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IL-2 Receptors in Adult T-Cell Leukemia: A Target for Immunotherapy

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A. Introduction

The induction of a T-cell immune response to a foreign antigen requires the activation of T-lymphocytes that is initiated by the interaction of the T-cell antigen receptor with antigen presented in the context of products of the major histocompatibility locus and the macrophage-derived interleukin-1. Following this interaction, T cells express the gene encoding the lymphokine interleukin-2 (IL-2) [1, 2]. To exert its biological effect, IL-2 must interact with specific high-affinity membrane receptors. Resting T cells do not express IL-2 receptors, but receptors are rapidly expressed on T cells after activation with an antigen or mitogen [3–5]. 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.

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 that recognizes the human receptor for IL-2 [6–8] and blocks the binding of IL-2 to this receptor.

Using quantitative receptor binding studies employing radiolabeled anti-Tac and radiolabeled IL-2, it was shown that activated T cells and IL-2 dependent T-cell lines express 5- to 20-fold more binding sites for the Tac antibody than for IL-2 [9, 10]. Employing high concentrations of IL-2, Robb et al. [11] resolved these differences by demonstrating two affinity classes of IL-2 receptors. One had a binding affinity for IL-2 in the range of 10^{-11}–10^{12} M, whereas the remaining receptors bound IL-2 at a much lower affinity, approximately 10^{-8} or 10^{-9} M. The high-affinity receptors appear to mediate the physiologic responses to IL-2, since the magnitude of cell responses is closely correlated with the occupancy of these receptors. As outlined below, the anti-Tac monoclonal antibody has been utilized to: (a) characterize the human receptor for IL-2; (b) molecularly clone cDNAs for the human IL-2 receptor; (c) analyze disorders of IL-2 receptor expression on leukemic cells; and (d) develop protocols for the therapy of patients with IL-2 receptor-expressing adult T-cell leukemia and autoimmune disorders, and for individuals receiving organ allografts.

B. Chemical Characterization of the IL-2 Receptor

Using the anti-Tac monoclonal antibody, the IL-2 binding receptor on phytohemagglutinin (PHA)-activated normal lymphocytes was shown to be a 55-kd glycoprotein [7, 8]. Leonard and coworkers [7, 8] showed that the IL-2 receptor is composed of a 33-kd peptide precursor that is cotranslationally N-glycosylated to 35-kd and 37-kd forms and then θ-glycosylated to the 55-kd mature form. Furthermore, the IL-2 receptor was shown to be sulfated [12] and phosphorylated on a serine residue [13].

There are a series of unresolved questions concerning the IL-2 receptor that are diffi-

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cult to answer when only the 55-kd Tac peptide is considered. These questions include: (a) what is the structural explanation for the great difference in affinity between high- and low-affinity receptors; (b) how, in light of the short cytoplasmic tail of 13 amino acids (see below), are the receptor signals transduced to the nucleus; and (c) how do certain Tac-negative cells (e.g., natural killer cells) make nonproliferative responses to IL-2? To address these questions, we have investigated the possibility that the IL-2 receptor is a complex receptor with multiple peptides in addition to the one identified by anti-Tac. A leukemic T-cell line was identified that binds IL-2 yet does not bind four different antibodies (including anti-Tac and 7G7) that react with the Tac peptide. This cell line manifests 6800 receptors per cell with an affinity of 14 nM (Tsudo, Kozak, Goldman, and Waldmann, unpublished observations). On the basis of cross-linking studies using [^{125}I]IL-2, this IL-2-binding receptor peptide was shown to be larger than the Tac peptide with an approximate M_r of 75000. When similar cross-linking studies were performed on human T-lymphotrophic virus I (HTLV-I)-induced T-cell lines (e.g., HUT 102) that manifest both high- and low-affinity receptors, IL-2 binding peptides of both 55 kd and 75 kd were demonstrated.

