Therapy of Patients With Human T-Cell Lymphotrophic Virus I–Induced Adult T-Cell Leukemia With Anti-Tac, a Monoclonal Antibody to the Receptor for Interleukin-2

By Thomas A. Waldmann, Carolyn K. Goldman, Kathleen F. Bongiovanni, Susan O. Sharrow, Michael P. Davey, Kemp B. Cease, Steven J. Greenberg, and Dan L. Longo

Human T-cell lymphotropic virus I (HTLV-I)-induced adult T-cell leukemia (ATL) cells constitutively express interleukin-2 (IL-2) receptors identified by the anti-Tac monoclonal antibody (MoAb), whereas normal resting cells do not. This observation provided the scientific basis for a trial of intravenous anti-Tac in the treatment of nine patients with ATL. The patients did not suffer untoward reactions and did not have a reduction in the normal formed elements of the blood, and only one of the nine produced antibodies to the anti-Tac MoAb. Three patients had transient mixed, partial, or complete remissions lasting from 1 to more than 8 months after anti-Tac therapy, as assessed by routine hematologic tests, immunofluorescence analysis of circulating cells, and molecular genetic analysis of HTLV-I provirus integration and of the T-cell receptor gene rearrangement. The precise mechanism of the antitumor effects is unclear; however, the use of a MoAb that prevents the interaction of IL-2 with its receptor on ATL cells provides a rational approach for the treatment of this malignancy.

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The use of chemotherapeutic agents has resulted in the cure of some types of human cancer. However, many types of cancer either initially are unresponsive or subsequently acquire resistance to chemotherapy. The hybridoma technique of Kohler and Milstein has rekindled interest in the use of antibodies as therapeutic agents in the treatment of cancer patients. Many in vitro studies have shown selective high-affinity binding of monoclonal antibodies (MoAb) to tumor cells. Furthermore, certain unconjugated murine MoAbs have had antitumor activity against human tumor xenografts in nude mice, and have, in certain cases, been curative in animal tumor models. MoAbs directed against tumor-associated antigens have been used since 1981 in at least 25 clinical trials that have involved 13 unconjugated murine MoAb. Twenty-three partial and three complete remissions were reported in the 185 patients included in these studies. In the remaining 86% of the cases, however, there were no beneficial clinical responses. There have been a number of explanations for the low therapeutic efficacy observed. These include the blocking effect of circulating antigen shed from the tumor, the modulation of the antigenic target from the surface of the tumor without cell death, the development of human anti-mouse antibodies in the patients treated with the mouse MoAb, and the emergence of tumor variants that do not express the antigenic target of the MoAb. Furthermore, many of the MoAbs used are not cytotoxic or cytostatic against neoplastic cells in humans. Specifically, the interaction of most of the antibodies with their target tumor cells did not lead to complement-mediated cytotoxicity, because the antibodies did not fix human complement. Furthermore, the antibodies used were not effective in antibody-mediated cellular cytotoxicity. Finally, in most cases the antibodies were not directed against a vital structure present on the surface of the malignant cells, such as a receptor for a factor required for tumor cell proliferation.

In this study we used the anti-Tac MoAb, an antibody directed against the receptor for a T-cell growth factor, interleukin-2 (IL-2), in the treatment of patients with adult T-cell leukemia (ATL), a leukemia of cells that constitutively express large numbers of IL-2 receptors. IL-2 plays a pivotal role in T-cell proliferation. Cellular immune responses require that T cells change from a resting to an activated state. Antigen-induced activation of resting T cells induces the synthesis of IL-2. To exert its biologic effect, IL-2 must interact with specific high-affinity membrane receptors. Most resting T cells do not express high-affinity IL-2 receptors, but receptors are expressed on T cells after activation. We have identified two IL-2 binding peptides, a 55Kd (Tac) and a 75Kd non-Tac IL-2 binding peptide. High-affinity IL-2 receptors are expressed when the p55 and p75 IL-2 binding peptides are associated in a multichain receptor complex. We have prepared a mouse monoclonal IgG2a antibody termed anti-Tac that recognizes the IL-2 binding site of the 55Kd peptide and thus blocks the binding of IL-2 to this receptor, thereby preventing T-cell proliferation. As noted previously, most resting T cells, B cells, and monocytes do not express the IL-2 receptor peptide identified by the anti-Tac monoclonal antibody. In contrast, the malignant T cells of patients with human T-cell lymphotrophic virus I (HTLV-I)-induced ATL constitutively express the Tac antigen. ATL is a malignant proliferation of mature T cells that have a propensity to infiltrate the skin, lungs, and numerous other organs. Cases of ATL are frequently associated with hypercalcemia, an immunodeficiency state, and a very aggressive clinical course. This form of leukemia is clustered within families and geographically, occurring in the southwest of Japan, in the Caribbean basin, and in certain areas of Africa. HTLV-I is the
primary etiologic agent in ATL. Furthermore, all populations of leukemic cells we have examined from patients with HTLV-I–associated ATL have expressed the Tac antigen. The observation that ATL cells constitutively express large numbers of IL-2 receptors identified by the anti-Tac MoAb (most normal resting cells and their precursors do not) provided the scientific basis for this therapeutic trial using the anti-Tac MoAb to eliminate IL-2 receptor-expressing cells. The elimination of Tac-expressing cells would theoretically lead to selective elimination of ATL cells and retention of the Tac-nonexpressing resting normal T cells and their precursors that express the full antigen recognition repertoire required for T-cell immune responses. This approach using an antibody to an activation antigen not expressed on resting T cells should be more tumor-specific than others using antibodies directed toward antigens expressed on all T cells (eg, the CD3 or CD2 antibodies) that would eliminate both normal and malignant cells. We now report on the use of unmodified anti-Tac antibody in the treatment of nine patients with HTLV-I–induced ATL.

