Chronic T Cell Leukemia With Unusual Cellular Characteristics in Ataxia Telangiectasia

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A 27-year-old male patient with ataxia telangiectasia (AT) developed atypical chronic lymphocytic leukemia with increasing bone marrow infiltration in the absence of organomegaly. One-third of the leukemia cells expressed a mature suppressor/cytotoxic T cell phenotype (T3+ T4− T6+ T8−T10+), two-thirds demonstrated additional helper/inducer T cell-associated antigens (T3+ T4+ T6+ T8− T10+), and a small fraction reacted with a natural killer (NK) cell-specific monoclonal antibody (Leu 11b). The proliferative response to stimulation in vitro with lectines and various monoclonal antibodies resembled the proliferation pattern of mature thymocytes: The cells responded to phytohemagglutinin (PHA), concanavalin A (ConA), stimulation of the T3− T8 receptor complex with Sepharose-bound anti-T3, and stimulation of the sheep erythrocyte receptor protein with anti-T112 and anti-T112 in conjunction with oxogenous interleukin-2 (IL-2); they failed, however, to proliferate after stimulation with anti-T112 and anti-T112 alone. There was no response in the mixed lymphocyte reaction (MLR) and no suppression of the MLR between two healthy donors. Antibody-dependent cell-mediated cytotoxicity and NK activity could not be demonstrated. Cytogenetic analysis revealed complex clonal aberrations, including an interstitial deletion of the long arm of chromosome 14 concerning bands q21−q31, loss of chromosome 20, and loss of the Y chromosome. Cytostatic chemotherapy was of little use and caused serious side effects, whereas leukapheresis proved effective in reducing the tumor load. The clinical data and laboratory findings in this case correspond to three previously described patients with AT who developed chronic T cell leukemia. Thus, in adult patients with AT, malignant proliferation of cytogenetically marked and phenotypically heterogeneous mature T cells seems to be a frequent complication.

A TAXIA telangiectasia (AT) is an autosomal recessive syndrome characterized by progressive cerebellar ataxia, oculocutaneous telangiectases, and a complex primary immune deficiency predisposing to frequent and severe respiratory infections; in addition, various endocrine, hepatic, and integumental abnormalities may be associated. Patients with AT have an elevated risk of developing malignancies (~10%), especially malignant lymphoma and leukemia. Because death usually occurs during childhood from respiratory failure or neoplastic diseases, few patients reach the third or fourth decade of life. Among these patients, development of chronic T cell leukemia (T-CLL) has repeatedly been observed.

In this report, we describe an adult male patient with AT complicated by T-CLL. The clinical data and laboratory findings in our patient reveal striking similarities with the previously reported cases. Thus, T-CLL in adults with AT may constitute a well-defined subentity in the heterogeneous group of the chronic T cell neoplasms.

MATERIALS AND METHODS

Case Report

K-D.B. is a male, born in 1955. From his second year of life, the patient developed a slowly progressive incoordination of gait, hand movements, and speech, which confined him to a wheelchair at the age of 6 years. When he was 20 years old, his parents first noticed ocular and cutaneous telangiectases, predominantly in the antecubital areas, on the back of the hands and on the aural regions. Apart from pneumonia following rubella in 1959 and herpes zoster of the left trigeminal nerve in 1981, there was no history of severe infections. A liver biopsy performed in 1983 because of elevated serum transaminase activities revealed diffuse fatty infiltration with minimal chronic inflammation.

In July 1983, the patient was first admitted to our hospital for evaluation of leukocytosis. He was of small stature (165 cm) and slightly underweight (55 kg). Physical examination showed several vitiligolike skin areas and mild bilateral gynecomastia in addition to oculocutaneous telangiectases. The right testicle was not palpable; the size of the left one was in the lower normal range. He had no enlarged lymph nodes and no splenomegaly. Neurological examination revealed severe cerebellar ataxia with ocular dysmetria, nystagmus, dysarthric speech, inability to stand or walk without help, intentional tremor, and dysdiadochokinesis. The tendon reflexes were absent; vibration sense was diminished in a socklike fashion.

Clinical diagnosis of AT was made and was corroborated by reduced serum concentrations of IgA (~42 mg/dL) and IgE (~5 U/mL), contrasting with increased concentrations of IgM (702 mg/dL), α1-fetoprotein (359 ng/mL) and carcinoembryonic antigen (3.0 ng/mL), and IgG levels in the upper normal range (1,762 mg/dL). The oral glucose tolerance test reflected impaired carbohydrate tolerance due to insulin resistance (fasting venous blood glucose 175 mg/dL, insulin >200 μU/mL). The result of the LH-RH test was compatible with primary hypogonadism (basal serum concentrations: LH 13.3 mU/mL, FSH 19.4 mU/mL; maximum values after stimulation: LH 45.3 mU/mL, FSH 28.9 mU/mL).

