S100-Positive, T-Cell Chronic Lymphoproliferative Disease: An Aggressive Disorder of an Uncommon T-Cell Subset

By Curtis A. Hanson, Paula L. Bockenstedt, Bertram Schnitzer, David A. Fox, Brian Kueck, and Daniel K. Braun

S100-positive T lymphocytes account for less than 3% of peripheral blood T cells. Rare cases of S100-positive T-cell lymphoma have been previously described. We report four such cases of S100-positive T-cell chronic lymphoproliferative disease. In all cases, hepatosplenomegaly was observed, without prominent lymphadenopathy. Central nervous system (CNS) involvement by the leukemic cells was suggested in three cases by physical symptoms and confirmed in two cases by cerebrospinal fluid studies. Despite treatment, three patients died at 3, 6, and 8 months after diagnosis. Although there was a leukemic presentation, only minimal bone marrow infiltration was evident. Splenectomy showed red pulp infiltration. Liver and lymph node biopsies showed sinusoidal leukemic involvement. In all cases, the leukemic cells expressed mature T-cell– and natural killer cell–associated antigens. Cytoplasmic S100 was detected in the leukemic cells in the blood, spleen, liver, and lymph node. Southern blot studies in two cases showed T-β, T-γ, and T-δ gene rearrangements. RNA Northern blots showed T-α and T-β chain transcripts with no T-γ or T-δ RNA identified. Southern blot analysis showed no hybridization to probes specific for Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus-1, or human T-cell lymphotropic virus type-1. These findings show that S100-positive T-cell chronic lymphoproliferative disorder is an aggressive, extramedullary-based disease frequently associated with CNS involvement and characterized by short survivals.

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CHRONIC lymphoproliferative disorders include a variety of entities having unique morphologic, clinical, and immunologic features.4 In general, these lymphoproliferative neoplasms correspond to particular subsets of normal B and T lymphocytes as defined by immunologic marker studies.5-7 The T-cell chronic lymphoproliferative disorders are not as frequently encountered as their B-cell counterparts and include “true” T-cell chronic lymphocytic leukemia,1 T-γ lymphoproliferative disorder (large granular lymphocytosis),4 peripheral T-cell lymphoma,6 adult T-cell leukemia/lymphoma (human T-cell lymphotropic virus type-1 [HTLV-1] associated),7 and mycosis fungoides/Sezary syndrome.8

A newly recognized and poorly understood subset of peripheral blood (PB) T lymphocytes has been shown to contain cytoplasmic S100β protein.5,9 These S100-positive T lymphocytes typically comprise less than 3% of circulating mononuclear cells and have a suppressor immunophenotype, with expression of CD2, CD8, and CD11b.10-14 In normal thymus, small lymphocytes with S100β immunoreactivity can be found in both the cortex and medulla.15 In addition, small numbers of S100β-positive small lymphocytes can be found in splenic red pulp, as well as in the mucosa of the small intestine.16 The nature and origin of S100β-positive lymphocytes remain obscure. Although these cells have a suppressor immunophenotype, their role in normal T-cell function remains an enigma.

Two cases of S100-positive T-cell lymphoma17,18 and one case of S100-positive T-cell leukemia have been previously reported.19 All three of these patients had organomegaly without prominent lymphadenopathy and their disease was characterized by an aggressive clinical course, central nervous system (CNS) involvement by the malignant cells, and relatively short survivals of 15 months or less.17-19

We have identified four more such cases of S100-positive T-cell chronic lymphoproliferative disease (S100-CLPD). In the present study, we have more extensively reported the morphologic features, immunophenotyping studies, molecular assays, functional studies, and virologic assays to more precisely characterize this fascinating yet poorly understood disorder. The initial diagnosis of an S100-positive lymphoproliferation in case no. 1 prompted the search for other similar cases; this patient’s case history is presented below to more extensively describe the clinical features of this disorder.

