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Interleukin 2 receptor gene expression in normal human T lymphocytes

(phorbol ester/phytohemagglutinin/nuclear transcription/nuclease S1)

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ABSTRACT **We have used cDNAs for the human interleukin 2 (IL-2) receptor to study IL-2 receptor gene expression in normal activated T cells. Resting T cells do not contain detectable IL-2 receptor mRNA. Within 1 hr after stimulation with phytohemagglutinin (PHA), a large, presumably nuclear precursor RNA species is seen, which then gradually disappears. Mature IL-2 receptor mRNA forms appear within 8 hr after stimulation, reach peak levels between 8 and 24 hr, and then decline. Thus, in PHA-activated lymphocytes the rise and fall in IL-2 receptor mRNA levels precede by more than 24 hr the peak and decline of IL-2 receptor protein expression occurring at the cell surface. 4β-Phorbol 12-myristate 13-acetate (PMA) also stimulates IL-2 receptor mRNA and protein expression by T cells. Combinations of optimal concentrations of PHA and PMA produce an additive effect on IL-2 receptor mRNA levels, suggesting that PHA and PMA may induce IL-2 receptor gene expression through different, complementary mechanisms. Nuclease S1-protection assays indicate that IL-2 receptor mRNAs may differ in length due to the use of three different polyadenylylation signals. Further, these assays demonstrate the presence of transcripts that lack a 216-base segment within the protein-coding region and thus do not encode a functional IL-2 receptor. Nuclear transcription assays indicate that the increase in IL-2 receptor mRNA is reflected at the level of transcription. Thus, IL-2 receptor gene regulation controls IL-2 receptor expression at the cell surface and is intimately linked to the control of T-cell proliferation.**

Resting T cells, when activated by antigen or mitogen in the presence of interleukin 1, synthesize and secrete interleukin 2 (IL-2, also known as T-cell growth factor) (1, 2). IL-2 is a glycoprotein with apparent M_r 15,500 that exerts growth-promoting effects on activated T cells through binding to high-affinity specific IL-2 receptors (3). IL-2 is not produced by resting T cells, and its synthesis is regulated at least in part at the level of transcription (4–6). Like IL-2, IL-2 receptors are not expressed in resting cells. After activation with phytohemagglutinin (PHA), the number of cell surface receptors peaks within 48–72 hr and then declines (7, 8). The rise and fall in IL-2 receptor expression is paralleled by changes in T-cell proliferation (3, 7–9). Thus, the regulation of both IL-2 and IL-2 receptor gene expression are critically involved in the control of T-cell growth and regulation of the normal immune response.

IL-2 receptors have been characterized as transmembrane glycoproteins with apparent M_r 55,000 (10–13) that may be sulfated (14) and phosphorylated (14, 15). cDNAs encoding the IL-2 receptor have been isolated, expressed, and sequenced (16–18), and the deduced amino acid sequence predicts a mature protein of 251 amino acids. In the present study, we have used these IL-2 receptor cDNAs to analyze the structure and kinetics of IL-2 receptor mRNA accumulation in normal T lymphocytes and to evaluate the role of transcription in the regulation of receptor gene expression.

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MATERIALS AND METHODS

Source and Stimulation of Cells. Peripheral blood lymphocytes were obtained from normal donors by leukapheresis and purified on Ficoll-Hypaque gradients. In some experiments, T cells were purified by E-rosette formation with sheep erythrocytes. Lymphocytes were activated with PHA (Burroughs Wellcome, Research Triangle Park, NC, 1 μg/ml) and cultured for 0, 1, 2, 4, 8, or 24 hr or 2, 3, 6, 9, or 12 days in RPMI 1640 medium supplemented with penicillin, streptomycin, and 10% fetal bovine serum. In some cases, cells were activated with 4β-phorbol 12-myristate 13-acetate (PMA) (Sigma, 50 ng/ml) alone or in combination with PHA.

