Abnormalities of Chromosome 7q and Tac Expression in T Cell Leukemias

By Vasantha Brito-Babapulle, Estela Matutes, Leonor Parreira, and Daniel Catovsky

Clonal chromosome abnormalities were demonstrated in 13 cases of T cell leukemia with a mature membrane phenotype. The cases comprised two adult T cell lymphoma leukemia (ATLL), five Sézary syndrome (SS), three T prolymphocytic leukemia (T-PLL), and three T chronic lymphocytic leukemia (T-CLL). The presence of clonal chromosome abnormalities in the three cases of T-CLL supports the view that although clinically benign, this disorder probably represents a neoplastic process.

Cells from six of 11 patients were tested and found to be reactive with mononclonal antibody (McAb) anti-Tac in the absence of mitogens (two ATLL, two SS, and two T-PLL).

PREVIOUS CYTOGENETIC studies on T cell leukemia have not shown any obvious relationship between chromosome abnormalities and the expression of factors that may be associated with T cell proliferation. Lectin-stimulated proliferation of normal mature T cells is associated with the production of T cell growth factor (TCGF) and the expression of its receptor (Tac antigen). T cells from human T cell leukemia virus (HTLV-I)-induced malignancies are known to express the Tac antigen in the absence of lectin stimulation, either in the resting phase or after culture. Cells from other T leukemias rarely express the Tac antigen in the absence of stimulation. It is also becoming increasingly clear that chromosomal regions bearing the T cell receptor genes may be significant in the generation of chromosome abnormalities in many T cell disorders.

We have carried out cytogenetic studies and demonstrated clonal chromosome abnormalities in 13 cases of T cell leukemia resulting from the expansion of populations of neoplastic T lymphocytes with a mature (postthymic) phenotype. Cells from 11 of these patients were tested for the membrane expression of the Tac antigen with a specific monoclonal antibody (McAb). A striking correlation was observed between abnormalities of 7q and expression of Tac.

MATERIALS AND METHODS

Patients. Cytogenetic studies and membrane marker analysis were carried out on mononuclear cells from peripheral blood samples of 13 patients with T cell leukemia. The T cell malignancies were classified according to clinical, morphological, and immunologic criteria. HTLV-I-induced malignancies were demonstrated by the presence of serum antibodies (kindly determined by Dr A. Dalgleish) and by the reactivity of the leukemic cells with McAb against the HTLV-I core proteins p19 and p24 (gift from Dr B. F. Haynes). Two patients (Nos. 1 and 2) were black and had HTLV-I-positive adult T cell lymphoma leukemia (ATLL), while all the others were HTLV-I-negative. Clinical details of the two ATLL patients have been reported elsewhere. Five patients (Nos. 3 to 7) had Sézary syndrome (SS), which was characterized by moderate lymphadenopathy, erythroderma, and the presence of typical cerebriform lymphocytes in the peripheral blood. Three patients (Nos. 8 to 10) had T prolymphocytic leukemia (T-PLL) with marked hepatosplenomegaly, high WBC count, and bone marrow infiltration by typical prolymphocytes. Three patients (Nos. 11 to 13) had T chronic lymphocytic leukemia (T-CLL). One of them was asymptomatic, and the other had neutropenia, infections, and splenomegaly. Two of the three had a past history of rheumatoid arthritis (RA) and had been splenectomized earlier. All three T-CLL patients had mature-looking granular lymphocytes in the peripheral blood.

Membrane marker analysis. This was carried out on mononuclear cells in suspension by indirect immunofluorescence. The membrane phenotype of the T cell malignancies was characterized by E rosettes and a panel of McAb: UCHT1 (T3;CD3), OKT4/Leu-3 (T4;CD4), OKT8 (T8;CD8), OKT6 (T6;CD6), and OKT17 (T17). Terminal transferase (TdT) was assessed on cytocentrifuge slides fixed in acetone-chloroform by an indirect immunoperoxidase method. The presence of receptors for TCGF was investigated with the McAb anti-Tac in fresh and short-term cultured (72 to 172 hours) cells. In four cases, the immunoglobulin method (IGM) was applied at electron-microscopic level in order to characterize the morphology of the cells reactive with anti-Tac.

