CONCISE REPORT

Functional Properties of Neoplastic T Cells in Adult T Cell Lymphoma/Leukemia Patients from the Caribbean


The neoplastic T cells from five patients with adult T cell lymphoma/leukemia (ATLL), born in the Caribbean, were studied with respect to immunoregulatory activity on pokeweed mitogen (PWM) driven immunoglobulin (Ig) synthesis as well as surface-marker phenotypes with monoclonal antibodies. The neoplastic T cells in all patients had an OKT1+ 8 11 M1 11 3A1 phenotype, but differed in the reactivity with OKT3. None of the patients' cells exerted helper activity on PWM-induced Ig synthesis. The neoplastic cells of three patients had suppressor activity on PWM-induced Ig synthesis. All patients were positive for human T cell leukemia/lymphoma virus (HTLV) or had antibodies against HTLV antigens. It has previously been shown that the neoplastic cells in Japanese ATLL patients and in patients from the Caribbean are indistinguishable by morphology and marker phenotype. We now show them to be also similar with respect to their functional properties.

In both Europe and the United States, T cell malignancies are rare and can be divided into two groups: (1) T-lymphoblastic leukemia or lymphoma, mostly in juveniles where the neoplastic T cells have immature phenotypes, and (2) proliferative disease involving more mature T cells, seen mainly in adults. The latter group includes chronic T cell lymphocytic leukemia (T-CLL), T-prolymphocytic leukemia (T-PLL), Sézary’s syndrome, and Tγ lymphocytosis. Surface-marker analysis shows helper phenotypes (OKT4+ 8) in Sézary’s syndrome and T-PLL, and suppressor phenotypes (OKT4- 8') in Tγ lymphocytosis and T-PLL.

In Japan, adult T cell lymphoma/leukemia (ATLL) is the predominant lymphoid neoplasm, with a marked clustering of the disease in Southwest Japan. It was shown that this clustering correlated with the presence of antibodies to antigens of a new C-type RNA leukemia virus (ATLV). Recently, adult T cell lymphoma/leukemia in blacks from the West Indies was described, and this disease is indistinguishable from Japanese ATLL. The neoplastic T cells in both groups of patients have the phenotype OKT3- 4 8 11, and in both groups, patients are positive for HTLV and/or antibodies to HTLV antigens. It has been reported that, by serology of the viral core proteins p19 and p24 and by nucleic acid homology, ATLV and HTLV are either identical or closely related to each other. This confirms the notion that the diseases are very similar and may have a common viral etiology.

Until now, the functional properties of neoplastic T cells of nine patients with Japanese ATLL were reported. In five patients, the neoplastic T cells exerted suppressor activity on pokeweed mitogen (PWM) driven Ig synthesis, whereas none of the patient cells displayed helper activity in this system. We now show that the neoplastic T cells from three of five black patients with ATLL from the Caribbean also suppress Ig synthesis.

MATERIALS AND METHODS

Patients

Functional studies and surface marker analyses were performed on peripheral blood lymphocytes obtained from five patients with ATLL. The main clinical findings at the time of blood sampling are summarized in Table 1.

Patient 1 is a black woman (31 yr old), born on Curacao, who lives in The Netherlands. She developed hypercalcemia following her fourth pregnancy and presented in July 1982 with lymphocytosis, eosinophilia, and splenomegaly. The morphology of her peripheral blood cells (PBL) was typical for ATLL by light and electron microscopic examination (DC). In her serum, antibodies to HTLV and ATLV antigens were demonstrated. At the time of study, she was not receiving any treatment.

Patient 2 is a 45-yr-old black woman, born in Guyana and a resident of the U.K. for 17 yr. She returned to Guyana on several occasions. She was admitted because of generalized itch, lymphadenopathy, lymphocytosis, and hypercalcemia. This patient was included in an earlier study on morphology, histology, and marker

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Table 1. Main Clinical Findings in Five Patients With ATLL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Origin*</th>
<th>Leukocytes (× 109/Liter)</th>
<th>Lymphocytes (× 109/Liter)</th>
<th>BM</th>
<th>Liver</th>
<th>Spleen</th>
<th>LN</th>
<th>Skin</th>
<th>Hypercalcemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Ke)</td>
<td>31</td>
<td>F</td>
<td>Black</td>
<td>WI</td>
<td>25.1</td>
<td>12</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2 (S)</td>
<td>45</td>
<td>F</td>
<td>Black</td>
<td>Gu</td>
<td>67</td>
<td>56</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3 (May)</td>
<td>29</td>
<td>M</td>
<td>Black</td>
<td>Sur</td>
<td>68</td>
<td>57</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4 (Sto)</td>
<td>52</td>
<td>M</td>
<td>Black</td>
<td>WI</td>
<td>103</td>
<td>90</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5 (Ste)</td>
<td>29</td>
<td>F</td>
<td>Black</td>
<td>Sur</td>
<td>28.5</td>
<td>26.2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*WI, West Indies; Gu, Guyana; Sur, Surinam.
†Prior to admission, this patient suffered from a transient skin rash of unknown etiology.