Hemoglobin concentration was 13.7 g/dL, leucocyte count was 52,500/μL with 92% atypical lymphocytes, and platelet count was 436,000/μL. Bone marrow aspirate was consistent with moderate patchy infiltration with medium-sized lymphocytes. Chest x-ray films, sonography of the abdomen, and total body computed tomography (CT) revealed neither lymphadenopathy nor other organomegaly. Further analysis of the atypical cells indicated chronic T cell leukemia. Antibodies against HTLV I could not be demonstrated by various techniques [enzyme-linked immunoassay (ELISA),...
radiouimmunoprecipitation). Because symptoms were lacking, a specific therapy was not initiated.

In March 1984, continuous increase of the leucocyte count up to 230,000/μL prompted us to start antineoplastic chemotherapy. Because the biweekly administration of chlorambucil and prednisone proved ineffective in preventing a further rise of leucocytes up to 300,000/μL, chemotherapy was intensified by continuous treatment with cyclophosphamide (100 to 150 mg daily) and prednisone (25 to 50 mg daily). After 4 weeks of therapy, the patient’s leucocyte count dropped to 170,000/μL, but a severe hemorrhagic cystitis with incomplete recovery forced us to discontinue this treatment. In November 1984, the leucocytosis exceeded 800,000/μL, and small pleural effusions developed; the patient complained of dyspnea, dizziness, and general fatigue. Bone marrow was now densely infiltrated, but apart from slight thrombocytopenia, the patient still had no important hematopoietic insufficiency, organomegaly, or significant change in serum immunoglobulin concentrations. Intermittent leukapheresis performed at intervals of 15 to 30 days led to a steady reduction of the WBC count to 400,000 to 500,000/μL, followed by a temporary improvement of the patient’s general condition. From March 1985 on, the cyclophosphamide-induced cystitis repeatedly worsened, and the patient was debilitated by nearly continuous urinary bleeding. He died on June 2, 1985, of severe hemorrhage from the bladder. Permission for autopsy was denied.

In accordance with institutional guidelines the patient was advised of all diagnostic and therapeutic procedures, including attendant risks, and gave informed consent.

Phenotypic Characterization

Cytomorphology and cytochemistry. Peripheral blood and bone marrow smears were stained with a modified May-Grünwald-Giemsa stain. Periodic-acid Schiff (PAS) and acid phosphatase reactions were performed using routine techniques.

Terminal deoxynucleotidyl transferase (TdT). TdT was determined in 2 patient samples from fixed mononuclear cells with specific rabbit anti-TdT antisera and fluorescein-conjugated goat anti-rabbit antibodies (BRL Terminal Transferase Immunofluorescent Assay Kit, Gaithersburg, Md.).

Surface marker analysis. Mononuclear cells from heparinized peripheral blood (PBMCs) and bone marrow samples (BMMCs) were separated by Ficoll sodium metrizoate gradient centrifugation (Lymphoprep, Nyegaard, Oslo) using standard techniques.10,11

Surface antigens defined by monoclonal antibodies were analyzed by indirect immunofluorescence. After incubation of 1 x 10⁶ cells with monoclonal mouse antibodies, cells were washed twice and then reincubated with an affinity-purified fluorescein-conjugated rabbit anti-mouse immunoglobulin as a second layer (Dakopatts, Copenhagen). The monoclonal antibodies used were commercially available products (Ortho Diagnostic Systems, Raritan, NJ; Becton Dickinson Laboratory Systems, Moutain View, Calif.; Coulter Immunology, Hialeah, Fla) that have been characterized in detail.12-14

In brief, pan-T antigens were detected by OKT 3, OKT 11 (E receptor), anti-Leu 1, and anti-Leu 4; inducer/helper T cell-associated antigens were detected by OKT 4 and anti-Leu 3a; suppressor/cytotoxic T cell-associated antigens were detected by OKT 8 and anti-Leu 2a; thymic antigens were detected by OKT 6, OKT 9 (transferrin receptor), and OKT 10; the HLA-DR antigen complex was detected by OKI a-1; the cALL antigen was detected by C-J 5; monocyte and macrophage-associated antigens were detected by OKM 1 and anti-Leu M 3; natural killer (NK) cell-associated antigens were detected by anti-Leu 11; and B cell-specific antigen was detected by C-E 1. These antibody studies were performed on fresh cells; only the anti-Leu 8 monoclonal antibody, which was tested for further dissection of the helper/inducer and suppressor/cytotoxic subsets,18 was applied to cryopreserved and thawed mononuclear cells.

Direct two-color immunofluorescence with fluorescein- and phycoerythrin-conjugated monoclonal antibodies was used in determining the percentage of cells simultaneously expressing Leu 2a and Leu 3a markers.

