TRANSMEMBRANE SIGNALING OF INTERLEUKIN 2 RECEPTOR

Conformation and Function of Human Interleukin 2 Receptor (p55)/Insulin Receptor Chimeric Molecules

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Interleukin 2 (IL-2) is best characterized as a lymphocyte growth factor. It induces cell proliferation by virtue of binding to a specific cell surface receptor that is expressed on activated T and B lymphocytes (1–3). Previously it has been shown that the IL-2 receptor (IL-2-R) is present at least in two forms, differing from each other on the basis of their affinity for ligand, and that IL-2-specific signals are delivered by the high-affinity IL-2-R (4, 5). We have demonstrated that the two forms of the IL-2-R can be reconstituted by expressing the gene encoding the human p55 (Tac antigen) in a mouse T cell line, EL-4 (6). Our study as well as those of others suggest that an additional molecule(s) present in lymphocytes is required for the formation of the high-affinity IL-2-R (6–10). While this work was in progress, several studies have demonstrated the presence of a cell surface molecule, p75, which is also capable of binding to IL-2, and indicated that the p75 constitutes the high-affinity IL-2-R in association with p55 (11–14). In view of those observations, elucidation of the structural basis of the high-affinity IL-2-R is one of the essential issues in understanding the mechanism of the IL-2-mediated signal transduction.

In growth factor receptors such as insulin receptor (Ins-R)[1] (15, 16), epidermal growth factor receptor (EGF-R) (17) and platelet-derived growth factor receptor (PDGF-R) (18), the receptor molecules contain a large cytoplasmic domain with tyrosine kinase activity that seems to play an essential role in the ligand-mediated signal transduction (19). In fact, the cytoplasmic domain of EGF-R has been shown to regulate high-affinity EGF binding of the extracellular domain (20). In

[1] *Abbreviations used in this paper:* EGF, epidermal growth factor; Ins, insulin; MPA, mycophenolic acid.

contrast to those receptors, the cytoplasmic domain of the human p55, consisting of only 13 amino acids (21–23), is too small to mediate any enzymatic activity. However, since amino acid sequences of the membrane-spanning and cytoplasmic domains are highly conserved between the human and mouse p55 molecules, it has been suggested that those regions play a role in IL-2-mediated signal transduction (24, 25). In this regard, our recent expression study using mutant cDNAs for human p55 showed no evidence for the role of two possible phosphorylation sites (Ser-247 and Thr-250) that are present within the cytoplasmic domain in the structure and function of the high-affinity IL-2-R (26).

To clarify further whether or not such a small cytoplasmic domain as well as the transmembrane region of the p55 play any role in IL-2 specific signaling, we constructed and expressed unique genes, each of which encodes a chimeric receptor consisting of the p55-derived extracellular domain and Ins-R-derived transmembrane/cytoplasmic domains (IL-2-R/Ins-R chimera). The structure of one of the expressed chimeric receptors mimics the kinase-type growth factor receptor with the ligand-binding domain being for IL-2. Here we present results of the structure and functional properties of the expressed chimeric molecules and discuss the significance of our findings in the IL-2-mediated signal transduction.

Materials and Methods

Construction of Expression Plasmids for IL-2-R/Ins-R Chimeric Receptor cDNAs. Plasmid constructions were carried out essentially following the general procedures (27).

To clone the cDNA for the human insulin receptor, a cDNA library was prepared from poly(A)$^+$ RNA of a human B cell line IM9 (28) by the standard procedure (29), and the library was screened with a synthetic oligonucleotide as the probe. One of the identified clones, designated pIns-R, contained within the cDNA a 1.6 kb Pst I–Pst I fragment encoding part of the β subunit of the human Ins-R (Fig. 1A) (15, 16). The Pst I fragment was then cloned into the Pst I site of M13 phage mp10 and the cDNA was excised out of the vector DNA by Eco RI and Hind III digestion. The resultant Hind III (5')–Eco RI (3') fragment was further digested by Ssp I, and the 1.4 kb Ssp I–Eco RI fragment was isolated (for the location of the unique restriction sites, see Fig. 1A) (15, 16). The Bam HI–Aat II fragment of the human IL-2-R (p55) cDNA (0.8 kb) was isolated from pSVIL2R-3 (6). In this procedure the Aat II site was rendered flush by T4 DNA polymerase. Then the 1.4 kb Ins-R cDNA and the 0.8 kb IL-2-R cDNA fragments were ligated and cloned into Eco RI–Bam HI–cleaved pUC19 backbone DNA. The resulting recombinant plasmid was digested by Xba I and a 2.2 kb DNA fragment containing the hybrid gene was isolated. It was then ligated with the Xba I–digested backbone fragment of pSVIL2R-3 to obtain pSVILIR-K (Fig. 1A). For the construction of pSVILIR-E, pSVILIR-K was digested with Xho I and subsequently treated by T4 DNA polymerase to generate flush ends. Then universal translation terminator sequences (GCTTAATTAAT-TAAGC) (Pharmacia Fine Chemicals, Piscataway, NJ) were inserted (Fig. 1A). The DNA sequences at the IL-2-R/Ins-R junction and at the terminator insertion site were confirmed by the sequence analysis (30).

