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# The interleukin 2 receptor (IL-2R): The IL-2R α subunit alters the function of the IL-2R β subunit to enhance IL-2 binding and signaling by mechanisms that do not require binding of IL-2 to IL-2R α subunit

**(affinity conversion/inter-chain interactions/leukemia)**

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**ABSTRACT** **Interleukin 2 (IL-2)-mediated signaling through its high-affinity receptor involves a complex interrelationship between IL-2 and two IL-2-binding chains, IL-2R α and β chains. Previously with the reagents available it was difficult to define functional interactions between these two IL-2R subunits involved in IL-2 binding and signal transduction. To extend our understanding of the interplay between the two binding subunits we have done studies with the monoclonal antibody HIEI, which interferes with interaction of IL-2R α and β chains (IL-2Rα and IL-2Rβ, respectively). Furthermore, we used two forms of IL-2, recombinant native IL-2 and F42A, an IL-2 analog (Phe-42 → Ala substitution) that binds only to IL-2Rβ. Analog F42A manifested 75–100% of the bioactivity of wild-type IL-2. This observation is inconsistent with the strict hierarchical IL-2-binding affinity conversion model previously proposed by Saito and coworkers [Saito Y., Sabe, H., Suzuki, N., Kondo, S., Ogura, T., Shimizu, A. & Honjo, T. (1988) *J. Exp. Med.* 168, 1563–1572] that predicted an ordered sequence of events in which IL-2 must first bind to IL-2Rα before its interaction with IL-2Rβ. Previous investigations using IL-2 variants were interpreted to show that IL-2Rα merely acts to concentrate IL-2 to the cell surface and that no other meaningful interaction occurred between IL-2Rα and IL-2Rβ. However, our data are inconsistent with this view. We draw this conclusion on the basis of our observation that antibody HIEI, which reacts with an epitope of IL-2Rα and interferes with interaction of this chain and IL-2Rβ, inhibits the IL-2-dependent proliferative effects mediated by analog F42A. Furthermore, by blocking interaction of IL-2Rα and IL-2Rβ with the antibody HIEI, a decrease in the affinity of radiolabeled analog F42A for IL-2Rβ was seen. In our proposed model IL-2Rα contributes several functions to IL-2-mediated signaling through the high-affinity IL-2R. These functions include concentration of IL-2 within the two-dimensional surface of the plasma membrane as well as alteration of the functional capacity of IL-2Rβ, an effect that does not require prior binding of IL-2 to IL-2Rα. The IL-2Rα-mediated augmentation of IL-2Rβ functions involves affinity conversion of IL-2Rβ, increasing its affinity for IL-2, and may involve facilitation of IL-2-mediated signaling after binding of IL-2 to this IL-2Rβ.**

The multichain IL-2R system is pivotal in the regulation and function of multiple cells in the immune system (1). There are three forms of cellular receptors for IL-2 based on their affinity for ligand, with $K_d$ values $10^{-11}$ M, $10^{-9}$ M, and $10^{-8}$ M. We and others (2–6) have used monoclonal antibodies (mAbs) and radiolabeled IL-2 in crosslinking studies to chemically characterize the multiple subunits of this receptor. Initially, with the mAb anti-Tac, a 55-kDa IL-2R protein [now termed IL-2R α chain (IL-2Rα)] was identified (2, 7). Subsequently, with crosslinking methods, a 70/75-kDa IL-2-binding protein [p75, IL-2R β chain (IL-2Rβ)] was defined (3, 4). We proposed a multichain model for the high-affinity IL-2R, in which the high-affinity receptors would be generated when both receptor subunits were expressed and noncovalently associated in a receptor complex (3).

Most resting T cells do not display the IL-2Rα protein (1, 2). However, most T lymphocytes can be induced to express IL-2Rα by antigenic stimulation. Resting large granular lymphocytes (LGL) express the IL-2Rβ protein, but the majority do not express the IL-2Rα protein. LGL not expressing IL-2Rα can be induced to express this subunit by culture in IL-2 (8).

