

**Brief Definitive Report****INTERLEUKIN 2 UPREGULATES EXPRESSION OF ITS  
RECEPTOR ON A T CELL CLONE**

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Recent studies (1–3) of polyclonally stimulated human peripheral blood lymphocytes have suggested that, after initial activation of the T cell, interleukin 2 (IL-2) may be necessary for optimal cell surface expression of the IL-2 receptor (IL-2-R). However, since these studies examined heterogenous populations of cells, it was not possible to determine whether the apparent upregulation of IL-2-R expression by IL-2 was the result of occupancy of the receptor by its ligand, or whether it reflected a more complex event that required the interaction of several cell types. In the current study, we have used a murine, untransformed, antigen-specific, class II-restricted T cell clone to analyze the effects of exogenous IL-2, as well as antigen, on the cell surface expression of the IL-2-R, and on the level of IL-2-R cytoplasmic messenger RNA (mRNA).

**Materials and Methods**

*Animals.* B10.A/SgSn (B10.A) mice were obtained from Harlan Sprague Dawley Inc. (Madison, WI).

*Medium.* Culture medium consisted of a 1:1 (vol/vol) mixture of RPMI 1640 (Biofluids, Inc., Rockville, MD) and Eagle's Hank's amino acid medium. This mixture was supplemented with 10% fetal calf serum, 4 mM glutamine, 100 U penicillin, and 50  $\mu$ M 2-mercaptoethanol.

*Monoclonal Antibodies.* Monoclonal antibodies specific for IL-2-R, (7D4) (4); L3T4, (GK1.5) (5); Thy-1, (JIJ) (6); and rat  $\kappa$  light chains (MAR 18.5) (7) have been previously described. Anti-Thy-1.2 was purchased from New England Nuclear (Boston, MA).

*Cell Culture.* The production and growth characteristics of the poly-(L-glutamic acid<sup>60</sup>-L-alanine<sup>30</sup>-L-tyrosine<sup>10</sup>)<sub>n</sub> (GAT)-specific, B10.A-derived, helper T cell clone, 11.4, have been described previously (8). This cell line was maintained by passage every 3–4 d in medium supplemented with unfractionated rat spleen supernatant fluid containing ~20 U/ml IL-2. For experimental cultures, 11.4 T cells ( $0.5$ – $1.0 \times 10^6$ ) were incubated in 24-well, flat-bottomed plates (3524; Costar Data Packaging Corp., Cambridge, MA) containing 1 ml of medium with various concentrations of GAT (Vega Biotechnologies, Inc., Tucson, AZ) and antigen-presenting cells ( $5 \times 10^6$ ) (APC) or IL-2. The APC were irradiated (3,300 rad), anti-Thy-1.2 and complement-treated syngeneic spleen cells (8). The sources of IL-2 were either unfractionated murine IL-2-containing culture fluids (4) supplemented with 10 mg/ml of  $\alpha$ -methylmannoside (Sigma Chemical Co., St. Louis, MO), affinity-purified human IL-2 (9) (kindly provided by R. Robb, E. I. DuPont Co., Glenolden, PA), or recombinant human IL-2 (10) (kindly provided by Cetus Corp., Emeryville, CA). Cytofluorometric analysis of the cultured cells was performed as previously described (4).

*Dot and Northern Blot Analysis.* Cytoplasmic RNA was isolated as previously described (11), and quantitated by spectrophotometric determination at 260 nm. For dot-blot analysis, the RNA was serial-diluted (twofold) with 15 $\times$  SSC (standard sodium citrate; 1 $\times$

SSC is 0.15 M NaCl in 0.015 M sodium citrate), blotted on nitrocellulose with a suction manifold, fixed by baking at 80°C for 1 h, and hybridized to nick-translated insert, purified from either pcEXV-mIL2R8 (12) or TM8 (13). In other experiments, unfractionated RNA was electrophoresed on 1% agarose-formaldehyde gels, transferred to nitrocellulose, and then hybridized to nick-translated insert from pcEXV-mIL2R8.