Isolation of EL-4 Cell Clones Expressing Chimeric Receptor cDNAs. Transfection of the expression plasmids into a mouse T lymphoma line, EL-4, was performed according to the method described previously (6). Transformants were selected in RPMI 1640 medium containing mycophenolic acid (4 μg/ml) and xanthine (250 μg/ml) for 10–14 d. Expression of the chimeric receptor molecules was examined by staining the cells with anti-Tac antibody (31), and Tac$^+$ cells were sorted by fluorescence-activated cell sorter (FACS440;
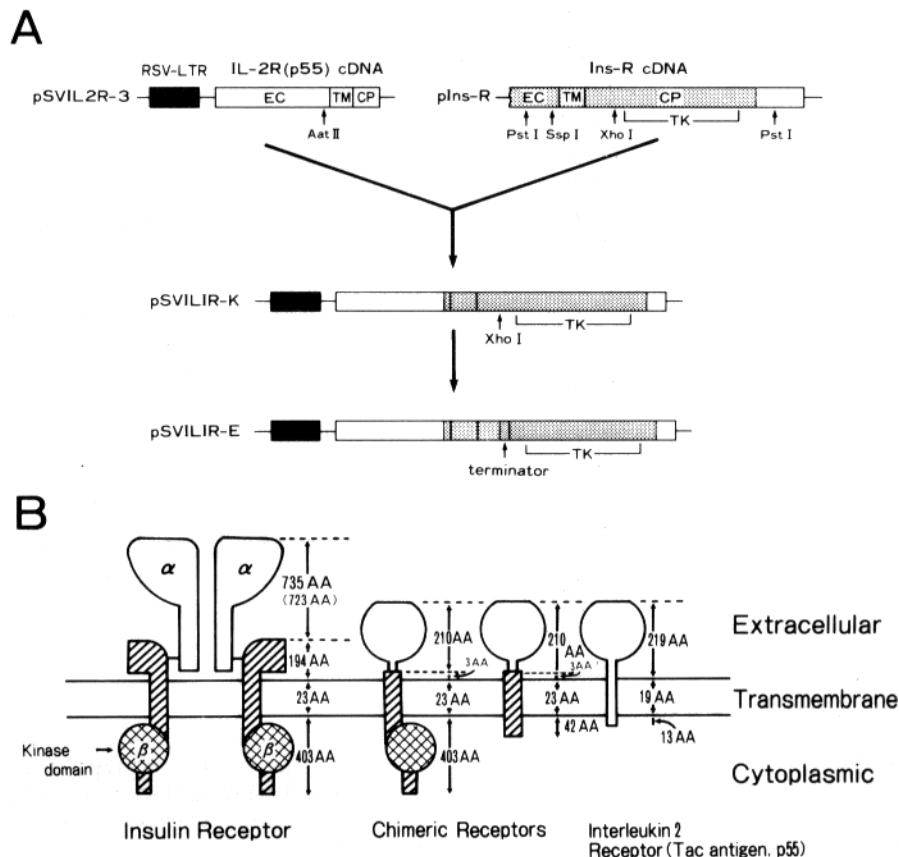
FIGURE 1.   Construction of the IL-2-R/Ins-R chimeric receptor cDNAs. (A) Construction of
the expression vectors for the chimeric receptors. See Materials and Methods for the detailed
procedure. EC, extracellular domain; TM, transmembrane domain; CP, cytoplasmic domain;
TK, tyrosine kinase domain. (B) The schematic view of the IL-2-R, Ins-R, and chimeric
receptors.

Beckton Dickinson Immunocytometry Systems, Mountain View, CA). Then the sorted
cells were cloned by seeding into each well of 96-well flat-bottom plates.