Kinetic binding studies with IL-2 provided an initial perspective on how the two IL-2-binding chains cooperate to form the high-affinity receptor. The kinetics of association and dissociation of IL-2 to IL-2Rα are rapid ($t_{1/2}$ = 4 and 6 sec, respectively), whereas the association and dissociation rates for IL-2 to the IL-2Rβ protein are markedly slower ($t_{1/2}$ = 45 and 290 min, respectively) (9, 10). These kinetic binding data suggested a binary complex model to define the relationship between IL-2 and the two IL-2R subunits, in which the association rate of the high-affinity receptor depends on its fast association with IL-2Rα, whereas the dissociation rate is derived from its slow dissociation from IL-2Rβ. Because the affinity of binding and equilibrium is determined by the ratio of dissociation and association rate constants, this kinetic cooperation between the low- and intermediate-affinity ligand-binding sites suggested a model for the receptor with a high affinity for IL-2 that did not require functional communication between IL-2Rα and -β subunits themselves.

There are a number of features inconsistent with this binary-complex model and that suggested a more complex relationship between IL-2, IL-2Rα, and IL-2Rβ. Critical experiments by Saito and coworkers (11, 12) focused on kinetic analyses of IL-2 binding to the high-affinity IL-2R on T lymphocytes expressing various numbers of IL-2Rα subunits and a relatively constant number of the IL-2Rβ subunits. Despite the fact that the number of IL-2Rα subunits far exceeded the number of IL-2Rβ subunits, they found that the

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Abbreviations: IL-2, interleukin 2; IL-2R, IL-2 receptor; IL-2Rα and IL-2Rβ, IL-2R α and β chains, respectively; LGL, large granular lymphocyte(s); mAb, monoclonal antibody.

expression of larger numbers of the IL-2Rα subunits accelerated the association of IL-2 to the high-affinity receptor. This result is not compatible with the binary-complex model that assumes a fixed number of high-affinity sites determined by number of the limiting chain. On the basis of these results they proposed a multistep affinity conversion model with an ordered sequence of events leading to binding and signaling (11, 12). In this model association of IL-2 with IL-2Rα is the mandatory first step followed by association of the IL-2–IL-2Rα complex with IL-2Rβ to generate the ternary complex that signals through IL-2Rβ.

To better understand the functional interactions between the two binding subunits, we did studies with two forms of IL-2, native recombinant IL-2 and F42A, an IL-2 analog (Phe-42 → Ala) that binds to IL-2Rβ alone (13). Furthermore, we used four mAbs that recognize distinct epitopes of IL-2R subunits in experiments that involved LGL expressing predominantly IL-2Rβ as well as IL-2-dependent, high-affinity IL-2Rα- and IL-2Rβ-coexpressing leukemic T-cell lines. Mik-β1, one of the mAbs used, inhibits IL-2 binding to IL-2Rβ (5). The anti-Tac mAb blocks interaction of IL-2 with IL-2Rα and disrupts IL-2 action through the high-affinity receptor (2, 7). HIEI, the third antibody used, is a mAb that recognizes a non-IL-2-binding site of IL-2Rα and can reduce the binding affinity of a leukemic cell line that has a high affinity for IL-2, supporting the view that this mAb interferes with interaction of IL-2Rα and IL-2Rβ (14, 15). Lastly, 7G7/B6 is a mAb that recognizes a non-IL-2-binding site on IL-2Rα (16).

The results obtained using mAb HIEI to interfere with interaction of the α and β chains together with those results from using IL-2 analog F42A, which does not bind to IL-2Rα, are inconsistent with the strict hierarchical affinity conversion model discussed above that mandates an initial interaction between IL-2 and IL-2Rα. We propose a model in which association of IL-2Rα alone with IL-2Rβ alters the function of IL-2Rβ to facilitate IL-2 binding and IL-2-mediated signaling. On the basis of studies using the F42A IL-2 analog, we conclude that certain effects mediated by IL-2Rα upon interaction with IL-2Rβ do not require the prior binding of IL-2 to IL-2Rα.

