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Interleukin 2 regulates its own receptors

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ABSTRACT **The cell surface density of high-affinity membrane receptors for the T-lymphocytotrophic hormone interleukin 2 (IL-2) determines the rate of T-cell-cycle progression. Since 10-fold greater numbers of IL-2 receptor molecules were found by using a radiolabeled monoclonal antibody reactive with IL-2 receptors (anti-Tac) compared with binding of IL-2, the functional relationship of the binding sites recognized by both of these ligands was assessed. In the presence of cycloheximide, IL-2 binding sites declined with a half-time ($t_{1/2}$) of 2.6 hr, whereas the decay of anti-Tac binding sites was much slower ($t_{1/2}$ = 6.4 hr). Moreover, after limited membrane proteolysis, the half-time for the reappearance of IL-2 binding sites was remarkably similar to its decay ($t_{1/2}$ = 2.2 hr), while Tac antigen reappearance was markedly retarded, returning to only 20% of original levels within 5 hr after proteolysis. Addition of homogeneous immunoaffinity-purified IL-2 to cell populations that expressed equivalent IL-2 and anti-Tac binding sites resulted in a time- and temperature-dependent 8- to 10-fold enhancement of Tac epitope expression and, simultaneously, a 20–30% diminishment of detectable high-affinity IL-2 binding sites. As the magnitude of the IL-2-dependent proliferative response correlated with the density of high-affinity IL-2 binding sites, rather than Tac antigen levels, quantitation of Tac epitope density does not provide a reliable indication of IL-2 responsiveness among activated T-cell populations. Instead, IL-2–receptor interactions actually promote the loss of IL-2 responsiveness by diminishing the density of high-affinity binding sites at the time that Tac antigen levels are increased.**

T lymphocyte DNA replication and mitosis is determined by a critical threshold of signals generated by the interaction between interleukin 2 (IL-2) (1–4) and its specific membrane receptors (5–7). Compared with other hormone–receptor systems, the IL-2–T-cell system is exceptional; T cells do not express IL-2 receptors unless activated by immunostimulatory ligands such as antigens, T-cell-specific monoclonal antibodies, mitogenic lectins, and phorbol esters, all of which trigger components of the T-cell surface antigen–receptor complex (4, 8). Moreover, once a cell population is exposed to these agents, individual cells express IL-2 receptors at different rates, giving rise to the asynchronous entry of cells into the proliferative phases of the cell cycle (6). If IL-2 is excluded from an antigen-activated T-cell population, IL-2 receptor expression still occurs, but DNA synthesis does not proceed, since it is the IL-2–receptor interaction that provides the crucial signal for initiation of DNA replication (6, 9). Furthermore, as IL-2 receptor expression is antigen-dependent, upon removal of the immunostimulatory signal, IL-2 receptor levels decline progressively. The proliferative rate of the cell population slows in parallel, and all of the cells eventually reaccumulate in the resting (G_0/G_1) phase of the cell cycle, even in the presence of saturating concentrations of IL-2 (6). Accordingly, the T-cell IL-2 hormone–receptor system is a distinctive example of a hormone–receptor mechanism, as it appears to be under the sole direction of signals that enter from the environment; thus far, there is no evidence for internal feedback regulatory controls that enhance or diminish the antigen-initiated, IL-2-dependent T-cell proliferative response.