Positive cells were enumerated by a fluorescein activated cell sorter (FACS II, Becton Dickinson) or by fluorescence microscopy (Orthoplan, Leitz, Wetzlar, FRG). By both techniques identical patterns of surface antigen expression were found (data not shown).

Spontaneous rosette formation with sheep erythrocytes (E rosettes) was assessed by adding a suspension of lymphoid cells to sheep cells in the presence of absorbed and inactivated fetal calf serum (FCS) at 37 °C. After cells were centrifuged and incubated overnight at 4 °C, the percentage of E rosette-forming cells was determined microscopically.

Functional Studies

Proliferative assays. Mitogen responsiveness was assayed by incubating 1.5 x 10⁶ PBMC with 4.21 μg of phytohemagglutinin (PHA-P, Difco, Detroit), 35.57 μg of concanavalin A (Con A, Difco), or 3.48 μg of pokeweed-mitogen (PWM; GIBCO, Grand Island, NY), respectively. The cells were cultured in RPMI 1629 with glutamine (GIBCO) and 13% inactivated FCS (final volume, 240 μL) at 37 °C for 68 hours. Two microcuits of 3H-thymidyline were added for an additional four hours. The cells were then harvested on paper strips, and 3H-thymidyline incorporation was determined by scintillation counting. A stimulation index (SI) was calculated as the ratio of cpm in stimulated and control cultures.18

To further characterize the proliferative behavior of the patient’s cells, a second set of experiments was performed. Proliferation was determined in response to PHA-P (Wellcome, Burgwedel, FRG), to Sepharose-bound anti-T3 monoclonal antibody,19,20 to stimulation of the E rosette receptor protein with anti-T11, and anti-T11, monoclonal antibodies21 and to stimulation with anti-T11, plus exogenous interleukin 2 (IL 2)22. All experiments were done both with the unseparated leukemia cells and after separation of the cells into the two main tumor cell subsets (T3+ T4+ T8+ and T3+ T4+ T8+). PBMC were obtained as described above. Separation of the T4+ T8+ was achieved either by indirect immunofluorescence on an EPICS C cell sorter (Coulter) or by selectively lysing the T4+ subset. For the latter purpose, the unseparated PBMCs were incubated with anti-T4 for one hour at 4 °C. Subsequently, rabbit complement was added, and the suspension was incubated for one hour at 37 °C in a shaking water bath; the cells were then washed repeatedly.

Recombinant IL 2 was a generous gift of Sandoz Research Institute (Vienna). Anti-T11 and anti-T11, monoclonal antibodies were kindly provided by Dr E.L. Reinherz (DFCI, Boston), and anti-T3, anti-T4, and anti-T8 were purchased from Coulter (Krefeld, FRG). For surface coupling of anti-T3, the monoclonal antibody was purified with Sepharose Protein A (Pharmacia, Uppsala, Sweden) and then covalently linked to CNBr-activated Sepharose 4 B (Pharmacia) at a concentration of 3 mg of purified antibody per milliliter of swollen Sepharose beads.19

Responder cells (3 x 10⁶) were incubated with various stimuli in round-bottomed microtiter wells (Costar, Cambridge, Mass) in 200 μL of final culture medium RPMI 1640, supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 2% glutamine (GIBCO, Paisley, Scotland). PHA was added at a concentration of 0.25 μg/mL, and anti-T3 carrying Sepharose beads were suspended at dilutions previously determined to yield optimal proliferative
responses. Stimulation with PHA and anti-T3 Sepharose was performed in the presence or absence of 5% adherent cells (6,000 rad irradiated) (Mφ) of a healthy donor. Anti-T11 and anti-T11, antibodies were used together at a final concentration of 1:300, with or without addition of recombinant IL 2 at a concentration of 5 ng/mL.

After three days of in vitro culture in a 7% CO₂, humidified atmosphere at 37 °C, wells were pulsed with 1 μCi of [³H]-thymidine for 16 hours and washed with a Titertek cell harvester (Flow, Meckenheim, FRG). [³H]-Thymidine uptake was measured in a Packard liquid scintillation spectrometer (Packard Instrument Co, Inc, Downers Grove, III). The results obtained for the patient's PBMCs were compared with those of purified T cells of a healthy donor. The method of T cell purification has been described previously and included Ficoll-Hypaque density centrifugation, removal of adherent cells on glass Petri dishes, separation of E rosette-positive cells, lysis of RBCs with ACK-buffer, and lysis of remaining monocytes by treatment with monocyte-specific monoclonal antibodies plus rabbit complement. Results were expressed as cpm, all values representing the mean of triplicate experiments. SDs were ± 15%.