### Results and Discussion

The 11.4 T cell clone is propagated in medium supplemented solely with IL-2 (i.e. in the absence of APC and antigen) and always bears low but detectable levels of IL-2-R 3–4 d after feeding with IL-2 (see kinetic data below). Since 11.4 proliferates when challenged with either IL-2 or GAT and the appropriate APC (in the absence of exogenous IL-2), it was possible to directly study the effect of exogenous IL-2 and antigen stimulation on IL-2-R expression. Stimulation of 11.4 with either unfractionated murine IL-2-containing supernatants (Fig. 1), purified human IL-2 (Fig. 1), or recombinant human IL-2 (Fig. 2), resulted in a 2–4-fold increase in IL-2-R expression, as judged by the change in the median fluorescent intensity. Since, at the start of culture, the cells bore levels of IL-2-R comparable to that detected 42 h after culture in medium alone, the observed increase in IL-2-R could not simply be due to a decay in IL-2-R expression on unstimulated 11.4 cells relative to stimulated cells, but reflect a true induction of cell surface receptor expression. Furthermore, unlike other IL-2-dependent T cells, such as CTLL or HT2, which rapidly die when cultured without IL-2, the majority of 11.4 survive a 42-h culture in the absence of IL-2 (always >90% viable cells recovered) and retain the capacity to proliferate to exogenous IL-2 or GAT and APC (not shown). Thus, IL-2 is at least sufficient for the induction of the IL-2-R on cells that already bear levels of IL-2-R. We cannot, however, rule out the participation of other soluble products secreted by 11.4 in response to IL-2.

Antigen stimulation of 11.4 resulted in a dose-dependent increase in IL-2-R expression (Fig. 1B). GAT-stimulated 11.4 expressed two to four times more IL-2-R than IL-2-stimulated 11.4. Time course studies (not shown) indicated

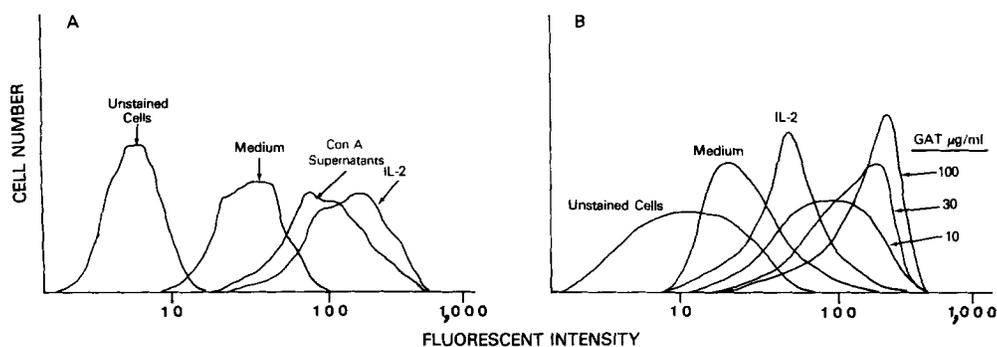


FIGURE 1. IL-2-R expression of 11.4 T cells. 11.4 T cells were cultured with medium alone, mouse concanavalin A (Con A)-supernatant fluids (100 U/ml), purified human IL-2 (200 U/ml), or GAT and B10.A APC, as indicated, for 42 h. Cytofluorometric profiles represent IL-2-R expression of the cultured cells analyzed by staining with fluorescein isothiocyanate (FITC)-7D4. The experiments shown in A and B were performed on different days. The staining was judged to be specific by the capacity of unlabeled 7D4 to inhibit the fluorescent signal of FITC-7D4 to a level comparable to unstained cells (data not shown).

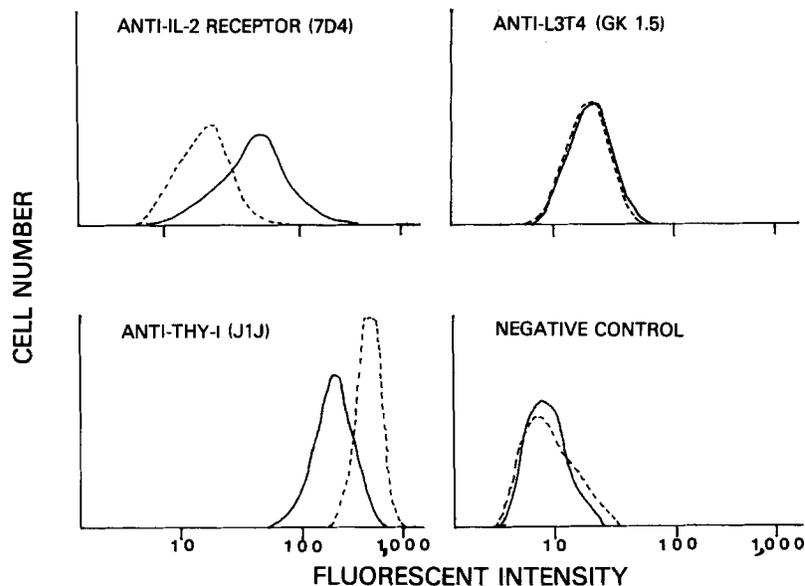


FIGURE 2. Effect of IL-2 on expression of cell surface antigens of 11.4. 11.4 T cells were cultured in medium alone (---), or medium supplemented with 500 U/ml recombinant human IL-2 (—) for 42 h. The cells were incubated with the indicated antibody, washed, then stained with FITC-MAR18.5 followed by cytofluorometric analysis. The negative control was cells stained with only FITC-MAR18.5.