Immunoprecipitation.   Immunoprecipitation was performed as described previously
(32). Briefly, cells (5 × 10⁷) were first suspended in 1 ml PBS containing 20 mM glucose.
After addition of 10 μl of lactoperoxidase solution (2 mg/ml), 20 μl of glucose oxidase
solution (10 U/ml) and 1 μl of Na¹²⁵I (0.5 mCi), the enzyme reaction was continued for
30 min at room temperature. Subsequently, the cells were washed five times with PBS
containing 1 mM PMSF and lysed in the cell extraction buffer (10 mM Tris HCl, pH 7.5;
0.5% NP-40; 0.15 M NaCl; 1 mM PMSF). The cell lysate was preabsorbed sequentially
with BSA-coupled Sepharose 4 B and protein A–coupled Sepharose 4 B (Pharmacia Fine
Chemicals). The preabsorbed lysate was incubated overnight with protein A–coupled
Sepharose 4 B and 10 μg/ml anti-Tac. Subsequently, the beads were extensively washed
as described by Wano et al. (32), and boiled for 5 min in the SDS sample buffer. The
supernatant was then subjected to SDS-PAGE analysis. SDS-PAGE standards (Bio-Rad
Laboratories, Richmond, CA) were run in parallel as size markers.

Purification of IL-2-R/Ins-R Chimeric Receptor.   The chimeric receptor was purified

according to the method described by Roth et al. (33). Briefly, cells (7.6 × 10^9) expressing chimeric receptor molecules were washed twice with PBS and lysed in the cell extraction buffer (5 × 10^7 cells/ml) for 60 min on ice. The lysate was centrifuged at 16,000 g for 30 min. After preabsorption with BSA-Sepharose column, the supernatant was applied on the anti-Tac Sepharose 4 B column. The column was washed with 10 mM Tris HCl, pH 7.4, 0.5% NP-40, 0.5 M NaCl. Materials were eluted with 0.12 M borate buffer, pH 6.5, 0.1% NP-40, 1.5 M MgCl$_2$, and immediately diluted with about five volumes of 50 mM Tris HCl (pH 7.5). The material was first dialyzed against 50 mM Tris HCl (pH 7.5) and then concentrated by ultrafiltration (Centricon; Milipore, Bedford, MA).

IL-2 Binding Assay.   Labeling of recombinant IL-2 (5 × 10^7 U/mg protein) and the IL-2 binding assay were carried out according to methods described previously (6). Briefly, cells were incubated with serial dilutions of radioiodinated human recombinant IL-2 (2.4 × 10^4 cpm/ng protein) in a total volume of 500 µl of RPMI 1640 containing 1% BSA, 0.1% NaN$_3$, and 25 mM Hepes (pH 7.4). After 30 min incubation at 37°C, cells were layered onto an oil cushion consisting of 80% di-n-butyl phthalate and 20% olive oil, and cell-bound and free IL-2 were separated by centrifugation. The tips of the tubes that contained cell pellets were cut and the remaining radioactivity was determined. Nonspecific binding was estimated by adding a 250-fold excess of unlabeled IL-2 in the binding assay.

Effect of IL-2 on Growth of EL-4 Transformants.   Cells (2 × 10^3) were seeded into each well of 96-well microculture plates and were cultured in RPMI 1640 medium supplemented with 10% FCS in the presence or absence of various concentrations of human recombinant IL-2 for 72 h. Subsequently, cells were pulsed with [^{3}H]thymidine (1 µCi/well) for 4 h and the [^{3}H]thymidine uptake was measured. The effect of IL-2 was demonstrated by stimulation index as previously described (6).

IL-2 Internalization Assay.   IL-2 internalization was examined by using a method described previously (34). Briefly, cells (4 × 10^6 cells/0.5 ml) were incubated with 500 pM ^{125}I-IL-2 for 30 min at 0°C. Incubation was continued at either 0°C or 37°C for another 30 min. Then equal volumes (0.5 ml) of PBS or 0.5 M acetic acid, pH 2.2, 0.5 M NaCl was added to the cell suspension and cells were immediately centrifuged through a 150 µl oil cushion. The tips of each tube were cut and the radioactivity was determined. Nonspecific binding level was determined by performing the assay in the presence of 200-fold excess of unlabeled IL-2. Specific binding level was determined by subtracting the nonspecific binding level.

In Vivo Phosphorylation of Chimeric Receptor.   Cells (2 × 10^7) were washed with phosphate-free MEM and incubated in the phosphate-free-MEM supplemented with 10% dialyzed FCS for 30 min at 37°C. Then cells were resuspended in the same medium and incubated for 2 h at 37°C in the presence of 1 mCi/ml [^{32}P]orthophosphate. Cells were then washed twice with PBS containing 1 mM PMSF, 5 mM EDTA, 10 mM Na$_4$P$_2$O$_7$ (PPi), and 2 mM NaVO$_4$, and lysed in a lysate buffer (10 mM Tris HCl, pH 7.4; 0.5% NP-40; 1 mM PMSF; 5 mM EDTA; 10 mM PPi; 2 mM NaVO$_4$). After immunoprecipitation by anti-Tac, the receptor was analyzed by 10% SDS-PAGE.

