The Structure, Function, and Expression of Interleukin-2 Receptors on Normal and Malignant Lymphocytes

THOMAS A. WALDMANN

Antigen or mitogen-induced activation of resting T cells induces the synthesis of interleukin-2 (IL-2) as well as the expression of specific cell surface receptors for this lymphokine. Failure of the production of either IL-2 or its receptor results in a failure of the T-cell immune response. The receptor is composed of a 33,000-dalton (251–amino acid) peptide precursor that is post-translationally glycosylated into the mature 55,000-dalton form. In contrast to resting T cells, human T-cell lymphotrophic virus I (HTLV-I)–associated adult T-cell leukemia cells constitutively express large numbers of IL-2 receptors. Because IL-2 receptors are present on the malignant T cells but not on normal resting cells, clinical trials have been initiated in which patients with adult T-cell leukemia are treated with a monoclonal antibody that binds to the IL-2 receptor.

THE VERTEBRATE IMMUNE SYSTEM HAS A VIRTUALLY UNLIMITED capacity to recognize and distinguish specific molecular patterns and thus to bind to millions of potential antigens. This immune response is mediated by two broad classes of cells that react specifically with antigens: B cells and thymus-derived lymphocytes or T cells. B cells react with soluble antigens and are the precursors of antibody-secreting cells of the humoral immune system. T cells encompass an array of cell subsets that includes those that mediate important regulatory functions such as help or suppression, as well as cells that are directly involved in effector functions such as the cytotoxic destruction of antigen-bearing cells and the production of soluble products termed lymphokines. The induction of a T-cell immune response to a foreign antigen requires the activation of T lymphocytes with receptors for the specific antigen. The human T-cell antigen receptor has been shown to be a 90-kilodalton (kD) polymorphic heterodimer of α and β chains, each of 40 to 50 kD associated with at least three 20- to 28-kD nonpolymorphic polypeptide chains identified by the T3 monoclonal antibody (*1*). T-cell activation is initiated after antigens, mitogens, or antibodies interact with this complex T-cell antigen receptor. When the receptor interacts with antigen in the context of products of a major histocompatibility locus and the macrophage-derived interleukin-1, T cells express the gene encoding the lymphokine interleukin-2 (IL-2), previously termed T-cell growth factor (*2*). To exert its biological effect, IL-2 must interact with specific high-affinity membrane receptors (*3*). Resting T cells do not express IL-2 receptors, but receptors are rapidly expressed on T cells after they are activated with an antigen or mitogen. Thus, the growth factor IL-2 and its receptor are absent in resting T cells, but after activation, the genes for both proteins become expressed. The failure of production of either the growth factor or its receptor results in failure of the T-cell immune response. Thus, both the production of IL-2 and the display of IL-2 receptors are pivotal events in the full expression of the T-cell immune response. Although the interaction of appropriately presented antigen with the antigen-receptor complex confers specificity for a given immune response, the interaction of IL-2 with IL-2 receptors determines its magnitude and duration.

The presence of receptors for IL-2 was first suggested by the observation that lectin- or alloantigen-activated T cells, but not resting T or B cells, could remove IL-2 from conditioned medium. Subsequently, Robb and Greene and their co-workers (*3*), utilizing purified, biosynthetically labeled IL-2, demonstrated specific, saturable, high-affinity binding sites on IL-2–dependent T-cell lines as well as mitogen- and alloantigen-activated T cells. Further progress in the analysis of the structure, function, and expression of the human IL-2 receptor was greatly facilitated by the production of the anti-Tac monoclonal antibody by Uchiyama, Broder, and Waldmann (*4*). This monoclonal antibody was selected on the basis of its ability to bind to activated T cells but not to resting T cells, B cells, or monocytes, a pattern of cellular reactivity identical to the distribution of IL-2 receptors reported by Robb and co-workers (*3*). We therefore hypothesized that anti-Tac recognizes the human receptor for IL-2 (*5*). Data in support of this hypothesis are: (i) anti-Tac blocks the IL-2–induced DNA synthesis of IL-2–dependent continuous T-cell lines but does not inhibit DNA synthesis of IL-2–independent T-cell lines; (ii) anti-Tac blocks more than 95 percent of the binding of ^{3}H-labeled IL-2 to HUT 102-B2 cells, a cell line derived from a patient with adult T-cell leukemia subsequently shown to be infected with human T-cell lymphotrophic virus I (HTLV-I), as well as to phytohemagglutinin (PHA)-activated lymphoblasts; and (iii) IL-2 at high concentration blocks the binding of ^{3}H-labeled anti-Tac to PHA-activated lymphoblasts. Furthermore, Robb and Greene (*6*) demonstrated that an initial passage of radiolabeled proteins from PHA-activated lymphoblasts through either an IL-2–coupled affinity support or a column of anti-Tac coupled to Sepharose effectively removed molecules reactive with the alternative support. Thus, under the conditions used, all anti-Tac–reactive molecules appeared capable of binding IL-2, and the ability to bind IL-2 was limited to the Tac protein.

Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Subsequently, other monoclonal antibodies to primate IL-2 receptors, as well as antibodies to rat and mouse IL-2 receptors, have been identified. Initial receptor binding studies with radiolabeled anti-Tac and radiolabeled IL-2 suggested that activated T cells and leukemic cell populations express 5- to 20-fold more binding sites for anti-Tac than for IL-2 (*7*). However, Robb, Greene, and Rusk (*8*), using high concentrations of IL-2, resolved this apparent difference in receptor markers by showing that there were two affinity classes of IL-2 receptors. On various cell populations, 5 to 15 percent of the IL-2 receptors had an apparent affinity in the range of 3 to 10 p*M*, whereas the remaining receptors bound IL-2 at the much lower apparent affinity of 3 n*M*. The high-affinity receptors appear to mediate the physiologic responses to IL-2, since the magnitude of the T-cell responses is closely correlated with the occupancy of these receptors (*3, 8, 9*). The structural basis for the difference in apparent IL-2 binding affinity is unknown. As outlined in the review below, the anti-Tac monoclonal antibody has been used to (i) characterize the human receptor for IL-2; (ii) molecularly clone complementary DNA's (cDNA's) for the human IL-2 receptor; (iii) define the cellular distribution of IL-2 receptors; (iv) determine the immunoregulatory effects that require the interaction of IL-2 with its receptor; (v) characterize disorders of IL-2 receptor expression on leukemic cells; and (vi) treat patients with IL-2 receptor–expressing adult T-cell leukemia, patients with autoimmune disorders, and individuals receiving organ allografts.

Chemical Characterization of the IL-2 Receptor

The anti-Tac monoclonal antibody was used to characterize the IL-2 receptor on PHA-activated normal lymphocytes (*5, 10*). When anti-Tac immunoprecipitations of PHA-activated normal lymphocytes surface-labeled with ^{125}I were analyzed on sodium dodecyl sulfate (SDS)–polyacrylamide gels under reducing conditions, a diffuse band of 55 to 60 kD was identified as the putative IL-2 receptor. This protein was also identified in immunoprecipitations from cells precultured with D-[^{3}H]glucosamine, thus demonstrating that it is a glycoprotein. Furthermore, when cells were biosynthetically labeled with [^{35}S]methionine and the immunoprecipitates analyzed on SDS gels, a similar band was identified. With anti-Tac, but not with certain other monoclonal antibodies to the IL-2 receptor, additional bands of approximately 115 kD and 180 kD were also identified. Since these two peptides were not labeled either with ^{125}I during lactoperoxidase labeling of intact cells or with radiolabeled glucosamine, they do not appear to be surface membrane receptors. The roles of these molecules have not been defined. It is possible that neither has any functional relation to the receptor identified by anti-Tac but rather contains shared antigenic determinants resulting in precipitation by anti-Tac. Alternatively, one or both may be part of a receptor complex and, therefore, may immunoprecipitate with the 55-kD peptide.

Leonard and his co-workers (*5, 10*) defined the post-translational processing of the 55-kD glycoprotein by using a combination of pulse-chase and tunicamycin experiments. These studies showed that the IL-2 receptor was composed of a 33-kD peptide precursor after cleavage of the hydrophobic leader sequence. This precursor was cotranslationally *N*-glycosylated to 35-kD and 37-kD forms. One hour after the addition of unlabeled amino acids, the 55- to 60-kD mature form of the receptor appeared, suggesting that *O*-linked carbohydrate was added to the IL-2 receptor. Furthermore, the IL-2 receptor was shown to be sulfated (*10*) and phosphorylated on a serine residue (*11*).

