Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 4127–4131, May 1993
Immunology

Reconstitution of functional interleukin 2 receptor complexes on fibroblastoid cells: Involvement of the cytoplasmic domain of the γ chain in two distinct signaling pathways

HIRONOBU ASAO, TOSHIKAZU TAKESHITA, NAOTO ISHII, SATORU KUMAKI, MASATAKA NAKAMURA, AND KAZUO SUGAMURA

Department of Microbiology, Tohoku University School of Medicine, Sendai 980, Japan

Communicated by Baruch S. Blumberg, February 12, 1993

ABSTRACT **We have previously shown that the interleukin 2 (IL-2) receptor γ chain is a member of the cytokine receptor superfamily and is indispensable for the formation of receptor complexes with high and intermediate affinities for IL-2. The present study demonstrates that the αβγ heterotrimer and βγ heterodimer complexes of IL-2 receptor reconstituted on murine fibroblast L929 cells can transduce IL-2-mediated signals for activation of tyrosine kinase and for induction of c-*myc*, c-*fos*, and c-*jun* expression. A mutant of the γ chain lacking the C-terminal 68 amino acids in its cytoplasmic region showed a loss of such signal-transducing ability when incorporated into the IL-2 receptor complexes but brought no effect on IL-2 binding and IL-2 internalization. Another mutant, with a C-terminal deletion of 30 amino acids, retained the ability to activate a tyrosine kinase and to induce c-*myc* expression but lost the ability to induce c-*fos* and c-*jun* expression. These results suggest that at least two distinct signals, one for c-*myc* induction, which parallels tyrosine kinase activation, and the other for c-*fos* and c-*jun* induction, can be transduced from the IL-2 receptor complexes reconstituted on fibroblastoid cells.**

Many cytokine receptors consist of a homodimer or heterodimer. Most of those components have been shown to have some consensus amino acid sequences characteristic of the cytokine receptor superfamily (1, 2). The interleukin 2 receptor α chain (IL-2Rα), which is not exclusively a member of the cytokine receptor superfamily, has only 13 amino acids in its cytoplasmic domain and forms the low-affinity ($K_d \approx 10$ nM) receptor (3–5). IL-2Rβ has 286 amino acids in its cytoplasmic domain, which is essential for intracellular signal transduction, and forms the intermediate-affinity receptor ($K_d \approx 1$ nM) and high-affinity receptor ($K_d \approx 10$ pM) in combination with IL-2Rα in lymphoid cells (6, 7). Unlike lymphoid cells, fibroblasts transfected with IL-2Rβ cDNA, however, had no significant affinity for IL-2, suggesting the existence of lymphoid cell-specific component(s) (6). We recently detected a 64-kDa molecule that coimmunoprecipitated with IL-2Rβ (8, 9). The cDNA sequence revealed that the 64-kDa molecule belongs to the cytokine receptor superfamily (10). In transfection experiments, the 64-kDa molecule formed the high- or intermediate-affinity IL-2R along with IL-2Rα and IL-2Rβ or with IL-2Rβ, respectively, in murine L929 fibroblasts and was involved in receptor-mediated IL-2 internalization (10). We thus concluded that the 64-kDa molecule is the third component, γ chain, of the IL-2R (IL-2Rγ).

Although the cytoplasmic domain of IL-2Rβ plays a crucial role in intracellular signal transduction (7), it remains to be solved whether IL-2Rγ participates in the mechanisms of intracellular signal transduction as well as IL-2 binding and IL-2 internalization. To answer this question, the present study examines IL-2Rγ mutants with deletions in the cytoplasmic region for their ability to transduce IL-2-mediated signals in transfected L929 cells, which were chosen because all the lymphoid cell lines tested spontaneously expressed IL-2Rγ (unpublished data). IL-2 stimulation induces various intracellular biochemical reactions, including activation of protein kinases such as $p56^{lck}$ (11), $p53/56^{lyn}$ (12), Raf-1 (13, 14), and S6 kinase (15, 16), possibly resulting in phosphorylation of some cellular proteins, including IL-2Rβ and IL-2Rγ (17–20); activation of $p21^{ras}$ (21); activation of phosphatidylinositol 3-kinase (37–39); glycosyl-phosphatidylinositol hydrolysis (22, 23); and expression of protooncogenes such as c-*myc*, c-*myb*, c-*fos*, and c-*jun* (24–27) in lymphoid cells. We therefore examined tyrosine phosphorylation of IL-2Rβ and induction of the protooncogene expression as parameters for signal transduction from the IL-2R complexes reconstituted on L929 cells. Our results demonstrate that IL-2Rγ is necessary for signal transduction that comprises at least two different signals.

