Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4636–4640, June 1991
Immunology

Localization in human interleukin 2 of the binding site to the α chain (p55) of the interleukin 2 receptor

(site-specific mutagenesis/structure–function analysis/lymphokines/competitive binding/Scatchard analysis)

K. SAUVÉ*, M. NACHMAN†, C. SPENCE†, P. BAILON†, E. CAMPBELL‡, W.-H. TSIEN‡, J. A. KONDAS‡, J. HAKIMI‡, AND G. JU*§

Departments of *Molecular Genetics, †Protein Biochemistry, and ‡Immunopharmacology, Roche Research Center, Hoffmann–La Roche Inc., Nutley, NJ 07110

Communicated by John J. Burns, March 7, 1991

ABSTRACT **Human interleukin 2 (IL-2) analogs with defined amino acid substitutions were used to identify specific residues that interact with the 55-kDa subunit (p55) or α chain of the human IL-2 receptor. Analog proteins containing specific substitutions for Lys-35, Arg-38, Phe-42, or Lys-43 were inactive in competitive binding assays for p55. All of these analogs retained substantial competitive binding to the intermediate-affinity p70 subunit (β chain) of the receptor complex. The analogs varied in ability to interact with the high-affinity p55/p70 receptor. Despite the lack of binding to p55, all analogs exhibited significant biological activity, as assayed on the murine CTLL cell line. The dissociation constants of Arg-38 and Phe-42 analogs for p70 were consistent with intermediate-affinity binding; the K_d values were not significantly affected by the presence of p55 in binding to the high-affinity IL-2 receptor complex. These results confirm the importance of the B α-helix in IL-2 as the locus for p55-receptor binding and support a revised model of IL-2–IL-2 receptor interaction.**

Human interleukin 2 (huIL-2) is a potent immunoregulatory cytokine, the biological effects of which are mediated through binding to specific receptors on the surface of target cells. These interleukin 2 (IL-2) receptors (IL-2Rs) are present in at least three forms that bind IL-2 with high (K_d = 10–50 pM), intermediate (K_d = 0.8–2 nM), or low (K_d = 10–30 nM) affinity (1). The high-affinity IL-2R complex consists of at least two receptor subunits, the α chain or p55 (IL-2Rα) and the β chain or p70 (IL-2Rβ). Molecular cloning and expression of these two subunits demonstrate that IL-2Rα corresponds to the low-affinity receptor (2–4), whereas IL-2Rβ is the binding component of the intermediate-affinity receptor (5). The complex of p55 and p70, with additional components, corresponds to the high-affinity IL-2R (5, 6).

The biological functions of each of these receptors have been determined by studying the effects of IL-2 binding to cells expressing natural receptor proteins or recombinant receptors. Binding of IL-2 to lymphoid cells expressing only p70 induces internalization of IL-2, expression of p55, and activation of lymphokine-activated killer (LAK) cells to an early effector phase of cytolytic activity (7–10). The interaction of IL-2 with p55 alone apparently does not result in any biological response (11), but binding to the high-affinity p55/p70 complex is essential for triggering a full proliferative response by T cells and for the late phase of effector LAK cell function (7).

To analyze the interactions between IL-2 and its receptors, we have used oligonucleotide-directed mutagenesis to map sites in IL-2 that interact with each receptor chain (12). We had previously determined that Asp-20 in the NH_2 terminus of IL-2 controls binding to the intermediate-affinity IL-2Rβ chain (13). These studies were extended to allow us to identify a cluster of specific amino acids that form the binding site in IL-2 for the low-affinity IL-2Rα subunit. Substitutions of these p55-specific residues created IL-2 analogs that interact only with p70; these analogs can help dissect the functions of the receptor components.

METHODS AND MATERIALS

Preparation of IL-2 Analog Proteins. The procedures for site-specific mutagenesis and for expression of IL-2 analog proteins in *Escherichia coli* were described (12, 13). Nucleotide sequence of the mutations was analyzed as described (12). Crude extracts of *E. coli* cells expressing analogs were prepared as described (12). Selected analogs were purified by immunoaffinity chromatography with a murine monoclonal antibody (5B1) that binds recombinant huIL-2 (14).

