Human T-Cell Lymphoma With Suppressor Effects on the Mixed Lymphocyte Reaction (MLR). I. Morphological and Cytogenetic Analysis

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The aggressive clinical course and the distinctive histologic, cytochemical, and cytogenetic features of an adult non-Sézary T-cell lymphoma with suppressor activity have been investigated. Morphological and ultrastructural analysis of neoplastic cells from peripheral blood and involved lymph nodes revealed cells with convoluted nuclei, prominent cytoplasmic azurophilic granules, well developed Golgi apparatus, short strands of endoplasmic reticulum, and moderate numbers of ribosomes and mitochondria. Cytochemical reactions showed acid phosphatase (ACP) positivity in virtually all of the neoplastic cells; in a substantial percentage of the cells, the tartrate-resistant acid phosphatase (T-ACP) isoenzyme was observed. Granular naphthyl acetate esterase (A-EST) reactivity was not present. The histologic and cytochemical features of these neoplastic suppressor cells were compared with those recently described for the suppressor T-cell fraction isolated from normal peripheral blood T-cell by Fcγ-receptor formation. The aneuploid clone had 47 chromosomes with multiple complex abnormalities, including a 14q+ chromosome formed by the tandem translocation of two no. 14 chromosomes and translocations involving the long arms of no. 2 and no. 9 at band 9q34. These latter changes are particularly common in T-cell disorders. The extensive analysis of the histologic, cytochemical, and cytogenetic features of this adult T-cell suppressor lymphoma should help to clarify the criteria for distinguishing among the subsets of T-cell neoplasms with definable immunologic function.

CONSIDERABLE evidence has accumulated indicating that the neoplastic cells of some T-cell lymphomas and leukemias retain certain functional properties of normal T lymphocytes. Neoplastic T cells that exhibit a T-helper cell function have been identified regularly in patients with Sézary syndrome; a Sézary cell population with potent suppressor activity has also been found. In a recent study, lymphoblasts from a child with T-cell acute lymphocytic leukemia (ALL) expressed suppressor activity only in the presence of cooperating normal T cells, suggesting that the blasts possessed a prosuppressor function. Saxon and coworkers have reported results suggesting that malignant cells from a patient with chronic T-cell leukemia and ataxia telangiectasia exhibited both helper and suppressor effects under in vitro conditions.

We report on the analysis of a human T-cell lymphoma with peripheral blood involvement that was characterized by distinctive histologic features and unique suppressor functional properties. The results of functional tests in vitro are detailed in an accompanying report. We have classified this lymphoproliferative disorder not only on clinical and morphological grounds, but also by cytogenetic and immune marker analysis. The results of this comprehensive analysis may provide the basis for future comparisons of related data on T-cell neoplasms that have similar histologic features.

CASE REPORT

Patient Wa, a 60-yr-old black female, was first admitted to the University of Chicago Hospitals and Clinics in July 1978, because of increasing lymphadenopathy, anorexia, 20-pound weight loss, and persistent diarrhea. The patient had been seen irregularly over 12 yr in the dermatology clinic for a recurrent nonspecific dermatitis with atopic features, and she was treated intermittently with topical steroids. Three months prior to her admission, the chronic dermatitis worsened, and generalized lymphadenopathy was noted for the first time.

On physical examination, the patient appeared chronically ill with dry and thickened skin, patchy alopecia, and generalized lymphadenopathy including large matted inguinal nodes bilaterally. The liver and spleen were not enlarged, and the remainder of the physical findings were normal.

