Hepatosplenic T-Cell Lymphoma: A Distinct Clinicopathologic Entity of Cytotoxic γδ T-Cell Origin

By C.B. Cooke, L. Krenacs, M. Stetler-Stevenson, T.C. Greiner, M. Raffeld, D.W. Kingma, L. Abruzzo, C. Frantz, M. Kaviani, and E.S. Jaffe

We identified eight cases of T-cell lymphoma with evidence of a γδ phenotype over a 13-year period. Seven of these cases conformed to a distinct clinicopathologic entity of hepatosplenic γδ T-cell lymphoma. Nearly all of these patients were young adult males (five of seven), with a median age at presentation of 20 years. They presented with marked hepatosplenomegaly, without lymphadenopathy or significant peripheral blood lymphocytosis. Thrombocytopenia was seen in all patients, and five of seven were mildly anemic. The clinical course was aggressive, and despite multiagent chemotherapy, the median survival duration was less than 1 year. The morphologic findings were uniform; a monomorphic population of medium-sized lymphoid cells with moderately clumped chromatin and a rim of pale cytoplasm infiltrated the sinusoids of the spleen, liver, and bone marrow. The cells had a characteristic immunophenotype: CD2+, CD3+, CD4−, CD5−, CD7+, CD16+, CD57−, CD25−, T-cell receptor (TCR)γδ+, βF1+. CD8 was positive in four of seven cases tested, and CD56 was positive in five of six. All cases expressed the cytotoxic granule-associated protein, TIA1, but perforin was detected in only one case. All cases with assessable DNA had a TCRγ gene rearrangement, and lacked Epstein-Barr virus sequences. Isochromosome 7q was identified in two cases with cytogenetic information. The one case of cutaneous γδ T-cell lymphoma differed in its clinical manifestations, histologic appearance, and immunophenotype. We conclude that hepatosplenic γδ T-cell lymphoma is a distinct clinicopathologic entity derived from cytotoxic γδ T cells, and should be distinguished from other lymphomas of T-cell and natural-killer cell (NK)-like T-cell derivation. This is a US government work. There are no restrictions on its use.

During the development of T cells, genes responsible for antigen recognition are assembled by the somatic recombination of variable (V), joining (J), diversity (D), and constant (C) regions, which ultimately results in the expression of an antigen receptor on the surface of the T cell. The rearrangement of these genes accounts for the clonal specificity of individual cells. The T-cell receptor (TCR) consists of either a γδ or αβ heterodimer expressed in association with the CD3 complex of proteins on the cell surface. The majority of mature T lymphocytes express the αβ TCR; however, a small group of normal T cells express the γδ TCR. In humans, reported percentages of γδ T cells vary from 1% to 10% of normal circulating peripheral blood T lymphocytes, 2% to 4% of T cells in normal and reactive lymph nodes, 3% to 5% of medullary thymocytes, 1% of cortical thymocytes, and 15% of splenic T lymphocytes.1-4 Studies have shown that during thymic development, cells that bear the γδ TCR appear earlier than those that bear the αβ heterodimer, and have suggested that δ gene rearrangement is a very early event, preceding the rearrangement of any other TCR gene.1,3

Human γδ T cells exhibit preferential homing to the sinusoidal areas (red pulp) of the spleen and into the epithelial layer of the intestine, whereas αβ T cells migrate preferentially to the splenic periarteriolar sheaths and intestinal lamina propria. This specialized anatomical distribution pattern suggests the selective expression of a homing molecule and important physiologic function for the γδ T cells.5 γδ cells have cytoxic capabilities and can respond to stimuli with lymphokine production and proliferation, which suggests that these cells are functionally mature, and not simply precursors to αβ T cells.5-6 However, the exact function of γδ T cells, as distinguished from other cells with cytotoxic activity, remains a mystery. Most γδ T cells in murine and avian systems lack the CD4 and CD8 subset markers that characterize mature αβ TCR-bearing T cells2,6; however, some γδ T cells in the human spleen and peripheral blood are CD8+.4