The dissection and identification of the variables controlling T-cell growth that led to the present model was made possible by the painstaking acquisition of unique cellular and molecular reagents, including (*i*) synchronized IL-2 receptor$^+$ clonal T-cell populations (6, 8), (*ii*) homogeneous immunoaffinity-purified IL-2 (10), (*iii*) the development of a quantitative radiolabeled IL-2 binding assay (5), and (*iv*) the identification of a monoclonal antibody reactive with IL-2 receptors (anti-Tac) (9). Cytofluorographic studies utilizing anti-Tac were especially revealing, since for the first time, it was discovered that IL-2 receptors have a logarithmic–normal distribution, not only within asynchronously proliferating polyclonal cell populations (7) but also within cloned and synchronized T-cell populations as well (7, 8). However, even through functional (9) and biochemical studies (11–13) indicated that anti-Tac reacted with IL-2 receptors, quantitative differences were found between the Tac antigen and IL-2 receptor densities (11–13), thus raising the possibility that the epitope recognized by anti-Tac did not include the IL-2 binding site. Accordingly, several fresh experimental approaches were undertaken to compare the binding of radiolabeled IL-2 vs. anti-Tac to activated human T-cell populations. The results reveal that IL-2 binding sites and anti-Tac-reactive epitopes differ markedly in their respective turnover characteristics; moreover, IL-2 actually regulates the expression of IL-2 receptor molecules as detected by radiolabeled IL-2 and anti-Tac binding. The IL-2–receptor interaction serves to diminish the density of high-affinity IL-2 receptors, at the very time it augments considerably Tac antigen expression. Consequently, IL-2 governs the magnitude of its own biologic response by a unique mechanism, manifested by an IL-2-promoted diminished expression of IL-2 binding sites, and an increased expression of IL-2 receptor molecules that are recognized by anti-Tac but do not bind IL-2 with high affinity.

MATERIALS AND METHODS

Cell Cultures. Human peripheral blood mononuclear cells (PBM cells), isolated by Ficoll-Hypaque discontinuous gradient centrifugation, were cultured (1×10^6 cells per ml) in RPMI 1640 medium (GIBCO) supplemented with 10% heat-inactivated (56°C, 45 min) fetal calf serum (FCS, Sterile Systems, Logan, UT) in the presence of phytohemagglutinin

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Abbreviations: IL-2, interleukin 2; anti-Tac, monoclonal antibody reactive with the IL-2 receptor; FCS, heat-inactivated fetal calf serum; PBM cells, human peripheral blood mononuclear cells; PBt_2, phorbol 12,13-dibutyrate; PHA, phytohemagglutinin; EGF, epidermal growth factor.

(PHA, 1 μg/ml, Wellcome) for 72 hr to ensure IL-2 receptor expression as described (6, 7). IL-2-dependent growth was maintained at cell concentrations between 1×10^5 and 1×10^6 cells per ml by re-exposure to PHA (0.5 μg/ml, 1 hr) at 10- to 14-day intervals and by supplementation with IL-2 every 2 days so that the IL-2 concentration was maintained at ≥100 pM. To ensure IL-2 receptor re-expression by G_0/G_1 synchronized cell populations in the absence of IL-2 production, phorbol 12,13-dibutyrate (PBt_2, 50 ng/ml, 6 hr, Consolidated Midland, Brewster, NY) was used as the immunostimulatory agent, since PBt_2 leads to IL-2 receptor re-expression without IL-2 production, as monitored by the IL-2 bioassay (sensitivity, 1 pM) and by [^{3}H]dThd incorporation of the PBt_2 stimulated cell population (unpublished observations).

[^{3}H]dThd incorporation was monitored (1 hr, 2.0 μCi/ml, specific activity = 1.9 Ci/mmol; 1 Ci = 37 GBq; Schwartz/Mann) by precipitation onto glass fiber filter paper followed by liquid scintillation spectroscopy.

IL-2 and Anti-Tac Binding Assays. Unlabeled and radiolabeled (with [^{3}H]leucine and [^{3}H]lysine) IL-2 derived from JURKAT T-leukemia cells was purified from culture media by immunoaffinity adsorption using a monoclonal antibody reactive with IL-2 (DMS-3) as described (6, 10). Both preparations were homogeneous within the limits of detection by $NaDodSO_4$/polyacrylamide gel electrophoresis, reversed-phase HPLC, and amino acid sequence analysis (6, 10). The specific activity of [^{3}H]IL-2 was 9.4×10^4 dpm/pmol. Anti-Tac monoclonal antibodies were purified from ascitic fluid by protein A-Sepharose (Sigma) affinity adsorption (10) and radiolabeled using Enzymobeads (Bio-Rad) (specific activity 4.0×10^5 dpm/pmol).

