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Immunology

Antigenic stimulation regulates the level of expression of interleukin 2 receptor on human T cells

(T-cell activation)

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ABSTRACT **Antigen-specific, interleukin 2 (IL-2)-dependent human T-cell lines and clones were utilized to study the relationship between IL-2 receptor expression and antigenic stimulation. T cells that had not been exposed to antigen for 2 wk or more expressed a stable low level of the IL-2 receptor. After reexposure to antigen, a 10- to 30-fold increase in the level of the IL-2 receptor was rapidly induced, with the peak level of IL-2 receptor expression occurring at 15–30 hr. This peak preceded the peak in cell proliferation ([^{3}H]thymidine incorporation), which was at 48–72 hr. Within 2–14 days after peak IL-2 receptor expression, it returned to a low base-line level. The transient elevation in IL-2 receptor level was antigen specific because it occurred in response to specific allogeneic stimulator cells but not after exposure to cells expressing irrelevant HLA allotypes. The levels of other cell-surface proteins, including those related to T-cell activation (HLA-DR, T10, 4F2, A-1A5) as well as T3, which has been proposed to be a component of the T-cell receptor complex for antigen, did not change in response to antigen exposure or deprivation. Because IL-2 was maintained at a consistently high level throughout these experiments, the antigen-induced changes in the IL-2 receptor appear to be independent of changes induced by IL-2 itself. Both cloned T cells and mixed populations containing T4 and T8 subsets showed similar IL-2 receptor responsiveness, indicating that this finding is generalizable to most, if not to all, antigen-responsive T cells.**

After interaction with specific antigen and interleukin 1 (IL-1), it is well established that T cells acquire receptors for IL-2, and some responding T cells then produce IL-2 as a nonspecific helper signal for proliferation. These events sequentially trigger the activation and propagation of immunocompetent T cells (1–4). The receptor for IL-2 has been identified and biochemically characterized in humans (5, 6) and mice (7). It is a M_r 55,000 monomer (6, 8) that is expressed along with several other "activation" antigens (9) on T cells in response to lectin or antigen stimulation. Resting T cells acquire the IL-2 receptor as a necessary step prior to DNA synthesis and cell expansion (10, 11). The availability of both cloned T-cell lines and monoclonal antibodies to the IL-2 receptor now make it feasible to analyze whether antigen can induce changes in the expression of the IL-2 receptor or other proteins expressed on antigen-responsive T cells.

MATERIALS AND METHODS

Derivation and Propagation of T-Cell Lines and Clones. Peripheral blood mononuclear cells (PBMC) were obtained from blood bank leukopheresis packs by density centrifugation on Ficoll diatrizoate (Litton Bionetics, Kensington, MD). A normal, responder donor (HLA-A1,A3; B8,B17; Cw6; DR3) was stimulated with irradiated (4,000 R) PBMC from another individual (HLA-A2,A9; B7,B27; Cw2; DR1,2). Six days later, the culture was cloned by limiting dilution on a feeder cell mixture composed of two different irradiated allogeneic PBMC KG (HLA-A3,Aw32; B7,B27; Cw1; DR1,2) or JC (HLA-A2,Aw23; Bw44,B27; Cw2; DR6,7). Cloning was performed in 96-well round-bottom mictotiter plates (Linbro, McLean, VA) in medium composed of RPMI 1640 supplemented with 10 mM Hepes, 2 mM L-glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml) (GIBCO) and 10% heat-inactivated human serum (BioBee, Boston, MA). Cloning medium was also supplemented with conditioned medium containing IL-2 activity. This was prepared by the method of Mier and Gallo (12) with 3-day phytohemagglutinin (PHA-P, Difco)-stimulated PBMC supernatant concentrated and partially purified by 50%, followed by 75% ammonium sulfate precipitations and dialysis in phosphate-buffered saline. Units of IL-2 activity were quantitated as defined by Gillis and Watson (13) by using a bioassay for [*methyl*-^{3}H]thymidine incorporation by the CTL-L2 IL-2-dependent mouse cell line. The cloning medium used contained a final concentration of 2–4 units of IL-2 activity. Positive wells that were seeded at five cells per well were expanded and are referred to as partially cloned lines. Positive wells that had initially been seeded at five cells per well and subsequently were recloned by limiting dilution at less than one cell per well and were phenotypically homogeneous are referred to as clones. All partially cloned lines and clones were expanded in 24-well plates (Linbro) and received cloning medium every 48 hr and irradiated allogeneic stimulator cells KG or JC once a week. These lines and clones have been maintained in culture for 11 months. The partially cloned line 5L10 had cytolytic activity, whereas the T-cell clones 3D4, 2B8, and 3G9 were T4 positive with no cytolytic activity. Uncloned T-cell lines (bulk cultures) were established by using PBMC as described above with stimulation by irradiated Epstein–Barr virus-transformed lymphoblastoid cell lines as described (14).