Admission blood counts revealed a WBC of 66,000/cu mm, Hb 9.9 g/dl, Hct 33.9%, and a platelet count of 666,000. A differential leukocyte count showed 11 bands, 69 polymorphonuclear leukocytes, 7 lymphocytes, 5 mononuclear cells, 7 eosinophils, and 1 basophil. Approximately half of the lymphoid cells were markedly atypical, with prominent azurophilic cytoplasmic granules and folded nuclei with loose chromatin. Hemoglobin electrophoresis revealed sickle cell trait with 34.5% S, 2.5% HbA2, and 63% HbA; no significant abnormalities were noted on routine battery of blood chemistries. Rectal biopsy and small-bowel biopsies were normal, as were proctoscopy and radiologic examination of the entire gastrointestinal tract.
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A skin biopsy showed no evidence for mycosis fungoides and was consistent with subacute psoriasiform dermatitis. A left inguinal lymph node biopsy revealed malignant lymphoma, composed of a mixture of large and small lymphocytes with hyperconvoluted nuclei. A bone marrow biopsy and aspirate showed rare scattered neoplastic lymphocytes. Immunologic marker analysis demonstrated that the abnormal peripheral blood lymphocytes were T-cell derived; similar studies were not performed on the lymph node.

The patient was diagnosed as having a T-cell lymphoma, stage IV, with peripheral blood involvement. She was started on monthly cycles of combination chemotherapy, receiving intravenous cytoxan 600 mg/sq m, vincristine 1.4 mg/sq m, and adriamycin 25 mg/sq m on days 1 and 8, with prednisone 40 mg/sq m given orally on days 1 through 8. Dramatic improvement in the lymphadenopathy and the dermatitis was seen during the first cycle, and total resolution of the lymphadenopathy and clearing of the peripheral blood had occurred by the third cycle. The patient received a total of 5 cycles of therapy, with some reduction in dose because of cytopenias.

The patient was readmitted in January 1979 with fever, periumbilical abdominal pain, and leukocytosis at the expected nadir in her chemotherapy cycle. On physical examination, she was acutely ill with a temperature of 39.5°C, pulse of 129, respiratory rate of 20, and blood pressure of 150/105. There was no significant lymphadenopathy, hepatosplenomegaly, or change in her skin.

Laboratory data included WBC 22,700/cu mm, Hb 10.4 g/dl, Hct 33.5%, and a platelet count of 167,000. Differential WBC showed 10 polymorphonuclear leukocytes, 5 bands, 1 monocyte, and 84 lymphocytes, most of which appeared malignant. Uric acid was 10.6 mg/dl and creatinine was 1.9 mg/dl with a normal BUN and serum electrolytes. The results of liver function tests were now abnormal, with the SGOT 111 IU (normal 0-35), SGPT 68 IU (normal 0-35), alkaline phosphatase 686 IU (normal 0-35), LDH 1920 IU (normal 12-55), and a bilirubin of 1.3 mg/dl (normal 0-1.2), with a direct bilirubin of 0.6 mg/dl (normal 0-0.3). Total protein was 5.1 g/dl, with a reduced albumin of 2.3 g/dl. The remainder of the laboratory data, chest x-ray, and abdominal flat plate were normal.

The patient received broad-spectrum antibiotics, with the biliary tree regarded as the presumed source of sepsis. Her fever defervesced promptly, and her abdominal pain resolved. Evaluation of the liver and biliary ducts by ultrasound revealed mild dilatation of the common bile duct; the findings were otherwise normal. Cultures were negative, but antibiotics were continued because of the observed clinical response.

After stabilization, the patient was begun on the COMLA chemotherapy regimen and received an initial intravenous cytoxan dose of 1.5 g/sq m. With this therapy, the patient's peripheral leukocytes fell from a high of 44,000 to 2100/cu mm within 10 days. However, the peripheral blood smear was never completely cleared of malignant lymphocytes.

The remainder of the patient's hospital course was complicated by progressive multifactorial renal failure, pseudomonas pneumonia with sepsis, and disseminated intravascular coagulation. Voluminous diarrheal stools, up to 4 liter/day, contributed to problems of fluid management. Although the patient's original skin disease did not worsen during this period, a toxic epidermal necrolysis and Herpes simplex infection supervened. Despite supportive therapy with platelet and white blood cell transfusions, appropriate antibiotic coverage, and peritoneal dialysis, the patient died on day 32 of hospitalization. Permission for autopsy was denied.