Isolation, Electrophoretic Fractionation, and Blot Transfer. Total cellular RNA was isolated by extraction in guanidinium thiocyanate and centrifugation of the extracts through cesium chloride gradients as described (19). RNA samples (10 μg per lane) were size-fractionated by electrophoresis in formaldehyde-containing agarose gels and transferred to nitrocellulose as described (20).

cDNA Probes. The IL-2 receptor cDNAs pIL2R2 and pIL2R4 were used in combination in these studies. Together these cDNAs constitute a full-length IL-2 receptor probe lacking the repetitive *Alu* sequences that are present within the 3′ untranslated region of pIL2R3. The nucleotide sequences of pIL2R3 and pIL2R4 have been published (16). The cDNA insert of pIL2R2 corresponds to the first 937 bases of the sequence of pIL2R3. The cDNA encoding the β chain of the human T-cell antigen receptor was the gift of T. Mak (University of Toronto). The HLA-B7 cDNA was the gift of S. M. Weissman (Yale University). cDNA probes were labeled with ^{32}P by the random-priming method of Feinberg and Vogelstein (21).

Hybridization of DNA and RNA. Hybridizations with ^{32}P-labeled IL-2 receptor cDNAs (100,000–500,000 cpm/ml) were at 42°C for 16 hr in 40% (vol/vol) formamide/0.6 M NaCl/0.06 M sodium citrate, pH 7.0/0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin/salmon sperm DNA (100 μg/ml)/10% (wt/vol) dextran sulfate. Nitrocellulose filters were washed in 0.3 M NaCl/0.03 *M* sodium citrate, pH 7.0/0.1% $NaDodSO_4$ three times for 20 min at room temperature and then in 15 mM NaCl/1.5 mM sodium citrate, pH 7.0/0.1% $NaDodSO_4$ twice for 20 min at 52°C.

Nuclease S1 Protection Assays. Appropriate cDNA fragments were subcloned in M13 bacteriophage, and radiola-

Abbreviations: PHA, phytohemagglutinin; PMA, 4β-phorbol 12-myristate 13-acetate; IL-2, interleukin 2.

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beled single-stranded DNA complementary to IL-2 receptor mRNA was synthesized using the 15-base M13 primer (P-L Biochemicals) and the large fragment of DNA polymerase I (New England Biolabs) in the presence of [α-^{32}P]dATP or [α-^{32}P]dCTP (Amersham, 400 or 3000 Ci/mmol; 1 Ci = 37 GBq). The DNA was digested with enzyme(s) appropriate to generate the desired radiolabeled probe, denatured, and electrophoresed in a 6% acrylamide/8 M urea gel in 133 mM Tris/80 mM boric acid/2.8 mM Na_2EDTA). The radiolabeled DNA fragment was recovered by electroelution, extracted with phenol/chloroform, and precipitated with ethanol. The purified DNA was denatured in 70% formamide, hybridized to mRNA from resting or activated E-rosette-positive cells for 16 hr at 50°C in a reaction volume of 50 μl (10^6 cpm/ml), and digested with nuclease S1 (5000 units, Boehringer Mannheim) for 30 min at 37°C. The reaction mixture was then extracted with phenol/chloroform, and the nucleic acids were ethanol-precipitated, denatured in formamide at 70°C, and electrophoresed in an acrylamide/urea gel as described above.

***In Vitro* Transcription Studies.** Nuclei were isolated from peripheral blood lymphocytes stimulated with PHA for varying periods of time, according to the method of McKnight and Palmiter (22) as modified by Krönke *et al.* (4). Nuclei ($4–5 \times 10^7$) were suspended in 200 μl of 100 mM KCl/4.5 mM $MgCl_2$/0.4 mM ATP/0.4 mM CTP/0.4 mM GTP/2 mM dithiothreitol/[^{32}P]UTP (0.2 mCi) and incubated for 30 min at 26°C. The reaction mixture was then treated with DNase I and proteinase K, and nuclear RNA was isolated by phenol/chloroform extraction and ethanol-precipitation as described (4). This ^{32}P-labeled nuclear RNA was hybridized for 36 hr to an excess of specific cDNA immobilized on nitrocellulose filters; the filters then were washed and autoradiographed. Hybridization cpm were determined by liquid scintillation spectrometry. Specific hybridization cpm were calculated by subtracting cpm associated with filters to which pBR322 was immobilized. This difference was divided by input cpm in ^{32}P-labeled RNA to determine specific relative transcription (ppm) for the IL-2 receptor and *HLA* genes.

