

INTERLEUKIN 2 HIGH-AFFINITY RECEPTOR EXPRESSION REQUIRES TWO DISTINCT BINDING PROTEINS

BY KEISUKE TESHIGAWARA, HUEY-MEI WANG, KOICHI KATO, AND
KENDALL A. SMITH

*From the Department of Medicine, Dartmouth Medical School,
Hanover, New Hampshire 03756*

IL-2, the lymphocytotropic hormone responsible for T cell cycle progression, initiates its effects by interacting with high-affinity, IL-2-specific membrane receptors (reviewed in 1). The development of the radiolabeled IL-2 binding assay (2) was a crucial step in the identification and characterization of IL-2-R, as it made possible the detection of mAbs that compete with IL-2 for binding (3-5). These antibodies (termed anti-Tac) subsequently proved instrumental in experimental approaches that ultimately led to the isolation of cDNA clones encoding a 28,500 M_r protein containing sites reactive with both IL-2 and anti-Tac (Tac cDNA) (6, 7). However, more recently anti-Tac and the IL-2 binding assay led to still further experiments uncovering a second class of binding sites with a much lower affinity for IL-2 than the high-affinity receptors described originally (i. e., $K_d = 10$ nM vs 10 pM; references 8, 9). In the light of these and subsequent experiments some uncertainty has evolved regarding the structural nature of biologically relevant high-affinity IL-2-R vs. low-affinity IL-2 binding sites. Seeking further data on both types of IL-2 binding, we have examined a neoplastic T cell line established originally from a patient with T cell acute lymphoblastic leukemia (T-ALL)¹ (10). This cell line, designated YT, attracted our attention because the low levels of Tac antigen that it expresses can be augmented considerably by treatment with conditioned medium, i. e., culture supernatants of cell lines derived from patients with adult T cell leukemia (ATL) (10, 11).

Here we report that YT cells express novel IL-2 binding sites, completely separate and distinguishable from the sites described previously (8, 9). Clones of YT cells that exclusively express this new variety of receptor do not contain mRNA detectable by hybridization with radiolabeled Tac cDNA, nor do they express cell surface Tac antigen. Moreover, these newly recognized sites bind IL-2 with a distinctive affinity, intermediate by comparison with previously identified characteristic high-affinity IL-2-R and low-affinity binding sites.

These findings indicated that an entirely separate gene encodes these curious IL-2-R, and prompted a reevaluation of the structural basis for high-affinity vs. low-affinity IL-2 binding. Using radioiodinated IL-2 and chemical crosslinking

This work was supported in part by grant CA-17643 from the National Cancer Institute, grant 1715 from The Council for Tobacco Research-U.S.A., Inc., and a grant from the Eli Lilly Corporation.

¹Abbreviations used in this paper: ATL, adult T cell leukemia; DSS, disuccinimidyl suberate; Pdbu, phorbol-12, 13-dibutyrate; T-ALL, T cell acute lymphoblastic leukemia.

approaches, the newly recognized IL-2 binding protein was found to be 75,000 M_r , readily distinguishable from the Tac antigen protein (M_r , 55,000). Remarkably enough, the expression of high-affinity IL-2-R by both YT cells and normal activated T cells correlated with the simultaneous expression of Tac antigen and this novel IL-2 binding site. Consequently, we propose that high-affinity IL-2-R are made up of these two IL-2 binding proteins, which cooperate to form authentic, fully functional receptors that can stimulate T cell growth.

Materials and Methods

Cell Cultures. Human leukemic cell lines YT (10), JURKAT clone 6.8 (2), HUT-102 (12), and ATL-2 (13) 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 μ g/ml gentamicin, and 300 μ g/ml L-glutamine in a humidified atmosphere of 5% CO₂ in air. ATL-conditioned medium was prepared by culturing ATL-2 cells at 3×10^5 cells/ml for 48 h. After removing the cells by centrifugation (1,000 \times g, 10 min), the supernatants were filtered (0.2 μ m) 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 PBMC with anti-T3 (Ortho Pharmaceutical, 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 (G₀/G₁) cell populations, phorbol-12, 13-dibutyrate (Pdbu, 50 ng/ml; Consolidated Midland Corp., Brewster, NY) was provided during the first 6 h, followed by incubation with IL-2 (125 pM) for 18 h as described previously (14).

Radiolabeled IL-2 and Anti-Tac Binding Assays. Homogeneous rIL-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. mAbs reactive with the Tac epitope were made available by Dr. Ellis Reinherz (1HT4; IgG2a; Dana-Farber Cancer Center, Boston, MA). IL-2 and anti-Tac were radioiodinated using lactoperoxidase/glucose oxidase (Enzymobeads; Bio-Rad Laboratories, Richmond, CA) according to the manufacturer's instructions. The specific activity of anti-Tac was 5.3×10^4 cpm/pmol. Radioiodinated IL-2 was prepared with two different specific activities (1.0×10^6 cpm/pmol and 1.0×10^5 cpm/pmol) so that binding assays could be carried out at IL-2 concentrations ranging from 1 pM to 100 nM. Binding of both ligands to whole cells was performed as described previously (2), except that the radioiodinated ligands were incubated together with the cell suspension (10^6 cells in 0.2 ml RPMI 1640 medium supplemented with 10% FCS), overlaid onto a 0.2 ml mixture of 80% silicone oil (Dexter Hysol 550 fluid; Dexter Corp., Orlean, NY) and 20% paraffin oil (0-119; Fischer Scientific Co., Pittsburgh, PA). After a 20-min incubation at 37°C, the tubes were centrifuged (8,500 g for 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 ligand. The lower limit of detection for both ligands was 50 binding sites per cell.