In Vitro Phosphorylation by Chimeric Receptor.   10 ng of the purified chimeric receptor molecule was incubated at 25°C for 1 h in 50 mM Tris HCl, pH 7.5, 0.1% NP-40 in the presence or absence of human recombinant IL-2. Autophosphorylation reaction was carried out in 30 µl reaction mixture containing γ-[^{32}P]ATP (1 µM) and MnCl$_2$ (2 mM) at 25°C for 1 h. For exogenous substrate phosphorylation, casein was added to the same reaction mixture at a final concentration of 1.5 mg/ml. The sample was then analyzed by 10 or 15% SDS-PAGE.

Results

Construction and Expression of Genes Encoding Chimeric Receptors.   Expression plasmids encoding IL-2-R (p55)/Ins-R chimeric receptors were constructed as illustrated in Fig. 1. In one of those chimeric receptors, the p55 protein was
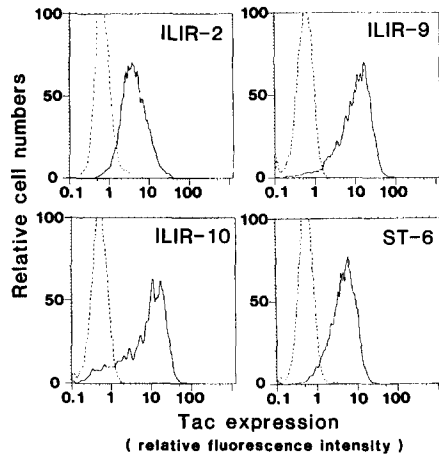
FIGURE 2.   Expression of Tac epitope in the EL-4 transformant clones. Each transformant clone was stained with anti-Tac as the primary antibody and FITC-conjugated goat anti–mouse IgG (Cappel Laboratories, Cochranville, PA) as the second antibody. Clones ILIR-2, ILIR-9, and ILIR-10 were established by transfecting pSVILIR-K, and ST-6, by pSVILIR-E. Dotted lines represent fluorescence pattern of cells treated with second-step antibody alone.

attached at residue 210 to the β subunit of the human Ins-R at residue 927, so that the extracellular domain of p55 is fused to the membrane-spanning and entire cytoplasmic region of the Ins-R (plasmid pSVILIR-K). A truncated version of this receptor was generated by introducing a universal translation terminator into the Xho I site located near the beginning of the kinase domain of Ins-R (plasmid pSVILIR-E). The cytoplasmic tail of the truncated form consists of 42 amino acid residues that contain a new Ala residue at the carboxyl terminus. Therefore, it is devoid of the tyrosine kinase domain. The predicted molecular sizes of the IL-2-R/Ins-R chimeric receptor proteins are 72 kD for pSVILIR-K and 31 kD for pSVILIR-E.

Transfection of the expression plasmids into mouse T cell line EL-4 was carried out by the bacterial protoplast fusion method as described previously (6), and the transformants were selected for mycophenolic acid (MPA) resistance. We then obtained MPA-resistant, stable EL-4 transformant clones that constitutively express the Tac epitope on the cell surface (Fig. 2). The clones, ILIR-2, ILIR-9, and ILIR-10 were obtained by transfection with pSVILIR-K, whereas clone ST-6 was obtained by transfecting pSVILIR-E. To examine whether or not the transformants express the chimeric molecules, cells were externally labeled with ^{125}I and the cell lysates were immunoprecipitated with anti-Tac antibody (31). As shown in Fig. 3, a 95–100 kD species was detected on the surface of ILIR-2, ILIR-9, and ILIR-10 cells and 55–60 kD species were detected on cells expressing either native p55 (ELT-5) (6) or the chimeric receptor without the tyrosine kinase domain (ST-6). While the observed molecular masses are 25–30 kD larger than those predicted from the protein sequence, it is most likely that posttranslational modification accounts for the difference, since massive glycosylation is known to occur in p55 processing (35).

