THE INTERLEUKIN 2 RECEPTOR

Functional Consequences of its Bimolecular Structure

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T lymphocytes undergo clonal expansion in response to antigenic stimulation of an autocrine-paracrine system based on IL-2, the lymphocytotrophic hormone responsible for signaling T cell cycle progression (1). One of the unique aspects of this autocrine T cell growth system stems from its inducibility, in that T cells are quiescent and remain unresponsive until stimulated via the T cell antigen receptor complex (2, 3). Thus, as a consequence of the T cell receptor–mediated transcriptional activation of both the IL-2 gene and IL-2 receptor genes, T cells become competent to receive cell cycle progression signals that are generated by subsequent IL-2-R interactions (2–4). Because the system is autocrine, it is likely that regulatory controls operate to prevent continuous cell growth. Indeed, upon the introduction of antigen, the production of IL-2 and the expression of IL-2-R are both transient events that lead inevitably to a self-limiting proliferative response (2, 5).

One approach to understanding the mechanisms by which IL-2-R function is to determine the structure of the receptor molecule(s). In this regard, it has recently been demonstrated that two distinct polypeptide chains express IL-2 binding sites (6–8). In conformity with the convention for nomenclature established for other cell surface receptors, the larger IL-2 binding protein (M_r 75,000) is designated as the α chain, whereas the smaller protein (M_r 55,000) is termed the β chain (9). The β chain was the first IL-2-binding protein to become recognized and characterized, owing primarily to its reactivity with mAb (10). It is now appreciated that isolated β chains bind IL-2 with a characteristic low affinity ($K_d \sim 10^{-8}$ M) (8). Individual α chains also bind IL-2 with a low affinity, but one that is clearly distinguishable from the β chain–IL-2 interaction ($K_d \sim 5 \times 10^{-10}$–$10^{-9}$ M) (8). By comparison, authentic, high-affinity IL-2-R binding ($K_d \sim 10^{-11}$ M) occurs only when both α chains and β chains are expressed simultaneously (8).

The manner in which α chains and β chains interact to form high-affinity IL-2-R, and the functional attributes of the individual chains compared with the proposed α/β heterodimeric IL-2-R are issues that have remained largely unexplored. Accordingly, to approach these questions directly, radiolabeled IL-2 was used as an affinity label and also for equilibrium and kinetic binding to cells that

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express each chain individually, versus cells expressing both α and β chains together. Our results, detailed in this report, indicate that both IL-2-binding proteins contribute their unique kinetic binding characteristics to form the true high-affinity IL-2-R, but that p75 α chains alone are responsible for stimulating T cell proliferation.

Materials and Methods

Cell Cultures. Human leukemic cell lines YT (11), HUT-102 (12), MT-1 (13), and ATL-2 (14) were cultured in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated FCS (56°C, 30 min) (Sterile Systems, Inc., Logan, UT), 50 U/ml penicillin, 50 μg/ml gentamicin, and 300 μg/ml L-glutamine in a humidified atmosphere of 5% CO_2 in air. ATL-2 conditioned medium was prepared by culturing ATL-2 cells at 3×10^5 cells/ml for 48 h (14). After removing the cells by centrifugation (1,000 *g*, 10 min), the supernatants were filtered (0.2 μm) and stored at 4°C before use. Individual lots of conditioned medium were standardized by titration with YT cells, and were adjusted so that 20% conditioned medium yielded maximum enhancement of Tac antigen expression within 24 h.

Activated human T cells were prepared by stimulating peripheral blood mononuclear cells with anti-T3 (1:10,000 dilution; Ortho Pharmaceutical Co., Raritan, NJ) for 3 d. For some experiments these cells were then cultured for an additional 10 d with 125 pM IL-2. To ensure maximal expression of both high-affinity and low-affinity IL-2 binding sites by these 13-d synchronized (G_0/G_1) cell populations, phorbol-12,13-dibutyrate (PDBu)[1] (50 ng/ml) (Consolidated Midland Co., Brewster, NY) was provided during the first 6 h followed by incubation with IL-2 (125 pM) for 18 h as described previously (8). Incorporation of [^{3}H]thymidine (2.0 μCi/ml) (Becton Dickinson and Co.) was monitored by liquid scintillation counting (2).

Affinity Labeling of IL-2-binding Proteins. Radioiodinated IL-2 was crosslinked to IL-2-binding proteins according to the following protocol: Cells ($5–20 \times 10^6$ cells/ml) were incubated with ^{125}I-IL-2 for 1 h at 4°C, then treated with 0.3 mM disuccinimidyl suberate (DSS) in HBSS for 15 min at 4°C, which was followed by the addition of a 50-fold molar excess of Tris HCl, pH 7.4, to quench the reaction (8). The cells were washed and solubilized using 0.5% NP-40, 10 mM NaCl, 1 mM $MgCl_2$, 1 mM PMSF in 10 mM Tris HCl, pH 7.4. The nuclear-free lysates were analyzed by SDS-PAGE using reducing conditions.

