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Signal transduction by interleukin 2 receptor β chain:
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receptors.

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Key words

interleukins
high-affinity receptor
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Abbreviations

DSS : disuccinimidyl suberate
EPO : erythropoietin
IL-2 : interleukin-2
IL-2R : interleukin-2 receptor
IL-3 : interleukin-3
T cells : T lymphocytes

Abstract

The functional interleukin-2 receptor(IL-2R) consists of at least two IL-2 binding cell surface molecules, IL-2R α and IL-2R β , the latter component is responsible for the intracellular growth signal transduction. In this study, we attempted to identify the critical amino acid residues in the cytoplasmic domain of the human IL-2R β for such signal transduction by expressing mutated IL-2R β cDNAs in a pro-B cell line, BAF-B03. We demonstrate that a single amino acid substitution within the "Serine-rich" cytoplasmic region of IL-2R β (i.e. leucine(Leu) 299 change to proline(Pro)) completely abrogates the receptor function in growth stimulation, but not in ligand binding. We also show that the murine erythropoietin receptor(EPO-R) is functional in BAF-B03, but that chimeric receptors, essentially possessing the IL-2R β extracellular and a homologous region derived from EPO-R cytoplasmic domain, are not capable of transducing the IL-2-induced signal.

Introduction

Antigen-specific clonal expansion of T lymphocytes (T cells) depends on the generation and response to IL-2. In fact, the regulated expression of IL-2 and its receptor (IL-2R) is one of the central events in the clonal T cell proliferation and differentiation (1,2,3,4). IL-2 is also known to affect the biological activity of other haematopoietic cells such as B lymphocytes (5,6), natural killer cells (7), macrophages (8), and cells of the central nervous system (9, 10). Two distinct components of IL-2R have been identified in humans and rodents; the IL-2R α chain (p55) (11,12,13), and the IL-2R β chain (p70-75) (14,15,16,17,18). The IL-2R α and IL-2R β components together form the high-affinity receptor which can transduce the ligand mediated proliferation signal (19).

Functional analyses of IL-2R α and IL-2R β chains expressed by cDNA transfection have revealed that only the latter is capable of transducing signal through a characteristic cytoplasmic domain (10,19,20). Expression studies of human IL-2R β cDNAs each carrying deletion mutation within the region for the cytoplasmic domain have allowed us to identify the critical region for the IL-2-induced growth signal transduction in a pro-B cell line, BAF-B03. The critical region includes a stretch of 46 amino acids, peculiar for the abundance of hydrophobic amino acids and serine residues, defined as "Serine-rich" region (18,19).

With the recent progress in the structural analysis of other cytokine receptors, it became clear that the IL-2R β is

structurally related to the receptors of IL-3(21), IL-4(22, 23,24), IL-6(25), IL-7(26), erythropoietin(EPO)(27), granulocyte-macrophage colony stimulating factor(GM-CSF)(28), granulocyte colony stimulating factor(G-CSF)(29), as well as to the receptors of growth hormone(GH)(30), and prolactin(PRL)(31).

It is important to note that a high degree of structural homology exists between the murine EPO-receptor(EPO-R) and IL-2R β chain(32). The shared homology between these two receptors is particularly characterized by the extracellular segment I which contains Trp-Ser-X-Trp-Ser (W-S-X-W-S) motif as well as the cytoplasmic, membrane proximal segment II(33). Interestingly, this latter shared homology segment II corresponds to the critical signal transducing domain of IL-2R β chain as described above. These observations thus indicate the possible functional similarity of the two receptors.

In order to analyse further the structure-function relationship of the IL-2R β cytoplasmic region, we generated mutations in the hydrophobic amino acid residues of the critical region and analysed the function of the mutants. We also examined whether the structurally related EPO-R is also functionally related to the IL-2R β , by expressing cDNAs encoding either the intact mouse EPO-R or the IL-2R β -EPO-R chimeric molecules.

Methods

Construction of expression plasmids.

The site-directed mutagenesis experiments were performed essentially according to a method described previously(34). Briefly, we first subcloned 1Kb SacI-BamHI fragment corresponding to IL-2R β chain cytoplasmic region from pIL-2R β 30(18) into M13mp11 vector. Using single strand DNA of this recombinant vector and three synthetic oligonucleotides each consisting of 1)5'TCCAGAAGTGGCCCTCTTCGCCCTT3', 2)5'CTGGCACCTGAGGTCTCGCCACCAGAAGTGCTGGAG3', and 3)5'GGCGGCCTGGCAGCTGAGATCTCGCCA3', a series of site directed mutations were introduced. The mutations were confirmed by nucleotide sequencing and the 170bp SacI-AflIII fragment was excised from each of the recombinant M13 vector. Each fragment containing a specific mutation was then replaced with the SacI-AflIII fragment of wild type IL-2R β cDNA which had been inserted into BamHI site of pUC19(pCU β 1). The mutated IL-2R β cDNAs were excised from the pUC19 vector with BamHI and inserted into BamHI site of p1013 expression vector(18).

For the construction of chimeric receptors, pUC β 1 was used. In the case of the chimeric receptor pBCE, the pUC β 1 was first cut with NcoI, filled both ends with T4 polymerase and then cut with SalI to isolate the 3.6Kb fragment containing pCU19 vector and IL-2R β cDNA corresponding to the extracellular, transmembrane, and the 8 amino acids of the

cytoplasmic domain. The EPO-R expression vector (pXM(ER)-190) (27) was first cut with BglII and after filling the ends, cut with XhoI to isolate the 880bp fragment corresponding to the cytoplasmic domain of EPO-R. These two 3.6Kb and 880bp fragments were purified by agarose gel electrophoresis and ligated. The resulting chimeric DNA was excised with EcoRI complete digestion and HindIII partial digestion. After filling both ends with T4 DNA polymerase, the chimeric DNA was ligated with p1013 vector whose BamHI site had been filled with T4 polymerase. In the case of pBEE, the pUC β 1 was cut with EcoT14I and after blunt-end conversion, cut with SalI to isolate the 3.5Kb DNA fragment contains pUC19 vector and IL-2R β cDNA corresponding to the extracellular domain. The pXM(ER)-190 was first cut with NheI and after filling ends, cut with XhoI to isolate the 980bp fragment corresponding to the C-terminal 6 amino acids of the extracellular domain, the transmembrane region, and the cytoplasmic domain of EPO-R. The resulting chimeric cDNA was cut with EcoRI completely and HindIII partially and then introduced into p1013 vector as described above.

DNA transfection and isolation of mutant receptor expressing cells.

The proB, BAF-B03 cells were cultured in RPMI-1640 medium supplemented with 10% FCS and 20% WEHI3B culture supernatant as source of IL-3. Each of cDNA expression vector and neo-resistance gene(pSTneoB(35)) were transfected into BAF-B03 cells by electroporation as previously described(18).

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After selection with G418(2mg/ml), IL-2R β positive cells were analyzed by flow cytometry using mouse anti-human IL-2R β monoclonal antibody, Mik- β 1(36).

Positive cells were propagated after cloning by limiting dilution.

IL-2 binding assay.

IL-2 binding assay was performed as described previously(18). Cells($3-5 \times 10^6$ /ml) were incubated with ^{125}I -labeled IL-2 (Takeda Chemical Co.) in the presence or absence of 250 fold excess cold IL-2. After incubation (at 4 $^{\circ}\text{C}$, for 30minutes), bound and free IL-2 were isolated by centrifugation through an oil cushion and specifically bound IL-2 was determined by γ -counter.

IL-2 internalization assay.

Cells (5×10^7 /ml) were incubated with 100pM of ^{125}I -IL-2 on ice for 20 minutes. Following one wash, the medium was changed and the incubation shifted to 37 $^{\circ}\text{C}$. To analyse the kinetics of IL-2 internalization, cells were harvested at the indicated times, washed, and treated with acid buffer. The internalized and surface bound IL-2 were separated by centrifugation through an oil cushion. The free, surface bound, and internalized IL-2 were measured.

Chemical cross-linking study.

Cells (5×10^6) were preincubated with or without excess amount of cold IL-2 for 1 hour at 4°C. Subsequently 200pM of ^{125}I -labeled IL-2 were added for another 1 hour at 4°C. The cells were then treated with the chemical cross-linker DSS (Pierce Chemical Co.) as described previously(18), and after extraction, the solublized membrane preparation was ran on a 8 % SDS-PAGE under reducing conditions.

Cell growth assay.

Cells (1×10^4) were cultured in RPMI-1640 medium with various concentrations of IL-2 for 24 hours in a 96-well microculture plate. $1 \mu\text{ci}$ of ^3H -thymidine was then added to each well, during the last 4 hours of incubation and the cells were harvested. The ^3H -thymidine uptake were measured and compared to that of cultured cells in RPMI-1640 medium supplemented with 20 % of WEHI3B supernatant.

Results

Construction of IL-2R β chain mutants.

To analyse the relationship between the structure of the critical cytoplasmic region and growth signal transduction function in detail, we introduced a series of mutations in this region using synthetic oligonucleotide primers. Since the region is characterized by the relative abundance of hydrophobic amino acids(19), we therefore focused on some of these residues as outlined in Fig.1. In fact, the Leu 278 (the number of amino acid is the same as previously described (18)) and Leu 299 residues are conserved in human and mouse IL-2R β chain, as well as in mouse EPO-R. These Leu residues were exchanged with Pro to generate the mutants SM1 and SM4, respectively. A mutation was also introduced by replacing the conserved Ile 296 with Val(SM3). A double mutation of Ile 296 and Leu 299 residues exchanged with Val and Pro, respectively, were also generated(SM2).

Another interesting structure was Ala(293)-Pro-Glu triplet which is highly conserved in the catalytic subdomain VIII of protein kinases(37). Although there is no evidence that IL-2R β chain has kinase activity, it was shown that IL-2 induces a rapid phosphorylation of cellular proteins (38,39,40) and IL-2R β chain itself(41,42). An exchange of Pro 294 with Ala was made to correspond to a mutation in this region (SM5)(Fig.1).

Expression and signal transduction of mutant IL-2R β chains.

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Each of the above mutant IL-2R β chain cDNA were inserted in the expression vector p1013(18) and transfected into BAF-B03 cells with neo-resistance gene (pSTneoB)(35) using electroporation. Following selection with G418 (2mg/ml), the neo-resistant clones were analysed by flowcytometry with mouse anti-human IL-2R β chain-specific monoclonal antibody Mik- β 1(36)(see Methods for details). Using cross-linking experiments, the molecular mass of the mutated IL-2R β chains were analysed, the size determined was similar with that of the wild type(data not shown).

As shown in Fig.2 a), Scatchard plot analyses revealed that the transformant cells expressing mutant IL-2R β chains formed high affinity receptor (dissociation constant(Kd): 53-176pM) and the number of expressed receptor sites varied between 4500-21000 sites/cell.

Since there was no drastic alteration in the affinity of the mutated IL-2R complex to IL-2, we next analysed growth signal transducing function. The growth promoting activity of IL-2 on the cell transformants was monitored on the basis of ³H-thymidine uptake as shown in Fig.2b). Cells expressing SM-1 mutant receptors (Leu 278 changed to Pro) showed lower growth response compared to the wild type receptor expressing cells(F7) at concentrations of 10pM and 100pM of IL-2, but the response was comparable at a higher ligand concentration, (1nM). Cells expressing SM-2 (Ile 296 and Leu 299 changed to Val and Pro, respectively) were not responsive at all the concentrations of IL-2 tested. Cells expressing SM-3

mutant (Ile 296 changed to Val) displayed an unchanged IL-2 growth response comparable to F7. Cells expressing SM-4 mutant (Leu 299 changed to Pro) had a similar growth response profile as SM-2; i.e. they were actually unresponsive to IL-2. On the other hand, the cells expressing SM-5 mutant (Pro 294 changed to Ala) showed the same growth response pattern as SM-1 expressing cells. The above site-specific mutagenized IL-2R β s strongly suggest the importance of Leu 299 in IL-2 mediated signal transduction. Moreover this Leu is located in the four amino acids stretch highly conserved in both human and mouse IL-2R β chain and mouse EPO-R(Fig.1).

Functional expression of EPO-R in BAF-B03 cells.

Because of the conserved homology in certain domains of IL-2R β and mouse EPO-R, the EPO-R expression plasmid pXM(ER)-190(27), (provided kindly by Dr. D'Andrea, Whitehead Institute, Boston) was cotransfected with the neo-resistance gene.

As shown in Fig.3, EPO-responsive transfectants were obtained, unlike the parental BAF-B03 cells or neo-resistant control cells. During the course of this study, the same observations have been reported by Li et al.(43) and Yoshimura et al.(44) using BAF-B03 parental cell line BA/F3. The above observations suggested further that IL-2 and EPO deliver growth signal to BAF-B03 cells through some common pathway, possibly involving the conserved homology domain(s) in their receptors.

As an approach to address this question, we proceeded in constructing chimeric IL-2R β molecules which contained various domains from EPO-R and analysed the signal transduction profile of the chimeric IL-2R β transfectants to IL-2 stimulation.

Construction and expression of chimeric receptors.