Cells that express γδ TCR have been reported in lymphoblastic lymphoma and T-cell lymphoblastic leukemia.1,3,7,8 However, most postthymic T-cell lymphomas (PTL) are derived from the predominant αβ T cells. Nevertheless, the tumor cells in a minority of PTL express a γδ phenotype and genotype. Several reported cases have presented with cutaneous disease, with either epidermal or subcutaneous involvement.9,10 One rare case of nasal T-cell lymphoma, with a γδ phenotype, has also been reported.11 Far cert et al in 199012 reported two cases of hepatosplenic T-cell lymphomas that expressed the γδ TCR, and proposed that this process represented a new form of PTL. In their cases, the tumor cells showed preferential tumoral infiltration of the hepatic sinusoids and splenic red pulp with no associated lymphadenopathy and minimal or absent peripheral blood lymphocytosis.13,14 Since their initial report, a number of individual case studies have been published.15,16 Recent cytogenetic studies have led to the identification of a common cytogenetic abnormality, further supporting the hypothesis that γδ PTL may be a distinct clinicopathologic entity.17 Nonetheless, the total number of cases in the literature is only 10. Therefore, the clinical distinctiveness of this syndrome is unproven.

We identified over a 13-year period (1983 to 1996) eight cases of peripheral T-cell lymphoma that demonstrated pre-

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sumptive evidence of a γδ phenotype/genotype. One case presented as a cutaneous T-cell lymphoma, with predomi-
nant epidermotropism. The remaining seven cases shared common clinicopathologic features, characteristic of those reported in hepatosplenic PTL. Our data suggest that hepa-
tosplenic γδ T-cell lymphoma represents a distinct clinicopathologic entity. In addition, we investigated our cases for the expression of perforin, and the cytotoxic granule-associ-
ated protein, TIA-1, to determine if the cells of γδ PTL resemble mature cytotoxic T lymphocytes.5,21,24

**MATERIALS AND METHODS**

**Clinicopathologic analysis.** Biopsies from all cases were sub-
mitted for review to the Hematopathology Section, Laboratory of
Pathology, National Cancer Institute (NCI). Two of the eight patients
had been referred for treatment to the NCI for either conventional
chemotherapy for relapsed lymphoma (case no. 4) or for immuno-
therapy using immunotuxin (case no. 8). The tissues were fixed in
buffered formalin or B5 and embedded in paraffin. Paraffin blocks
were sectioned at a thickness of 5 mm and sections were stained
with hematoxylin and eosin (H&E). Bone marrow aspirates were
stained with Wright-Giemsa.

**Immunophenotypic analysis.** Immunophenotypic character-
ization of cases no. 1 to 5 and no. 8, was performed using either
frozen-section immunohistochemistry or flow cytometry, or both,
employing a panel of monoclonal antibodies (Table 1). Frozen tissue
sections were analyzed using the Avidin-Biotin-Complex Technique
(Vector Laboratories, Burlingame, CA) as described previously.22,25
Rabbit secondary antibodies were obtained from Vector Labora-
tories, Burlingame, CA. Mouse secondary antibodies for frozen-
section immunoperoxidase stains were obtained from TAGO, Burlin-
game, CA.

Paraffin sections only were available for cases no. 6 and 7. These
cases were analyzed with a more limited panel of monoclonal anti-
bodies (Table 1), and the ABC immunoperoxidase technique, as
previously described.26 Antigen retrieval for most antibodies
was performed in a microwave pressure cooker (Nordic Ware, Minneapo-
lis, MN) in 0.01 citrate buffer (pH 6.0) with heating at maximum
power (800 W for 40 minutes). For CD56 with 323C3, the sections
were heated for 8 minutes. For CD4/1F6, heating was performed in
0.5 mol/L Tris base buffer (pH 10) for 40 minutes.

Immunophenotypic analysis of peripheral blood and/or bone mar-
row was performed in cases no. 2 through 5 by flow cytometry using
a whole-blood lysis technique as described.27 In one of four bone
marrow specimens that received on case no. 5, mononuclear cells were
isolated by density gradient analysis and stained as described.28 All
analyses were performed on a FACScan flow cytometer (Becton
Dickinson, San Jose, CA). The sensitivity of fluorescent detectors
was set and monitored using Autocomp software and Autocomp
beads (Becton Dickinson, San Jose, CA). Unstained cells and
isotypic negatives were analyzed as controls. For two-color combi-
nations that contained the antibody TCRγδ, at least 3,000 TCRγδ-
positive events were collected. Multiparametric analysis was per-
formed using two or three fluorescent signals (antibodies conjugated
to fluorescein isothiocyanate [FITC], phycoerythrin [PE], and peridi-
nin chlorophyll [PerCP], using forward scatter and orthogonal scat-
ter. Forward versus orthogonal scatter was used to select the lympho-
cyte population in peripheral blood and bone marrow specimens
from cases no. 2 through 4.29 CD45 versus orthogonal scatter was
used to select the lymphocyte population in the bone marrow speci-
mens from case no. 5.30 Gates were verified by staining with CD45
versus CD14, as was the distribution of CD3+ and CD19+ cells.29

