S-100β Positive T Cell Leukemia

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We reported a peculiar case with T cell leukemia. The patient was a 34-year-old woman showing extensive splenomegaly and marked leukemic cell proliferation and running a rapid fatal clinical course. The leukemic cells were morphologically ordinary lymphocytes showing suppressor/cytotoxic (s/c) T cell phenotypes and containing S-100b protein. Southern blot analysis revealed rearrangement of the β chain genes of the T cell receptor (TcR) of the leukemic cells. Because these phenotypic and morphologic features were identical with those of S-100β T lymphocytes (S-100β TL) in normal human peripheral blood, we regarded this case as S-100β T cell leukemia. We discussed clinicopathological features of S-100β T cell leukemia/lymphoma by assessing similar cases reported so far. S-100β T cell leukemia/lymphoma is a new type of s/c T lymphocytic leukemia/lymphoma with aggressive features.

THE FUNCTIONS OF THE newly identified human peripheral blood T lymphocytes of suppressor/cytotoxic (s/c) subtype containing S-100b protein (a dimer of β subunit) in their cytoplasm and comprising 2% to 10% of human peripheral blood mononuclear cells (PBM), S-100β T lymphocytes (S-100β TL), remain as yet undetermined.1,2 Two cases with S-100β T cell lymphoma have been reported so far.4,5 However, the nature of neoplastic S-100β TL and the clinicopathological features of the disease remain almost unknown. In the present study, we reported a case with S-100β T cell leukemia and precisely examined the nature of neoplastic cells in relation to morphology, phenotypes, and genotype. We also attempted to clarify the clinicopathologic features of S-100β T cell leukemia/lymphoma by assessing our case and reported cases.

CASE HISTORY

The patient was a 34-year-old woman who experienced the onset of fever following a stillbirth on August 24, 1986. Two weeks later, she was admitted to a university hospital where she was noted to have marked splenomegaly. There were moderate anemia and thrombocytopenia (RBC, 278 x 10^6/L; platelet [Plt], 3.6 x 10^9/L). Her leukocyte count was 6.6 x 10^9/L with 17% leukemic cells. A bone marrow aspirate showed 36.4% leukemic cells. She was administered several combinations of chemotherapy (cyclophosphamide, doxorubicin, vincristin, and predonisolone) to no avail. In January of 1987, splenectomy was performed. The spleen, which was 2,650 g in weight, showed diffuse infiltration of medium-sized leukemic cells. Postoperatively, the patient’s leukocyte count increased to 204 x 10^9/L with 95% leukemic cells; she developed disseminated intravascular coagulation (DIC) syndrome and died of cerebral bleeding on February 5, 1987. Serology for HTLV-I and HIV was negative. An autopsy was performed two hours after death. The patient was advised of procedures and attendant risks, in accordance with institutional guidelines, and gave informed consent.

MATERIALS AND METHODS

Morphology and enzymatic activities of leukemic cells. Smear whole peripheral blood from the patient was stained with Wright-Giemsa and periodic acid-Shiff (PAS). Smearred cells were also stained for the enzymes such as acid anaphthyl-acetate esterase (ANAE), β glucuronidase, and acid phosphatase by routine methods. PBM were obtained by Ficoll-Hypaque density gradient centrifugation as described elsewhere.1 Splenic tissues and PBM were also examined by electron microscopy by routine method.

Immunocytochemistry at light microscope level. PBM were examined with each polyclonal antibody against S-100β subunit,4 or terminal deoxynucleotidyl transferase (TdT; Bethesda Research Laboratory, MD), and various monoclonal antibodies including OKT3 (CD3), OKT4 (CD4), OKT6 (CD1), OKT8 (CD8), OKT11 (CD2), OKM1 (CD11) (Ortho Diagnostic, Raritan, NJ), Leu-7, Leu-11b (CD16), HLA-DR, and interleukin-2 receptor (CD25) (Becton Dickinson Monoclonal Center Inc, Mountain View, CA). For immunostaining, we used indirect immunoperoxidase method and ABC method for polyclonal or monoclonal antibodies, respectively, as described elsewhere.2

Immunocytochemistry at electron microscopic level. To further clarify ultrastructural features of the leukemic cells, PBM were examined by an indirect immunoperoxidase method at electron microscopic level for S-100β protein as described elsewhere.1,2

Analysis of sheep erythrocyte rosette formation. PBM were tested for spontaneous sheep erythrocyte rosette (E-rosette) formation at 4°C as described previously.1 Analysis of IgG-Fc receptor of leukemic cells. PBM were also tested for IgG-coated bovine erythrocyte rosette formation (E-rosette) as described previously.2