CASE REPORT OF CASE NO. 1

R.N. was a 29-year-old man who was well until January 1988, when he presented with epistaxis, easy bruisingability, thrombocytopenia (30 × 10⁹/L), and splenomegaly. In March 1988, a bone marrow (BM) biopsy was normal; a liver-spleen scan showed a massively enlarged spleen. The patient underwent splenectomy for treatment of his refractory thrombocytopenia. The spleen was enlarged to 980 g with no evidence of malignancy. Physical examination in June 1988 showed massive hepatomegaly, but no lymphadenopathy. Laboratory findings included a hemoglobin (Hb) of 17.0 g/dL, hematocrit (Hct) of 50.8%, white blood cell count (WBC) of 40 × 10⁹/L with 67% atypical lymphocytes, and 15 nucleated red blood cells (RBCs) per 100 WBCs. Liver function and coagulation tests were elevated. A serum calcium was low-normal at 8.8 mg/dL. A BM aspirate and biopsy were hypercellular without evidence of leukemia. Flow cytometry studies supported the diagnosis of a chronic T-cell lymphoproliferative disorder. Epstein-Barr virus (EBV) serologies were consistent with past EBV infection, while cytomegalovirus (CMV), hepatitis B, and hepatitis A serologies were negative. A liver biopsy showed marked sinusoidal infiltration of the liver with atypical lymphocytes. The patient clinically improved with prednisone (60 mg/d) with a

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Blood and BM try Laboratories at the University of Michigan. Two patients were blood culture was positive for Staphylococcus black-B, nonspecific esterase, and periodic acid-Schiff. BM trephine sections were obtained for morphologic evaluation. PB and Monoclonal Antibodies (MoAbs) being diagnosed with a chronic leukemia.

In all cases, PB smears, BM aspirate smears, and BM trephine biopsy sections were obtained for morphologic evaluation. PB and BM aspirate smears were evaluated after Wright's or Wright's-fixed in B-5 and/or neutral buffered formalin. The tissues were then processed routinely, and sections stained with hematoxylin and eosin. A head computerized tomography (CT) scan showed increased ventricular size. The cerebrospinal fluid (CSF) showed an increase in liver span and a reduction in the WBC to 12 × 10^9/L. In addition, coagulation tests and liver function tests normalized. In September 1990, a palpable lymph node was biopsied, with findings consistent with a chronic lymphoproliferative disorder. Because of increasing fatigue, recurring night sweats, and an increasing lymphocyte count, the patient subsequently received three doses of vincristine and a 3-day course of daunomycin.

The patient was readmitted in November 1988 with fever. One blood culture was positive for Staphylococcus aureus. He subsequently experienced an acute episode of confusion with bitemporal headaches. A head computerized tomography (CT) scan showed an acute episode of confusion with bitemporal headaches. The patient died on January 4, 1989, approximately 1 year after seeking medical care and 6 months after being diagnosed with a chronic leukemia.

MATERIALS AND METHODS

Case Selection

The case population for this study consisted of four patients selected from the files of the Hematopathology and Flow Cytometry Laboratories at the University of Michigan. Two patients were treated and followed-up at the University of Michigan Hospital; the other two patients were referral cases sent to the Laboratory for consultation and further characterization.

Blood and BM

In all cases, PB smears, BM aspirate smears, and BM trephine biopsy sections were obtained for morphologic evaluation. PB and BM aspirate smears were evaluated after Wright's or Wright's-Giemsa stain. Cytotoxicological stains were performed on blood or BM aspirate smears (cases no. 1 and 2) and included myeloperoxidase, sudan black-B, nonspecific esterase, and periodic acid-Schiff. BM trephine biopsy specimens were fixed in either B-5 or neutral buffered formalin and processed by routine methods. Ultrastructural studies on PB were performed in case no. 1 using standard procedures, as previously described.

Tissues

Lymph node (cases no. 1 and 2), spleen (case no. 1), and liver (case no. 1) tissues were submitted fresh and were immediately fixed in B-5 and/or neutral buffered formalin. The tissues were then processed routinely, and sections stained with hematoxylin and eosin. A portion of each lymph node and spleen were snap-frozen in liquid nitrogen and then stored at -70°C for later use. The tissues were placed in a ventricular-peritoneal shunt, the patient subsequently received three doses of vincristine and a 3-day course of daunomycin.

In September 1990, a palpable lymph node was biopsied, with findings consistent with a chronic lymphoproliferative disorder. Because of increasing fatigue, recurring night sweats, and an increasing lymphocyte count, the patient subsequently received three doses of vincristine and a 3-day course of daunomycin.

After a lengthy course in the intensive care unit without significant improvement, the patient died on January 4, 1989, approximately 1 year after seeking medical care and 6 months after being diagnosed with a chronic leukemia.

Flow Cytometry

Immunophenotypic analysis of PB was performed by flow cytometry in all cases; single cell suspensions from lymph node biopsies were also evaluated in cases no. 1 and 2. Flow cytometric methods have been previously described.