MATERIALS AND METHODS

Patient population. Nine patients with a histologic confirmed diagnosis of ATL were studied (Tables 1–3). Each of the patients fulfilled the eligibility requirements by manifesting the following features: (1) a leukemia of mature T cells with polymorphic, indented, or lobulated nuclei; (2) expression of Tac antigen on at least 10% of the circulating lymphocytes; (3) antibodies to HTLV-I demonstrable in the serum; (4) a monoclonal expansion of T cells, as demonstrated by molecular genetic analysis of the T-cell receptor β gene rearrangement and by a clonal integration of the retrovirus HTLV-I into the genome of circulating T cells; and (5) omission of cytotoxic chemotherapy for at least 4 weeks. Patients with and without previous chemotherapy were eligible for inclusion in the study.

Therapeutic study plan. In the basic study plan, 20 mg of unmodified anti-Tac was administered intravenously (IV) on two occasions during the first week of therapy, and 40 mg was administered on two occasions during the second week of therapy. Approximately 80 mg of anti-Tac was required to saturate the Tac receptors in patients with Tac-expressing leukemias. Dose schedules were modified slightly, and additional 20 mg or 50 mg doses were administered during subsequent weeks, as indicated in Table 3, to maintain saturation of the IL-2 receptors expressed on the leukemic cells with the anti-Tac monoclonal. The leukemic cells were felt to be saturated when they were no longer reactive with fluorochrome-labeled anti-Tac. Furthermore, in such cases, sufficient anti-Tac was administered to yield measurable levels of mouse IgG in the circulation.

The criteria for responses were as follows: (1) complete response—disappearance of all measurable and evaluable disease lasting more than 1 month; (2) partial response—reduction by 50% of leukemic cell count or 50% reduction in the size of a measurable lesion and no increase in size of any measurable or evaluable lesion or appearance of a new lesion for 1 month; (3) mixed response—identical to partial response with the exception that there is the appearance of a new lesion within a month in a tissue other than that involved initially.

Production of anti-Tac MoAb. The anti-Tac MoAb was produced as described previously by fusion of NS-1 mouse myeloma cells with spleen cells of mice that had been immunized with a cell line derived from an ATL patient. The antibody does not function in complement-mediated cytotoxicity with human plasma but does not induce antibody-mediated cellular cytotoxicity. However, it does block the interaction of IL-2 with the high-affinity receptors for this lymphokine. Large quantities of the antibody were produced by inoculating hybrid cells into the peritoneal cavity of BALB/c mice and then purifying the IgG2a anti-Tac MoAb from the resulting ascites fluid by diethylaminoethyl cellulose (DEAE) chromatography. The material was dialyzed against saline, centrifuged, filtered, and precipitated with 20% sodium sulfate, and then diluted in saline at pH 7.4 to a concentration of 2 mg/mL. Each lot of the product was assayed by immunoelectrophoresis and diffusion in agar plates using antisera to IgG2a, IgG, and IgM as well as polyvalent antibodies to most mouse proteins. Lots greater than 98% pure as assessed by these analyses, as well as by high-performance liquid chromatography (HPLC) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), were used. The MoAb preparation was sterilized by passage through a 0.22 mm millipore filter by the Pharmaceutical Development Section of the Clinical Center of the National Institutes of Health (NIH) and was shown to be nonpyrogenic and sterile by the Bureau of Biologics.

Immunofluorescence analysis of cell surface phenotype. The phenotype of the leukemic cell population was defined by indirect and direct immunofluorescence performed with mouse MoAbs, using a fluorescence-activated cell sorter, as described elsewhere. Cells were tested with MoAbs to HLA-DR (Ia-1, Ortho, Raritan, NJ); human T-cell–associated antigens (CD2, CD3, CD4, CD5, CD8, and antitransferrin receptor antibodies from Ortho, and Becton Dickinson, Mountain View, CA); 3A1 (CD7) (a gift from Dr

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>ATL Involvement of Skin</th>
<th>Other Clinical Features</th>
<th>Serum Calcium (mEq/L)</th>
<th>Abnormal Liver Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>F</td>
<td>B</td>
<td>—</td>
<td>Thrombophlebitis, chronic Isospora belli infestation, posthepatitis</td>
<td>5.6</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>M</td>
<td>B</td>
<td>—</td>
<td>Thrombophlebitis</td>
<td>5.0*</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>M</td>
<td>B</td>
<td>—</td>
<td>Thrombophlebitis</td>
<td>6.9</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>M</td>
<td>B</td>
<td>—</td>
<td>Pneumonia</td>
<td>9.2</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>M</td>
<td>B</td>
<td>—</td>
<td>Pneumonia</td>
<td>8.0</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>F</td>
<td>B</td>
<td>—</td>
<td>Pneumocystis pneumonia</td>
<td>7.6</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>F</td>
<td>B</td>
<td>—</td>
<td>Pneumocystis pneumonia</td>
<td>8.7</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>M</td>
<td>W</td>
<td>—</td>
<td>Pneumocystis pneumonia</td>
<td>7.2</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>F</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: B, black; W, white.