IL-2 Binding Activity of IL-2-R/Ins-R Chimeric Molecules Expressed on EL-4.   We next examined the IL-2 binding activity of the chimeric receptor by using radiolabeled human recombinant IL-2. Scatchard plot analysis of the IL-2
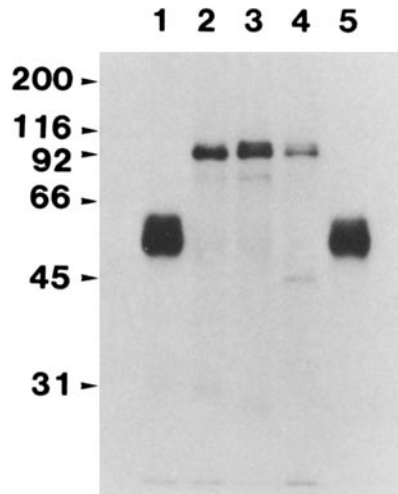
FIGURE 3. Immunoprecipitation of the native and chimeric IL-2-R molecules by anti-Tac antibody. Cells were membrane-labeled with ^{125}I by lactoperoxidase method. Cell lysates were prepared, subjected to immunoprecipitation by anti-Tac and precipitated molecules were analyzed on a 10% SDS-PAGE as described in Materials and Methods: Lane *1*, ELT-5 (an EL-4 transformant expressing the native p55 [6]; *2*, ILIR-2; *3*, ILIR-10; *4*, ILIR-9; *5*, ST-6. Arrows indicate positions of size markers (kD).
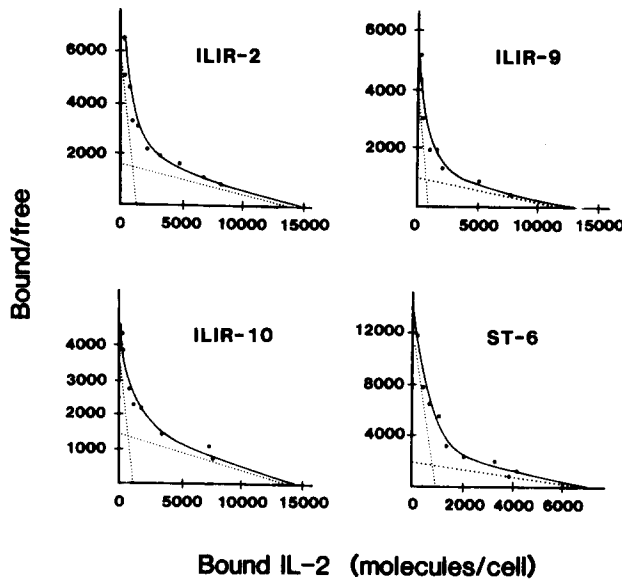

FIGURE 4. Scatchard analysis of ^{125}I-IL-2 binding assay in EL-4 transformants. The number of high- and low-affinity IL-2-R was estimated by computer-assisted analysis of the two linear components of the Scatchard plots. For the experimental details, see Materials and Methods.

binding data gave evidence of two classes of binding sites in transformant clones expressing IL-2-R/Ins-R chimeric molecules either with or without the kinase domain (Fig. 4). The dissociation constants (K_d) of the high- and low-affinity IL-2 binding sites were 110–240 pM and 6–10 nM, respectively, and the receptor numbers were ~900–1,500 and 6,000–12,000 per cell, respectively. The comparable affinities and densities on the EL-4 cells transfected with native p55 cDNA are 160–220 pM and 3,000–4,500 per cell, and 2.1–2.2 nM and 6,000–7,500 per cell for high- and low-affinity sites, respectively (6). Transfection and expression of the same chimeric receptor cDNAs in mouse L cells resulted in the appearance only of low-affinity IL-2-R (results not shown). MPA-resistant clones
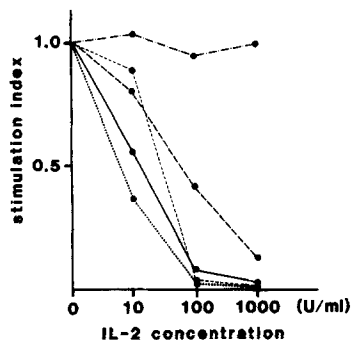
FIGURE 5.   Effect of human recombinant IL-2 on the growth of EL-4 transformants. Each of the EL-4 transformants (2 × 10³) was grown in RPMI 1640 containing 10% FCS and indicated concentrations of human recombinant IL-2 for 72 h, then pulsed with [³H]thymidine for another 4 h and the [³H]thymidine uptake was measured. ILIR-2 (――), ILIR-9 (· · · ·), ILIR-10 (――), ST-6 (– – – –). ST-2 (·――·――·) is an MPA-resistant EL-4 transformant clone that does not express Tac epitope on the cell surface. Result was demonstrated by stimulation index, which was calculated as follows: [³H]thymidine uptake of cells cultured in the presence of IL-2/[³H]thymidine uptake of cells cultured in the absence of IL-2.

that do not express the Tac epitope, as well as parental EL-4 cells showed no significant IL-2 binding (data not shown).