## MATERIALS AND METHODS

**mAbs.** Mik-β1, anti-Tac, 7G7/B6, and HIEI were prepared as described (2, 5, 14, 16).

**IL-2 Analog Proteins.** The procedures for site-specific mutagenesis and for expression of IL-2 analog proteins in *Escherichia coli* have been described (17). The selected analog was purified by immunoaffinity chromatography with a murine mAb (5B1) that binds recombinant human IL-2 (13). The F42A IL-2 analog, which does not bind to isolated IL-2Rα subunits, contains a Phe-42 → Ala conversion.

**Cells.** YTS, a cell line expressing ≈20,000 IL-2Rβ subunits per cell and small numbers of IL-2Rα, was obtained from J. Yodoi (Kyoto University). Kit-225, a human T-lymphotropic virus-negative leukemic T-cell line that expresses high-affinity IL-2 receptors was from T. Uchiyama (Kyoto University). LGL depleted of T cells, B cells, and monocytes were prepared as described (18, 19). This LGL population was depleted of IL-2Rα-expressing cells by coating the cells with anti-Tac on ice for 30 min and then removing the mAb-binding cells by adding anti-mouse IgG on magnetic beads followed by magnetic extraction (Collaborative Research).

**Functional Assays.** Cytotoxicity ($^{51}$Cr release), proliferation ([$^{3}$H]thymidine incorporation), and binding ($^{125}$I-labeled IL-2) assays were done as described (5, 18).

## RESULTS

**Effects of mAbs on Proliferation of IL-2Rβ-Expressing LGL to IL-2.** The initial functional and mAb-blocking studies were done on LGL predominantly expressing IL-2Rβ. In 72-hr proliferation experiments with IL-2Rα-depleted IL-2Rβ-expressing LGL, the addition of mAb Mik-β1 at 20 μg/ml inhibited IL-2-induced proliferation by ≈70%, whereas anti-Tac at 20 μg/ml yielded ≈30% inhibition (data not shown). The inhibition by anti-Tac suggests that, as in previous studies, the IL-2Rα subunit is rapidly expressed by LGL on IL-2 exposure (8). Combination of mAbs Mik-β1 and anti-Tac resulted in almost complete inhibition in these proliferation studies.

**Effects of mAbs on Cytolytic Activity of IL-2Rα-Nonexpressing, IL-2Rβ-Expressing LGL.** To assess the effects of mAbs on IL-2-dependent cytotoxicity, LGL were activated for 24 hr with or without IL-2 and mAbs before 4-hr coculture with $^{51}$Cr-labeled Daudi cells. Addition of mAb Mik-β1 at 20 μg/ml to IL-2Rα-depleted LGL cultured with 1 nM IL-2 for 24 hr decreased the generation of activated cytolytic activity against Daudi targets by ≈80% when compared with the cytotoxicity seen after incubation of LGL with recombinant IL-2 without mAb (Fig. 1). Addition of anti-Tac had no effect on IL-2-activated killing of Daudi targets. Addition of both Mik-β1 and anti-Tac completely inhibited IL-2-induced killing. From these studies on LGL proliferation and cytotoxic activity, we conclude that an antibody that blocks IL-2-binding to IL-2Rβ inhibits IL-2-mediated events when the cells examined initially express IL-2Rβ alone. Phillips and coworkers (20) reported similar results. Thus, LGL expressing predominantly IL-2Rβ can respond functionally to IL-2 without any need for IL-2Rα.

**Effects of Anti-IL-2R mAbs on $^{125}$I-Labeled IL-2 Binding to Kit-225, an IL-2Rα/IL-2Rβ-Expressing T-Cell Line.** To address the central issue of the present study, the functional interactions between the α and β subunits of the multichain IL-2R, the effects of different mAbs on IL-2-mediated events were studied by using Kit-225, a human T-lymphotropic virus-negative T-cell line that expresses high-affinity IL-2