*In previous admissions, the patient’s serum calcium was elevated with a value as high as 7.4 mEq/L. The normal range for serum calcium was 4.5-5.3 mEq/L.
mustard (medorethamine). Oncovin (vincristine), prednisone,

was determined from the product of the circulating WBC from frozen cell suspensions containing approximately

number of rearrangements and for HTLV-I integration were performed as

ANTI-TAC THERAPY OF ATL 1807

and 7G7/B6) to different epitopes of the 55-kD IL-2

receptor

were integrated
to determine

reagent was obtained from Coulter. Histograms for each cell
type

peptide. The fluorescein isothiocyanate (FITC) antimouse IgG fluorography.

er/inducer

activity, respecitvely; FL), technology, Hialeah, Barton Haynes);? 2H4 (CD45R) and 4B4 (CD29) (Coulter Immu-
nology, Hialeah, FL), associated with suppressor/inducer and help-
er/inducer activity, respectively; and with two antibodies (anti-Tac
and 7G7/B6) to different epitopes of the 55-kD IL-2 receptor
peptide. The fluoroescein isothiocyanate (FITC) antimouse IgG
reagent was obtained from Coulter. Histograms for each cell
type were integrated to determine the percentage of the mononuclear
cells that reacted with individual MoAbs. The absolute number of
cells in the circulation per cubic millimeter expressing a particular
antigen was determined from the product of the circulating WBC

count per cubic millimeter, the proportion of these circulating WBCs
that were mononuclear cells as determined by routine hematologic
analysis, and the proportion of these mononuclear cells that
expressed the antigen under study as assessed by immunoce-tyco-
fluorography.

Molecular genetic analysis of T-cell receptor gene rearrange-
ment and HTLV-I integration. Assays for clonal T-cell receptor gene
rearrangements and for HTLV-I integration were performed as
described previously.36 High molecular weight DNA was extracted
from frozen cell suspensions containing approximately 10⁶ cells. The

Table 2. Hematologic and Phenotypic Analysis of ATL Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Previous Therapy</th>
<th>Dose of Anti-Tac Administered</th>
<th>Level of Circulating Mouse IgG (ng/mL)*</th>
<th>Toxicity Observed</th>
<th>Reduction in Normal Formed Elements of Blood</th>
<th>Development of Anti-Tac Antibodies</th>
<th>Clinical Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ProMACE MOPP</td>
<td>20, 60 mg doses; total dose 180 mg over eight days</td>
<td>5,740</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Mixed; WBC reduced from 26,500 (56% normal) to 4,300 (none abnormal). Number of Tac-positive cells reduced from 8,200 to less than 200/μL. Lymph node enlargement involved with neoplastic cells developed 13 days after initiation of therapy.</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>20, 50 mg doses; total dose 490 mg over 51 days</td>
<td>5,530</td>
<td>None</td>
<td>400 ng/mL of anti-Tac 5 months after anti-Tac initiated, compared with 120 ng/mL prior to therapy</td>
<td>Partial remission persistent for over 8 months; number of Tac-positive cells reduced from 23,300 to 340/μL; developed Kaposis sarcoma</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ProMACE MOPP</td>
<td>20, 40 mg doses; total dose 220 mg over 445 days</td>
<td>6,300</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Complete clinical remission lasting 5 months; partial remission lasting about 6 weeks on retreatment.</td>
</tr>
<tr>
<td>4</td>
<td>Deoxycoformycin</td>
<td>20, 40 mg doses; total dose 140 mg over ten days</td>
<td>12,160</td>
<td>None</td>
<td>Platelet count reduced to 87,000/μL.</td>
<td>None</td>
<td>No response; stable disease</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>1, 20, 50 mg doses; total dose 141 mg over 16 days</td>
<td>1,060</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>No response; stable disease; progressive pulmonary Pneumocystis carini</td>
</tr>
<tr>
<td>6</td>
<td>Cyclophosphamide, etoposide, Adriamycin methotrexate</td>
<td>20, 40 mg doses; total dose 100 mg over five days</td>
<td>1,030</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>7</td>
<td>ProMACE MOPP</td>
<td>20, 50, 100 mg doses; total dose 220 mg over seven days</td>
<td>12,230</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>No response; stable disease</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>20, 50 mg doses; total dose 120 mg over five days</td>
<td>690</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>No response; progressive disease</td>
</tr>
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</table>
| 9       | None             | 20, 50 mg doses; total dose 220 mg over nine days | 595                                    | None              | None                                       | None                             | No response; stable disease; Staphylo-
coccus aureus septicemia |

Abbreviations: ProMACE MOPP, Prednisone, methotrexate, adriamycin (doxorubicin), cytoxan (cyclophosphamide)-etoposide (VP-18), nitrogen mustard (medorethamine), oncovin (vincristine), prednisone, and procarbazine; COP, cyclophosphamide-ovcin (vincristine) prednisone.  *24 hours after completion of first course of therapy.

Table 3. Effect of Anti-Tac Therapy

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DNA samples were digested with the restriction enzymes BamHI, EcoRI, and HindIII (International Biotechnologies, New Haven, CT, and New England Biolabs, Beverly, MA) and were size fractionated on 0.5% to 0.9% agarose gels. They were transferred by Southern blot technique to nitrocellulose paper. Hybridizations to randomly primed 32P-labeled DNA probes of the constant regions of the Tβ gene and the HTLV-I gene were performed, followed by washing at appropriate stringency and radioautography. Nonlymphoid control DNA was run simultaneously to identify the germline positions of the T-cell receptor genes examined. The Tβ gene probe used was a 700-basepair (bp) EcoRI fragment containing the mouse Cβ region that recognized both human Cβ regions. The HTLV-I probe used was the 9-kb SalI fragment containing the entire viral genome (a gift from Dr Flossie Wong-Staal).