RESULTS

Histopathology, Including Transmission Electron Microscopy and Cytochemical Staining

The biopsy from the skin showed a modest perivascular infiltrate of small, round lymphocytes and of histiocytes, concentrated in the upper dermis. Moderate hyperkeratosis, focal parakeratosis, and moderate regular acanthosis was also observed, as was absence of the granular layer. These findings were felt to be consistent with subacute psoriasiform dermatitis (Fig. 1).

Histologic sections of the inguinal lymph node biopsy showed diffuse replacement of the architecture by large and small neoplastic lymphoid cells (Figs. 2

Fig. 1. The H & E sections of skin shows moderate hyperkeratosis, focal parakeratosis, and moderate regular acanthosis. There is absence of the granular layer. Some inter- and intracellular edema are also seen focally. One to two rows of the upper malpighian layer show perinuclear vacuolization. The upper dermis reveals a moderately dense perivascular lymphohistiocytic infiltrate. These findings are those of subacute psoriasiform dermatitis with epidermal changes suggestive of nutritional deficiency (x 250).

MATERIALS AND METHODS

Routine histologic sections of the lymph node biopsy were obtained from formalin-fixed tissue and stained with hematoxylin-eosin, methyl green pyronine, periodic-acid Schiff (PAS), reticulin, and alcin blue; naphtho AS-D chloroacetate esterase reactions (NASDCA) were also performed. Material for transmission electron microscopy (TEM) was obtained from glutaraldehyde-fixed tissue. Cytochemical reactions performed on peripheral blood included PAS, alpha-naphthyl acetate esterase (A-EST) with and without NaF, NASDCA, acid phosphatase with and without tartrate inhibition, and peroxidase.

Chromosome analyses were performed on cells derived from bone marrow and peripheral blood as previously described. Mitotic cells were stained with conventional Giemsa stain and photographed. After destaining, the same metaphases were restained with quinacrine mustard and photographed again. Duplicate photographs of metaphases were used for precise identification of each chromosome according to the Paris nomenclature.
and 3). The large cells had vesicular, often lobulated nuclei with prominent nucleoli, whereas the nuclei of the smaller cells tended to be convoluted. Pyroninophilia was present in the cytoplasm of both cell populations. Numerous large, phagocytic histiocytes with ingested nuclear debris were scattered throughout the lymph node. A moderate proliferation of capillary-sized vessels was also observed. Touch imprints of the lymph node confirmed the convoluted nature of the lymphoma cell nuclei; impressive azurophilic granules, \(1-2\mu\) in diameter, were aggregated toward one pole of the cells (Fig. 4). These granules were present in both the large and small cell populations, although the smaller cells tended to have a larger number of granules. The cells seen in the touch imprints were similar to those observed in peripheral blood smears; cytospin preparations prepared from E-rosette suspensions showed that these cells from the peripheral blood formed E rosettes, confirming their T-cell nature (Fig. 5).

Transmission electron microscopy (TEM) performed on the lymph node biopsy specimen demonstrated that the granules in the neoplastic cells were surrounded by a limiting membrane with a dense inner...
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Variability in size and structure of the membrane-enclosed granules is illustrated at higher magnification (x 19,500).

The results of cytochemical reactions performed on cells from the patient's peripheral blood are presented in Table 1. One-hundred percent of the circulating malignant cells showed acid phosphatase activity, and more than one-third of these cells had tartrate-resistant acid phosphatase activity (T-ACP). A-EST activity was faint and occurred in a diffuse rather than a granular distribution. In addition, the NASDCA reaction performed on tissue sections of the lymph nodes showed a definite, although weak reaction in some of the neoplastic cells, and an alcian blue stain at pH 2.5 demonstrated intense granular positivity.

Cytogenetic Analysis

Chromosome analyses were performed on cells obtained from bone marrow and peripheral blood (Table 2). In the initial sample (8/78), only cells with a normal karyotype were seen in the bone marrow. However, one of three cells from a 24-hr unstimulated blood culture obtained on the same date had an abnormal karyotype similar to that observed in all cells of the later (1/79) sample. No normal karyotypes were seen in the bone marrow aspirate received on 1/10/79. The modal chromosome number was 47. Sixteen cells were karyotyped with the use of multiple banding techniques. Every cell showed essentially the same karyotype, which was one of the most complex to be reported in a T-cell malignancy, and included the apparent loss of 5 chromosomes associated with 17 structural rearrangements. The detailed karyotype is described in the legend for Fig. 8.