that optimal cell surface IL-2-R levels were seen 30 h after stimulation with either IL-2 or GAT, and by 72 h, IL-2-R expression had decreased to levels comparable to that shown for unstimulated 11.4 (Fig. 1, A and B). The basis for the ability of antigen stimulation to induce more cell-surface IL-2-R than IL-2 stimulation is not known. It is unlikely that the effect of antigen stimulation on IL-2-R expression can be due simply to large amounts of endogenous IL-2 production and subsequent IL-2 upregulation. If this were the case, we would expect that IL-2-R induction by IL-2 would precede induction stimulated by antigen. However, this was not observed in kinetic experiments.

The effect of IL-2 on IL-2-R expression was selective. The addition of recombinant human IL-2 caused no increase in L3T4, and it actually caused a decrease in Thy-1, whereas IL-2-R increased by more than twofold (Fig. 2). Therefore, it is unlikely that the increase observed for IL-2-R can be accounted for simply by a general cell-cycle-dependent fluctuation in expression of cell surface proteins whose levels are maintained during the growth of 11.4.

The increased level of surface IL-2-R after IL-2 stimulation could be due to increased synthesis of this protein, or could reflect other effects on IL-2-R, such as a decrease in turnover, or a redistribution of molecules already present in the cell. To distinguish between these possibilities, IL-2-R cytoplasmic mRNA from 11.4 was quantitated by dot-blot analysis, using a  $^{32}\text{P}$ -labelled complementary DNA probe containing the entire coding region of the mouse IL-2-R. Cell equivalents were not compared when assessing the specific induction of mRNA, since activated cells contained more total RNA than unactivated cells (usually

1.5–2-fold more in the case of 11.4). Instead, we applied identical quantities ( $\mu\text{g}$ ) of RNA from each group to the nitrocellulose, to ensure that any changes observed in mRNA levels were specific increases, not simply the result of the different percentage of cells in each group moving through the cell cycle. Stimulation of 11.4 with purified human IL-2 resulted in a dose-dependent increase in IL-2-R mRNA (Fig. 3A), with maximum IL-2-R mRNA detected 7–24 h after addition of an optimal dose of purified human IL-2 (an increase of about eightfold) (Fig. 3B). In another experiment, IL-2 stimulation increased IL-2-R mRNA  $\sim 32$ -fold, as did antigen stimulation (Fig. 3D), whereas Thy-1 mRNA increased only twofold (Fig. 3C). Thus, unlike the changes detected in cell surface IL-2-R (Fig. 1), the dramatic increase in IL-2 mRNA seen after IL-2 stimulation appeared to be comparable to that seen after antigen stimulation. One possible cause of this dichotomy, an intracellular location for newly synthesized protein, is currently under investigation. The significance of the small increase in Thy-1 mRNA is not clear. It may reflect an increase in synthesis to compensate for the decrease in cell surface expression of Thy-1 (Fig. 2), or the necessity of dividing cells to produce those proteins that must be maintained in the daughter cells. In any case, the large increase in IL-2-R mRNA after IL-2 stimulation suggests that it was not due to a generalized increase, within lymphocytes preparing to divide, of cytoplasmic mRNA encoding surface antigens.

Northern blot analyses confirmed that the poststimulation increase in hybridization of the cDNA probe in the dot blots was in fact due to an increase in IL-2-R mRNA. Each of the five mRNA species that are characteristic for IL-2-R mRNA (14) was easily seen in 11.4 cells after IL-2 stimulation, and each band was substantially increased when compared to RNA from unstimulated 11.4

