Interleukin 2 Receptor (Tac Antigen) Expression in HTLV-I-associated Adult T-Cell Leukemia

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Abstract

Interleukin-2 (IL-2) is a lymphokine synthesized by some T-cells following activation. Resting T-cells do not express IL-2 receptors, but receptors are rapidly expressed on T-cells following interaction of antigens, mitogens, or monoclonal antibodies with the antigen-specific T-cell receptor complex. Using anti-Tac, a monoclonal antibody that recognizes the IL-2 receptor, the receptor has been purified and shown to be a Mr 33,000 peptide that is posttranslationally glycosylated to a Mr 55,000 mature form. Normal resting T-cells and most leukemic T-cell populations do not express IL-2 receptors; however, the leukemic cells of the 11 patients examined who had human T-cell lymphotropic virus-associated adult T-cell leukemia expressed the Tac antigen. In human T-cell lymphotropic virus-I infected cells, the Mr 42,000 long open reading frame protein encoded in part by the pX region of this virus may serve as a transacting transcriptional activator that induces IL-2 receptor gene transcription, thus providing an explanation for the constant association of IL-2 receptor expression with adult T-cell lymphotropic virus-I-infected cells. The constant expression of IL-2 receptors may be aberrant may play a role in the uncontrolled growth of adult T-cell leukemia cells. Two patients with Tac-positive adult T-cell leukemia have been treated with the anti-Tac. One of the patients had 6- and 3-mo remissions of his leukemia following two courses of therapy with this monoclonal antibody directed toward this growth factor receptor.

Introduction

The induction of an immune response to a foreign antigen requires the activation of T-lymphocytes with receptors for the specific antigen. The human antigen-specific T-cell receptor has been shown to be a Mr 90,000 polymorphic heterodimer of α and β chains of approximately molecular weight of 40,000 to 50,000 associated with three nonpolymorphic peptide chains of molecular weight of 20,000 to 28,000 identified by the T-3 monoclonal antibody (1–3). T-cell activation is initiated following the interaction of antigen with this complex antigen-specific T-cell receptor. Two principal events occur at this point which are required for T-cell proliferation and the development of functionally active effector T-cells. First, following interaction with antigen and the macrophage-derived interleukin-1, T-cells synthesize and secrete the lymphokines IL-2 and IL-1. In order to exert its biological effects, IL-2 must interact with high-affinity-specific membrane receptors (6). Resting T-cells do not express IL-2 receptors, but receptors are rapidly expressed on T-cells following activation with antigen or mitogen. Thus, both the growth factor interleukin-2 and its receptor are absent in resting T-cells, but following activation, the genes for both proteins become expressed. Thus, both the production of interleukin-2 and the expression of the interleukin-2 receptors are pivotal events in the full expression of the human immune response. While the antigen confers specificity for a given immune response, the interaction of IL-2 and IL-2 receptors determines its magnitude and duration.

The specific membrane receptor for IL-2 on human lymphocytes has been identified using a monoclonal antibody (anti-Tac) directed towards this molecule (7–9). The anti-Tac monoclonal antibody, prepared by Uchiyama et al. (7), was selected on the basis of its ability to bind to activated T-cells but not to resting T-cells, B-cells, or monocytes. This pattern of cellular reactivity was identical with the distribution of IL-2 receptors reported by Robb et al. (6). We therefore hypothesized that anti-Tac recognizes the human receptor for IL-2 (8, 9). Data in support of this hypothesis are as follows: 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; anti-Tac blocks over 95% of the binding of [3H]IL-2 to HUT 102-B2 cells and PHA-activated lymphoblasts; and IL-2 at high concentration blocks the binding of [3H]-labeled anti-Tac to PHA-activated lymphoblasts. The requirement for high concentrations of IL-2 is explained by the observation that anti-Tac identifies two species of IL-2 receptors, one with a high (10−12 M) and one with a lower affinity of IL-2 binding. Furthermore, Robb and Greene (10) demonstrated that an initial passage of radiolabeled proteins from PHA 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.

Utilizing the anti-Tac monoclonal antibody, we have defined a variety of T- and B-lymphocyte functions that require an interaction of IL-2 with its inducible receptor on activated lymphocytes (11, 12). The addition of anti-Tac to in vitro culture systems blocked the IL-2-induced DNA synthesis of IL-2-dependent T-cell lines and inhibited soluble auto- and alloantigen-induced T-cell proliferation. Furthermore, it abrogated the generation of cytotoxic and suppressor effector T-cells but did not inhibit their action once generated. The anti-receptor antibody also inhibited the proliferation and immunoglobulin synthesis of purified B-cells stimulated with Staphylococcus Cowan strain I organisms.