MATERIALS AND METHODS

Cell Lines. The cell lines used were previously established by stable transfection of the human IL-2Rα and IL-2Rβ genes into murine L929 cells (10); Lαβ-2, Lαβγ-4, and Lβγ-9 transfectants express the human IL-2R α and β chains, α, β, and γ chains, and β and γ chains, respectively. Lαβγ68-6 and Lαβγ68-10 cell lines are sublines of Lαβ-2 transfected stably with pSRGdC68 plasmid DNA containing a mutant IL-2Rγ gene. Similarly, Lαβγ30-3 and Lαβγ30-5 cell lines are sublines of Lαβ-2 carrying pSRGdC30 plasmid with another mutant IL-2Rγ gene. All the cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum and antibiotics.

Plasmid Construction. For the truncation of the γ chain, an in-frame nonsense codon was introduced into pSRG1, a cDNA expression plasmid for human IL-2Rγ (10); by site-directed mutagenesis, GAA (Glu^{302}) was changed to TAA, yielding pSRGdC68, which produces a γ mutant with deletion of the C-terminal 68 amino acids. The other truncated γ mutant, with a 30-amino acid deletion at the C terminus and addition of 2 amino acids (Ala-Arg) to the C end, is encoded by plasmid pSRGdC30. For the construction of this plasmid, nucleotides for the C-terminal residues of the γ chain were deleted up to nucleotide 1031 with exonuclease III and mung bean nuclease, and then a multiple-termination linker (pGCTAGGTAGGTAGTCTAGACTACCTACCTAGC) was inserted.

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

Abbreviations: IL-2, interleukin 2; IL-2R, IL-2 receptor; SH2, Src homology 2.

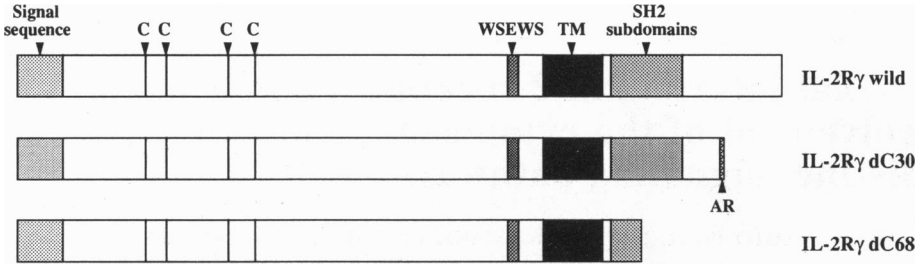

FIG. 1. Schematic structure of wild-type and mutant IL-2R γ chains. The γdC30 and γdC68 mutants lack 30 and 68 amino acids, respectively, at the C termini. In the γdC30 mutant, alanine (A) and arginine (R) residues are added to the C terminus. The possible signal sequence, cysteine residues (C), WS motif (WSEWS), transmembrane domain (TM), and Src homology 2 (SH2) subdomains are shown.

Radioimmunoprecipitation. Radioimmunoprecipitation was carried out as described (28). In brief, 10^7 cells were metabolically radiolabeled with [^{35}S]methionine/cysteine (EXPRE^{35}S^{35}S protein labeling mix, catalogue number NEG-072, NEN) at 0.1 mCi/ml (1 mCi = 37 MBq) for 8 hr, treated with 30 nM IL-2 for another 15 min, lysed in cell extraction buffer [1% (wt/vol) Nonidet P-40, 25 mM Tris·HCl, pH 7.5/140 mM NaCl/10 mM EDTA/1 mM Na_3VO_4/2 mM phenylmethylsulfonyl fluoride with aprotinin (Sigma) at 20 μg/ml], and immunoprecipitated with TU11 monoclonal antibody, which is specific for human IL-2Rβ (29). The immunoprecipitates were analyzed by two-dimensional PAGE.

IL-2 Binding and Internalization. Recombinant human IL-2 (Ajinomoto, Kanagawa, Japan) was radiolabeled with Na^{125}I (ICN) by the chloramine-T method. IL-2 binding and internalization assays were carried out with ^{125}I-IL-2 as reported previously (30). In the IL-2 binding assay, 10^6 cells were incubated with ^{125}I-IL-2 (1.5×10^6 dpm/pmol) for 1.5 hr at 4°C. Radioactivities in the supernatants and cell pellets were measured separately, and bound/unbound fractions were analyzed by Scatchard plots. In the IL-2 internalization assay, 2×10^6 cells were incubated with 200 pM ^{125}I-IL-2 on ice for 30 min. They were then washed three times and further incubated at 37°C for the indicated time. The suspension was centrifuged, and the culture supernatant was harvested. The cell pellet was then treated with chilled 20 mM citric acid in 150 mM NaCl (pH 2.8) for 10 min on ice. The radioactivities of the culture supernatant fraction, acid-soluble fraction, and acid-insoluble cell precipitate fraction were measured with a γ counter.