Tritiated Thymidine Incorporation Assays. Responder T-cell clones (2×10^4 cells per well) were plated alone or with one of several irradiated (4,000 R) allogeneic stimulator PBMC at 1×10^5 cells per well in 96-well, round-bottom microtiter plates (Linbro). Cultures were performed in cloning medium with or without supplemental conditioned medium containing IL-2 activity; 24 hr before termination of culture, 1 μCi of [*methyl*-^{3}H]thymidine (6.7 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) was added to each well. Cultures were harvested on an automatic cell harvester PHD (Cambridge Technology, Cambridge, MA). Results are expressed as net cpm of radioactivity, defined as cpm of stimulated cultures − cpm of irradiated stimulator PBMC. Results shown represent the means of triplicate samples. Standard errors were <20% of means in all cases.

Monoclonal Antibodies (MAbs). The MAb B1.49.9 (6) has the same cell distribution and reacts with the same M_r 55,000

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Abbreviations: MAb, monoclonal antibody; PBMC, peripheral blood mononuclear cells; IL-1 and IL-2, interleukins 1 and 2; FACS, fluorescein-activated cell sorter.

protein as does the MAb anti-TAC. The latter MAb has clearly been shown to react with the IL-2 receptor (5, 8). The MAbs LB3.1 (anti-HLA-DR monomorphic determinant), 4F2 (15, 16), and OKT10 (17, 18) all react with cell-surface proteins found on activated T cells but not on resting T cells. The MAb A-1A5 reacts with a determinant that is both elevated and associated with additional proteins on activated T cells (14). The MAb 0KT3 recognizes a M_r 20,000 protein on 95% of T cells (19, 20).

Analysis by Fluorescence-Activated Cell Sorter (FACS). Cell surface binding by MAb was determined by using fluoresceinated goat anti-mouse $F(ab')_2$, specific for heavy and light chains, and was analyzed on a FACS II (Becton Dickinson). The median fluorescence intensity from a log scale was converted to linear median fluorescence intensity units by using sheep erythrocyte standards as described (21), and fluorescence due to background binding with the control antibody P3 (IgG1) was subtracted. Background binding by other control antibodies of IgG2a and IgG2b subclasses was identical to P3 binding.

RESULTS

During the long-term culture of antigen-stimulated, IL-2 dependent T-cell clones, levels of the IL-2 receptor were noted to vary in response to antigenic stimulation. A T4-positive T-cell clone (3D4), which had been maintained in culture for 11 months, was deprived of stimulator alloantigen for 1, 2, 7, or 22 days and then analyzed by a FACS II for expression of the IL-2 receptor (Fig. 1). One day after exposure to irradiated allogeneic cells, a broad FACS profile corresponding to a high fluorescence intensity was seen (MAb B1.49.9 binding), but after longer intervals (2, 7, and 22 days) after antigen exposure, the fluorescence intensity diminished. At the same time, expression of the T-cell surface antigen T3 did not vary. Results of quantitative analysis of median fluorescence intensities for 3D4 and another T4-positive T-cell clone, 3G9 (Fig. 2), showed that B1.49.9 binding diminished several fold during a 2-wk period and then stabilized at a low level by 3 wks after antigenic stimulation. In contrast, expression of T3 and T10 (an activation antigen on T cells) showed little if any change during the same 3-wk period. Throughout the time period of this experiment, including the unstimulated resting phase, conditioned medium containing IL-2 was added at 48-hr intervals to maintain T-cell viability. Thus, the observed changes in IL-2 receptor expression were independent of a specific effect induced by IL-2 itself.