In the present study the anti-Tac monoclonal antibody was used to characterize IL-2 receptor expression in HTLV-I-associated adult T-cell leukemia. Furthermore, we report the initial results of a clinical trial to evaluate the efficacy of i.v. administered leucovorin rescue.
anti-Tac monoclonal antibody in the treatment of patients with the adult T-cell leukemia.

Materials and Methods

Patient Population. T-cells were studied from 17 normal individuals, 10 patients with the Sézary leukemia who did not have antibodies to HTLV-I, 11 patients with the ATL; 5 patients with acute T-cell leukemia, and the lines CCRF-CEM, Molt-4, RPMI-84027, and CCRF-HSB-2 derived from patients with acute T-cell leukemia (13). The patients with the diagnosis of the Sézary syndrome presented with exfoliative dermatitis, lymphadenopathy, and a white blood count ranging from 12,600 to 124,000 cells/mm³. The patients with ATL had skin involvement usually in the form of plaque-like lesions and had white blood counts ranging from 11,200 to 105,000/mm³. Six of the patients were hypercalcemic. Each of the patients with ATL had serum antibodies to the M, 19,000 core protein of HTLV-I and had incorporated this virus into the genome of the leukemic cells (14).

Immunofluorescence Analysis of Cells with Fluorescence-activated Cell Sorter. Peripheral blood mononuclear cells were analyzed using immunofluorescence and fluorescence-activated cell sorter analysis by a slight modification of a procedure described previously (15). The cells of patients who were not being treated with the monoclonal antibody were analyzed by indirect immunofluorescence using anti-Tac and 7G7, two monoclonal antibodies to different epitopes of the IL-2 receptor; 3A-1, an antibody to an antigen expressed on normal T-cells but not on Sézary or adult T-cell leukemic cells (16); and the T4, T8, and T9 antibodies (Ortho Pharmaceuticals, Raritan, NJ) and the Leu 4 antibody (Becton-Dickenson & Co., Sunnyvale, CA) as the first antibody and FITC-conjugated goat anti-mouse immunoglobulin (Coulter Electronics, Hi-ateah, FL) as the second antibody. Following therapy with anti-Tac, the T-cells of the patients were analyzed by direct immunofluorescence using FITC conjugates of goat anti-mouse immunoglobulin, anti-Tac, 7G7, 3A-1, T9, and Leu 4. FITC-conjugated goat IgG was used as the negative control.

Determination of Tβ Gene Configuration. The arrangement of the Tβ genes in the leukemic T-cells of a patient with ATL at various periods prior to and following therapy with anti-Tac was analyzed and compared to the arrangement of these genes in peripheral blood buffy coat cells of normal individuals, and in a B-cell line that was established from the patient using the Epstein-Barr virus. High molecular weight DNA was extracted from the leukemic and normal cells and was digested to completion with BanHI, size fractionated over agarose gels by electrophoresis, and transferred to nitrocellulose paper (17, 18). Such paper-bound DNA fragments were then hybridized to a random primed [32P]DNA probe to the constant domain of the Tβ gene (19) obtained from Mark Davis. After washes at the appropriate stringency, the Tβ gene configurations were visualized on autoradiograms.

Results and Discussion

IL-2 Receptor Expression in Adult T-Cell Leukemia. Peripheral blood mononuclear cells from patients with three forms of T-cell leukemia—acute T-cell leukemia, the Sézary leukemia, and adult T-cell leukemia—were analyzed for IL-2 receptor expression using indirect immunofluorescence using the anti-Tac monoclonal antibody. The acute T-cell leukemic populations and lines derived from such cells did not express IL-2 receptors. Furthermore, nine of the ten populations of Sézary leukemic T-cells not associated with HTLV-I examined were Tac antigen negative (13). In contrast, all of the populations of leukemic cells from the 11 patients studied with the adult T-cell leukemia associated with HTLV-I expressed the Tac antigen. Thus, the demonstration of IL-2 receptors on leukemic T-cells may aid in differentiating those leukemias caused by HTLV-I, which are Tac antigen positive, from other forms of T-cell leukemia which are, in general, Tac antigen negative.