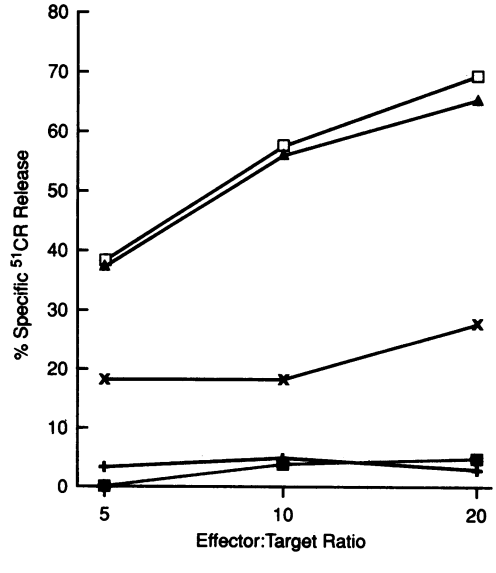


FIG. 1. Representative $^{51}$Cr-release cytotoxicity assay showing effects of IL-2 (□), IL-2 plus anti-Tac (▲), IL-2 plus mAb Mik-β1 (×), IL-2 plus anti-Tac plus mAb Mik-β1 (+), or medium alone (■) on LGL-induced killing of Daudi targets. All antibodies (20 μg/ml) were added to LGL at time 0 just before IL-2 (1 nM) was added for a 24-hr incubation. The various populations of effector cells were cocultured with $^{51}$Cr-labeled Daudi targets for 4 hr. Each point represents an average of triplicates.

receptors. The initial studies were directed toward defining the effects of the three IL-2R mAbs on $^{125}$I-labeled IL-2 binding by this cell line as assessed by Scatchard analysis. Addition of anti-Tac, which recognizes the ligand-binding site of IL-2R$\alpha$, abolished specific binding of $^{125}$I-labeled IL-2 to Kit-225 cells. Addition of mAb Mik-$\beta$1 to these cells abolished high-affinity binding, measured at 4°C, while retaining residual low-affinity IL-2 binding (data not shown). Addition of antibodies to two distinct epitopes of the IL-2R$\alpha$ subunit not involved in direct ligand binding led to quite different effects. Addition of mAb 7G7/B6 had no effect on radiolabeled IL-2 binding to Kit-225 cells (Fig. 2*A*). In contrast, addition of mAb HIEI reduced high-affinity binding markedly, but there was no effect on low-affinity IL-2 binding. In parallel studies mAb HIEI did not affect IL-2 binding to a cell line (MT-1) that expresses IL-2R$\alpha$ alone and that manifests low-affinity binding. Furthermore, mAb HIEI does not bind to cells expressing IL-2R$\beta$ alone. These data support the view that mAb HIEI blocks high-affinity binding by inhibiting interaction of IL-2R$\alpha$ and IL-2R$\beta$.

**Effects of mAbs on IL-2-Dependent Proliferation of Kit-225 Cells.** To assess the functional effects of mAbs on proliferation mediated by IL-2 through the high-affinity IL-2Rs expressed on T cells, Kit-225 cells were washed free of IL-2 and cultured for 3–4 days without IL-2. These rested, IL-2-responsive Kit-225 cells manifest a sigmoid proliferation dose–response to IL-2 (Fig. 3). When mAbs to different epitopes on the two IL-2R subunits were added to cultures, distinct patterns of inhibition of IL-2-mediated proliferation were observed. Addition of anti-Tac alone was associated with a profound, yet incomplete, inhibition of proliferation. In contrast, addition of mAb Mik-$\beta$1, which at 4°C abolished high-affinity IL-2 binding to these cells, only very modestly inhibited IL-2-induced proliferation, as has been reported (5). Although mAb Mik-$\beta$1 had only a modest effect when used alone, it significantly augmented the capacity of anti-Tac to inhibit IL-2-mediated events (Fig. 3). Addition of mAb HIEI alone only slightly inhibited IL-2-induced proliferation of Kit-225 (Fig. 3). However, when mAb Mik-$\beta$1, incapable of meaningful inhibition alone, was added with mAb HIEI, profound inhibition was seen. We conclude from these studies that when the interaction between IL-2R$\alpha$ and IL-2R$\beta$ is blocked by mAb HIEI, subsequent interference of the direct binding of IL-2 to IL-2R$\beta$ is associated with marked inhibition of IL-2-mediated proliferation.