Molecular Cloning of cDNA's for the Human IL-2 Receptor

Three groups have succeeded in cloning cDNA's for the IL-2 receptor protein (*12*). Each of the groups has purified IL-2 receptors from HTLV-I–infected adult T-cell lines by affinity chromatography with monoclonal antibodies to the IL-2 receptor covalently coupled to Sepharose. The NH_2-terminal amino acid sequence of the purified protein was determined, and oligonucleotide probes were synthesized and used to screen cDNA libraries prepared with messenger RNA (mRNA) isolated from Tac-expressing HTLV-I–infected T-cell lines. The deduced amino acid sequence of the IL-2 receptor indicates that this peptide is composed of 272 amino acids, including a 21–amino acid signal peptide. The receptor contains two potential *N*-linked glycosylation sites and multiple possible *O*-linked carbohydrate sites. Finally, there is a single hydrophobic membrane region of 19 amino acids and a very short (13–amino acid) cytoplasmic domain. The cytoplasmic domain of the IL-2 receptor peptide identified by anti-Tac thus appears to be too small for enzymatic function. Potential phosphate acceptor sites (serine and threonine, but not tyrosine) are present within the intracytoplasmic domain. Thus, this receptor differs from other known growth-factor receptors that have large intracytoplasmic domains with tyrosine kinase activity. The possibility that the IL-2 receptor is a complex receptor with multiple peptides in addition to the one identified by anti-Tac is being investigated.

Leonard *et al.* (*13*) demonstrated that the single gene encoding the IL-2 receptor consists of eight exons on chromosome 10p14. However, mRNA's of two different sizes, approximately 1500 and 3500 bases long, have been identified. These mRNA classes differ because of their use of two or more polyadenylation signals (*12*). Receptor gene transcription is initiated at two principal sites in normal activated T lymphocytes (*13*). Furthermore, sequence analyses of the cloned DNA's also indicate that the alternative mRNA splicing may delete a 216–base pair segment in the center of the protein-coding sequence encoded by the fourth exon (*12, 13*). Both the spliced and unspliced forms of the mRNA were demonstrated in all IL-2 receptor–expressing T-cell lines examined. Using expression studies of cDNA's in COS-1 cells, Leonard and his co-workers (*12*) demonstrated that the unspliced but not the spliced form of the mRNA was translated into the cell surface receptor that binds IL-2 and anti-Tac.

Induction of IL-2 Receptor Expression

IL-2 receptors are induced on T cells when appropriately presented antigens, mitogens, or antibodies interact with the complex T-cell antigen receptor. This IL-2 receptor induction is inhibited by cycloheximide and actinomycin D but not by mitomycin C or x-irradiation, indicating that receptor expression requires de novo RNA and protein synthesis but not DNA synthesis. Activation through the T-cell receptor complex is associated with an increase in intracellular calcium and the involvement of protein kinase C, with translocation of this enzyme from the cytoplasm to the membrane (*14*). IL-2 receptors may also be induced without interaction with the T-cell antigen receptor by the use of the calcium ionophore A23187, which increases intracellular calcium, or by phorbol myristic acetate, which acts on protein kinase C. After activation with mitogenic lectins, the number of IL-2 receptors reaches a maximum at 48 to 72 hours. Following this period, there is a progressive decline in the number of receptors, so that at 7 to 21 days in culture the number of receptors is fewer than 20 percent of the number during peak expression (*7, 15*). These observations have supported

the view that IL-2 receptor expression in combination with IL-2 secretion controls the expansion and subsequent termination of the normal cellular immune response. The expression of receptors on PHA-activated lymphoblasts can be reinduced by addition of the initial stimulus, mitogenic lectins, or antigens. Depper and his coworkers (*7*) demonstrated that the addition of phorbol diesters, phospholipase C, or the diacylglycerol congeners that activate protein kinase C also resulted in augmented IL-2 receptor expression.

Several observations have shown that IL-2 upregulates the expression of its specific receptor as quantitated by anti-Tac. Using dexamethasone to inhibit IL-2 production during lectin activation of human lymphocytes, Reem and Yeh (*16*) observed that IL-2 receptor expression was augmented by the addition of IL-2. Similarly, we demonstrated that the addition of IL-2 to a cloned, normal IL-2 receptor-expressing B-cell line leads to the induction of increased numbers of receptors identified by anti-Tac (*17*). A similar upregulation of receptors identified by monoclonal antibodies to the IL-2 receptor was demonstrated in T cells exposed to submitogenic concentrations of a monoclonal antibody to T3 (*18*). Depper *et al.* (*19*) demonstrated that purified IL-2 can directly upregulate IL-2 receptor expression on PHA-activated T lymphocytes maintained in culture until IL-2 receptor expression markedly declines. IL-2 stimulation of such cells resulted in an increase in IL-2 receptor gene transcription detectable within 30 minutes in isolated nuclei, indicating that IL-2 can directly upregulate transcription of mRNA for the receptor peptide identified by anti-Tac.