Flow Cytofluorometry. 10^6 cells/ml were incubated (45 min, 4°C) with a saturating concentration of anti-Tac (100 nM), washed three times, and then incubated with a 1:20 dilution of fluorescein-conjugated F(ab')₂ fragments of rabbit anti-mouse Ig (Dakopatts; Accurate Chemical & Scientific Corp., Westbury, NY). Samples were passed through a cytofluorograph (system 50H; Ortho Diagnostic Systems, Inc., Westwood MA) using an argon ion laser at 488 nm excitation wavelength. Green fluorescence was observed using a 630-nm long-pass filter. A minimum of 10^4 cells, gated to exclude nonviable cells, were accumulated for each histogram. All data were analyzed using the 2150 Data Handler

System (Ortho Diagnostics Systems, Inc.), and the percentage of positive cells was calculated against a background of nonspecific labeling with normal mouse Ig (1–3%).

cDNA/RNA Hybridization. Total RNA was isolated from cells by solubilization in 6.0 M guanidine hydrochloride, 20 mM sodium acetate (pH 5.0), followed by ultracentrifugation through a 5.7 M cesium chloride cushion (16). Poly(A)⁺ RNA was prepared by oligo(dT) affinity column chromatography as described previously (17). Serial twofold dilutions of RNA (10 µg) were applied to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH) using a dot-blot apparatus (Bethesda Research Laboratories, Bethesda, MD). The filters were baked at reduced pressure for 2 h at 80°C and were prehybridized overnight at 42°C with 6× SSC (1× SSC: 0.15 M sodium chloride, 15 mM sodium citrate, pH 7.0) containing 1% SDS, poly adenosine (50 µg/ml), sheared salmon sperm DNA (100 µg/ml) and 1× Denhardt's solution. Hybridization was effected at 40°C for 24 h in 50% deionized formamide, 6× SSC, 0.1% SDS with 100 ng ³²P-nick-translated probes (1–2 × 10⁶ CPM/µg DNA) from Tac cDNA (7) and HLA-B7 cDNA (18). After high stringency washes with 0.1× SSC, 0.1% SDS (30 min, 20°C; 30 min, 56°C), radioactivity bound to the filters was detected by autoradiography. Hybridization intensity was quantified by densitometry.

Gel Electrophoresis of IL-2-binding Proteins. Radioiodinated IL-2 was crosslinked to IL-2 binding proteins according to the following protocol: YT cells (20 × 10⁶ cells/ml) were incubated with 10 nM ¹²⁵I-IL-2 for 1 h at 4°C, then treated with 1.0 mM disuccinimidyl suberate (DSS) (19) in HBSS. After washing with HBSS, the cells were disrupted using a Dounce homogenizer and the nuclear-free lysate was enriched for plasma membranes as described previously (20). This membrane fraction was solubilized using 1% sodium deoxycholate, 10 mM Tris-HCl (pH 7.4), 0.15 M NaCl, 1 mM PMSF, then clarified by centrifugation (100,000 g, 60 min). The supernatant was electrophoresed using a 7.5% acrylamide gel under reducing conditions (20 mM DTT). Similar conditions were used for normal activated T cells, except that 10⁷ cells were exposed to ¹²⁵I-IL-2 concentrations of 4 nM and 50 pM. After crosslinking with DSS, membrane proteins were solubilized with 0.5% NP-40 before electrophoresis under reducing conditions on a 7–15% acrylamide gradient gel.

For immunoprecipitation of YT cell membrane proteins, the cells were iodinated using lactoperoxidase as described (21), then radiolabeled proteins were extracted with a lysis buffer (RIPA: 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 1 mM PMSF) containing 1% Triton X-100. Proteins were precipitated with anti-Tac (100 nM) and formalin-fixed *Staphylococcus aureus* Cowan I cells, and electrophoresed under reducing conditions on a 7.5% acrylamide gel.

Results

The Effect of ATL-conditioned Medium on YT Cell IL-2 Receptor Expression. Previous experiments had already established that ATL-conditioned medium contained an activity that augmented the expression of the Tac antigen (11), but the nature of the IL-2-R expressed by YT cells remained unexplored, particularly as regards the affinity of their IL-2 binding. Accordingly, YT cells were exposed to ATL-conditioned medium for 24 h, after which IL-2-R expression was assessed with radiolabeled IL-2 binding assays and anti-Tac immunocytofluorography. As shown in Fig. 1, ATL-conditioned medium promoted a sixfold increase in Tac antigen expression (Fig. 1, A and C). By comparison, as displayed by Scatchard plots (Fig. 1, B and D), the number of high-affinity IL-2-R was augmented to a similar degree by the ATL-derived conditioned medium (1,120 sites per cell to 7880 sites per cell), but the number of IL-2 binding sites with lower affinity remained unchanged (32,200 sites per cell vs. 33,300 sites per cell). These results pointed to the activity contained within ATL-conditioned medium as promoting selectively the expression of high-affinity IL-2-R that also

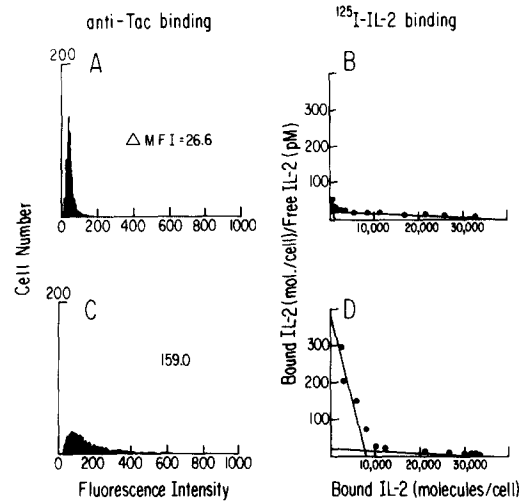


FIGURE 1. The effect of ATL-conditioned medium on YT cell Tac antigen and IL-2-R expression. (A) Control YT cell Tac antigen expression as detected by immunocytofluorography with anti-Tac. Δ MFI indicates the mean fluorescence intensity after subtraction of the MFI obtained using control Ig (20.0 U). (B) Scatchard plot of ^{125}I -IL-2 binding to control YT cells. (C) Anti-Tac immunocytofluorography of YT cells exposed to 20% ATL-conditioned medium for 24 h. (D) Scatchard plot of ^{125}I -IL-2 binding to an aliquot of the cells exposed to 20% ATL-conditioned medium.

bear the Tac epitope. However, the data also suggested that YT cells express sites with a lower IL-2-binding affinity that do not contain the Tac epitope, and that do not increase upon exposure to ATL-conditioned medium.