Analysis of IL-2 Analog Proteins. Each analog was characterized for bioactivity on the IL-2-dependent murine T-cell line CTLL-2 as described (12, 13). Competitive binding to the human high-affinity IL-2R was done at 37°C as described (12) by using a subline of constitutively activated YT cells that express both IL-2Rα and IL-2Rβ. Competitive binding to the human low-affinity p55 and the intermediate-affinity p70 receptors was determined as described (13), except that a subline of YT-1 cells expressing ≈13,000 p70 molecules per cell was used. The YT-1 cells were obtained from T. Waldmann and J. Burton (National Institutes of Health). Competitive binding to the murine IL-2R complex was determined by using EL4J-3.4 cells, a subline of the murine thymoma EL4 cell line transfected with a plasmid expressing recombinant murine low-affinity p58 receptor, as well as the high-affinity receptor (15). For direct binding studies, Scatchard analysis was done with IL-2 proteins radiolabeled with $Na^{125}I$ by using Enzymobeads (Bio-Rad) or Iodo-Gen (Pierce) to specific activities of ≈50 μCi/μg (1 Ci = 37 GBq).

RESULTS

In previous studies, we had characterized the biological activity and binding of a series of IL-2 analogs that included mutations of individual residues from over two-thirds of the IL-2 molecule (12, 13). In a preliminary analysis, none of the analogs with decreased biological activity seemed to be affected in binding to the IL-2Rα chain while still maintaining binding to the IL-2Rβ chain. To determine the amino acid(s)

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Abbreviations: IL-2, interleukin 2; huIL-2, human IL-2; IL-2R, interleukin 2 receptor; IL-2Rα, interleukin 2 receptor α chain or p55 subunit; IL-2Rβ, interleukin 2 receptor β chain or p70 subunit; LAK, lymphokine-activated killer cells; R38A, Arg-38 → Ala; F42A, Phe-42 → Ala.

§To whom reprint requests should be addressed.

that interacts with IL-2Rα, a secondary analysis was undertaken to test analogs that had retained bioactivity in the competitive binding assays (16). In these studies, we concentrated on the region of amino acids 30–60 because reports (17) had demonstrated that neutralizing antibodies that block IL-2 binding to p55 recognize epitopes mapped to this region.

In this secondary analysis, we identified an analog with the desired phenotype. Crude extracts of *E. coli* cells expressing IL-2 with a substitution of Arg-38 by Ala (R38A) contained analog protein that had <1% competitive binding to the p55 receptor (ref. 16; Table 1). This analog, designated IL-2 R38A, was capable of substantial competitive binding to p70 (≈50%) and partially bound to the high-affinity receptor (p55/p70). Although binding of IL-2 R38A to p55 substantially decreased, the analog retained full biological activity.

Additional mutations were inserted into the IL-2 protein to analyze the function of the residue at position 38 (Table 1). Substitution of Arg-38 with a negatively charged residue (Glu), polar residues (Gln, Ser), or hydrophobic residues (Phe, Leu) resulted in analogs incapable of interaction with p55 (<1% competitive binding) but fully active in the p70-binding assay as well as the CTLL bioassay. In contrast to previous analogs (12), bioactivity did not correlate with competitive binding to the high-affinity receptor, which varied from 2% to 17%. Surprisingly, the inability to bind to p55 seemed to have little, if any, effect upon biological activity.

Another residue adjacent to Arg-38 was also analyzed for its contribution to the p55-binding site (Table 1). Substitution of Phe-42 with Ala (F42A) resulted in an analog (IL-2 F42A) identical to IL-2 R38A—i.e., significantly decreased ability to interact with p55, while retaining full binding to p70. Four additional substitutions at position 42 confirm that this residue is essential for p55 binding but dispensable for p70 binding. Both hydrophobic (Leu) and polar (Ser) residues could be tolerated at this position without any significant diminution in biological activity; however, a charged residue (Lys) drastically decreased the bioactivity (to 2%), whereas substitution of a second polar residue (Gln) resulted in a smaller decrease. Again, no correlation between bioactivity and binding to the high-affinity receptor was noted for the multiple substitutions at position 42.