C. Molecular Cloning of cDNAs for the Human 55-kd Tac IL-2 Receptor Peptide

Three groups [14–17] have succeeded in cloning cDNAs for the IL-2 receptor protein. The deduced amino acid sequence of the IL-2 receptor indicates that this peptide is composed of 251 amino acids and a 21-amino acid signal peptide. The receptor contains two potential N-linked glycosylation sites and multiple possible 0-linked carbohydrate sites. Finally, there is a single hydrophobic membrane region of 19 amino acids and a very short (13-amino acid) cytoplasmic domain. Potential phosphate acceptor sites (serine and threonine, but not tyrosine) are present within the intracytoplasmic domain. However, the cytoplasmic domain of the IL-2 receptor peptide identified by anti-Tac appears to be too small for enzymatic function. Thus, this receptor differs from other known growth factor receptors that have large intracytoplasmic domains with tyrosine kinase activity. Leonard and coworkers [15] have demonstrated that the single gene encoding the IL-2 receptor consists of eight exons on chromosome 10p14. However, mRNAs of two different sizes approximately 1500 and 3500 bases long have been identified. These classes of mRNA differ because of the utilization of two or more polyadenylation signals [14]. Receptor gene transcription is initiated at two principal sites in normal activated T-lymphocytes [15]. Furthermore, sequence analyses of the cloned DNAs also indicate that alternative messenger RNA splicing may delete a 216-base pair segment in the center of the protein coding sequence encoded by the fourth exon [14, 15]. Using expression studies of cDNAs in COS-1 cells, Leonard and coworkers [14] demonstrated that the unspliced but not the spliced form of the mRNA was translated into the cell surface receptor that binds IL-2 and the anti-Tac monoclonal antibody.

D. Distribution of IL-2 Receptors

As discussed above, the majority of resting T cells, B cells, or macrophages in the circulation do not display IL-2 receptors. Specifically, less than 5% of freshly isolated, unstimulated human peripheral blood T-lymphocytes react with the anti-Tac monoclonal antibody. The majority of 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, IL-2 receptors have also been demonstrated on activated B-lymphocytes [18, 19].

Rubin, Nelson, and their coworkers [20] have demonstrated that in addition to cellular IL-2 receptors, activated normal peripheral blood mononuclear cells and certain lines of T- and B-cell origin release a soluble form of the IL-2 receptor into the culture medium. Using an enzyme-linked immunoabsorbent assay, which employs two monoclonal antibodies that recognize distinct epitopes on the human IL-2 receptor, it was shown 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 a variety of 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 an important new approach to the analysis of lymphocyte activation in vivo.

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

A distinct form of mature T-cell leukemia was defined by Takasuki and coworkers [21] and termed adult T-cell leukemia (ATL). ATL is a malignant proliferation of mature T cells that have a propensity to infiltrate the skin. Cases of ATL are associated with hypercalcemia and have a very aggressive course in most cases. They are clustered within families and geographically, occurring in the southwest of Japan, the Caribbean basin, and in certain areas of Africa. Human T-cell lymphotrophic virus I has been shown to be a primary etiologic agent in ATL [22]. All the populations of leukemic cells we have examined from patients with HTLV-I-associated ATL expressed the Tac antigen [23]. The expression of IL-2 receptors on ATL cells differs from that of normal T cells. First, unlike normal T cells, ATL cells do not require prior activation to express IL-2 receptors. Furthermore, when a ^{3}H-labeled anti-Tac receptor assay was used, HTLV-I-infected leukemic T-cell lines characteristically expressed five- to tenfold more receptors per cell (270 000–1 000 000) than did maximally PHA-stimulated T-lymphoblasts (30 000–60 000). In addition, whereas normal human T-lymphocytes maintained in culture with IL-2 demonstrate a rapid decline in receptor number, adult ATL lines do not show a similar decline. Leonard et al. [12] and Wano et al. [24] also demonstrated that some but not all HTLV-I-infected cell lines display aberrantly sized IL-2 receptors owing to differences in glycosylation. It is conceivable that the constant presence of high numbers of IL-2 receptors on ATL cells and/or the aberrancy of these receptors may play a role in the pathogenesis of uncontrolled growth of these malignant T cells.