IL-2-R Expressed by YT Cell Clones. To define further the types of IL-2-R expressed by YT cells, the YT cell line was cloned by limiting dilution (≤ 0.3 cell per well) (22). Of nine clones isolated, five resembled the original YT cell line, in that they expressed both high and lower affinity IL-2 binding sites; here too, the ATL-conditioned medium enhanced only the number of high-affinity IL-2-R. By comparison, four of the nine clones differed from the others, in that no high-affinity IL-2-R expression could be discerned. Moreover, the lower affinity IL-2 binding sites expressed by these clones contained no Tac epitopes, as monitored using either immunocytofluorography or radioiodinated anti-Tac binding. Furthermore, as displayed by the Scatchard plots in Fig. 2 for a representative clone (2C2), there was no response discernible to ATL-derived conditioned medium monitored by radiolabeled IL-2 binding. Analysis of IL-2 binding to clone 2C2 cells revealed still another perplexing characteristic of these binding sites; the K_d was found consistently to be 1.5 ± 0.17 nM (mean \pm SEM; $n = 9$), clearly different from high-affinity IL-2-R, but also distinguishable from low-affinity IL-2 binding sites expressed by normal T cells and ATL cell lines. For illustration and comparison, the data from a representative IL-2 binding experiment performed with normal T cells are also plotted in Fig. 2. As the slopes of the data points are indicative of the binding affinities, it can be readily discerned that normal T cells bind IL-2 with two distinct affinities ($K_d = 10.6$ pM, 1,700 sites per cell; $K_d = 10.0$ nM, 13,000 sites per cell), whereas the YT clone 2C2 cells bind IL-2 with a single intermediate affinity ($K_d = 1.7$ nM;

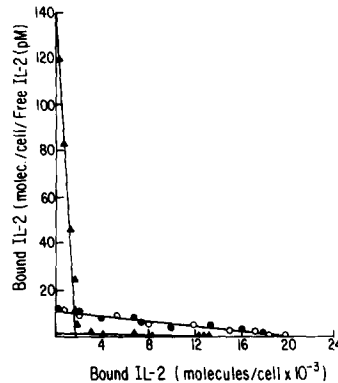


FIGURE 2. IL-2-R expression by YT clone 2C2 cells compared with normal activated T cells. Scatchard plots of ^{125}I -IL-2 binding data to YT clone 2C2, before (○) and after (●) exposure to 20% ATL-conditioned medium for 24 h. A similar plot of human peripheral T cell ^{125}I -IL-2 binding after 72 h culture with anti-T3 (▲) is shown for comparison. ^{125}I -anti-Tac binding to the YT clone 2C2 cells was negative before and after exposure to ATL-conditioned medium. The normal T cells expressed 13,500 anti-Tac binding sites/cell. *Molec.*, molecules.

20,000 sites per cell). Consequently, these data implied that this YT cell IL-2 binding site could be entirely different from the one identified using anti-Tac, especially considering that anti-Tac does not react with the YT-2C2 cells.

Tac mRNA Expression by YT Cell Clones. An obvious but trivial explanation for the lack of Tac antigen expression by YT-2C2 cells would be a simple point mutation within the region encoding the Tac epitope. However, in this instance, Tac mRNA should still be detectable. Accordingly, RNA samples from two of the representative YT clones were extracted at intervals before and after exposure to ATL-derived conditioned medium, then hybridized with a probe containing the entire coding sequence for the Tac protein. Using serial dilutions of total RNA (10–2.5 μg), quantitative analysis of densitometric scans of dot blots proved to correlate directly with the expression of Tac antigen after exposure to ATL-conditioned medium. Thus, ATL-conditioned medium augmented YT clone 2D7 expression of both detectable Tac mRNA (Fig. 3A) and cell surface Tac antigen (Fig. 3B), whereas YT clone 2C2 Tac mRNA and Tac antigen expression were undetectable before, and at every interval monitored after exposure to ATL-conditioned medium. The actual dot blots from which the densitometric scans were performed are presented in Fig. 3C. It is noteworthy that YT clone 2D7 Tac mRNA expression was augmented 10-fold by ATL cell-conditioned medium, whereas YT clone 2C2 Tac mRNA expression remained undetectable, identical to that found with RNA from JURKAT clone 6.8, a T-ALL cell line that does not express IL-2-R or Tac antigen. Moreover, as shown by the same RNA samples hybridized to a cDNA probe for HLA-B7 (Fig. 3D), equivalent amounts of mRNA from both clone 2D7 and 2C2 were analyzed at each time interval.

To seek further substantiation that YT-2C2 cells do not express Tac mRNA, RNA extracted from clones 2D7, 2F6 and 2C2 was enriched for poly(A)⁺ RNA using oligo(dT) cellulose, so as to improve the sensitivity of the hybridization assay. As is evident in Fig. 4, even the 20-fold increase in sensitivity achieved by

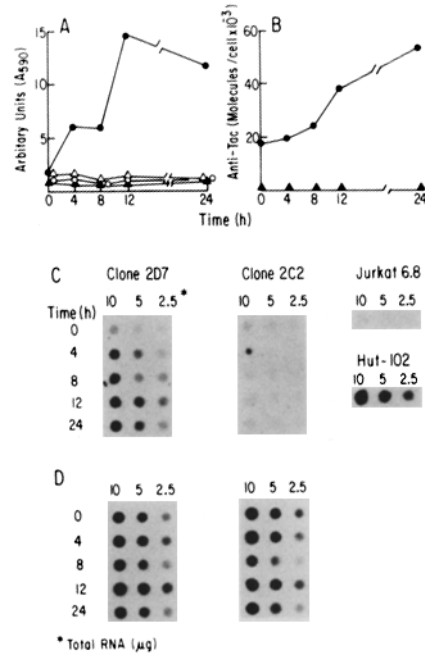


FIGURE 3. Tac mRNA and Tac antigen expression by YT clone 2D7 and clone 2C2 before and after exposure to ATL-conditioned medium. (A) Densitometric scans of the total RNA dot blots shown in C and D. Hybridization was performed with the Tac 2 cDNA probe (7) (solid symbols) and an HLA-B7 probe (18) (open symbols). Clone 2D7 (circles); clone 2C2 (triangles). Time intervals denote exposure of cells to ATL-conditioned medium. For comparison of the hybridization intensities, the densitometric scanning results were expressed as 1.0 arbitrary unit using Tac cDNA and Jurkat 6.8 RNA. For HLA-B7 hybridization, the value obtained with YT clone 2D7 at time 0 was assigned 1.0 AU. (B) ¹²⁵I-anti-Tac binding to clone 2D7 (●) and to clone 2C2 (▲) assayed on cell aliquots at the same time intervals for A. (C) Dot blots of Tac cDNA hybridization to 10, 5, and 2.5 μg of total RNA from YT clone 2D7, clone 2C2, Jurkat 6.8 (negative control) and HUT-102 (positive control). (D) The same filters shown in C after washing and hybridization to the HLA-B7 probe (18).

selecting 4–5% of the total RNA failed to yield a positive signal when 2C2 poly(A)⁺ RNA was hybridized with Tac cDNA. By comparison, even in the absence of exposure to ATL-conditioned medium, YT clone 2D7 and clone 2F6 both contained easily detectable Tac mRNA, as did HUT-102 cells. Once again, the JURKAT clone 6.8 hybridization was negative and indistinguishable from YT clone 2C2.