As depicted in Fig.4, two kinds of chimeric receptor expression plasmids were constructed. The pBCE construct contained the extracellular, transmembrane, and 8 amino acids of the cytoplasmic region derived from IL-2R β chain, the remaining cytoplasmic region was derived from EPO-R. The pBEE construct had the extracellular domain derived from IL-2R β chain and the remaining 6 amino acids of extracellular, the transmembrane, and the cytoplasmic regions derived from EPO-R.

Each of these two expression plasmids was transfected into BAF-B03 cell line with the neo-resistance gene pSTneoB. Following selection with G418, Mik- β 1 reactive transfectants were identified using flowcytometric analysis, since these chimeric receptors had the extracellular IL-2R β chain and the epitope for Mik- β 1 antibody. Expression of the predicted size chimeric receptors was confirmed using chemical cross-linking study with ^{125}I -labeled IL-2. These two chimeric receptors showed slightly smaller size as predicted and had a gel migration pattern relatively faster than the wild type IL-2R β chain(Fig.5 a)).

Chimeric receptors form high affinity IL-2R complex.

The affinity and the number of expressed chimeric IL-2R complex was measured. As shown in Fig.5 b), Scatchard plot analysis confirmed that even though these transfectants expressed a chimeric receptor, their extracellular ligand binding domain was strictly derived from IL-2R β .

In fact, high affinity IL-2 binding was observed with a Kd of 13-58pM, with 1800-6000 binding sites/cell. Moreover, these observations indicated that the extracellular domain of IL-2R β was sufficient for high affinity receptor formation.

IL-2 internalization and signal transduction through chimeric receptors.

In a previous study using IL-2R β chain deletion mutants, it was observed that internalization is not sufficient for signal transduction(19). The profile of IL-2 internalization mediated by chimeric receptor expressing cells was measured and compared to the wild-type expressing cells. As shown in Fig.6, cells expressing each of these chimeric receptors internalized IL-2 as efficiently as the wild type.

Although, each transfectant expressing the specific chimeric receptor could bind IL-2 with high affinity, and efficiently internalize IL-2, as shown in Fig.7 , they failed to transduce

the growth signal as measured by the uptake of ³H-thymidine.

Discussion

The unique feature of the IL-2R system is the presence of two distinct receptor components designated IL-2R α chain (p55) and IL-2R β chain (p70-75). Evidence has been provided that only a combination of IL-2R α and β might carry a physiological signal(14,15,16,17), although in some cell lines only IL-2R β might transduce the IL-2 signal, provided that IL-2 is present at high concentrations($\geq 1nM$) (45,46). We have reported previously that the IL-2R β is of critical importance in IL-2-dependent signal transduction (10,19,20). Furthermore, a certain level of homology has been observed among other hormone and cytokine receptors(47). However, this homology is confined mainly to the extracellular region particularly the position of four Cys residues and the Trp-Ser-X-Trp-Ser(W-S-X-W-S) conserved motif. To date only IL-2R β and EPO-R seem to have an apparent cytoplasmic conserved homology domain(32,33).

Careful dissection of the different domains in the cytoplasmic region of IL-2R β has revealed that this region can be divided into the so-called a) Serine-rich, b) Acidic, and c) Proline-rich domains(18). It has been found that the Serine-rich domain is essential for growth signal, and this domain shows a shared homology with EPO-R cytoplasmic region(19,32,33).

The conservation of the hydrophobic amino acids in the homology region between IL-2R β and EPO-R was one of the obvious sites to explore. The various single site-directed

mutants starting with SM-1 (Leu 278 changed to Pro) and SM-3 (Ile 296 changed to Val) had no dramatic effect on signal transduction. In the case of SM-1, the profile of growth response to IL-2 was rather low at concentrations of 10pM and 100 pM compared to the wild-type. However, the response was indistinguishable at high concentrations of the ligand. It is hard to give an explanation for this difference in response, such a change in response pattern can be expected only if there were affinity differences. But, no alterations were introduced in the extracellular, ligand-binding domain of the receptor. It will remain to clarify this difference. It could be speculated that this difference is due to the choice of the amino acid residue, Pro, which lacks the side chains similar to the native Leu residue. It could also be that the threshold of ligand-mediated signal might be different in the mutant compared to the wild type.

The SM-3 mutant (Ile 296 to Val) did not affect the ligand-mediated signal transduction. Although it is understood that by its nature Val has many physico-chemical similarities with the replaced Ile residue. However, a replacement of Leu 299 to Pro (SM-4) resulted in a receptor structure which could not transduce the IL-2 signal. This is indicative that this amino acid residue, which is conserved in both human and murine IL-2R β chains, is essential in the signal pathway. Moreover, this residue is also conserved in mouse EPO-R(Fig.1).

Other mutations involving two amino acid residues as in the example of SM-2, replacing Ile 296 with Val and Leu 299 with Pro abolished the biological signal, again indicating the

importance of the latter residue.

Our findings point out to the essential role of Leu 299 and it is possible that due to its nature this amino acid imparts a certain stability to this particular domain by affecting the tertiary conformation of the intracellular domain.

In fact, Chou-Fasman's program(48) predict that the Leu 299 residue might participate in the helix formation of the IL-2R β chain(unpublished data). Substitution of this Leu residue to Pro might disrupt the helical structure, thus a distortion of the helical configuration can result in the interruption of the IL-2 signal, indicating the importance of the helical integrity of the IL-2R β chain.

The Ala(293)-Pro-Glu motif is shared in human and murine IL-2R β , it has also been found to be conserved in the catalytic subdomain VIII of protein kinases(37). It might therefore have a potential enzymatic activity. The SM5 mutant was constructed (Pro 294 to Ala) to measure the importance of this motif. As in the case of SM-1, the profile of IL-2 response was rather weak at low ligand concentration, but indistinguishable from the wild-type at very high concentrations of IL-2. Furthermore, even if the Ala-Pro-Glu stretch takes part in kinase activity, it is apparently not critical. As in the case of SM-1, it affects the magnitude of response at low ligand concentrations. The decreased sensitivity may be the result of low enzymatic activity or a shift in the equilibrium of interaction with the putative signal transducing molecule which may be located downstream of

IL-2R β .

Unlike the homology with the extracellular domain of many cytokine receptors, only EPO-R and IL-2R β chain are found to share a conserved segment in their cytoplasmic component. With the conserved homology in the cytoplasmic region and the biological effect on BAF-B03 cells, it was assumed that the region of homology might be the site for a common catalytic action, or the site of interaction with a possibly common intracellular signal mediator. In order to address this question, two kinds of chimeric IL-2R β -EPO-R constructs were made and transfected into BAF-B03 cells. In fact, chimeric receptors are convenient tools to analyse the structural and functional role of particular domains. It has been reported that a chimeric epidermal growth factor receptor (EGF-R) and its homologous proto-oncogene product erb-B2 or neu can actually transduce EGF-mediated growth signal(49,50). Similarly EGF-R and insulin receptor (IR) chimeric molecule can activate IR tyrosine kinase when stimulated with EGF(51). Although, the above examples use homologous receptors, in fact, unrelated receptor structure can actually be functional, as in the case of bacterial aspartate and IR chimeras which respond to aspartate signal(52). Unlike EGF or insulin mediated signal transduction, the signal mechanism of recently identified cytokines remains a mystery.

A strict replacement/exchange of the conserved homology domains between IL-2R β and EPO-R cytoplasmic domains (Fig.4 pBCE) could not mediate IL-2 growth signal. It is

possible that the lack of signal might be due to improper conformation of the replaced domain. In order to address this possibility the entire cytoplasmic domain, including the transmembrane region, were exchanged with that of EPO-R(pBEE). Such transfectants displayed no effect in their interaction with the ligand, but failed to transduce the signal.

As indicated above, the lack of signal might be due (1) to the nature of the chimeric receptor and/or (2) to an interruption in the flow of the signal at the junction of IL-2R β and EPO-R components. It remains to elucidate if the signal pathway mediated by either of these cytokine converge further downstream.

In the present study, we have explored in detail the critical cytoplasmic domain of IL-2R β in mediating IL-2 growth signal. The site-directed mutagenesis experiments have revealed the presence of specific amino acid residues essential for the biological signal. The chimeric receptors have revealed that the extracellular domain of IL-2R β is sufficient for high affinity IL-2R formation and IL-2 internalization. But these chimeric receptors were insufficient for signal transduction. The above results indicate that the structure or the integrity of IL-2R β chain is important for the competence of signal transduction.

Figure legends

Figure 1. Schematic structure of IL-2R β mutants.

Boxed area is the homology segment II of mouse EPO-R, mouse IL-2R β and human IL-2R β . The alignment is the same as in Kono et al.(33). Asterisks and open circles represent identical amino acids (single letter) and conservative substitutions, respectively. The part between the two arrowheads is the critical region for growth signal transduction of human IL-2R β chain identified previously(19). The lower part depict the structure of mutant of IL-2R β . The amino acid sequences are illustrated. Triangles represent target amino acids for substitution. (-) represent the same amino acid as the wild type human IL-2R β .

Figure 2. Expression and signal transduction of IL-2R β mutants.

a) Scatchard plot analysis of mutant IL-2R β expressing cells. BAF-B03 transformants expressing the mutant IL-2R β (SM1-5) were examined for their IL-2 binding activity using ^{125}I -labeled recombinant human IL-2 as described(18). The calculated high affinity receptor numbers (No) and dissociation constants (Kd) are as follows: SM1-1; (No:6210, Kd:165pM), SM1-2; (No:4470, Kd:176pM), SM2-1; (No:14230, Kd:130pM), SM2-2; (No:21250, Kd:170pM), SM3-1; (No:7190, Kd:53pM), SM3-2; (No:4290, Kd:83pM), SM4-1; (No:8110, Kd:67pM), SM4-2;(No:5650, Kd:73pM), SM5-1; (No:6940, Kd:74pM), SM5-2;

(No:13180, Kd:161pM).

b) IL-2-dependent growth of BAF-B03 transformants expressing wild type and mutant human IL-2R β s. Transformant clones were cultured with various concentrations of human IL-2 and analysed for their ability to incorporate ^3H -thymidine into DNA. The results are expressed as the percentage of incorporation of ^3H -thymidine in the same cells incubated with 20% WEHI3B-conditioned medium. Data are the average of triplicate counts. F-7 is the wild type IL-2R β expressing cell line. N is the IL-2R β negative cell line. SM1-5 are the mutant IL-2R β expressing cells.

Figure 3. EPO-dependent growth of parental BAF-B03 and transformant cells.

Cells were cultured in various concentrations of EPO.

^3H -thymidine incorporation was measured in the same way as in Figure 2 b). BAF-B03 (\square) is the parental cell line of transformants. N (\triangle) is the neo-resistant cell line. 1, 5, and 12 (\bullet) are neo-resistant and EPO-responsive transfectants.

Figure 4. Schematic structure of IL-2R β (pUC β -1), EPO-R(pXM(ER)-190), and chimeric receptor plasmids(pBCE and pBEE).

EC : extracellular domain, TM : transmembrane domain, CP : cytoplasmic domain, respectively. See Methods for details of construction.

Figure 5. Expression of chimeric receptors.

a) Chemical cross-linking of IL-2R β and chimeric molecules with ^{125}I -IL-2. Cells were incubated in the absence (-) or presence (+) of cold IL-2 (250 fold excess). Subsequently cells were incubated with 200pM of ^{125}I -IL-2 and the cross linker DSS. Cell lysates were analysed on 8% SDS-PAGE. Complex for ^{125}I -IL-2 and mouse IL-2R α (\blacktriangleright) and for ^{125}I -IL-2 and wild type IL-2R β or chimeric receptors (\blacktriangleright) are indicated. The cell clones used were: N, IL-2R β negative; F-7, expressing wild type IL-2R β ; BCE1, expressing BCE chimeric molecule; BEE19, expressing BEE chimeric molecule.

b) Scatchard plot analysis of BAF-B03 transformant cell lines expressing chimeric receptors. The calculated numbers(N_0) and dissociation constants(Kd) of high affinity IL-2R expressed on each of transformant are as follows: BCE1; (N_0 :6000, Kd:58pM), BCE6; (N_0 :4200, Kd:38pM), BEE6; (N_0 :4250, Kd:43pM), BEE19; (N_0 :1830, Kd:13pM).

Figure 6. IL-2 internalization of the transformant cells. IL-2 internalization was measured as described(18). (\bullet) internalized IL-2, (\bullet \square \bullet) cell-surface bound IL-2, (\blacktriangle) free IL-2 and (\blacktriangle) the sum of all three fractions are graphed.

Figure 7. IL-2 dependent growth of BAF-B03 transformant cell lines expressing the chimeric receptor.

^3H -thymidine incorporation is expressed in the same manner as in Figure 2 b). F-7 is the wild type IL-2R β expressing cells. N is the IL-2R β negative cells. BCE are expressing BCE chimeric molecules. BEE are expressing BEE chimeric

molecules.