Cytogenetic studies. Mononuclear cells were seeded at a concentration of 1.5 to 2.0 x 10^6 cells/mL in medium RPMI-1640 supplemented with fetal calf serum (20%). The cells were cultured in the presence of phytohemagglutinin (PHA) 0.2 μg/mL and TCGF (1 μL/10 mL culture) for 5 to 10 days. Cells were harvested after incubation with 0.05 μg/mL colcemid (Gibco) for one hour, followed by hypotonic treatment and fixed in one part glacial acetic acid to three parts methanol. Giemsa banding was carried out on air-dried slides by routine methods.

RESULTS

Membrane markers and reactivity with anti-tac. The cells in all cases had a mature (postthymic) phenotype: E', TdT', T6', T3', and T17'. In addition, these cells were T4' or T8', except in patient 2 (ATLL), whose cells were unreactive with the McAb against the two major lymphocyte subsets (Table 1).

Fresh and/or unstimulated lymphoid cells from six cases (two ATLL, two SS, and two T-PLL) of 11 cases tested showed reactivity with the McAb anti-Tac (8% to 59% of positive cells).

The IgM method demonstrated Tac positivity in the

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CHROMOSOME 7q AND Tac IN T-LEUKEMIAS

Table 1. Correlation Between Abnormalities of Chromosome 7 and Expression of IL 2 Receptors

<table>
<thead>
<tr>
<th>Disease</th>
<th>Case No.</th>
<th>Phenotype</th>
<th>Anti-Tac (%)</th>
<th>Chromosome 7q Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATLL</td>
<td>1</td>
<td>T4+</td>
<td>52</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>T4-, T8-</td>
<td>10</td>
<td>Present</td>
</tr>
<tr>
<td>SS</td>
<td>3</td>
<td>-</td>
<td>0</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>T4+</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>20</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-</td>
<td>8</td>
<td>Present</td>
</tr>
<tr>
<td>T-PLL</td>
<td>8</td>
<td>T4+</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>T8+</td>
<td>59</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>T4+</td>
<td>11</td>
<td>Present</td>
</tr>
<tr>
<td>T-CLL</td>
<td>11</td>
<td>T4+</td>
<td>0</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>T8+</td>
<td>0</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>T8+</td>
<td>0</td>
<td>Absent</td>
</tr>
</tbody>
</table>

*Demonstrated by reactivity with McAb anti-Tac without mitogenic stimulation.
†The percentage of anti-Tac-positive leukemic cells was possibly higher in patients 2, 6, and 7 since the proportion of neoplastic cells in these samples ranged from 20% to 30% of the mononuclear fraction.

Table 2. Chromosome Abnormalities in 13 Cases of T Cell Leukemia

<table>
<thead>
<tr>
<th>Disease</th>
<th>Case No.</th>
<th>Chromosome Abnormalities</th>
<th>Significant Clonal Abnormalities*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATLL</td>
<td>1</td>
<td>50XXY</td>
<td>+ 3, + del 7(p14), 1q+, .6q- (q21), t(11;13)(q14;q22-24)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47XY</td>
<td>+ del 7(p14)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>46XXY</td>
<td>- 15.11(11;17), 2p- (p23)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>88XXXY</td>
<td>2p- (p14), 2p- - (p14), 6q- (q21), 6q- (q21), i7q(i7q)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>44XX/88XX</td>
<td>2p- (p14-15)2p- (p14-15), 6q- (q21)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>46XY</td>
<td>i7q(i7q)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>46XY</td>
<td>t8;8p12(11;11), 5q+ (14;11-32)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>46XY</td>
<td>t8;8p12(11;11), 5q+ (14;11-32)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>46Xq+Y</td>
<td>inv.del(7)(q21-35), t7;7(15)(q35-36), M</td>
</tr>
<tr>
<td>T-PLL</td>
<td>10</td>
<td>42XX</td>
<td>1p+, 7p+, .6q- , + 7p+ (q35)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>46XXX</td>
<td>t(4;17)(p15-16;q23)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>46XXO</td>
<td>6q- (q21), t11;15(q13;q22-24)</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>46XXO</td>
<td>int.del (14)(q22-24)</td>
</tr>
</tbody>
</table>

*Chromosome 7 abnormalities are underlined.
†See Fig 2.