Analysis of YT Cell IL-2-binding Proteins. The only interpretation that explains logically the expression of $2-3 \times 10^4$ IL-2 binding sites in the absence of both cell surface Tac antigen and Tac mRNA expression predicts that the gene product responsible for IL-2 binding by these cells should be distinct from the Tac antigen protein. Since YT clone 2C2 cells did not react with anti-Tac, even when immunoprecipitation experiments were attempted, the cell surface proteins reactive with IL-2 were analyzed by forming covalent crosslinkages between ¹²⁵I-IL-2 and YT clone 2C2 cells. The results from a representative experiment, displayed in Fig. 5, reveal a single major band migrating at 90,000 M_r (p90), as analyzed by autoradiography after SDS-PAGE (Fig. 5, lane 1). When the chemical crosslinking reagent was omitted (lane 2) no crosslinked proteins were discernible.

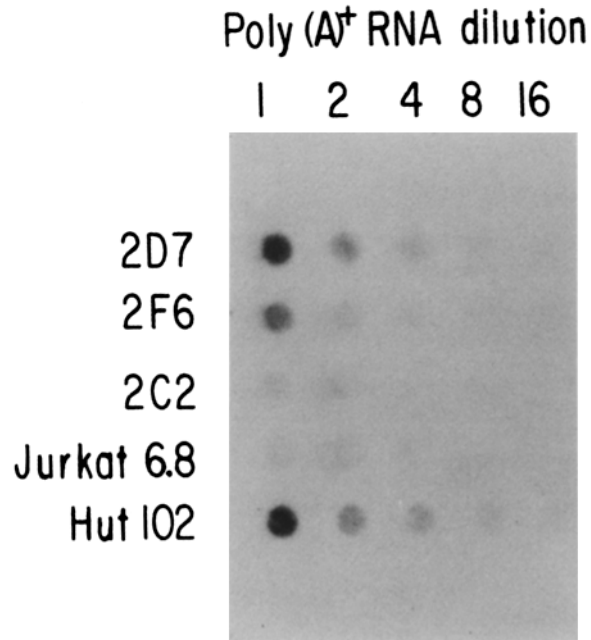


FIGURE 4. Tac mRNA expression. Dot blot hybridization of cellular poly(A)⁺ RNA with the Tac 2 cDNA probe (7). Selected RNA was applied to the filters in twofold dilutions from 10 μ g. HUT-102 RNA was diluted from a 20-fold lower amount (0.5 μ g).

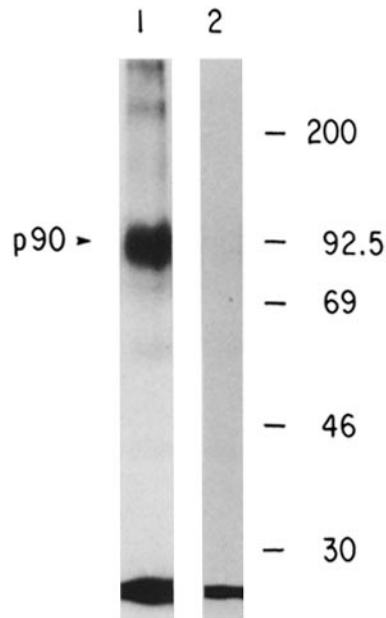


FIGURE 5. SDS-PAGE of YT clone 2C2-IL-2-binding protein as detected by ¹²⁵I-IL-2 cross-linkage. Lane 1, ¹²⁵I-IL-2 bound and crosslinked (with DSS) to YT-2C2 cells. Lane 2, DSS was omitted. $M_r \times 10^{-3}$ shown at right.

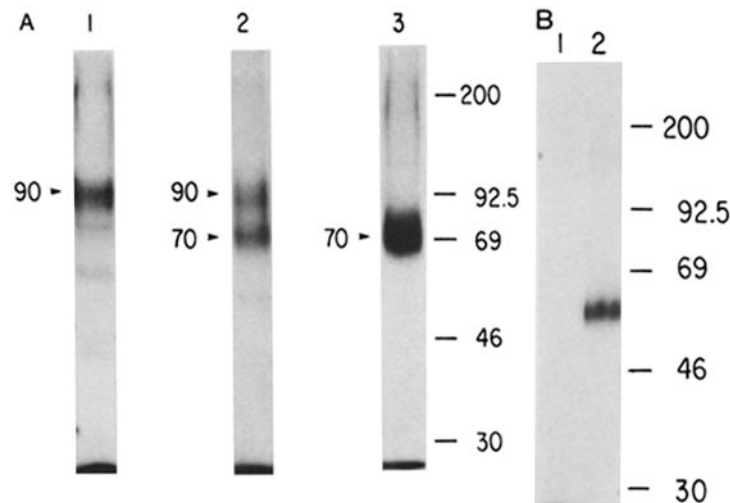


FIGURE 6. SDS-PAGE of IL-2-binding proteins as analyzed by ^{125}I -IL-2 crosslinkage (A) and by immunoprecipitation with anti-Tac (B). (A) Lane 1, control YT cells; lane 2, YT cells exposed to 20% ATL-conditioned medium for 24 h; lane 3, HUT-102 cells. (B) Anti-Tac immunoprecipitation of YT cells before (lane 1) and 24 h after exposure to ATL-conditioned medium (lane 2). $M_r \times 10^{-3}$ are shown.

Moreover, although not shown, p90 was also undetectable when a 100-fold excess of unlabeled IL-2 was included in the binding mixture, thus providing the appropriate IL-2 specificity control. Accordingly, the size of the YT clone 2C2 cell membrane protein calculated to be crosslinked to the 15,000 M_r IL-2 is 75,000 M_r (p75), quite different from Tac antigen, which migrates at 55,000 M_r (p55) (5).