Equilibrium IL-2 Binding. Homogeneous recombinant IL-2 (15) was provided by Takeda Chemical Industries, Ltd., Osaka, Japan as a 1.0 mg/ml solution in ammonium acetate buffer, pH 5.0. IL-2 was radioiodinated using lactoperoxidase glucose oxidase (Enzymobeads; Bio-Rad Laboratories, Richmond, CA) according to the manufacturer's instructions. Radioiodinated IL-2 was prepared with two different specific activities (10^6 cpm/pmol and 10^5 cpm/pmol) so that binding assays could be carried out at IL-2 concentrations ranging from 1 pM to 100 nM. Binding of IL-2 to whole cells was performed as described previously (16), except that the radiolabeled ligands were incubated together with the cell suspension (10^6 cells in 0.2 ml RPMI 1640 medium supplemented with 10% FCS), overlayed onto a 0.2 ml mixture of 80% silicone oil (Dexter Hysol 550 fluid; Dexter Corp., Orlean, NY) and 20% paraffin oil (O-119; Fischer Scientific Co., Fair Lawn, NJ). After a 20-min incubation at 37°C, the tubes were centrifuged (8,500 *g*, 90 s), the tips of the tubes containing the cell pellet were severed, and the cell-bound and free radioactivity were determined by solid scintillation counting. The calculated values for the number of binding sites per cell were derived by Scatchard analysis of equilibrium binding data after subtraction of nonspecific binding determined in the presence of 150-fold molar excess of unlabeled IL-2. The lower limit of detection for IL-2-R was 50 binding sites/cell. The Scatchard plots shown are based on data analyzed by the "Ligand" computer program (17).

Kinetic IL-2 Binding. Isolated membranes from the induced YT cells and YT-2C2 cells were used to analyze ^{125}I-IL-2 binding to high-affinity IL-2-R and to isolated α chains,

[1] *Abbreviations used in this paper:* NGF, nerve growth factor; PDBu, phorbol dibutyrate.

respectively, since α chains mediate a rapid internalization of bound IL-2 ($t_{1/2}$ = 15 min, our unpublished observations) (18); β chain–IL-2 binding was analyzed using whole cells, as the rate of IL-2 internalization after binding to β chains is negligible (18). Cell membranes were prepared by the method of Jett et al. (19) using lysis by Dounce homogenization in 10 mM Tris HCl, pH 7.4, containing 1 mM $MgCl_2$ and 1 mM PMSF. After removing nuclei by centrifugation, the membrane fraction was enriched by centrifugation on a 38% sucrose cushion (100,000 *g*, 2 h), then by pelleting (100,000 *g*, 30 min). For dissociation, membrane or whole cells were preequilibrated with ^{125}I-IL-2 for 20 min at 37°C. Dissociation was initiated by the addition of 1 μM unlabeled IL-2, and the remaining bound ^{125}I-IL-2 was determined at various intervals afterward. The concentrations of ^{125}I-IL-2 used varied from 5×10^{-11} M (α/β dimers), 10^{-9} M (α chains) to 10^{-8} M (β chains). Association was performed by exposing membranes or whole cells to the same concentrations of ^{125}I-IL-2, dependent upon receptor type. At intervals, bound versus free ^{125}I-IL-2 were determined by centrifugation of the cells through oil, whereas membranes were centrifuged through a 19% sucrose cushion (30,000 *g*, 15 min at 4°C). The association rate constants (k) and dissociation rate constants (k') were calculated from natural log plots of the data. The slope of the dissociation plot is $-k'$ and that of the association plot is $k' + k(H)$. The half-time for dissociation is $0.693(1/k')$.

Results

Induction of Low- and High-affinity IL-2-binding Sites. Seeking to elucidate further the structure-function relationship of the α/β heterodimer model of high-affinity IL-2-R (8), we exploited two different experimental systems where it is possible to induce p55 β chain expression, resulting in either the selective augmentation of the expression of low-affinity binding sites or solely high-affinity IL-2-R. One system capitalized on the finding that IL-2 stimulation of normal T cells markedly stimulates the expression of low-affinity binding sites (5). Peripheral T cells were synchronized into G_0/G_1 by a transient proliferative expansion, then induced to reexpress IL-2-R by a brief exposure to phorbol dibutyrate (4). Control cells cultured without IL-2 displayed 3,370 high-affinity receptors per cell and 9,100 low-affinity binding sites per cell (Fig. 1 *A*). By comparison, cells cultured with IL-2 for 18 h are distinctly different: there are 30% fewer high-affinity receptors expressed, while an increase in the number of low-affinity binding sites is found (23,000 sites/cell, Fig. 1 *B*). Therefore, as a consequence of IL-2 exposure, the ratio of high-affinity receptors to low-affinity binding sites changed from 1:3 to 1:9.

The membrane proteins responsible for IL-2 binding in each of these cell populations were examined by polyacrylamide gel electrophoresis after the formation of covalent bonds with bound ^{125}I-IL-2 (8). The data obtained by this approach further support the interpretation that high-affinity receptors are formed by a combination of α chains and β chains (6–8), and also show that IL-2 binding to high-affinity receptors augments p55 β chain expression preferentially (Fig. 1 *C*). Thus, for both cell populations (i.e., with or without IL-2), as the ^{125}I-IL-2 concentration used for binding before crosslinking decreased from 5×10^{-8} M (Fig. 1, lane *1*) to 5×10^{-10} M (lane *2*) and to 5×10^{-11} M (lane *3*), the 75,000 M_r α chain crosslinked with IL-2 (M_r 15,000) to form a 90,000 M_r complex became progressively more discernible. Moreover, for the cell population exposed to IL-2, the density of the 55,000 M_r β chain crosslinked with IL-2 to form a 70,000 M_r complex was more apparent compared with the uninduced cells, particularly at the highest IL-2 concentration tested (Fig. 1, compare lane