**Assay for human antimouse anti-Tac antibody.** The sera used in all assays were separated from peripheral blood and stored at −20°C until used. For detection of human antimouse antibodies, the technique described by Schroff et al11 was used with the exception that anti-Tac was substituted for the T101 or 9.2.27 antibodies. Serum antiglobulin levels for a given patient were considered significantly increased when antiglobulin levels after therapy were greater than twice that in the sample obtained before administration of mouse MoAbs.

**Assay for mouse Ig.** Murine IgG2a anti-Tac levels in the sera of patients were assayed in an enzyme-linked immunosorbent assay (ELISA) technique using affinity-purified goat antimouse immunoglobulin (Ig) absorbed onto polyvinyl plates at 100 ng per well and washed. Bound murine anti-Tac was detected with a goat antimagglum Ig conjugated with alkaline phosphatase (Sigma, St Louis) and compared with a standard curve of anti-Tac antibody.

**Assays for antibodies to HTLV-I.** The sera of ATL patients were analyzed for antibodies to disrupted and inactivated HTLV-I by use of an ELISA.22

## RESULTS

Nine patients with histologically confirmed HTLV-I–induced ATL were treated with IV administered anti-Tac MoAb. The T-cell leukemic populations were shown to be monoclonal by use of molecular genetic analysis of the rearrangement of the genes encoding the β chain of the antigen-specific T-cell receptor. Southern blot analysis of the T-cell β receptor gene rearrangement, using a radiolabeled probe that hybridizes with the constant region of the T-cell β chain, revealed a band that was not present in germline tissues, the hallmark of a clonally expanded population of T lymphocytes.

A summary of the remaining demographic factors and pertinent medical history for the study population is shown in Table 1. Five of the patients were men and four were women; eight were black and one was white. The patients ranged in age from 24 to 60 years (mean, 42 years). Three of the patients manifested involvement of the skin. Six were hypercalcemic, with the serum calcium ranging from 6.9 to 9.2 mEq/L. Mild to moderate liver function abnormalities were demonstrable in eight of the cases.

Results of flow cytometric phenotypic analysis of the circulating mononuclear cells were shown in Table 2. The WBC count ranged from 3,800 to 102,800/μL with 10% to 80% of these circulating cells defined as abnormal (malignant) lymphocytes on routine light microscopic examination. In eight cases, the predominant leukemic cell population expressed the CD3+/CD4+/CD8− phenotype. The phenotype in the remaining case was CD3+/CD4+/CD8−. Forty percent to 90% of the circulating mononuclear cells expressed the Tac antigen. The transferrin receptor was expressed on 13% to 71% of the circulating cells. In five of the six cases examined, the cells expressed the 4B4 antigen.

In eight of the nine cases, the abnormal cell population did not react with a CD7 (3A1) MoAb, an antibody that reacts with normal T-cell precursors and with 70% of normal mature T lymphocytes.19

Response of ATL patients to treatment with anti-Tac MoAb. The basic protocol for anti-Tac therapy involved the administration of 20 mg per patient on two occasions during the first week and 40 mg of anti-Tac on two occasions during the second week. The dosage was altered in some cases to achieve rapid saturation of the IL-2 receptors as assessed by flow cytometric immunofluorescence analysis (see information that follows). After the second week of therapy, additional doses of 20 mg or 50 mg of anti-Tac were administered to patients who had made an initial clinical response to anti-Tac therapy. Anti-Tac was administered IV in 100 mL of saline containing 5% albumin over a two-hour period. The maximum levels of mouse antibody achieved in the serum 24 hours after the last therapeutic dose ranged from 595 to 12,230 ng/mL in the different patients (Table 3).

The nine patients did not have any untoward acute reactions. One patient manifested a transient increase in plasma uric acid to 19.8 mg/dL, without sequelae, and one patient had progression of a Pneumocystis carinii infection during therapy with anti-Tac and subsequent chemotherapy. Anti-Tac would inhibit cell-mediated immune responses to antigens presented to the host during the period of therapy. However, it is much less immunosuppressive than MoAbs (eg, CD3 or antithymocyte globulin) that react with all T cells with their full repertoire of antigen receptors. There was no reduction below the limits of the control range in any of the normal formed elements of the blood, including platelets, granulocytes, or RBCs, with the exception of a reduction of the platelet count to 87,000/μL in one patient. Only one of the nine patients produced antibodies to anti-Tac, as assessed with an ELISA procedure. The patient who produced anti-Tac antibodies manifested these antibodies at low concentrations (400 ng/mL v the pretreatment value of 120 ng/mL) during an anti-Tac–induced clinical remission.

Six of the patients, most with a very rapidly developing form of ATL, had either a very transient response (less than 1 week) or no response to this therapy. The other three patients manifested a mixed, partial, or complete remission, as assessed by routine hematologic tests, by immunofluorescence analysis of tumor-associated antigens expressed by circulating T cells, and by molecular genetic analysis monitoring the presence of cells with clonally integrated HTLV-I provirus and monitoring the clonal pattern of T-cell antigen receptor β chain rearrangements in the leukemic cells. Before anti-Tac therapy, patient no. 1 had 8,200 circulating Tac-expressing malignant T cells/μL, as assessed by immunofluorescence analysis using the anti-Tac MoAb (Fig 1A). Furthermore, 9,300 of the circulating cells/μL reacted with an antibody to the transferrin receptor, a receptor expressed
on malignant T cells but not on normal circulating cells. After anti-Tac therapy, there was a sustained decline in the number of circulating T cells bearing the Tac antigen to less than 200/μL, and there was a decline in the number of transferrin receptor-expressing T cells from 9,300 to less than 200/μL. Normal individuals have up to 500 Tac-expressing circulating T cells/μL.