The results obtained with the 2C2 clone indicated that the original YT cells should express at least two cell surface proteins capable of binding IL-2, the p55 Tac antigen and the p75 protein, both of which should be especially evident after augmentation of Tac antigen expression via ATL-conditioned medium. Therefore, the original YT cell line was examined for the type of IL-2-binding proteins expressed in the presence and in the absence of exposure to ATL-conditioned medium. As shown in Fig. 6A, in the absence of ATL-conditioned medium, ^{125}I -IL-2 is crosslinked predominantly to form a membrane IL-2 complex indistinguishable from the p90 observed for YT clone 2C2 cells (Fig. 6, lane 1). It is noteworthy that ^{125}I -IL-2 and ^{125}I -anti-Tac binding assays performed on aliquots of these cells revealed 32,000 intermediate-affinity IL-2 binding sites but only 600 high-affinity IL-2-R and 700 anti-Tac-reactive sites. For comparison, the SDS-PAGE pattern of YT cells exposed to ATL-conditioned medium for 24 h before ^{125}I -IL-2 binding and crosslinking is shown in lane 2; clearly there are two major bands visible, at 90,000 M_r and 70,000 M_r . Moreover, in contrast to the binding studies performed on the uninduced cells, assays performed on these cells yielded 6,000 high-affinity IL-2-R and 9,800 anti-Tac-reactive sites, in addition to 25,400 intermediate-affinity IL-2 binding sites.

Using the same methods, only the 70,000 M_r band was detectable after crosslinking ^{125}I -IL-2 to HUT-102 cells, which express 98–99% Tac antigen–

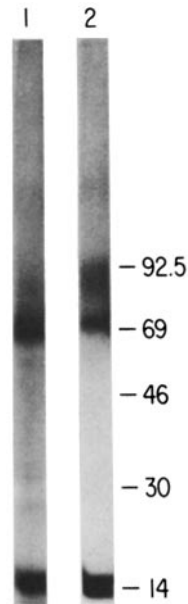


FIGURE 7. SDS-PAGE of ^{125}I -IL-2 crosslinked to activated normal human T cells. Lane 1, 4 nM ^{125}I -IL-2 bound; lane 2, 50 pM ^{125}I -IL-2 bound. $M_r \times 10^{-3}$ are shown.

positive low-affinity IL-2 binding sites (Fig. 6, lane 3). Accordingly, these results are consonant with the interpretation that YT cells can express two proteins; one, p75, does not contain the Tac epitope, whereas the other, p55, is indistinguishable from Tac antigen as expressed by both normal T cells and ATL cell lines. A final confirmation that YT cells induced by ATL-conditioned medium express the typical Tac antigen protein is shown in Fig. 6B. The single protein, p55, immunoprecipitated by anti-Tac is in sharp contrast to the two proteins bound and crosslinked to ^{125}I -IL-2 (Fig. 6A, lane 2).

Analysis of High- and Low-Affinity Receptors Expressed by Activated Normal T Cells. The discovery that immature leukemic cells express an entirely new IL-2-binding protein was intriguing. However, the correlation of high-affinity receptors with the coordinate expression of p55 and p75 implied an even more fundamental finding: i. e., both IL-2-binding proteins may well be essential for the formation of functional, high-affinity receptors. If so, then normal activated T cells should also coexpress p55 and p75. To assess this possibility, G_0/G_1 -synchronized normal human T cells were activated by Pdbu, and cultured with IL-2 for 18 h, since previous studies had established that this activation protocol results in a ratio of 10:1 low-affinity to high-affinity IL-2 binding sites (9). To reveal p75, which was anticipated to be expressed in limited amounts compared with the easily detectable amounts of p55, two different ^{125}I -IL-2 concentrations were used for binding before crosslinking; a high IL-2 concentration calculated to saturate a majority of the low-affinity binding sites, and a low IL-2 concentration that would saturate only a few low-affinity binding sites but all of the high-affinity receptors. The results, shown in Fig. 7, are entirely consistent with those already found for YT cells, and substantiate that high-affinity binding correlates

with IL-2 binding to both p75 and p55. Accordingly, as shown in Fig. 7, lane 1, when low-affinity binding predominates, only one ^{125}I -IL-2-crosslinked protein is visible at 70,000 M_r . In contrast, at a low ^{125}I -IL-2 concentration (lane 2), which favors the proportion of high-affinity receptors relative to low-affinity binding sites occupied, two bands emerge distinctly, M_r 70,000 and 90,000.

Discussion

Fortuitously, we have uncovered clones of a T-ALL cell line that express an IL-2-binding protein, but do not express detectable levels of Tac mRNA nor Tac antigen. As these cells bind IL-2 with an intermediate affinity, and the binding protein is larger than Tac antigen, the summative evidence shows that this novel IL-2-binding protein is encoded by a gene entirely separate from Tac. However, even more striking is the discovery that high-affinity IL-2 binding correlates with the dual expression of Tac antigen together with this new IL-2-binding protein, thereby suggesting that the binding sites on both cell surface molecules are required to cooperate to form biologically relevant, high-affinity IL-2-R. Accordingly, to conform with the terminology of other receptor systems, and to facilitate expression, we propose that p75 be referred to as the IL-2-R α chain, and p55 (Tac antigen) as the IL-2-R β chain.

Soon after the development of the IL-2 binding assay, the very first experiments performed with Tac antibodies (5) revealed a large discrepancy between the number of binding sites detectable using radiolabeled IL-2 vs. radiolabeled anti-Tac. In particular, ATL cell lines such as HUT-102 express 50–100-fold more anti-Tac binding sites than high-affinity IL-2-R. Subsequent experiments defined a second class of sites that bind IL-2 with a 1000-fold lower affinity (i. e., $K_d = 10$ pM vs. 10 nM) (8, 9), and the great majority ($\geq 98\%$) of the IL-2 binding sites expressed, especially by ATL cell lines, were found to be of the low-affinity category. These findings became increasingly disturbing, considering that ATL cell lines and the amino acid sequence of anti-Tac-purified protein were used to identify cDNA clones that were purported to encode the IL-2-R (6, 7). After cDNA transfection experiments revealed solely low-affinity IL-2 binding by non-T cells (23, 24), but both high- and low-affinity IL-2 binding by T cells (25, 26), it became apparent that an additional, T cell-specific protein was necessary to form high-affinity IL-2-R. Thus far, investigators have searched for a component that would not of itself bind IL-2, but that would serve to convert the IL-2 binding of the Tac protein from low affinity to high affinity. Consequently, the discovery of another IL-2-binding protein, and the evidence that Tac antigen and this new IL-2-binding protein are coexpressed by cells that have detectable high-affinity receptors, now make essential an entirely new conceptual formulation of the structural basis for functional, high-affinity IL-2-R.