To accurately quantitate ligand binding, especially on cells exposed to IL-2, any receptor-bound IL-2 was dissociated by a 60-sec exposure to RPMI 1640 medium/25 mM sodium acetate, pH 4.0, followed by three washes with cold (4°C) RPMI 1640 medium/10% FCS. For equilibrium binding analysis, serial dilutions of [^{3}H]IL-2 or ^{125}I-labeled anti-Tac (^{125}I-anti-Tac) were incubated with $1–10 \times 10^5$ cells (200 μl) for 20 min at 37°C or 60 min at 4°C. Cell-bound and free radioactivity was determined after separation by centrifugation (13,000 × *g*, 90 sec) through a mixture of 84% silicone oil and 16% paraffin oil as described (5, 6). The number of binding sites per cell was determined by Scatchard analysis after subtraction of nonsaturable binding, as detected in the presence of a 150-fold molar excess of unlabeled ligand.

IL-2 Receptor Turnover Characteristics. To ascertain the contribution of protein synthesis to membrane levels of IL-2 receptors and Tac antigen, IL-2 receptor$^+$ cells were harvested, maintained in IL-2-free conditions for 4–6 hr to allow dissociation of endogenously bound IL-2, and incubated without IL-2 supplementation (to study turnover in the absence of ligand) at 37°C in RPMI 1640 medium/10% FCS either in the presence or absence of 0.5 mM cycloheximide (Sigma). The levels of IL-2 and anti-Tac binding sites were determined at various intervals.

To establish the characteristics of renewal of IL-2 and anti-Tac binding sites following Pronase treatment, the same IL-2 receptor$^+$ cell populations were incubated at 37°C for 30 min in the presence and absence of Pronase (200 μg/ml, Sigma), then washed three times with cold (4°C) RPMI 1640 medium/10% FCS, and replaced in culture (1×10^6 cells per ml) without exogenous IL-2. Here too, the levels of IL-2 and anti-Tac binding sites were determined at various time intervals.

RESULTS

IL-2 Receptor Turnover Characteristics. The turnover of membrane proteins, generally determined by the half-time for disappearance or appearance of a population of molecules, is equivalent to their median functional lifetime (14). Therefore, to investigate what was perceived as a subtle relationship between the binding sites for IL-2 and for anti-Tac, the turnover characteristics for the sites recognized by these ligands were assessed in the absence of IL-2. Lectin-activated IL-2 receptor$^+$ human peripheral T cells were examined for decay of [^{3}H]IL-2 and for ^{125}I-anti-Tac binding in the presence of cycloheximide. Simultaneously, the kinetics of reappearance of binding capacity was studied subsequent to limited membrane proteolysis. The results of six experiments, performed in the absence of exogenous IL-2, are illustrated in Fig. 1; the findings reveal that IL-2 binding sites and Tac antigen manifest markedly different turnover characteristics. IL-2 binding declined rapidly in the presence of cycloheximide: receptor density was reduced by 50% within 2.6 hr, and only 30% of original levels remained after 5 hr. In contrast, Tac antigen density, which was 10-fold greater than IL-2 receptor density at the beginning of the experiments, persisted at >90% of original levels for 3 hr, and by 5 hr had only decreased gradually to 70% (Fig. 1*A*). It is noteworthy that these decay characteristics were mirrored by the kinetics of reappearance of binding capacity following limited membrane proteolysis (Fig. 1*B*). IL-2 receptor density returned to 50% of control levels by 2.2 hr and attained 90% of