Monoclonal Antibodies (MoAbs)

Surface immunophenotyping used a wide panel of lymphoid- and myeloid-associated MoAbs. B-cell-associated MoAbs used included: CD19 (B4); CD20 (B1); CD22 (Leu-14); CD24 (BA-1); and antisurface Ig heavy and light chains. T-cell-associated MoAbs used included: CD1 (T6); CD2 (T11); CD3 (T3); CD4 (T4); CD5 (Leu-1); CD7 (Leu-9); and CD8 (T8). Myeloid-associated MoAbs used included: CD13 (My7); CD14 (My4); and CD33 (My9). Natural killer (NK) cell-associated MoAbs used included: CD11b (Leu-15); CD11c (Leu-M5); CD16 (Leu-11); CD56 (NKH-1); and CD57 (Leu-7). Other MoAbs used included: CD10 (J5); CD25 (interleukin-2 receptor [IL-2R]); CD38 (T10); Leu-8; and HLA-DR, T-cell receptor (TCR) MoAbs used included: WT31 (TCR-1); CD3 (TCR-8); and TCR-γ-1.

Immunohistochemistry

Immunohistochemical analyses of paraffin sections were performed on B5 or formalin-fixed tissues. The antisera used included: CD45 (LCA); L-26; CD74 (LN2); CDw75 (LN1); Ig heavy and light chains; CD45RO (UCHL-1); MT1; CD15 (Leu-M1); CD68 (KP1); lysozyme; α-antichymotrypsin; desmin; vimentin; cytokeratin; epithelial membrane antigen (EMA); and anti-S100B. An avidin-biotin-peroxidase complex technique was used with all antisera. Immunoalkaline phosphatase studies with anti-S100B were performed on PB smears in cases no. 1, 3, and 4. Appropriate positive and negative controls were used for all immunohistochemical studies.

Molecular Analyses

The DNA used for gene rearrangement studies and RNA for Northern blot studies were extracted from blood and tissue specimens from cases no. 1 and 2 according to previously described standard procedures.

Molecular Gene Probes

Ig gene probes were analyzed in this study consisted of a 6.0-kb BamHI/HindIII fragment containing the joining region of the Ig heavy chain gene (JH). Analysis of JH was performed with DNA digested with BamHI and HindIII. The TCR gene probes used included a 0.77-kb cDNA from the T-p chain gene constant regions, 0.6-kb chains; CD45RO (UCHL-1); MT1; CD15 (Leu-M1); CD68 (KP1); myeloid-associated MoAbs. Other MoAbs used included: CD1 (T6); CD2 (T11); CD3 (T3); CD4 (T4); CD5 (Leu-1); CD7 (Leu-9); and CD8 (T8). Myeloid-associated MoAbs used included: CD19 (B4); CD20 (B1); CD22 (Leu-14); CD24 (BA-1); and antisurface Ig heavy and light chains. T-cell-associated MoAbs used included: CD1 (T6); CD2 (T11); CD3 (T3); CD4 (T4); CD5 (Leu-1); CD7 (Leu-9); and CD8 (T8). Myeloid-associated MoAbs used included: CD13 (My7); CD14 (My4); and CD33 (My9). Natural killer (NK) cell-associated MoAbs used included: CD11b (Leu-15); CD11c (Leu-M5); CD16 (Leu-11); CD56 (NKH-1); and CD57 (Leu-7). Other MoAbs used included: CD10 (J5); CD25 (interleukin-2 receptor [IL-2R]); CD38 (T10); Leu-8; and HLA-DR, T-cell receptor (TCR) MoAbs used included: WT31 (TCR-1); βF1; TCR-8-1; and TCR-γ-1.

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Viral Genomic Studies

DNA obtained from PB lymphocytes of cases no. 1 and 2 was probed via Southern blot hybridization for the presence of viral genomic DNA of the human retroviruses, human immunodeficiency virus-I (HIV-1), and HTLV-I. Probes corresponded to the highly conserved gag regions of both viruses. Binding to positive controls were used with both probes.

DNA hybridization studies for two herpesviruses were also performed. An EBV probe was derived from the 9.0-kb BamHI W fragment of the B95-8 strain of EBV. The CMV probe used was constructed from the 32-kb Xba I fragment of the Towne strain of CMV. Control DNA obtained from an EBV-positive lymphoma and CMV-positive brain tissue showed binding of EBV- and CMV-specific probes, respectively.

Cytotoxicity Assay

A standard ¹²⁵I cytotoxicity assay was performed to determine if the neoplastic cells exhibited any non-major histocompatibility complex (MHC)-restricted, NK cell function. An NK-sensitive cell line, K562, and a relatively NK-insensitive cell line, Daudi, were used as target cells. Two different concentration ratios (50:1 and 25:1) of PB mononuclear cells (case no. 1) to target cells were
incubated with $1 \times 10^6 \text{Cr}$-labeled target cells (K562 and Daudi). Cytotoxicity assays were performed in duplicate. After 3 hours of incubation, the supernatant was harvested and the radioactivity was determined with a gamma counter. The results were expressed as the mean percent of $\text{Cr}$ release.