Analysis by two dimensional western blot method. The soluble protein fraction extracted from PBM, which was obtained by the modified method of Isobe and Okuyama,1 was examined by a two dimensional western blot method using indirect immunoperoxidase method for S-100β subunit as described by Manabe et al.3 Analysis of rearrangement of β TcR gene. High molecular weight DNA was obtained from fresh PBM as described.4 DNA, completely digested with EcoRI, BamHI or XbaI (New England Biolabs, Beverly, MA) were examined by Southern blot method.5

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As a probe, we used the DNA fragment containing the Cβ2 constant region gene segment prepared as described previously.11

**Histopathological analysis.** Routine paraffin sections of formalin-fixed various organs including extirpated spleen and autopsy materials were stained with hematoxylin-eosin (H&E) and Giemsa. Tissues were also stained with polyclonal antibodies against immunoglobulin light chains, muramidase, alpha-1 antichymotrypsin (Dakopatts, Denmark) or S-100β subunit and monoclonal antibodies, MT-1, MB-1 (Bioscience, Switzerland) or leukocyte common antigen (LCA) (Dakopatts, Denmark) using ABC method as described.2

**RESULTS**

**Characterization of neoplastic lymphocytes by morphology.** The leukemic cells were medium to large-sized lymphocytes 10 to 20 μm in diameter having monotonous, round to oval or singly indented nuclei with conspicuous nucleoli and scanty cytoplasm with no cytoplasmic granules (Fig 1A). The leukemic cells showing erythrophagocytosis were occasionally, but not frequently, observed. The leukemic cells showed localized fine granular cytoplasmic staining for ANAE, but were negative for PAS, β glucuronidase and acid phosphatase. They were strongly positive for S-100β protein (Fig 1B). Ultrastructurally, the leukemic cells had round or slightly indented nuclei with conspicuous nucleoli and scant to relatively abundant cytoplasm showing diffuse S-100β immunostaining and poorly developed cellular organelles (Figs 2A and B). No cytoplasmic granule revealing parallel tubular arrays was observed. Phenotypes of the leukemic cells are summarized in Table 1 (Figs 1C and D).

**Histopathological findings.** The spleen was markedly enlarged and almost entirely filled with medium-sized atypical lymphocytes (Figs 1E and 2C), which were intensively positive for S-100β (Fig 1F). They were positive for LCA and MT-1 (plan T cell marker), but not for other antigens tested. The leukemic cells were heavily infiltrated in sinusoidal spaces in the liver, mesenteric lymph nodes, bone marrow, kidneys, lungs, and heart, but not in the CNS.

**Western blotting.** S-100β subunit in the soluble protein fraction extracted from the leukemic cells was clearly identified around the point of 10 kd and pl 4.5 (Fig 3), where S-100β subunit extracted from human brain tissue was always detected (data not shown).

**Rearrangement of β TcR gene.** Patterns of Southern blot hybridization were interpreted as described elsewhere.11 As shown in Fig 4, the arranged DNA band containing Cβ2 region was found in DNA from the leukemic cells, indicating the deletion of the Cβ1 region and rearrangement at the Jβ2 region in single allele.
Fig 3. Western blot using two dimensional electrophoresis. Arrow indicates S-100β subunit in the soluble protein fraction extracted from the leukemic cells around the point of mol. wt. 10 kd and pI 4.5.

Table 1. Phenotypes of PBM of the Patient With S-100β+ T cell Leukemia

<table>
<thead>
<tr>
<th>Items Tested</th>
<th>Cluster</th>
<th>Positive cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemic cells</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>S-100β</td>
<td>CD3</td>
<td>90</td>
</tr>
<tr>
<td>E-rosette</td>
<td>CD4</td>
<td>90</td>
</tr>
<tr>
<td>EA-rosette</td>
<td>CD1</td>
<td>75</td>
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<tr>
<td>Surface immunoglobulin</td>
<td>CD2</td>
<td>2</td>
</tr>
<tr>
<td>TdT</td>
<td>CD11</td>
<td>0</td>
</tr>
<tr>
<td>OKT3</td>
<td>CD1</td>
<td>85</td>
</tr>
<tr>
<td>OKT4</td>
<td>CD8</td>
<td>86</td>
</tr>
<tr>
<td>OKT6</td>
<td>CD2</td>
<td>75</td>
</tr>
<tr>
<td>OKT8</td>
<td>CD11</td>
<td>95</td>
</tr>
<tr>
<td>OKT11</td>
<td>CD12</td>
<td>85</td>
</tr>
<tr>
<td>OKM1</td>
<td>CD25</td>
<td>2</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>CD16</td>
<td>85</td>
</tr>
<tr>
<td>IL-2R</td>
<td>Leu-7</td>
<td>1</td>
</tr>
<tr>
<td>Leu-7</td>
<td>Leu-11b</td>
<td>1</td>
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</tbody>
</table>