Mixed lymphocyte reaction (MLR). PBMCs obtained from the patient and two healthy donors as described above were used as stimulators and responders. In addition, response to stimulation with an irradiated Epstein-Barr Virus (EBV)-transformed human lymphoblastoid B cell line (B-LCL) was determined. After irradiation (6,000 rad) of stimulator cells, 5 x 10⁵ stimulator and 5 x 10⁴ responder cells were incubated in standard medium under the above described conditions for five days. After being pulsed with 1 μCi of [³H]-thymidine for 18 hours, cells were harvested, and [³H]-thymidine uptake was measured as above. Results were expressed as cpm (means of triplicate experiments). SDs were ± 15%.

Cytotoxic activity. Antibody-dependent cell-mediated cytotoxicity (ADCC) was measured by ⁵¹Cr-labeled rabbit-antibody sensitized RL 8 1 lymphoblastoid cells. Natural killer (NK) activity was determined by adding PBMCs to ⁵¹Cr-labeled K 562 target cells in the presence or absence of human sera containing NK-stimulating factors. Specific cytotoxicity was calculated as the ratio of cpm in the supernatant (after subtraction of controls) and total cpm in the target cells, multiplied by 100.⁴[^4]

<table>
<thead>
<tr>
<th>Cells</th>
<th>PBMCs</th>
<th>BMMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan-T cell antigens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>97 (79-98)</td>
<td>46</td>
</tr>
<tr>
<td>T11</td>
<td>98 (94-99)</td>
<td>40</td>
</tr>
<tr>
<td>Leu 1</td>
<td>97 (94-98)</td>
<td>ND</td>
</tr>
<tr>
<td>Leu 4</td>
<td>98 (96-99)</td>
<td>ND</td>
</tr>
<tr>
<td>Helper/inducer T cell subset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>56 (48-64)</td>
<td>6</td>
</tr>
<tr>
<td>Leu 3a</td>
<td>61 (56-69)</td>
<td>ND</td>
</tr>
<tr>
<td>Suppressor/cytotoxic T cell subset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T8</td>
<td>98 (93-98)</td>
<td>40</td>
</tr>
<tr>
<td>Leu 2a</td>
<td>98 (96-99)</td>
<td>ND</td>
</tr>
<tr>
<td>Thymocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>2 (1-3)</td>
<td>4</td>
</tr>
<tr>
<td>T9</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>T10</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Subdivisions of T4⁺ and T8⁺ cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu 8</td>
<td>1 (0-3)</td>
<td>ND</td>
</tr>
<tr>
<td>NK cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu 11</td>
<td>7 (3-9)</td>
<td>ND</td>
</tr>
<tr>
<td>HLA-DR complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DR complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu 1</td>
<td>4 (1-5)</td>
<td>6</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>2 (1-3)</td>
<td>ND</td>
</tr>
<tr>
<td>Monocytes/macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>2</td>
<td>53</td>
</tr>
<tr>
<td>Leu M3</td>
<td>1 (1-2)</td>
<td>ND</td>
</tr>
<tr>
<td>CALL antigen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J5</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Peripheral blood mononuclear cell (PBMC) profile is median and range of seven analyses; bone marrow mononuclear cell (BMMC) profile was determined once. ND, not determined.

Cytogenetic Studies

Cultures of PHA-stimulated lymphocytes were set up in RPMI 1640 medium containing 20% FCS, and were harvested after 72 hours, which included a treatment with colcemide (0.02 μg/mL) for the last two hours. Thereafter, cells were treated with hypotonic KCl (0.075 mol/L) and fixed in methyl alcohol-acetic acid (3:1). G-banding was performed according to conventional methods after short-term treatment with trypsin. Karyotypic analysis was done according to the guidelines of the Paris Conference.⁵[^5]

RESULTS

Phenotypic Characterization of Leukemic Cells

The peripheral blood smear showed a predominance of medium-sized, pleomorphic lymphocytes with irregularly shaped, partly indented nuclei and moderate cytoplasm (Fig 1). Cytochemically, most cells displayed a dotlike activity of acid phosphatase; the PAS reaction and immunofluorescent staining of TdT were negative.