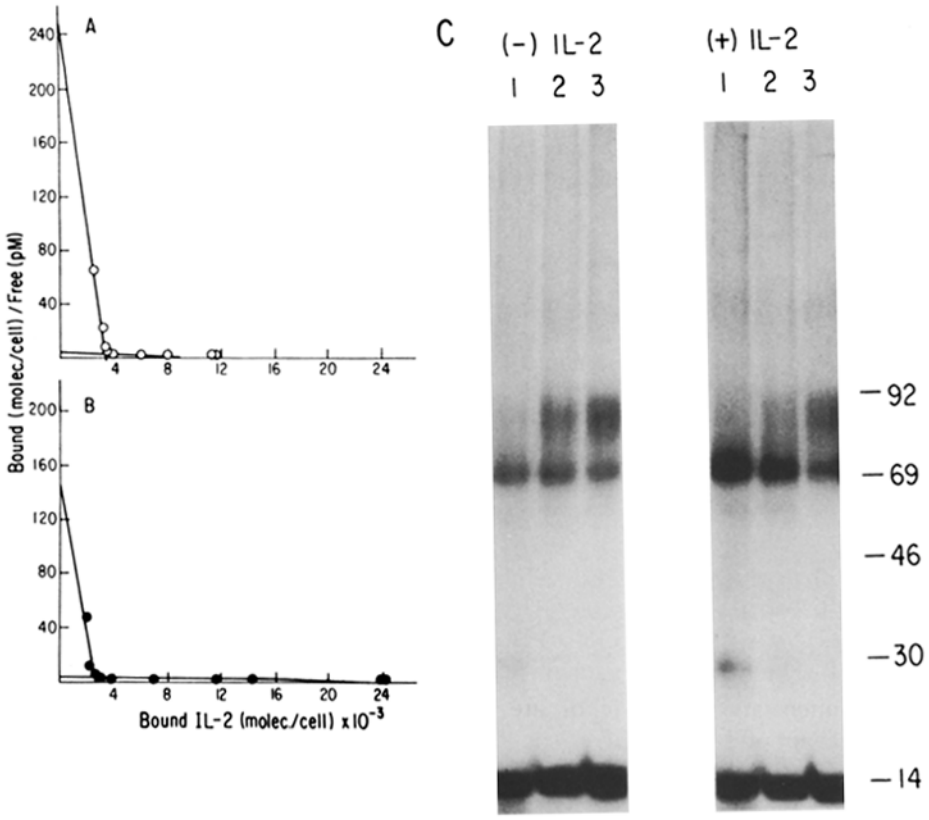

FIGURE 1. IL-2 induction of low-affinity IL-2-binding sites on peripheral T cells. PDBu-restimulated normal T cells were cultured in fresh medium with or without IL-2 for 18 h. Scatchard analyses display the data from ^{125}I-IL-2 binding to control cells (*A*) and IL-2-induced cells (*B*). (*C*) Crosslinking of ^{125}I-IL-2 to the binding proteins ($M_r \times 10^{-3}$). Control cells (*left*) and IL-2-induced cells (*right*) were incubated with ^{125}I-IL-2 at concentrations of 5×10^{-9} M (lane *1*), 5×10^{-10} M (lane *2*), and 5×10^{-11} M (lane *3*). After the equilibrium binding, bound IL-2 was crosslinked to the cells using disuccinimydal suberate, and the crosslinked complexes were analyzed by SDS-PAGE with reducing conditions.

1 from the two populations). Therefore, high-affinity IL-2 binding is associated with the formation of two ^{125}I-IL-2 crosslinked complexes, and the IL-2 induction of low-affinity IL-2-binding sites correlates with an increase in the density of p55 β chains crosslinked.

For comparison with the results obtained using normal T cells, we examined another p55 β chain–inducible system, but one that results in the stimulation of high-affinity receptor expression rather than an excess of low-affinity binding sites. Moreover, to facilitate the interpretation of these binding experiments we used anti-Tac, which reacts with an epitope expressed exclusively on the p55 β chain. A cell line (designated YT) derived from a child with acute lymphoblastic leukemia that expresses predominantly p75 α chains can be induced to express p55 β chains by exposure to conditioned medium derived from adult T leukemia (ATL) cell lines (8, 11, 14). An example of such an experiment is shown in Fig. 2. As shown in Fig. 2*A*, ^{125}I-IL-2 binding to control uninduced YT cells reveals