RESULTS

Total cellular RNA was isolated from peripheral blood lymphocytes that had been activated with PHA for 0–12 days. Ten micrograms of each RNA sample was electrophoresed in a formaldehyde/agarose gel, transferred to nitrocellulose, and hybridized with ^{32}P-labeled IL-2 receptor cDNAs. As shown in Fig. 1, within 8 hr of PHA stimulation, both the 3500- and 1500-base families of IL-2 receptor mRNA were detected (corresponding to 25 S and 16 S, respectively). These levels peaked between 8 and 24 hr and then declined. In contrast, cell surface IL-2 receptor protein expression peaks between 48 and 72 hr after stimulation with PHA and then declines (7). Thus, the peak and decline in IL-2 receptor mRNA levels precede those of cell surface IL-2 receptor expression by more than 24 hr. At time points as early as 1 hr, an additional, larger mRNA species was detected. We hypothesize that this may represent a nuclear precursor form of IL-2 receptor mRNA, as levels of this species declined as the mature forms appeared.

Since total cellular RNA rather than mRNA was used in the blot in Fig. 1, it was possible that the differences in IL-2 receptor mRNA levels merely reflected an induction of all mRNA species in the cells in response to PHA activation, rather than a more specific increase in IL-2 receptor mRNA levels. However, the changes in IL-2 receptor mRNA levels were not paralleled by those in mRNA encoding the T-cell antigen receptor β chain (data not shown).

The 3500-base mRNA species differ from the 1500-base forms because of utilization of a more 3′ polyadenylylation signal sequence (ref. 16; see below for detailed nuclease S1-protection assays). As shown in Fig. 1, activation of cells with PHA alone resulted in preferential expression of the 3500-base mRNA form(s). In contrast, stimulation with both PHA and PMA resulted in more equal accumulation of the 1500- and 3500-base mRNA forms. To further evaluate this observation, we prepared RNA from peripheral blood resting non-T cells, resting T cells, and T cells activated for 24 hr with PHA, PMA, or PHA plus PMA. These RNAs were then size-fractionated in formaldehyde/agarose gels, transferred to nitrocellulose, and hybridized with ^{32}P-labeled IL-2 receptor cDNAs. As shown in Fig. 2, either PHA or PMA alone appeared to stimulate preferentially the accumulation of the 3500-base mRNAs, but cells stimulated with both signals expressed nearly equivalent amounts of the 3500- and the 1500-base mRNA classses. Thus, it is possible that PHA and PMA stimulate IL-2 receptor expression through complementary mechanisms. We have also noted a predominance of

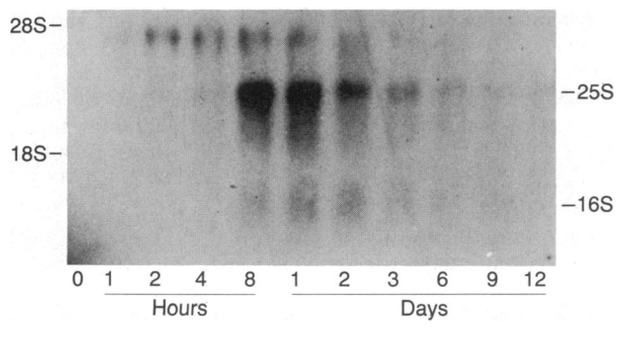

FIG. 1. Time course of IL-2 receptor mRNA accumulation in PHA-stimulated peripheral blood lymphocytes. RNA was isolated from peripheral blood lymphocytes stimulated with PHA for the indicated time, electrophoresed in formaldehyde/agarose gels, and transferred to nitrocellulose paper, and the blot was hybridized with ^{32}P-labeled pIL2R2 plus pIL2R4. Positions of ribosomal RNA markers are indicated at left.