Effect of IL-2 on Growth of EL-4 Transformants Expressing Chimeric Receptors.   We have shown previously that EL-4 transformants expressing the native human p55 on their cell surface respond to human IL-2 (6). In those cells, IL-2 delivered negative growth signals through the reconstituted high-affinity IL-2-R. The p55 molecule was also shown to deliver positive growth signals when it was expressed in an IL-2-dependent mouse T cell clone (9). As demonstrated in Fig. 5, the growth of EL-4 transformants expressing the chimeric receptors was inhibited by IL-2. The inhibitory effect was detectable at the IL-2 concentration of 10 U/ml (~13 pM), indicating that the signaling occurs via the high-affinity receptor. The inhibitory effect was completely abolished by the addition of 1 μg/ml anti-Tac antibody, which inhibits binding of IL-2 to human IL-2-R, while addition of the same amount of anti–mouse IL-2-R (AMT-13), which inhibits the binding of IL-2 to mouse IL-2-R (36), had no effect (Fig. 6).

Ligand-mediated Internalization of Chimeric Receptor Molecules.   It has been shown that IL-2 molecules bound to the high-affinity IL-2-R are selectively internalized (34, 37). To determine whether or not the internalization of IL-2 occurs via the chimeric receptor molecules, an acid-wash method described by Weissman et al. (34) was applied. Cells were incubated with ^{125}I-IL-2 (500 pM) at 0 or 37°C. Subsequently, the acid-resistant, cell-bound radioactivity was determined to measure the degree of the IL-2 internalization. Like the EL-4 cells expressing the native human p55, the EL-4 transformants expressing chimeric receptors either with or without the kinase domain mediated the IL-2 internalization at 37°C (Fig. 7).

Protein Kinase Activity of IL-2-R/Ins-R Chimeric Molecule.   Native insulin receptor has been shown to undergo insulin-mediated autophosphorylation both in vivo and in vitro (38–40). We therefore examined in vivo phosphorylation of the chimeric receptor molecule possessing the tyrosine kinase domain. Cells (ILIR-2) expressing the chimeric receptor were incubated for 2 h with [^{32}P]-orthophosphate, lysed, and the receptor was immunoprecipitated by anti-Tac. As shown in Fig. 8A, a predominant band of 95–100 kD was detected by autoradiography, indicating that the chimeric receptor molecule is phosphorylated. However, this in vivo phosphorylation level was not altered by the addition
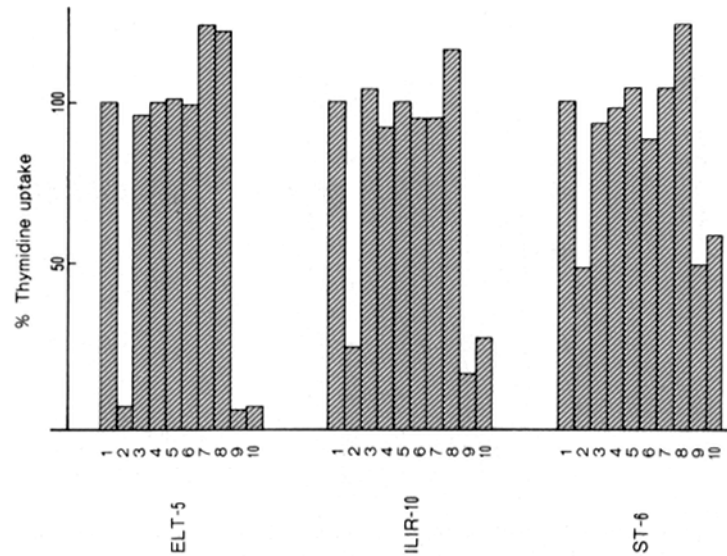
FIGURE 6. Effect of human and mouse anti-IL-2-R antibody on the cell growth inhibition by human IL-2. Each of the EL-4 transformants was cocultured for 72 h with the agents as described below, and thymidine uptake was measured. Agents used were: *1*, none; *2*, human recombinant IL-2 (h-rIL-2) 100 U/ml; *3*, anti-Tac (10 μg/ml); *4*, anti-Tac (1 μg/ml); *5*, h-rIL-2 (100 U/ml) and anti-Tac (10 μg/ml); *6*, h-rIL-2 (100 U/ml) and anti-Tac (1 μg/ml); *7*, AMT-13 (10 μg/ml); *8*, AMT-13 (1 μg/ml); *9*, h-rIL-2 (100 U/ml) and AMT-13 (10 μg/ml); *10*, h-rIL-2 (100 U/ml) and AMT-13 (1 μg/ml).
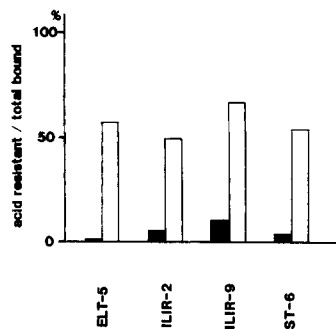