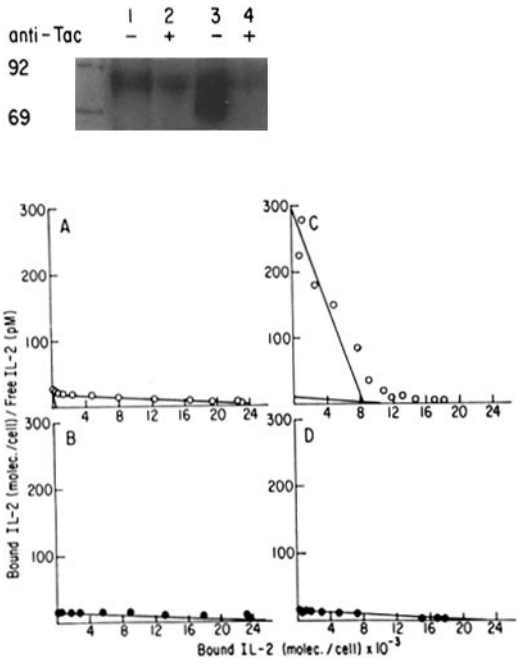

FIGURE 2. The induction of high-affinity IL-2-R expression by YT cells and the effect of anti-Tac. Scatchard plots of ^{125}I-IL-2 binding data on YT cells cultured in control medium (*A* and *B*) and in 20% ATL-conditioned medium (induced, *C* and *D*) for 24 h. The assays were performed in the absence (○) and presence (●) of 5×10^{-8} M anti-Tac. The top panel shows the SDS-PAGE analysis of ^{125}I-IL-2 crosslinking experiments using 10^{-8} M ^{125}I-IL-2, with anti-Tac (5×10^{-8} M) absent or present during the binding reaction before crosslinkage. Lanes *1* and *2*, uninduced YT cells; lanes *3* and *4*, induced YT cells. Only the portion of the autoradiogram containing 90,000 and 70,000 M_r complexes is shown.

a very small number of high-affinity receptors ($K_d = 1.6 \times 10^{-11}$ M; 170 sites/cell), and a much larger number of sites (24,000 sites/cell) that bind IL-2 with an intermediate affinity characteristic for p75 α chains ($K_d = 1.4 \times 10^{-9}$ M). When anti-Tac is present during the IL-2-binding reaction, high-affinity IL-2-binding sites are no longer detectable, whereas binding to the p75 α chains remains essentially unchanged (Fig. 2*B*). By comparison, the results of ^{125}I-IL-2 binding to YT cells induced to express p55 β chains are much more distinct and impressive, as shown in Fig. 2, *C* and *D*. After induction (Fig. 2*C*), there is a marked increase in high-affinity IL-2-R expressed ($K_d = 2.9 \times 10^{-11}$ M; 8,400 sites/cell), and a commensurate decrease in the number of intermediate-affinity binding sites ($K_d = 10^{-9}$ M; 9,800 sites/cell). These data are consistent with some of the p75 α chains combining with p55 β chains to form high-affinity IL-2-R, an interpretation that is substantiated further by the data obtained from ^{125}I-IL-2 binding to this cell population when the assays are performed in the presence of anti-Tac (Fig. 2*D*). Anti-Tac abolishes all detectable high-affinity IL-2 binding, and returns the number of p75 α chain binding sites to the high-density (23,000 sites/cell) observed before the induction of p55 β chain expression (Fig. 2, compare *D* with *B*).

The kind of IL-2-binding proteins detectable on these cells by ^{125}I-IL-2 crosslinkage experiments performed in the presence and absence of anti-Tac is shown at the top of Fig. 2. As anticipated, only a single 90,000 M_r ^{125}I-IL-2–crosslinked complex was observed using uninduced control YT cells (Fig. 2, lane *1*), and anti-Tac had no effect on the intensity of the band observed on the autoradiograph (Fig. 2, lane *2*). By comparison, YT cells induced to express high-affinity receptors display two distinct ^{125}I-IL-2–crosslinked complexes M_r 90,000 and 70,000 (Fig. 2, lane *3*), and the presence of anti-Tac prevented the

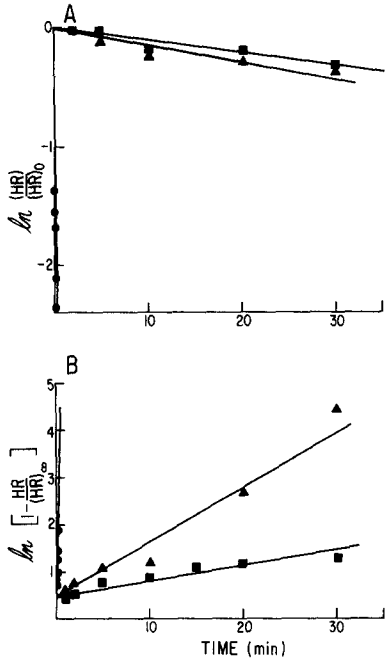

FIGURE 3. Kinetics of IL-2 binding at 37°C. Natural log plots of data are shown for dissociation (*A*) and association (*B*) of ^{125}I-IL-2 at 37°C to isolated membranes for the analysis of high-affinity α/β dimers (induced YT cells, ▲) and isolated α chains (YT-2C2 cells, ■), whereas isolated β chains were examined using whole cells (MT-1 cells, ●). For dissociation, the specific binding at $t = 0$, $(HR)_0$, was measured in quadruplicate, while at intervals during dissociation, duplicate determinations were made to determine HR. For association, at the intervals indicated, duplicate aliquots were determined for: HR = bound ^{125}I-IL-2 at time t and $(HR)_\infty$ = bound ^{125}I-IL-2 at equilibrium. All the data shown are corrected for nonspecific binding. The duplicate values obtained for all points varied <10–15% from the mean.

formation of the 70,000 M_r complex, but not the formation of the 90,000 M_r complex (Fig. 2, lane *4*). Accordingly, from these data it is apparent that IL-2-R p75 α chains and p55 β chains cooperate to form high-affinity receptors, and that this cooperative interaction can be readily disrupted, as shown so distinctly by the effect of anti-Tac on both the binding and crosslinking experiments.