Eighty-nine percent of the PBMCs formed rosettes with sheep erythrocytes. From July 1983 to August 1984, the surface antigens of the PBMCs were analyzed on seven occasions. Despite an increase in the absolute number of leukocytes from 52,500 to 406,000/µL, all examinations yielded comparable results (Table 1, Fig 2). Approximately 98% of the PBMCs reacted strongly with pan-T and suppressor/cytotoxic T cell reagents. Approximately 60% of the cells showed a (weaker) reaction with anti-helper/inducer T cell antibodies. Double staining with anti-Leu 3a and anti-Leu 2a
was performed on four occasions; 95% (range 93% to 95%) of the PBMCs were positive for Leu 2a, and 55% were positive (52% to 58%) for Leu 3a. All cells staining for Leu 3a simultaneously reacted with anti-Leu 2a; about one-third of the cells, however, exhibited only positivity for Leu 2a. The fraction of cells that reacted with anti-Leu 11 was in the normal range; with regard to the increased absolute number of PBMCs, however, the number of Leu 11+ cells was distinctly raised whenever examined (Fig 2). The panel of monoclonal antibodies tested failed to demonstrate an increased expression of antigens associated with thymocytes, B lymphocytes, and monocytes.

The surface marker profile of the BMCCs was analyzed only once. Due to a high proportion of monocytes (OKM 1+ cells: 53%) the number of cells staining for anti-Leu 2a antibodies was correspondingly lower. The relation of the BMMCs carrying pan-T cell, suppressor/cytotoxic T cell, and thymocyte-associated antigens was comparable to the PBMCs, whereas the proportion of cells expressing helper/inducer T cell-associated antigens was lower.

**Functional Characterization of Leukemic Cells**

At the time of investigation, the absolute count of PBMCs varied between 300,000 and 400,000/µL, with 98% T 8+ cells; admixture of normal cells was therefore assumed to be very small.

**Proliferative response to various stimuli.** Proliferation after stimulation with ConA was normal (SI 11.6; normal range 7 to 90). Response to PHA was slightly reduced (SI 13.9; normal range 14 to 112). Response to PWM was markedly reduced (SI 0.8; normal range 2 to 80).

The proliferative behavior was further investigated in a second set of experiments (Table 2). The response to PHA of both the patient's cells and purified T cells derived from a healthy donor was grossly enhanced after addition of MΦ to the culture medium. Stimulation with Sepharose-bound anti-T3 through the T3-Ti receptor complex also required the presence of macrophages, under these conditions resulting in a high proliferative response of both the patient's and the healthy donor's cells. Although the patient's leukemia cells showed virtually the same response to PHA and Sepharose-bound anti-T3 as did the healthy donor's purified T cells, triggering of the E rosette receptor protein with anti-T112 and anti-T113 induced proliferation only of the healthy donor's cells, not the patient's cells. However, if exogenous IL-2 was added to the patient's anti-T112-treated cells, substantial proliferation was observed. In contrast, proliferation of the healthy donor's anti-T112-treated cells could not be further enhanced by addition of IL 2. IL 2 alone proved to be a very weak stimulus for the donor's purified T cells and was not capable of inducing proliferation of the patient's cells. The proliferation pattern was identical in the patient's unseparated cells and in the T3+T4+T8+ and T3+T4+T8- subsets, respectively.

**Mixed lymphocyte reaction.** In the first set of experiments, the MLR between the patient and two healthy donors was studied. As shown in Table 3, the patient's PBMCs

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### Table 2. Proliferative Response of the Patient's Unseparated and Separated PBMCs (T4+ and T8 Subsets) to Various Stimuli in Comparison to Response of Purified T Cells of Healthy Donor

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Unseparated Cells</th>
<th>Cell Sorter-Separated Cells</th>
<th>Anti-T4 + Complement-Treated Cells</th>
<th>Healthy Donor Purified T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T3+T4+T8+</td>
<td>T3+T4+T8+</td>
<td>T3+T4+T8+</td>
<td>T3+T4+T8+</td>
</tr>
<tr>
<td>Medium</td>
<td>204</td>
<td>195</td>
<td>113</td>
<td>86</td>
</tr>
<tr>
<td>IL 2</td>
<td>174</td>
<td>142</td>
<td>97</td>
<td>147</td>
</tr>
<tr>
<td>PHA</td>
<td>334</td>
<td>ND</td>
<td>ND</td>
<td>224</td>
</tr>
<tr>
<td>PHA + MΦ</td>
<td>23,023</td>
<td>19,272</td>
<td>27,663</td>
<td>33,550</td>
</tr>
<tr>
<td>Anti-T3-Sepharose</td>
<td>194</td>
<td>104</td>
<td>151</td>
<td>219</td>
</tr>
<tr>
<td>Anti-T3-Sepharose + MΦ</td>
<td>11,484</td>
<td>10,042</td>
<td>11,977</td>
<td>10,873</td>
</tr>
<tr>
<td>Anti-T112 + anti-T113</td>
<td>356</td>
<td>262</td>
<td>179</td>
<td>100</td>
</tr>
<tr>
<td>Anti-T112 + anti-T113 + IL 2</td>
<td>5,516</td>
<td>4,187</td>
<td>5,220</td>
<td>5,060</td>
</tr>
</tbody>
</table>
neither responded to stimulation with the donors' PBMCs nor to stimulation with B-LCL. Similarly, the patient's irradiated cells could not stimulate the responder cells of either donor. The MLR between the two donors and each of them vs B-LCL, however, yielded high stimulative responses.