**RESULTS**

**Clinical and Laboratory Findings**

The major clinical and laboratory findings are summarized in Table 1. The four patients included three men and one woman, with ages ranging from 29 to 62 years (median, 45 years). Three of the four patients had constitutional symptoms of fatigue and fever. The fourth patient (case no. 1) had epistaxis and a petechial rash. None of the four patients had lymphadenopathy, although two patients (cases no. 1 and 2) eventually developed small palpable nodes during the course of their disease. In contrast, massive splenomegaly was present in all four patients and three of the four patients had significant hepatomegaly. One patient (case no. 1) underwent splenectomy to treat a presumed immune thrombocytopenic purpura, refractory to steroids. No cutaneous involvement was noted on physical examination in any of these patients. No signs of CNS involvement were noted at presentation.

Leukocytosis was present in all four patients with the WBC ranging from 18.5 to 411.0 x $10^9$/L (median, 45.5 x $10^9$/L). A relative and absolute lymphocytosis was present in all four patients with the lymphocyte percentage ranging from 62% to 95% of the overall WBC (median, 73.5%). No anemia was noted in three of the four patients, with the Hct ranging from 45% to 51% (median, 48%); the Hb ranged from 12.5 gm/dL to 14.0 gm/dL (median, 13.5 gm/dL). In contrast, all had thrombocytopenia at presentation, ranging from 9 to 122 x $10^9$/L (median, 41 x $10^9$/L). Liver function tests were elevated in all four patients to varying degrees. Serum calcium levels were normal to slightly decreased, ranging from 8.8 to 10.0 mg/dL.

CSF studies were performed in three of the patients at the time of diagnosis; all three were unremarkable at this time. However, three of the four patients (cases no. 1, 2, and 3) eventually developed signs of CNS involvement with symptoms of headache, confusion, and drowsiness. Two of these three patients had lumbar puncture studies performed. Both CSF samples showed a significant lymphocytosis, 55 and 95 WBC/mm$^3$. Cytologically, the lymphocytes were atypical in appearance and consistent with neoplastic involvement.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (y)</th>
<th>Survival*</th>
<th>Sex</th>
<th>Symptoms†</th>
<th>WBC (10$^9$/L)</th>
<th>ALC (10$^9$/L)</th>
<th>Hb (g/dL)</th>
<th>Pt (10$^9$/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29/6</td>
<td>M</td>
<td>N</td>
<td></td>
<td>51.0</td>
<td>34.2</td>
<td>12.5</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>48/8</td>
<td>F</td>
<td>Y</td>
<td></td>
<td>411.0</td>
<td>390.5</td>
<td>13.3</td>
<td>122</td>
</tr>
<tr>
<td>3</td>
<td>62/3</td>
<td>M</td>
<td>Y</td>
<td></td>
<td>18.5</td>
<td>14.8</td>
<td>13.7</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>42/—</td>
<td>M</td>
<td>Y</td>
<td></td>
<td>40.0</td>
<td>32.0</td>
<td>14.0</td>
<td>9</td>
</tr>
</tbody>
</table>

Abbreviations: ALC, absolute lymphocyte count; Pt, platelet count. *Survival in months from diagnosis. †"B" symptoms.

All four patients underwent treatment. Patients no. 1 and 3 received a combination of prednisone, vincristine, and daunomycin. Patients no. 2 and 4 received various combinations of prednisone, vincristine, and cytoxan. Three of the four patients (cases no. 1, 2, and 3) have died at 6, 8, and 3 months after diagnosis of leukemia, respectively, all with multisystem failure. The fourth patient (case no. 4) was lost to follow-up 16 months after presentation of disease.

**Morphology**

PB. Morphologic examination of the PB showed a moderate lymphocytosis in three of the four cases (nos. 1, 2, and 3). The lymphocytes in all cases were heteromorphic in appearance and varied from small to intermediate in size (Fig 1). The nuclei were basically round in shape but occasionally exhibited an irregular nuclear outline, ranging from slight nuclear folding to obvious nuclear convolutions. The nuclear chromatin appeared coarsely clumped in these lymphocytes. Some of these atypical lymphocytes had a monocytoid-like appearance. Only rare hyperlobulated or mulberry-shaped nuclei typical of adult T-cell leukemia/lymphoma were seen. Nucleoli were small and inconspicuous. Cytoplasm was relatively abundant and amphophilic to slightly basophilic in appearance. No cytoplasmic granules were seen in any of the leukemic cells, although rare granular lymphocytes could be identified on the smears. During the course of disease in patient no. 1, the morphologic characteristics of the lymphocytes changed and acquired more of a prolymphocytoid appearance (Fig 1). These cells had finely reticular chromatin and contained one or two prominent nucleoli. All cases had striking thrombocytopenia. Interestingly, mild anemia was noted in only one case; the other three cases had normal Hb/Hct values. RBC morphology was normal in all cases.