Two other residues in the vicinity of Arg-38 and Phe-42 also appear to affect p55 binding but in a different manner. Substitution of the Lys at positions 35 or 43 with the nonconservative amino acid Glu produced analogs with characteristics similar to R38A and F42A analogs—i.e., complete loss of p55 binding but significant p70 binding (Table 1). However, replacement of Lys-35 with Ala had no significant effect on binding to the IL-2Rα chain. There was also only a minimal effect upon binding to the high-affinity receptor even when Glu was substituted at these positions. Therefore, in contrast to residues 38 and 42, positions 35 and 43 may tolerate some side chains but not a negative charge.

The results described above were obtained with protein from crude extracts. To validate these results, two analogs were purified to homogeneity and reassayed (Table 1, Fig. 1). Comparison of the IC_{50} values of purified IL-2 R38A and IL-2 F42A with wild-type IL-2 in each of the three competitive binding assays confirmed that both residues are essential for p55 binding (Fig. 1*C*). With some preparations of purified IL-2 R38A, a very low amount of competitive binding to p55 could sometimes be detected (Table 1). Both analogs exhibited 50–100% competitive binding to p70 (Fig. 1*B*) and low but detectable binding to the p55/p70 complex (Fig. 1*A*). The specific activity of the purified analogs was $\approx 1–2 \times 10^7$ units per mg, not significantly different from wild-type IL-2 (Table 1).

Direct binding studies were used to determine the affinity of IL-2 R38A for the IL-2R (Fig. 2, Table 2). Scatchard analysis with ^{125}I-labeled R38A protein binding to human YT-1 cells expressing p70 receptors indicated that this analog bound to the β chain with intermediate affinity (K_d = 1 nM). When tested on YT cells expressing the high-affinity receptors, Scatchard analysis revealed that the affinity of R38A was only slightly altered (K_d = 0.79 nM), whereas wild-type IL-2 had a

Table 1. Bioactivity versus competitive binding of huIL-2 analogs

	Bioactivity		Competitive binding, %		
	Units/mg	% wild-type	p55/p70	p55	p70
Crude extract					
Wild type	ND	100	100	100	100
Arg-38 substitution					
→ Ala (R38A)	ND	37–150	3–50	no IC_{50}	50–54
→ Glu	ND	25–50	2–3	no IC_{50}	2–130
→ Gln	ND	133	12	no IC_{50}	73
→ Phe	ND	133	9	no IC_{50}	59
→ Ser	ND	133	10	no IC_{50}	99
→ Leu	ND	37	17	no IC_{50}	52
Phe-42 substitution					
→ Ala (F42A)	ND	19–75	1.5–7	no IC_{50}	96–195
→ Lys	ND	2	2.5	no IC_{50}	100
→ Leu	ND	75	21	no IC_{50}	30
→ Ser	ND	50	8	no IC_{50}	80
→ Gln	ND	12.5	5	no IC_{50}	74
Lys-35 substitution					
→ Glu	ND	200	47	1.25	40–175
→ Ala	ND	75	ND	64–76	170
Lys-43 substitution					
→ Glu	ND	25	38	no IC_{50}	52–300
Purified protein					
Wild type	2×10^7	100	100	100	100
R38A	$1–2 \times 10^7$	50–100	5–17	<0.05, 2.7	50–100
F42A	$0.8–1 \times 10^7$	40–50	6	<0.05	100

Percent bioactivity and competitive binding were calculated from IC_{50} values as described (12, 13) and in the legend to Fig. 1. Bioactivity was measured on murine CTLL-2 cells. Competitive binding to p55/p70, p70, and p55 receptors was assayed on human YT cells, human YT-1, and immobilized purified recombinant human p55, respectively. ND, not determined.