Given the understanding that IL-2-R α chains and β chains are coexpressed by cells with high-affinity IL-2-R, it is somewhat puzzling that the α chain was not discovered earlier. In retrospect, it is now easy to perceive that the large excess of β chains (10–100-fold) expressed by the only cells available for analysis obscured the presence of the α chains. Consequently, the isolation of YT cell clones that solely express α chains was pivotal for the present, new interpretation

that two distinct IL-2-binding proteins are actually operative. Of equal importance, the ability to induce selectively the expression of β chains expressing Tac antigen, while simultaneously monitoring the appearance of high-affinity IL-2-R, made the conclusion inevitable and incontrovertible that both of these IL-2-binding proteins must be important for the formation of high-affinity IL-2-R. In this respect, the stoichiometry of β chain expression as monitored by anti-Tac and the expression of high-affinity receptors by YT cells stimulated with ATL-conditioned medium is especially convincing. Moreover, this interpretation is even more compelling when it is appreciated that either chain expressed separately makes solely for lower affinity IL-2 binding sites.

The size of the IL-2-R α chain, being 75,000 M_r , rather than only 55,000 M_r , provides for the expectation that the cytoplasmic domain of the α chain will be larger than the rather short 13 residues of the β chain, and thus yields some insight on possible pathways activated by IL-2-R. If the α chain contains all the structures necessary for signal transduction, cells expressing only the α chain, exemplified by the YT clone 2C2 cells, should respond to IL-2. Thus far, we have yet to detect IL-2-mediated signal transduction via the YT-2C2 α chain, but we are mindful that these cells are neoplastic and can proliferate without IL-2. Accordingly, additional experimental manipulations will be required to investigate fully the functional attributes of these IL-2 binding sites. Moreover, it remains a distinct possibility that both α and β chains are required for detectable signal transduction. Certainly, those cells that express only one of the chains, such as YT-2C2 cells as reported here, or Tac cDNA transfected non-T cells (23, 24), are unresponsive to IL-2.

The functional role of the β chain also requires reconsideration in the light of these new findings. In earlier experiments we had found that IL-2 itself induced the expression of the β chain (9), and others found this induction to result from enhanced transcription (27). The IL-2-mediated transcriptional activation becomes manifest ultimately as a 10-fold increase in the cell surface density of Tac antigen-positive low-affinity IL-2 binding sites (9). Concomitantly, high-affinity IL-2-R decline by as much as 50% in the presence of IL-2 (9, 28). Our own metabolic studies have traced the disappearance of the high-affinity IL-2-R to an IL-2-mediated accelerated internalization of ligand-receptor complexes (Cantrell, D. A., and K. A. Smith, manuscript in preparation). Accordingly, these observations are in harmony with the interpretation that the α chain contains the structural information necessary for ligand-mediated internalization, whereas the β chain does not. Consistent with this view, our previous studies (9) found a very slow turnover of Tac antigen in comparison with high-affinity IL-2-R. Therefore, IL-2 binding to high-affinity receptors promotes rapid internalization of α chains (and perhaps associated β chains), while simultaneously, signals are generated that result ultimately in enhanced expression of β chain mRNA and low-affinity cell surface binding sites. The uncertainty underlying these observations relates to the functional importance, if any, of the expression of 10–20-fold more β chains than α chains. Consideration of kinetic and equilibrium binding characteristics of each binding site may well provide clues to this enigma, and point the way to further experimentation.

Although the present studies indicate that α chains and β chains are expressed

together on the cell surface, as yet we have no data to indicate whether these two chains associate with one another. However, it is likely that the α and β chains are linked physically, considering the effect of anti-Tac; even though the Tac epitope is expressed only on the β chain, anti-Tac prevents IL-2 binding both to low-affinity sites and to high-affinity receptors (5, 8). In this regard, we have recently completed an extensive study of β chains under both nonreducing and reducing conditions, and have found that these chains form preferentially disulfide-linked homodimers that can bind two IL-2 molecules.² In contrast, α chains expressed in the absence of β chains by YT clone 2C2 cells do not form homodimers (Kato, K., and K. A. Smith, unpublished observations). The behavior of these IL-2-binding proteins, especially the homodimer formation by β chains, is reminiscent of the behavior of the T6 and T8 glycoproteins, which are structurally homologous members of the Ig family. On immature thymocytes T8 is found disulfide-linked to T6, whereas T8 homodimers are expressed by mature T cells, which lack T6 molecules (29). Thus, like the Ig family, whose members preferentially associate with themselves, or other members of the same group (e. g., Ig H and L chains), the α and β chains of the IL-2-R may well prove to associate with one another as members of a family of homologous, but separate and distinct lymphokine receptor molecules.

Since α chains and β chains bind IL-2 with distinctly different affinities ($K_d = 1$ nM and 10 nM, respectively), the question arises whether the same or different sites on the IL-2 molecule interact with each binding protein. If the same configuration on the IL-2 molecule were responsible for binding to both chains, one would anticipate some measure of competition between the two binding proteins, which would be evident as negative cooperation in the analysis of IL-2 binding curves. Actually, quite the opposite occurs, i. e., the expression of both proteins is required for high-affinity IL-2 binding, thereby pointing to two distinct active sites on the IL-2 molecule responsible for interacting separately with each chain. This interpretation is particularly persuasive, as it hints that high-affinity IL-2 binding sites are formed from the combination of the α and β chains in a manner similar to the formation of the antigen binding site from residues contributed by both the H and L chains of Ig (30). Particularly convincing in this regard is the finding that the chain present in limiting amounts dictates the number of high-affinity receptors, and either α or β chains can function in this role. For example, normal activated T cells and ATL cell lines display excess β chains as evidenced by the ¹²⁵I-IL-2 crosslinking experiments, and consequently the number of high affinity receptors depends upon the absolute number of α chains expressed. In contrast, YT cells express α chains in excess, and high-affinity receptors are detected only when the cells are induced to express β chains.