The IL-2 receptor expression on adult T-cell leukemic cells differed from that on normal T-cells. First, unlike normal T-cells, ATL cells do not require prior activation to express the IL-2 receptors. Furthermore, using a [3H]-labeled anti-Tac receptor assay, HTLV-I infected leukemic T-cell lines characteristically expressed 5- to 10-fold more receptors per cell (270,000 to 640,000) than did maximally PHA-stimulated T-lymphoblasts (30,000 to 60,000) (20). Since the cell volume of these leukemic cells was only 13% greater than that of PHA lymphoblasts, the density of IL-2 receptors on these leukemic cells was correspondingly increased. In addition, whereas normal PHA-stimulated human T-lymphocytes maintained in culture with IL-2 demonstrated a rapid decline in receptor number from a maximum of 20,000 receptors per cell on Day 3 to approximately 5,000 on Day 13, the ATL lines did not show a similar decline. Furthermore, we have noted that some, but not all, HTLV-I infected continuous T-cell lines display aberrantly sized IL-2 receptors (21, 22). For example, the receptor on HTLV-I infected HUT 102-B2 cells is approximately 5,000 daltons smaller than that on PHA lymphoblasts. Using pulse chase, tunicamycin, endoglycosidase, and neuraminidase analyses, the difference in receptor size was shown to be due to differences in posttranslational modification of the M, 33,000 protein backbone (21, 22). Furthermore, the receptors on the HUT 102-B2 cells manifested less sulfation than did normal receptors. Finally, Uchiyama and coworkers (23, 24) have reported that IL-2 receptors on ATL cells are modulated (down-regulated) by anti-Tac (unlike normal activated T-cells) and that IL-2 receptors on ATL cell lines were spontaneously (IL-2 independently) phosphorylated, whereas the phosphorylation of receptors on PHA-stimulated T-cells requires the addition of IL-2. It is conceivable that the constant presence of high numbers of IL-2 receptors on the adult T-cell leukemic cells and/or the aberrancy of these receptors may play a major role in the pathogenesis of uncontrolled growth of these malignant T-cells.

As noted above, the T-cell leukemias caused by HTLV-I express the Tac antigen. In addition, all T-cell and the five B-cell lines we examined that were infected with HTLV-I universally expressed large numbers of IL-2 receptors. A recent report by Sodorski et al. (25) 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. (26). In addition to the gag, pol, env, and LTR sequences characteristic of other groups of retroviruses, HTLV-I was shown to contain an additional genomic region between env and the LTR referred to as pX. Sodorski et al. (25) demonstrated that a M, 38,000 to 42,000 protein termed LOR is encoded, in large measure, by the 3' 1000 bases of the pX region and that this LOR protein may act as a transacting regulator of transcription. They demonstrated that the LOR protein acts on regulatory sequences in the LTRs of HTLV-I, -II, and -III stimulating transcription. The LOR protein could theoretically 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 leukemogenesis. With the cloning of the gene encoding the IL-2 receptor (27), this hypothesis that the LOR protein acts as a transacting regulator of transcription of the IL-2 receptor gene can now be readily tested.
Treatment of Patients with Adult T-Cell Leukemia with the Anti-Tac Monoclonal Antibody. We have initiated a clinical trial to evaluate the efficacy of i.v. administered anti-Tac monoclonal antibody in the treatment of patients with the adult T-cell leukemia. The scientific basis for these studies is the observation that adult T-cell leukemia cells express the Tac antigen, whereas normal resting T-cells and their precursors do not. Two patients with adult T-cell leukemia have been treated with i.v. administered anti-Tac. Neither patient suffered any untoward reactions nor did they produce antibodies reactive with mouse immunoglobulin or the idiotype of the anti-Tac monoclonal. One patient with a very rapidly developing form of adult T-cell leukemia had a very transient response. However, therapy of the other patient was followed by a 6-mo remission as assessed by regression of skin lesions, routine hematomatological tests, immunofluorescence analysis, and molecular genetic analysis of the arrangement of T-cell receptor β chain genes. The patient, a 24-yr-old black man, developed cryptococcal meningitis and papular neoplastic skin lesions that showed diffuse mixed lymphoma on histological examination. The patient was treated with nine cycles of Pro-MACE-MOPP chemotherapy and had a complete remission lasting 26 mo. He relapsed with recurrent papular adenopathy, hypercalcemia, and a peripheral blood WBC count of 26,800/mm³. He achieved a second complete remission on Pro-MACE-MOPP chemotherapy and had a series of remissions and relapses. Five mo after the last course of Pro-MACE-MOPP chemotherapy, the patient had a relapse with 2,200 malignant T-cells/mm³ in the circulation as assessed by immunofluorescence analysis using the anti-Tac monoclonal antibody. Furthermore, 1,200 of these circulating lymphocytes/mm³ reacted with T9, an antibody to the transferrin receptor, which is a receptor expressed on the malignant but not on normal circulating T-cells.