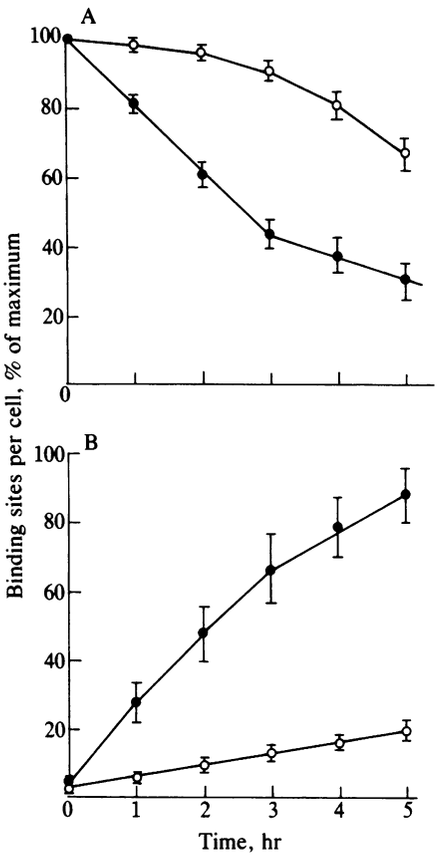

FIG. 1. IL-2-independent disappearance and reappearance of binding sites for IL-2 and anti-Tac. Human PHA-activated (72 hr) IL-2 receptor$^+$ T cells were examined for [^{3}H]IL-2 (●) and ^{125}I-anti-Tac (○) binding in the presence of cycloheximide (*A*) and after limited membrane proteolysis (*B*). The data are presented as the percent of IL-2 and anti-Tac binding sites compared to control cells cultured in medium alone. Symbols represent the mean ± SEM of six separate experiments in which the maximal levels of IL-2 receptors and anti-Tac binding sites at $t = 0$ were 5813 ± 648 and 59,000 ± 19,000, respectively. The recovery of IL-2 and anti-Tac reactivity following Pronase treatment was inhibited by cycloheximide (data not shown), indicating that receptor renewal required *de novo* synthesis of protein.

the original levels by 5 hr, whereas the reappearance of anti-Tac binding remained markedly retarded, reaching only 20% of pretreatment levels during this interval. Especially enlightening are the half-times ($t_{1/2}$) for the decay and reappearance of IL-2 and anti-Tac binding derived from these data. Although the half-times required for the decline and reappearance of IL-2 binding sites are similar (2.6 hr and 2.2 hr, respectively), the values for the decline and reappearance of anti-Tac binding, obtained by extrapolation from the plots, proved to be much longer and, moreover, discordant: the decay of Tac antigen is slower by a factor of 2.5 than IL-2 binding ($t_{1/2}$ = 6.4 hr), and its reappearance is prolonged inordinately compared either to its decay or the kinetics of reappearance of IL-2 binding. Although the theoretical half-time for Tac antigen reappearance extrapolated from the plot is 14.2 hr, the density of anti-Tac binding sites was observed to plateau 5 hr after proteolysis and to actually decline over the succeeding 12 hr. Thus, as a consequence of the 10-fold discrepancy between Tac antigen and IL-2 binding site levels prior to proteolysis, 8–10 hr after enzyme exposure the absolute levels of IL-2 binding sites and Tac antigen are equivalent, provided the cells have not been supplemented with IL-2.

Effect of IL-2 on Expression of IL-2 Receptors and Tac Antigen. In previous studies, IL-2 receptor and Tac antigen expression had been found to be dependent upon lectin or antigen stimulation and proved to be transient in nature: upon removal of the immunostimulatory signals, IL-2 receptor levels decline progressively (6, 8). Consequently, in view of the marked difference between the turnover characteristics for anti-Tac and IL-2 binding sites, especially the slow decay and reappearance of Tac antigen, it became essential to ascertain IL-2 receptor and Tac antigen expression sequentially following removal of the receptor-inducing signal provided by lectin. IL-2 receptor$^+$ T cells harvested after 3 days of culture were washed and replaced in culture with a saturating concentration of immunoaffinity-purified homogeneous IL-2 (500 pM) (10). Cells cultured with IL-2 were maintained in exponential growth at cell concentrations between 1×10^5 and 1×10^6 cells per ml by replacement of IL-2 every 2 days; control cells, cultured without IL-2, were washed every 2 days and the medium was replaced. To quantitate accurately the IL-2 binding sites on cells cultured with IL-2, residual membrane-bound IL-2 was eluted by brief exposure to acid (pH 4.0, 60 sec), a procedure found to remove ≥95% of receptor-associated IL-2. The results of this approach, given in Fig. 2, reveal a striking paradox of