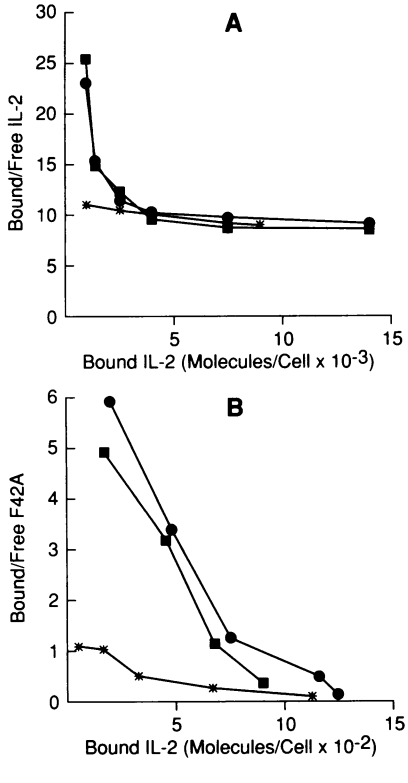


FIG. 2. Effect of mAb HIEI on high-affinity IL-2 binding to Kit-225. Scatchard plot analysis of $^{125}$I-labeled IL-2 (*A*) and $^{125}$I-labeled F42A (*B*) to Kit-225 cells with 7G7/B6 (●) or mAb HIEI (*) (each at 20 $\mu$g/ml) or medium (■).

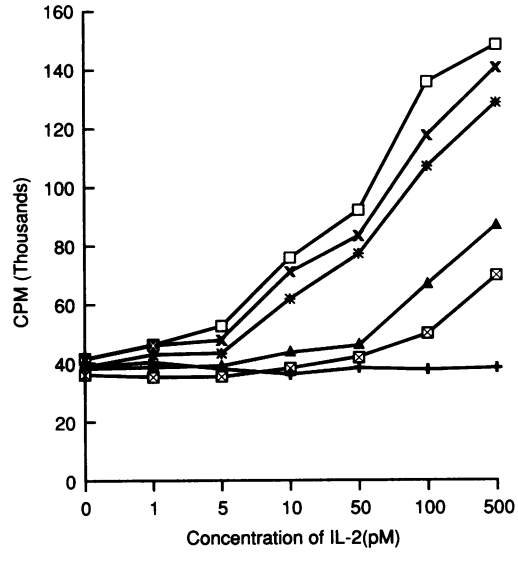


FIG. 3. Proliferative response of Kit-225 cells to IL-2 (□), IL-2 plus mAb HIEI (*), IL-2 plus anti-Tac (▲), IL-2 plus mAb Mik-$\beta$1 (×), IL-2 plus mAb Mik-$\beta$1 plus mAb HIEI (⊠), IL-2 plus mAb Mik-$\beta$1 plus anti-Tac (+). mAbs were added at the beginning of the assay at 5 $\mu$g/ml.

**Effects of Anti-IL-2R mAbs on Binding and Function of F42A, an IL-2 Analog That Binds to IL-2R$\beta$ but Not to Cells Expressing the IL-2R$\alpha$ Subunit Alone.** The studies just discussed indicate that interaction of IL-2R$\alpha$ and IL-2R$\beta$ facilitates IL-2-mediated proliferation. These studies do not, however, distinguish between two alternative models. In the first, the hierarchical IL-2-binding affinity conversion model proposed and developed by Saito and coworkers (11, 12) proposes a step-wise binding of IL-2, in which IL-2 must first bind to IL-2R$\alpha$, and then the resultant complex becomes associated with IL-2R$\beta$, thereby forming a high-affinity ternary complex.