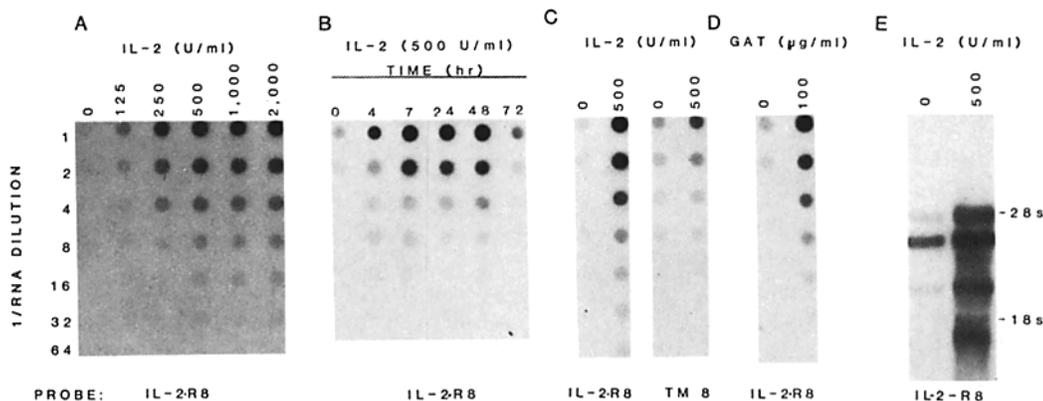


FIGURE 3. Analysis of RNA from 11.4 T cells. Cytoplasmic RNA was isolated from 11.4 cells after culture with the indicated amount of purified human IL-2 or GAT and APC. The first dot in blots (A–D) contained  $2 \mu\text{g}$  of RNA, and the rest represent twofold serial dilutions. Northern blot analysis was performed on  $5 \mu\text{g}$  of cytoplasmic RNA. Ethidium bromide staining of the gel to visualize 28 S and 18 S ribosomal RNA confirmed that each lane contained equivalent amounts of RNA, and that the RNA was not significantly degraded (not shown). The cells in A and C–E were cultured for 24 h. The RNA was hybridized with  $^{32}\text{P}$ -labelled probes, IL-2-R8 (for the IL-2-R) and TM8 (for Thy-1), as indicated. Blots were washed with  $0.2\times$  SSC containing 0.1% sodium dodecyl sulfate at  $60^\circ\text{C}$ . Autoradiographs were exposed for 72 h with an intensifying screen at  $-70^\circ\text{C}$ . The blots shown in A–E represent data from five independent experiments.

cells. These results are consistent with transcriptional control of IL-2-R upregulation by antigen or IL-2. However, it is possible that increased cytoplasmic IL-2-R mRNA in 11.4 is the result of mRNA stabilization, rather than increased transcription. Nuclear RNA runoff experiments must be performed to distinguish between these possibilities.

Our study extends the analysis of polyclonally-stimulated human T cells (1-3) by demonstrating that the induction of the IL-2-R is the result of IL-2-R occupation by IL-2 on a single cell type. We do not know whether IL-2 upregulation of the IL-2-R is a property of all IL-2-R-bearing T cells. However, the results already reported for mitogen-activated human T cells (1-3) suggest that IL-2 regulation of IL-2-R is a property not solely expressed by long-term-cultured T cells.

The ability of IL-2 to upregulate its own receptor may have implications for in vivo immune response. The specificity of the induction, at both the mRNA and protein levels, suggests that the increased levels of IL-2-R play a role in the activation of immune T cells. One might expect, for example, that it would permit antigen-independent amplification of recently antigen-activated T cells. However, such speculations must be made cautiously, in view of the recently reported observation (3) that IL-2 induces only low-affinity IL-2-R on mitogen-activated human peripheral blood lymphocytes. Whether this is also the case for antigen-specific T cells remains to be determined. Thus, the potential immunoregulatory role of IL-2 upregulation of the IL-2-R will require further investigation.

### Summary

Stimulation of a class II-restricted, antigen-specific T cell clone with interleukin 2 (IL-2) resulted in substantial increases in both cell surface IL-2 receptor (IL-2-R) and cytoplasmic IL-2-R messenger RNA (mRNA), whereas no increase was observed for cell-surface expression of Thy-1 and L3T4 antigens, and only a modest increase in Thy-1 mRNA was observed. These experiments demonstrate that, after initial acquisition of the IL-2-R, IL-2 as well as antigen is able to directly upregulate both the level of IL-2-R mRNA and cell surface IL-2-R molecules.

We thank Ms. S. Starnes for preparation of this manuscript; Drs. R. Schwartz, E. Shevach, J. Miller, and R. Germain for critical reading of this manuscript; Dr. T. Chused and Ms. L. Edison for cytofluorometric analysis; and Drs. F. Fitch, R. Robb, and Cetus Corp. for kindly providing reagents, antibodies, and cell lines.

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