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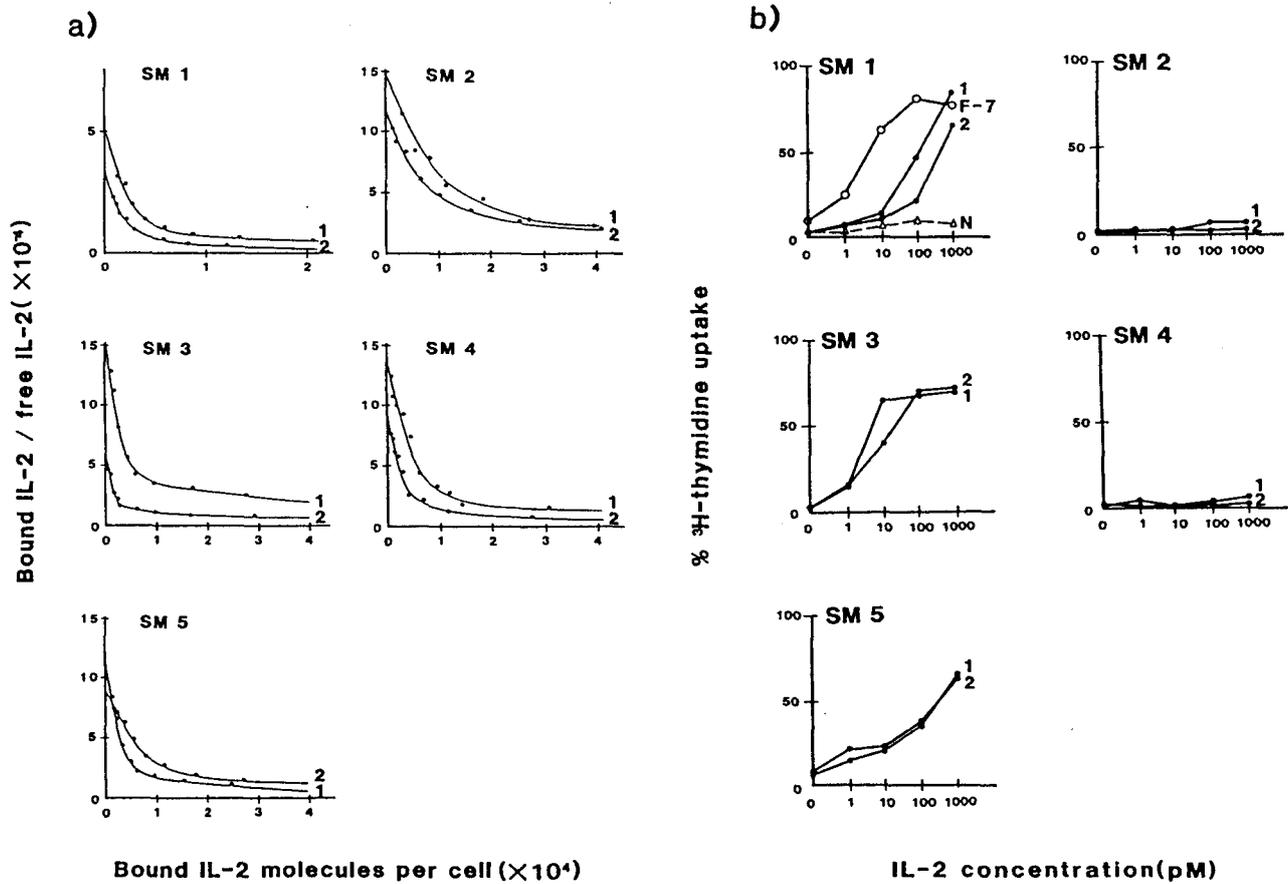


Figure 2.

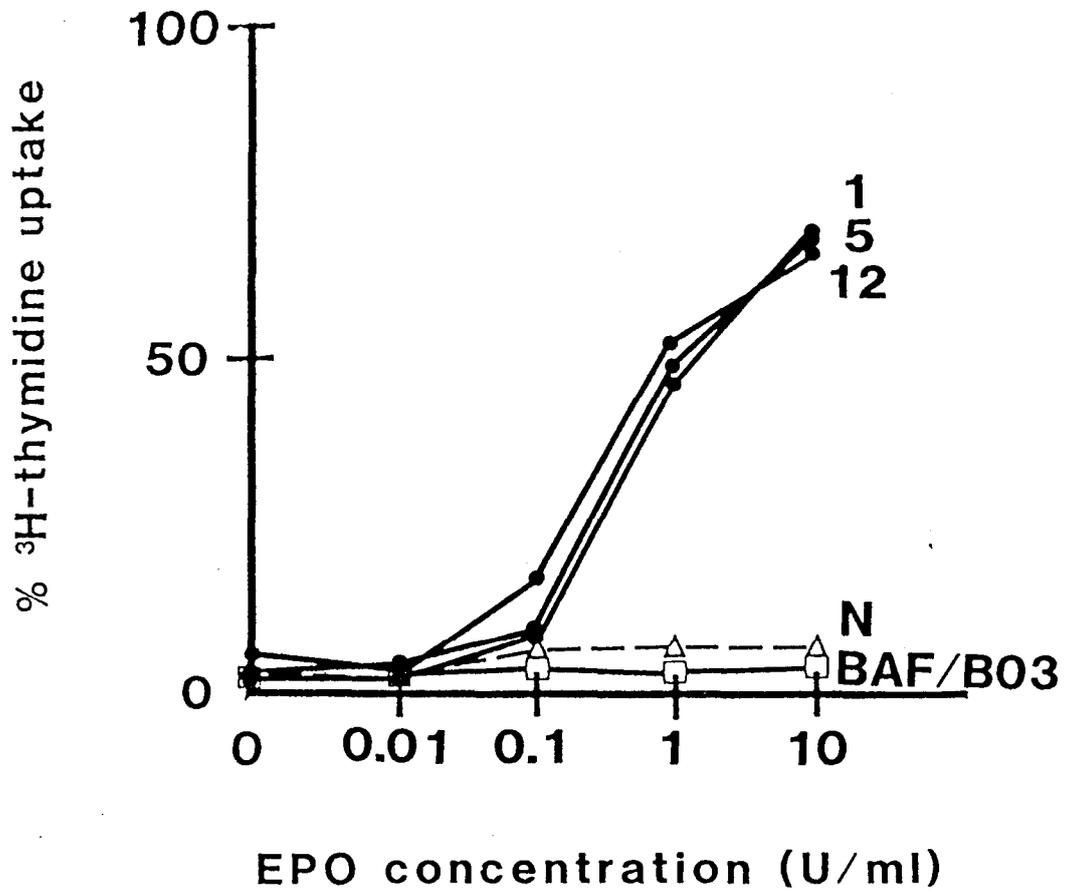


Figure 3

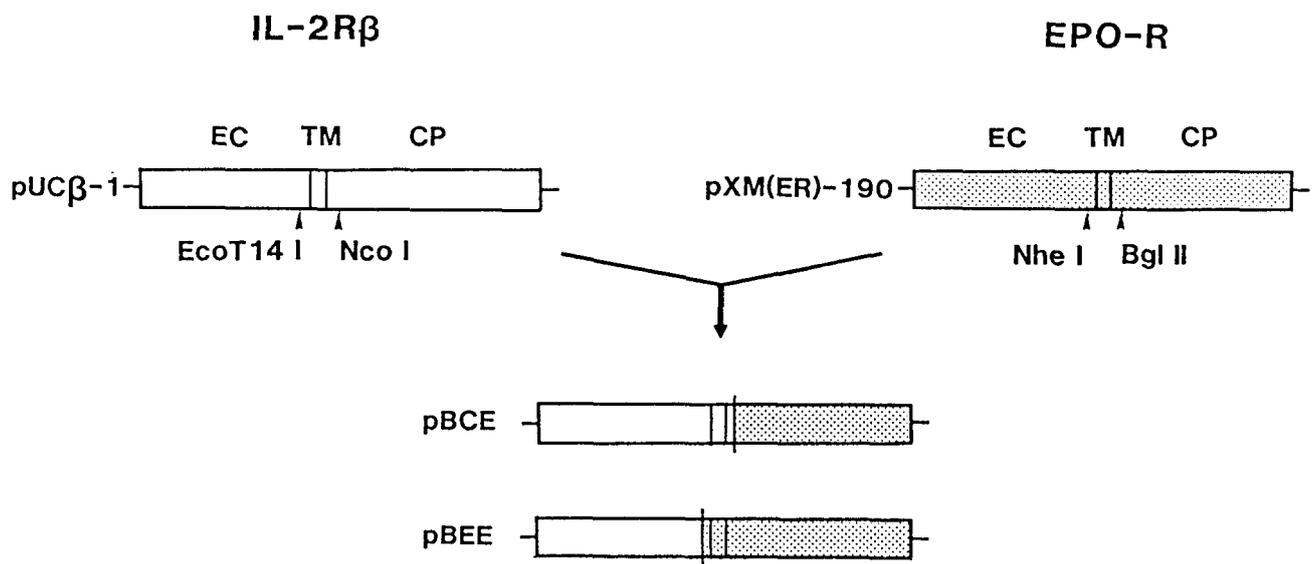


Figure 4.

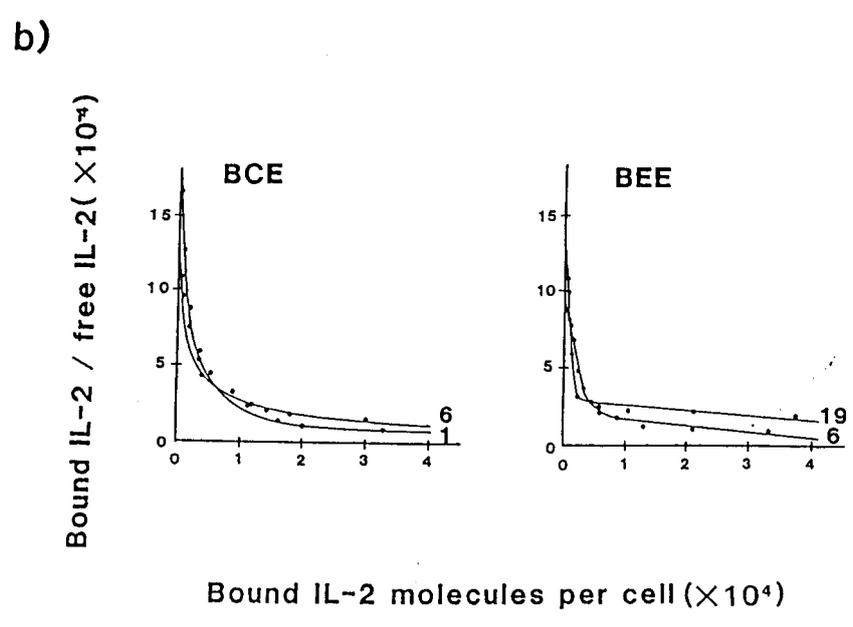
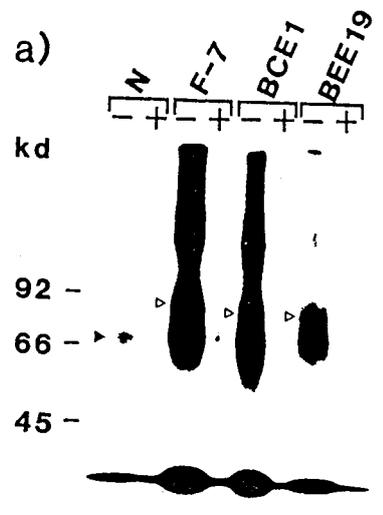