BM aspirate and trephine biopsy sections. BM aspirates in all cases appeared normocellular to slightly hypercellular. Only a minimal increase in lymphocytes (up to 10%) was identified. Erythroid and granulocytic precursors appeared unremarkable and showed normal maturation. The BM trephine biopsies from all cases appeared slightly hypercellular with only scattered interstitial lymphocytes seen. Megakaryocytes were easily identified, suggesting that the thrombocytopenia was due to peripheral destruction. No marrow involvement with leukemia could be definitively identified in the biopsy sections.

CSF. Evaluation of the CSF in cases no. 1 and 2 showed a lymphocytosis with lymphocytes similar to those described in the PB. These cells were quite distinct from the background, normal lymphocytes and monocytes in the CSF.

Spleen, liver, and lymph node tissue sections. The spleen from patient no. 1, which was removed 3 months before his leukemic presentation, was enlarged to four times normal size (980 g) with an expanded red pulp and normal-appearing white pulp. Microscopic sections showed congestion of the red pulp with an inconspicuous lymphoid infiltrate that was difficult to recognize morphologically. A needle biopsy of liver from case no. 1 showed a diffuse, sinusoidal infiltrate composed of small, lymphoid-appearing cells having round to slightly irregular nuclei, coarse...
chromatin, and faint nucleoli (Fig 2). No distinct lymphoid nodules were identified. The hepatocytes in this biopsy appeared unremarkable with some bile stasis noted.

Lymph node biopsies in cases no. 1 and 2 showed diffuse sinusoidal infiltrates in both subcapsular and medullary sinuses. The sinusoidal infiltrates encroached on the cortical areas of the lymph node with some compression of normal follicles. The neoplastic cells within the lymph node sinuses had a similar appearance to those described in the PB smears and liver biopsy (Fig 2).

**Ultrastructure.** Ultrastructural studies of the PB lymphocytes in case no. 1 showed cells with abundant cytoplasm and round to slightly irregular nuclei. Only small nucleoli were discerned. Occasional electron-dense granules with pale matrix material and poorly defined tubuloreticular structures were seen within the cytoplasm (Fig 3). Higher resolution of these particles did not confirm the presence of any capsid-like structure. No parallel tubular arrays were identified, as have been characteristically found in large granular lymphocytes.4

**Immunophenotyping**

*Flow cytometry.* Flow cytometric immunophenotyping results are summarized in Table 2. Two pan–T-cell antigens, CD2 and CD3, were identified in the majority of leukemic cells in all four cases; CD7 was identified in two of two cases. Another pan–T-cell marker, CD5, was identified in two of four cases. CD4 and CD8 were essentially negative in all cases with only residual normal lymphocytes showing staining. Interestingly, no staining was seen with WT31 (TCR-γ). This antibody recognizes a conformational epitope of the T-cell α/β receptor.23 The combination of CD3 positivity and WT31/CD4/CD8 negativity has usually been equated with cells expressing a γ/δ TCR rather than the more common α/β TCR.23,24 However, MoAbs directed against the T-γ (TCRγ) and T-δ (TCRδ) receptor chains failed to show any staining.

Several NK cell-associated markers were expressed in these cases. CD11b and CD16 were expressed in the majority of leukemic cells in all three cases studied. CD56 was identified in two cases and CD11c in one case, respectively. CD57 was absent in all three cases studied. All B-cell–associated and myeloid-associated MoAbs failed to show any significant reactivity.

**Lymph node frozen section immunophenotyping.** A similar immunophenotype as described above was also found with frozen section studies of lymph node biopsies from cases no. 1 and 2. These biopsies included reactivity with

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The image includes a figure (Fig 1) showing leukemic cells from S100-CLPD (cases no. 1 and 3). Cells are heterogeneous in appearance and occasionally exhibit an irregular nuclear outline (Wright's-Giemsa, original magnification x 1,000). (C) Leukemic cells from case no. 1, late in the course of his disease. These cells have a prolymphocytoid appearance with fine reticular chromatin and prominent nucleoli (Wright's-Giemsa, original magnification x 1.250).
Fig 2. Sections of liver (A) and lymph node (B) from case no. 1 show a lymphoid infiltrate that expands the hepatic sinusoids and both the subcapsular and medullary sinususes of the lymph node (H & E, original magnification x 200). Higher magnification of the hepatic (C) and lymph node (D) sinusoids shows that the cells have a round to slightly irregular nuclear appearance with coarse chromatin and faint nucleoli (H & E, original magnification x 1,000).