As noted above, T-cell leukemias caused by HTLV-I, as well as all T-cell and B-cell lines infected with HTLV-I, universally 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. In addition to the presence of typical long-terminal repeats (LTRs), *gag*, *pol*, and *env* genes, and retroviral gene sequences common to other groups of retroviruses, HTLV-I and HTLV-II contain an additional genomic region between *env* and the LTR referred to as *pX* or more recently as *tat*. Sodroski and colleagues [25] demonstrated that this *pX* or *tat* region encodes a 42-kd protein, now termed the *tat* protein, that is essential for viral replication. These authors demonstrated that the *tat* protein acts on a receptor region within the LTRs of HTLV-I and -II, stimulating transcription. Greene and co-workers [26] have demonstrated that this *tat* protein could also play a central role in directly or indirectly 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.

F. The IL-2 Receptor as a Target for Therapy in Patients with ATL and Patients with Autoimmune Disorders, and Individuals Receiving Organ Allografts

The observation that ATL cells constitutively express large numbers of IL-2 receptors identified by the anti-Tac monoclonal antibody, whereas normal resting cells and their precursors do not, provides the scientific basis for therapeutic trials using agents to eliminate the IL-2 receptor-expressing cells. The agents that have been used or are being prepared include: (a) unmodified anti-Tac monoclonal; (b) toxin (e.g., *Pseudomonas* toxin) conjugates of anti-Tac; and (c) conjugates of alpha-emitting isotopes (e.g., ^{212}Bis) with anti-Tac.

We initiated a clinical trial to evaluate the efficacy of intravenously administering anti-Tac monoclonal antibody in the treatment of patients with ATL [27]. None of the five patients treated suffered any untoward reactions and none produced antibodies to the mouse immunoglobulin or to the idiotype of the anti-Tac monoclonal antibody. Three of the patients with a very rapidly developing form of ATL had a very transient response. Two of the patients had a temporary partial or complete remission following anti-Tac therapy. In one of these patients, therapy was followed by a 5-month remission, as assessed by routine hematologic 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 5-month remission, the patient's disease relapsed, but a new course of anti-Tac infusions was followed by a virtual disappearance of skin lesions and an over 80% reduction in the number of circulating leukemic cells. Two months later, leukemic cells were again demonstrable in the circulation. At this time, although the leukemic cells remained Tac-positive and bound anti-Tac in vivo, 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 initially when he responded to anti-Tac therapy wherein 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 ability of toxins coupled to anti-Tac to selectively inhibit protein synthesis and viability of Tac-positive ATL lines. The addition of anti-Tac antibody coupled to *Pseudomonas* exotoxin inhibited protein synthesis by Tac-expressing HUT 102-B2 cells, but not that by the Tac-negative acute T-cell line MOLT-4, which does not express the Tac antigen [28].

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 is required for its action. 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. It was shown that ^{212}Bi, an alpha-emitting radionuclide conjugated to anti-Tac by use of a bifunctional chelate, was well suited for this role [29]. Activity levels of 0.5 μCi or the equivalent of 12 rad/ml of alpha radiation targeted by ^{212}Bi-anti-Tac eliminated over 98% 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 IgG. Thus, ^{212}Bi-anti-Tac is a potentially effective and specific immunocytotoxic agent for the elimination of IL-2 receptor-positive cells.

In addition to being used 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 and coworkers [30], who have demonstrated that select patients with aplastic anemia have increased 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. Furthermore, we have demonstrated that anti-Tac inhibits the generation of activated suppressor T cells (Oh-ishi and Waldmann, unpublished observations). 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 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 and coworkers [31], the survival of renal and cardiac allografts was prolonged in rodent recipients treated with an anti-IL-2 receptor monoclonal antibody. Thus, the development of monoclonal antibodies directed toward 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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