The patient was then treated with three i.v. infusions (20, 40, and 40 mg) of the anti-Tac monoclonal antibody over a 10-day period. Following the anti-Tac therapy, there was a decline in the number of circulating T-cells bearing the Tac antigen from 2,200 to less than 100/mm³ and of transferrin receptor expressing T-cells from 1,200 to less than 100/mm³. During the 4-wk period following the anti-Tac infusions, there were no cells with free IL-2 receptors, i.e., with receptors unblocked by the infused anti-Tac monoclonal, whereas small numbers of circulating cells with anti-Tac blocking the IL-2 receptor were identified. Cells with blocked IL-2 receptors were identified as cells that were not reactive with FITC-conjugated anti-Tac but were reactive with FITC-conjugated anti-mouse IgG and with the 7G7 monoclonal antibody, an antibody that identifies an epitope of the IL-2 receptor peptide other than that identified by anti-Tac. The remission of the T-cell leukemia in this patient was confirmed utilizing molecular genetic analysis of the arrangement of the genes encoding the β chain of the antigen-specific T-cell receptor. Southern analysis of the arrangement of the T-cell receptor β chain genes was performed on BamHI digests of DNA from peripheral blood mononuclear cells of the patient using a radio-labeled probe to the constant region of the Tβ chain. The constant Tβ genes were universally present on a 24-kilobase BamHI fragment in germ-line tissues of normal individuals and in a B-cell line derived from the patient. However, prior to therapy, there was an additional 22-kilobase BamHI band hybridizing with the constant Tα probe when digests of the patient’s circulating T-cells were examined, a hallmark of a clonal expansion of T-lymphocytes. This band reflecting the clonally rearranged T-cell receptor gene was not demonstrable on specimens obtained following anti-Tac therapy when the patient was in remission. Six mo following the initial remission, the leukemia recurred with a reappearance of circulating leukemic cells, identified by immunofluorescence and molecular genetic analysis, and with the development of large (5 x 7 x 1 cm) malignant skin lesions. A second course of three i.v. infusions of 20 mg each of anti-Tac over a 10-day period was followed by the virtual disappearance of the skin lesions and an over 90% reduction in the number of circulating leukemic cells. Three mo subsequently leukemic cells were again demonstrable in the circulation. At this time the leukemia was no longer responsive to infusions of anti-Tac, and the patient required further chemotherapy.

There was no toxicity associated with the anti-Tac therapy nor was there a reduction in the number of any of the normal formed elements of the blood including normal T-cells. Normal T-cells could be identified by immunofluorescence analysis, since they expressed the antigen defined by the 3A-1 monoclonal antibody, whereas the leukemic cells did not express this antigen. The patient did not produce antibodies to mouse IgG nor to the idiotype of anti-Tac as assessed using an enzyme-linked immunosorbent assay procedure.

These therapeutic studies have been extended *in vitro* by examining the efficacy of toxins coupled to anti-Tac in selectively inhibiting the protein synthesis and the viability of Tac-positive adult T-cell leukemic cell lines. The addition of anti-Tac antibody coupled to the A chain of the toxin ricin effectively inhibited protein synthesis by the HTLV-I-associated, Tac-positive adult T-cell leukemia line HUT 102-B2. In contrast, conjugates of ricin A with a control monoclonal of the same isotype did not inhibit protein synthesis when used in the same concentration (10⁻⁸ M) (28). The inhibitory action of anti-Tac conjugated with ricin A could be abolished by the addition of excess unlabeled anti-Tac or IL-2. In parallel studies performed in collaboration with D. FitzGerald et al. (29), Pseudomonas exotoxin conjugates of anti-Tac inhibited the protein synthesis by HUT 102-B2 cells but not that of the acute T-cell leukemia line Molt-4 that does not express the Tac antigen. Again, the toxicity of the anti-Tac toxin conjugates could be inhibited by adding excess unlabeled anti-Tac. Thus, the development of toxin conjugates of the monoclonal anti-Tac that is directed toward the IL-2 receptor expressed on adult T-cell leukemic cells may permit the development of a rational approach for the treatment of this almost uniformly fatal form of leukemia.

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