To test the generality of the results found utilizing T-cell clones in Figs. 1 and 2, further experiments were carried out with heterogeneous T cells composed of both T4- and T8-positive populations. An uncloned T-cell bulk culture (activated in a mixed lymphocyte reaction) and a partially cloned T-cell line (5L10) were deprived of stimulator antigen for various times (Fig. 3). The level of IL-2 receptor expression (B1.49.9 binding) decreased to 1/20th to 1/40th, whereas the T3 antigen and the A-1A5 antigen did not vary. At the end of the antigen deprivation experiment, the uncloned T-cell bulk culture was in week 4 and was approximately 51% T4- and 45% T8-positive, suggesting that both of these subpopulations respond similarly with respect to antigen-induced changes in the IL-2 receptor.

Two weeks after antigen stimulation, when the IL-2 receptor had diminished, another T4-positive T-cell clone 2B8 was restimulated with antigen, and the rapid reappearance of the IL-2 receptor was monitored. From an initial low level, IL-2 receptor expression increased 15- to 20-fold, with peak expression in the interval of 15–30 hr, and then decreased again by 49 hr (Fig. 4). In contrast, the expression of T3, HLA-DR (LB3.1-binding), and the 4F2 antigen did not change markedly. Whereas IL-2 receptor expression diminished 3-fold between days 1 and 2 (Fig. 4*A*), proliferation of clone 2B8 increased 3-fold between days 1 and 2 and peaked between days 2 and 3 (data not shown). Proliferation was measured by [^{3}H]thymidine incorporation, except that cells were pulsed with [^{3}H]thymidine for 8 hr preceding each time point.

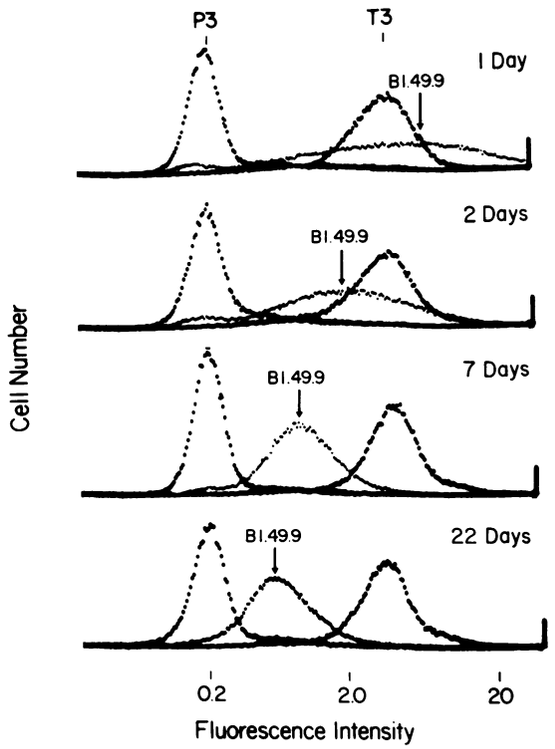

FIG. 1. Change in the level of IL-2 receptor expression on the T-cell clone 3D4 in response to stimulation by target antigen. Different samples of the T-cell clone 3D4 (T4 positive), which had last been stimulated with antigen at 1, 2, 7, or 22 days prior to assay, were all harvested simultaneously for FACS analysis. Fluorescence profiles due to cell surface binding of the MAbs anti-T3 and B1.49.9 (anti-IL-2-receptor) are compared to the binding of the control antibody P3.

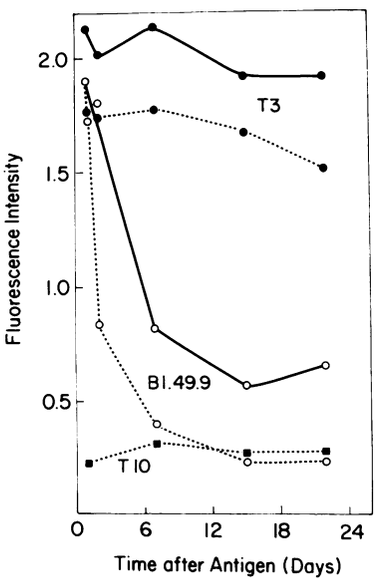

FIG. 2. Quantitation of the changes in IL-2 receptor expression on two different T4-positive T-cell clones during antigen deprivation. T-cell clones 3D4 (----) and 3G9 (—), which had been deprived of antigen for various times, were harvested simultaneously and analyzed by FACS for binding by MAbs anti-T3 (●), B1.49.9 (○), and anti-T10 (■). Quantitation of median fluorescence intensity relative to control P3 binding was carried out as described.