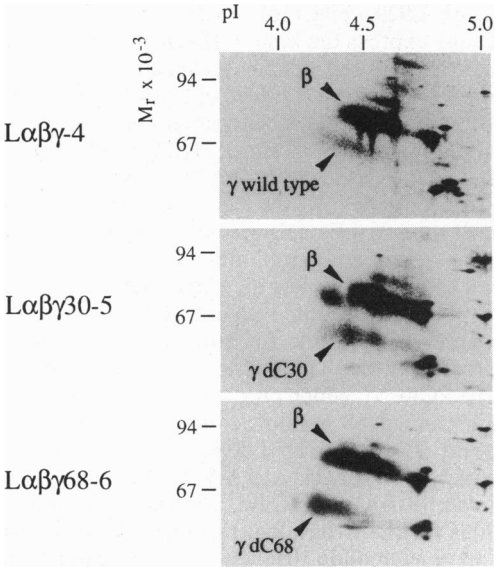

FIG. 2. Association of mutant and wild-type IL-2Rγ molecules with IL-2Rβ. The transfectants were labeled with [^{35}S]methionine and [^{35}S]cysteine, treated with 30 nM IL-2, and immunoprecipitated with TU11 antibody. Immunoprecipitates were analyzed by two-dimensional PAGE followed by autoradiography. The β and γ molecules are indicated by arrowheads.

Phosphorylation of IL-2Rβ in Cultured Cells. Cells were labeled with [^{32}P]orthophosphate (NEN) as described (19). Briefly, 10^8 cells were suspended in phosphate-free Eagle's minimum essential medium supplemented with 20 mM Hepes (pH 7.5) and 10% dialyzed fetal bovine serum. [^{32}P]Orthophosphate was added at 1 mCi/ml and cells were cultured for 2 hr. Various concentrations of IL-2 were then added, and the cells were further cultured for 10 min. They were then lysed in the cell extraction buffer and immunoprecipitated with TU11 antibody. The immunoprecipitates were analyzed by two-dimensional PAGE. Phospho amino acid analysis of ^{32}P-labeled IL-2Rβ was performed as described (19).

Northern Blot Analysis. Total RNA was isolated by extraction with guanidinium isothiocyanate from cells stimulated with 10 nM IL-2. Northern blot hybridization and probes were described previously (31). The c-*jun* probe was a 1-kbp *Pst* I fragment from pGEM4-jun.

RESULTS

Two mutant genes deleted in the cytoplasmic domain of IL-2Rγ were constructed and introduced by stable transfection with the expression vector pSRα into an L929 cell variant, Lαβ-2, expressing human IL-2R α and β subunits. One mutant, expressed from pSRGdC68, lacks 68 amino acid residues at the C terminus, resulting in impairment of the putative SH2 subdomain (Fig. 1). Although the other mutant, expressed from pSRGdC30, has 30 amino acids deleted from the C terminus, the putative SH2 subdomain is intact (Fig. 1). These plasmid DNAs were transfected into Lαβ-2 cells, and four transfectant clones were obtained: Lαβγ30-3, Lαβγ30-5, Lαβγ68-6, and Lαβγ68-10. Expression of the mutant γ chains was examined by the anti-IL-2Rβ monoclonal antibody (TU11)-directed coprecipitation with IL-2Rβ from radiolabeled cells treated with IL-2. We found coimmunoprecipitation with IL-2Rβ of 61-kDa and 57-kDa molecules with the same isoelectric point as that of the wild-type γ chain with Lαβγ30-5 and Lαβγ68-6, respectively (Fig. 2). Similar results were observed in the other cell lines, Lαβγ30-3 and Lαβγ68-10 (data not shown). The 61- and 57-kDa proteins are strong candidates for the truncated γ

Table 1. Binding profiles of IL-2R on L-cell transfectants

Transfectant	High affinity		Intermediate affinity	
	K_d, pM	Binding sites per cell	K_d, nM	Binding sites per cell
Lαβγ-4	170	1380	1.3	2400
Lαβ-2	710*	2000		
Lβγ-9			4.6	8800
Lαβγ30-3	170	370	1.7	1300
Lαβγ30-5	150	930	1.1	1830
Lαβγ68-6	160	2200	1.2	7900
Lαβγ68-10	170	1800	3.2	9270

*Lαβ-2 cells exhibit the high-affinity receptor, but its affinity is slightly lower than that of heterotrimeric IL-2R.