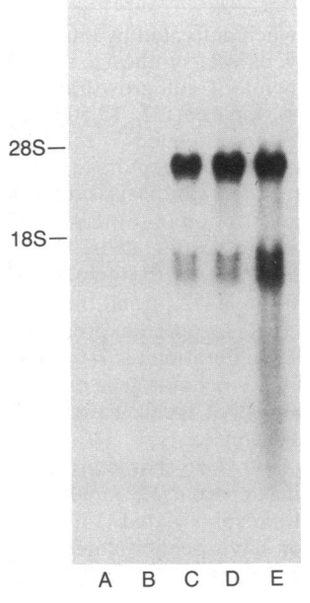

FIG. 2. IL-2 receptor mRNA expression in resting and activated T cells. RNAs from resting peripheral blood non-T (lane A) or T cells (lane B) or from T cells activated with PHA (lane C), PMA (lane D), or PHA plus PMA (lane E) were electrophoresed in formaldehyde/agarose gels, transferred to nitrocellulose, and hybridized with ^{32}P-labeled pIL2R2 plus pIL2R4.

the 3500-base form(s) in other cell populations expressing low levels of IL-2 receptors (including PMA-induced Jurkat and HSB-2 acute lymphocytic leukemia cell lines and hairy-cell leukemia cells), suggesting that the 1500-base mRNAs may vary with the magnitude of IL-2 receptor expression.

Although blot hybridization had indicated two major size classes of mRNA, the nucleotide sequence of a full-length IL-2 receptor cDNA suggested that there might be three distinct polyadenylylation signals. Furthermore, the blot in Fig. 2 appeared to resolve the 1500-base mRNA into at least two species, consistent with this hypothesis. We used nuclease S1-protection assays to determine whether all potential polyadenylylation signals were used. We directionally subcloned the insert of pIL2R3 (16) into the polylinker of M13mp11, synthesized sense-strand DNA homogeneously labeled with ^{32}P, and purified the *Bgl* I–*Eco*RI fragment corresponding to bases 785–2335 of pIL2R3. This fragment overlapped the two putative proximal polyadenylylation signal sequences (ATTAAA at bases 1298–1303 and AATAAA at bases 1523–1528), which are 3′ to the termination codon. Following hybridization to mRNA from resting or activated T cells, nuclease S1-protection assays were performed. Two bands, corresponding to mRNAs utilizing both proximal predicted polyadenylylation signals, and a third band (the length of the *Bgl* I–*Eco*RI fragment), corresponding to mRNA species using more distal polyadenylylation site(s), were identified (Fig. 3). Thus, at least three different polyadenylylation signals are utilized.

In the cloning of IL-2 receptor cDNAs from HUT-102B2 cells, which are infected with human T-cell lymphotropic virus type I, two clones (16, 18) were identified in which an internal 216-base-pair segment had been removed. When transfected into COS-1 cells (16) or L cells (26), this spliced cDNA did not direct the synthesis of functional IL-2 receptors. Nuclease S1-protection assays were used (*i*) to confirm that the spliced cDNAs corresponded to mRNAs and did not simply represent cloning artifacts; (*ii*) to evaluate whether such alternatively spliced mRNAs existed in normal activated T cells in addition to HUT-102 cells; and (*iii*) to study potential differences in splicing as a mechanism of IL-2 receptor regulation. For these experiments, the 5′ *Eco*RI–*Nae* I fragment of pIL2R3, corresponding to bases 1–910 of the published pIL2R3 sequence (16) was subcloned in M13mp10. As shown in Fig. 4, three fragments, approximately 910, 550, and 155 bases long, were generated by nuclease S1 digestion. The 910-base fragment corresponded to functional IL-2 receptor mRNA, whereas the other two fragments resulted from the presence of alternatively spliced IL-2 receptor mRNA in normal activated T cells. The alternatively spliced mRNA appeared to be less prevalent