DISCUSSION

We have described clonal chromosome abnormalities in 13 cases of T cell leukemia, and the presence of chromosome 7 rearrangements were found to correlate with Tac expression in the leukemic cells.

The demonstration of clonal abnormalities in the three cases of T-CLL in our study is of interest. Although it has been recognized that T-CLL constitutes a distinct clinical entity, its chronic course and difficulty in demonstrating clonality have led to the use of the uncommitted term chronic T cell lymphocytosis. We are aware of only four cases where clonal chromosome abnormalities have been demonstrated in this disease. The findings in this study, together with the possibility of demonstrating rearrangements of the T cell receptor genes, strengthens the concept that T-CLL is clonal in nature and probably represents a neoplastic process.

One patient in this study had an inversion involving 14q11 and 14q32. Abnormalities of chromosome 14 with q11-13 as break point have been reported in T-ALL, T-PLL, SS, chronic T cell leukemias, and T cell leukemias in patients with ataxia telangiectasia (AT). The rearrangements are usually inversions or tandem translocations involving 14q11 and 14q32 and translocations between other autosomes and 14q11-13. In this respect the recent localization to 14q11 of the T cell receptor α-chain gene, which rearranges during T cell differentiation, is significant, and a possible oncogene has been postulated on 14q32 proximal to the IgH locus. The rearrangements are thought to act in a manner analogous to translocations in Burkitt’s lymphoma, where the immunoglobulin genes and the c-myc oncogene are brought adjacent to each other.

The presence of a chromosome 7 abnormality in seven of
Fig 1. Anti-Tac reactivity is shown by colloidal gold particles attached to the cell membrane. (A), ATLL-lymphoid cell (case 1); (B) Sézary cell (case 8); (C) T prolymphocyte (case 9); (D) large granular lymphocyte characteristic of T-CLL (case 12). The strongest reactivity is seen in ATLL cells whereas T-CLL lymphocytes are negative. Insets in B and C are higher magnifications of the regions indicated by the double arrows (uranyl acetate and Reynolds’s lead citrate stain).
Fig 1 (Cont’d).
13 cases in our study is of particular interest. Based on the frequent rearrangements between 7p14, 7q35, 14q11, and 14q32 in T cells of patients with AT, Fiorelli et al. have speculated that T cell receptor genes may be present on 7p14 and 7q35. In fact, the T cell receptor \( \beta \)-chain gene has been mapped to 7q35, while the \( \gamma \) chain gene has been assigned to 7p (T. H. Rabbitts, personal communication). The involvement of 7p14 and 7q35 in six of seven cases with chromosome 7 abnormalities in our study is significant. The most important and intriguing finding in the present study has been the correlation observed between abnormality of 7q and the expression of the Tac antigen in the absence of mitogenic stimulation. Of the six cases showing anti-Tac reactivity, five had trisomy or partial trisomy for 7q. Trisomy for 7q has been described in one third of patients with ATLL, especially in cases with an acute clinical course. Since marked anti-Tac reactivity is observed in leukemic cells of the majority of ATLL patients, it would appear that infection with HTLV-I induces Tac expression independently of chromosome 7q abnormalities. The exact mechanism and role of Tac expression associated with HTLV-I are still not clear, since most T cell lines derived from ATLL patients grow in the absence of exogenous TCGF and have no detectable messenger RNA for endogenous TCGF production. This suggests that HTLV-I infection induces cell proliferation in these T-cell lines by a mechanism that bypasses TCGF-Tac binding.

The structural gene for the Tac antigen has been mapped to chromosome 10p14-15. Therefore, the association between 7q abnormalities and Tac expression is unlikely to result from an alteration of the Tac gene. Since the gene for the T cell receptor \( \beta \) chain is mapped to 7q35, activation of this antigen receptor may result indirectly in Tac expression. Thus it is possible to speculate that 7q abnormalities produce an activated phenotype by alteration of the T cell receptor locus. Alternatively, there may be other genes on 7q that are deregulated and amplified by the chromosome abnormalities, leading to the activation of the Tac gene by a transregulatory mechanism.

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