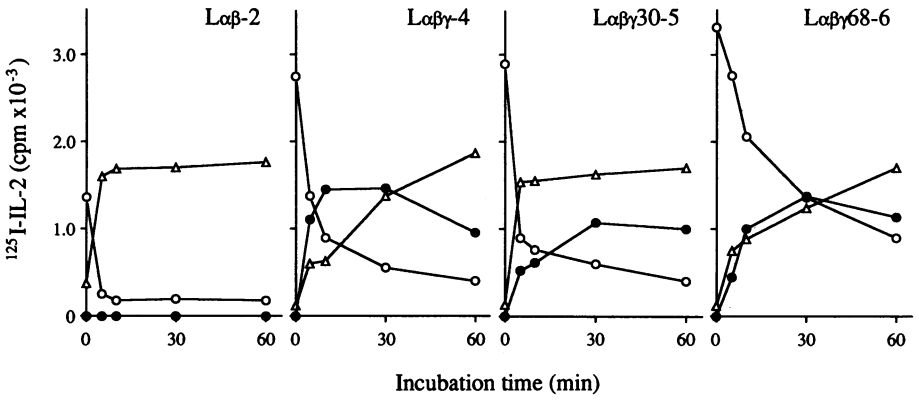

FIG. 3. Internalization of IL-2 in the transfectants. Lαβ-2, Lαβγ-4, Lαβγ30-5, and Lαβγ68-6 were examined for receptor-mediated internalization of IL-2. Radioactivities associated with IL-2 that was unbound (△), bound to cells but removed by acid wash (○), and bound to cells even after acid wash (●) were determined separately. The acid wash-resistant radioactivity is considered to be from internalized IL-2.

chains, γdC30 and γdC68, because their detection is dependent on IL-2 treatment and molecular masses of these molecules are consistent with those calculated.

Effects of truncation of the C terminus of the γ chain on IL-2 binding and IL-2 internalization were analyzed. The transfectant cell lines, Lαβγ30-3, Lαβγ30-5, Lαβγ68-6, and Lαβγ68-10, expressing mutant γ chains, exhibited intermediate ($K_d \approx$ 1.1–3.2 nM) and high (150–170 pM) affinities for IL-2, similarly to Lαβγ-4 transfectant expressing the wild-type γ chain (Table 1). Internalization of IL-2 bound to IL-2R was detected in Lαβγ30-5 and Lαβγ68-6 cells as well as in Lαβγ-4 cells but not in Lαβ-2 cells (Fig. 3). These results indicate that the cytoplasmic domain of IL-2Rγ is required neither for formation of high- and intermediate-affinity receptors nor for IL-2 internalization.

We attempted to manifest the roles of the γ chain in activation of tyrosine kinase in response to IL-2 (19). To analyze tyrosine phosphorylation of IL-2Rβ in response to IL-2, IL-2Rβ was immunoprecipitated with TU11 from lysates of Lαβγ-4 cells or Lαβ-2 cells which had been labeled with [^{32}P]orthophosphate and then stimulated with IL-2. Phosphorylation of IL-2Rβ in Lαβγ-4 cells increased in response to IL-2 stimulation, whereas little if any increase was seen with Lαβ-2 cells (Fig. 4). Phosphorylated IL-2Rβ molecules were then examined for their phospho amino acids. In Lαβγ-4 cells, increased tyrosine phosphorylation was prominent and serine/threonine phosphorylation was also responsive to IL-2 stimulation (Fig. 4). These results are very similar to those seen with lymphoid cells (19, 20). However, no IL-2-induced increase in tyrosine and serine/threonine phosphorylation was seen in Lαβ-2 cells.

Implication of the cytoplasmic domain of IL-2Rγ in the phosphorylation was examined with the transfectants of the IL-2Rγ mutants. Lαβγ68-6 cells did not show an appreciable increase in tyrosine phosphorylation or serine/threonine phosphorylation of IL-2Rβ upon IL-2 stimulation. On the other hand, an IL-2 dose-dependent increase was observed with Lαβγ30-5 cells; tyrosine phosphorylation after IL-2 stimulation at 3 nM was 6-fold higher than the control (Fig. 5). These results indicated that a 56-amino acid subregion containing the SH2 subdomains was essential and sufficient for mediating IL-2 action to activate a tyrosine kinase possibly associated with the receptor.

In lymphoid cells, IL-2 can induce expression of c-*myc*, c-*fos*, and c-*jun* (24, 26, 27). We examined by Northern blot whether the reconstituted IL-2R complexes including the IL-2Rγ mutants could transduce signals to induce expression of these genes in response to IL-2. In Lαβγ-4 and Lβγ-9 cells, IL-2 induced expression of c-*myc*, c-*fos*, and c-*jun* mRNA (Fig. 6), indicating that even in L929 cells, the reconstituted IL-2R complexes with the αβγ heterotrimers and βγ heterodimers are able to transduce the signals for induction of such protooncogenes. On the other hand, in Lαβ-2, Lαβγ68-6, and Lαβγ68-10 cells, no appreciable change in expression of these genes was detected after IL-2 treatment. Lαβγ30-3 and Lαβγ30-5 cells, which are capable of IL-2-dependent tyrosine kinase activation, showed significant induction of c-*myc* mRNA expression in response to IL-2, but showed little, if any, induction of c-*fos* and c-*jun* expression.