During the 4-week period following anti-Tac infusions, there were <100/μL circulating cells with free IL-2 receptors (•——•) defined with FITC-anti-Tac, i.e., with receptors unblocked by the infused anti-Tac monoclonal. Cells with blocked IL-2 receptors were identified as cells that were not reactive with fluorochrome-labeled anti-Tac but were reactive with FITC-conjugated goat antimouse immunoglobulin.

surgery, which was performed five days after the completion of the course of therapy. The cells of this lymph node manifested the same HTLV-I integration pattern and T-cell receptor β chain gene arrangement as pretherapy peripheral blood lymphocytes, thus confirming that they were from a single clone rather than two independent clones of malignant cells. The patient returned to her referring physician and received chemotherapy; therefore, the effect of anti-Tac therapy could not be evaluated further.

Patient no. 2 had a WBC count of 38,000/μL with 69% abnormal cells, including 23,300 circulating Tac-expressing malignant T cells/μL, as assessed by immunofluorescence analysis. During the weeks after the initiation of anti-Tac

Fig 1.  (A) Effect of anti-Tac therapy on patient no. 1 with a Tac-expressing HTLV-I-associated ATL.  (B) Effect of anti-Tac therapy on patient no. 2.  (C) Effect of anti-Tac therapy on patient no. 3.  (A) Anti-Tac monoclonal antibody (20 mg to 60 mg per dose; total dose 180 mg) was administered IV to the patient on the days indicated by the solid bars. There was a marked reduction in the number of circulating leukemic cells as assessed by routine hematologic tests and immunofluorescence analysis of circulating T cells. On indirect immunofluorescence analysis of the circulating cells after anti-Tac therapy, there was a decline in the number of circulating T cells bearing the Tac antigen from 8,200/μL to less than 200/μL (●——●), defined by anti-Tac followed by FITC antimouse Ig. There was also a decline in the number of transferrin receptor-expressing T cells from 9,300/μL to less than 200/μL (○——○).  (B) Anti-Tac (20 mg to 50 mg per dose; total dose 490 mg) was administered IV to this patient on the days indicated by the solid bars. After anti-Tac therapy, there was a decline in the number of circulating T cells bearing the Tac antigen from 23,300/μL to less than 400/μL.  (C) Anti-Tac (20 mg to 40 mg per dose; total dose 220 mg) was administered as indicated by the solid bars. Before anti-Tac therapy, the patient had 1,800 Tac-expressing malignant T cells/μL in the circulation, as assessed by immunofluorescence analysis using the anti-Tac MoAb. After three IV infusions (20 mg, 40 mg, and 40 mg) of anti-Tac MoAb, there was a decline in the number of circulating T cells bearing the Tac antigen from 1,800/μL to less than 400/μL (●——●). During the 4-week period following anti-Tac infusions, there were <100/μL circulating cells with free IL-2 receptors (●——●) defined with FITC-anti-Tac, i.e., with receptors unblocked by the infused anti-Tac monoclonal. Cells with blocked IL-2 receptors were identified as cells that were not reactive with fluorochrome-labeled anti-Tac but were reactive with FITC-conjugated goat antimouse immunoglobulin.
MoAb therapy, the patient's WBC count declined from 38,000 to 3,700, and the number of Tac-positive cells declined to 340/μL, <3% of the peak value (Fig 1B). The patient remains clinically well at the time of this report, over 8 months following the initiation of the remission, with the exception that he has developed skin lesions that were shown to be Kaposi's sarcoma. No ATL cells were noted in the lesions. Before anti-Tac therapy, this patient was anergic; however, 1 month after completion of anti-Tac therapy and at 4 months when the Kaposi's sarcoma was first identified, the patient manifested a positive skin test response to all seven recall skin test antigens used.

Therapy of patient no. 3 with anti-Tac was followed by a 5-month remission, as assessed by routine hematologic tests, immunofluorescence analysis (Fig 1C), and molecular genetic analysis of the arrangement of T-cell β receptor genes (Fig 2). Before anti-Tac therapy, the patient's WBC count was 3,800/μL, including 10% malignant cells as defined by routine hematologic examination. Circulating Tac-expressing malignant T cells (1,810/μL) were identified by immunofluorescence analysis using the anti-Tac MoAb. Furthermore, some (1,500/μL), but not all, of these circulating leukemic lymphocytes reacted with an antibody to the transferrin receptor, a receptor expressed on the malignant T cells but not on normal circulating cells. Anti-Tac therapy was followed by a decline in the number of circulating T cells bearing the Tac antigen from 1,810/μL to less than 400/μL, as well as a decline in the number of transferrin receptor-expressing T cells from 1,500/μL to less than 100/μL. During the 4-week period following the anti-Tac infusions, there were no cells that expressed free IL-2 receptors, ie, with IL-2 receptors unblocked by the infused anti-Tac monoclonal. Cells with unblocked IL-2 receptors were identified as cells that are reactive with FITC-conjugated anti-Tac. Cells with blocked IL-2 receptors were identified as cells that were not reactive directly with fluorochrome-labeled anti-Tac but were reactive with FITC-conjugated goat antimouse IgG. Approximately 5 months after the initial remission, the leukemia recurred with the reappearance of circulating leukemic cells identified by immunofluorescence and molecular genetic analysis. Large (5 × 7 × 1 cm) malignant skin lesions developed over the subsequent 4 months. A new course of IV infusions of anti-Tac was followed by a >80% reduction in the number of circulating leukemic cells. Furthermore, there was a virtual disappearance of the skin lesions; only a nonraised discoloration of the skin remained. Within 2 months, circulating leukemic cells were again identified. At this time, although the leukemic cells remained Tac positive and there was no circulating antibody to the anti-Tac MoAb, the leukemic cells were no longer responsive to these further infusions of anti-Tac, and the patient required chemotherapy.

