3. Sperm was collected by centrifugation and then washed three times in PBS (*p*H 7.2). A drop of sperm suspension, untreated or treated with Triton X-100 [T. C. Rodman *et al.*, *Gamete Res.* **8**, 129 (1983)], was placed on a slide, air-dired, and prepared (unfixed) for indirect immunofluorescence study with human serum.
4. The fluorescein isothiocyanate (FITC)–conjugated antibodies to human Ig's were selected to provide evidence of specific antibody recognition: $F(ab')_2$ goat antibody to human IgM (μ chain–specific); $F(ab')_2$ goat antibody to human IgG (Fc fragment–specific); and affinity-purified goat antibody to human serum IgA (α chain–specific). Use of $F(ab')_2$ fragments as the fluoresceinated probe eliminates the possibility of nonspecific reactivity of the probe with Fc receptors on sperm.
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6. A fraction of sperm heads, cleanly separated from tails, was treated with Triton X-100 without mechanical disruption of the heads (*1*). The proteins that were soluble in Triton X-100 were recovered in PBS (*p*H 7.2) as described [P. W. Holloway, *Anal. Biochem.* **53**, 304 (1973)] and subjected to electrophoresis on a sodium dodecyl sulfate gel. Blots of the separated proteins were tested with human sera and peroxidase-conjugated (goat) antibody to human IgM or IgG.
7. Total concentrations of IgM in six control sera (Table 1) were 55 to 103 mg per 100 ml and in four antibody-negative sera from patients at risk for AIDS (Table 1) were 39 to 99 mg per 100 ml. The IgM titer for the pediatric serum (Fig. 1B), which showed reactivity with sperm head proteins, was 55 mg per 100 ml; that for the AIDS serum (Fig. 1E), which showed no sperm head immunofluorescence, was 69 mg per 100 ml. Similarly, the IgM titer for the pediatric serum (Fig. 2, lane d) was 83 mg per 100 ml and that for the AIDS serum (Fig. 2, lane f and Fig. 3B) was 88 mg per 100 ml.
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Deregulation of Interleukin-2 Receptor Gene Expression in HTLV-I–Induced Adult T-Cell Leukemia

Abstract. *Infection of human T cells by human T-lymphotropic virus, type I (HTLV-I), a retrovirus, is uniformly associated with the constitutive expression of large numbers of cellular receptors for interleukin-2 (IL-2). Comparison with normal T cells shows that neither IL-2 receptor gene organization nor IL-2 receptor messenger RNA processing are altered in the leukemic cells. However, mitogenic stimuli activate IL-2 receptor gene expression in normal T cells, whereas these stimuli paradoxically inhibit IL-2 receptor gene transcription in HTLV-I–infected leukemic T cells.*

The type C retrovirus, human T-lymphotropic virus, type I (HTLV-I), has been identified as the etiologic agent in adult T-cell leukemia (ATL) (*1*). HTLV-I infection of human T cells is uniformly associated with expression of large numbers of cellular receptors for interleukin-2 (IL-2) (*2*, *3*). Together, IL-2 and its cellular receptor play an essential role in the control of normal T-cell growth (*4*). The relation of IL-2 receptor expression to HTLV-I infection is still unexplained. However, since most ATL cell lines do not transcribe IL-2 messenger RNA (mRNA) nor secrete IL-2, an autocrine growth model based on the continuous interaction of IL-2 with its receptor is unlikely (*5*). Furthermore, there is evidence that the IL-2 receptor is not the cellular receptor mediating entry of the HTLV-I virus (*6*). The finding that HTLV-I is not integrated at unique sites within the human genome argues against IL-2 receptor gene activation by adjacent insertion of HTLV-I promoter-enhancer sequences (*7*). We (*8*) and others (*9*) have recently isolated complementary DNA's (cDNA's) encoding the human IL-2 receptor. Using these IL-2 receptor cDNA probes, we have studied the deregulated expression of the IL-2 receptor gene in HTLV-I–infected T lymphocytes.