CD2, CD3, CD7, CD11b, and CD56. The staining pattern was primarily sinusoidal. CD4 and CD8 reactivity was limited to uninvolved paracortical regions. The germinal centers showed polyclonal Ig staining. In addition, several TCR MoAbs were used on frozen sections of cases no. 1 and 2. These results are summarized in Table 3. As was found in the PB, no staining with WT31 was identified. However, βF1-staining was seen in the neoplastic cells in frozen section studies. This antibody recognizes a different antigenic epitope on the T-β chain than that identified by WT31. In addition, antibodies against the T-γ and T-δ chains (TCRγ-1 and TCRδ-1) failed to show any reactivity by these immunoperoxidase studies.

Paraffin section immunohistochemistry. Figure 4 shows the intense cytoplasmic positivity seen with anti-S100B in the leukemic cells in the liver and lymph node paraffin sections. The anti-S100B staining pattern accentuated the sinusoidal pattern of involvement in the liver and lymph node and showed a more significant involvement in the spleen than could be recognized morphologically. A similar staining pattern was seen with UCHL-1 (CD45RO), a pan-T-cell-associated marker, showing that the neoplastic cells were staining with both antibodies. Other lymphoid-associated, histiocytic-associated, epithelial, and soft tissue antibodies were negative on these paraffin section immunoperoxidase studies. Anti-S100B staining was identified in the BM trephine biopsy sections of case no. 2.

PB immunocytochemistry. Immunohistochemistry of the PB smears in cases no. 1, 3, and 4 showed intense cytoplasmic positivity with anti-S100B in the majority of the circulating lymphoid cells (Fig 4).

Ig/TCR DNA Studies

Clonal TCR gene rearrangements were seen in both cases (no. 1 and 2) studied with TCR β, γ (Fig 5), and δ (data not shown) chain gene probes. The marked intensity of the rearranged bands indicated that the majority of the cells contained the clonal rearrangements. All rearrangements were confirmed with at least two restriction endonucleases. No Ig gene rearrangements were seen.
**TCR RNA Gene Studies**

TCR RNA expression was evaluated by Northern blotting with T-α, T-β, T-γ, and T-δ gene probes in cases no. 1 and 2 to determine which of the clonal rearrangements just described were indeed productive and capable of encoding for an intact TCR protein. These Northern blot studies showed normal-sized T-α and T-β gene RNA transcripts, indicating that both T-α and T-β underwent a productive gene rearrangement (Fig 6). No T-γ or T-δ RNA were identified by Northern blot studies (Fig 6). These results confirmed that the α/β TCR genes were effectively transcribed despite having a CD3-positive/wT31, CD4, CD8-negative immunophenotype.

**Table 2. Flow Cytometric Immunophenotyping Percentages**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Case No. 1</th>
<th>Case No. 2</th>
<th>Case No. 3</th>
<th>Case No. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1 (T6)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>CD2 (T11)</td>
<td>92</td>
<td>85</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>CD3 (T3)</td>
<td>66</td>
<td>81</td>
<td>60</td>
<td>85</td>
</tr>
<tr>
<td>CD4 (T4)</td>
<td>8</td>
<td>15</td>
<td>0</td>
<td>7</td>
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<td>CD5 (Leu-1)</td>
<td>14</td>
<td>80</td>
<td>99</td>
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<tr>
<td>CD7 (Leu-9)</td>
<td>53</td>
<td>43</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>CD8 (T8)</td>
<td>3</td>
<td>9</td>
<td>10</td>
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<td>TCR-1 (WT31)</td>
<td>15</td>
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<td>18</td>
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<td>TCRγ-1</td>
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<td>TCRβ-1</td>
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<td>2</td>
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<tr>
<td>CD11b (Leu-15)</td>
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<td>75</td>
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<td>CD16 (Leu-11)</td>
<td>66</td>
<td>70</td>
<td>42</td>
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</tr>
<tr>
<td>CD56 (NKH-1)</td>
<td>72</td>
<td>68</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD57 (Leu-7)</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>ND</td>
</tr>
<tr>
<td>CD19 (B4)</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>CD20 (B1)</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>CD22 (Leu-14)</td>
<td>1</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD24 (BA-1)</td>
<td>2</td>
<td>ND</td>
<td>1</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

**Viral DNA and RNA Studies**

HIV-1, HTLV-1, EBV, and CMV. DNA from PB lymphocytes of cases no. 1 and 2 was probed via Southern blot hybridization for the presence of human retrovirus (HIV-1 and HTLV-1) and herpesvirus (EBV and CMV) genomic DNA. No hybridization was seen in either case with the respective probes.