**In situ hybridization.** In situ hybridization for Epstein-Barr virus
(EBV) RNA was performed in all cases. The methods have been
previously described in detail.31 Briefly, 5-mm paraffin embedded
sections were prepared on sialinated slides. The tissue sections
were then deparaffinized, rehydrated, permeabilized with nonionic deter-
gent, and digested with proteinase K (10 mg/mL). The EBV EBER1
riboprobe was prepared as described previously.31 The RNA integrity
was evaluated using a digoxigenin-labeled riboprobe directed at an
abundant cellular mRNA transcript of a small nuclear riboprotein,
rNRP U6. Tissues that showed adequate hybridization signal using the
U6 probe were judged to have adequate cellular RNA.

Each riboprobe was applied separately in a formamide buffer and
the slides were hybridized overnight. Following stringent posthy-
bridization washes, an antidigoxigenin alkaline phosphatase anti-
body-conjugate was used. The slides were then washed and placed
overnight into a color-developing solution that consisted of nitro-
bluetetrazolium and X-phosphate. The reaction was stopped by a

**Table 1. Monoclonal Antibodies Used in Immunophenotypic Analysis**

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<th>Antigen</th>
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<td>CD11b</td>
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<td>*</td>
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<td></td>
<td>123C3*</td>
<td>*John Hilken's, The Netherlands Cancer Institute, Amsterdam, The Netherlands</td>
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<td>CD38</td>
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<td>TCR αβ+</td>
<td>(λF11)</td>
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<td>P-8</td>
<td>H. Yagita, Juntendo University, Tokyo, Japan</td>
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<td>CGP*</td>
<td>TIA-1</td>
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<td>CD5+</td>
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<td>CD6+</td>
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<tr>
<td>TDT</td>
<td></td>
<td>Bethesda Research Laboratories, Bethesda, MD</td>
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</table>

Abbreviations: CGP, cytotoxic granule-associated protein; TDT, termi-
nal deoxynucleotidyl transferase.
* Used in paraffin sections.
† Used in paraffin and frozen sections.
‡ Used in flow cytometry only.

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brief wash in an appropriate buffer. The slides were then counterstained with eosin and coverslipped.

Formalin fixed, paraffin-embedded tissue blocks were prepared from cultured RAJI and MOLT4 cell lines, which served as positive and negative controls, respectively. As with case materials, 5-mm sections were prepared on sialinated slides, and a positive and negative control accompanied each in situ hybridization run. Hybridization runs using hybridization buffer that contained sense probe, antisense probe, and absence of probe were separately performed to confirm proper transcription of the digoxigenin-labeled riboprobe.

**TCR gene analysis.** TCRγ gene rearrangements were identified by a polymerase chain reaction (PCR) assay coupled with denaturing gradient gel electrophoresis (DGGE) in all seven cases of hepatosplenic lymphoma. DNA was extracted from frozen tissue or cells by proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation as described previously. Individual primers were synthesized via a solid-phase triester method on a DuPont synthesizer (Wilmington, DE) for each of the four main groups (I through IV) of variable (Vγ) region genes that contain Vγ1-8, Vγ9, Vγ10, and Vγ11 genes, respectively. In addition, primers were chosen for the joining region (Jγ) genes Jγ1 and Jγ2, Jγp, Jγpl, and Jγp2. A 40-nt GC clamp, which does not anneal to target sequences in the genome, was synthesized at the 5' end of the Vγ primers. These primers will form products of 300, 220, 200, and 180 nt long for groups I to IV of the Vγ-Jγ gene rearrangements, respectively.

Genomic DNA (0.5 to 0.6 mg) was used to perform PCR as described. Forty microliters of PCR reactions with identical products on agarose electrophoresis were analyzed on a denaturing gradient gel for 7 hours at 150 V. For DGGE 15-cm × 20-cm × 0.75-mm gels with a 30% to 60% denaturing gradient range were poured using 8% polyacrylamide (acrylamide: bis-acrylamide at 37:1) at pH 7.4 (1 × TAE). The presence of a prominent band was regarded as a positive TCRγ gene rearrangement.