Among these abnormalities, those affecting nos. 2, 5, 7, 9, and 14 are particularly significant. The cells contained one normal and two abnormal no. 14s. The abnormal no. 14s appeared to result from a tandem translocation between two no. 14s, with a long marker chromosome (14q+) containing almost all of the long arm of both chromosomes and a small chromosome...
Table 2. Summary of Chromosome Analyses

<table>
<thead>
<tr>
<th>Date</th>
<th>Source</th>
<th>No. of Chromosomes</th>
<th>Total No. of Cells</th>
<th>Percent Abnormal†</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-11-78</td>
<td>Bone marrow</td>
<td>5 (3) 1 (0) 2 (0) 5 (3) 9 (7)</td>
<td>22 (13)</td>
<td>0</td>
</tr>
<tr>
<td>8-11-78</td>
<td>Peripheral blood (24 hr)</td>
<td>1 (1) 2 (2) 3 (3) 33</td>
<td>3 (3)</td>
<td>33</td>
</tr>
<tr>
<td>9-7-78</td>
<td>Peripheral blood (5 day) + PHA</td>
<td>1 (1) 5 (5) 6 (6) 0</td>
<td>6 (6)</td>
<td>0</td>
</tr>
<tr>
<td>1-10-79</td>
<td>Bone marrow</td>
<td>1 (0) 2 (2) 4 (2) 7 (0) 6 (4) 15 (8)</td>
<td>35 (16)</td>
<td>100</td>
</tr>
</tbody>
</table>

*Number in parentheses indicates number of cells examined with banding.
†Calculated percent of number of cells examined with banding.
‡One of these cells shows abnormality seen in sample on 1-10-79.

that represented the centromere region of the translocated no. 14. Each chromosome no. 2 had an abnormality affecting the end of the long arm; in one, this was associated with an extra pale band, and in the second, a complex pattern of pale and dark bands was added to the middle of 2q. One chromosome, no. 5, had an interstitial deletion of the long arm, and both no. 7 chromosomes were involved in the formation of a single long marker that consisted of all of one no. 7 and about one-half of the second no. 7. Another rearrange-
ment involved the addition of unknown material at the end of the long arm of one no. 9 (9q+). The origin of two marker chromosomes could not be determined.

DISCUSSION

This patient had a lymphoproliferative disorder in which the malignant cell was a functionally competent suppressor T cell. The aspects of the morphology, cytochemistry, and cytogenetics support the proposal that there are specific characteristics that are clearly associated with particular subsets of malignant T cells.

Morphology

The neoplastic lymphocytes from patient Wa are similar in appearance to normal suppressor T cells described by Grossi et al., who report that the helper and suppressor T-cell fractions of normal human peripheral blood lymphocytes appear to be distinguishable according to their morphological characteristics. Their previous studies indicated that helper T cells possess membrane Fc receptors for IgM and that suppressor T cells bear membrane Fc receptors for IgG. These cells were isolated and studied separately. The helper T cells (Th or Ts cells), isolated by rosette formation with IgM-coated erythrocytes, were found on routine microscopy to be small lymphocytes with scant basophilic cytoplasm. Although generally devoid of organelles, the cytoplasm of Th cells had one or two large distinctive concentrations of nonspecific esterase activity. Transmission electron microscopy (TEM) demonstrated a small Golgi apparatus and rare mitochondria. In contrast, suppressor T cells (TTh or Ts cells), isolated by rosette formation with IgG-coated lymphocytes, were shown to be larger cells, with greater amounts of cytoplasm that contained a more complex system of organelles, including prominent azurophilic granules. TEM of Ts cells revealed a well developed Golgi apparatus, rough endoplasmic reticulum, and clusters of mitochondria. The azurophilic granules in those studies were not PAS-positive or alcian-blue-positive and did not contain lysosomal enzyme markers. Grossi noted that the appearance of suppressor activity in culture supernatants of Ts cells coincided with loss of the cytoplasmic granules, suggesting that the granules contained suppressor substances.