To investigate the possibility that IL-2 receptor expression reflects constitutive synthesis of IL-2 receptor mRNA, we used ^{32}P-labeled IL-2 receptor cDNA to analyze total cellular RNA from five ATL cell lines by Northern blotting. Each of these ATL lines constitutively expressed IL-2 receptor mRNA species similar in size to those present in mitogen-activated normal T cells (Fig. 1).

In an attempt to detect subtle differences between IL-2 receptor mRNA species from ATL cells and those from normal T cells, which might not have been evident in the Northern blotting analyses, we performed S1 nuclease protection studies with IL-2 receptor mRNA obtained from normal T cells and ATL cells (Fig. 2). As reported earlier (*8*), the formation of mature IL-2 receptor mRNA involves extensive post-transcriptional processing, including alternate splicing and the use of at least two, and probably three, separate polyadenylation [poly(A)] sites. In a first set of experiments, we used the Eco RI–Nae I cDNA fragment of pIL-2R3, corresponding to 910 base pairs (bp) at the 5′ end of the published sequence of pIL-2R3 (*8*). This fragment contains an internal 216-bp segment that may be removed by alternate splicing (*8*, *9*). Each of the ATL cell lines, like normal activated T cells, expressed both spliced and unspliced forms of IL-2 receptor mRNA's (Fig. 2). The spliced mRNA was detected in the S1 nuclease protection assay by identifying two fragments of sizes 549 bp and 155 bp, indicating the lack of protection within the 216-bp segment of the labeled Eco RI–Nae I fragment. The unspliced mRNA species is translated into an IL-2 binding receptor (*8*), but the function and the protein product encoded by the alternately spliced mRNA species are still undefined.

In a second series of S1 nuclease experiments, the 3′ Bgl I–Eco RI fragment of pIL-2R3 corresponding to base pairs

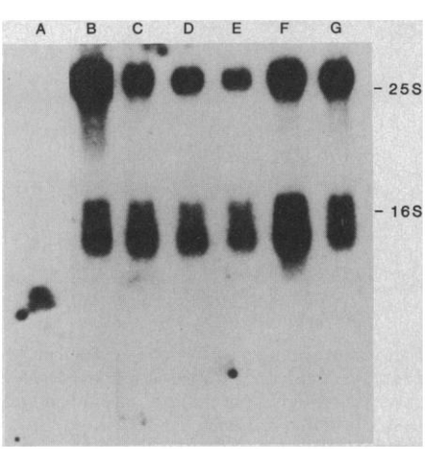


Fig. 1. Northern blot analysis of IL-2 receptor mRNA expression in normal T cells and ATL cell lines. Normal T cells were incubated in RPMI 1640 culture medium and stimulated for 18 hours with PHA (1 μg/ml) and PMA (50 ng/ml). Total cellular RNA (10 μg) from unstimulated T cells (A), T cells stimulated with PHA and PMA (B), and ATL cell lines HUT 102 (C), PL/P6 (D), C91/PL (E), MJ (F), and C5/MJ (G) were size-fractionated on formaldehyde-agarose gels, transferred to nitrocellulose filters, and hybridized to pIL2R2 and pIL2R4 cDNA probes (*8*) labeled with ^{32}P by nick translation. The origin and cell surface phenotype of these cell ATL lines have been described (*3*).