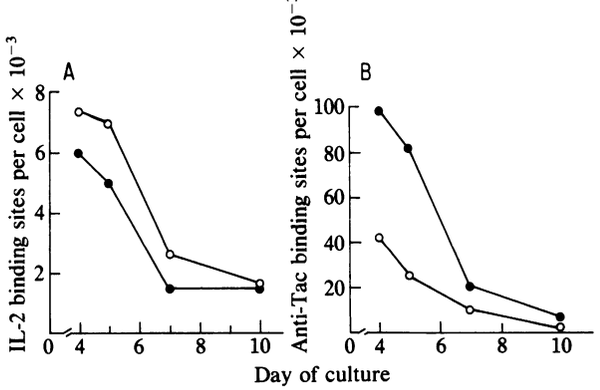

FIG. 2. Effect of IL-2 on the decline of binding sites for IL-2 and for anti-Tac after removal of lectin. PHA-stimulated (72 hr) human PBM cells were harvested, washed, and replaced into culture in fresh medium with receptor-saturating concentrations of affinity-purified IL-2 (500 pM). (*A*) IL-2 binding sites per cell in the presence (●) and absence (○) of exogenous IL-2. (*B*) Anti-Tac binding sites per cell in the presence (●) and absence (○) of exogenous IL-2.

Table 1. Inverse effect of IL-2 on IL-2 receptor vs. Tac antigen expression after PBt_2 stimulation

IL-2	[^{3}H]dThd incorporation, cpm	IL-2 binding sites per cell	Anti-Tac binding sites per cell
Without	527 ± 148	7185 ± 1184	8,934 ± 2437
With	3139 ± 294	5050 ± 472	41,502 ± 2960

Data shown represent the mean ± SEM of six separate experiments after both primary and secondary (11 days) activation of human PBM cells by PBt_2. In three primary stimulation experiments, freshly isolated PBM cells were cultured with 50 ng of PBt_2 per ml in the presence of affinity-purified IL-2 (500 pM) for 72 hr prior to the binding assay. In three secondary stimulation experiments, binding assays were performed after 24 hr of culture with or without IL-2, the time required for maximal receptor expression after a secondary stimulation (6). [^{3}H]dThd data are expressed as cpm per 10^4 cells ± SEM.

IL-2 receptor and Tac antigen expression that was already evident within 24 hr of culture (day 4). Cells cultured in the presence of IL-2 expressed 20% fewer IL-2 binding sites than those deprived of IL-2 (6000 sites per cell vs. 7500 sites per cell) (Fig. 2*A*) but expressed more than twice the density of anti-Tac binding sites compared to cells cultured without IL-2 (96,000 sites per cell vs. 42,500 sites per cell) (Fig. 2*B*). Thus, IL-2–receptor interaction functioned to diminish IL-2 binding sites even as it enhanced the magnitude of Tac antigen expression. Subsequently, in the 6 days of culture after removing the immunostimulatory signal provided by lectin, both IL-2 and anti-Tac binding sites declined progressively, although the cell population exposed to IL-2 lost IL-2 binding sites more rapidly but retained Tac binding sites longer than the cell population cultured without IL-2.