Several other morphological and clinical features are shared by patient Wa and other series of patients with T-cell lymphoproliferative disorders. The lymphocytes of patient Wa had hyperconvoluted nuclei similar to those seen in Sézary cells. In the Sézary syndrome, malignant lymphomas of hyperconvoluted T cells arise in association with a prolonged dermatologic disorder. The skin lesions in Sézary syndrome show involvement with these malignant cells, characteristically with formation of Pautrier microabscesses. Our patient’s skin biopsies did not show these changes, however. Prominent nuclear irregularities were also seen in the neoplastic T lymphocytes involving the lymph nodes of a series of patients described by Waldron et al. The diffuse pattern of lymph node involvement, variation in size of the malignant lymphocytes, increased numbers of large acidophilic macrophages, and proliferation of capillary-sized vessels that were seen in our patient were also seen in Waldron’s series. Several clinical features in patient Wa were also shared by Waldron’s patients: generalized lymphadenopathy, relative bone marrow sparing, and poor survival despite combination chemotherapy.

Functional Analysis

The functional analysis of Wa lymphocytes is described in detail in the accompanying paper. The Wa T cells showed suppressor effects in the mixed lymphocyte reaction (MLR) between allogeneic normal controls. The Wa cells exhibited no suppressor activity on the in vitro Ig synthesis of pokeweed-mitogen-stimulated normal peripheral blood lymphocytes, thus providing evidence that the Wa suppressor function was selective for certain subsets of immune-reactive T cells. MLR suppressor T cells can be induced routinely by concanavalin-A activation of normal peripheral blood lymphocytes; therefore, this subset of T-suppressor cells with MLR suppressor activity must be commonly represented in the T-suppressor cell pool of normal peripheral blood. Thus, the Wa suppressor lymphocytes may have been derived from neoplastic transformation of a normal lymphoid clone whose original T-cell function centered on suppressing T cells normally reactive to allogeneic histocompatibility antigens.

Cytochemistry

The cytochemical reactions performed on peripheral blood cells (Table 1) yielded some findings consistent with a T-suppressor cell origin. A single globule of acid phosphatase activity in lymphoblasts is considered by some to be a fairly reliable marker of the T-cell origin of the cells. Tartrate-resistant acid phosphatase activity was seen in a substantial number of our patient’s cells. Wa T-cells are the first example, to our knowledge, of T-ACP in a proven T-cell lymphoma. Rare cases of T-cell hairy cell leukemia, several cases of B-cell lymphoma, and two cases of lymphoma that were not classified as to B-cell or T-cell origin have been described with this isoenzyme. In most of our patients’ cells, the A-EST reaction was faint and inhibited with NaF. Although such a pattern might indicate a monocytic origin, the
faint reaction product was so much less intense than in the monocytic cells of the peripheral blood, that it was probably nonspecific. Grossi et al.\textsuperscript{9} have reported the presence of single granules of A-EST activity in T-helper cells and their absence in T-suppressor cells and in most B-lymphoid cells. The lack of A-EST in the form of granules in the neoplastic cells of our patient, which were shown by E-rosette formation to be T cells, is a negative finding that supports the T-suppressor cell origin of the cells. In contrast to the findings of Grossi et al.,\textsuperscript{9} however, both periodic-acid Schiff-positive and alcian-blue-positive granules were seen, indicating the presence of glucosaminoglycans in the cytoplasm of the malignant cells. The NASDCA reaction, a cytochemical test useful in identifying the neutrophilic myeloid series as well as mast cells, showed faint, but unequivocal granules of positivity in the neoplastic cells of the peripheral blood and lymph node; the significance of this finding is not known. In summary, the lack of punctate granules of A-EST activity and the presence of T-ACP may be characteristic findings for a neoplasm of T-suppressor cell origin.