Kinetic Binding Studies Reveal a Unique Form of Cooperative Ligand Binding. Experiments that provide information as to the rates of ligand-receptor interactions often can lead to insights on reaction mechanisms unobtainable by equilibrium measurements. Therefore, to investigate further the nature of the α/β heterodimer, we next examined in detail the kinetics of ^{125}I-IL-2 binding to the three distinct kinds of binding sites (i.e., α chains, β chains, and α/β heterodimers). To perform kinetic binding experiments unambiguously, we exploited leukemic cell lines expressing solely either p75 α chains (YT-2C2 cells) (8), or p55 β chains (MT-1 cells) (13) to compare with cells displaying the α/β high-affinity receptor complex (induced YT cells and HUT-102 cells) (12). Moreover, to provide an independent assessment of the equilibrium binding constants, which were determined at 37°C, all kinetic binding determinations were also performed at this temperature, thereby generating data of physiologic relevance with respect to the reaction rates.

Natural log plots of data representative of three separate kinetic binding experiments are displayed in Fig. 3, and the kinetic binding constants calculated on the basis of these data are listed in Table I. From these results it is evident that the kinetics of IL-2 binding to isolated p75 α chains are markedly different compared with those obtained using isolated p55 β chains, even though the equilibrium dissociation constants obtained for binding to these two chains differ

TABLE I
Kinetic and Equilibrium IL-2 Binding Constants

Chain*	Kinetic binding constants‡			Equilibrium dissociation constants	
	Dissociation (k')		Association (k)	Kinetic (k'/k)	Equilibrium§
	s^{-1}	$t_{1/2}$	$M^{-1} \cdot s^{-1}$	M	
α	2.5×10^{-4}	46 min	3.8×10^{5}	0.7×10^{-9}	$1.2 (\pm 0.1) \times 10^{-9}$
β	4.0×10^{-1}	1.7 s	1.4×10^{7}	2.9×10^{-8}	$1.4 (\pm 0.1) \times 10^{-8}$
α, β	2.3×10^{-4}	50 min	3.1×10^{7}	0.7×10^{-11}	$1.3 (\pm 0.1) \times 10^{-11}$

* α Chain, YT-2C2 membranes; β chain, MT-1 cells; α, β chains, induced YT membranes.
‡ Calculated from the data shown in Fig. 3.
§ Mean ± SEM from eight separate equilibrium binding experiments.

only 10-fold: IL-2 binds to and dissociates from p55 β chains much more rapidly than to p75 α chains. The magnitude of the differences are readily appreciated by examination of the plot of dissociation rate data (Fig. 3*A*), where it is obvious that the slope of the data for IL-2 p55 β chain dissociation is very steep compared with the data for IL-2 p75 α chain dissociation. Because ligand dissociation follows first-order kinetics, the half-times ($t_{1/2}$) for dissociation can be used to obtain meaningful comparisons. Therefore, the $t_{1/2}$ for IL-2 dissociation from p55 β chains calculated from the dissociation rate constant differs by three orders of magnitude compared to the value obtained with p75 α chains (1.7 s versus 2,770 s [46 min] respectively) (Table I).

Because IL-2 dissociates from p55 β chains much more rapidly than from α chains, we expected the rates of IL-2 association to the two chains to reflect the dissociation rates. When determined directly, IL-2 was found to associate with the p55 β chain binding site very rapidly, at a rate that was especially noteworthy when compared with the much slower association of IL-2 to p75 α chains (Fig. 3*B*). The association rate constants (k) calculated from these data revealed that the rate constant for IL-2–p55β chain association is 100 times greater than the rate constant for IL-2–p75α chain association, and approaches the values for association rate constants that are determined by diffusion alone ($k = 1.4 \times 10^7/\text{M} \cdot \text{s}$; Table I). Accordingly, for the first time it could be seen that IL-2 binds to and dissociates from p55 β chains very rapidly, while the kinetics of IL-2 binding to p75 α chains are much slower, thereby contributing further data indicating that p75 α chains and p55 β chains have separate and distinct attributes.

Even more distinctive and provocative were the results obtained from kinetic binding determinations analyzing α/β heterodimers. From the data shown in Fig. 3, it is readily appreciated that IL-2 binding to the α/β chain complex is a synthesis of the rates of binding to each isolated chain. Thus, IL-2 binding to high-affinity receptors takes on the characteristics of the p55 β chain for the forward reaction, so that it associates rapidly to the α/β dimer. It should be emphasized that the slope of the experimentally determined association rate (Fig. 3*B*) is a function of the sum of the dissociation rate constant and the product of the association rate constant and hormone concentration ($k' + k[H]$). Therefore,

even though the natural log plot of the association data shown in Fig. 3*B* has a slope for IL-2 association with α/β dimers that is intermediate compared with those of individual p75 α chains and individual p55 β chains, the association rate constant calculated from these data is equivalent to the p55 β chain–IL-2 association rate constant ($k = 3.1 \times 10^7$/M·s; Table I). In contrast, the reverse reaction assumes the behavior of IL-2 as if it were bound solely to p75 α chains (Fig. 3*A*). Actually, the dissociation rate constant calculated from these data is essentially identical to the dissociation rate constant calculated for isolated p75 α chains (i.e., $k' = 2.5 \times 10^{-4}$/s for p75 α chains and $k' = 2.3 \times 10^{-4}$/s for α/β dimers). As expected, since the equilibrium dissociation constant is an expression of the ratio of the dissociation rate constant and association rate constant (i.e., $K_d = k'/k$), the biologic consequence of combining a slow dissociation rate ($k' = 2.3 \times 10^{-4}$/s) with a very fast association rate ($k = 3.1 \times 10^7$/M·s) is a very high affinity, with a calculated equilibrium dissociation constant ($K_d = 0.74 \times 10^{-11}$ M) that differs 100-fold from that calculated for IL-2 binding to the p75 α chain ($K_d = 0.7 \times 10^{-9}$ M), and 1,000-fold from the K_d calculated for IL-2 binding to individual p55 β chains (2.9×10^{-8} M).