In the second model considered, the capacity of IL-2R$\beta$ to respond to IL-2 alters by interaction with IL-2R$\alpha$, as in the affinity conversion model. However, this second model does not require any binding of IL-2 to IL-2R$\alpha$. To distinguish between these alternatives, we used an IL-2 analog protein, F42A. In previously reported competitive binding studies, this analog exhibited 50–100% of wild-type binding capacity to IL-2R$\beta$ and no demonstrable binding to IL-2R$\alpha$ (13). We have confirmed that this analog binds to IL-2R$\beta$ but not to isolated IL-2R$\alpha$ by using fluorescein-activated cell sorter analysis, competitive binding inhibition, as well as radiolabeled binding studies that involved cell lines bearing IL-2R$\beta$ alone (MLA144) or IL-2R$\alpha$ alone (MT-1). In studies assessing IL-2-dependent proliferation, F42A manifested 75–100% of the bioactivity of wild-type IL-2 in IL-2R$\alpha$- and -$\beta$-expressing Kit-225 cells. However, a pattern of inhibition distinct from that of wild-type IL-2 was seen when mAbs to IL-2R subunits were added. Addition of mAb Mik-$\beta$1 alone profoundly inhibited proliferation of Kit-225 cells stimulated by F42A in contrast to its very modest inhibition when wild-type IL-2 was used (Fig. 4). Furthermore, although F42A does not bind to the isolated IL-2R$\alpha$ subunit, addition of anti-Tac was

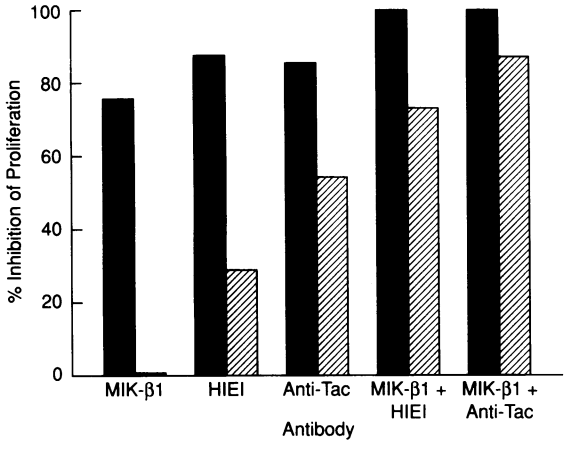


FIG. 4. Inhibition of IL-2 (▨) or analog F42A (■) induced proliferation of Kit-225 cells in the presence of indicated antibodies at 5 μg/ml. mAb 7G7/B6 produced <15% inhibition for IL-2 and for F42A.

associated with a profound inhibition of F42A-mediated proliferation. Finally, we observed that the proliferation induced by F42A through the high-affinity receptor was inhibited by addition of mAb HIEI, which interacts solely with IL-2Rα and which inhibits its close association with IL-2Rβ (Fig. 4). This result suggested that IL-2Rα contributes functionally to the signaling of F42A through IL-2Rβ.

To evaluate the nature of the effect that IL-2Rα exerts upon IL-2Rβ we used $^{125}$I-labeled F42A for binding studies. In Scatchard analysis done at 4°C, radiolabeled F42A bound with intermediate affinity to both IL-2Rβ-expressing YTS cells (2.1 nM) and with 10-fold higher affinity (0.16 nM) to IL-2Rα- and IL-2Rβ-expressing Kit-225 cells (Table 1). We then defined its binding affinity for Kit-225 cells in the presence or absence of various antibodies that bind to the IL-2R. The addition of anti-Tac or mAb Mik-β1 produced complete inhibition of binding (data not shown). mAb HIEI reduced the affinity of F42A for Kit-225 from 0.16 nM to 1.10 nM, whereas 7G7/B6 had no effect (Fig. 2*B*, Table 1). On the basis of these studies with F42A, which binds solely to IL-2Rβ, and those with mAb HIEI, which inhibits IL-2Rα and IL-2Rβ interaction, we propose that some functional contributions of IL-2Rα- to IL-2Rβ-mediated signaling do not require the binding of IL-2 to IL-2Rα subunit.