In general, the types of routine hematologic analyses and immunofluorescence analyses just discussed provide valid measures of the efficacy of treatment with MoAbs. Care, however, must be taken in interpreting the immunofluorescence analysis, because a MoAb could theoretically cause modulation of its antigenic target from the cell surface without leading to the death of the cell. Furthermore, small numbers of malignant circulating cells can be difficult to detect by these conventional techniques because the malignant T cells in ATL are mature T cells that may be hard to distinguish from normal T lymphocytes and because a small number (0-500/μL) of Tac-positive cells can be identified in the circulation of normal individuals. Therefore, the remission of the T-cell leukemia in patient no. 3 was confirmed by use of molecular genetic analysis of the rearrangement of the genes encoding the β chain of the α-β heterodimer of the antigen-specific T-cell receptor.

The evaluation of clonality via molecular genetic approaches involves an analysis of the rearrangement of the T-cell receptor genes. The rationale for this approach involves the fact that, during differentiation of a pluripotent stem cell into a mature cell of the T-cell lineage, rearrangements of the genetic subsegments of the antigen-specific T-cell receptor genes must take place. These DNA rear-
rangements generate changes in the location of restriction endonuclease sites surrounding the antigen receptor genes (Fig 2). This can be used in Southern blot analyses to distinguish the rearranged from the germline form of the genes. For example, all cells in a monoclonal T-cell tumor have the same rearrangement, yielding a new single-sized fragment bearing the T-cell β chain receptor gene, which can be identified as a new nongermline band on a radioautograph of a Southern gel. In contrast, a population of polyclonal T cells manifests many different rearrangements that yield different sized nongermline fragments that bear the T-cell receptor gene. None of these fragments is represented in sufficiently high proportion to be identified as a new band on a Southern gel. This approach to define clonality is sufficiently sensitive to be used to detect a monoclonal expansion of cells in a mixed cell population when it represents only 1% of these cells.22-25

The remission of the T-cell leukemia in patient no. 3 was confirmed by use of molecular genetic analysis of the rearrangement of the genes encoding the β chain of the antigen-specific T-cell receptor. With use of a radiolabeled probe to the constant regions of the Tβ chain gene, Southern analysis of the T-cell receptor β chain gene rearrangement was performed on BamHI digests of DNA from peripheral blood mononuclear cells of this patient. The constant Tβ chain genes are universally present on a 24-kb BamHI fragment in germline tissues of normal individuals and in a B-cell line derived from the patient (Fig 2). However, before therapy, an additional nongermline 22-kb BamHI band hybridizing with a constant Tβ probe was demonstrable in the BamHI digests of the patient's circulating T cells, a hallmark of a clonal expansion of T lymphocytes. This band, reflecting the clonally rearranged T-cell receptor gene, was not demonstrable on peripheral blood mononuclear specimens obtained during the remission that followed anti-Tac therapy. On relapse, the

22-kb BamHI band was again demonstrable. These results, showing that the clonal leukemic cells were eliminated from the circulation during the clinical remission, were confirmed in a parallel molecular genetic search for integrated HTLV-I proviruses in the circulating mononuclear cells (Fig 3). Two copies of HTLV-I were integrated into the circulating leukemic cells, as assessed on EcoRI digests of the peripheral blood T-cell DNA examined with an HTLV-I provirus probe. No integrated HTLV-I was demonstrable in the peripheral blood cells when the patient was in remission, whereas virus integration was again demonstrable during the final relapse. The positions of the bands identified by the HTLV-I probe on DNA digests of leukemic cells obtained during the final relapse were identical to those observed before therapy. There are no EcoRI restriction sites within the viral genome. Therefore, the generation of restriction-length fragments containing HTLV-I depends on the recognition of EcoRI sites in host DNA adjacent to viral integration. The identity of pretherapy and final relapse HTLV-I integration patterns confirms the reappearance of the original clone of transformed T cells.

This observation of a common clonal origin by demonstration of an identical integration pattern of the virus HTLV-I in a particular chromosomal site is of importance in this case. The patient achieved an initial 5-month remission following anti-Tac administration and then relapsed with a clone that was identical to the pretreatment clone by both Tβ gene rearrangements and sites of HTLV-I proviral integration. This clone remained sensitive to treatment with anti-Tac, and the patient achieved a second relapse lasting about 2 months. However, after a second relapse, the tumor was no longer responsive to anti-Tac. The leukemic cells obtained during the initial relapse when the patient was still responsive to anti-Tac therapy manifested Tβ gene rearrangements in EcoRI digests of leukemic cells that differed from those