DISCUSSION

Our previous study documented that IL-2Rγ is essential for the ligand binding and internalizing activities of IL-2R in L929 cells transfected with the human IL-2R α, β, and γ genes (10). To determine whether IL-2Rγ also plays a critical role in intracellular signal transduction, in the present study we constructed IL-2Rγ cytoplasmic deletion mutants and reconstituted the IL-2R complexes on L929 cells by transfection with the wild-type or mutant IL-2Rγ gene along with the α and β genes. All the transfectants with IL-2R α, β, and γ subunits, either wild type or mutants (dC30 and dC68), expressed receptors that bound IL-2 with high affinities and mediated internalization of IL-2, suggesting that the cytoplasmic domain of IL-2Rγ is not required for high-affinity IL-2 binding and internalization of IL-2. Moreover, internalization of IL-2 seems independent of induction of intracyto-

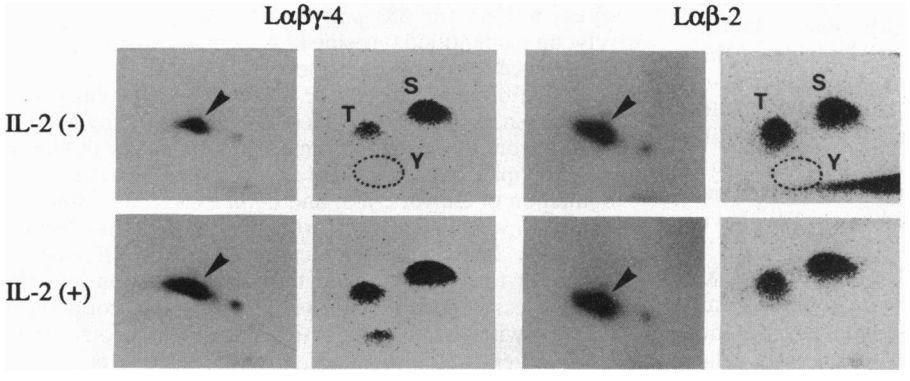

FIG. 4. IL-2-induced tyrosine phosphorylation of IL-2Rβ in the transfectants. Lαβγ-4 and Lαβ-2 cells were labeled with [^{32}P]orthophosphate, stimulated with or without 30 nM IL-2 for 10 min, and immunoprecipitated with TU11 antibody. Immunoprecipitates were analyzed by two-dimensional PAGE. Phosphorylated IL-2Rβ is indicated by arrowheads. Phospho amino acids of IL-2Rβ spots are shown in the righthand panels of each group. Positions of phosphoserine (S), phosphothreonine (T), and phosphotyrosine (Y) are indicated.

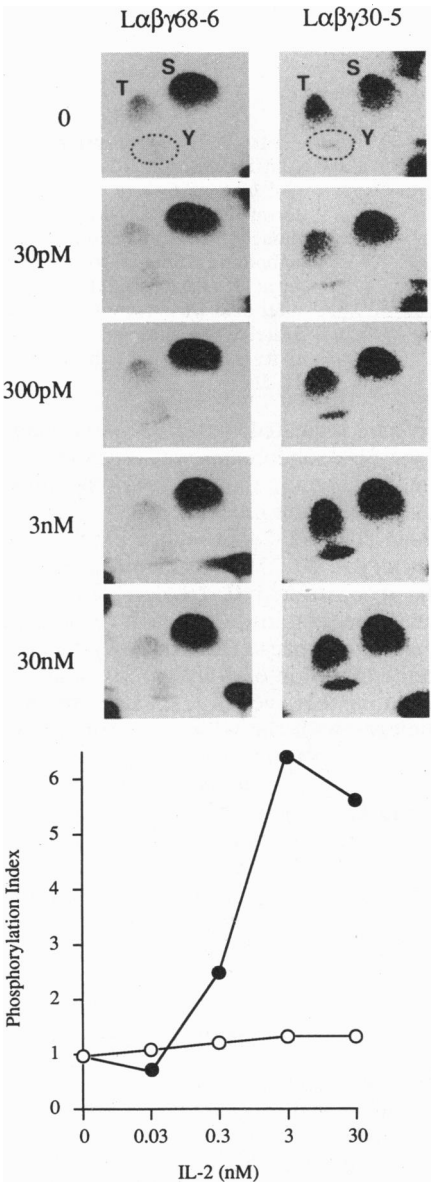

FIG. 5. IL-2-induced tyrosine phosphorylation of IL-2Rβ in the transfectants expressing mutant γ chains. Lαβγ68-6 and Lαβγ30-5 were labeled with [^{32}P]orthophosphate, stimulated with the indicated concentrations of IL-2 for 10 min, and immunoprecipitated with TU11 antibody. (*Upper*) Phospho amino acid analyses of phosphorylated IL-2Rβ. Positions of phosphoserine (S), phosphothreonine (T), and phosphotyrosine (Y) are indicated. (*Lower*) Densities of phosphotyrosine spots from Lαβγ68-6 (○) and Lαβγ30-5 (●) measured with a Bio Image analyzer (BAS2000, Fuji).