### RESULTS

**Clinical features.** The clinical features of the hepatosplenic γδ T-cell lymphoma are summarized in Table 2. In the current series, six of seven patients were male, and the age range was 15 to 65 years (median, 20). Four of seven were black, one was Hispanic, and two were white. All patients had splenomegaly and five of seven had hepatomegaly at presentation. The presenting symptoms included fever, abdominal pain, and weakness. All patients had a decreased platelet count, usually markedly so, with no discreet nodules, and no discernible expansion of the white pulp. In each case, the tumor cells diffusely invaded the red pulp, but with preferential infiltration throughout the red pulp, but with preferential infiltration.

**Liver biopsies were available in four of six cases. The sinusoids were markedly expanded by an infiltrate of tumor cells, with sparing of portal triads. No hepatocyte destruction was noted.**

Splenectomy specimens were examined in five of seven cases. The spleens were enlarged (weight, 570 to 1,700 g). The gross appearance was beefy red in color, with no discreet nodules, and no discernible expansion of the white pulp. In each case, the tumor cells diffusely invaded and expanded the red pulp with occasional mitotic figures and occasional single-cell necrosis. Tumor cells were distributed throughout the red pulp, but with preferential infiltration.
of the sinusoids. Blood lakes were not observed. Each spleen had preserved residual islands of normal white pulp.

Four cases had involved bone marrow biopsies. The bone marrow was moderately hypercellular, with residual normal hematopoiesis. Small clusters of abnormal lymphoid cells were observed in dilated sinuses. The cells were similar in appearance to those in the liver and spleen. The infiltrate was difficult to observe in H&E-stained sections, and demonstration of malignant cells was greatly facilitated with immunohistochemical techniques. The Wright-Giemsa-stained bone marrow smears showed atypical, medium-sized lymphocytes with moderately dispersed nuclear chromatin, minimal nuclear irregularity, and a moderate amount of basophilic cytoplasm. Azurophil granules were not seen in the four cases examined. Both the absence of granules and the basophilia of the cytoplasm distinguish these cells from large lymphocytes with dispersed nuclear chromatin, and negative staining with βF1, we believe they represent hepatosplenic γδ T-PTL.

One case (no. 8) presented with disease restricted to the skin. There was a dense band-like infiltrate in the superficial dermis, with marked epidermotropism. The abnormal lymphocytes were medium in size, with densely clumped chromatin, and irregular, but not cerebriform nuclear contours (Fig 2). Nucleoli were inconspicuous, and cytoplasm was sparse. The infiltrate lacked admixed inflammatory cells.

**Immunophenotypic studies.** In five of five hepatosplenic cases with complete immunophenotypic characterization, the neoplastic cells were CD4−, CD3+, CD4−, CD5−, CD25−, and positive for TCRγδ, but negative for TCRαβ (CD25 was not examined in one case) (Fig 3). Initially, all cases expressed CD7 either by flow cytometry of single-cell suspensions or frozen-section immunoperoxidase studies. A repeat posttreatment evaluation of the bone marrow in case no. 3 showed loss of expression of CD7 by the neoplastic cell population. Two of four cases expressed CD8, although staining was weak in one case. All cases expressed one or more natural-killer cell (NK) markers, including positivity for CD56 in all assessable cases but one. All cases were positive for CD16, although staining was weak and variable in two. However, CD57 was absent in all cases. CD38 was expressed in cases no. 1 and 3. CD11b was detected in the bone marrow but not peripheral blood of case no. 3, and 50% of the γδ T cells in case no. 4 also expressed CD11b. All cases were negative for all pan-B-cell markers tested (Table 3).

The one case of cutaneous γδ T-cell lymphoma (case no. 8) had an immunophenotype similar to that of the hepatosplenic cases, but differed in the complete absence of NK markers (CD16, CD56, and CD57) and CD38. CD8 was positive.

Limited paraffin section immunohistochemistry was available in cases no. 6 and 7. The neoplastic cells were positive for polyclonal CD3, and positive for CD45RO and CD43. βF1 stained admixed small lymphocytes in paraffin sections, but did not stain the neoplastic cells. Studies for TCRα were unsuccessful in paraffin sections. One of the two cases stained for CD8 with the 144B monoclonal antibody in paraffin sections. Results of frozen-section and paraffin-section immunohistochemistry were concordant with the Leu-2a and 144B antibodies, respectively. Therefore, a total of eight cases expressed CD8. Both cases were negative for CD4 using 1F6 to detect CD4 in paraffin sections, with positivity observed in residual normal lymphocytes. CD56 was successful in paraffin sections in one case (no. 6), and was positive. The cells were negative for B-cell (CD20, k.