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**Fig 3.** Southern analysis of HTLV-I proviral integration in EcoRI digests of the DNA obtained from patient no. 3. Two bands are observed on the Southern gels of mononuclear cell DNA obtained prior to therapy (day 0) and during relapse (day 316) that define two copies of clonally integrated HTLV-I proviruses. No HTLV-I was demonstrable in the DNA of the circulating cells during remission (day 351), whereas two bands at identical positions to those observed in the pretreatment sample were identified during the terminal relapse (day 422). Each of the lanes was shown to have comparable quantities of DNA, as assessed by ethidium bromide staining. A schematic diagram of the virus showing the EcoRI restriction endonuclease sites is shown below; because the EcoRI restriction sites are outside of the viral genome, the band pattern depends on the position of EcoRI restriction sites within the host DNA adjacent to the site of viral integration. Thus, the identity of pretherapy and final relapse HTLV-I integration banding patterns confirms the reappearance of the original clone of transformed T cells.
obtained during the final relapse when the patient was no longer responsive to this form of therapy (Fig 4). It appears that a form of tumor progression had occurred in the transformed clone between these analyses, leading either to a loss of a subclone of T cells with a particular T-cell receptor β chain gene rearrangement or further internal deletions in the T-cell receptor β gene locus. The identical proviral integration in cells from this second (and anti-Tac unresponsive) relapse rules out the possibility that a second T-cell transformation (ie, a second HTLV-I–induced tumor) had occurred. Although progression of Ig gene rearrangements and especially point mutations within the genes encoding Igs are very common in certain B-cell malignancies, they have not been observed with T-cell receptor genes in malignant T cells.

**DISCUSSION**

MoAbs are tailor-made homogeneous antibodies that may be specifically directed at particular antigens. To be of value in tumor therapy, the antibodies must be directed toward antigens expressed on tumor cells and must have antitumor activity; that is, there must be tumor cell destruction or inhibition of tumor cell growth after interaction of the MoAb with the tumor cell. The anti-Tac MoAb appears to fulfill the first requirement when patients with ATL are considered. ATL cells constitutively express from 5,000 to 35,000 IL-2 receptors identified by the anti-Tac MoAb, whereas most normal resting cells and their precursors do not. Furthermore, in terms of the second requirement, in three of nine cases, the IV administration of the anti-Tac MoAb was followed by a transient mixed, partial, or complete remission. It is of value to consider the potential mechanisms leading to the antitumor activity observed in the three patients and its failure in the remaining six. Such an analysis may be useful in designing strategies for using anti-Tac in other clinical circumstances where one might wish to eliminate IL-2 receptor-expressing cells. For example, it may be useful in eliminating Tac-expressing activated T cells that participate in certain autoimmune disorders or in organ allograft protocols where activated IL-2 receptor-expressing cells may be involved in the rejection of the allograft. Such an analysis may also be of value in developing altered anti-Tac molecules that have greater antitumor activity. There was no apparent relationship between the clinical response and previous therapy, tumor burden, or level of circulating mouse Ig achieved.

One mechanism that can lead to tumor destruction is complement-mediated cytotoxicity in which the complement cascade is triggered by the antibody on the tumor cells. However, cells of the HTLV-I–induced ATL cell line HUT 102 are not killed in vitro by anti-Tac in the presence of human complement-containing serum or plasma, although they are killed in the presence of rabbit complement (T.A.W., unpublished observations, 1987).

An alternative mechanism of antitumor activity is antibody-mediated cellular cytotoxicity wherein host effector cells bind to the fragment (Fc) portion of an antibody after its reaction with its antigenic target on the surface of the tumor cells. However, anti-Tac did not induce antibody-mediated cellular cytotoxicity when bound to HUT 102 cells that were cultured with appropriate peripheral blood effector cells from normal individuals.

A third potential mechanism of therapeutic antitumor action is a direct effect of the MoAb on a vital structure present on the surface of the malignant cell, such as a growth factor receptor or a receptor necessary for cellular attachment. This possible mode of antitumor activity provided the scientific rationale for the present study. It was known that ATL is caused by the human type C retrovirus HTLV-I and that this virus can transform normal human T cells to immortalized growth. In addition, we and others had shown that leukemic cells in vivo and normal T cells transformed with HTLV-I in vitro express IL-2 receptors. Furthermore, in certain cases, the cells produced IL-2 as well. Thus, an autocrine model for T-cell growth was considered in which the same leukemic cell produced IL-2 and the receptor required for its action. However, since the initiation of the study, it was shown that, although HTLV-I in vitro immortalized T-cell lines express IL-2 receptors in all cases, they release detectable IL-2 and transcribe mRNA for this lymphokine in only a minority of cases. In those cases...
of leukemia where the malignant cells do not produce IL-2, an IL-2/IL-2 receptor-mediated autocrine model is not possible. However, there is another subset of cases in which the available evidence still supports an IL-2-mediated autocrine model for the proliferation of the ATL cells. In such cases, anti-Tac might function to inhibit the growth of the leukemic cells by preventing IL-2 from interacting with the Tac peptide receptor required for its action.

A number of the obstacles preventing antitumor activity by other MoAbs can be excluded as the cause for the limitation of anti-Tac activity. One obstacle for effective therapeutic activity observed in other systems is tumor release of antigen into the circulation that blocks and prevents the interaction of the antibody with its antigenic target displayed on the tumor cell surface. A soluble form of the Tac peptide of the IL-2 receptor that reacts with the anti-Tac MoAb is indeed released by Tac-expressing T cells. Furthermore, the concentration of this serum form of the Tac peptide is elevated in patients with ATL, providing a marker of value in the diagnosis and monitoring the therapy of this condition. However, the circulating Tac antigen did not block the anti-Tac MoAb from reacting with the circulating tumor cells and thus blocking the sites of IL-2 binding in the patients studied. Specifically, using immunofluorescence analysis of the cells obtained after administration of IV anti-Tac, we were able to demonstrate the anti-Tac MoAb on the surface of the tumor cells by using FITC-conjugated antimouse IgG (data not shown). Furthermore, sufficient anti-Tac was administered to block the Tac peptide receptors for IL-2 during the initial course of therapy. Cells expressing blocked IL-2 receptors were identified as cells that were reactive with antimouse Ig but not with fluorochrome-labeled anti-Tac. In patient no. 1, the lymph node cells obtained 13 days after initiation of the anti-Tac therapy were not reactive with antimouse IgG but were reactive in vitro with FITC-anti-Tac, suggesting that insufficient antibody to block the IL-2 receptors on these lymph node cells had been administered in this case. In the remaining cases, sufficient anti-Tac was administered during the initial phase of therapy to saturate the Tac sites on the malignant cells so that no unblocked IL-2 receptors could be identified with fluorochrome-labeled anti-Tac. Furthermore, sufficient anti-Tac was administered to yield measurable levels of mouse IgG in the circulation (Table 3).