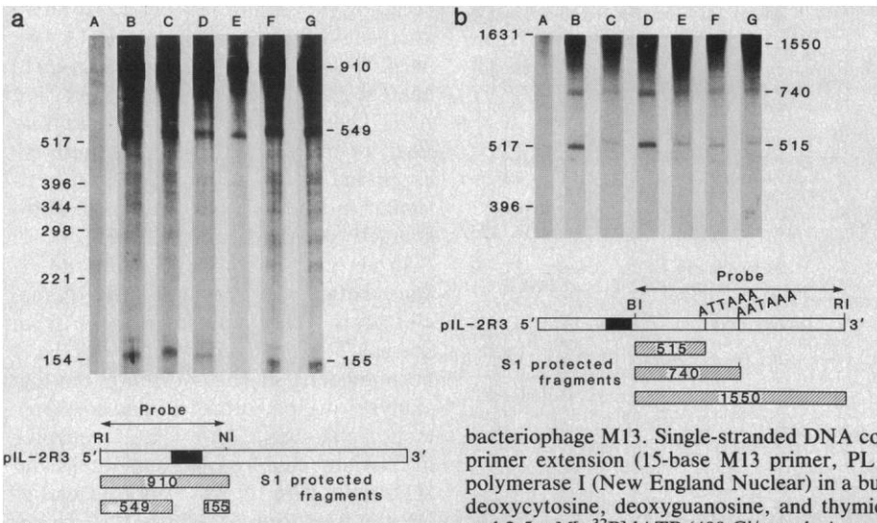
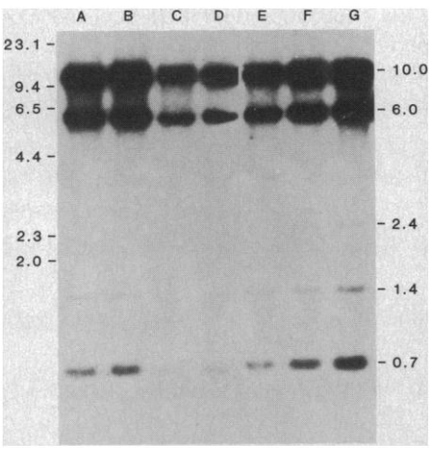


Fig. 2. S1 nuclease protection assay of IL-2 receptor mRNA in normal T cells and ATL cells. Total RNA from resting T cells (A), T cells stimulated with PHA and PMA (B), HUT 102 (C), PL/P6 (D), C91/PL (E), MJ (F), and C5/MJ (G) was hybridized either to the ^{32}P-labeled single-stranded M13 IL-2 receptor "5′ probe" (Eco RI–Nae I fragment, base pairs 1 to 910, left panel) or "3′ probe" (Bgl I–Eco RI fragment, base pairs 785 to 2335, right panel) and subsequently digested with S1 nuclease. The solid region depicted within the IL-2 receptor cDNA clone pIL-2R3 represents the 216-bp segment that may be removed by alternate splicing (*8*). Size markers indicate the migration of ^{32}P-phosphorylated pBR322 Hinf I fragments. Each of the respective cDNA probes was subcloned into bacteriophage M13. Single-stranded DNA complementary to mRNA was uniformly labeled by primer extension (15-base M13 primer, PL Laboratories) using the large fragment of DNA polymerase I (New England Nuclear) in a buffer containing 1 m*M* each of the triphosphates of deoxycytosine, deoxyguanosine, and thymidine; 5 μ*M* deoxyadenosine triphosphate (dATP); and 2.5 μ*M*[α^{32}P]dATP (400 Ci/mmol, Amersham). After digestion with Hind III, the homogeneously labeled DNA was electrophoresed on a gel containing 5 percent polyacrylamide, 8*M* urea, and single-strength TBE (180 m*M* tris-borate, 180 m*M* boric acid, and 2 m*M* EDTA). The band corresponding in size to the desired probe was excised, electroeluted, and precipitated with ethanol. Total cellular RNA (5 μg) was then hybridized at 50°C for 16 hours to the probe (50,000 cpm) in a buffer containing 0.2*M* NaCl, 0.2*M* Pipes (*p*H 6.8), 5 m*M* EDTA, and 70 percent formamide. The reactants were then digested with 5000 units of S1 nuclease (Boehringer-Mannheim) in a buffer containing 0.25*M* NaCl, 30 m*M* sodium acetate (*p*H 4.4), and 1 m*M* $ZnCl_2$ for 30 minutes at 37°C. The samples were then analyzed on a sequencing gel prepared with 5 percent polyacrylamide, 8*M* urea, and single-strength TBE.

785 to 2335 (*8*) was used to study the RNA poly(A) signal sequences. These studies revealed three major bands, indicating the use of three different poly(A) signals. We had earlier proposed three potential poly(A) sites, including the sequence ATTAAA (A, adenine; T, thymine) at position 1298, AATAAA at position 1523, and a third site 3′ to the end of the isolated pIL-2R3 clone (*8*). As shown in the S1 nuclease protection assays (Fig. 2), ATL cells utilize each of these three possible poly(A) signals. Furthermore, the frequency of use does not differ substantially from that occurring in normal activated T cells. Thus, IL-2 receptor mRNA processing appears to be quite similar in normal T cells and ATL cells.