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<th>BM</th>
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<td>3 mo</td>
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Abbreviations: cr, current report; Dec. Plts., decreased platelets; H/S, hepatosplenomegaly; LN, lymphadenopathy; BM, positive bone marrow; PB, peripheral blood involved; LTF, lost to follow-up; CR, complete remission; allo BMT, allogeneic bone marrow transplant; AWD, alive with disease, currently undergoing chemotherapy.

* Cases no. 18 and 19 were published as lymphomas of large granular T lymphocytes. However, based on characteristic clinicopathologic features, presence of isochromosome 7q, and negative staining with βF1, we believe they represent hepatosplenic γδ T-PTL.
HEPATOSPLENIC γδ T-CELL LYMPHOMA

Fig 1. Pathology of hepatosplenic gamma/delta lymphoma. (A) Splenic sinusoids are markedly expanded by a monomorphic infiltrate of atypical lymphoid cells. (H&E; original magnification × 400.) (B) The cells have round to oval nuclei, with slightly dispersed chromatin, and inconspicuous nucleoli. They have abundant pale cytoplasm. Rare mitotic figures are seen. (H&E; original magnification × 630.) (C) The bone marrow biopsy is moderately hypercellular. Tumor cells infiltrate the sinusoids (arrows), but are difficult to discern with H&E stain. (H&E; original magnification × 200.) (D) Immunoperoxidase stain for CD3 in paraffin section highlights tumor cells filling sinusoids. (ABC immunoperoxidase technique, hematoxylin counterstain; original magnification × 200.) (E) Atypical lymphoid cells are seen in bone marrow aspirate smear. (Wright-Giemsa; original magnification × 1,000.)

λ markers, and negative for CD 57 (Leu-7), CD68 (KP1), lysozyme, and S100.

Studies for perforin and TIA-1 were performed in seven of eight cases. All seven cases tested were positive for TIA-1, but only one case, a hepatosplenic lymphoma, showed weak focal staining for perforin in the neoplastic cells (Fig 4). Rare scattered small lymphocytes positive for perforin were seen in all other cases tested, assuring adequacy of the staining method.

Molecular studies. The tumor cells were negative for
Fig 2. \(\gamma \delta\) T-cell lymphoma involving the skin. The cells have more clumped chromatin, and more irregular nuclei than those of hepatosplenic \(\gamma \delta\). Prominent epidermotropism is seen. (H&E; original magnification \(\times 400\).)

EBV RNA transcripts in all seven cases studied by in situ hybridization. All cases with successful amplification of DNA had rearrangement of the TCR\(\gamma\) gene by PCR coupled with DGGE (Fig 5). Case no. 6 was rearranged but is not illustrated; DNA could not be amplified from cases no. 5 and 7.

DISCUSSION

We report eight cases of \(\gamma \delta\) PTL, seven of which conform to the clinicopathologic picture previously reported as hepatosplenic T-cell lymphoma. Our study provides additional evidence that hepatosplenic \(\gamma \delta\) T-cell lymphoma is a distinct disease entity. These patients exhibit striking similarities in clinical presentation and course. All but one of the patients were young, ranging from 15 to 32 years of age at diagnosis; one patient was 65. There appears to be a marked predilection for males; only 2 of the 19 reported subjects has been female. Our study included seven patients, four of whom were black and one Hispanic, which suggests a possible racial predisposition as well. The patients presented with marked splenomegaly, hepatomegaly in all but one, with no associated lymphadenopathy, and absent or only modest peripheral blood lymphocytosis. Clinical symptoms at presentation were largely attributable to the hepatosplenomegaly and anemia, and included fatigue, fever, and abdominal pain. The disorder carries a poor prognosis, with most patients dying of the disease within 2 years, despite multiagent chemotherapy. None of our patients achieved a complete remission with conventional chemotherapy, and the median survival duration was less than 1 year. The only patient in continuous complete remission underwent allogeneic bone marrow transplantation.