To determine the effect of IL-2 on the acquisition of IL-2 receptors and Tac antigen, lymphocytes were activated with PBt_2 an agent that stimulates IL-2 receptor expression in the absence of IL-2 production (unpublished). This made it feasible to supply IL-2 exogenously; its effect on IL-2 receptor and Tac antigen densities could then be measured at the precise time of maximal receptor expression. As is evident in Table 1, the results of six experiments attest to the magnitude of the difference between Tac antigen density and IL-2 receptor density being attributable to IL-2. Accordingly, in the absence of exogenous IL-2, PBt_2-activated cells incorporated low levels of [^{3}H]dThd but expressed equivalent levels of IL-2 and Tac antigen binding sites. In contrast, cells supplemented with IL-2 proliferated but expressed 30% fewer IL-2 binding sites and 5-fold higher densities of Tac antigen compared to control cell populations.

To investigate the time course and the effect of temperature on the IL-2 augmentation of Tac epitope expression, G_0/G_1 synchronized, IL-2 receptor$^+$ cells were exposed to IL-2 at 4°C and at 37°C. No change in IL-2 or anti-Tac binding occurred within 3 hr at 4°C. Rather, the enhancement of Tac epitope expression required 37°C and became evident slowly over 24 hr (Fig. 3). Thus, as these differences precede the onset of cellular division (6–8), the reciprocal switch in IL-2 receptor expression is not attributable to proliferative changes in the cell population over this time interval. Moreover, an apparent change in Tac epitope and IL-2 binding affinity is also excluded: the equilibrium dissociation constants of both ligands remained unchanged before and after the IL-2 induced switch in IL-2 receptor expression (Fig. 3 *Insets*).

T-Cell Proliferation and IL-2 Receptor vs. Tac Antigen Density. Although previous studies have established that the extent of IL-2-mediated T-cell proliferation was dictated by the affinity and density of IL-2 receptors (5–8), the discovery that IL-2 induces a marked enhancement of the density of

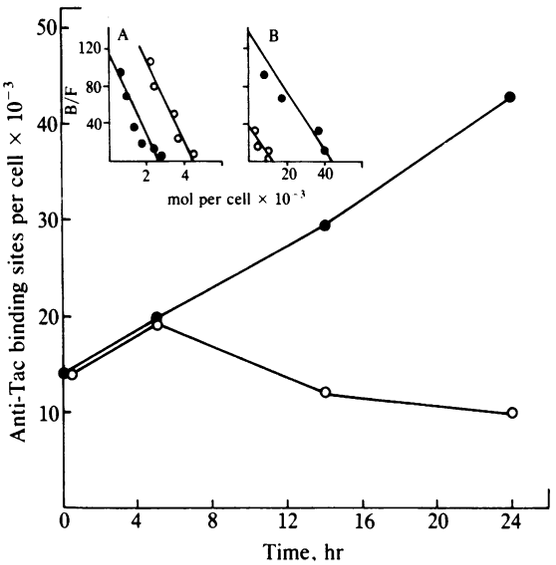

FIG. 3. IL-2 enhancement of Tac epitope and diminution of IL-2 binding site expression. PHA-activated (72 hr) T cells were harvested from IL-2-dependent cell growth after 11 days of culture, washed, and maintained in IL-2-free medium for 24 hr. Re-expression of IL-2 receptors was ensured by exposure to PBt_2 (50 ng/ml, 6 hr). Thereafter, the cells were cultured with or without IL-2 (500 pM) for 24 hr and anti-Tac binding was determined at the times indicated on cells cultured in the presence (●) and absence (○) of IL-2. (*Inset A*) Scatchard plots of [^{3}H]IL-2 binding performed after 24 hr in the presence (●, K_d = 21 pM, 2800 sites per cell) and absence (○, K_d = 23 pM, 4400 sites per cell) of IL-2. (*Inset B*) Scatchard plots of ^{125}I-anti-Tac binding performed after 24 hr in the presence (●, K_d = 0.3 nM, 43,000 sites per cell) and absence (○, K_d = 0.3 nM, 10,000 sites per cell) of IL-2. B/F, bound/free.