**Cytotoxicity Assay**

Cytotoxicity assays were performed to evaluate the function of the neoplastic cells in case no. 1. The patient’s cells showed strong cytotoxicity for K562 target cells as compared with normal donor lymphocytes (Table 4). Only minimal cytotoxic activity was seen when Daudi cells were used as the target cell. These results are consistent with non–MHC-restricted cytotoxicity, which is a characteristic and defining feature of NK cell function. The non–MHC-restricted cytotoxicity of the patient’s mononuclear cells was effectively inhibited by the addition of either CD2 or CD3 MoAbs.

**Table 3. TCR Immunophenotype From Frozen Section Lymph Nodes**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Case No. 1</th>
<th>Case No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT31 (TCR-1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>βF1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TCRγ-1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TCRβ-1</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The S100 protein is an acidic, calcium-binding protein with a molecular weight of approximately 10 to 20 Kd that has structural and functional homology to calmodulin, another calcium-binding protein. The S100 protein is a dimer composed of at least three distinct forms based on the dimer composition (α and/or β chains): α/α (S100αα), α/β (S100αβ), and β/β (S100β). This protein was initially
thought to be specific for neural tissue, but is now known to be present in a variety of cells including myoepithelium, melanocytes, chondrocytes, adipocytes, Langerhans cells, interdigitating reticulum cells, and various carcinoma cells. The gene encoding the S100 antigen has recently been localized to chromosome 21 band q22.2, but has not been further characterized. Although we know this ubiquitous protein belongs to the family of calcium-binding proteins, its precise role in cellular function remains an enigma.

Recently, several reports have described reactivity of anti-S100β in a small subset of human PB T lymphocytes. These cells account for approximately 1% to 4% of PB mononuclear cells and have been immunologically classified as suppressor/cytotoxic T cells based on the expression of CD2, CD8, and CD11b antigens. The difficulty in characterizing and evaluating these cells is that the S100β antigen is located within the cellular cytoplasm with the resulting effect that these cells cannot be directly sorted by a flow cytometer.

The S100-CLPD represents a unique malignancy having characteristic clinical and laboratory features. The four patients from our study, along with the three previously reported patients, uniformly had massive hepatosplenomegaly without significant lymphadenopathy, and exhibited an aggressive clinical course with relatively short survivals. Interestingly, three of our patients developed documented CNS disease and two of the previously reported patients had signs and symptoms of CNS disease at the time of death. The PB findings varied from a normal WBC to a frank leukemic picture. These patients also had thrombocytopenia without significant anemia or neutropenia. Morphologically, the tumor cells showed a characteristic predilection for the lymph node and hepatic sinususes and splenic red pulp.

The above features are quite distinct from other T-cell lymphoproliferative disorders. T-γ lymphoproliferative disorder (TγLPD), or large granular lymphocytosis, is typically an indolent disease that is usually associated with long survivals and few clinical symptoms. Although the immunophenotype of S100-CLPD is similar to that found in TγLPD, TγLPD is seldom associated with massive organomegaly. More importantly, the large granular lymphocytes of TγLPD have a unique cytologic appearance on
both PB smears and ultrastructural studies, features that were absent from the S100-positive lymphoproliferations.

Although the aggressive clinical course for these patients was reminiscent of an HTLV-1–positive, adult T-cell leukemia/lymphoma, the lymphocytes in S100-CLPD lacked the characteristic hyperlobulated nuclei of adult T-cell leukemia. In addition, this CLPD did not have any obvious BM involvement and was not a CD4-positive, T-cell proliferation. More importantly, serologic and DNA assays for HTLV-1 were negative. Although these cases could be considered as “peripheral T-cell lymphomas” in a broad sense, the histologic pattern of involvement in the liver, spleen, and lymph node, the predilection for splenic involvement, and the presence of a leukemic proliferation without a BM infiltrate would not be considered characteristic of a typical peripheral T-cell lymphoma. Previous studies of anti-S100β staining in non-Hodgkin’s lymphomas failed to show any S100-positive reactivity in peripheral T-cell lymphomas. Thus, the S100-CLPD have many morphologic and clinical features that are unique as compared with other T-cell malignancies.