**Cytogenetics**

Although chromosomes from fewer than 20 patients with malignant disorders of T-cell origin have been analyzed with banding,\textsuperscript{19} some differences in the frequency of certain chromosome changes can be seen in T-cell disorders compared to other malignant lymphomas.\textsuperscript{19}

Three aberrations merit special attention: the 14q + chromosome and the abnormalities involving 2q and 9q. This patient had a 14q + chromosome, apparently derived from a tandem translocation of most of one no. 14 to another, as well as one normal no. 14. The patient was thus trisomic for no. 14. Of 18 patients with T-cell malignancies, including the present one, whose cells have been karyotyped with banding, 9 had a structural rearrangement of no. 14, usually involving band 14q32 and leading to a 14q + chromosome.\textsuperscript{19} In four cases, a tandem translocation similar to that noted in the present patient was found; the break point occurred in q11–12 in one no. 14 and in q32 in the other no. 14 [t(14;14)(q11–12;q32)]. Our patient was the only one with one normal no. 14 as well as the translocation. Three of the earlier patients had ataxia telangiectasia (AT) and had the 14q + translocation in the stable phase of the disease; this chromosomally marked clone was involved in the leukemic transformation to chronic lymphocytic leukemia (2 patients)\textsuperscript{20} or to atypical subacute lymphocytic leukemia (1 patient).\textsuperscript{21} Four of the 9 patients had a 14q + chromosome derived from a presumably balanced translocation\textsuperscript{22,25} and the ninth patient had mycosis fungoides and a complex three-way translocation involving chromosomes 2, 8, and 14.\textsuperscript{24} Two of the patients with a 14q + chromosome had adult T-cell leukemia. In a recent report from Japan, five of seven patients had a Dq + or a 14q + chromosome.\textsuperscript{25} Therefore, although the 14q + chromosome was first described in malignant B cells, e.g., Burkitt lymphoma, multiple myeloma, and B-cell ALL, it is also found in at least one-half of the patients with malignant T-cell diseases.\textsuperscript{19}

Among the other structural rearrangements noted, those affecting chromosome no. 2 are noteworthy, since this chromosome is rarely involved in abnormalities in myeloid disorders or in other non-Hodgkin lymphomas. In a series of 18 T-cell lymphomas, on the other hand, cells from 2 patients showed a gain of one no. 2 and 8 had a translocation involving the long arm of one or both no. 2. The short arm of no. 2 was not affected.\textsuperscript{19} Eight patients had abnormalities of no. 9 including a gain of no. 9 in one patient, and structural rearrangements involving primarily the long arm in seven others, two of whom had no normal no. 9 chromosome. In three patients, there was a 9q + chromosome due to a translocation to 9q34, the same band affected in the Ph\textsuperscript{1} translocation in chronic myelogenous leukemia.\textsuperscript{19}

A comparison of the profile of gains, losses, and rearrangements of chromosomes in T-cell disorders, poorly differentiated lymphocytic lymphoma, and diffuse histiocytic lymphoma has revealed that the pattern of abnormalities in each of these disorders is unique and is distinctly different from that seen in the other two.\textsuperscript{19} Although abnormalities of chromosome no. 14 are common in all three, those affecting 2q and 9q are much more common in T-cell disorders. In the future, we will be able to relate specific structural aberrations to the genes that are affected and thus to understand their influence on the functional immunologic activity of various subclasses of these cells.

Subsets of the heterogeneous polyclonal, and multifunctional T-cell pool have only recently been delineated because, in part, they are ordinarily few in number. The malignant transformation of this particular subset suppressor clone has allowed us to define its unique features. The malignant cells maintained some morphological and cytochemical aspects of normal suppressor T cells and also expressed the limited function of the clone, MLR suppression. More importantly, histologic, cytochemical, and cytogenetic homologies have been shown between the case described and many other cases of T-cell malignant disorders. By means of comparable multifaceted analyses of other lymphoproliferative disorders,
further significant correlations will be made that will lead to the identification of other subsets of normal lymphocytes, thus broadening our understanding of immune function.

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