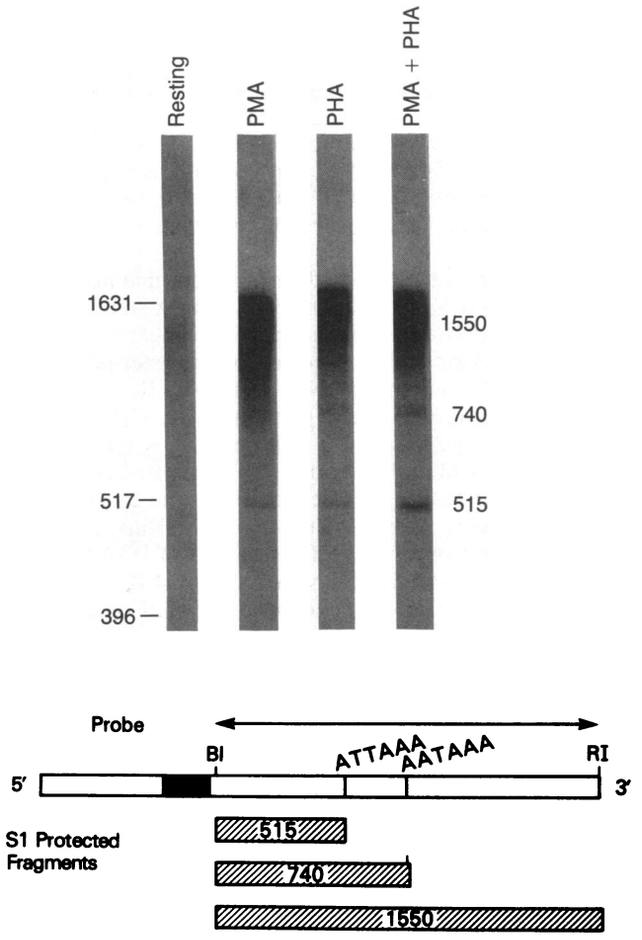

FIG. 3. IL-2 receptor mRNAs may utilize three different polyadenylylation signal sequences. Nuclease S1-protection assays using the *Bgl* I–*Eco*RI fragment of pIL2R3 and RNA from resting T cells or T cells activated with PMA, PHA, or PMA plus PHA were performed as described in *Materials and Methods*. Markers at left represent positions of fragments generated by digesting pBR322 with *Hin*fI. The 515-, 740- and 1550-base protected fragments correspond to the use of three different polyadenylylation signals, as illustrated in the diagram below the autoradiographs. Restriction sites: Bl, *Bgl*I; RI, *Eco*RI.

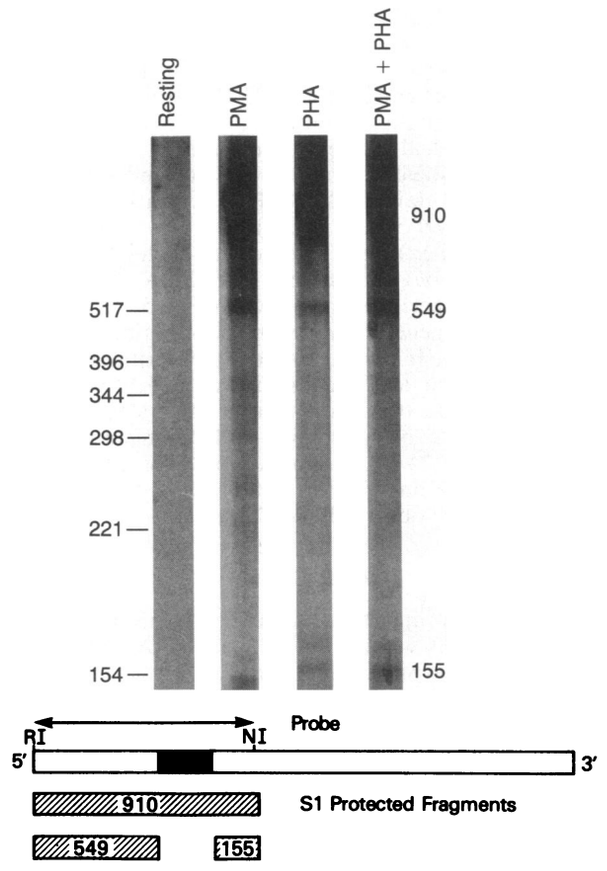

FIG. 4. IL-2 receptor mRNAs may either retain or not retain a 216-base spliceable segment. Nuclease S1-protection assays using the *Eco*RI–*Nae* I fragment of pIL2R3 and RNA from resting T cells or T cells activated with PMA, PHA, or PMA plus PHA were performed. Markers at left represent positions of fragments generated by digesting pBR322 with *Hin*fI. The 549- and 155-base protected fragments correspond to mRNA from which a 216-base segment has been removed. The 910-base mRNA corresponds to mRNA that retains this segment. RI, *Eco*RI; NI, *Nae* I.