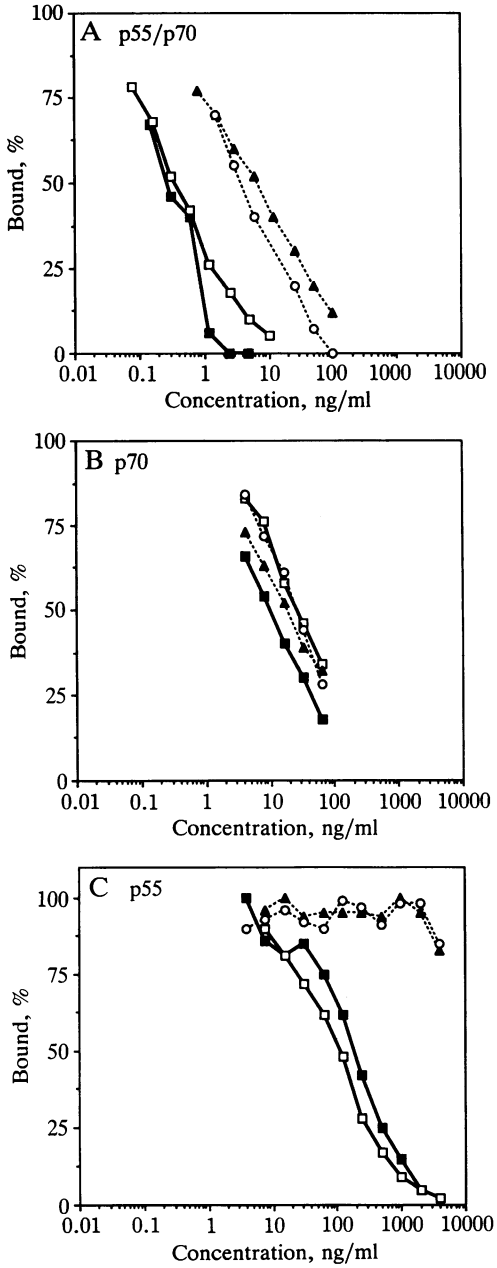

FIG. 1. Competitive binding of IL-2 R38A and F42A analogs to the human IL-2R. Human activated YT cells (*A*) or YT-1 cells (*B*) were incubated with 40 pM or 800 pM ^{125}I-labeled huIL-2, respectively, with various concentrations of purified IL-2 R38A protein (▲) or IL-2 F42A protein (○). In the solid-phase p55 assay (*C*), immobilized purified human p55 receptor protein was incubated with biotinylated huIL-2 (100 ng/ml) with various concentrations of analog proteins. The analogs were tested in separate assays. In each experiment, competitive binding with purified wild-type IL-2 protein was also determined and compared with the analog [R38A compared with wild type I (■) and F42A compared with wild type II (□)]. Data represent binding of percentage of that seen without any competing ligand. In each assay, IC_{50} (concentration of protein required to inhibit binding of labeled IL-2 by 50%) was determined and used to calculate percent competitive binding of each analog as compared with wild type protein (percent values are shown in Table 1). Competitive binding to high-affinity p55/p70 (*A*), intermediate-affinity p70 (*B*), and low-affinity p55 (*C*) components of human IL-2R are shown.

much higher affinity in the presence of p55 (K_d = 54 pM vs. 360 pM). These results confirm that the IL-2 R38A analog recognizes only the p70 component of the IL-2R complex. Moreover, the presence of p55 IL-2Rα chains does not significantly affect the binding affinity of IL-2 R38A for p70.