Figure 5

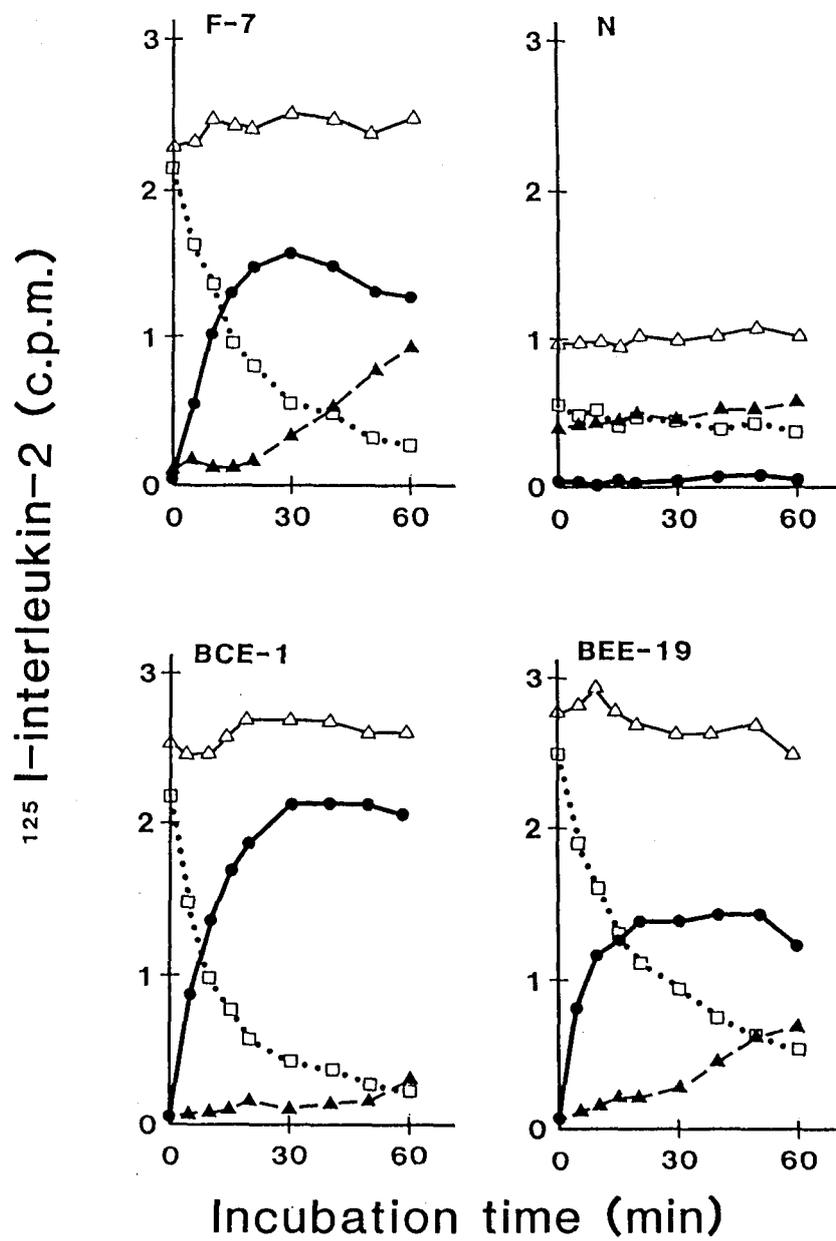


Figure 6

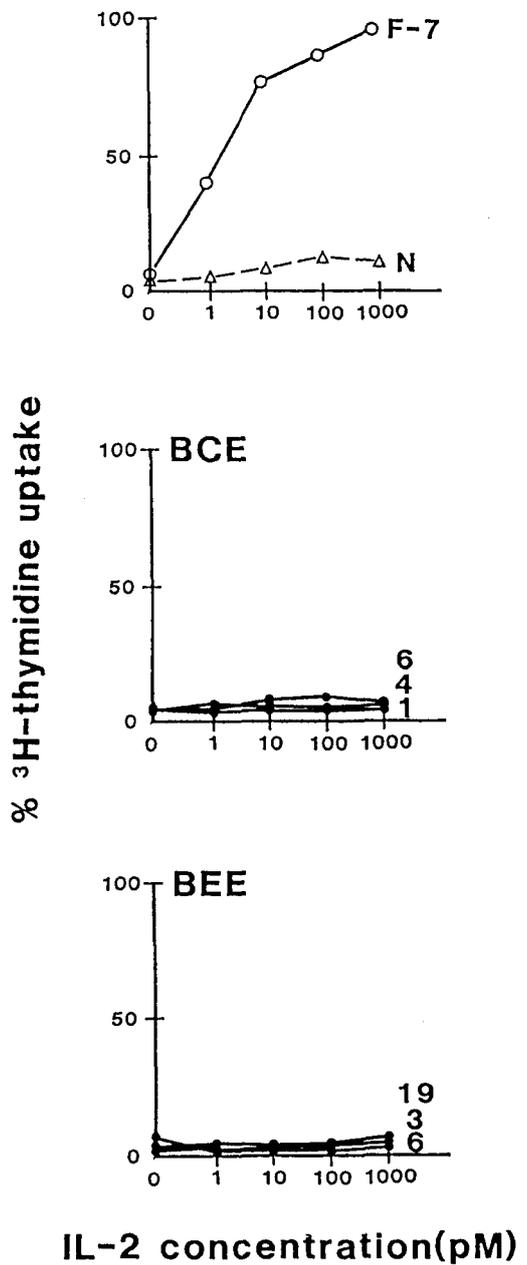


Figure 7.

Short paper



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Human interleukin 2 (IL 2) receptor β chain allows transduction of IL 2-induced proliferation signal(s) in a murine cell line*

Interleukin 2 (IL 2) delivers cell growth signal by virtue of its interaction with the high-affinity receptor complex, which consists of two distinct IL 2-binding molecules: the IL 2 receptor α (IL 2R α) and β (IL 2R β) chains. Unlike many known growth factor receptors, neither of the IL 2R chains seems to contain a tyrosine kinase domain. In this report, we have shown that the human IL 2R β chain expressed in a murine IL 3-dependent, non-lymphoid cell line can transduce IL 2-induced cell proliferation signal(s) in combination with the autologous mouse IL 2R α chain. This observation should provide a tool to dissect IL 2-induced signal transduction pathway in lymphoid and non-lymphoid cells.

1 Introduction

IL 2 plays a key role in the clonal proliferation of T lymphocytes (T cells) and also displays biological activities on other cells [1–3]. The cell proliferation signal is thought to be transduced by the interactions of IL 2 with a specific cell surface receptor complex which manifests high-affinity to IL 2 (K_d : 10–50 pM) [4–6]. The IL 2R complex consists of two distinct ligand-binding molecules; IL 2R α (p55, Tac antigen) and IL 2R β (p70–75) [7–9]. It has been recently demonstrated that coexpression of the cDNA each encoding, respectively, the human IL 2R α and IL 2R β chains in a receptor-negative, human leukemic T cell line results in the formation of the high-affinity IL 2R complex [10]. The human IL 2R β chain has a large intracytoplasmic region consisting of 286 amino acid (aa) residues, whereas the corresponding region of IL 2R α chain is only 13 aa long and is thought not to be essential for signal transduction [11, 12]. Unlike other growth factor receptors, the cytoplasmic region of the IL 2R β chain does not contain an apparent tyrosine kinase domain [10]. In addressing the issue of whether IL 2R β mediates the IL 2-induced growth signal(s), we introduced the expression plasmid for the human IL 2R β chain into a murine IL 3-dependent cell line IC2 [13]. Our results demonstrate that the IL 2R β chain forms high-affinity IL 2R heterodimeric complex with the endogenous murine IL 2R α chain and transduces IL 2-induced cell growth signal.

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2 Materials and methods

2.1 Isolation of transformant expressing human IL 2R β chain

The IL 3-dependent cell line, IC 2 (Thy-1⁻, Ly-1⁻, Ly-2⁻, Ly-5.1⁺, I-A^{d+}, I-E^{d+}, Fc γ R⁺, Fc ϵ R⁺, asialo GM₁) [13] was kindly provided by Dr. I. Yahara (Tokyo Metropolitan Institute for Medical Science, Tokyo). It was cultured in RPMI 1640 medium containing 10% (v/v) FCS and 20% (v/v) conditioned medium from WEHI-3B cells. For the transformation, pLCKR β was introduced together with neomycin-resistance gene into IC2 cells by electroporation as described previously [10]. The neo-resistant clones were selected in the above described culture medium containing 2 mg/ml of G418. The human IL 2R β -positive clones were selected by staining the cells with anti-human IL 2R β mAb, Mik- β 1 [14]. FACS analysis and IL 2-binding assay were performed as described previously [10, 14].

2.2 Cell proliferation assays

For [³H]dThd measurement, the cells were seeded at 1×10^3 in 96-well microtiter plate in 100 μ l of RPMI 1640 medium with 10% FCS in the presence of various concentrations of murine rIL 3 (Genzyme, Boston, MA) or human rIL 2 (Takeda Chemical Co., Osaka, Japan), and cultured for 72 h. During the last 4 h, 1 μ Ci = 37 kBq of [³H]dThd (New England Nuclear, Boston, MA) was added to each well, and the incorporated radioactivities were measured as described previously [11]. To measure the effect of antibodies on the [³H]dThd uptake, IC2 β -2 cells (1×10^3) were cultured for 72 h with human IL 2 or murine IL 3 in the presence or absence of either anti-human IL 2R β antibody, Mik- β 1 (50 μ g/ml), anti-mouse IL 2R α antibody, AMT-13 (50 μ g/ml) [15] or both. Both antibodies were purified by affinity chromatography. Proliferation was measured by the incorporation of [³H]dThd during the last 4 h of culture. The cell number increase was monitored as follows: cells were seeded at 1×10^5 /ml in RPMI 1640 medium containing

10% FCS, with 360 pM of murine rIL 3 or 200 pM of human rIL 2. Cells were transferred at 1×10^5 cells/ml every 2 days. Viable cell number was determined daily by trypan blue exclusion method.

2.3 RNA blotting analysis

RNA blotting analysis was performed as described previously [10] using the Pvu II-digested 530-bp fragment of the mouse IL 2R β cDNA from λ MIL2R β -26 (T. Kono, unpublished results). Specific activity of the probe DNA was 8×10^8 cpm/ μ g. The X-ray film was exposed for 24 h at -70°C .

3 Results and discussion

The mouse cell line IC2 was transfected by pLCKR β , an expression plasmid for the human IL 2R β cDNA (Fig. 1A). IC2 is an IL 3-dependent P cell clone (mast cell progenitor) [13]. The IC2 cells express autologous IL 2R α chain at high levels, but their growth can not be supported by IL 2 (see below and [13]). Following the isolation of a transformant clone, IC2 β -2, expressing the human IL 2R β chain (Fig. 1B), binding properties of human IL 2 to the expressed receptors were analyzed. The Scatchard plot analyses of the IL 2-binding data obtained from IC2 β -2 revealed the expression of high-affinity IL 2R with a K_d of about 46 pM (1200 binding sites for IC2 β -2), that is not detectable in the parental IC2 cells (Fig. 1C). Treatment of IC2 β -2 cells by Mik- β 1, an mAb specific to the human IL 2R β chain, abrogated the appearance of this IL 2R (Fig. 1C). Next, the response of the transformant clone to IL 2 was monitored on the basis of [^3H]dThd uptake and increase in the cell number. As shown in Fig. 2A, the parental IC2 cells responded well to IL 3 as measured by [^3H]dThd uptake, but very poorly to IL 2, even at high concentrations of the latter. The poor response to IL 2 may be due to the expression of autologous mouse IL 2R β at a marginal level, which was not detectable by Scatchard plot analysis (*i.e.* < 100 sites/cell). In fact, expression of the mouse IL 2R β mRNA was detected at very low levels by RNA blotting analysis (Fig. 3). In contrast, IC2 β -2 cells responded to IL 2 efficiently. In fact, the maximum [^3H]dThd uptake levels induced by IL 2 are comparable to those by IL 3 (Fig. 2A). Moreover, the IC2 β -2 cells acquired proliferative properties in the presence of IL 2, whereas the parental cells stopped dividing after a few days in the same culture conditions (Fig. 2B). As shown in Fig. 3, the low-level expression of the autologous IL 2R β mRNA observed in the parental IC2 cells remained essentially the same in the IC2 β -2 cells both before and after IL 2 stimulation. Thus, it appears that in IC2 β -2 cells, growth signals are transduced not only by IL 3 but also by IL 2 as a result of the expression of human IL 2R β cDNA. This observation suggests that the human IL 2R β allows transduction of IL 2-induced proliferative signal(s) in this murine IL 3-dependent cell line. Essentially the same results were obtained with another IL 3-dependent cell line, BA/F3 (a pro-B cell line, [16]; and M. Hatakeyama and T. Doi, unpublished observations). To examine the contribution of the autologous IL 2R α and human IL 2R β in the growth of IC2 β -2 cells by IL 2, the effects of mAb specific to mouse IL 2R α (AMT-13) and human IL 2R β (Mik- β 1) were examined. Both antibodies have been

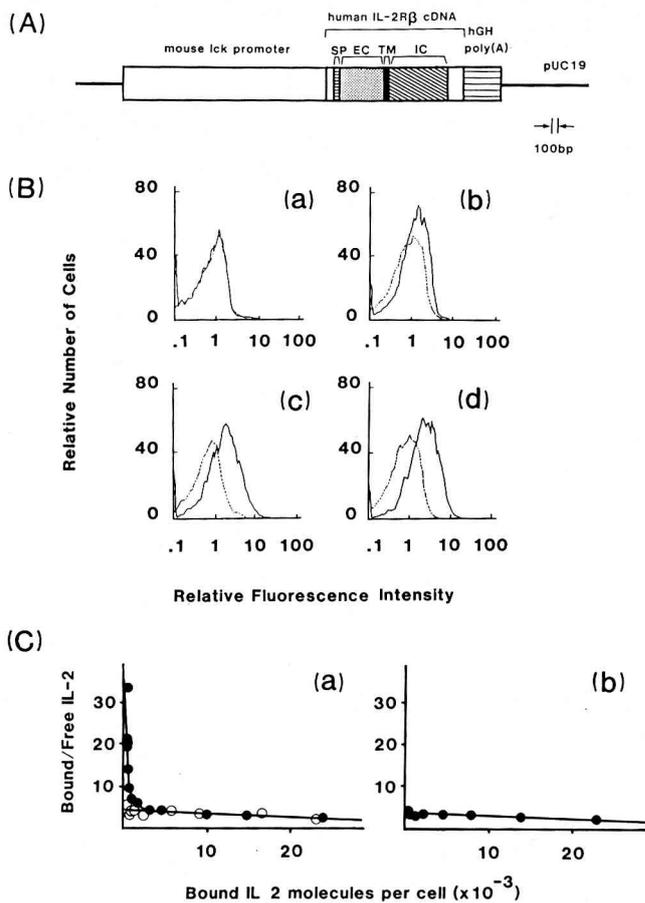


Figure 1. (A) Illustration of the expression plasmid pLCKR β for the human IL 2R β chain cDNA. Construction procedure of expression vector has been described elsewhere [10]. Essentially, the human IL 2R β cDNA encodes 26 aa signal peptide sequence (SP), extracellular region (EC; 214 aa), transmembrane region (TM; 25 aa), intracellular region (IC; 286 aa). The cDNA is flanked by the mouse *lck* promoter and the poly(A) addition sequence of the human growth hormone gene (hGH). (B) FACS analysis of IL 2R expression. IC2 (a, c) and IC2 β -2 (b, d) were each stained with either Mik- β 1 (a, b) or AMT-13 (c, d). The dotted lines represent the fluorescence profile of cells stained with fluorescein-conjugated 2nd-step antibodies. (C) Scatchard plot analyses of [^{125}I]-labeled IL 2 binding to IC2 and IC2 β -2 cells. Binding profile of IL 2 to IC2 β -2 (a) or IC2 (b) in the absence (●) or presence (○) of Mik- β 1 (100-fold dilution of ascites). The number of IL 2-binding sites per cell and dissociation constants (K_d) were determined by computer-assisted analysis of IL 2-binding data. The calculated receptor numbers and K_d are as follows: IC2; no detectable high-affinity receptor and low-affinity receptor (130 000 sites/cell, K_d : 20 nM), IC2 β -2; high-affinity receptor (1200 sites/cell, K_d : 46 pM) and low-affinity receptor (160 000 sites/cell, K_d : 20 nM).