As noted above, IL-2 receptors have two affinities; both high- and low-affinity binding sites for IL-2 are identified by anti-Tac. Smith and Cantrell (*9*) added purified IL-2 to activated T cells brought to rest by IL-2 deprivation. The addition of IL-2 to these cultures resulted in an eight- to tenfold increase in the expression of the Tac epitope, but a simultaneous 20 to 30 percent diminution in detectable high-affinity IL-2 binding sites. The addition of IL-2 thus may only stimulate the synthesis of low-affinity IL-2 receptors. Alternatively, the addition of IL-2 may induce the synthesis of both high- and low-affinity receptors but may mediate the rapid internalization of the high-affinity receptor alone by specific ligand-mediated receptor endocytosis. Such IL-2 receptor–mediated internalization limited to high-affinity receptors has been demonstrated by Weissman *et al.* (*20*).

Cellular Distribution of IL-2 Receptors

As discussed above, most resting T cells, B cells, or macrophages in the circulation do not display IL-2 receptors. Specifically, less than 5 percent of freshly isolated, unstimulated human peripheral blood T lymphocytes react with anti-Tac. Most T lymphocytes, however, can be induced to express IL-2 receptors by interaction with lectins, monoclonal antibodies to the T-cell antigen receptor complex, or alloantigen stimulation. Furthermore, thymocytes can be induced to express the IL-2 receptors by the simultaneous exposure to two T11 antibodies that identify two different epitopes of the sheep red blood cell receptor at a time when these cells do not express the T-cell antigen receptor complex (*21*). These findings indicate that there is an alternative antigen-independent mechanism for IL-2 receptor induction. Tac-positive cells have been demonstrated in the circulation after immunization in vivo with tetanus toxin; however, this represents a very transient appearance of such cells. Miyawaki *et al.* (*22*) showed that approximately 20 percent of the T cells in the normal pericortical and interfollicular regions of lymph nodes and tonsils express the Tac antigen.

IL-2 receptor expression is not limited to activated T cells but has been observed with other activated mononuclear cell populations. Korsmeyer *et al.* (*23*) showed that hairy cell leukemic cells express the Tac antigen. These hairy cell leukemic cells had rearranged heavy and light chain immunoglobulin genes, produced mRNA for immunoglobulins, displayed the appropriate surface immunoglobulin, and were thus shown to be B cells expressing the Tac antigen. Subsequently, we demonstrated Tac antigen expression by many B-cell lines derived from patients with Burkitt's lymphoma and on all B-cell lines containing the HTLV-I genome. Normal peripheral blood B lymphocytes activated by *Staphylococcus aureus* Cowan I organisms, pokeweed mitogen, phorbol myristic acetate, or anti-μ immunoglobulins can be induced to express IL-2 receptors (*17, 24*). In addition, cloned Epstein-Barr virus–transformed human B-cell lines derived from Tac-positive, activated, normal B cells continued to express the Tac antigen in long-term cultures. Such Tac-positive cells manifested both high- and low-affinity IL-2 receptors at a ratio of 1 to 10, comparable to that observed with IL-2–dependent T-cell lines and activated T lymphocytes. The size of the IL-2 receptors on Tac-positive, cloned, normal B cells was comparable (53 to 57 kD) to that of receptors on PHA-stimulated T lymphoblasts (*17*). Furthermore, Tac-positive B cells transcribed 1500 and 3500 base mRNA's for the IL-2 receptor. Thus, certain malignant as well as activated normal B cells display the Tac antigen and manifest high-affinity receptors for IL-2.

IL-2 receptors identified with the anti-Tac monoclonal antibody have been detected on cells of the monocyte-macrophage series, including cultured monocytes, Kupffer cells of the liver, cultured lung macrophages, Langerhans' cells of the skin, and Reed-Sternberg cells in Hodgkin's disease (*25*).