It is noteworthy that the validity and accuracy of the association and dissociation rate constants calculated from the natural log plots of the experimental data (Fig. 3) for each type of IL-2-binding site (i.e., α and β, and α/β) are confirmed by the values of the equilibrium constants obtained by calculating the ratio of the rate constants. Because the values calculated from the rate constants are comparable with the experimentally derived equilibrium dissociation constants (Table I), it is unlikely that any one of the individual rate constants calculated from the kinetic data obtained experimentally is erroneous. Consequently, the construction of this kinetic hybrid ligand receptor interaction using two distinct IL-2-binding proteins makes for a biologically relevant hormone receptor of exceptional functional efficiency: Because the 50% effective concentration (EC_{50}) is determined by the K_d ($\sim 10^{-11}$ M), very low IL-2 concentrations (i.e., $<10^{-10}$ M) will trigger a T cell proliferative response.

α Chains Are Responsible for Stimulating Cell Cycle Progression. Even though the kinetic and equilibrium binding studies revealed how α and β chains cooperate to form a very-high-affinity IL-2-R, these data alone did not indicate which chain is actually responsible for transducing a growth signal to the cell. However, in this regard, transfection experiments where non-T cells were made to express p55 β chains in the absence of p75 α chains showed that p55 β chains alone are incapable of stimulating cell proliferation (20, 21). Therefore, it remained to be determined whether the p75 α chain itself could function in this regard, or whether the complete α/β heterodimer is essential. To approach this question directly, we capitalized on our findings with YT cells showing that anti-Tac prevents IL-2 binding to p55 β chains and disrupts high-affinity α/β dimer binding, leaving isolated p75 α chain IL-2 binding intact. A similar approach was tested using normal T cells that were first activated by a 3-d culture with anti-T3 to render the cell population IL-2 responsive (4). As shown in Fig. 4*A*, these T cell blasts express both high-affinity receptors ($K_d = 2.7 \times 10^{-11}$ M; 1,900 sites/cell) as well as an excess of low-affinity IL-2-binding sites ($K_d = 1.4 \times 10^{-8}$ M; 11,500 sites/cell). However, when binding is performed with anti-Tac present

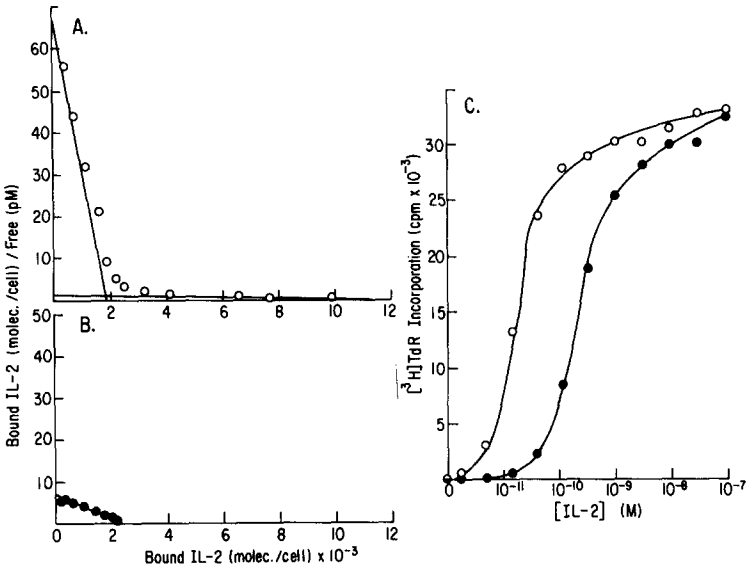

FIGURE 4. The effect of anti-Tac on ^{125}I-IL-2 binding and the IL-2-dependent proliferative response of anti-T3–activated normal T cells. Peripheral mononuclear cells were activated with anti-T3 for 3 d, then washed before ^{125}I-IL-2 binding assays. *A* and *B* are Scatchard plots of binding data in the presence of control normal mouse IgG (5×10^{-8} M) (*A*), and in the presence of anti-Tac (5×10^{-8} M) (*B*). (*C*) [^{3}H]TdR incorporation by aliquots of the anti-T3–activated cells. The cells were cultured (10^5 cells/ml) in RPMI 1640 medium 10% FCS, with 5.0×10^{-8} M normal mouse IgG (○), and with 5.0×10^{-8} M anti-Tac (●) in the presence of rIL-2 from 10^{-12}–10^{-7} M. The data shown are from day 3 of culture and are representative of data also obtained on days 1 and 2. The standard error of the mean values shown were <5%, determined on quadruplicate samples.

in 5–10-fold excess of the concentration necessary to saturate all Tac epitopes (5×10^{-8} M), neither high-affinity receptors nor low-affinity IL-2-binding sites are occupied. Instead, the only detectable ^{125}I-IL-2–binding occurs to sites that express an intermediate affinity ($K_d = 3.6 \times 10^{-10}$ M), even though the assay was performed with very high ^{125}I-IL-2 concentrations ($\leq 10^{-7}$ M) (Fig. 4*B*). Moreover, the number of sites occupied (2,100 sites/cell), is indistinguishable experimentally from the number of high-affinity IL-2-R detectable in the absence of anti-Tac (1,900 sites/cell). Therefore, in a fashion similar to its effect on YT cells, on normal T cell blasts anti-Tac functions to completely prevent IL-2 binding to p55 β chains and α/β dimers, and in doing so it reveals the IL-2 binding characteristic for isolated p75 α chains.