known to inhibit the IL 2 binding to the respective receptor component [14, 15]. FACS analyses revealed that Mik- β 1 did not cross-react with the mouse β chain (S. Minamoto, unpublished data) and had no effect on IL 2-induced growth of mouse CTLL-2 cells (data not shown). As shown in Fig. 4, presence of AMT-13 or Mik- β 1 inhibited cell growth by IL 2. Furthermore, simultaneous presence of both AMT-13 and Mik- β 1 had more pronounced effects. These observations indicate that IL 2 binding to both receptor components is important for the signal transduction. As shown in Fig. 2C, IL 2 and IL 3 can act in an

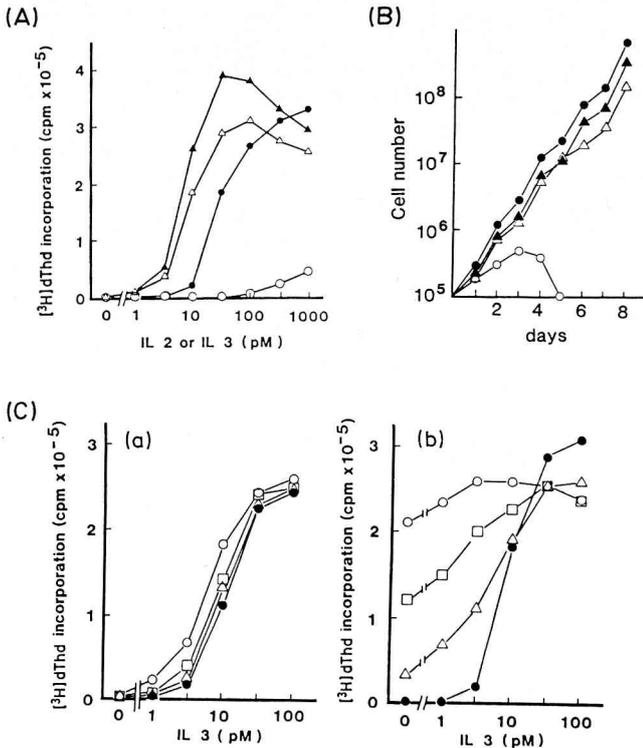


Figure 2. (A) Effects of human IL 2 or murine IL 3 on $[^3\text{H}]\text{dThd}$ uptake of IC2 and IC2 β -2 cells. Human IL 2 (\circ , Δ) or murine-IL 3 (\bullet , \blacktriangle)-induced incorporation of $[^3\text{H}]\text{dThd}$ was examined for IC2 (\circ , \bullet) or IC2 β -2 (Δ , \blacktriangle) cells. Data represent the mean values of triplicate samples. (B) Cell number increase of the IC2 and IC2 β -2. Cells were seeded at $1 \times 10^5/\text{ml}$ in RPMI 1640 medium containing 10% FCS, with 360 pM of murine IL 3 (\bullet , \blacktriangle) or 200 pM of human IL 2 (\circ , Δ). IC2 (\circ , \bullet), IC2 β -2 (Δ , \blacktriangle). (C) Proliferative response of IC2 and IC2 β -2 cells cultured in the presence of IL 3 and IL 2. IC2 and IC2 β -2 ($1 \times 10^3/\text{well}$) were cultured with various concentrations of IL 3 in the presence or absence of IL 2. Sixty-eight hours after the onset of culture, cells were pulsed with $[^3\text{H}]\text{dThd}$ for 4 h. (a) IC2, (b) IC2 β -2. no IL 2 (\bullet); IL 2, 10 pM (Δ); 30 pM (\square); 100 pM (\circ).

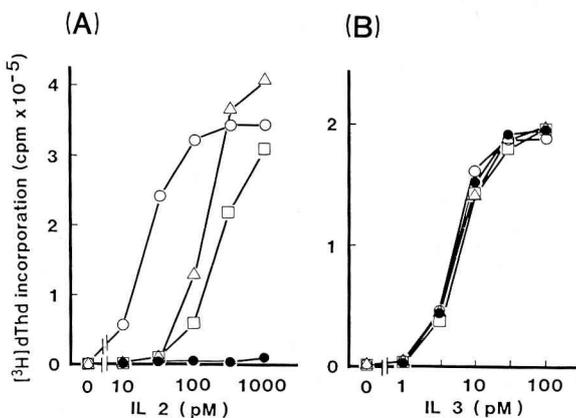


Figure 4. Effects of anti-IL 2R antibodies on the IL 2-dependent and IL 3-dependent growth of IC2 β -2 cells. IC2 β -2 cells (1×10^3) were cultured for 72 h with various concentrations of human IL 2 (A) or murine IL 3 (B) in the absence (\circ) or presence of either anti-human IL 2R β antibody, Mik- β 1 (50 $\mu\text{g}/\text{ml}$) (\square), anti-mouse IL 2R α antibody, AMT-13 (50 $\mu\text{g}/\text{ml}$) (Δ) or both (\bullet). Both antibodies were purified by affinity chromatography. Proliferation was measured by the incorporation of $[^3\text{H}]\text{dThd}$ during the last 4 h of culture.

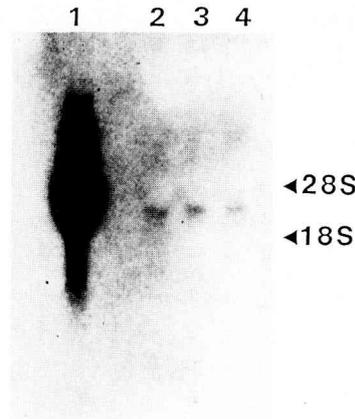


Figure 3. RNA blot analysis for the expression of mouse endogenous IL 2R β chain. Total cellular RNA (20 μg) from each cell clones were subjected to the RNA blot analysis as described in Sect. 2.3. Lane 1: IL 2-dependent mouse T cell line, CTLL-2, lane 2: IC2, lane 3: IC2 β -2, lane 4: IC2 β -2 cultured in the presence of 1 nM human rIL 2 for 24 h.

additive manner in the growth of IC2 β -2 cells at their suboptimal concentrations. When IL 2 was present at a higher concentration (100 pM), it seemed to provide the necessary magnitude of growth signal to the cells and the further addition of IL 3 did not augment the $[^3\text{H}]\text{dThd}$ -uptake levels and *vice versa* (Fig. 2C).

It has been reported that some of the IL 3-dependent cell lines can be switched to IL 2-dependent state [17]. Hence, one may postulate that the signal pathways for IL 2 and IL 3 are convergent at a certain stage, in which a second messenger(s) possibly carries the signal(s) to the nucleus. If we assume that the levels and/or functions of such a second messenger(s) are limiting, saturating levels of one ligand-receptor interaction may mobilize the entire activity of the messenger(s), thus making the latter inaccessible to the other ligand-receptor system, as observed in the case of IC2 β -2 cells (Fig. 2C).

Our results are, thus, consistent with the idea that IL 2 delivers growth signals via the high-affinity α/β receptor complex, although the possibility cannot be ruled out that an additional membrane component(s) is present which couples with the receptor complex in facilitating the signal pathway(s) [1-3, 7-10]. Our findings suggest that the cytoplasmic region of the β chain delivers further downstream in signal pathway(s) even in non-lymphoid cells. These observations may provide a clue as to the identity of the domain(s) within the β chain that is responsible for signal transduction and how such a domain(s) interplays with the conjectured second messenger(s).

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A Restricted Cytoplasmic Region of IL-2 Receptor β Chain Is Essential for Growth Signal Transduction but Not for Ligand Binding and Internalization

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Summary

The functional, high affinity form of interleukin-2 receptor (IL-2R) is composed of two receptor components, the IL-2R α (p55) and IL-2R β (p70-75) chains. Unlike the IL-2R α chain, the IL-2R β chain contains a large cytoplasmic domain that shows no obvious tyrosine kinase motif. In the present study, we report the establishment of a system in which the cDNA-directed human IL-2R β allows growth signal transduction in a mouse pro-B cell line. This system enabled us to identify a unique region within the cytoplasmic domain of the human IL-2R β chain essential for ligand-mediated signal transduction. We also demonstrate that certain cytoplasmic deletion mutants in the IL-2R β chain, although deficient in signal transduction, can still form high affinity IL-2R in conjunction with endogenous mouse IL-2R α chain; the mutants are still able to internalize the ligand as well.

Introduction

Antigen-specific, clonal proliferation of T lymphocytes (T cells) is initiated through a process of signal transduction wherein the specific interaction of antigen-major histocompatibility complex (MHC) molecules and CD3 complex/T cell receptor complex (TCR) triggers the expression of interleukin-2 (IL-2) and its homologous receptor (IL-2R). Subsequent to IL-2-IL-2R interaction, the T cells undergo proliferation (for reviews, see Waldmann, 1986; Taniguchi et al., 1986; Greene and Leonard, 1986; Smith, 1988). Although IL-2 was originally identified as a T cell growth factor (Morgan et al., 1976), it has been shown to manifest biological activities on other hemopoietic cells, such as B lymphocytes (B cells; Waldmann et al., 1984; Zubler et al., 1984), natural killer cells (NK cells; Henny et al., 1981), thymocytes (Raulet, 1985), and macrophages (Malkovsky et al., 1987).

One of the unique features of the IL-2R is the presence of two distinct receptor components designated IL-2R α chain (p55) (Leonard et al., 1984; Nikaido et al., 1984; Cosman et al., 1984) and IL-2R β chain (p70-75) (Sharon et al., 1986; Tsudo et al., 1986; Teshigawara et al., 1987; Dukovich et al., 1987; Hatakeyama et al., 1989). In fact, the IL-2R exists in three different forms on the basis of its affinity to IL-2, i.e., low, intermediate, and high affinity forms with respective dissociation constants (K_D s) of 10^{-8} M, 10^{-9} M, and 10^{-11} M. The high affinity IL-2R is composed of IL-2R α and IL-2R β chains, each of which manifests low and

intermediate affinities, respectively, when expressed singly (Robb et al., 1984; Tsudo et al., 1986; Teshigawara et al., 1987). Evidence has been provided that IL-2 internalization and signal transduction are mediated by both high and intermediate affinity forms of IL-2R, but not by low affinity IL-2R; actually, IL-2-mediated cellular responses occur only in cells bearing either of the two IL-2R forms (Fujii et al., 1986; Weissman et al., 1986; Robb and Greene, 1987). In addition, cDNA expression studies revealed that replacement of the cytoplasmic as well as transmembrane regions of the IL-2R α chain with the corresponding regions of structurally unrelated receptors did not affect the conformation and function of the reconstituted high affinity IL-2R (Hatakeyama et al., 1987; Kondo et al., 1987). These observations suggest the importance of the IL-2R β chain in the intracellular signal transduction pathway(s). We have recently isolated cDNAs encoding the human IL-2R β chain and reconstituted the three forms of IL-2R by expressing the IL-2R α and IL-2R β chain cDNAs in a human leukemic T cell line, Jurkat; the high affinity form of IL-2R is generated by coexpressing both cDNAs (Hatakeyama et al., 1989). However, IL-2 inhibited the growth of this leukemic line through the reconstituted receptor as demonstrated previously (Hatakeyama et al., 1985; Sugamura et al., 1985).

To date, only limited information has been available concerning the mechanism of growth signal transduction following the extracellular ligand-receptor interactions. Many of the growth factor receptors contain a tyrosine kinase domain within their cytoplasmic region essential for the signal transduction (for review, see Hunter and Cooper, 1985). Unlike the IL-2R α chain, the human IL-2R β chain contains a large cytoplasmic region consisting of 286 amino acids (a.a.) that lacks an obvious tyrosine kinase domain (Hatakeyama et al., 1989). Furthermore, the structure as a whole is well conserved between the human and mouse chains (T. Kono and T. Taniguchi, unpublished data).

In the present study, we report the establishment of a cDNA expression system in which the human IL-2R β transduces a positive growth signal upon IL-2 stimulation in a mouse IL-3-dependent pro-B cell line. Utilizing this expression system, we identified a restricted cytoplasmic region of the human IL-2R β chain essential for IL-2 signal transduction. Our results also demonstrate that mutant receptor molecules incapable of signal transduction still form high affinity IL-2R, together with the host cell-derived mouse IL-2R α chain, and internalize IL-2. These findings are discussed in light of the structure and function of the IL-2R complex.