Rubin *et al.* (*26*) demonstrated that activated normal peripheral blood mononuclear cells and certain lines of T- and B-cell origin release a soluble form of the IL-2 receptors into the culture medium. Using an enzyme-linked immunosorbent assay with two monoclonal antibodies that recognize distinct epitopes on the human IL-2 receptor, they showed that normal individuals have measurable amounts of IL-2 receptors in their plasma and that certain lymphoreticular malignancies are associated with elevated plasma levels of this receptor. The release of soluble IL-2 receptors appears to be a consequence of cellular activation of various cell types that may play a role in the regulation of the immune response. Furthermore, the analysis of plasma levels of IL-2 receptors may provide a new approach to the analysis of lymphocyte activation in vivo.

Lymphocyte Functions Regulated by the Interaction of IL-2 with Its Receptor

The anti-Tac monoclonal antibody has been used to define those lymphocyte functions that require an interaction of IL-2 with its inducible receptor on activated T and B lymphocytes. The addition of anti-Tac to cultures of human peripheral blood mononuclear cells inhibited various immune reactions. Anti-Tac profoundly inhibited the proliferation of T lymphocytes stimulated by soluble antigens and by cell surface antigens (autologous and allogeneic mixed lymphocyte reactions) (*27*). This anti-Tac inhibition of antigen-induced proliferation was reversed by the addition of purified IL-2. Since anti-Tac did not inhibit endogenous IL-2 production, this result indicated that the antibody functions by competition with IL-2 for receptor binding.

Upon activation, human T cells acquire surface structures that are not easily detectable during their resting stage (*28*). This series of activation antigens, which in large measure represent receptors for growth factors, appears on T cells after lectins, antigens, or the T3 antibody interact with the T-cell antigen receptor complex. The

addition of anti-Tac at the initiation of cultures of T cells stimulated by mitogens, antigens, or the T3 antibody inhibited the expression of the late-appearing activation antigens examined, the insulin and transferrin receptors, and Ia antigens (*28*). Anti-Tac also inhibited a series of T-cell functions, including the generation of both cytotoxic and suppressor T lymphocytes in allogeneic cell cultures, but did not inhibit their action once generated. The interaction of IL-2 with its receptor is also involved in the control of the production of other lymphokines. Although IL-2 does not appear to regulate its own synthesis, as demonstrated by the failure of anti-Tac to block IL-2 production (*27*), IL-2 enhances the production of granulocyte-macrophage colony-stimulating factor (GM-CSF), γ-interferon, and B-cell growth factor–I (BCGF-I or BSF) (*29*).

B cells, like T cells, are regulated in their proliferative and differentiative responses to antigen by a series of lymphokines, including BCGF-I or BSF and such B-cell differentiation factors as BCDF or TRF. There has been controversy regarding the possible involvement of IL-2 as well in B-cell responses and specifically the ability of this growth factor to act directly on B lymphocytes. We and others have recently made the observations that support the view that IL-2 may play a direct role in B-cell proliferation and differentiation. Anti-Tac inhibited immunoglobulin production by B lymphocytes activated by polyclonal activators (*17*). This inhibition of immunoglobulin synthesis by B cells could theoretically reflect either an inhibition of the synthesis of T cell–derived lymphokines that act on B cells, or a direct action on the B cells themselves, or both. As indicated above, certain malignant as well as activated normal B cells transcribe mRNA for the IL-2 receptor and manifest high-affinity receptors for IL-2. In functional studies, peripheral blood B cells from normal individuals activated with *Staphylococcus aureus* Cowan strain I organisms could be induced to proliferate and to synthesize immunoglobulin molecules by the addition of recombinant IL-2 (*30*). These responses were abolished when anti-Tac was added to the cultures. Although the role of IL-2 in normal immunoglobulin and antibody production is still controversial, these data support the view that IL-2 may play a role in the proliferation of activated B cells as well as in their differentiation into immunoglobulin-synthesizing and -secreting cells.

Disorders of IL-2 Production and IL-2 Receptor Expression

Diminished production of IL-2 or the response to this lymphokine has been described in association with a number of primary and acquired immunodeficiency diseases, as well as in drug-induced immunosuppression. Diminished IL-2 production has been reported in patients with acquired immunodeficiency syndrome (AIDS), patients with common variable immunodeficiency, patients who have had bone marrow transplantation, as well as in some patients with primary immunodeficiencies of T-cell function, including those with severe combined immunodeficiency disease (*29*). The T cells from most patients with primary and secondary immunodeficiency disorders can be induced to express IL-2 receptors. However, in rare cases, IL-2 receptor expression cannot be induced in the circulating lymphoid cells of patients with severe combined immunodeficiency disease. Theoretically, a primary defect in the ability to express the IL-2 receptor could underlie such a syndrome.