Varying degrees of pancytopenia have been reported in patients with hepatosplenic \(\gamma \delta\) T-cell lymphoma. Thrombocytopenia is usually the most striking finding, and has been reported in nearly all cases. Reduction in platelets may be due in part to the marked splenomegaly, but in many patients platelet counts do not return to normal following splenectomy, or rebound only briefly. In our patients, thrombocytopenia correlated closely with disease activity, and was often a harbinger of relapse. Evidence of platelet destruction may suggest a diagnosis of ITP, as occurred in one of our cases.22

Fig 3. Liver biopsy involved by hepatosplenic \(\gamma \delta\) T-cell lymphoma. (A) Sinusoids are markedly expanded by atypical lymphoid cells with rim of pale cytoplasm. (H&E; original magnification \(\times 400\).) (B) Frozen section stained for TCR\(\gamma\) shows strong staining of tumor cells, which were negative for \(\mu\)F1. (ABC immunoperoxidase technique, methyl green counterstain; original magnification \(\times 400\).)
HEPATOSPLENIC \( \gamma \delta \) T-CELL LYMPHOMA

Table 3. \( \gamma \delta \) T-Cell Lymphomas: Immunophenotypic Findings

<table>
<thead>
<tr>
<th>Marker</th>
<th>Case No.</th>
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<td></td>
<td>1 2 3 4 5 6 7 8</td>
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<tr>
<td>CD1a</td>
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<tr>
<td>CD2</td>
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<tr>
<td>CD3</td>
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<tr>
<td>CD4</td>
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<tr>
<td>CD5</td>
<td>– – – – – – – –</td>
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<tr>
<td>CD7</td>
<td>+ + + + + + + +</td>
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<tr>
<td>CD8</td>
<td>– + – + – + + +</td>
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<tr>
<td>CD11b</td>
<td>nd +/- +/- +/- nd</td>
</tr>
<tr>
<td>CD11c</td>
<td>+ + +/- +/- +/-</td>
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<tr>
<td>CD16</td>
<td>+ + + + + + + +</td>
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<tr>
<td>CD56</td>
<td>+ + + + + + + + u</td>
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<td>CD57</td>
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<td>CD20*</td>
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<td>CD25</td>
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<td>CD38</td>
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<tr>
<td>TCR( \gamma \delta )</td>
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<tr>
<td>TCR( \alpha \beta )</td>
<td>– – – – – – – –</td>
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<tr>
<td>Perforin</td>
<td>– +/- – nd – – – –</td>
</tr>
<tr>
<td>TIA-1</td>
<td>+ + + + nd + + + +</td>
</tr>
<tr>
<td>TDT</td>
<td>– nd – nd – – – –</td>
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<tr>
<td>EBV in situ</td>
<td>– nd – nd – nd – –</td>
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</tbody>
</table>

* Includes results of other B-cell markers, CD19, CD22, and \( \kappa \) and \( \lambda \) light chains; paraffin sections only available for cases 5 and 6, with limited immunophenotypic panel.

Abbreviations: nd, not determined; u, unsatisfactory.

The exact mechanism of the thrombocytopenia is uncertain. In addition to thrombocytopenia, most patients have a modest anemia. In one case of a subcutaneous \( \gamma \delta \) T-cell lymphoma, high levels of interferon gamma were produced by the tumor cells, causing suppression of hematopoiesis without tumoral infiltration of the bone marrow.\(^{10,34}\) Thus, humoral factors may contribute to the peripheral blood cytopenias in hepatosplenic \( \gamma \delta \) T-cell lymphoma.

Notably, suppression of normal hematopoiesis has been described in leukemias of cytotoxic T-cell derivation.\(^{35}\) Although it has been suggested that normal \( \gamma \delta \) T cells may function as cytotoxic T cells,\(^ {5,25}\) \( \gamma \delta \) T-cell lymphomas have not been previously studied for markers associated with cytotoxic T-cell function. We found positivity for the cytotoxic granule-associated protein TIA-1 in all seven cases studied. This protein is expressed in NK cells, cytotoxic CD8\(^+\) T cells, and normal \( \gamma \delta \) T cells. However, perforin, which is associated more directly with cytotoxic T-cell function, was positive in only a few cells of one case. PeripheraI blood \( \gamma \delta \) T cells contain abundant perforin in their cytoplasmic granules.\(^{36}\) However, the cells of \( \gamma \delta \) PTL may be functionally immature, and not yet capable of cytotoxic function.