Having created normal T cells capable of binding IL-2 solely via p75 α chains, it was possible to test the hypothesis as to whether an IL-2-p75α chain interaction can promote growth, or alternatively, whether binding to high-affinity α/β heterodimers is required. To this end, aliquots of the anti-T3–activated T cells were washed free of any IL-2 produced in situ, then cultured with anti-Tac (5×10^{-8} M) in the presence of a broad range of IL-2 concentrations (10^{-12}–10^{-7} M). Parenthetically, it should be emphasized that even the highest concentration of IL-2 used (10^{-7} M) was insufficient to compete with anti-Tac for binding to high-affinity receptors and isolated p55 β chains, as shown by the binding assay (Fig.

4*B*). Therefore, if IL-2 binding to high-affinity receptors is necessary for growth signal transduction, T cell blasts cultured with anti-Tac should remain unresponsive to IL-2. Alternatively, if individual p75 α chains can function to stimulate T cell growth, we expected that the presence of anti-Tac would function simply to shift the IL-2 dose-response curve, so that the effective IL-2 concentrations would be predictable from those necessary to saturate the p75 α chains. The results, depicted in Fig. 4*C*, show that the IL-2–p75α chain interaction alone is sufficient to signal the complete T cell growth response: The presence of anti-Tac does not inhibit IL-2 stimulation of proliferation, despite completely preventing IL-2 binding to both isolated p55 β chains and high-affinity α/β dimers. Instead, the IL-2 concentrations required to trigger the response are simply increased 15-fold by the presence of anti-Tac, a result anticipated by the 13-fold higher concentrations of IL-2 necessary to bind to isolated p75 α chains versus high-affinity α/β dimers.

Discussion

From the experimental findings accumulated in the course of these studies, it is evident that the characteristic high-affinity receptor for IL-2 is formed by a unique cooperative functional interaction between the two separate and distinct binding sites expressed on p75 α and p55 β chains. The most intriguing aspect of this bimolecular receptor system relates to the synergistic manner in which the association rate is contributed by the rapidly reacting p55 β chain, whereas the dissociation rate is determined by the slowly reacting p75 α chain. These findings relegate the p55 β chain to function essentially as a helper binding site with no signaling capacity of its own, particularly as we now realize that the larger p75 α chain is capable of stimulating cell division without the assistance of the p55 β chain.

The formation of high-affinity IL-2-R by the combination of distinct binding sites contributed by two separate chains thus far represents a unique example of a way to form specific, high-affinity ligand-receptor reaction complexes. However, the marked efficiency of ligand binding that results from the kinetic hybrid α/β dimer IL-2-R leads to the expectation that other lymphokine and growth factor receptor systems will prove to be similar to the one detailed here for IL-2. In this regard, it is particularly noteworthy that IL-3 crosslinkage experiments have also revealed two binding proteins, identical in size to the IL-2-R α and β chains (22). Moreover, it may be more than mere coincidence that nerve growth factor (NGF) receptors appear to be so similar to IL-2-R. For example, two classes of NGF receptors are expressed (i.e., $K_d = 10^{-11}$ and 10^{-9} M), but no structural explanation for the 100-fold difference in binding affinities has yet been disclosed (23). Recently, genomic and cDNA clones encoding a single NGF receptor chain have been isolated: Transfectants expressing this chain bind NGF with only the characteristic "fast-reacting" low affinity (24–26). Based on our findings regarding the dynamic cooperative interaction between IL-2-R p75 α and p55 β chains in the formation of high-affinity receptors, it is entirely conceivable that high-affinity NGF receptors are likewise made up of two ligand-binding chains. Indeed, recent crosslinkage studies suggest that there may be an additional protein that is also capable of binding NGF (27).

It is worthy of emphasis that there is no indication that the interaction between the α and β chains involves a covalent disulfide linkage. Instead, all of the data point to a noncovalent association as being responsible for the formation of the high-affinity binding site. Thus, anti-Tac has the effect of actually exposing IL-2 binding to individual p75 α chains, which on normal T cells can only be derived from α/β dimers. Therefore, as displayed so convincingly by the combined results of the ^{125}I-IL-2 binding and crosslinking experiments performed in the presence of anti-Tac, the net effect of the disruption of high-affinity binding is intermediate-affinity binding to the 75,000 M_r α chain. Moreover, the realization that the α chain–β chain interaction occurs via noncovalent forces that are easily disruptable by anti-Tac indicates that the interaction between these two chains is itself likely to be dynamic, and thus subject to association and dissociation. It follows that the marked induction of β chains upon IL-2 binding to high-affinity IL-2-R would favor the formation of α/β heterodimers, especially as the mass action of a higher density of p55 β chains would promote association with p75 α chains. This reasoning is especially compelling, as there is no evidence that p55 β chains have any biologic role beyond functioning to cooperate with p75 α chains to make high-affinity IL-2-binding sites.