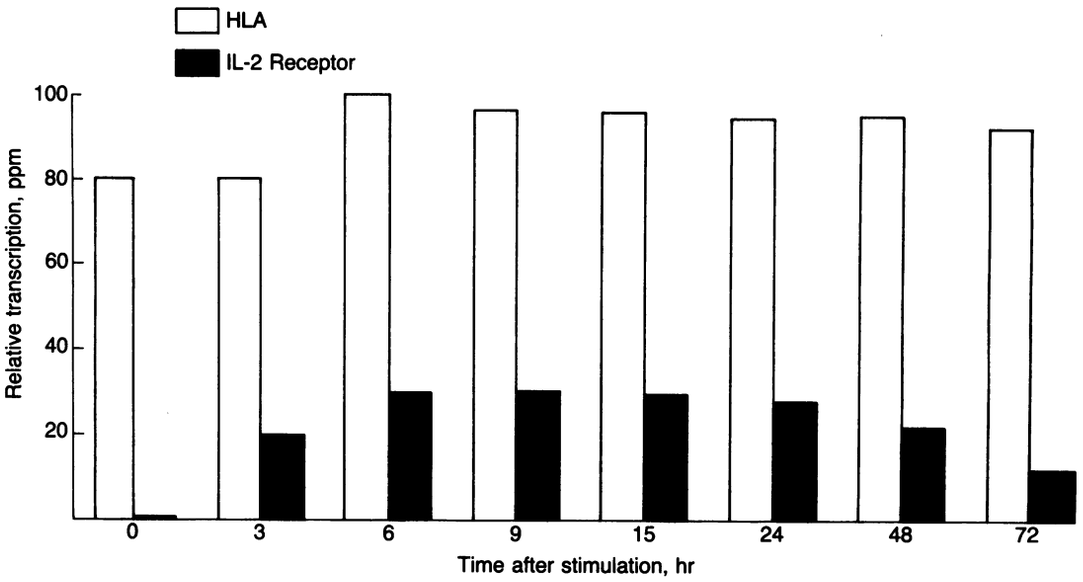

FIG. 5. Nuclear transcription studies of IL-2 receptor and *HLA-B7* gene transcription. The IL-2 receptor gene is actively transcribed within 3 hr of PHA activation; transcription peaks after 15–24 hr and then declines. *HLA-B7* gene transcription is constitutive and is minimally augmented by PHA activation.

than the functional IL-2 receptor mRNA. We have found no evidence that this alternative splicing event is involved in regulation of IL-2 receptor expression. Specifically, the relative amounts of alternatively spliced IL-2 receptor-associated mRNAs and functional IL-2 receptor mRNAs did not change in long-term-cultured T cells in which IL-2 receptor number had markedly declined.

We next investigated whether the rise and fall in IL-2 receptor mRNA levels after PHA activation reflected changes in the level of gene transcription. *In vitro* transcription assays were performed with isolated nuclei from peripheral blood lymphocytes activated with PHA. Results of a representative experiment are shown in Fig. 5. As anticipated, the *HLA-B7* gene was constantly transcribed at a high level. In contrast, IL-2 receptor-gene transcription was not detected in unstimulated T cells but was evident within 3 hr of PHA activation (the earliest time point studied). Peak transcription was rapidly achieved and then maintained at a plateau level for 24–48 hr. In two of three experiments, decreases of 30% and 50% in relative IL-2 receptor gene transcription were observed after 72 hr.