plasmic signals, because even the IL-2Rγ mutants incapable of transducing signals could internalize IL-2 similarly to the wild type. These results are in accord with those obtained previously by deletion of the cytoplasmic domains of the other cytokine receptor subunits for IL-2, IL-6, and granulocyte/macrophage-colony-stimulating factor (7, 32, 33). In addition, the soluble form of IL-6 receptor α subunit, in combination with IL-6R β chain (gp130), can bind and internalize IL-6 (32), but it remains unknown whether similar soluble forms of the other subunits, including IL-2Rγ, have such ability.

There is accumulating evidence that IL-2 induces activation of tyrosine kinases which phosphorylate some cellular proteins, including IL-2Rβ (18, 19). IL-2-induced tyrosine phosphorylation of IL-2Rβ is detectable in lymphoid cells

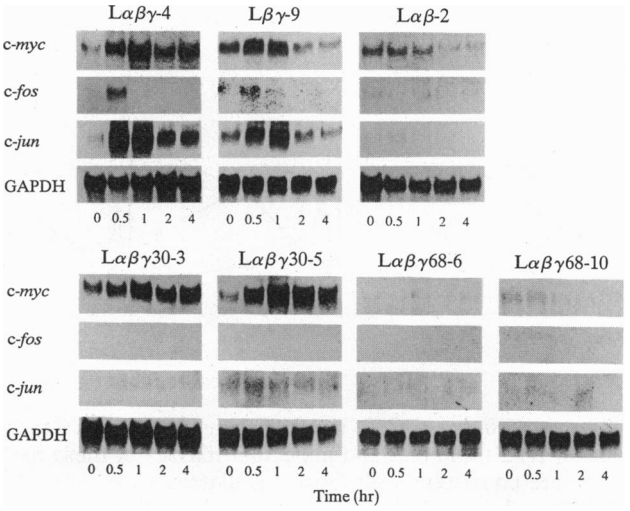

FIG. 6. Northern blot analysis of IL-2-stimulated transfectants for expression of protooncogenes encoding nuclear proteins. Cells were stimulated with 10 nM IL-2 for the indicated time and harvested for RNA preparation. The relative amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was monitored to check the amount of total RNA applied to each lane.

expressing IL-2Rβ but not in L cells (Lαβ-2) transfected with the IL-2R α and β genes (34). Our results in this study clearly show that even L cells, when transfected with the IL-2Rγ gene along with the α and β genes (Lαβγ-4), undergo tyrosine phosphorylation of IL-2Rβ in response to IL-2. Accordingly we conclude that the γ chain as well as the β chain is absolutely required for IL-2-induced activation of tyrosine kinase(s). The tyrosine phosphorylation of IL-2Rβ was also detected in the L-cell transfectant expressing the γdC30 mutant (Lαβγ30-5), but no significant phosphorylation was seen with the L subclone expressing the γdC68 mutant (Lαβγ68-6), implying that the IL-2Rγ cytoplasmic region encompassing the SH2 subdomains is involved in signal transduction through activation of tyrosine kinase, but that the C-terminal 30 amino acids of IL-2Rγ are not. Furthermore, the Lβγ-9 transfectant provides evidence that IL-2Rα is not essential for activation of the tyrosine kinase(s) that phosphorylates IL-2Rβ, as shown previously with lymphoid cells; weak but significant tyrosine phosphorylation of IL-2Rβ was seen with IL-2-stimulated Lβγ-9 cells (data not shown). It has been reported that IL-2 induces activation of p56^{lck} and p53/56^{lyn} tyrosine kinases, which are thought to be physically associated with IL-2Rβ (11, 12, 35). However, our observation of IL-2-induced tyrosine phosphorylation of IL-2Rβ in p56^{lck}-negative lymphoid cell lines (20, 34), and even in L-cell transfectants which do not express p56^{lck} at all, argues that a tyrosine kinase different from p56^{lck} may participate in the tyrosine phosphorylation of IL-2Rβ. p53/p56^{lyn} is a possible candidate because the L-cell transfectants express mRNA for p53/p56^{lyn} (data not shown). Alternatively, an unidentified tyrosine kinase may be responsible for IL-2-induced tyrosine phosphorylation of IL-2Rβ in lymphoid and fibroblastoid cells, or the tyrosine phosphorylation of IL-2Rβ in fibroblastoid cells may be mediated by a kinase different from that used in lymphoid cells, and both kinases may participate in IL-2-mediated signal transduction.