## DISCUSSION

The high-affinity receptor for IL-2 requires interaction of the IL-2Rα and IL-2Rβ subunits (4, 5). IL-2Rβ is a member of the hemopoietin receptor family, which includes receptors for interleukins 3, 4, 6, 7; granulocyte/macrophage colony-stimulating factor; prolactin; growth hormone; and erythropoietin (21–24). Although conclusions cannot be drawn about the nature of the signaling pathways for any of these systems, in many of these cases two cooperating receptor proteins are required to initiate signaling. This situation appears true for the IL-2Rβ subunit. Transfection of IL-2Rβ cDNA into nonlymphoid NIH 3T3 or Chinese hamster ovary (CHO) cells decreases the $K_d$ for IL-2 binding on this subunit by a factor of 50–100, suggesting that additional lymphoid-specific factors are required for the intermediate affinity-binding profile characteristic of lymphoid cells (21). A putative γ chain has been considered as the lymphoid factor involved in augmenting the capacity of IL-2Rβ to bind IL-2. However, IL-2Rα might also contribute to this enhanced IL-2 binding by IL-2Rβ. COS cells expressing both IL-2Rα and IL-2Rβ have receptors with an increased $K_d$ when compared with cells expressing IL-2Rα alone; yet these cells have a diminished $K_d$ when compared with high-affinity IL-2 receptors on T cells, adding further evidence for a more complex receptor subunit composition (21). Numerous studies have supported the view that the IL-2Rα subunit plays a major role in IL-2-mediated biological effects. Saito and coworkers (11, 12) proposed a hierarchical-binding affinity conversion model that predicts an ordered sequence of events leading to binding and signaling, in which IL-2 must first bind to the IL-2Rα subunit before its interaction with IL-2Rβ. With native IL-2 it is difficult to distinguish this hierarchical IL-2-binding affinity conversion model from an alternative model in which binding of IL-2 to IL-2Rα is not required for cooperative interaction of IL-2Rα and IL-2Rβ. To help analyze the relationship between IL-2Rα and IL-2Rβ we used an IL-2 analog protein F42A, which has 50–100% of wild-type binding capacity to IL-2Rβ and yet no demonstrable binding to IL-2Rα (13). On the basis of the hierarchical IL-2-binding affinity conversion model one would predict that this mutant IL-2 molecule, incapable of binding to IL-2Rα, would not effectively signal through cells expressing high-affinity IL-2 receptors. In contrast to this prediction, F42A manifested 75–100% of the bioactivity of wild-type IL-2, as assessed by proliferation using IL-2Rα- and IL-2Rβ-coexpressing Kit-225 cells. These results agree with the observations made with R38A, a second IL-2 analog in which amino acid 38 of the second α-helix is altered (13), a comparable amino acid 38 analog (Arg → Glu) defined by Weigel and coworkers (25), as well as a murine IL-2 analog with a disrupted fifth α-helix described by Zurawski and Zurawski (26). In each case the IL-2 variants could not bind to IL-2Rα and yet manifested 30–100% of the bioactivity of wild-type IL-2. These observations are not in accord with the strict, hierarchical IL-2-binding affinity conversion model.

Table 1. Scatchard analysis of binding

| Cell | mAb | $K_d$ (nM) IL-2 | $K_d$ (nM) F42A |
|---|---|---|---|
| MT-1 | | 27.00 | None |
| YTS | | 1.50 | 2.10 |
| Kit-225 | | 0.04, 12.5* | 0.16 |
| Kit-225 | HIEI | 2.50 | 1.10 |
| Kit-225 | 7G7/B6 | 0.04, 15.5* | 0.18 |

MT-1 cells carry only low-affinity IL-2Rα, YTS cells express predominantly intermediate-affinity IL-2Rβ, and Kit-225 expresses IL-2Rα and IL-2Rβ high-affinity IL-2 receptors.
*Values represent both high-affinity binding of complexed IL-2Rα with IL-2Rβ and low-affinity binding of excess IL-2Rα.

The observations discussed, with IL-2 variants incapable of binding to IL-2Rα, have been interpreted as showing that IL-2Rα merely acts to concentrate IL-2 to the cell surface plasma membrane and that no relevant role exists for an interaction between IL-2Rα and IL-2Rβ (26). However, our data are not consistent with this model relegating IL-2Rα to such a modest role. In contrast to the predictions of this latter model, addition of either anti-Tac or mAb HIEI, which binds to IL-2Rα, profoundly inhibited the proliferation mediated by F42A, which binds solely to IL-2Rβ. The inhibition mediated by anti-Tac might be from stearic inhibition of access of F42A to IL-2Rβ if the two complexed chains form a receptor pocket. Alternatively, anti-Tac might interfere with the interaction of IL-2Rα and IL-2Rβ. In accord with this second possible contribution, proliferation mediated by F42A was inhibited by adding mAb HIEI, which prevents association of IL-2Rα and IL-2Rβ. Taken together, our studies support the view that, in addition to its capacity to bind IL-2, IL-2Rα alone interacts with IL-2Rβ and alters its IL-2-induced functions. Furthermore, we conclude that certain contributions of IL-2Rα to IL-2Rβ do not require IL-2 binding to IL-2Rα.