A third cause for low therapeutic activity in other systems, antigenic modulation, wherein the antibody leads to the loss of its antigenic target of the cell surface without destruction of the tumor cell, can also be excluded in the present cases. In contrast to antibodies to such antigens as common acute lymphoblastic leukemia antigen (CALLA) or the transferrin receptor, anti-Tac has been shown to be very ineffective in the modulation of its target peptide from normal cells and even less efficient in the modulation of the Tac peptide from the HTLV-I–induced tumor cells. The studies just discussed, with FITC-conjugated anti-Tac, antimouse IgG, and 7G7/B6 MoAb, also indicate that anti-Tac did not lead to the modulation of the 55-Kd Tac peptide from the surface of the ATL cells. Finally, and of greater significance, the molecular biologic studies analyzing the rearrangement of the T-cell receptor β gene and the HTLV-I provirus integration confirm that the elimination of Tac-positive cells is paralleled by the elimination of clonal cells identified by this approach, and thus these studies speak against antigenic modulation.

Another common obstacle to therapeutic activity is the emergence of tumor variants that do not express the antigen identified by the MoAb. However, we have not observed Tac-nonexpressing HTLV-I–induced ATL variants. Specifically, the leukemic cells of patient no. 3, who was responsive to IV administered anti-Tac on the two initial therapeutic trials but not on the third, continued to express the Tac antigen during the final relapse, yet did not respond to therapy with anti-Tac. Finally, with the exception of the patient still in remission, the ATL patients did not produce human antinouse antibodies that for other systems may block antitumor activity. This failure to make antibodies does not appear to be due to a universal inhibition of such antibody production by anti-Tac itself, because such human antinouse antibodies were demonstrable in the three patients with aplastic anemia treated with anti-Tac and in the cynomolgus monkeys receiving this MoAb (T.A.W., unpublished observations, 1987). Thus, the observation that ATL patients did not produce antinouse antibodies appears to reflect the immunodeficiency state observed in these patients. In this regard, patients with ATL appear to be analogous to patients with chronic lymphocytic leukemia, who have an immunodeficiency state and an associated failure to produce human antinouse antibodies after MoAb therapy.

Another possible explanation for the variable therapeutic effect of treatment of ATL patients with the anti-Tac MoAb is suggested by a hypothetical model of progression of HTLV-I–induced leukemia outlined in Fig 5. An initial event is the integration of HTLV-I into the genome of the infected T cells. In addition to the presence of retroviral gene sequences common to other retroviruses, integrated HTLV-I was shown to contain an additional genomic region referred to as pX by Seiki et al.16 Sodroski et al17 demonstrated that a 42-Kd protein, transacting transcriptional activator (tat), encoded predominantly by this pX region functions as a tat and is essential for viral replication. This tat protein acts on a receptor region within the long terminal repeat (LTR) of HTLV-I, stimulating transcription. This protein has also been shown to play a central role in directly or indirectly increasing the transcription of the host genes, including the IL-2 and IL-2 receptor genes involved in T-cell activation and HTLV-I–mediated T-cell leukemogenesis. Thus, during this first phase of leukemic transformation, one may have a polyclonal, oligoclonal, and perhaps ultimately a monoclonal expansion of T lymphocytes whose proliferation is associated with autocrine signals provided by IL-2 and IL-2 receptors produced and expressed on the same cell. At this phase of the leukemic transformation, anti-Tac might inhibit HTLV-I–induced T-cell proliferation and thus lead to a remission as these no longer proliferating T cells with anti-Tac on their surface die or are eliminated by host mechanisms. HTLV-I–infected cells have a high incidence of mutations and manifest chromosomal abnormalities.
T-cell: Autocrine IL-2 dependent proliferation

Monoclonal Tac+ ATL: IL-2 independent proliferation

Oligoclonal Tac + T-cell: Autocrine IL-2 dependent proliferation

Undefined second event leads to Tumor Progression

Fig 5. Possible model for the mechanism of T-cell transformation induced by HTLV-I proviral integration. During the first phase of leukemic transformation, the expression of polyclonal, oligoclonal, or monoclonal T lymphocyte proliferation may involve induction by the tet protein of autocrine growth signals provided by IL-2 and IL-2 receptors produced and expressed on the same cell. At this phase, the leukemic cells may be responsive to anti-Tac therapy. Ultimately, second-stage events occur that lead to leukemic cells that express the IL-2 receptor peptide identified by anti-Tac but no longer produce IL-2. Following the second-stage events, the leukemic cells are autonomous, no longer requiring the growth factor/growth factor receptor interaction for their proliferation. At this autonomous phase, the leukemia may not be responsive to therapy with unmodified anti-Tac.

In this autonomous phase, the leukemia may only be responsive to therapy with modified anti-Tac. This suggests that the autocrine growth signals provided by IL-2 can promote the development of autonomous leukemia.

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Therapy of patients with human T-cell lymphotrophic virus I-induced adult T-cell leukemia with anti-Tac, a monoclonal antibody to the receptor for interleukin-2

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