Tac epitopes led to a reassessment of the apparent correlation between IL-2 receptor levels and the T-cell proliferative response, particularly in relationship to Tac antigen levels. To compare cell populations with a high and low Tac antigen:IL-2 receptor ratio, IL-2 receptor$^+$ T cells cultured with IL-2 for short (4 days) or prolonged (40 days) periods were subjected to limited proteolysis to remove both IL-2 receptors and Tac antigen. After an 8-hr equilibration period to facilitate recovery and equivalent expression of IL-2 and Tac antigen binding sites, these cell populations were monitored for their IL-2 concentration-dependent proliferative response and compared with untreated cells, which expressed a high Tac antigen:IL-2 receptor ratio. The results, given in Table 2, indicate that a high Tac antigen density on IL-2 receptor$^+$ T cells does not lead to any discernible suppression or enhancement of the IL-2 response, this despite large differences between the Tac antigen:IL-2 receptor ratios of the cell populations. Maximal levels of [^{3}H]dThd incorporation and the IL-2 concentration required for a half-maximal proliferative response proved to be similar, thus corresponding to the equivalent densities of high-affinity IL-2 receptors rather than to Tac antigen levels.

DISCUSSION

The results from the various experimental approaches devised for this investigation all lead to the conclusion that the magnitude of Tac epitope expression is dependent upon the interaction of IL-2 with high-affinity IL-2 binding sites. Consequently, the IL-2-induced variability of Tac antigen:IL-2 receptor ratios, and the absence of correlation between Tac antigen density and IL-2-promoted T-cell proliferation, in effect obviate Tac antigen quantitation as a physiologically pertinent indicator of IL-2 receptor density or IL-2 responsiveness among T-cell populations. For the present, the functional significance of the IL-2-induced expression of the new anti-Tac reactive molecules that do not bind IL-2 with high affinity remains obscure. From the results obtained thus far, a high density of Tac epitopes does not alter the magnitude of the IL-2-mediated proliferative response, provided adequate levels of high-affinity receptors are present. However, it is conceivable that the newly expressed molecules play a role unrelated to T-cell growth and are associated with differentiative aspects of T-cell function. If so, the newly expressed molecules may interact with a separate lymphokine, analogous but not identical to IL-2. In this regard, unpublished results indicate that the IL-2-induced, newly expressed Tac antigen$^+$ molecules can still bind IL-2, albeit with much lower affinity.

The most compelling observation emerging from these studies is the reciprocal effect on the expression of high-affinity, biologically relevant IL-2 binding sites mediated by immunostimulatory signals—e.g., antigen or lectin—and that of IL-2 itself. Triggering of the T-cell antigen–receptor complex results in the expression solely of high-affinity IL-2 binding sites, whereas IL-2 binding to these very same high-affinity sites results in a marked change in the IL-2 receptor molecules expressed. Further studies will determine whether the IL-2-promoted decrease in detectable high-affinity IL-2 binding sites results from the combination of the induction of new molecules that are incapable of binding IL-2 with the same high affinity, in addition to an IL-2-promoted accelerated internalization and degradation of cell surface high-affinity binding sites. However, the marked IL-2-induction of Tac antigen expression indicates that an IL-2-promoted

Table 2. IL-2 proliferative effect on cell populations expressing different Tac antigen:IL-2 receptor ratios

Experiment	Pronase	Tac antigen sites per cell	IL-2 receptor sites per cell	Ratio*	Maximal [^{3}]dThd	IL-2 ED_{50}, pM
1	With	14,465	10,021	1.4:1	7633	14
	Without	64,255	10,052	6.4:1	8677	11
2	With	11,959	14,357	0.8:1	4410	17
	Without	81,340	11,536	7.1:1	5162	20

Data shown represent IL-2 and anti-Tac binding sites per cell, as determined by Scatchard analysis performed on short-term (4 days, experiment 1) and long-term (40 days, experiment 2) cultured cells with and without Pronase treatment and an 8-hr equilibration period. The IL-2 concentration-dependent proliferative responses were determined by [^{3}H]dThd incorporation during the last 4 hr of an 18-hr culture (3×10^4 cells) after subtraction of values obtained in the absence of IL-2. Each cell population gave a typical sigmoid logarithmic dose–response to IL-2; the concentration of IL-2 that yielded 50% of the maximal [^{3}H]dThd incorporation of each population was determined by probit analysis using a homogeneous immunoaffinity-purified standard IL-2 preparation of known protein concentration.