The results derived from the kinetic binding experiments with isolated α chains, isolated β chains, and α/β heterodimers also provide exceptional mechanistic information pertaining to the structure-activity relationships of the respective IL-2-binding proteins. IL-2 binding to isolated p55 β chains, which are generally expressed in 10–20-fold excess of α/β heterodimers on actively growing T cells, can now be envisioned to convert the actual interaction between IL-2 and α/β heterodimers from a three-dimensional search to a two-dimensional search in the plane of the membrane, a concept proposed originally by Adam and Delbruck (28). A similar, low-affinity, high-capacity reaction can be demonstrated to be operative in the interaction between endonucleases and nonspecific sites on DNA, as compared with the high-affinity specific cleavage sites determined by the DNA sequence (29, 30). A low-affinity, rapidly reversible reaction occurs between the endonuclease and nonspecific DNA sequences. The search for the specific sites, where the enzyme binds with high affinity, is thus made linear and is reduced from an infinitely complex three-dimensional problem to a relatively simple two-dimensional quest. The singular aspect of the structure-activity relationships of the IL-2-binding proteins, when interpreted in the light of this concept, resides in the probability that the noncovalent formation of authentic, high-affinity IL-2-R, and the induction of an excess of rapidly reacting, low-affinity p55 β chains, may well delineate the principle guiding the selection and conservation of lymphokine-receptor interaction systems.

The finding that the p75 α chain alone appears to transduce a growth signal to the cell is entirely consistent with the previous results obtained with p55 β chain transfectants, which proved to be nonfunctional (20, 21). Moreover, the data obtained with normal T cells using anti-Tac are identical to those obtained previously using the MLA-144 cell line. This cell line both produces and responds to IL-2, as shown by our studies using glucocorticoids, which block proliferation by inhibiting IL-2 production (31). Subsequent to the report by Tsudo et al. (7) showing that MLA-144 cells express solely p75 α chains, we repeated our earlier

studies and confirmed that MLA-144 cells express a single class of intermediate-affinity receptors that are capable of transducing a growth signal (32). It is worthy of emphasis that the effect of anti-Tac on normal T cells, in causing a shift to higher IL-2 concentrations for a growth response, is entirely consistent with our originally reported data (10). Thus, at low IL-2 concentrations (e.g., 10^{-11} M), anti-Tac completely abrogates T cell growth (Fig. 4*C*). However, as the IL-2 concentration is increased, the inhibitory effect of anti-Tac becomes progressively less evident. The competitive effect of anti-Tac on IL-2-binding and proliferation of T cells was previously attributed to a simple mass action equilibrium between IL-2 and anti-Tac both vying for a single high-affinity receptor molecule (33). However, in these earlier experiments, the effect of anti-Tac to decrease the number as well as the affinity of IL-2-binding sites went unnoticed. From the ^{125}I-IL-2 binding and crosslinking results reported here, it is evident that by binding to β chains, anti-Tac prevents IL-2 binding to the α/β dimer. Therefore, anti-Tac disrupts the cooperation between p75 α chains and p55 β chains, so that there can be no simple competitive relationship between anti-Tac, IL-2, and α/β dimers. Instead, the presence of saturating concentrations of anti-Tac guarantees that only p75 α chains are available for IL-2 binding.

Confirmation of the results regarding the signaling capacity of the p75 α chain must now be sought using alternative approaches, such as transfection methods and reconstitution experiments using purified p75 α chains. Meanwhile, the realization that IL-2-R p75 α chains convey a biologic response in the absence of IL-2 binding to p55 β chains provides an acceptable explanation for several of the heretofore perplexing findings where IL-2 stimulates a biologic response, yet IL-2-R are undetectable using antibodies reactive solely with the p55 β chain. For example, immature human thymocytes proliferate in response to IL-2, provided contact with thymic stromal cells is maintained in vitro (B. F. Haynes, personal communication). Of considerable importance regarding the present findings, these "double negative" fetal thymocytes lack detectable Tac antigen when initially isolated, even though they are IL-2 responsive. Accordingly, it appears that during ontogeny, IL-2-R p75 α chains are expressed before p55 β chains, and could be responsible for the marked proliferative activity of this thymic subset as it occurs in vivo. The IL-2 reactivity of natural killer cells might also be attributable to the expression of p75 α chains, particularly since high IL-2 concentrations are generally required to activate this cell type (34). A similar argument is persuasive for explaining a role for IL-2 in B cell responses, especially as activated human and murine B cells express small numbers of lower-affinity IL-2-binding sites (35, 36). Thus, we look forward to further, more definitive studies dealing with these issues, once p75 α chain–specific reagents have been generated.

Summary

High-affinity IL-2-R binding results from an exceptional type of cooperative interaction between two IL-2-binding proteins termed α and β. When expressed together on the cell surface, these two distinct chains form a noncovalent kinetic hybrid receptor complex that exploits a rapid association rate contributed by the p55 β chain and a slow dissociation rate characteristic for the p75 α chain. The

p75 α chains signal cell growth, whereas the p55 β chains only facilitate IL-2 binding by serving as helper binding sites, having no discernible signaling role themselves. The unique functional implications of this structural organization indicate that this cooperative bimolecular arrangement reflects a general mechanism by which the efficiency of surface receptors can be enhanced markedly.

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