The NK markers CD56 and CD16 have not been analyzed in all previously reported cases. However, our data, plus that of previous reports,\(^ {16,19}\) suggest that expression of these antigens is characteristic of hepatosplenic \( \gamma \delta \) T-cell lymphoma. As noted, \( \gamma \delta \) T cells share some cytotoxic functions with NK cells, and therefore, expression of NK-associated markers is not surprising. These observations suggest that hepatosplenic \( \gamma \delta \) T-cell lymphomas represent NK-like T cells. Although the vast majority of NK-like T cells express an \( \alpha \beta \) TCR, a rare population of \( \gamma \delta \)-expressing NK-like T cells has been described in the mouse.\(^ {23,27}\) Notably, the one cutaneous \( \gamma \delta \) lymphoma in our study was negative for NK markers, although it is premature to draw any conclusions from a single case.

TCR\( \gamma \delta \) T cells have been generally regarded as double negative (CD4\(^-\), CD8\(^-\)); however, expression of CD8 does occur in \( \gamma \delta \) T cells in peripheral lymphoid tissues.\(^4\) In addition, a minor population of \( \gamma \delta \) in murine thymus has been shown to be CD8\(^-\).\(^ {35,37}\) In the current study, four cases were CD8\(^-\), while three hepatic cases and one cutaneous case each expressed CD8. The hepatic tumors had an otherwise identical immunophenotype (CD2\(^+\), CD3\(^+\), CD4\(^-\), CD5\(^+\), CD16\(^+\), CD25\(^-\), CD56\(^+\), and CD57\(^+\)) with expression of the \( \gamma \delta \) TCR detected with monoclonal antibodies TCR\( \gamma \delta \) or TCR\( \delta \). All cases reported to date have lacked expression...
of one or more pan-T antigens, most commonly CD5 or CD7. Absence of one or more pan-T antigens is a common feature of peripheral T-cell lymphomas, and has been used to support to the malignant nature of the cells. Alternatively, this phenotype might be reflective of minor normal populations. The neoplastic cells in all of our cases tested lacked CD5, but did express CD7, at least before therapy.

Recently, a recurring cytogenetic abnormality has been reported in γδ hepatosplenic T-cell lymphoma. Three cases, all containing an isochromosome 7q, were reported from Taiwan.23 The investigators also noted a fourth case reported with this cytogenetic lesion that conformed to the characteristic clinicopathologic picture, but was not fully characterized immunophenotypically.39 Notably, two of our cases (nos. 3 and 5) also contained an isochromosome 7q (Alonsozana E., Stamberg J., Kumar D., manuscript in preparation). The finding of a primary, nonrandom, chromosomal abnormality in these patients further supports hepatosplenic γδ T-cell lymphoma, as a distinct disease entity.

Hepatosplenic γδ T-cell lymphoma is remarkably uniform in its histologic characteristics. Involved tissues show a striking localization of tumor cells within sinuses. Infiltration is most conspicuous in the splenic red pulp and liver, but a similar pattern can be seen in the bone marrow. This sinusoidal localization in the bone marrow makes detection of tumor-cell infiltration more difficult. We suspect that prior reports may have underestimated the degree of bone marrow involvement, because it may be difficult to identify without immunohistochemical stains, which highlight the abnormal cells. Both the subtle pattern of bone marrow involvement, and the minimal cytologic atypia may contribute to delay in diagnosis.23 Even if liver biopsies or a splenectomy is performed, the diagnosis may be missed, as was the case in one of our patients. Perhaps earlier therapeutic intervention may lead to an improved outlook for these patients.

Cytologically, the cells are medium in size, and monomorphic in appearance. The monomorphic cytology is in contrast to the pleomorphism usually observed in PTL. In the four cases with Wright-Giemsa–stained smears, the basophilia of the cytoplasm, and the absence of granules, distinguished these cells from large granular lymphocytes. Notably, the one case of cutaneous γδ T-cell lymphoma differed in its cytologic appearance. The cells contained densely clumped chromatin and had irregular nuclear contours.

The tumor cells from each case successfully studied had a γTγ gene rearrangement, which although not specific for γδ T cells, does provide evidence of both T-cell lineage and clonality. We were able to prove a γδ phenotype in all five hepatosplenic lymphomas fully analyzed. In two cases, the absence of viable cells or frozen-tissue samples precluded complete analysis. However, the absence of staining for βF1, with positivity for CD3, was suggestive of a γδ origin. In addition, one case was characteristically double negative for CD4/CD8, whereas the other was CD8+, as seen in three additional γδ cases. Finally, on clinicopathologic grounds, these two cases appeared to conform to the entity of hepatosplenic PTL.