*Tac antigen: IL-2 receptor ratio.

switch to the appearance of new, low-affinity receptor molecules is a previously unrecognized phenomenon that could contribute to the apparent disappearance of detectable high-affinity IL-2 binding sites. In this regard, were it not for the singular nature of the anti-Tac reactivity, the IL-2-induced change in IL-2 receptor expression would have remained undetected; indeed, there would have been no reason to suspect an induction in the expression of different receptor molecules. Thus, the absence of evidence for a similar mechanism operative in other hormone–receptor systems could be due to the lack of application of additional methods of receptor detection other than ligand binding.

It may well be significant that two classes of receptors have been demonstrated in other growth-promoting polypeptide hormone systems, including epidermal growth factor (EGF) (15) and nerve growth factor (16, 17). Since the biologically relevant receptor in both of these systems has been thought to be embodied in the binding site with the highest affinity, studies of receptor turnover and ligand-associated changes in levels of receptor expression have focused exclusively on the high-affinity sites. Consequently, receptor turnover data are not available for the second class of receptors in these systems. Thus, the importance of the T-cell IL-2 hormone–receptor studies resides in the probability that growth factor-mediated reciprocal changes in receptor expression could be a general phenomenon, which is likely to become evident only when both classes of receptors are quantitated after ligand binding. Also, information regarding the mechanism by which the ligand–receptor interaction promotes a switch in receptor expression should be forthcoming from investigations of receptor gene expression. Recent studies have shown that there are two IL-2 receptor mRNA transcripts that arise from one gene (18, 19). Accordingly, all of the present findings are explicable on the basis that only *one* of the two transcripts translates molecules containing a high-affinity IL-2 binding site, whereas *both* transcripts encode receptor molecules that express the Tac epitope. For purposes of comparison, the EGF receptor gene is the only other polypeptide hormone receptor that has been cloned (20). Thus, it is especially germane that normal EGF receptor$^+$ cells also contain one EGF receptor gene, contain two mRNA transcripts, and express two classes of receptors for EGF (15, 20). In both the IL-2 and EGF systems, quantitative binding assays have yet to be performed using cells transfected with cDNAs corresponding to each of the two receptor transcripts. Consequently, the construction of stable, high-expression transfectants now becomes a key requirement for further, more directed analyses of the implied structural and functional relationship of both receptor mRNA transcripts and their translation products.

Regardless of the precise mechanism responsible for the variable nature of IL-2 receptors expressed, the present findings lead to an inescapable conclusion that the intracellular signals generated by triggered antigen receptors and triggered IL-2 receptors function to switch membrane IL-2 receptor expression in a reciprocal fashion. Accordingly, there is a critical interplay between the positive effect of antigen receptor triggering and the negative influence of IL-2 receptor triggering, both harmonizing to regulate precisely the density of high-affinity IL-2 binding sites, and thereby determine the ultimate proliferative potential of activated T cells. The immunologic consequences of such reciprocal regulation are profound; it offers all of the requisite attributes for providing an elegant fail-safe form of intrinsic control over antigen-initiated, but IL-2-dependent, T-cell clonal expansion. Since high-affinity IL-2 receptors are expressed *only* upon antigen receptor activation, and as antigen is cleared progressively *in vivo*, the IL-2 receptor-mediated switch in IL-2 receptor expression inevitably favors the loss of IL-2 binding sites and regression to a resting G_0 cell.

Having perceived the remarkable functional implications of such a regulatory system, it is considered likely that this constitutes a basic means by which hormones regulate the level of their receptors and, in this distinctive fashion, exercise strict control over their own biologic responses.

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