To define additional actions that do require IL-2 binding to IL-2Rα in IL-2-mediated signaling, we compared the function of native IL-2, which can bind to both subunits, with F42A, which can bind solely to the IL-2Rβ chain. When IL-2Rα- and -β-expressing Kit-225 cells were used, affinity of F42A was 10-fold lower than that seen for native IL-2 ($K_d$ = 0.16 nM vs. 0.04 nM). Furthermore, the proliferative functions mediated by F42A in cells expressing the high-affinity receptor were markedly inhibited by adding mAb Mik-β1 alone. This result contrasted with observations with native IL-2, which required coaddition of a mAb to IL-2Rα—either anti-Tac or mAb HIEI—as well as mAb Mik-β1 to profoundly inhibit IL-2-induced proliferation.

From the functional studies, IL-2Rα clearly contributes in several ways to IL-2-mediated signaling through the high-affinity IL-2 receptor, in addition to its proposed role in concentrating IL-2 onto the plasma membrane surface. Interaction of IL-2Rα alone with IL-2Rβ alters the ability of IL-2Rβ to enhance IL-2-mediated signaling. On the basis of studies using the F42A IL-2 analog, we conclude that certain functions facilitated by this interaction between IL-2Rα and IL-2Rβ are independent of the binding of IL-2 to IL-2Rα subunit. The nature of the contribution mediated by the interaction of IL-2Rα alone with IL-2Rβ has not been completely defined. This contribution appears due, in part, to affinity conversion, wherein IL-2Rβ has a higher affinity for IL-2 when near IL-2Rα. Using an analog of IL-2, Lys-20 (Asp-20 → Lys substitution), which binds solely to IL-2Rα, Arima and coworkers (27) also proposed that IL-2Rα alters the binding of IL-2 to IL-2Rβ. Evidence that IL-2Rα increased the affinity of IL-2Rβ for IL-2 was obtained in the current study by exploring the effect of mAb HIEI on the affinity of analog F42A to IL-2Rα/IL-2Rβ-expressing cells.

Greater binding affinity of F42A was seen with Kit-225 cells (0.16 nM), which manifest IL-2Rα and IL-2Rβ, than to YTS cells (2.1 nM), which express IL-2Rβ predominantly. Furthermore, mAb HIEI reduced the affinity of F42A binding to the IL-2Rα/IL-2Rβ-expressing cells by 7-fold (0.16 vs. 1.10 nM) in our studies with this IL-2 variant that cannot bind to isolated IL-2Rα. The 7-fold increase in affinity that IL-2Rα exerts upon IL-2Rβ is not sufficient to explain the functional contributions of IL-2Rα to IL-2Rβ as seen in proliferation assays. Therefore, we suggest that the proximity of IL-2Rα to IL-2Rβ may, in addition, facilitate IL-2/IL-2Rβ-mediated signaling as well.

In addition to their contribution to our understanding of the functional interactions of IL-2Rα and IL-2Rβ in IL-2-mediated signaling, the above studies offer a scientific basis for designing rational IL-2R-mediated therapies of human disease. From the studies on cells expressing predominantly IL-2Rβ it would seem logical to use antibodies blocking IL-2 binding to IL-2Rβ for treating patients with leukemia of cells (e.g., LGL leukemia) that express IL-2Rβ alone (8). From the data showing complementary activity of antibodies to both IL-2Rα and IL-2Rβ, when cells express the high-affinity receptors, use of antibodies to IL-2Rβ in association with antibodies to IL-2Rα may provide better treatment of individuals with diseases caused by abnormal T cells expressing high-affinity IL-2Rs (e.g., patients with adult T-cell leukemia/lymphoma, autoimmune disorders, graft vs. host disease, as well as those receiving allografts) (1, 28).


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