To exclude a suppressive effect of the patient's cells on the MLR, a second set of experiments was performed in which 5 x 10^6 nonirradiated cells of the patient were added to the mixed lymphocyte culture set up between the two donors or between each of them vs B-LCL; however, yielded high stimulative responses.

The patient's cells did not influence the stimulative response of either healthy donor (Table 3).

**Cytotoxic activity.** As compared with those of a healthy control the patient's ADCC and NK activities were extremely low. Specific cytotoxicity for ADCC was 2.5% (control 47.5%), for NK activity in the absence of stimulat-

**Cytogenetic Characterization of Leukemic Cells**

Chromosome analysis revealed an abnormal hypodiploid clone with <10% normal cells left. The karyotype was described as 44X, Y, 20q-, 6p-, 14q-, 19p+. 20q+, 22q-, the structural anomalies were rec t(4;20)(q13;q12), interstitial del14(q21;q31), t(6;19;22)(p11;p13;q13). Although the 14q1 region seemed untypically condensed in three karyotypes, the breakpoint at 14q was clearly below 14q11. The Y chromosome was lost only in abnormal cells.

**DISCUSSION**

Subacute or chronic leukemias of mature T cells constitute a heterogeneous group of diseases. Clinical and laboratory findings allow the definition of several subentities, such as Sézary's syndrome and HTLV I-associated adult T cell leukemia of Japan.27,28 both phenotypic helper cell disorders.29,30 The term "chronic lymphocytic leukemia of T cell origin" has been applied to a number of derangements with variable clinical features.31,32 Some of these are now being subsumed under the term of Tγ-lymphoproliferative disease, a proliferation of cytotoxic lymphocytes of monoclonal or polyclonal origin.33,34

Our patient's leukemia does not correspond to any of the previously defined categories. Phenotypically, at least two different T cell subsets could be defined: Although a minor proportion of cells exhibited the mature suppressor/cytotoxic T cell phenotype (T3+T4−T8−, Leu 4+ Leu 3a− Leu 2a+), about two-thirds of the PBMCs carried both suppressor/ cytotoxic and helper/inducer T cell associated antigens (T3+T4−T8−, Leu 4+ Leu 3a− Leu 2a+). Further characterization was achieved by analyzing the proliferation pattern in response to various stimuli. T cell activation with Sepharose-bound anti-T3 mimics triggering of the T cell receptor by antigen9,20 and is known to induce changes similar to those induced by stimulation with PHA.21 Although resting T cells proliferate in response to PHA or stimulation of the T3−Ti receptor complex only if monocytes are present to provide an additional signal (possibly IL1), preactivated HLA-DR positive T cells may proliferate even in the absence of monocytes.21 In keeping with the lack of expression of HLA-DR antigens, our patient's cells behaved like resting T cells, proliferation being observed only if monocytes were added to the culture medium.

Apart from stimulation of the T3−Ti receptor complex, T cells can also be activated by the T11 sheep erythrocyte receptor protein.21 This mode of activation is antigen independent and requires simultaneous triggering of two distinct epitopes (T112, T113) of the sheep erythrocyte binding protein by means of anti-T112 and anti-T113 monoclonal antibodies.21 The physiological role of this "alternative pathway" of T cell activation and the natural ligand of the T11 receptor are still unknown.27 Although peripheral T cells proliferate in response to both the antigen-dependent mechanism and the alternative path of activation by an IL 2-dependent autocrine mechanism,9,21 it has been shown that thymocytes, although expressing the T11 receptor in almost all maturational stages, fail to proliferate after stimulation with anti-T112 and anti-T113.22 However, if exogenous IL 2 is added to the anti-T112-treated cells, substantial proliferation occurs, indicating that—in contrast to peripheral T lymphocytes— triggering of T112 and T113 epitopes of thymocytes results only in expression of IL 2 receptors, but not in IL 2 secretion.22 Although the T10 antigen that is usually encountered in late stages of thymic maturation12 was not present on the patient's leukemia cells, their stimulation pattern was characteristic of mature thymocytes, proliferation being induced by PHA, Sepharose-bound anti-T3, and anti-T112/3 in conjunction with exogenous IL 2, but not by anti-T112/3 alone. No differences were found between the T3+T4−T8− and the T3+T4+T8+ subsets.

The phenotype of the largest tumor cell subset (T3+T4−T6−T8+T10−T11+) has repeatedly been observed in T cell-derived non-Hodgkin lymphomas36,37 and leukemias.38,39 It is apparently not linked to a homogeneous clinical picture, because the patients' survival times ranged from a few weeks38 to many years.39 None of the other patients with a T cell neoplasm of this phenotype suffered from AT, and no details were given as to additional tumor cell populations.