The present study clearly indicated that the leukemic cells with S-100β immunoreactivity were morphologically ordinary lymphocytes and showed mature CD4+/CD8+ T lymphocyte phenotype. Western blotting analysis confirmed the presence of S-100b protein in the leukemic cells. The T cell nature of the leukemic cells was definitely confirmed by demonstrating the rearranged β TcR gene. Therefore, it was concluded that the leukemic cells were s/c subtype T cells containing S-100b protein in their cytoplasm. S/c T chronic lymphocytic leukemia (T-CLL) is a rare disease and is commonly known to correspond to proliferative disorder of large granular lymphocytes (LGL). The surface antigen phenotype of the present leukemic cells resembled that of LGL leukemia previously reported. Cases with LGL leukemia in which leukemic cells showed similar splenic or sinusoidal infiltration of the liver have been reported. Moreover, an aggressive variant of LGL leukemia has been reported. Although this case resembles the clinical course of the aggressive variant of LGL leukemia, it differs in morphology and expression of S-100b protein. The most reliable criterion for LGL is its characteristic morphologic features rather than surface phenotypes. As shown in the present study, the leukemic cells were morphologically different from LGL but were ordinary lymphocytes with definite T cell natures. In

DISCUSSION

The present study clearly indicated that the leukemic cells with S-100β immunoreactivity were morphologically ordinary lymphocytes and showed mature CD4+/CD8+ T lymphocyte phenotype. Western blotting analysis confirmed the presence of S-100b protein in the leukemic cells. The T cell nature of the leukemic cells was definitely confirmed by...
human peripheral blood, S-100b protein is found exclusively in a subset of CD4⁻/CD8⁺ T cells with ordinary lymphocyte morphology but not in LGL. Thus, the phenotypic and morphologic features of the leukemic cells were virtually identical with those of S-100b⁺ TL in normal human peripheral blood. Moreover, we examined two cases with LGL leukemia, in which the leukemic cells were morphologically LGL and showed strong β glucuronidase and acid phosphatase activities but were negative for S-100b protein (unpublished data). Furthermore, among several reported cases with S-100b⁺ T cell lymphoma, none of the leukemic cells showed LGL features. Judging from these facts, it is reasonable to assume that this case is different from proliferative LGL disorder and corresponds to neoplasia of S-100b⁺ TL. Two cases with S-100b⁺ T cell lymphoma have been reported. The phenotypic features of the case by Ruco et al were virtually identical to those of our case. However, in the Chen et al case, S-100b⁺ leukemic cells showed helper/inducer (h/i) T cell phenotype. They considered this discrepancy to be caused by aberrant marker expression, which has been known to often occur in T cell lymphoma. Similarly, aberrant expression of OKM1 antigen, which is not expressed on normal S-100b⁺ 'TL', occurred in the present leukemic cells. The presence of IgG-Fc receptor and CD16 (Leu-11b) antigen, which is usually expressed on granulocytes and natural killer cells, on the present leukemic cells is not inconsistent with their S-100b⁺ 'TL' origin since these antigens are partially expressed on normal S-100b⁺ 'TL'. Despite such phenotypic differences, these three cases with S-100b⁺ T cell leukemia/lymphoma showed very similar clinicopathological features: (1) the leukemic cells showed mature T cell phenotype of usually s/c and sometimes h/i subsets and contained S-100b⁺ protein; (2) all cases run rapid and fatal clinical courses; (3) all cases showed marked splenomegaly accompanied by pancytopenia, anemia, or thrombocytopenia during early onset; and (4) marked leukemic cell proliferation occurred in terminal stages (our case and the Chen et al case). Motoi et al also reported three adult cases with S-100b⁺ T cell lymphoma showing marked splenomegaly, extensive leukemic change, and aggressive clinical courses. In contrast to these cases, patients with common s/c T-CLL, which is a proliferative LGL disorder, usually show very mild and prolonged clinical courses often accompanied by severe neutropenia and autoimmune diseases. Judging from these facts, the aggressive nature of S-100b⁺ T cell leukemia/lymphoma should be emphasized.

It is of interest that the clinical course and some of the features of the tumor cells of this case are similar to those of erythrophagocytic Ty lymphoma described by Kadin et al, which is characterized by a systemic proliferation of erythrophagocytic Ty lymphocytes whose nature and origin remain unclear. Indeed, a small number of the leukemic cells in peripheral blood of our case showed erythrophagocytosis. Ruco et al also pointed out the similarities between their case of S-100b⁺ T-lymphoma and erythrophagocytic Ty lymphoma. Therefore, for the elucidating the origin of erythrophagocytic Ty lymphoma, it is important to clarify whether the tumor cells in such cases contain S-100b⁺ protein. Finally, further investigations are needed to clarify the clinicopathological features of a neoplasia of this poorly understood S100b⁺ T cell subset, in conjunction with their functions.

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