Isolation of Lymphocytes and Separation of Subsets

Mononuclear cells (PBL) from healthy donors and patients were isolated from either defibrinated or heparinized blood by Ficoll-Isoopaque density gradient centrifugation. T and non-T lymphocytes were separated by E-rosette sedimentation, using neuraminidase-treated sheep red blood cells. For the separation of T cell subsets, based on their reactivity with anti-T cell monoclonal antibodies, a "panning" technique was used to obtain OKT4- or OKT8-depleted T cell subsets. OKT4-depleted T cells were <5% OKT4, and OKT8-depleted T cells >95% OKT8; these populations are designated as OKT8 and OKT4, respectively.

Surface Marker Analysis

Phenotypic analysis of PBL of patients was performed by indirect immunofluorescence using OK monoclonal antibodies (a kind gift from Dr. G. Goldstein), the monoclonal antibody 3A11, and fluorescein-conjugated goat anti-mouse IgG (Nordic, Tilburg, The Netherlands). Analysis was performed on an Ortho FC-200 cytofluorometer.

Help and Suppression of In Vitro IgM Synthesis

Helper and suppressor activity of healthy donor OKT4+ and OKT8+ cells and patient cells was evaluated on PWM-driven IgM synthesis in a microculture system, as described in detail before. For assay of helper activity, graded numbers of the cells to be tested were added to 20 × 10³ healthy donor B cells in 170 µl Iscove’s modified Dulbecco’s medium (IMDM) with 50 µg/ml PWM (GIBCO, Grand Island, NY, lot no. C477102) and cultured in round-bottomed wells (Cooke 220 M-24 AR) for 7 days. The culture medium was supplemented with 20% fetal calf serum (FCS) (GIBCO, cat. no. 629 H1); IgM production in the supernatants was measured with an enzyme immunoassay, as described before. Non-T cells alone synthesize background levels of IgM in this system. For assay of suppressor activity, graded numbers of the cells to be tested were cocultured with 20 × 10³ normal B cells and 10 × 10³ normal OKT4+ cells. This mixture was chosen because it allowed an optimal distinction between help and suppression. Helper and suppressor tests for patient cells were performed 2–3 times. Suppression is expressed as:

\[
\left(1 - \frac{\text{ng IgM/20,000 B cells + 10,000 OKT4+ cells + patient cells}}{\text{ng IgM/20,000 B cells + 10,000 OKT4+ cells}}\right) \times 100\%
\]

Statistical Analysis

Suppressor activity of patient cells and normal OKT4+ or OKT8+ cells was evaluated by analysis of variance and the Student’s t test.

RESULTS

Surface Markers of Patient Cells

The surface marker profiles of PBL from the five patients with adult T cell lymphoma/leukemia show that all patients have a homogeneous outgrowth of OKT1+ 8 11+ M1+ 11 3A1+ T cells, whereas they differed in their reactivity with OKT3 (Table 2). It appears that the neoplastic T cells in ATLL patients and in Sézary’s syndrome patients have the same T cell phenotype.

Helper Activity of Patient Cells

The helper activity in PWM-driven IgM synthesis of patients’ PBL was compared with that of healthy donor T cells. Normal OKT4+ , but not OKT8+, cells had helper activity (Table 3). Patient cells did not display any helper activity. Even after irradiation (4,000 rad) to abolish the possible masking of helper
activity by suppressor activity, patient cells still lacked helper activity (data not shown). Cells of patients 1–4 were unable to provide help for PWM-induced IgM synthesis, as was previously reported for the cells of patient 5. This is in agreement with the findings with T cells of Japanese ATLL patients.

**Suppressor Activity of Patient Cells**

Suppressor activity of patient cells and normal OKT8⁺ cells is shown in Fig. 1. The cells of patients 1 and 3 were found to suppress PWM-induced Ig synthesis, comparable to healthy donor OKT8⁺ cells. Analysis of variance showed that there was a significant difference between the cells tested in the suppressor assay (p < 0.003). Suppression by 20,000 normal OKT8⁺ cells and by 20,000 cells of patients 1 and 3 was significant (p < 0.003, Student’s t test). The cells of patients 2 and 4 did not mediate suppressor activity. The suppression by the patient cells was fully abrogated by gamma irradiation at a dose of 4,000 rad, like the suppression by normal OKT8⁺ cells (data not shown). Normal OKT4⁺ T cells do not suppress in this system (Fig. 1, exp. 1 and 2). In a previous study, the cells of patient 5 were shown to exert strong suppressor activity, which was also sensitive to irradiation.