DISCUSSION

We have demonstrated that IL-2 receptor mRNAs achieve high cellular levels within 8 hr following PHA activation of peripheral blood lymphocytes. Further, we have provided evidence consistent with the presence of a larger nuclear precursor form of receptor mRNA as early as 1 hr after stimulation. Isolated nuclei from unstimulated T cells did not transcribe IL-2 receptor mRNA; however, nuclei from cells stimulated with PHA for as little as 3 hr actively transcribed the IL-2 receptor gene. These data show that IL-2 receptor gene expression is dynamic and regulated, at least in part, at the level of transcription. Our data suggest that the subsequent decline in IL-2 receptor mRNA levels occurs more rapidly than changes in transcription rates; thus changes in the rates of mRNA processing and degradation may also be important to the regulation of IL-2 receptor expression. Fig. 6 summarizes the time course of IL-2 receptor gene transcription, IL-2 receptor mRNA levels achieved, and IL-2 receptor protein expression, indicating the natural progression of transcription of the gene and translation of the mRNA. These data underscore the tightly regulated nature of IL-2 receptor gene expression, which is intimately linked with T-cell proliferation. The reason for the decline in IL-2 receptor gene transcription is unknown. One possible explanation is that the transcription is under control of a repressor whose synthesis or function is augmented following T-cell activation.

We have no direct data regarding variations that may occur in the rates of mRNA processing or in mRNA stability. It is quite possible that alterations in these parameters may also serve as important control mechanisms in determining the levels of IL-2 receptor mRNA. Whether IL-2 receptor expression is also controlled in part at the level of translation or posttranslationally remains unknown. It is conceivable that sequences within the various 3′ untranslated regions of the different mRNAs are critical to either the efficiency of mRNA processing, stability, or translation. Thus, it is possible that expression of the different forms of IL-2 receptor mRNA may serve as another level of control of IL-2 receptor expression (see ref. 23 for general discussion of different levels of control of gene expression in eukaryotic cells).

Leonard *et al.* (16) observed that both human T-cell lymphotropic virus type I-infected HUT-102B2 cells and

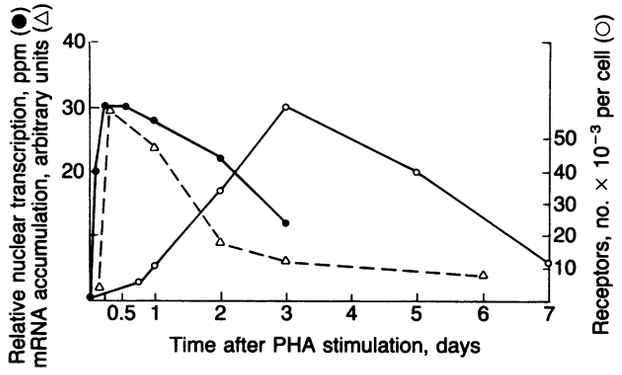

FIG. 6. Time-course summary of IL-2 receptor gene transcription, IL-2 receptor mRNA accumulation, and IL-2 receptor protein expression in PHA-activated T lymphocytes.

PHA-plus-PMA-stimulated peripheral blood lymphocytes contained similar levels of both 3500- and 1500-base classes of IL-2 receptor mRNA. In contrast, in PMA-activated HSB-2 acute lymphocytic leukemic cells, which express considerably fewer IL-2 receptors (24), preferential accumulation of the 3500-base forms was detected. In the present study, we found that in normal T cells, stimulation with either PMA or PHA alone results in the preferential accumulation of the 3500-base forms. However, when combined, these reagents appear to be additive in terms of their stimulatory effect on accumulation of the 1500-base species. Thus, we suggest that PHA and PMA may stimulate the IL-2 receptor gene through different complementary mechanisms and that full IL-2 receptor gene expression may not be achieved with either reagent alone. These findings are also supported in ^{3}H-labeled anti-Tac binding assays measuring surface receptor expression (unpublished observations).

We present evidence that three different polyadenylylation signals may be utilized in the processing of IL-2 receptor mRNAs. In addition, we demonstrate that an alternatively spliced form of IL-2 receptor mRNA, which lacks a 216-base segment within the protein-coding region, exists in normal activated T cells and that previously identified cDNAs corresponding to this mRNA do not represent cloning artifacts. We have obtained no evidence that IL-2 receptor expression is regulated via changes in the relative quantities of normally spliced versus alternatively spliced IL-2 receptor mRNA. Thus far, no protein product has been identified that corresponds to the spliced form of the IL-2 receptor mRNA.

Previous studies have shown the presence of both high- and low-affinity IL-2 receptors (25). The high-affinity receptors appear to mediate all the mitogenic effects of IL-2 (3, 25), whereas the biologic function of the low-affinity receptors remains unknown. We have not excluded the possibility that different mRNA forms may preferentially give rise to high- or low-affinity IL-2 receptors. However, we believe it more likely that the determination of high- versus low-affinity IL-2 receptors relates solely to posttranslational events.