FIGURE 7. Chimeric receptor–mediated IL-2 internalization. The specific binding was measured as described in Materials and Methods. 100% activity represents a total IL-2 bound at 0 or 37°C, which is obtained by washing cells with PBS. Solid bars show specific IL-2 internalizations at 0°C, and open bars show specific IL-2 internalizations at 37°C. No specific binding was observed when parental EL-4 cells were used in this assay (<50 cpm per 4 × 10⁶ cells).

of IL-2, anti-Tac or 12-*o*-tetradecanoylphorbol-13-acetate (TPA) (data not shown). To determine if the chimeric receptor still possesses autophosphorylation activity, the molecule was purified by anti-Tac immunoaffinity chromatography and subsequently incubated in the presence of γ-[³²P]ATP and MnCl₂. The result shown in Fig. 8*B* demonstrates that the 95–100 kD affinity-purified receptor manifests autophosphorylating kinase activity. The phosphorylation seems to be tyrosine-specific, as the phosphorylated residues were resistant to treatment with KOH (data not shown). The receptor-associated kinase also had an ability to phosphorylate an exogenous substrate, casein (Fig. 8*C*). Although we do not at present have direct evidence that the purified chimeric receptor
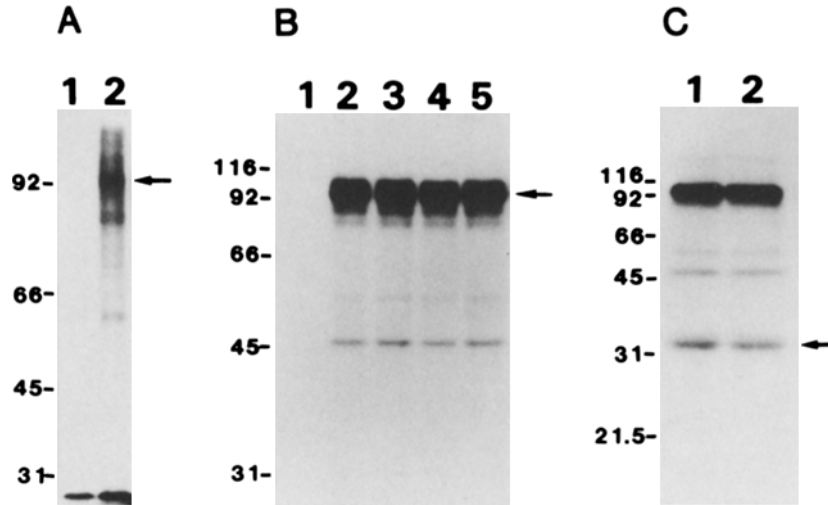
FIGURE 8.   In vivo and in vitro phosphorylation of the chimeric receptor molecules. (A) Cells (ILIR-2) were labeled with [^{32}P]orthophosphate for 2 h at 37°C. Receptor molecules were solubilized and immunoprecipitated with anti-Tac (lane 2) or control mouse monoclonal antibody (lane 1) and analyzed by 10% SDS-PAGE. (B) Affinity-purified chimeric receptor molecule of 95–100 kD was analyzed for its specific autophosphorylation activity in the presence (lanes 2–5) or absence (lane 1) of MnCl$_2$. Samples were pretreated with IL-2 for 1 h at 25°C, and the phosphorylation reactions were carried out by adding γ-[^{32}P]ATP and were analyzed by 10% SDS-PAGE. The position of the chimeric receptor molecule of 95–100 kD is indicated by an arrow: Lanes 1 and 2, no IL-2 added; 3, 100 pM IL-2; 4, 10 nM IL-2; 5, 1 μM IL-2. (C) Auto- and exogenous substrate phosphorylations were measured by adding casein as a substrate for tyrosine kinase in the autophosphorylation assay as described in B. The reaction mixture was analyzed by 15% SDS-PAGE. Lane 1, no IL-2 added; 2, 1 μM IL-2. Arrow indicates the position of phosphorylated casein.

still retains IL-2 binding activity, the kinase activity was neither enhanced nor suppressed by the addition of IL-2 in vitro at concentrations up to 1 μM (Fig. 8 B).

Discussion

To gain insight of the structural nature of IL-2-R and the signal transduction mechanism operating in the IL-2/IL-2-R system, we have constructed two genes each encoding an extracellular human IL-2-R (p55 or Tac antigen) domain fused to transmembrane and cytoplasmic domains of the human Ins-R with or without a tyrosine kinase domain. When transfected into mouse T lymphoma EL-4 cells, both genes directed the expression of chimeric receptor molecules, which specifically bound IL-2, underwent internalization, and mediated IL-2-specific inhibition of cell growth in a manner indistinguishable from the native p55. Cells transfected with the chimeric receptor cDNA manifested both high- and low-affinity IL-2 binding sites, and the K_d values were comparable to those of native p55 expressed on EL-4 (6).