The patient's cells did not respond to allogeneic leukocytes in the mixed lymphocyte culture. Because the MLR between
two normal donors was not influenced by the patient's PBMCs, the negative proliferative response could not be ascribed to a suppressive effect of the tumor cells. Moreover, it is unlikely that the general immunodeficiency associated with AT was responsible for the negative MLR because in contrast to other T cell-dependent functions, the MLR is normal in most patients with AT. Because chromosome analysis showed that >90% of the PHA-stimulated blood cells had the same pathological karyotype and because ~98% of PBMCs expressed the T3+ T4+ T8- phenotype, it appears conceivable that the proportion of normal lymphocytes was too low to give a positive MLR, the tumor cells themselves being unable to recognize the MHC-antigens on the stimulator cells.

Because the absolute number of Leu 11+ cells was constantly increased, it appears possible that a third tumor cell subset existed. Since pan-T and suppressor/cytotoxic T cell epitopes were demonstrable on 98% of PBMCs, most Leu 11+ cells probably reacted with anti-Leu 1, anti-Leu 4, and anti-Leu 2a as well, a possible hint to their T cell origin. Normally, only ~1.5% of PBMCs coexpress Leu 11 and Leu 2a, and no significant overlap is known to occur between Leu 11 and Leu 4, and Leu 3a. Under physiological conditions, the Leu 11- subpopulation is functionally associated with ADCC and NK activity. Our assays, however, failed to detect any cytotoxic activity of the patient's tumor cells.

Phenotypic heterogeneity of a tumor cell population is thought to reflect either the existence of multiple cell clones or the retained capacity of a monoclonal cell line for further differentiation. In our observation, several arguments are in favor of the latter assumption. First, cytogenetic analysis failed to demonstrate any chromosomally marked subclones within the tumor cell population. Second, regardless of the enormous variations of the WBCs, the ratio of Leu 2a+ Leu 3a+, Leu 2a+ Leu 3a-, and Leu 11+ cells remained comparable throughout the observation period, suggesting that the tumor cell population as a whole responded to some regulatory mechanism; likewise the proportion of all subsets remained constant following initiation of antineoplastic chemotherapy. If multiple neoplastic cell clones had been present, such an observation could only be explained by the unlikely assumption of identical proliferation kinetics and identical sensitivity toward cytotoxic drugs of all clones. If a monoclonal tumor cell origin is hypothesized, the existence of multiple cell subsets could be explained either by different maturational stages on the way to a single terminal cell type, by multiple terminal cell types evolving from a common precursor, or by different functional states of the same cell population. Although in our case both the T3+ T4+ T8+ and the T3+ T4+ T8- subset displayed the proliferation pattern of mature thymocytes, it is conceivable that the T4+ subset was the precursor of the T4- subset, as has been suggested with regard to other mature T cell neoplasms coexpressing T4 and T8. However, our results do not exclude the possibility that both subsets originated from the same precursor with further differentiation into different paths. The ratio between Leu 2a+ Leu 3a+ and Leu 2a+ Leu 3a- cells in our patient (56% to 69%; 29% to 43%) reflected the ratio between helper/inducer and suppressor/cytotoxic T cells (47% to 78%; 22% to 46%) in healthy individuals. Similarly, the proportion of Leu 11+ cells (3% to 9%) was comparable to the percentage of these cells (5% to 15%) under physiological conditions. Although these similarities may be fortuitous, one is tempted to speculate that further differentiation of the incessantly proliferating tumor cells was controlled by regulatory mechanisms similar to those maintaining the physiological balance between the T cell subpopulations.

Chronic T cell leukemias have been reported in at least three other adult patients with AT (Tables 4 and 5). All cases showed comparable clinical findings. Apart from mild liver enlargement in two cases, organomegaly was not found at diagnosis. In spite of a more or less distinct bone marrow infiltration and a steadily increasing leukocytosis, bone marrow function remained sufficient for months or years. In the previously described cases, death resulted from respiratory failure either due to pleuropulmonary leukemic involvement in terminal stages (cases 1 and 3) or to bronchopneumonia facilitated by cytostatic therapy (case 2). The response to cytotoxic treatment was modest or absent; its tolerance was poor, with major complications arising from infections (cases 1 and 2) or cyclophosphamide-induced cystitis (present observation). A reduced tolerance to radiotherapy and chemotherapy is a well-known feature of AT, possibly resulting from defective DNA repair. An extensive phenotypic and functional analysis of the leukemic cells has only been done in the case described by Saxon et al. Their findings reveal some resemblances to our own: The leukemic cells were subdivisible into two subpopulations carrying Fc-receptors for either IgM or IgG. Depending on the ratio