In certain cases, drugs that act to induce immunosuppression do so by inhibiting IL-2 production. For example, glucocorticoids such as dexamethasone prevent IL-2 production but do not prevent IL-2 receptor expression (*16, 31*). Similarly, it has been shown that cyclosporin A inhibits IL-2 production by inhibiting T-cell IL-2 gene expression at the level of mRNA transcription (*32*). Cyclosporin A does not prevent IL-2 receptor expression. Thus, a major mode of action of cyclosporin A may be on IL-2 gene transcription.

Disorders of IL-2 Expression in Adult T-Cell Leukemia

The expression of IL-2 receptors has been analyzed in various immature and mature forms of T-cell leukemia. IL-2 receptors were not present in the leukemic cells of most patients with acute T-cell leukemias, although cell lines from some such patients could be induced to express IL-2 receptors on addition of PHA and phorbol myristic acetate (*33*). Furthermore, the circulating malignant T cells from patients with the Sézary leukemia, a leukemia of mature T cells with a propensity to infiltrate the skin, lacked the Tac antigen (*34*). A distinct form of mature T-cell leukemia was defined by Takasuki and co-workers (*35*) and termed adult T-cell leukemia (ATL). Like the Sézary leukemia, ATL is a malignant proliferation of mature T cells that have a propensity to infiltrate the skin. Cases of ATL in contrast to those of the Sézary syndrome are associated with hypercalcemia and have a very aggressive course in most cases. The ATL cases are clustered within families and geographically, occurring in southwest Japan, the Caribbean basin, and in certain areas of Africa. Furthermore, HTLV-I has been shown to be a primary etiologic agent in ATL (*36*), whereas patients with the Sézary syndrome usually do not have circulating antibodies to this virus. All the populations of leukemic cells we have examined from patients with HTLV-I–associated ATL expressed the Tac antigen (*34*). The expression of IL-2 receptors on ATL cells differs from that of normal T cells (*7, 10, 37, 38*). First, unlike normal T cells, ATL cells do not require prior activation to express IL-2 receptors. Furthermore, in a ^{3}H-labeled anti-Tac receptor assay, HTLV-I–infected leukemic T-cell lines characteristically expressed five to ten times more receptors per cell (270,000 to 1,000,000) than did maximally PHA-stimulated T lymphoblasts (30,000 to 60,000). In addition, whereas normal human T lymphocytes maintained in culture with IL-2 show a rapid decline in receptor number, adult ATL lines do not show a similar decline. Leonard and Wano and their co-workers (*10*) also demonstrated that some but not all HTLV-I–infected cell lines display aberrantly sized IL-2 receptors as a result of differences in glycosylation. In addition, in studies by Tsudo *et al.* (*37*), IL-2 receptors on ATL cells, unlike those on normal activated T cells or Tac-positive T-CLL cells, were not modulated (downregulated) by anti-Tac. Finally, IL-2 receptors on ATL cells were spontaneously (without IL-2) phosphorylated, whereas the phosphorylation of receptors on PHA-stimulated T cells required the addition of IL-2 (*38*). It is conceivable that the constant presence of large numbers of both high- and low-affinity IL-2 receptors on ATL cells or the aberrance of these receptors (or both) may play a role in the pathogenesis of uncontrolled growth of these malignant T cells.

As stated above, T-cell leukemias caused by HTLV-I, as well as all T-cell and B-cell lines infected with HTLV-I, express large numbers of IL-2 receptors. An analysis of this virus and its protein products suggests a potential mechanism for this association between HTLV-I and IL-2 receptor expression. The complete sequence of HTLV-I has been determined by Seiki *et al.* (*39*). In addition to typical long terminal repeats (LTR's), *gag*, *pol*, and *env* genes—gene sequences common to other groups of retroviruses—HTLV-I and HTLV-II contain a genomic region between *env* and the LTR referred to as pX or more recently as *tat*. Sodroski *et al.* (*40*) demonstrated that this pX or *tat* region encodes a 42-kD protein, initially termed LOR (long open reading frame) and more recently the *tat* protein, which is essential for viral replication. This protein is produced by a double splicing event not present in other retroviruses. These authors