The existence of an IL-2-binding protein that does not contain the Tac epitope but that nonetheless appears to be necessary for the formation of fully functional IL-2-R now calls into question the significance of finding solely Tac antigen-positive, low-affinity IL-2 binding sites on cells other than the T cell lineage, e. g., B cells and mast cells (31, 32). Since the β chain of the IL-2-R appears to

² Kato, R., and K. A. Smith. Tac antigen forms disulfide-linked heterodimers. Submitted for publication.

have a much wider tissue distribution than do high-affinity IL-2-R, it is likely that the simultaneous expression of α chains and β chains is rigorously restricted to T cells stimulated via the T cell antigen receptor complex (33). Moreover, as IL-2 itself causes such a marked induction of β chain expression (9) it is possible that the α chain gene expression is triggered solely via the T cell antigen receptor complex, whereas β chain expression is regulated by the IL-2- α chain interaction. Such a model predicts that during the T cell proliferative response to antigen, IL-2-R α chains are expressed before β chains. A sequential activation of α chains followed by β chains is attractive, in that the IL-2- α chain-mediated induction of β chains would have the effect of promoting formation of the more biologically efficient high-affinity receptors, thereby lowering by 100-fold the IL-2 concentration required for binding and receptor activation.

The phenotype of the YT cells, particularly of the 2C2 clone, deserves comment, as it may well be of importance for the biologic relevance of the IL-2-R α chain. The YT cells resemble "double negative," very immature (class I) thymocytes (34), in that analysis by immunocytofluorometry using T cell-specific mAbs results consistently in the following pattern: T3⁻, T4⁻, T6⁻, T8⁻, T9⁺, T11⁺ (Teshigawara, K., and K. A. Smith, unpublished observations). Consequently, the expression of these newly recognized IL-2 binding sites by leukemic cells that resemble normal, immature, class I thymocytes, may well be a clue that this binding site could play some role in normal thymocyte proliferation or differentiation.

It is particularly noteworthy that many other polypeptide hormone receptors are known to be present in both high-affinity and low-affinity forms (35), yet no structural explanations for these distinct receptor classes have been uncovered. Consequently, it is likely that the proposed model system, which involves the generation of high-affinity IL-2-R via an interaction between two distinct low-affinity binding proteins, will be found to reflect a general aspect of hormone/receptor systems. For studies relating to T cell proliferation, the discovery of the IL-2-R α chain is particularly timely, in that all attempts thus far to attribute functional, high-affinity IL-2-R to the Tac protein alone have been futile. Now, with the insight afforded by the knowledge that two IL-2-binding proteins are involved, and that both must be expressed for the formation of high-affinity IL-2-R, it should be possible to move forward once again to the experimental dissection of the biochemical events triggered by IL-2-R that initiate T cell cycle progression.

Summary

A cell line established from a patient with acute lymphoblastic leukemia was found to express IL-2 binding sites with a novel, intermediate affinity compared with the characteristic high-affinity IL-2-receptors and low-affinity IL-2 binding sites described previously. Clones were isolated from this cell line that displayed solely this new IL-2-binding protein, and were found to be unreactive with anti-Tac, the mAb that competes with IL-2 for binding. Moreover, these same cloned cells did not express mRNA detectable by hybridization with radiolabeled cDNA encoding the Tac protein. In contrast, the original cell line and similar clones expressed low levels of Tac mRNA and cell surface Tac antigen, both of which

could be augmented by exposure to medium conditioned by adult T leukemia cell lines. Particularly noteworthy, induction of Tac antigen expression was paralleled by an increase in the number of high-affinity IL-2-R detectable. Since the expression of the Tac antigen protein by itself makes only for low-affinity IL-2 binding, these data prompted a reevaluation of the structural composition of high-affinity IL-2-R. Analysis of the IL-2-binding proteins expressed by leukemic cell lines lacking high-affinity receptors revealed only a single protein, larger than the Tac antigen protein ($M_r = 75,000$ vs. 55,000). In contrast, clones induced to express high-affinity receptors had clearly both of these IL-2-binding proteins. Moreover, when IL-2 binding to normal T cells was performed under conditions that favored the proportion of high-affinity receptors occupied, two distinct proteins identical to those already identified on the leukemic cells could be crosslinked covalently to radiolabeled IL-2. The interpretations derived from these varied, assembled data, point to two IL-2-binding proteins, both of which are required for high-affinity IL-2 binding.

We are grateful to Dr. J. Yodoi for the YT cell line, to Dr. E. Reinherz for the mAbs reactive with Tac antigen, and to Dr. M. Landy for editorial comments.

Received for publication 21 August 1986.

References

1. Smith, K. A. 1984. Interleukin 2. *Annu. Rev. Immunol.* 2:319.
2. Robb, R. J., A. Munck, and K. A. Smith. 1981. T-cell growth factor receptors: quantitation, specificity, and biological relevance. *J. Exp. Med.* 154:1455.
3. Uchiyama, T., S. Broder, and T. A. Waldmann. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac(+) cells. *J. Immunol.* 126:1393.
4. Uchiyama, T., D. L. Nelson, T. A. Fleisher, and T. A. Waldmann. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. II. Expression of Tac antigen on activated cytotoxic killer T cells, suppressor cells, and on one of two types of helper T cells. *J. Immunol.* 126:1398.
5. Leonard, W. J., J. M. Depper, T. Uchiyama, K. A. Smith, T. A. Waldmann, and W. C. Greene. 1982. A monoclonal antibody, anti-Tac, blocks the membrane binding and action of human T-cell growth factor. *Nature (Lond.)*. 300:267.
6. Leonard, W. J., J. M. Depper, G. R. Crabtree, S. Rudikoff, J. Pumphrey, R. J. Robb, M. Kronke, P. B. Svetlik, N. J. Pfeffer, T. A. Waldmann, and W. C. Greene. 1984. Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. *Nature (Lond.)*. 311:626.
7. Nikaido, T., A. Shimizu, N. Ishida, H. Sabe, K. Teshigawara, M. Maeda, T. Uchiyama, J. Yodoi, and T. Honjo. 1984. Molecular cloning of cDNA encoding human interleukin-2 receptor. *Nature (Lond.)*. 311:631.
8. Robb, R. J., W. C. Greene, and C. M. Rusk. 1984. Low and high affinity cellular receptors for interleukin 2: implications for the level of Tac antigen. *J. Exp. Med.* 160:1126.
9. Smith, K. A., and D. A. Cantrell. 1985. Interleukin 2 regulates its own receptors. *Proc. Natl. Acad. Sci. USA.* 82:864.
10. Yodoi, J., M. Maeda, Y. Wano, M. Tsudo, K. Teshigawara, and T. Uchiyama. 1984. TCGF (IL-2) receptor (Tac Ag) on ATL and non-ATL leukemia cells. *In* Thymic