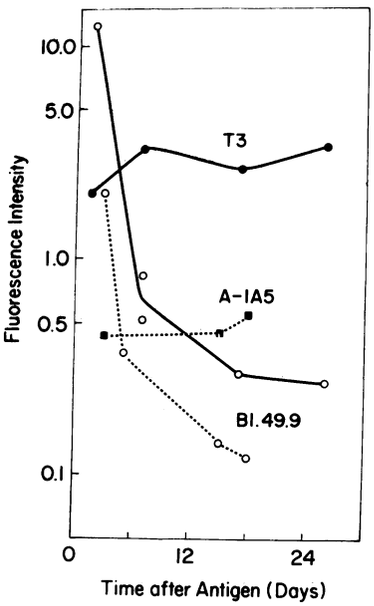

FIG. 3. Quantitation of the changes in IL-2 receptor expression on heterogeneous T-cell populations after antigen deprivation. An alloantigen-stimulated T-cell culture was deprived of stimulator antigen for various times during weeks 2–4 after culture initiation (····). Also, a partially cloned T-cell line (5L10, 90% T4, 10% T8) was deprived of antigen (—), and these cultures were analyzed for cell surface binding by MAbs anti-T3 (●), A-1A5 (■), and B1.49.9 (○). In contrast to Fig. 2, the *y* axis in this figure is a log scale to better accommodate the wide range of median fluorescence intensity values reported for these cells.

To determine if these increases in IL-2 receptor expression were antigen specific, the T4-positive T-cell clone 3D4 was analyzed after stimulation with appropriately and inappropriately irradiated allogeneic cells (JC and KG, respectively). Specific antigen-expressing stimulator cells (JC) caused a major increase in the IL-2 receptor at 17 and 26 hr (Table 1), correlating with antigen-specific induction of cell division ([^{3}H]thymidine uptake; Table 2). However, other irradiated stimulator cells (KG) did not stimulate IL-2 receptor expression nor cell division and had an effect comparable to not adding stimulator cells. Conversely, another T4-positive T-cell clone (2B8) that was responsive to irradiated KG but not irradiated JC did show IL-2 receptor induction (20-fold) and [^{3}H]thymidine uptake corresponding to cell growth in response to KG cells (data not shown).

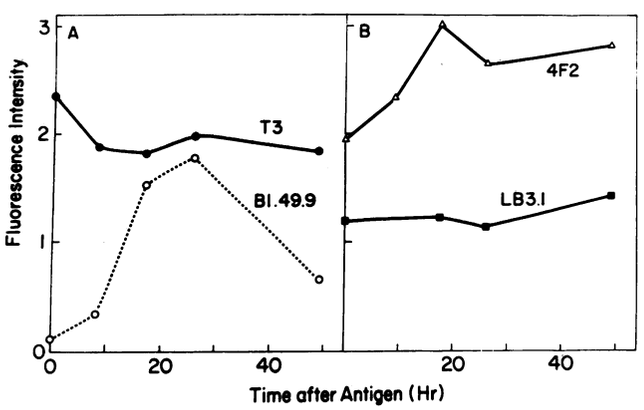

FIG. 4. Increase in IL-2 receptor expression on T-cell clone 2B8 compared to other antigens after reexposure to antigen stimulation. The T4-positive T-cell clone 2B8 was deprived of antigen for 2 wk, and then reexposed to antigen stimulator cells at time 0. At various times thereafter, the cloned T cells were tested for binding by the MAbs T3 (●) and B1.49.9 (○) in *A* and 4F2 (△) and LB3.1 (■) in *B*.

Table 1. Effect of specific and irrelevant antigenic stimulation on IL-2 receptor expression of T-cell clone 3D4

Stimulator*	Time, hr	MFI† OKT3	MFI† B1.49.9
JC	17	1.0	1.47
JC	26	0.7	1.97
KG	17	1.2	0.17
KG	26	1.2	0.16
None	26	1.2	0.13

*Clone 3D4 was stimulated at time 0 in 24-well culture plates with 3 × 10^6 irradiated PBMC JC (HLA-A2,Aw23; B27,Bw44; Cw2; DR6,7) or KG (HLA-A3,Aw32; B7,B27; Cw1;DR1,2) or unstimulated.
†Median fluorescence intensity (MFI) for binding by OKT3 and B1.49.9 was determined as described.