demonstrated that the *tat* protein acts on a receptor within the LTR's of HTLV-I and HTLV-II, stimulating transcription. This *tat* protein could also play a central role in increasing the transcription of host genes such as the IL-2 receptor gene involved in T-cell activation and HTLV-I–mediated T-cell leukemogenesis. The mechanism by which the *tat* protein activates the expression of IL-2 receptor genes is unclear, with either a direct or indirect interaction with the IL-2 receptor gene being possible. Yodoi *et al.* (*41*) isolated and characterized a soluble factor termed adult T-cell leukemia–derived factor (ADF) that is present in the supernatants of ATL cell cultures. This factor augments the expression of IL-2 receptors on the surface of certain target T-cell lines. It is possible that the *tat* protein activates the expression of the ADF gene, which could in turn stimulate IL-2 receptor expression. Alternatively, the *tat* protein may act directly to activate IL-2 receptor gene expression. With the cloning of the genes encoding the *tat* protein and the IL-2 receptor, the hypothesis that the *tat* protein acts as a transacting regulator of transcription of the IL-2 receptor gene can be readily tested.

The IL-2 Receptor as a Target for Therapy

The observation that ATL cells constitutively express large numbers of IL-2 receptors identified by anti-Tac, whereas normal resting cells and their precursors do not, provides the scientific basis for therapeutic trials with agents to eliminate the IL-2 receptor–expressing cells. Such agents could theoretically eliminate Tac-expressing leukemic cells or activated T cells involved in other disease states, while retaining the mature normal T cells and their precursors that express the full repertoire for T-cell immune responses. The agents that have been used or are being prepared include: (i) unmodified anti-Tac; (ii) toxin (for example, the A chain of ricin toxin or *Pseudomonas* toxin) conjugates of anti-Tac; (iii) conjugates of alpha-emitting isotopes (for example, bismuth-212) with anti-Tac; (iv) antibodies, prepared by recombinant DNA techniques, that express the antigen-binding domains of anti-Tac associated with the constant domains of human immunoglobulin light and heavy chains; and (v) recombinant peptides, with the ligand IL-2 associated with a toxin.

We have initiated a clinical trial to evaluate the efficacy of intravenously administering anti-Tac monoclonal antibody in the treatment of patients with ATL (*42*). None of the three patients treated suffered any untoward reactions, and none produced antibodies to the mouse immunoglobulin or to the idiotype of anti-Tac. Two of the patients with a very rapidly developing form of ATL had a transient response. However, therapy of the other patient was followed by a 6-month remission, as assessed by routine hematological tests, immunofluorescence analysis of circulating T cells, and molecular genetic analysis of arrangement of the genes encoding the β chain of the T-cell antigen receptor. After the 6-month remission the patient's disease recurred, but a new course of anti-Tac infusions was followed by a virtual disappearance of skin lesions and more than 90 percent reduction in the number of circulating leukemic cells. Three months later, leukemic cells were again demonstrable in the circulation. At this time, although the leukemic cells continued to express the Tac antigen, the leukemia was no longer responsive to infusions of anti-Tac, and the patient required chemotherapy. This patient may have had the smoldering form of ATL in which the leukemic T cells may still require IL-2 for their proliferation. Alternatively, the clinical responses may have been mediated by host cytotoxic cells reacting with the tumor cells bearing the anti-Tac mouse immunoglobulin on their surface by such mechanisms as antibody-dependent cellular cytotoxicity.

These therapeutic studies have been extended in vitro by examining the efficacy of toxins coupled to anti-Tac for selectively inhibiting protein synthesis and viability of Tac-positive ATL lines. The addition of anti-Tac coupled to the A chain of the toxin ricin effectively inhibited protein synthesis by the HTLV-I–associated, Tac-positive ATL line HUT 102-B2 (*43*). In contrast, conjugates of ricin A with a control monoclonal antibody of the same isotype did not inhibit protein synthesis when used in the same concentration. In parallel studies performed in collaboration with FitzGerald *et al.* (*44*), *Pseudomonas* exotoxin conjugates of anti-Tac inhibited protein synthesis by HUT 102-B2 cells but not that by the Tac-negative acute T-cell line MOLT-4, which does not express the Tac antigen.