Results

Generation of the Functional High Affinity IL-2R by Mouse α and Human β Chains in a Mouse IL-3-Dependent Pro-B Cell Line

To explore the role of the IL-2R β chain in IL-2-mediated signal transduction, we developed a cDNA expression

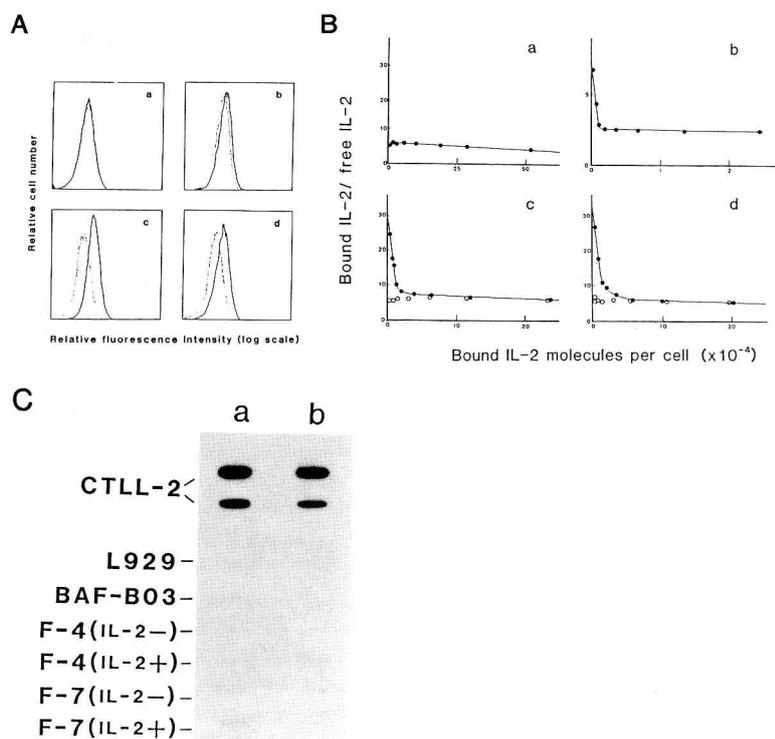


Figure 1. Properties of the IL-2R Expressed in the BAF-B03 and Transformant Clones

(A) FACS profile of human IL-2R β expression on BAF-B03 transformants. (a) Control BAF-B03 cells; (b-d) BAF-B03 transformant clones expressing the wild-type human IL-2R β cDNA; (b) clone F-4, (c) clone F-7, (d) clone F-10. Cells were incubated with the anti-human IL-2R β monoclonal antibody Mik- β 1 and then with FITC-conjugated goat anti-mouse IgG (solid lines). Dotted lines indicate the fluorescence profile of the cells stained with FITC-conjugated goat anti-mouse IgG alone.

(B) Scatchard analysis of IL-2R expression of the control cells and transformant clones profiled in (A). The IL-2 binding assay was carried out in the absence (\bullet) or presence (\circ) of Mik- β 1 (see Experimental Procedures).

(C) Slot blot analysis of mRNAs for mouse endogenous IL-2R β . Ten micrograms (lane a) or 5 μ g (lane b) of total RNA obtained from each cell line was loaded onto the slot blotter and analyzed as described in Experimental Procedures. CTLL-2 was used as a positive control: 10 μ g (upper row, lane a), 5 μ g (upper row, lane b), 1.25 μ g (lower row, lane a), and 0.625 μ g (lower row, lane b) of total RNAs were applied.

system that allows functional studies of the human IL-2R β chain. For the cDNA expression, we isolated a subclone of BA/F3, a mouse IL-3-dependent pro-B cell line (Palacios and Steinmetz, 1985; Collins et al., 1988; Daley and Baltimore, 1988). The isolated subclone, designated BAF-B03, expressed a large number of mouse endogenous IL-2R α (\sim 1,000,000 sites per cell) as did the parental BA/F3 cells, but the cell line showed an absolute requirement for IL-3, and not IL-2, for its survival (see below). The BAF-B03 clone exhibited the following profile of cell surface marker expression: Mac-1 $^{+}$, Bp1 $^{-}$, B220 $^{-}$, sIgM $^{-}$, Lyb1 $^{-}$, Thy1 $^{-}$, Ly1 $^{-}$, L3T4(CD4) $^{-}$, Lyt2(CD8) $^{-}$, and IL-2R α^{+} (data not shown). By cotransfection of an expression plasmid, pLCKR β , which contains the entire protein coding region of human IL-2R β cDNA under the control of a promoter sequence derived from mouse *lck* gene (Garvin et al., 1988) and a plasmid carrying the neo-resistance gene into the BAF-B03 cells, we selected for G418-resistant stable transformants and isolated clones F-1, F-2, F-3, etc. Subsequently, each clone was examined for the expression of human IL-2R β using a monoclonal antibody, Mik- β 1, specific to human IL-2R β (Tsuda et al., 1989; S. Minamoto and M. Hatakeyama, unpublished data) (Figure 1A).

Using the IL-2R β^{+} transformant clones, we examined the IL-2 binding properties of the receptor (Figure 1B; Table 1). In contrast to the parental cells, which express exclusively the endogenous low affinity IL-2R, each of the human IL-2R β^{+} transformant clones displayed both high and low affinity forms of IL-2R (Figure 1B; Table 1). The number of high affinity IL-2R expressed on each of the

clones ranged from 1350–9800 sites per cell (Table 1). Pretreatment of the cells with Mik- β 1 completely abolished ligand binding to high affinity IL-2R, but not to low affinity IL-2R, as displayed in human high affinity IL-2R (Figure 1B) (Tsuda et al., 1989; Hatakeyama et al., 1989). These results demonstrate the reconstitution of high affinity IL-2R by human IL-2R β and endogenous mouse IL-2R α chains.

Next, we examined the effect of recombinant human IL-2 on the growth properties of these clones. As shown in Figure 2A, human IL-2R β^{+} transformant clones displayed a significant response to recombinant human IL-2 as measured by [3 H]thymidine uptake. The response increased in a concentration-dependent manner, and the response

Table 1. High and Low Affinity IL-2R Numbers in BAF-B03 Transformants Expressing Wild-Type Human IL-2R β

Cell	IL-2R Number (sites per cell)	
	High Affinity	Low Affinity
BAF-B03	<50	1,086,000 (18.3 nM) ^a
F-2	2,750 (14.6 pM) ^a	976,000 (23.3 nM)
F-4	1,350 (21.3 pM)	720,000 (27.9 nM)
F-7	9,260 (28.9 pM)	797,300 (12.8 nM)
F-10	9,800 (36.1 pM)	1,156,000 (15.5 nM)
F-11	4,000 (15.6 pM)	695,800 (11.0 nM)
F-15	4,110 (12.2 pM)	1,100,000 (19.9 nM)

^a Numbers in parentheses show dissociation constant (K_D) of the receptor.

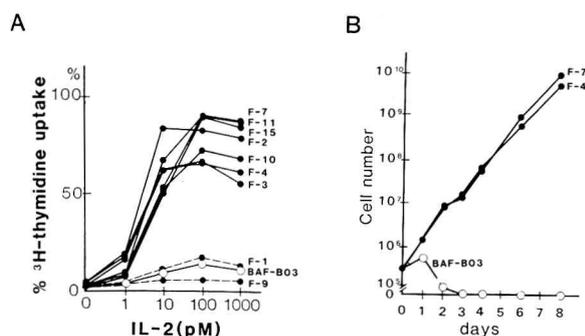


Figure 2. IL-2-Dependent Growth of BAF-B03 Transformants Expressing Full-Length Human IL-2R β cDNA

(A) BAF-B03 and its transformant clones positive or negative for the human IL-2R β selected by FACS were independently cultured with various concentrations of human IL-2 and analyzed for their ability to incorporate [³H]thymidine into DNA. The results are expressed as the percentage of incorporation of [³H]thymidine in the same cells incubated with WEHI conditioned medium. Data are the average of triplicate determinations. Human IL-2R β ⁺ transformants: —●—●—; neo-resistant, human IL-2R β ⁻ transformants: -●-●-.

(B) Increase in cell number of the transformants F-4 and F-7 by recombinant human IL-2.

to 1 nM of IL-2 was comparable to that observed with medium containing 20% WEHI-3B culture supernatant as the IL-3 source. Furthermore, these cell clones acquired IL-2-dependent proliferative properties independent of IL-3 (Figure 2B). In contrast to the human IL-2R β ⁺ transformants, the parental cells as well as the G418-resistant, human IL-2R β ⁻ cell clones barely responded to IL-2 at its very high concentrations, and this weak response was insufficient to sustain cell growth (Figure 2B). The weak response may be attributed to very low-level expression of the mouse endogenous IL-2R β chains, which cannot be detected by the IL-2 binding assay. In fact, RNA blot analysis with the use of mouse IL-2R β cDNA (T. Kono and T. Taniguchi, unpublished data) as the probe revealed the expression of mouse IL-2R β mRNA at very low levels in the BAF-B03 cells (approximately 500- to 1000-fold lower compared with the levels in an IL-2-dependent cell line, CTLL-2; Figure 1C). The expression levels of mouse IL-2R β mRNA were not significantly different between parental and transformed cells before and after IL-2 stimulation, further supporting the notion that the human IL-2R β , but not the endogenous mouse IL-2R β , is responsible for the IL-2-dependent cell growth of the transformants.

It was previously shown that the anti-human IL-2R β antibody Mik- β 1 did not inhibit the IL-2-dependent growth of a human T cell line, kit 225, which expresses 3400 sites/cell of high affinity IL-2R unless it was added together with anti-Tac, an anti-human IL-2R α monoclonal antibody (Tsuda et al., 1989). Similarly, Mik- β 1 did not block the IL-2-dependent growth of a BAF-B03 transformant clone (F-7) expressing as many as 9300 high affinity IL-2 binding sites, but it caused almost complete inhibition when added together with an anti-mouse IL-2R α antibody, AMT-13 (Osawa and Diamantstein, 1984) (Table 1). Interestingly, IL-2-dependent growth of the clone F-4 expressing a

Table 2. Effect of Anti-IL-2R Antibodies on the IL-2-Dependent Growth of BAF-B03 Transformants

Cells	Additions	[³ H]Thymidine Incorporation (cpm)
BAF-B03	None	599 \pm 377
	IL-2	2,861 \pm 1,147
	IL-2 + Mik- β 1	2,478 \pm 694
	IL-2 + AMT-13	4,605 \pm 1,013
	IL-2 + Mik- β 1 + AMT-13	818 \pm 221
	IL-3	81,605 \pm 1,028
	IL-3 + Mik- β 1 + AMT-13	70,479 \pm 8,113
F-4	None	805 \pm 455
	IL-2	31,003 \pm 8,412
	IL-2 + Mik- β 1	8,181 \pm 1,720
	IL-2 + AMT-13	8,896 \pm 1,804
	IL-2 + Mik- β 1 + AMT-13	938 \pm 661
	IL-3	61,570 \pm 2,320
F-7	None	1,906 \pm 1,034
	IL-2	46,422 \pm 1,260
	IL-2 + Mik- β 1	65,501 \pm 4,512
	IL-2 + AMT-13	48,079 \pm 499
	IL-2 + Mik- β 1 + AMT-13	6,981 \pm 458
	IL-3	91,746 \pm 1,189
CTLL-2	None	1,498 \pm 397
	IL-2	100,423 \pm 2,265
	IL-2 + Mik- β 1	106,736 \pm 4,652
	IL-2 + AMT-13	118,046 \pm 5,210
	IL-2 + Mik- β 1 + AMT-13	114,080 \pm 4,208
	IL-2 (1 nM)	114,679 \pm 3,359
	IL-2 (1 nM) + Mik- β 1 + AMT-13	128,758 \pm 5,292

Cells were cultured for 24 hr as indicated in the Experimental Procedures, with or without recombinant human IL-2 (final concentration of 10 pM) or mouse IL-3 (10% WEHI conditioned media) in the presence or absence of the affinity-purified Mik- β 1 (50 μ g/ml) and/or AMT-13 (50 μ g/ml). The results represent mean \pm standard deviation of triplicated samples.

smaller number of high affinity IL-2R (1350 sites) was efficiently inhibited by either Mik- β 1 or AMT-13 alone under the same assay conditions (Table 2). Thus, the human IL-2R β chain appears to allow IL-2-induced growth signal transduction by forming the high affinity IL-2R with the mouse IL-2R α chain in these transformant clones.