Induction of c-*myc*, c-*fos*, and c-*jun* expression after IL-2 stimulation was detected in Lαβγ-4 and Lβγ-9 transfectants but not in Lαβ-2 cells, indicating that IL-2Rβ alone is insufficient for signals linked to gene expression and that IL-2Rγ is essential for the signals; presumably, cooperation of IL-2Rγ with IL-2Rβ may result in the expression of those protooncogenes. Interestingly, the γdC30 mutant has the

ability to phosphorylate IL-2Rβ on tyrosine and to induce c-*myc* expression but has lost the ability to induce c-*fos* and c-*jun* expression. On the other hand, the γdC68 mutant does not show any ability to induce protooncogene expression. Collectively, these results indicate that the SH2 subdomains may be responsible for activation of c-*myc* expression and tyrosine phosphorylation of IL-2Rβ, but the induction of c-*fos* and c-*jun* expression requires the region containing the C-terminal 30 amino acids. These observations, in part in concert with results for lymphoid-lineage BAF-B03 cells expressing two IL-2Rβ intracytoplasmic deletion mutants (27, 36), further suggest that at least two distinct pathways are implicated in signals transduced from the IL-2R complexes in L cells; one is for c-*myc* induction and correlates with tyrosine phosphorylation of IL-2Rβ, and the other is for c-*fos* and c-*jun* induction and is independent of IL-2Rβ phosphorylation. In the BAF-B03 cell system, activation of tyrosine kinase parallels c-*fos* and c-*jun* induction but not c-*myc* induction (27, 36). This seems inconsistent with our data. This discrepancy may be due to the difference in cell types or the difference in assay system for tyrosine kinase activation. It is obvious from previous and present studies that both IL-2Rβ and γ chains are essential for intracellular signal transduction, but we do not know whether the two subunits cooperatively combine to transduce at least two different signals or whether each subunit transduces a different signal independently.

This study shows that the IL-2R αβγ heterotrimer and βγ heterodimer complexes reconstituted on L cells retain the ability to transduce signals for tyrosine kinase activation and c-*myc*, c-*fos*, and c-*jun* induction, similar to lymphoid cells transfected with the receptor subunits; however, unlike the lymphoid transfectants, no IL-2-promoted growth of the L-cell transfectants was seen even in serum-starved conditions (data not shown). Therefore, the IL-2-induced signals in fibroblastoid cells may not be exactly the same as those in lymphoid cells, and perhaps (and more probably) some additional signals that fibroblasts lack may be required for cell-growth-signal transduction mediated by IL-2R. Alternatively, as growth signals in L cells are constitutive without IL-2, effects of IL-2 on the growth promotion of L cells may not be seen.

IL-2 is known to activate putative second messengers other than tyrosine kinase, such as Raf-1 (13, 14), S6 kinase (15, 16), $p21^{ras}$ (21), phosphatidylinositol 3-kinase (37–39), and glycosyl-phosphatidylinositol hydrolysis (22, 23) in lymphoid cells. Further investigation of these signal-transducing effector molecules in conjunction with the signal-transducing ability of various mutants of IL-2R β and γ chains would shed light on the mechanisms of IL-2-induced signal transduction.

This work was supported in part by grants-in-aid for General Science Research from the Ministry of Education, Science, and Culture; by a grant-in-aid from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control; by a grant from Special Coordination Funds of the Science and Technology Agency of the Japanese Government; and by grants from the Tokyo Biochemical Research Foundation and the Ichiro Kanahara Foundation.