The action of toxin conjugates of monoclonal antibodies depends on their ability to be internalized by the cell and released into the cytoplasm. Anti-Tac bound to IL-2 receptors on leukemic cells is internalized slowly into coated pits and then endosomic vesicles. Furthermore, the toxin conjugate does not pass easily from the endosome to the cytosol, as required for its action on elongation factor 2. To circumvent these limitations, an alternative cytotoxic reagent was developed that could be conjugated to anti-Tac and that was effective when bound to the surface of leukemic cells. In studies performed in conjunction with Kozak *et al.* (*45*), it was shown that bismuth-212 (^{212}Bi), an alpha-emitting radionuclide conjugated to anti-Tac by use of a bifunctional chelate, was well suited for this role. Activity levels of 0.5 μCi (equivalent to 12 rad of alpha radiation per milliliter) targeted by ^{212}Bi-labeled anti-Tac eliminated more than 98 percent of the proliferative capacity of the HUT 102-B2 cells, with only a modest effect on IL-2 receptor–negative lines. This specific cytotoxicity was blocked by excess unlabeled anti-Tac, but not by human immunoglobulin G. Thus, ^{212}Bi-labeled anti-Tac is a potentially effective and specific immunocytotoxic agent for the elimination of IL-2 receptor–bearing cells.

In addition to its use in the therapy of patients with ATL, antibodies to the IL-2 receptors are being evaluated as potential therapeutic agents to eliminate activated IL-2 receptor–expressing T cells in other clinical states, including certain autoimmune disorders, and in protocols involving organ allografts. The rationale for the use of anti-Tac in patients with aplastic anemia is derived from the work of Zoumbos *et al.* (*46*), who demonstrated that some patients with aplastic anemia have large numbers of circulating Tac-positive cells. In this group of patients, the Tac-positive but not Tac-negative T cells were shown to inhibit hematopoiesis when cocultured with normal bone marrow cells. We demonstrated that anti-Tac inhibits the generation of activated suppressor T cells. Studies have been initiated to define the value of anti-Tac in the therapy of patients with aplastic anemia. The rationale for the use of an antibody to IL-2 receptors in recipients of renal and cardiac allografts is that anti-Tac inhibits the proliferation of T cells in response to foreign histocompatibility antigens expressed on the donor organs and prevents the generation of cytotoxic T cells in allogeneic cell cocultures. Furthermore, in studies by Strom *et al.* (*47*), the survival of renal and cardiac allografts was prolonged in rodent recipients treated with a monoclonal antibody to the IL-2 receptor. Thus, the development of monoclonal antibodies directed to the IL-2 receptor expressed on ATL cells, on autoreactive T cells of certain patients with autoimmune disorders, and on host T cells responding to foreign histocompatibility antigens on organ allografts may permit the development of rational new therapeutic approaches in these clinical conditions.

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Laboratory Experiments in Economics: The Implications of Posted-Price Institutions

CHARLES R. PLOTT

In recent years a methodology for performing laboratory experiments in economics has been developed. The object of the methodology is to integrate clearly motivated but largely subjectively determined human decisions with the organizational features of markets. The nature of the incentive system and the use of market organization as an independent variable are described. Initial results of basic research that involved assessment of the effects of the "posted-price" institution demonstrated that the effect of the institution is to raise prices and lower market efficiency. The existence of such effects and the close proximity of the laboratory posted-price institution to the rate-posting institution required by the government in several industries has led to a series of policy-related experiments. The results have also led to more basic research efforts on seemingly unrelated topics.

EXPERIMENTAL RESEARCH IN ECONOMICS HAS BEEN RAPIDly expanding for several years. The number of papers published annually has increased from two or three in the 1960's to over 70. Laboratory experimental research in economics was being done at no more than one U.S. university at a given time in the early 1970's, while more than 20 universities are involved now. Major topics have expanded from one area of applied game theory (the oligopoly problem) to include almost every subfield of economics and some of the management sciences. Research that was purely basic a few years ago has already had policy applications.

In this article I examine the experimental treatment of one topic that has contributed to the increased interest in the methods: the implications of posted-price institutions. The experimental methodology is explained and then the results and the applications are summarized.

Traditionally, economics has not enjoyed the benefits of an experimental methodology. Naturally occurring economic processes are so complex that complete experimental control with multiple replications defies the imagination. Yet, in spite of that seemingly insurmountable obstacle, the methodological posture taken by experimentalists is straightforward. General theories intended for application in complex markets should be expected to work when applied to the simple special cases. Such theories that do not work in the special cases should be discarded or modified. In order to create the necessary simple special cases, significant financial incentives are used to create markets in which buying and selling take place and in which people actually keep the profits they make. General theories

Charles R. Plott is a professor of economics, California Institute of Technology, Pasadena, CA 91125.