Expression of a full-length IL-2 receptor cDNA isolated from HUT 102 cells in either COS-1 (*8*) or L cells (*10*) resulted in the display of IL-2 receptors that recapitulated the abnormal receptor size characteristic of HUT 102 cells (molecular size 50 kD, compared to 55 kD in normal activated T cells). The IL-2 receptor aberrancy in the HUT 102 cell line is secondary to altered post-transcriptional processing but specific for the IL-2 receptor since other cell surface glycoproteins are processed normally (*11*). These findings suggest the possibility that differences in the primary structure of the normal and HUT 102 IL-2 receptor may exist. However, no unexpected fragments were obtained in the S1 nuclease protection assays with either mRNA from normal activated T cells or mRNA from two M13 subclones derived from the HUT 102 cDNA that spanned the entire protein coding region of the IL-2 receptor. These data suggest that the amino acid sequence of the IL-2 receptor in normal T cells and ATL cells are identical. Notwithstanding, it is possible that the S1 nuclease protection assays may fail to detect single base pair mismatches. Final resolution of the question of potential differences in the primary sequence of the IL-2 receptor in



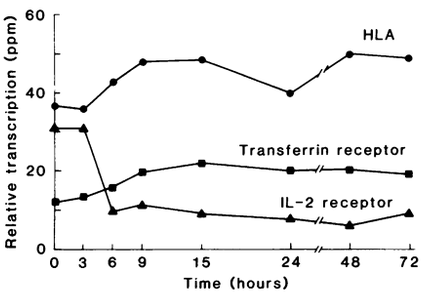


Fig. 3 (left). Southern blot analysis of HTLV-I–infected T cells from ATL patients. DNA from normal T cells (G) and from T-cell lines established from the peripheral blood of six ATL patients [YO (A), TO (B), TA (C), ST (D), SK (E), and HA (F)] was digested with Eco RI, size-fractionated on agarose gels, transferred to nitrocellulose paper, and hybridized with ^{32}P-labeled pIL2R2 and pIL2R4 cDNA probes (*8*). Size markers indicate migration of lambda DNA–Hind III fragments. Fig. 4 (right). Nuclear transcription assays in HUT 102 cells. HUT 102 cells were stimulated with PHA (1 μg/ml) and PMA (50 ng/ml). At the indicated times, nuclei were isolated and assayed for specific transcriptional activities of genes encoding HLA (●), IL-2 receptor (▲), and transferrin receptor (■). In vitro transcription assays with isolated nuclei were performed as described (*17*). Briefly, nascent RNA chains were allowed to elongate in isolated nuclei in the presence of α-^{32}P-labeled uridine triphosphate. The labeled nuclear RNA was then purified by deoxyribonuclease and proteinase K digestion, phenol extraction, and ethanol precipitation. Equivalent amounts of ^{32}P-labeled nuclear RNA were then hybridized to excess amounts of specific cDNA probes immobilized on nitrocellulose filters. The amount of labeled nuclear RNA bound to the filters was determined by liquid scintillation counting. The transcriptional activity is expressed as parts per million. The specific parts-per-million value was determined by subtracting the background hybridization to pBR322 DNA from the actual radioactivity measured. This specific value was then divided by the total input radioactive nuclear RNA (in counts per minute) and multiplied by 10^6.

HUT 102 and normal T cells must await complete nucleotide sequence analysis of the normal IL-2 receptor gene.

To investigate whether the constitutive high-level expression of IL-2 receptor mRNA was secondary to HTLV-I–induced perturbation of the IL-2 receptor gene structure, we performed Southern blot analyses of restricted DNA extracted from leukemic T-cell lines established from the peripheral blood of six ATL patients (Fig. 3). The five Eco RI restriction fragments for the IL-2 receptor were identical in size in both the ATL and normal T cells. These data suggest that HTLV-I–associated IL-2 receptor expression is probably not due to, or associated with, IL-2 receptor gene rearrangement. Furthermore, these studies provided no evidence for selective IL-2 receptor gene amplification in the ATL cell lines studied. The single-copy IL-2 receptor gene is located on the short arm of chromosome 10 (10p14-15) (*12*). Karyotype analysis of several ATL cell lines has not revealed consistent translocations involving chromosome 10, suggesting that chromosomal breakage is not involved in the high-level expression of IL-2 receptors characteristic of ATL cells.