Construction and Expression of Mutant cDNAs for the Human IL-2R β Chains with Altered Cytoplasmic Domain

To delineate and identify the various functional domains of the IL-2R β chain, we constructed and expressed a series of cDNAs with specific deletion mutations in the cytoplasmic domain and monitored receptor affinity, ligand internalization, and growth signal transduction. As reported previously, the cytoplasmic domain has several characteristic features: it contains serine-rich, acidic, and proline-rich regions (Hatakeyama et al., 1989). As depicted in Figure 3, a number of cDNA mutants were constructed and introduced into the expression vector p1013 (Garvin et al., 1988). Briefly, the cDNA of pLCKR β -H encodes the human IL-2R β with a deletion of 147 amino acids from the carboxyl terminus (designated H-mutant). The cDNA of

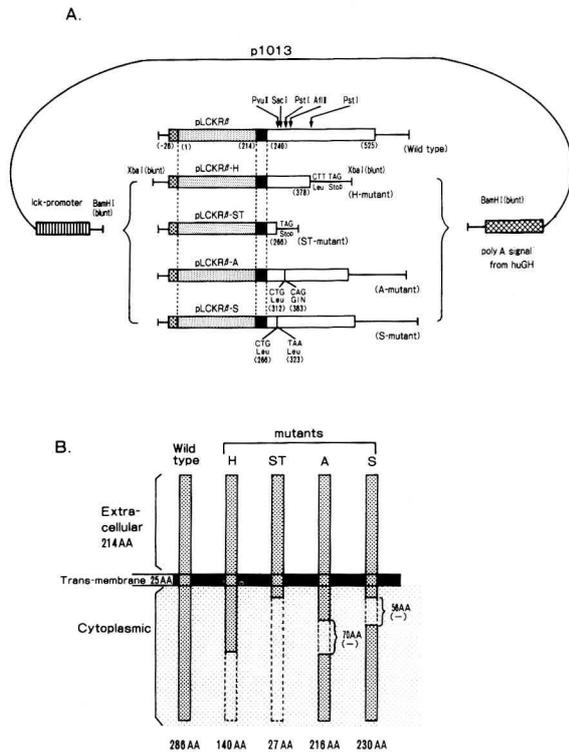


Figure 3. Construction of the Mutant Human IL-2R β cDNAs
(A) Construction of expression vectors encoding mutant human IL-2R β chains. The mutant cDNAs were constructed as described in Experimental Procedures and inserted into an expression vector, p1013, which contains the mouse *lck* downstream promoter sequence and the polyadenylation signal from human growth hormone (huGH) gene. Dotted, stippled, closed, and open areas represent, respectively, the cDNA regions encoding the signal peptide, extracellular, transmembrane, and cytoplasmic regions. The numbers in parentheses correspond to amino acid residues.
(B) Schematic view of the mutant IL-2R β molecules. Shown are Wild-type: wild-type, full-length human IL-2R β molecule encoded by pLCKR β ; H: H-mutant, mutant IL-2R β encoded by pLCKR β -H; ST: ST-mutant, mutant IL-2R β encoded by pLCKR β -ST; A: A-mutant, mutant IL-2R β encoded by pLCKR β -A; and S: S-mutant, mutant IL-2R β encoded by pLCKR β -S. In the H-mutant, a leucine residue is added to the carboxyl terminus as a result of the *Bst*XI linker sequence.

pLCKR β -ST codes for mutant IL-2R β , which retains only 27 a.a. of the cytoplasmic domain (ST-mutant). The cDNAs of pLCKR β -A and pLCKR β -S, respectively, encode IL-2R β molecules lacking the internal acidic region (a.a. 313–382) and the serine-rich region (a.a. 267–322) (designated A-mutant and S-mutant, respectively). These plasmid DNAs were transfected into BAF-B03 cells, and transformants expressing cell surface epitopes recognized by the Mik- β 1 were obtained.

Detection of Mutant IL-2R β Chains by IL-2 Cross-Linking

To confirm that these Mik- β 1 reactive transformant clones indeed expressed the expected protein products encoded by the mutant cDNAs, representative cell clones for each mutant were subjected to IL-2 cross-linking analysis by using the radioiodinated IL-2 (¹²⁵I-IL-2) and noncleavable cross-linker disuccinimidyl suberate (DSS). The protein

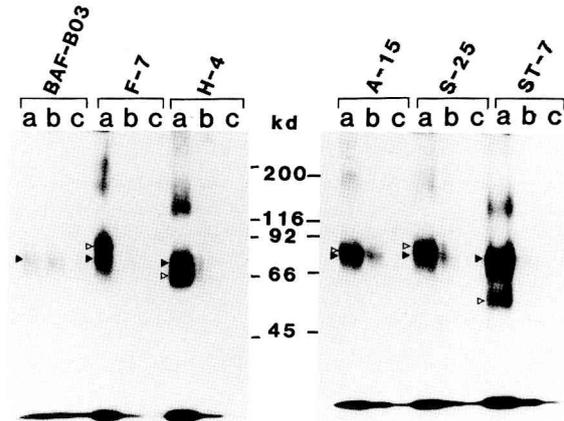


Figure 4. Chemical Cross-Linking of IL-2Rs with ¹²⁵I-IL-2
Cells were incubated for 1 hr in the absence (a) or presence of Mik- β 1 (b), or in cold IL-2 (c). Subsequently, cells were incubated with 200 pM ¹²⁵I-IL-2 and then cross-linked by DSS. The cell lysates were analyzed on 8% SDS-PAGE. The cell clones used were: F-7, expressing wild-type human IL-2R β ; H-4, expressing H-mutant; A-15, expressing A-mutant; S-25, expressing S-mutant; and ST-7, expressing ST-mutant. Complexes for ¹²⁵I-IL-2 and mouse IL-2R α (\blacktriangleright) and for ¹²⁵I-IL-2 and human wild-type or mutant IL-2R β (\blacktriangleright) are indicated.

products from pLCKR β -H, pLCKR β -A, pLCKR β -S, and pLCKR-ST were predicted to be approximately 16 kd, 8 kd, 6 kd, and 28 kd smaller than that of native IL-2R β , respectively. As shown in Figure 4, the results of the chemical cross-linking experiments revealed that each of the representative cell clones expressed the IL-2R β molecules with the predicted molecular size.

In the cross-linking analysis, a band corresponding to a trimolecular complex by IL-2, IL-2R α , and IL-2R β can also be detected (Figure 4). In fact, the complex migrates faster in cells expressing truncated IL-2R β chains. In addition to the trimolecular complex, a specific band with very high molecular weight was noticed, but its nature is unclear at present.

Deletions within the Cytoplasmic Region of the IL-2R β Do Not Affect the Formation of High Affinity IL-2R

Using the transformant clones expressing the mutant IL-2R β chain, we addressed the issue of whether the mutations affect the ligand binding properties of the IL-2R. As summarized in Table 3, Scatchard plot analyses of the IL-2 binding with those transformants revealed that they all expressed high affinity IL-2R, the appearance of which was completely inhibited by Mik- β 1 (data not shown). The dissociation constants (K_D s) of high affinity receptors expressed in each of the transformants were between 10–50 pM, indicating that truncations in the cytoplasmic domain did not affect the extracellular IL-2 binding properties of the IL-2R β chain.

Function of the Mutant IL-2R β Chains in Growth Signal Transduction

The observation that all of the mutant IL-2R β chains formed high affinity IL-2R with the endogenous mouse IL-2R α

Table 3. Numbers of High and Low Affinity IL-2R Receptors Expressed on BAF-B03 Transformants Expressing Mutated Human IL-2R β

Cells	Receptor Numbers (sites per cell)	
	High Affinity	Low Affinity
H-mutant		
H-1	14,010 (36.3 pM) ^a	896,700 (12.8 nM) ^a
H-4	19,000 (37.0 pM)	811,300 (13.4 nM)
A-mutant		
A-3	1,690 (14.0 pM)	795,800 (16.1 nM)
A-15	4,810 (17.1 pM)	1,227,000 (17.4 nM)
A-17	1,490 (12.0 pM)	1,247,000 (35.5 nM)
S-mutant		
S-2	4,300 (27.7 pM)	518,300 (35.6 nM)
S-25	2,800 (18.6 pM)	747,800 (30.8 nM)
S-28	2,170 (21.3 pM)	ND
ST-mutant		
ST-7	15,960 (36.9 pM)	ND
ST-14	4,890 (18.7 pM)	697,900 (23.2 nM)
ST-23	6,130 (21.2 pM)	577,300 (14.7 nM)

^a Numbers in parentheses show dissociation constant (K_D) of the receptor. ND, not determined.

chain has led us to examine whether they are able to transduce the IL-2-induced growth signal. As shown in Figure 5, transformant clones, which express the H-mutant lacking 147 COOH-terminal amino acids, responded to IL-2. In contrast, clones expressing the ST-mutant, a more severely truncated IL-2R β containing only a 27 a.a. cytoplasmic tail, failed to respond. These observations provide evidence that the cytoplasmic domain of the IL-2R β is in fact involved in the IL-2 signaling, and that an essential region for signal transduction is located between a.a. residues 267 and 378, corresponding to the region that shows abundance in serine and acidic amino acid residues (Hatakeyama et al., 1989). Further characterization of this region was carried out by examining the functional response of A- and S-mutants. As shown in Figure 5, all of the clones expressing the A-mutant exhibited good response to IL-2, whereas none of the S-mutants responded to IL-2 above background levels. All of the clones for both S- and ST-mutant IL-2R β s expressed more than 2000 of the high affinity IL-2R (Table 3). In the case of the wild-type IL-2R β , expression of as few as 1350 high affinity IL-2R sites in the BAF-B03 was found to be sufficient for IL-2-induced cell proliferation (Figure 2; Table 1). It is therefore unlikely that the nonresponsiveness of the S- or ST-mutant clones to IL-2 is due to a difference in receptor density. Hence, these observations suggest the importance of a restricted region of the IL-2R β cytoplasmic domain for the IL-2-induced growth signal transduction (see Discussion). The IL-2-mediated cell growth through the high affinity receptor, composed of H- or A-mutant IL-2R β and mouse IL-2R α , was also inhibited by the simultaneous presence of Mik- β 1 and AMT-13 (data not shown).

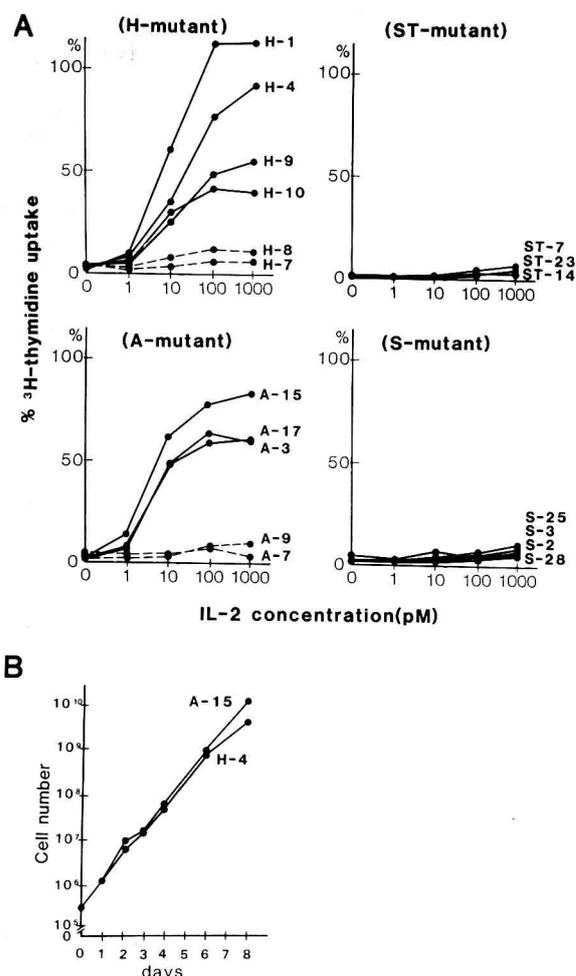


Figure 5. IL-2-Dependent Growth of BAF-B03 Transformants Expressing the Mutant IL-2R β s

(A) [³H]thymidine incorporation. Mutated human IL-2R β ⁺ transformants: —●—●—; neo-resistant mutated human IL-2R β negative transformants: —●—●—.

(B) Cell growth curve. For details, see Experimental Procedures and the legend to Figure 2.

Mutant Receptor-Mediated IL-2 Internalization in the Absence of Signal Transduction

In the case of IL-2R, IL-2R β is responsible for IL-2 internalization (Fujii et al., 1986; Weissman et al., 1986; Robb and Greene, 1987; Hatakeyama et al., 1989). In this context, we also addressed the question as to whether the above mutant receptor molecules have the ability to internalize IL-2. As demonstrated in Figure 6, all of the IL-2R β mutants including S- and ST-mutants are capable of internalizing IL-2. For all of the cell clones tested, the internalization is rapid and reaches a peak 5–10 min following a shift to a physiological temperature (37°C). These results indicate that the deletions affecting the signal transducing domain(s) of IL-2R β do not significantly affect the internalization properties. However, our results do not strictly rule out the possibility that there may be subtle differences among these receptors: e.g., the fate of the internalized IL-2.