Thus, regulation of the IL-2 receptor gene occurs at least in part at the level of transcription. In addition, the use of the multiple polyadenylylation signals and alternative mRNA splicing may also contribute to the control of IL-2 receptor expression.

1. Morgan, D. A., Ruscetti, F. W. & Gallo, R. C. (1976) *Science* **193,** 1007–1008.
2. Smith, K. A. (1980) *Immunol. Rev.* **51,** 337–351.
3. Robb, R. J., Munck, A. & Smith, K. A. (1981) *J. Exp. Med.* **154,** 1455–1474.
4. Krönke, M., Leonard, W. J., Depper, J. M., Arya, S. K., Wong-Staal, F., Gallo, R. C., Waldmann, T. A. & Greene, W. C. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5214–5218.
5. Arya, S. K. & Gallo, R. C. (1984) *Biochemistry* **23,** 6685–6690.
6. Krönke, M., Leonard, W. J., Depper, J. M. & Greene, W. C. (1985) *J. Exp. Med.* **161,** 1593–1598.
7. Cantrell, D. A. & Smith, K. A. (1983) *J. Exp. Med.* **158,** 1895–1911.
8. Depper, J. M., Leonard, W. J., Krönke, M., Noguchi, P. D., Cunningham, R. E., Waldmann, T. A. & Greene, W. C. (1984) *J. Immunol.* **133,** 3054–3061.
9. Robb, R. J. (1984) *Immunol. Today* **5,** 203–209.
10. Leonard, W. J. Depper, J. M., Uchiyama, T., Smith, K. A., Waldmann, T. A. & Greene, W. C. (1982) *Nature (London)* **300,** 267–269.
11. Leonard, W. J., Depper, J. M., Robb, R. J., Waldmann, T. A. & Greene, W. C. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6957–6961.
12. Wano, Y., Uchiyama, T., Fukui, K., Maeda, M., Uchino, H. & Yodoi, J. (1984) *J. Immunol.* **132,** 3005–3010.
13. Urdal, D. A., March, C. J., Gillis, S., Larsen, A. & Dower, S. K. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6481–6485.
14. Leonard, W. J., Depper, J. M., Krönke, M., Robb, R. J., Waldmann, T. A. & Greene, W. C. (1985) *J. Biol. Chem.* **260,** 1872–1880.
15. Shackleford, D. A. & Trowbridge, I. S. (1984) *J. Biol. Chem.* **259,** 11706–11712.
16. Leonard, W. J., Depper, J. M., Crabtree, G. R., Rudikoff, S., Pumphrey, J., Robb, R. J., Krönke, M., Svetlik, P. B., Peffer, N. J., Waldmann, T. A. & Greene, W. C. (1984) *Nature (London)* **312,** 626–631.
17. Nikaido, T., Shimizu, A., Ishida, N., Sabe, H., Teshigawara, K., Maeda, M., Uchiyama, T., Yodoi, J. & Honjo, T. (1984) *Nature (London)* **312,** 631–635.
18. Cosman, D., Cerretti, D. P., Larsen, A., Park, L., March, C., Dower, S., Gillis, S. & Urdal, D. (1984) *Nature (London)* **312,** 768–771.
19. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 202–203.
21. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
22. McKnight, G. S.. & Palmiter, R. D. (1979) *J. Biol. Chem.* **254,** 9050–9058.
23. Darnell, J. E. (1982) *Nature (London)* **297,** 365–371.
24. Greene, W. C., Robb, R. J., Depper, J. M., Leonard, W. J., Drogula, C., Svetlik, P. B., Wong-Staal, F., Gallo, R. C. & Waldmann, T. A. (1984) *J. Immunol.* **131,** 1042–1047.
25. Robb, R. J., Greene, W. C. & Rusk, C. M. (1984) *J. Exp. Med.* **160,** 1126–1146.
26. Greene, W. C., Robb, R. J., Svetlik, P. B., Rusk, C. M., Depper, J. M. & Leonard, W. J. (1985) *J. Exp. Med.* **162,** 363–368.