DISCUSSION

These results are consistent with a scheme of antigen-specific T-cell clonal expansion, whereby stimulation with specific antigen induces an increase in the level of IL-2 receptor expression, so that the responding clones can then selectively proliferate to the nonspecific signal IL-2. Thus, the expression of the IL-2 receptor appears to be directly coupled to specific antigen binding to the T-cell receptor. When stimulating antigen is no longer present, activated cells then return to a state of low IL-2 receptor expression. Although these cells are not actively expanding, they presumably need low levels of IL-2 to maintain viability. It would be interesting to determine if the high (antigen stimulated) and low (antigen deprived) levels of IL-2 receptor expression have a correlation with different IL-2 receptors hypothesized to have high and low affinities (8). Although it is assumed that changes in IL-2 receptor expression on T cells are induced by responding T-cell interactions with stimulator antigen, it is notable that T3, which appears to be stoichiometrically associated with the T-cell receptor (22, 23), did not change markedly in response to antigen. This result is different from previous results, which showed that the T3 protein on T-cell clones can be modulated in response to specific antigen stimulation (24) or in response to anti-T3 antibody, which presumably mimics stimulator antigen (24, 25). However, the former study (24) was carried out with high "tolerizing" doses of soluble antigen, and lower doses of antigen may be enough to stimulate T cells without modulating T3.

Mitogenic lectins can trigger lymphocytes to gain the ability to respond to growth factors (including IL-2) (3, 26) and, at the same time, trigger expression of the IL-2 receptor (6,

Table 2. Effect of specific and irrelevant antigenic stimulation on cell proliferation of T-cell clone 3D4

Stimulator*	[^{3}H]Thymidine, net cpm	Exogenous IL-2†
JC	3,980	−
KG	0	−
None	301	−
JC	18,094	+
KG	6,908	+
None	6,731	+

*Clone 3D4 at 2 × 10^4 cells per well was stimulated in 96-well round-bottom microtiter plates with 1 × 10^5 irradiated JC or KG per well or was unstimulated for 72 hr and pulsed with 1 μCi of [^{3}H]thymidine 24 hr prior to harvest.
†[^{3}H]Thymidine incorporation was determined in the presence or absence of exogenous IL-2 containing conditioned media with a final activity of 4 units/ml.

10). The observation that the peak level of IL-2 receptor expression after antigenic stimulation (15–30 hr, Fig. 4) preceded the peak level of T-cell proliferation measured by [^{3}H]thymidine uptake (>48 hr) is consistent with previous results showing that IL-2 receptor expression does not require DNA synthesis (10, 11). However, expression does appear to require protein synthesis (10), although this latter point has been controversial (27). Several other activation antigens also are triggered by mitogens to appear on activated T cells, including HLA-DR (28–31), T10 (17, 18), B1.19.2 (32), the insulin (33) and transferrin (34, 35) receptors, and the 4F2 antigen (15). However, in this study using long-term T-cell clones and lines, alloantigen stimulation influenced the levels of the IL-2 receptor but not HLA-DR, T10 antigen, 4F2 antigen, and A-1A5 antigen (another T-cell activation-related structure (14)). Thus, regulation of IL-2 receptor expression appears to be unique and readily distinguishable from a variety of other antigens that appear on activated T cells. In this regard, an *in vivo* study has shown transient IL-2 receptor expression but more persistent HLA-DR expression on T cells after antigenic challenge (36).

The IL-2 receptor has been shown to be expressed on all or most activated T cells (6), including helper, suppressor, and cytotoxic cells (37). The results in this paper suggest that all or most activated T cells that express the IL-2 receptor undergo similar antigen-induced fluctuations in the amount of that receptor.

This study does not address questions concerning the levels of IL-2 receptor expression on antigen-independent T cells (38) or on IL-2-dependent natural killer cells (39), although it is expected that such cells might show a more constant level of IL-2 receptor expression. In fact, it is possible that antigen-independent expression of the IL-2 receptor may be part of the mechanism that facilitates antigen-independent proliferation. In this regard, continuously growing, antigen-independent human T-cell leukemia virus-infected T-cell lines appear to maintain continuously high levels of IL-2 receptor expression (40).

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