The immunophenotypic features and functional studies in our cases were consistent with a proliferation of NK-like cells, expressing CD11b, CD11c, CD16, and CD56 without CD57. It is now clear that this immunophenotype is not solely restricted to “true” NK cells, but can also be found in non-MHC-restricted, cytotoxic T lymphocytes. As compared with the “true” NK cells, which lack any TCR, the non-MHC-restricted cytotoxic T lymphocytes may express either an α/β or γ/δ TCR. Thus, the use of the term “NK cell” is quite ambiguous and refers to a heterogeneous group of cells, all exhibiting NK cell activity but with differing antigenic and genotypic features. The molecular studies in the S100-CLPD cases in this report clearly showed multiple TCR gene rearrangements and, more importantly, showed RNA transcripts of both the T-α and T-β genes. Thus, it would appear that these S100-positive cells are representative of the non-MHC-restricted, cytotoxic T lymphocytes rather than “true” NK cells.

The initial immunophenotype of CD3-positive/WT31, CD4, CD8-negative cells was strong presumptive evidence for a γ/δ TCR. However, subsequent staining with βF1, lack of reactivity with TCRγ-1 and TCRδ-1, and the Northern blot studies showing α and β TCR transcripts were evidence that these cells truly had an α/β TCR rather than a γ/δ TCR. Although unexpected, other reports have also suggested that such a phenotype may exist. Several studies have shown a lack of concordance between CD3, WT31, and/or βF1 staining in T-cell malignancies.

Other investigators have characterized a unique subset of circulating T cells in mice that were CD3-positive, CD4-negative, and CD8-negative that bore an α/β TCR. Thus, the unexpected finding of a CD3, βF1-positive/CD4, CD8, WT31-negative phenotype and an α/β TCR transcript could represent: (1) conformational change of the α/β heterodimer, leading to loss of anti-WT31 expression while still maintaining at least β-chain (βF1) antigenicity; (2) expansion of a heretofore poorly recognized, minor subset of circulating T cells; or (3) aberrant loss of surface antigen(s) by a neoplastic process.

Many features of this disorder suggest a possible association with a viral transformation. The relative young age group, as compared with most non-Hodgkin’s lymphomas, the incidence of CNS involvement, and an aggressive disease course are all characteristics that can be seen in a viral-induced lymphoid process. Although HTLV-1 was a
Fig 6. Northern blot hybridization analysis with Tα, Tβ, Tγ, and Tδ gene probes of PB lymphocytes from cases no. 1 and 2. Jurkat and Peer cell lines represent positive controls for Tα/Tβ RNA and Tγ/Tδ RNA, respectively. U937 cell line served as a negative control for Tα and Tβ.
primary consideration in this case initially, subsequent studies effectively ruled out that retrovirus. In addition to its well-established relationship with infectious mononucleosis, EBV has long been associated with many lymphoproliferative disorders, including endemic Burkitt’s leukemia/lymphoma, posttransplantation lymphoproliferative disorder, rare cases of T-cell lymphoma, and, recently, a report of TyLPD. Serologic studies in case no. 1, however, were consistent with past infection, and DNA hybridization studies in cases no. 1 and 2 failed to show any evidence of EBV genomes. Human herpesvirus-6 (HHV-6), originally termed human B-lymphotropic virus, is a newly recognized herpesvirus first isolated from patients with lymphoproliferative disorders. A recent report from our laboratory has shown preliminary evidence that S100-CLPD may be associated with HHV-6. Further laboratory studies are in progress to evaluate the potential role that this herpesvirus may have in the development of this uncommon, yet aggressive lymphoproliferative disorder.

ACKNOWLEDGMENT

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**Table 4. Non-MHC-Restricted Cytolytic Activity**

<table>
<thead>
<tr>
<th>Target Cell</th>
<th>E:T = 50:1 (%)</th>
<th>E:T = 20:1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-562</td>
<td>62</td>
<td>41</td>
</tr>
<tr>
<td>Daudi</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>K-562 + CD3</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>K-562 + CD2</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

Numbers represent percent 51Cr release.
Abbreviation: E:T, effector to target cell ratio.
S100, T-CELL LYMPHOPROLIFERATIVE DISEASE

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S100-positive, T-cell chronic lymphoproliferative disease: an aggressive disorder of an uncommon T-cell subset

CA Hanson, PL Bockenstedt, B Schnitzer, DA Fox, B Kueck and DK Braun

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