**DISCUSSION**

The similarity between the five black patients with ATLL described here and the Japanese cases with ATLL, was observed earlier with respect to histology, morphology, surface marker analysis with monoclonal antibodies, and clinical features. The present study extends this similarity to the functional properties exerted by the neoplastic T cells. In both groups of patients, the cells do not help, but often suppress PWM-induced Ig synthesis (Fig. 1). It has recently been reported that healthy donor OKT4⁺ cells can be divided into OKT4⁺, T-Q1 cells that do, and OKT4⁺, T-Q1 cells that do not, have helper activity. It was also shown that activated healthy donor OKT4⁺ T cells suppress PWM-driven Ig synthesis, whereas activated OKT4⁺ T-Q1 cells do not. Moreover, it was shown that, in normal blood, virtually all helper activity was contained within the OKT4⁺ 3A1⁺ T cell population, and OKT4⁺ 3A1⁺ T cells possess only marginal helper activity. Hence, it may well be that

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**Table 2. Surface Markers of PBL From ATLL Patients**

<table>
<thead>
<tr>
<th>Binding of Monoclonal Antibodies*</th>
<th>E-Rosettes</th>
<th>T1</th>
<th>T3</th>
<th>T4</th>
<th>T8</th>
<th>T11</th>
<th>T17</th>
<th>M1</th>
<th>I1</th>
<th>3A1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient E-Rosettes</td>
<td>70</td>
<td>37</td>
<td>39</td>
<td>48</td>
<td>2</td>
<td>55</td>
<td>35</td>
<td>6</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>2 (5)</td>
<td>82</td>
<td>NT</td>
<td>16</td>
<td>84</td>
<td>2</td>
<td>92</td>
<td>NT</td>
<td>NT</td>
<td>1</td>
<td>NT</td>
</tr>
<tr>
<td>3 (May)</td>
<td>87</td>
<td>82</td>
<td>90</td>
<td>84</td>
<td>5</td>
<td>86</td>
<td>85</td>
<td>3</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>4 (Sto)</td>
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<td>91</td>
<td>88</td>
<td>90</td>
<td>6</td>
<td>90</td>
<td>90</td>
<td>6</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>5 (Ste)</td>
<td>90</td>
<td>95</td>
<td>0</td>
<td>97</td>
<td>2</td>
<td>98</td>
<td>NT</td>
<td>3</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Healthy donor (mean, n = 3)</td>
<td>70</td>
<td>83</td>
<td>80</td>
<td>66</td>
<td>21</td>
<td>91</td>
<td>85</td>
<td>23</td>
<td>15</td>
<td>70</td>
</tr>
</tbody>
</table>

*See ref. 19 for description of OKT monoclonal antibodies. OKM1 binds monocytes and Tγ lymphocytes.

NT, not tested.

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**Table 3. Lack of Helper Activity of PBL From ATLL Patients**

<table>
<thead>
<tr>
<th>Helper Activity*</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T4⁺</td>
</tr>
<tr>
<td>Patient</td>
<td>ATLL Cells</td>
</tr>
<tr>
<td></td>
<td>Control†</td>
</tr>
<tr>
<td>1 (Ke)</td>
<td>1 &lt;19‡</td>
</tr>
<tr>
<td></td>
<td>2 &lt;19</td>
</tr>
<tr>
<td></td>
<td>3 &lt;19</td>
</tr>
<tr>
<td>2 (5)</td>
<td>1 &lt;19</td>
</tr>
<tr>
<td></td>
<td>2 &lt;19</td>
</tr>
<tr>
<td></td>
<td>3 &lt;19</td>
</tr>
<tr>
<td>3 (May)</td>
<td>1 74</td>
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<td></td>
<td>2 &lt;19</td>
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<tr>
<td></td>
<td>3 &lt;19</td>
</tr>
<tr>
<td>4 (Sto)</td>
<td>1 &lt;19</td>
</tr>
<tr>
<td></td>
<td>2 &lt;19</td>
</tr>
</tbody>
</table>

*Expressed as the IgM production induced by 10 × 10³ patient cells or healthy T4⁺ or T8⁺ cells.
†Healthy donor non-T cells alone.
‡Nanograms of IgM per culture, determined by ELISA on pooled supernatants from quadruplicate cultures. Variation between duplicate ELISAs was always less than 5%.
the neoplastic T cells in ATLL patients originate from the OKT4+ T cells, which are unable to mediate helper activity, and upon activation, may act as suppressor cells on PWM-induced Ig synthesis. The OKT17 marker, expressed by the cells of patients 1 and 3, fits with this hypothesis, because it acted as a suppressor. However, in a series of Sézary’s syndrome patients, the OKT17 marker did not discriminate between functionally distinct neoplastic OKT4+ cells (Miedema et al., submitted).

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