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Table 4. Clinical Data of Patients with AT and T-CLL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr), Sex</th>
<th>Survival (mo)</th>
<th>Organ Involvement</th>
<th>Response to and Adverse Effects of Chemotherapy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29, F</td>
<td>29</td>
<td>Bone marrow, liver?</td>
<td>Lymph nodes, liver, lungs, pleura (effusions), skin, hematopoietic insufficiency</td>
<td>Resistance to VCR + PRD; septic burns after CBL</td>
</tr>
<tr>
<td>2</td>
<td>25, F</td>
<td>14</td>
<td>Bone marrow</td>
<td>Mild hematopoietic insufficiency</td>
<td>Bronchopneumonia after CBL + PRD</td>
</tr>
<tr>
<td>3</td>
<td>47, F</td>
<td>42</td>
<td>Bone marrow, liver?</td>
<td>Pleura (effusions)</td>
<td>Resistance to CTX, CBL</td>
</tr>
<tr>
<td>4</td>
<td>27, M</td>
<td>24</td>
<td>Bone marrow</td>
<td>Pleura (effusions), mild hematopoietic insufficiency</td>
<td>Resistance to CBL + PRD, hemorrhagic cystitis after CTX + PRD</td>
</tr>
</tbody>
</table>

It is concluded from the literature that refs. 3 (case V) and 4 deal with the same patient.

AT, ataxia telangiectasia; T-CLL, chronic T cell leukemia; VCR, vincristine; PRD, prednisone; CBL, chlorambucil; CTX, cyclophosphamide.
between leukemic T cells and normal B cells, the leukemic cells provided either help for or suppression of immunoglobulin synthesis in vitro. Response to PHA and ConA stimulation was normal; ADCC was diminished.

Complex clonal chromosome changes were found in all cases with T-CLL in AT, the unifying element being a structural anomaly of the long arm of chromosome 14. The three previously reported patients showed a tandem translocation t(14;14) involving band q11 or q12 of one chromosome 14 and band q31 or q32 of the other. Structural changes in bands q11–12 of chromosome 14 seem to occur in most chronic T cell neoplasms in patients with and without AT.46 This chromosome region has recently been shown to code for the α-chain of the human T cell receptor47,48 and is one of the “hot spots” for chromosome breakage even in lymphocytes of normal individuals.49 Croce et al.50 state that malignant transformation in T-CLL may be related to juxtaposition of the α-chain gene locus to chromosome region 14q32.3, which they suspect to be the site of a proto-oncogene. Although our results do not exclude rearrangements at a molecular level, the karyotype in the present case differs from the previously reported observations: Whereas chromosome region q11–12 seemed to be conserved in both chromosomes 14, one chromosome 14 had an interstitial deletion affecting bands q21–31. Thus, it appears unlikely that the T cell receptor α-chain genes were involved in malignant cell transformation in our patient. Because the interstitial deletion concerned the region to which the c-fos oncogene has been mapped,51,52 this oncogene may play a role in the pathogenesis of our case.

Clonal anomalies affecting chromosome 14 occur equally in lymphocytes of patients with AT not suffering from leukemia.53,54,55 Because the proportion of these chromosomally marked cells tends to increase gradually with time,53,54,55 one must consider the possibility that these patients will all sooner or later develop overt leukemia unless they die from other causes before. Various findings, however, support the view that a second event occurs, converting the “premalignant” state characterized by a sole anomaly of 14q into a neoplastic process.54,55 First, although many cases with clonal anomalies of both chromosomes 14 have been followed up for several years,53,54,55 only three patients with AT have been reported to develop T-CLL (apart from our observation).56,57 Second, once T cell leukemia is diagnosed, deterioration is rather rapid in comparison to the benign clinical course before manifestation of lymphocytosis. Third, all cases with T-CLL and AT showed other complex chromosome anomalies in addition to those affecting chromosome 14.53,58 Consistent cytogenetic abnormalities, however, have not been demonstrated yet in these patients. The malignant clones in the present case and in two of the three observations previously published53,54 were marked by a loss of one chromosome 20, a finding that has repeatedly been observed in acute lymphoblastic leukemia.56 However, the general significance of this aberration for the malignant transformation in AT appears questionable, because the same anomaly in connection with a rearrangement in chromosome 14 has been described in AT without leukemia.55

Chronic leukemias of phenotypically heterogeneous mature T cells may develop more often in AT than is yet known. To our knowledge, a comparable leukemia has never been described in patients without this hereditary disorder. Therefore, it is tempting to assume a genetic predisposition for the development of this particular type of T cell leukemia.

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This paper is dedicated to Prof Dr Karl Lennert on the occasion of his sixty-fifth birthday.

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