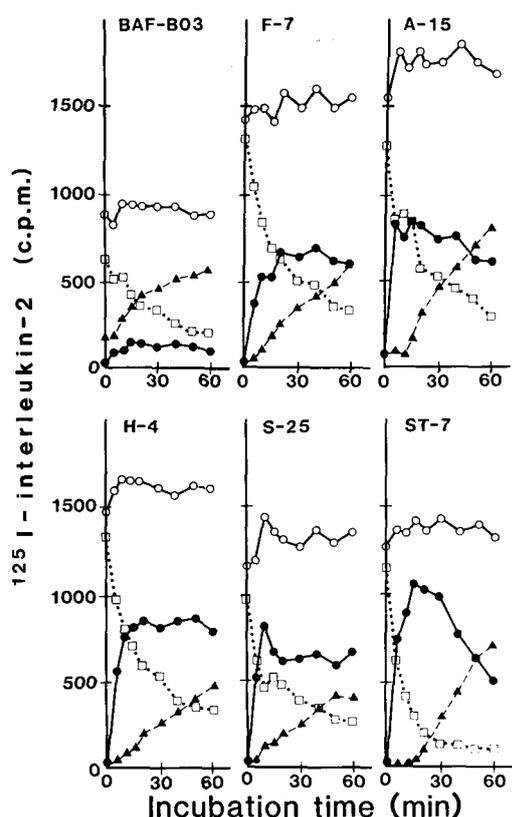


Figure 6. IL-2 Internalization in the Parental and Various Transformants Expressing the Wild-Type and Mutant IL-2R β s

The IL-2 internalization was monitored by the procedure described by Robb and Greene (1987). At each time indicated, the level of radioactivity in the cell supernatant was measured (—▲—▲—). The cells were then resuspended in pH 4 buffer and centrifuged through a layer of oil cushion as described previously (Robb and Greene, 1987). The radioactivity in the cell pellet was measured to determine the level of pH 4-resistant, internalized IL-2 (—●—●—). The radioactivity in the supernatant was measured to determine the amount of cell surface-bound IL-2 dissociated in the pH 4 buffer (—□—□—). The sum of the radioactivities found in all three fractions is also graphed (—○—○—). Decrease of the radioactivity in the cytoplasm after prolonged incubation, accompanied by the increase of the radioactivity in the cell supernatant, is mainly due to the degradation of ¹²⁵I-IL-2 after internalization (K. Smith, Dartmouth University, personal communication). Essentially the same results were obtained with other transformant clones expressing the wild-type or mutant IL-2R β .

Discussion

In the present study, we provide evidence that the human IL-2R β chain allows transduction of the IL-2-induced cell proliferation signal(s) when expressed in a murine IL-3-dependent pro-B cell line, BAF-B03. Essentially the same findings were made when the IL-2R β was expressed in another IL-3-dependent mast cell precursor line, IC-2 (Koyasu et al., 1986; T. Doi and M. Hatakeyama, unpublished data). In the system described here, the IL-2-mediated cell proliferation signal(s) seems to be derived primarily through the high affinity IL-2R, which consists of the endogenous mouse IL-2R α and the cDNA-directed human IL-2R β . In fact, the presence of antibodies specific

to both IL-2R chains efficiently inhibited the IL-2-mediated cell growth (Table 2).

It has been shown that some of the IL-3-dependent cell lines can switch to IL-2 dependence (Le Gros et al., 1985, 1987). These observations, as well as our present study, suggest the presence of a common growth signal transduction pathway(s) in a wide range of hemopoietic cells. It is interesting to note that in the BA/F3 cell line, as well as in other IL-3-dependent cell lines, cDNA-directed expression of the epidermal growth factor (EGF) receptor, which contain a tyrosine kinase domain, renders the cells responsive to EGF, but the EGF stimulation cannot support long-term cell growth (Collins et al., 1988; Pierce et al., 1988; von Ruden and Wagner, 1988). EGF stimulation of the receptor tyrosine kinase has been shown to result in autophosphorylation of the receptor and the phosphorylation of phospholipase C (Margolis et al., 1989; Meisenhelder et al., 1989). Although evidence for rapid tyrosine phosphorylation of unidentified intracellular proteins by IL-2 has been provided (Saltzman et al., 1988; Morla et al., 1988), no obvious tyrosine kinase domain has been identified in the IL-2R β chain by which the growth signal can be transduced. Taking these observations together with the recent reports that protein kinase C, calcium mobilization, and phosphatidylinositol turnover are not directly involved in signal transduction by IL-2 (Mills et al., 1986, 1988; Valge et al., 1988; LeGrue, 1988), the role of tyrosine phosphorylations in IL-2R β -mediated signal transduction remains to be determined.

As revealed from the present study, the cytoplasmic region of the IL-2R β chain appears to have a "critical domain" for signal transduction. This cytoplasmic domain was found to be located proximal to the inner surface of the cell membrane, which includes the serine-rich region (Hatakeyama et al., 1989). Because the IL-2 signal is transduced by the A-mutant (lacking a.a. 313–382), but not by the S-mutant (lacking a.a. 267–322) and the ST-mutant (lacking a.a. 267 and thereafter), the 46 a.a. residues spanning a.a. 267–312 are essential for growth signal transduction (Figure 7). The fact that the H-mutant, which retains only 138 a.a. of the cytoplasmic region, is still competent in signal transduction indicates that the carboxy-end region (i.e., a.a. 383–525) of the A-mutant is perhaps dispensable. However, the role of the other regions in the overall function of the IL-2R β remains to be determined. It is important to note that the primary sequence of this region shows about 80% homology with mouse IL-2R β , in which 6 out of 8 serine residues and 8 out of 9 hydrophobic a.a. residues (i.e., leucine, isoleucine, and valine) are conserved (T. Kono and T. Taniguchi, unpublished data). We postulate that this "critical domain" constitutes an active site for as yet unknown enzymatic function and/or a domain that is required for association with a second messenger(s) in the cascade of signal transduction. This critical domain is unique among regions for growth signaling in growth factor receptors in that it apparently lacks a typical tyrosine kinase structure. Recently it has been shown that the human IL-2R β is structurally related to the murine erythropoietin receptor (EPO-R; D'Andrea et al., 1989). It is interesting to note that the highest degree of homology

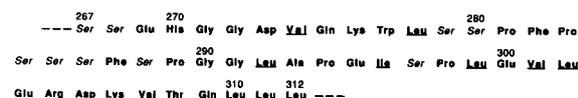


Figure 7. Primary Sequence of the 46 Amino Acid Residues Constituting the Functional Signal Transduction Domain

Serine residues are indicated in italics, and hydrophobic amino acid residues are underlined.

between the two molecules is present in this region as indicated below:



(data from D'Andrea et al., 1989).

The biological significance of this homology between the two receptors is yet to be determined.

Expression studies with the mutant IL-2R β chains showed another interesting feature of the IL-2R β function: drastic deletions of the cytoplasmic domain have no effect on the formation of high affinity IL-2R. Our observations are consistent with previous findings with mutant IL-2R α , which indicated that high affinity IL-2R is generated by noncovalent association of both chains solely at the extracellular regions (Hatakeyama et al., 1987; Kondo et al., 1987). Furthermore, truncations in the cytoplasmic region of the IL-2R β chain do not seem to affect the ligand internalization in the assay system employed in the present study (Figure 6). Although functional linkage between internalization and signal transduction in the IL-2R remains obscure at present, our findings indicate that the internalization process may not directly couple with the intracellular signal transduction pathway. Further studies will be required to analyze and distinguish in more detail these two phenomena. The system described in this study may provide a model to identify the downstream cellular components involved in the intracellular cascade of growth induction.

Experimental Procedures

Cells and Cell Culture

B0 cells, a subline of BA/F3 (kindly provided by Dr. Mary Collins, IRC-Chester Beatty Laboratories, London), were maintained in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum (FCS) and 20% (v/v) conditioned medium from the WEHI-3B cell line (as a source of IL-3) (20% WEHI conditioned medium). For the transfection of wild-type and mutant cDNAs, a subclone of the B0 cells, designated BAF-B03, was isolated by limiting dilution technique.

Plasmid Construction

The construction of pLCKR β , a cDNA expression vector for human IL-2R β , was reported previously (Hatakeyama et al., 1989). For the construction of plasmid pLCKR β -H, a 1.3 kb cDNA insert was excised from pIL-2R β 9 by XbaI and, after filling in both ends with T4 DNA polymerase, inserted into BamHI-cleaved p1013 vector whose cleavage sites were filled in similarly. For the construction of pLCKR β -ST, pIL-2R β 30 was first digested with PvuII. Subsequently, an XbaI linker (pCTCTAGAG, New England Biolabs, Inc.), which allows the insertion of an in-frame non-sense codon, was inserted, and the resulting plasmid was cut by XbaI. The XbaI-digested cDNA fragment was then inserted into p1013 as described above. To construct pLCKR β -A, the SacI-XbaI cDNA fragment from pIL-2R β 30 was first subcloned into CDM8, and

the obtained plasmid was digested with SacI, PstI, and BamHI. After the digestion, SacI-PstI and PstI-BamHI fragments were recovered by agarose gel electrophoresis and inserted into a backbone fragment of pIL-2R β 30, which was generated by digestion of the plasmid with SacI and BamHI. The resulting plasmid was digested with XbaI and inserted into p1013 as described above. For the construction of pLCKR β -S, pIL-2R β 30 was digested with SacI and AflIII, and after filling in both ends, the plasmid was cut by XbaI. Subsequently, the XbaI-SacI and AflIII-XbaI fragments were recovered. Those fragments were ligated with XbaI-cleaved CDM8 vector. The resulting plasmid was cloned and digested with XbaI. The XbaI-cleaved fragment was inserted into the p1013 vector as described above. The introduced mutations were confirmed by DNA sequence analysis.

DNA Transfection

Plasmid DNAs were transfected into BAF-B03 cells by an electroporation procedure as described previously (Doi et al., 1989). Selection was initiated 24 hr after the transfection using 2 mg/ml G418 in the 20% WEHI conditioned medium as described above. Wells containing a single colony were expanded.

Flow Cytometric Analysis

Cells were treated with the anti-human IL-2R β monoclonal antibody Mik- β 1 (1:400 dilution of ascites) for 30 min at 4°C. After washing, cells were stained with 1:20 dilution of fluorescein-conjugated goat anti-mouse IgG (Cappel) as a second antibody. Each stained sample was analyzed on a FACS440 cell sorter (Beckton-Dickinson).

IL-2 Binding Assay and Chemical Cross-Linking

Radiolabeling of recombinant human IL-2 (provided by Takeda Chemical Co.) and the ¹²⁵I-IL-2 binding assay were carried out according to the methods described previously (Fujii et al., 1986; Tsudo et al., 1986). Briefly, cells (3 × 10⁶/ml) were incubated with serial dilutions of ¹²⁵I-labeled IL-2 for 30 min at 4°C. After incubation, cells were layered onto an oil cushion and cell-bound, and free IL-2 was separated by centrifugation. Nonspecific binding was estimated by adding a 250-fold excess of unlabeled IL-2 in the binding assay. In some experiments, cells were pretreated with Mik- β 1 (1:100 dilution of ascites) for 30 min at 4°C before the IL-2 binding assay. For cross-linking experiments, cells (5 × 10⁶) were treated with the noncleavable cross-linker DSS (Pierce Chemical Co.) as described previously by Sharon et al. (1986). The sample was run on 8% SDS-PAGE under reducing conditions.

IL-2 Internalization

Cells (5 × 10⁷/ml) in RPMI-1640 containing 1% bovine serum albumin (BSA) and 25 mM HEPES were treated with 100 pM ¹²⁵I-IL-2 for 20 min at 4°C. After washing, cells were incubated at 37°C, and the kinetics of IL-2 internalization was measured as described previously (Robb and Greene, 1987).

Measurement of [³H]Thymidine Uptake Levels

Cells (1 × 10⁴) were cultured with various concentrations of recombinant human IL-2 or mouse IL-3 (20% WEHI conditioned medium) in RPMI-1640 supplemented with 10% FCS in a 96-well microculture plate for 24 hr. Cells were pulsed with 1 μ Ci of [³H]thymidine for 4 hr prior to harvest. To examine the effect of anti-IL-2R monoclonal antibodies on the [³H]thymidine incorporation, cells were cultured in the presence of 50 μ g/ml affinity-purified Mik- β 1 or AMT-13 (an anti-mouse IL-2R α monoclonal antibody) (Osawa and Diamantstein, 1984) or in the presence of both antibodies at the IL-2 concentration of 10 pM. [³H]-thymidine uptake was measured as described previously (Hatakeyama et al., 1985).

Measurement of the Cell Number Increase

Cells were cultured in 25 cm² culture flasks at a density of 1 × 10⁵/ml (5 ml of culture) in RPMI-1640/10% FCS containing human recombinant IL-2 at a final concentration of 1 nM. Culture media were changed every 2 days. Viable cell counts were determined by trypan blue staining.

RNA Blot Analysis

Total cellular RNA was prepared by the guanidine isothiocyanate/CsCl ultracentrifugation procedure and dissolved in 4.6 M formamide, 7.5x

SSC. After incubation at 65°C for 15 min, the sample was loaded onto nitrocellulose filter with the use of a Minifold II slot blotter (Schleicher & Schuell). A PvuII-digested 530 bp fragment of the mouse IL-2R β cDNA derived from λ MIL2R β -26 was labeled with 32 P by a random priming method (8×10^8 cpm/ μ g) and used as the probe. After hybridization, the filter was washed with 0.5 \times SSC, 0.2% SDS for 60 min at 50°C.

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Note Added in Proof

The work cited in the text as T. Doi and M. Hatakeyama, unpublished data, is now in press.

Doi, T., Hatakeyama, M., Minamoto, S., Kono, T., Mori, H., and Taniguchi, T. (1989). Human IL-2 receptor β chain allows transduction of IL-2-induced proliferation signal(s) in a murine cell line. *Eur. J. Immunol.*, in press.