Comparison of the human and mouse p55 cDNAs has revealed the conserva-

tion of the amino acid sequences in the transmembrane and cytoplasmic domains between the two species, suggesting that those regions may have important roles for signal transduction by the IL-2-R (24, 25). Our results, however, demonstrate that both transmembrane and cytoplasmic domains of the human p55 can be replaced with that of a heterologous receptor to reconstitute the functional high-affinity IL-2-R. Because the amino acid sequences of the transmembrane and cytoplasmic domains of human p55 and human Ins-R do not show any significant homology, it seems quite unlikely that the region derived from Ins-R mimics the corresponding region of p55. Therefore, the amino acid conservation may reflect other roles, such as regulation of synthesis or degradation, or subtle forms of signaling not measured in these experiments. It may be worth noting that we could not observe any significant differences in their property as IL-2-R between the receptor molecules with or without the Ins-R-derived kinase domain. This indicates the existence of a large cytoplasmic domain with active kinase does not affect the IL-2-R structure and function to a detectable degree.

Previously, we as well as others have presented evidence for a lymphocyte-specific molecules(s) whose interaction with p55 creates the high-affinity IL-2-R (6–10). In view of the finding presented here, such a molecule(s) would have direct or indirect interactions with p55 outside the cell. The primary structure of the extracellular domain of the chimeric receptor molecule is slightly shorter (six amino acids) than that of native p55, but this difference does not impair the formation of a functional IL-2-R complex. While this work was in progress, existence of a 75 kD membrane protein (p75) that might be involved in the formation of the high-affinity human IL-2-R was reported (11–14). p75 by itself binds IL-2 with intermediate affinity (K_d, 1 nM) (12–14). If p75 is the p55-associated molecule we mentioned above, then the high-affinity IL-2-R expressed on the EL-4 transformants should be composed of human p55 and mouse p75. Our recent affinity crosslink studies with ^{125}I-IL-2 support this idea; i.e., ^{125}I-IL-2 was crosslinked to a mouse p75 protein in the cells expressing native or chimeric receptors (data not shown). The formation of such a receptor complex can explain why chimeric receptor molecules that do not use the transmembrane and cytoplasmic domains of p55 can transduce an IL-2-specific signal. It seems likely that p75 is primarily responsible for the IL-2-specific signal transduction, and that the role of p55 is to increase the affinity of p75 for IL-2. The K_d values of the high-affinity IL-2-R expressed on EL-4 transformants are slightly higher than those expressed on human T lymphocytes (50–80 pM in our experimental condition, result not shown). This observation implies that binding of mouse p75 to human IL-2 is weaker than that of human p75 to human IL-2. From this viewpoint, it is worth noting that we were unable to crosslink the human IL-2 to any surface molecule on EL-4 parental cells, suggesting that the mouse p75 has very low if any affinity to the human IL-2 (our unpublished data).

Insulin stimulates the tyrosine kinase activity of Ins-R and potentiates the autophosphorylation of the β subunit of the receptor molecule (38–40). In contrast, the kinase activity of the chimeric receptor was not modulated by IL-2 binding inasmuch as measured by receptor autophosphorylation in vitro. No significant increase in cellular glucose uptake or amino acid uptake was observed

after treatment of EL-4 transformants with IL-2 (results not shown). The chimeric receptor thus appears incapable of converting an IL-2 signal into an insulin-specific cellular action.

The results presented here further emphasize the notion that the signal transduction mechanism operating in the IL-2/IL-2-R system is quite unique as compared to other growth factor receptors. Elucidation of the molecular nature of the component(s) other than p55 that would constitute the high-affinity IL-2-R in association with p55 will uncover the novel features concerning the ligand affinity and signal transduction in the IL-2/IL-2-R system.

Summary

Chimeric genes were constructed which gave rise to the expression of novel receptor molecules consisting of the extracellular domain of the human interleukin 2 receptor (IL-2-R; p55 or Tac antigen) joined to the transmembrane domain and either full-length or truncated cytoplasmic domain of the human insulin receptor (Ins-R). Expression studies using mouse T cell line EL-4 revealed that the chimeric receptors are able to manifest properties indistinguishable from the authentic IL-2-R. On the other hand, stimulation of the tyrosine kinase activity by IL-2 was not observed in the chimeric receptor with the entire cytoplasmic domain of the Ins-R. These findings thus shed light on the structural conformation and functioning of the IL-2-R complex.

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