To further study the deregulation of IL-2 receptor gene expression in ATL cells, we used nuclear transcription assays with isolated HUT 102 nuclei (Fig. 4). In contrast to normal T cells, which must be activated with antigen or mitogen before IL-2 receptors are expressed (*13*), the IL-2 receptor gene was constitutively transcribed in HUT 102 cells. This constitutive expression of the IL-2 receptor gene may reflect direct or indirect *trans*-acting transcriptional activation by the "LOR protein" encoded by the long open reading (LOR) frame associated with the pX region of HTLV-I. Sodroski *et al.* (*14*) showed that the LOR region of HTLV-I encodes a 42-kD protein that can enhance the transcription of genes under the control of the HTLV-I long terminal repeat (LTR) in a *trans*-acting manner. These authors have speculated that the LOR protein enhances HTLV-I replication and also may activate cellular genes involved in neoplastic transformation.

In normal T cells, stimulation with phytohemagglutinin (PHA) and phorbol myristate acetate (PMA) induces transcription of the IL-2 receptor gene within 3 hours (*15*). Paradoxically, stimulation of HUT 102 cells with PHA and PMA resulted in rapid and selective inhibition of IL-2 receptor gene transcription (Fig. 4). This effect was not the result of a generalized nonspecific inhibition of gene expression, since PHA and PMA addition did not alter active transcription of genes encoding histocompatibility antigen (HLA), transferrin receptor (Fig. 4), or c-*myc* (not shown). IL-2 receptor gene transcription is a transient event in normal PHA- and PMA-activated T cells (*15*). After peak transcription at 6 hours, IL-2 receptor gene transcription gradually declines, perhaps secondary to the action of a co-induced repressor mechanism similar to that described for the IL-2 gene (*16*). The constitutive expression of the IL-2 receptor gene in ATL cells may indicate that this putative repressor system is silent. We are intrigued by a model of ATL whereby the LOR protein selectively stimulates IL-2 receptor gene transcription but fails to activate the repressor mechanism. The addition of PHA and PMA, however, may activate the repressor mechanism and result in diminished IL-2 receptor transcription. This hypothesis can be formally tested when the promoter region of the IL-2 receptor gene is isolated. Furthermore, the IL-2 receptor promoter region can be compared with the LTR of HTLV-I for possible sequence homologies and functional LOR protein-binding capacity.

Deregulated expression of genes controlling cell growth and differentiation, including cellular oncogenes, has been associated with the genesis of various neoplasms. Further study of the abnormal regulation of IL-2 receptor gene expression in ATL cells may provide important insights into the molecular mechanisms of HTLV-I–mediated leukemogenesis.

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"Where" and "What" in Vision

Abstract. *The mixture of a few horizontal and vertical line segments embedded in an aggregate of diagonal line segments can be rapidly counted and their positions rapidly determined by a parallel (preattentive) process. However, the discrimination between horizontal and vertical orientation (that is, discrimination of a single conspicuous feature) requires serial search by focal attention. Under recent theories of attention, focal attention has been assumed to be required for the recognition of different combinations of features. According to the findings of this experiment, knowing "what" even a single feature is requires time-consuming search by focal attention. Only knowing "where" a target is is mediated by a parallel process.*

Traditionally, psychologists differentiate between detection and discrimination tasks. Detection is considered an easier task since the observer requires less information, whereas discrimination entails some further computation. Here we suggest that detection and localization can be done in parallel, and that the further computation required for discrimination is done by a serial process. By measuring processing time for detection and discrimination of orientation, we found that processing time is independent of the number of targets to be detected but depends on the number of targets to be identified. Furthermore, the processing time that is required to detect a small number of targets is sufficient to locate them quite accurately. That processing time is independent of the number of targets indicates that the targets are processed in parallel, whereas the dependence of processing time on the number of targets indicates serial processing. This serial process is required for even such a simple task as discrimi-