1. Bazan, J. F. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6934–6938.
2. Cosman, D., Lyman, S., Idzerda, R., Beckmann, M. P., Park, L. S., Goodwin, R. G. & March, C. J. (1990) *Trends Biochem. Sci.* **15,** 265–270.
3. Leonard, W. J., Depper, J. M., Crabtree, G. R., Rudikoff, S., Pumphrey, J., Robb, R. J., Krönke, M., Svetlik, P. B., Peffer, N. J., Waldmann, T. A. & Greene, W. C. (1984) *Nature (London)* **311,** 626–631.
4. Nikaido, T., Shimizu, A., Ishida, N., Sabe, H., Teshigawara, K., Maeda, M., Uchiyama, T., Yodoi, J. & Honjo, T. (1984) *Nature (London)* **311,** 631–635.
5. Cosman, D., Cerretti, D. P., Larsen, A., Park, L., March, C., Dower, S., Gillis, S. & Urdal, D. (1984) *Nature (London)* **312,** 768–771.
6. Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. & Taniguchi, T. (1989) *Science* **244,** 551–556.
7. Hatakeyama, M., Mori, H., Doi, T. & Taniguchi, T. (1989) *Cell* **59,** 837–845.
8. Takeshita, T., Asao, H., Suzuki, J. & Sugamura, K. (1990) *Int. Immunol.* **2,** 477–480.
9. Takeshita, T., Ohtani, K., Asao, H., Kumaki, S., Nakamura, M. & Sugamura, K. (1992) *J. Immunol.* **148,** 2154–2158.
10. Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M. & Sugamura, K. (1992) *Science* **257,** 379–382.
11. Horak, I. D., Gress, R. E., Lucas, P. J., Horak, E. M., Waldmann, T. A. & Bolen, J. B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1996–2000.
12. Torigoe, T., Saragovi, H. U. & Reed, J. C. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2674–2678.
13. Turner, B., Rapp, U., App, H., Greene, M., Dobashi, K. & Reed, J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1227–1231.
14. Zmuidzinas, A., Mamon, H., Roberts, T. M. & Smith, K. A. (1991) *Mol. Cell. Biol.* **11,** 2794–2803.
15. Evans, S. W. & Farrar, W. L. (1987) *J. Biol. Chem.* **262,** 4624–4630.
16. Kuo, C. J., Chung, J., Fiorentino, D. F., Flanagan, W. M., Blenis, J. & Crabtree, G. R. (1992) *Nature (London)* **358,** 70–73.
17. Ishii, T., Sugamura, K., Nakamura, M. & Hinuma, Y. (1986) *Biochem. Biophys. Res. Commun.* **135,** 487–494.
18. Saltzman, E. M., Thom, R. R. & Casnellie, J. E. (1988) *J. Biol. Chem.* **263,** 6956–6959.
19. Asao, H., Takeshita, T., Nakamura, M., Nagata, K. & Sugamura, K. (1990) *J. Exp. Med.* **171,** 637–644.
20. Asao, H., Kumaki, S., Takeshita, T., Nakamura, M. & Sugamura, K. (1992) *FEBS Lett.* **304,** 141–145.
21. Satoh, T., Nakafuku, M., Miyajima, A. & Kaziro, Y. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3314–3318.
22. Merida, I., Pratt, J. C. & Gaulton, G. N. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9421–9425.
23. Eardley, D. D. & Koshland, M. E. (1991) *Science* **251,** 78–81.
24. Reed, J. C., Sabath, D. E., Hoover, R. G. & Prystowsky, M. B. (1985) *Mol. Cell. Biol.* **5,** 3361–3368.
25. Stern, J. B. & Smith, K. A. (1986) *Science* **233,** 203–206.
26. Farrar, W. L., Cleveland, J. L., Beckner, S. K., Bonvini, E. & Evans, S. W. (1986) *Immunol. Rev.* **92,** 49–65.
27. Shibuya, H., Yoneyama, M., Tsuji, J., Matsumoto, K. & Taniguchi, T. (1992) *Cell* **70,** 57–67.
28. Asao, H., Takeshita, T., Nakamura, M., Nagata, K. & Sugamura, K. (1990) *Int. Immunol.* **2,** 469–472.
29. Suzuki, J., Takeshita, T., Ohbo, K., Asao, H., Tada, K. & Sugamura, K. (1989) *Int. Immunol.* **1,** 373–377.
30. Fujii, M., Sugamura, K., Sano, K., Nakai, M., Sugita, K. & Hinuma, Y. (1986) *J. Exp. Med.* **163,** 550–562.
31. Nagata, K., Ohtani, K., Nakamura, M. & Sugamura, K. (1989) *J. Virol.* **63,** 3220–3226.
32. Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T. & Kishimoto, T. (1989) *Cell* **58,** 573–581.
33. Sakamaki, K., Miyajima, I., Kitamura, T. & Miyajima, A. (1992) *EMBO J.* **11,** 3541–3549.
34. Kumaki, S., Asao, H., Takeshita, T., Kurahayashi, Y., Nakamura, M., Beckers, T., Engels, J. W. & Sugamura, K. (1992) *FEBS Lett.* **310,** 22–26.
35. Hatakeyama, M., Kono, T., Kobayashi, N., Kawahara, A., Levin, S. D., Perlmutter, R. M. & Taniguchi, T. (1991) *Science* **252,** 1523–1528.
36. Satoh, T., Minami, Y., Kono, T., Yamada, K., Kawahara, A., Taniguchi, T. & Kaziro, Y. (1992) *J. Biol. Chem.* **267,** 25423–25427.
37. Remillard, B., Petrillo, R., Maslinski, W., Tsudo, M., Strom, T. B., Cantley, L. & Varticovski, L. (1991) *J. Biol. Chem.* **266,** 14167–14170.
38. Augustine, J. A., Sutor, S. L. & Abraham, R. T. (1991) *Mol. Cell. Biol.* **11,** 4431–4440.
36. Merida, I., Diez, E. & Gaulton, G. N. (1991) *J. Immunol.* **147,** 2202–2207.