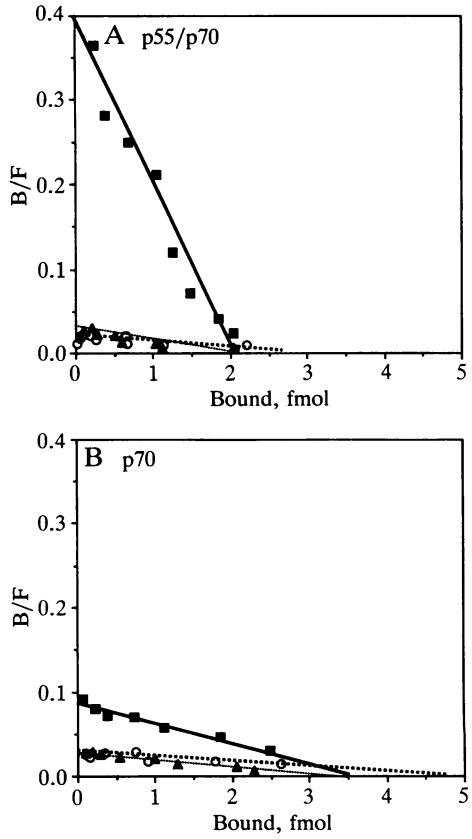

FIG. 2. Direct binding of IL-2 analog proteins to human high- and intermediate-affinity receptors. huIL-2 and IL-2 analogs R38A and F42A were labeled with ^{125}I and then incubated with human activated YT (*A*) or YT-1 cells (*B*). Labeled protein bound to cells was separated from protein that remained free in solution. Ratio of bound versus free ligand (B/F) was then calculated. Direct binding to high-affinity p55/p70 (*A*) and intermediate-affinity p70 (*B*) components of human IL-2R are shown. ■, Wild-type IL-2; ○, IL-2 F42A; and ▲, IL-2 R38A.

Similar results were obtained with ^{125}I-labeled IL-2 F42A analog protein (Fig. 2, Table 2). Scatchard analysis revealed that this analog also bound to the β-chain p70 with intermediate affinity (K_d = 1.9 nM, Fig. 2*B*). On YT cells (Fig. 2*A*), in the presence of p55, binding to IL-2R was not altered (K_d = 1.5 nM). The increase in B_{max} for the F42A analog on YT and YT-1 cells compared with wild-type IL-2 or the R38A analog was observed several times (data not shown); the significance of these data are unclear.

We examined the basis for discordance between the significant bioactivity of the IL-2 R38A and F42A analogs and their decreased binding to the high-affinity receptor. The possibility of species differences between the murine CTLL cells used for the bioassay and the human IL-2R subunits present in the competitive binding assays was considered. Competitive binding was determined (Fig. 3) by using the murine EL4J-3.4 cell line, a derivative of EL4 transfected with the recombinant

Table 2. Scatchard analysis of IL-2 analog proteins

	YT(p55/p70)*		YT-1(p70)†	
Analog	K_d, nM	B_{max}, sites/cell	K_d, nM	B_{max}, sites/cell
Wild type	0.054	5100	0.36	8,700
R38A	0.79	5280	1.0	8,700
F42A	1.5	7900	1.9	13,000

The K_d and B_{max} were determined from data shown in Fig. 2.
*Determined from Fig. 2*A*.
†Determined from Fig. 2*B*.

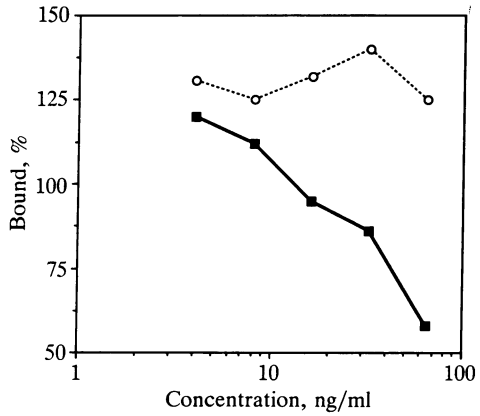

FIG. 3. Inability of IL-2 F42A to compete for binding to mouse high-affinity IL-2R. Competitive binding assays were done as described in Fig. 1, except that murine cell line EL4J-3.4 was used; these cells express recombinant murine low-affinity IL-2R (p58) and 1000–3000 murine high-affinity IL-2Rs per cell (15). EL4J-3.4 cells (6×10^6) were incubated with 800 pM of ^{125}I-huIL-2 and various concentrations of wild-type IL-2 (■) or IL-2 F42A analog (○).