- Hormones and Lymphokines. A. L. Goldstein, editor. Plenum Publishing Corp., New York. 273-277.
11. Teshigawara, K., M. Maeda, K. Nishino, T. Nikaido, T. Uchiyama, M. Tsudo, Y. Wano, and J. Yodoi. 1985. Adult T leukemia cells produce a lymphokine that augments interleukin 2 receptor expression. *J. Mol. Cell. Immunol.* 2:17.
 12. Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA.* 77:7415.
 13. Okada, M., M. Maeda, Y. Tagaya, Y. Taniguchi, K. Teshigawara, T. Yoshiki, T. Diamantstein, K. A. Smith, T. Uchiyama, T. Honjo, and J. Yodoi. 1985. TCGF(IL-2)-receptor reducing factor(s). II. Possible role of ATL-derived factor (ADF) on constitutive IL-2 receptor expression of HTLV-I(+) T cell lines. *J. Immunol.* 135:3995.
 14. Gullberg, M., and K. A. Smith. 1986. Regulation of T cell autocrine growth: T4⁺ cells become refractory to interleukin 2. *J. Exp. Med.* 163:270.
 15. Kato, K., T. Yamada, K. Kawahara, H. Onda, T. Asano, H. Sugino, and A. Kakinuma. 1985. Purification and characterization of recombinant human interleukin-2 produced in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* 130:692.
 16. Stern, J. S., and K. A. Smith. 1986. Interleukin 2 induction of T cell G₁ progression and c-myc expression. *Science (Wash. DC).* 233:203.
 17. Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. USA.* 69:1408.
 18. Sood, A. K., D. Pereira, S. M. Weissman. 1981. Isolation and partial nucleotide-sequence of a cDNA clone for human histocompatibility antigen HLA-B by use of an oligodeoxynucleotide primer. *Proc. Natl. Acad. Sci. USA.* 78:616.
 19. Pilch, P. F., and M. P. Czech. 1978. Interaction of cross-linking agents with the insulin effector system of isolated fat cells. *J. Biol. Chem.* 254:3375.
 20. Jett, M., T. M. Seed, and G. A. Jamieson. 1977. Isolation and characterization of plasma membranes and intact nuclei from lymphoid cells. *J. Biol. Chem.* 252:2134.
 21. Ritz, J., T. J. Campen, R. E. Schmidt, H. D. Royer, T. Hercend, R. E. Hussey, and E. L. Reinherz. 1985. Analysis of T-cell receptor gene rearrangement and expression in human natural killer clones. *Science (Wash. DC).* 228:1540.
 22. Baker, P. E., S. Gillis, and K. A. Smith. 1979. Monoclonal cytolytic T cell lines. *J. Exp. Med.* 149:273.
 23. Greene, W. C., R. J. Robb, P. B. Svetlik, C. M. Rusk, J. M. Depper, and W. J. Leonard. 1985. Stable expression of cDNA encoding the human interleukin 2 receptor in eukaryotic cells. *J. Exp. Med.* 162:363.
 24. Sabe, H., S. Kondo, A. Shimizu, Y. Tagaya, J. Yodoi, N. Kobayashi, M. Hatanaka, N. Matsunami, M. Maeda, T. Noma, and T. Honjo. 1986. Properties of human interleukin 2 receptors expressed on non-lymphoid cells by cDNA transfection. *Mol. Biol. Med.* 2:379.
 25. Hatakeyama, M., S. Minamoto, T. Uchiyama, R. R. Hardy, G. Yamada, and T. Taniguchi. 1985. Reconstitution of functional receptor for human interleukin 2 in mouse cells. *Nature (Lond.).* 318:467.
 26. Kondo, S., A. Shimizu, M. Maeda, Y. Tagaya, J. Yodoi, and T. Honjo. 1986. Expression of functional human interleukin 2 receptor in mouse T cells by cDNA transfection. *Nature (Lond.).* 320:75.
 27. Leonard, W. J., M. Kronnke, N. J. Peffer, J. M. Depper, and W. C. Greene. 1985.

- Interleukin 2 receptor gene expression in normal human T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 82:6281.
28. Fujii, M., K. Sugamura, K. Sano, M. Nakai, K. Sugita, and Y. Hinuma. 1986. High-affinity receptor-mediated internalization and degradation of interleukin 2 in human T cells. *J. Exp. Med.* 163:550.
 29. Snow, P. M., and C. Terhorst. 1983. The T8 antigen is a multimeric complex of two distinct subunits on human thymocytes but consists of homomultimeric forms on peripheral blood T lymphocytes. *J. Biol. Chem.* 258:14675.
 30. Amit, A. G., R. A. Mariuzza, S. E. V. Phillips, and R. J. Poljak. 1986. Three-dimensional structure of an antigen-antibody complex at 2.8 Å resolution. *Science (Wash. DC)* 233:747.
 31. Muraguchi, J. H., J. H. Kehrl, D. L. Longo, D. J. Volkman, K. A. Smith, and A. S. Fauci. 1985. Interleukin 2 receptors on human B cells. Implication for the role of interleukin 2 in B cell function. *J. Exp. Med.* 161:181.
 32. Koyasu, S., J. Yodoi, T. Nikaïdo, Y. Tagaya, Y. Taniguchi, T. Honjo, and I. Yahara. 1986. Expression of interleukin 2 receptors on interleukin 3-dependent cell lines. *J. Immunol.* 136:984.
 33. Meuer, S. C., R. E. Hussey, D. A. Cantrell, J. C. Hodgdon, S. F. Schlossman, K. A. Smith, and E. L. Reinherz. 1984. Triggering of the T3-Ti antigen-receptor complex results in clonal T cell proliferation through an interleukin 2-dependent autocrine pathway. *Proc. Natl. Acad. Sci. USA.* 81:1509.
 34. Reinherz, E. L., and S. F. Schlossman. 1980. The differentiation and function of human T lymphocytes. *Cell.* 19:821.
 35. James, R., and R. A. Bradshaw. 1984. Polypeptide growth factors. *Annu. Rev. Biochem.* 53:259.