic region which most likely is involved in driving the IL-2 signal pathways. The particular acidic nucleus found in the cytoplasmic region may suggest coupling to other cytoplasmic signal transducers. Alternatively, in view of a previous report on the presence of IL-2 within the nucleus (*33*), the possibility exists that the acidic as well as the proline-rich regions of the IL-2Rβ cytoplasmic component may participate in activation of the genetic programming. The availability of the expression system in which the cDNA-encoded β chain can deliver growth signals should allow us to dissect further the functional domains of the receptor. Furthermore, the mouse homolog of the IL-2Rβ chain has been cloned recently (*32*), and therefore it should now be possible to study the essential role of IL-2 in the development and regulation of the immune system.

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30. Expression vectors were constructed by the following procedures: pIL-2Rβ 30 was digested with Hind III (the cleavage site is located within the polylinker regions of CDM8) and, after fill-in both ends, a Bam HI linker was attached and religated. The resulting plasmid was then digested with Bam HI, and the 1.8-kb DNA fragment which contains the entire coding sequence for the β chain was introduced into Bam HI–cleaved p1013 vector containing the mouse *lck* promoter (provided by R. Perlmutter, University of Washington) to construct pLCKRβ. The Bam HI–digested cDNA fragment was also introduced into a retrovirus vector, pZipSV(X) (*29*), to construct pMLVRβ. The human IL-2Rα expression vector, pSVIL2Rneo, was obtained from pSVIL2R-3(*14*) by replacing the Eco-gpt gene with the neo-resistance gene.
31. The expression plasmids were transfected into Jurkat and EL-4 by electroporation as described (*39*). Transfected cells were selected in the RPMI 1640 medium containing 10 percent fetal calf serum (FCS) and G418 (1 mg/ml for EL-4 and 1.5 mg/ml for Jurkat). To obtain cells expressing cDNA's for human IL-2Rα and IL-2Rβ chains simultaneously, a Jurkat-derived clone Jα-5, transfected with pSVIL2Rneo, was co-transfected with pLCKRβ and a plasmid containing the hygromycin resistance gene, pHyg (provided by F. Grosveld, MRC, London). The transfected cells were selected with hygromycin at 200 μg/ml. Transfection of pMLVRβ into Ψ2 cells was carried out by calcium-phosphate method (*14*) and the cells were selected by G418 at 700 μg/ml. For flow cytometric analysis, 5×10^5 cells were treated with antibody (1:500 dilution of ascites) at 4°C for 30 minutes. After washing, cells were stained with fluorescein-conjugated goat antibody to mouse immunoglobulin G. The stained cells were analyzed on a FACS440 flow cytometer (Beckton Dickinson). The ^{125}I-labeled IL-2 binding assay and Scatchard plot analysis were carried out as described (*12*).
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"Your urine sample contained traces of malaise and cynicism."