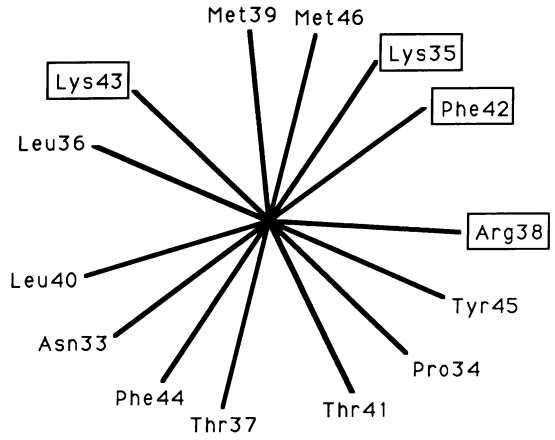

FIG. 4. Helical-wheel projection of the B α-helix of huIL-2. The B helix of IL-2 contains residues Asn-33–Met-46, as determined by x-ray crystallography (18). Residues important for binding to p55 (see text) are boxed.

murine low-affinity IL-2R subunit; these cells express ≈1000–3000 high-affinity murine IL-2Rs per cell. Wild-type human IL-2 was effective at competing for binding to these cells, as reported (15). In contrast, purified IL-2 F42A analog protein was unable to compete for binding, even at concentrations >4 nM. Species specificity, therefore, probably does not account for the surprising lack of correlation between bioactivity and high-affinity binding seen with these analogs.

DISCUSSION

Using site-specific mutagenesis, we have identified a cluster of four amino acids—Lys-35, Arg-38, Phe-42, and Lys-43—that form at least part of the binding site of IL-2 for its low-affinity receptor. IL-2 analog proteins containing substitutions of each of these residues were unaffected in binding to the p70 intermediate-affinity receptor but had dramatically reduced ability to compete for binding to the p55 low-affinity receptor. Direct Scatchard analysis confirmed that two of these analogs exhibited intermediate-affinity binding only, even on cells expressing both α and β chains of the high-affinity receptor.

The location and position of these residues that interact with p55 are consistent with antibody epitope mapping as well as with structural studies on IL-2. Neutralizing antibodies to IL-2 have been shown to recognize epitopes located in two distinct regions, either the NH_2-terminal 20 amino acids or an internal region composed of residues 30–60. Those antibodies that block binding of human IL-2 to the p55 subunit recognize epitopes in this internal region (17), which confirms our results that the α chain-specific residues lie between Lys-35 and Lys-43. The three-dimensional structure determination by Brandhuber *et al.* (18) indicated that IL-2 is a globular protein composed of six α-helices, labeled A through F. All four α chain-specific residues are located in a cluster in the middle of the second or B helix. Most strikingly, three of the four residues—i.e., Lys-35, Arg-38, and Phe-42—are positioned such that their side chains would be exposed on the same face of the α-helix, as predicted by helical wheel analysis (Fig. 4). Thus, the binding site of IL-2 for p55 appears to be a linear epitope composed of at least four residues held in an α-helical structure. Additional substitutions in this helix indicate that other residues may contribute to helical stability or have minor effects upon p55 binding (data not shown).

That substitutions at positions 35, 38, 42, or 43 created analogs unable to bind p55 because of drastic alterations in conformation or stability of the mutant protein is unlikely. (*i*) These analogs retained substantial biological activity and were completely unaffected in competitive binding to p70 (Table 1). (*ii*) Multiple substitutions confirmed the phenotype of R38A and F42A analogs; these substitutions included both conservative and nonconservative amino acids. (*iii*) Circular dichroism of purified IL-2 R38A and F42A indicated that the overall conformations of these two analogs were identical to that of wild-type IL-2 (data not shown). CD spectra have been used (13) to differentiate mutations that affected binding to the p70 receptor from those mutations that affected the conformation of IL-2.