The differential diagnosis of γδ hepatosplenic lymphoma includes aggressive NK leukemia/lymphoma.40–42 This disease also presents with marked hepatosplenomegaly, and has an aggressive clinical course. The tumor cells in NK-cell lymphoproliferative disorders are immunophenotypically similar to our cases (CD2+, CD56+, CD16+), but they lack expression of a TCR and CD3.42 However, rare aggressive malignancies of NK-like large granular T lymphocytes also have been reported.43 γδ T cells share many functional and phenotypic similarities with NK cells and NK-like cytotoxic T cells, and therefore, clinical overlap among these malignancies is perhaps not surprising. Morphologic overlap is seen as well, although the neoplastic cells in hepatosplenic γδ T-cell lymphoma usually lack azurophil granules. In addition, the often exquisite localization of the tumor cells within sinusoids in spleen and bone marrow is a feature we have not seen in other large granular lymphocyte disorders.

The difficulties in differential diagnosis are exemplified by a recent report, which described two cases felt to be NK-like T-cell lymphomas of large granular lymphocyte derivation.44 Both patients were young males who presented with marked hepatosplenomegaly, fever, and thrombocytopenia. The clinical course was acute, with death within a few months. Notably, the neoplastic cells from both cases had an isochromosome 7q abnormality, and were negative for βF1, despite the presence of CD3. The cells were not studied for TCRβ. Nevertheless, these cases almost certainly represent hepatosplenic γδ T-cell lymphomas, and confirm the unique aspects of this syndrome. Notably, azurophil granules were
HEPATOSPLENIC γδ T-CELL LYMPHOMA

seen in both cases, and were prominent in one. Finally, S100 T-cell lymphoproliferative disorder should be included in the differential diagnosis. That aggressive T-cell malignancy is of αβ phenotype, and presents with hepatosplenomegaly, but usually conspicuous peripheral blood involvement. It may be of cytotoxic T-cell derivation, and is usually positive for CD8 and CD56.

Rare lymphomas of γδ T-cell derivation present with disease in other extranodal sites, in all cases but one involving the skin or subcutaneous tissue. Of the eight reported cutaneous cases, including the present report, four involved the subcutaneous tissue, and four were epidermotropic, as in our case. Notably, in each case of the latter group, the investigators noted marked epidermal infiltration with replacement of the epidermis, resembling pagetoid reticulosis. This pattern was present in our case as well. Although the clinical course has been variable, in general it has been more aggressive than classic mycosis fungoides, but not associated with either hepatosplenomegaly or lymphadenopathy. Our patient died of disease approximately 5 years after presentation.

In our series, the one cutaneous case also differed from the hepatosplenic cases both cytologically and phenotypically. It was negative for the NK-associated markers CD16 and CD56, as well as for CD3, and differed in its cytologic appearance, exhibiting greater chromatin clumping and less abundant cytoplasm. None of the other epidermotropic cases reported were studied for CD56; one case was negative for CD16. These observations suggest that hepatosplenic γδ T-cell lymphoma can be distinguished from other rare forms of γδ PTL presenting in other extranodal sites.

One previously reported case of γδ T-cell lymphoma presenting as a nasal lymphoma was positive for EBV-associated EBER1 RNA transcripts. Nearly all cases of nasal T-cell lymphoma are EBV-positive, although the finding of a γδ phenotype or genotype is exceptional. All of our cases were negative for EBV transcripts, suggesting no association of EBV with this disease.

In summary, γδ hepatosplenic T-cell lymphoma is a rare disease, but is remarkably distinctive, with characteristic clinical, pathologic, and cytogenetic features. γδ T-cell lymphomas most likely represent the neoplastic counterpart of the small population of T cells expressing the γδ TCR found in normal lymphoid tissues. By immunophenotype, they appear to be NK-like cytotoxic T cells. The striking propensity for involvement of the splenic red pulp by the neoplastic γδ T cells resembles the normal distribution of γδ T cells and reinforces the concept of preferential homing of these cells to the spleen.

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Hepatosplenic T-cell lymphoma: a distinct clinicopathologic entity of cytotoxic gamma delta T-cell origin

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