Our mutagenesis results can be compared with similar studies by other groups. Weigel *et al.* (19) have described an Arg-38 → Gln substitution in IL-2 that reduced p55 binding by 20-fold but had no effect upon proliferative activity; Arg-38 was postulated to be a contact residue for p55, although no results were described for p70 binding. Weir *et al.* (20) proposed a key role for Phe-42 from their observation that a Phe-42 → Ala substitution reduced binding to the high-affinity receptor by 12-fold without any conformational perturbation. However, they reported a corresponding 6-fold reduction in bioactivity of the F42A analog that we did not observe with purified protein (Table 1). Substitution of Lys-35 or Lys-43 with Gln has been reported (19) not to affect p55 binding; these reports confirm our result that other side chains may be tolerated at position 35 and suggest that position 43 may be similar. The differences seen between position 38 or 42 analogs and the position 35 or 43 analogs may indicate that Arg-38 and Phe-42 contribute significantly to the free energy of binding to p55; therefore, any substitution at these latter positions will decrease binding. In contrast, adjacent residues (e.g., Lys-35 or Lys-43) that make a minor contribution to the interaction with p55 may require a drastic substitution before any loss of p55 binding is seen. Multiple substitutions at positions 35 and 43 may resolve this point.

Perhaps the most intriguing observation with all p55-negative analogs described here is their retention of substantial biological activity. Unlike most described mutants of human and mouse IL-2 (12, 13, 21), the biological activity of these analogs did not correlate with their significantly reduced binding to the human high-affinity receptor. This discordance does not reflect any species difference between the receptors on the mouse CTLL cell line used to assay bioactivity and the human IL-2Rs, as the IL-2 F42A analog also did not appear to compete for binding to the murine high-affinity IL-2R. Preliminary results indicate that the

p55-negative analogs also exhibit full bioactivity on human cells. Mouse IL-2 analog proteins with mutations in the fifth α-helix (the E helix) have been described that are also bioactive but unable to bind murine IL-2Rα; however, no residues in this helix were concluded to be important for biological activity (21). It is interesting that, although the four residues identified here as important for interaction with the human p55 receptor are absolutely conserved in mouse IL-2 (22), mouse IL-2 still has very low biological activity on human cells (23).

The discordance between binding and biological activity of these analogs has important implications for our understanding of IL-2 interactions with the IL-2R complex. Saito *et al.* (24) have proposed an affinity conversion model in which IL-2 binds first to p55 and the IL-2/p55 complex then interacts with p70 to form the high-affinity ternary complex; the large numbers of p55 molecules (in excess over the p70 molecules) should accelerate the high-affinity binding. The affinity conversion model would predict that an IL-2 analog unable to bind to p55 would have no or reduced biological activity. However, specific activities of both R38A and F42A are 50–100% of that of wild-type IL-2, confirming that binding to p55 is not a prerequisite event for bioactivity. On activated YT cells, the α chain probably does not alter affinity of the β chain or the α/β complex for these particular IL-2 analogs because the K_d values of IL-2 R38A and F42A for p70 vs. p55/p70 did not differ significantly (Table 2).

The molecular basis for the unexpected lack of correlation between bioactivity and high-affinity binding deserves further study. Preliminary results indicate that the interaction of IL-2 analogs with only the β chain induces cointernalization of the ternary complex of IL-2, the β chain, and the α chain (W. Kuziel, G.J., and W. C. Greene, unpublished work). These observations can explain how binding of IL-2 to the β chain alone is sufficient for induction of bioactivity on T cells. Our results also support a model in which at least some of the IL-2 receptor molecules exist in a preformed complex of α and β chains; such a complex has been detected by crosslinking on the surface of mouse T cells (15).

We thank Warren McComas for oligonucleotide synthesis, Lisa Collins for mutagenesis, and Bogda Wegrznski and Voldemar Toome for CD analysis. We thank Tom Waldmann and Jack Burton for the YT-1 cell line and Tom Malek for providing the EL4J-3.4 cell line.

1. Waldmann, T. A. (1989) *Annu. Rev. Biochem.* **58,** 875–911.
2. 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.
3. Nikaido, T., Shimizu, A., Ishida, N., Sabe, H., Teshigawara, K., Maeda, M., Uchiyama, T., Yodoi, J. & Honjo, T. (1984) *Nature (London)* **311,** 631–635.
4. Cosman, D., Cerretti, D. P., Larsen, A., Park, L., March, C., Dower, S., Gillis, S. & Urdal, D. (1984) *Nature (London)* **312,** 768–771.
5. Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. & Taniguchi, T. (1989) *Science* **244,** 551–556.
6. Herrmann, T. & Diamantstein, T. (1988) *Eur. J. Immunol.* **18,** 1051–1057.
7. Seigel, J. P., Sharon, M., Smith, P. L. & Leonard, W. J. (1987) *Science* **238,** 75–78.
8. Tsudo, M., Goldman, C. K., Bongiovanni, K. F., Chan, W. C., Winton, E. F., Yagita, M., Grimm, E. A. & Waldmann, T. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5394–5398.
9. Robb, R. J. & Greene, W. C. (1987) *J. Exp. Med.* **165,** 1201–1206.
10. Wang, H.-M. & Smith, K. A. (1987) *J. Exp. Med.* **166,** 1055–1069.
11. Kondo, S., Kinoshita, M., Shimizu, A., Saito, Y., Konishi, M., Sabe, H. & Honjo, T. (1987) *Nature (London)* **327,** 64–67.
12. Ju, G., Collins, L., Kaffka, K. L., Tsien, W.-H., Chizzonite, R., Crowl, R., Bhatt, R. & Kilian, P. L. (1987) *J. Biol. Chem.* **262,** 5723–5731.
13. Collins, L., Tsien, W.-H., Seals, C., Hakimi, J., Weber, D., Bailon, P., Hoskings, J., Greene, W. C., Toome, V. & Ju, G. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7709–7713.
14. Bailon, P., Weber, D. V., Keeney, R. F., Fredericks, J. E., Smith, C., Familletti, P. C. & Smart, J. (1987) *BioTechnology* **5,** 1195–1198.
15. Saragovi, H. & Malek, T. R. (1987) *J. Immunol.* **139,** 1918–1926.
16. Ju, G., Sauvé, K., Bailon, P., Tsien, W.-H., Lin, P., Hakimi, J., Toome, V. & Greene, W. C. (1990) in *The Biology and Clinical Applications of Interleukin-2*, ed. Rees, R. C. (Oxford Univ. Press, Oxford), pp. 7–14.
17. Kuo, L.-M. & Robb, R. J. (1986) *J. Immunol.* **137,** 1538–1543.
18. Brandhuber, B. J., Boone, T., Kenney, W. C. & McKay, D. B. (1987) *Science* **238,** 1707–1709.
19. Weigel, U., Meyer, M. & Sebald, W. (1989) *Eur. J. Biochem.* **180,** 295–300.
20. Weir, M. P., Chaplin, M. A., Wallace, D. M., Dykes, C. W. & Hobden, A. N. (1988) *Biochemistry* **27,** 6883–6892.
21. Zurawski, S. M. & Zurawski, G. (1989) *EMBO J.* **8,** 2583–2590.
22. Yokota, T., Arai, N., Lee, F., Rennick, D., Mosmann, T. & Arai, K.-I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 68–72.
23. Mosmann, T. R., Yokota, T., Kastelein, R., Zurawski, S. M., Arai, N. & Takeba, Y. (1987) *J. Immunol.* **138,** 1813–1816.
24. Saito, Y., Sabe, H., Suzuki, N., Kondo, S., Ogura, T., Shimizu, A. & Honjo, T. (1988) *J. Exp. Med.* **168,** 1563–1572.