Hepatosplenic T-Cell Lymphoma: Sinusal/Sinusoidal Localization of Malignant Cells Expressing the T-Cell Receptor γδ

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Peripheral T-cell lymphomas consist of a clinically heterogeneous group of malignant disorders whose immunophenotype usually corresponds to that of normal mature T cells. We describe and correlate the clinical, histopathologic, phenotypic, and genotypic findings in two patients with malignant lymphoma presenting with hepatosplenic disease. The morphologic pattern of lymphoma was that of a sinusoidal/sinusoidal infiltration in spleen, marrow, and liver. This morphologic characteristic was associated with the presence of a productive clonal rearrangement of the T-cell receptor (TCR) δ gene. Lymphoma cells expressed a CD3 TCR-δ phenotype. They were also double negative (ie, CD4, CD8) and lacked the CD5 and CD7 antigens. In one patient, tumor progression was associated with phenotypic changes that resulted in a CD3 TCR-δ phenotype with the same δ-gene rearrangement as initially. These observations suggest the existence of a new type of peripheral T-cell lymphoma characterized by its hepatosplenic presentation, and by the sinusosal/sinusoidal tropism and the TCR-δ phenotype of the malignant cells.

Within the clinical spectrum of malignant lymphomas, cases presenting predominantly with hepatosplenic involvement are rare. Liver and spleen involvement may be found as a result of the staging procedure performed in patients with lymphadenopathy, and the neoplastic cells are usually found in the liver in the portal tracts, and in the spleen mainly in the white pulp. Preferential infiltration of the splenic red pulp and of liver sinusoids has been noted only rarely in T-cell lymphoma or in large granular lymphocytic leukemia, a T-cell related proliferative disorder.

The development of monoclonal antibodies (MoAbs) and DNA probes has contributed significantly to the phenotypic and genotypic characterization of lymphomas as clonal malignancies of several cell subsets of the immune system. The recent identification of the two rearranging genes encoding the α and β chains of the heterodimeric T-cell receptor (TCR-αβ) has been rapidly followed by the discovery of two novel rearranging genes encoding the γ and δ chains of a distinct heterodimeric receptor, termed TCR-γδ, and by the development of several MoAbs specifically directed against their products.

We have previously described the pathologic features of peripheral T-cell lymphoma (PTCL) in three cases presenting as hepatosplenic lymphoma. We now present in detail the clinical characteristics and the phenotypic and genotypic findings in two of these cases in which we were able to study material obtained at various times during the course of the disease. We propose that a distinct entity can be delineated within PTCL on the basis of the clinical presentation, the sinusosal/sinusoidal pattern of histologic involvement, and the TCR-γδ phenotype of tumor cells.

CASE REPORTS

Two young adult white patients (23 and 36 years old) were referred because of marked liver enlargement, splenomegaly, and thrombocytopenia below 50 × 10⁹/L. Physical examination and blood cell counts were otherwise normal. Serologic tests for HTLV I and human immunodeficiency virus infection were negative. Liver tests and lactate dehydrogenase levels were normal. Chest x-ray and abdominal CT scan showed no enlarged lymph nodes. Both patients underwent a splenectomy, and the absence of lymphadenopathy was confirmed at laparotomy. Histologic examination of the liver and spleen showed malignant lymphoma (see below). The platelet count rose to normal values after splenectomy.

In patient 1, the spleen weighed 3,000 g. Before splenectomy, examination of blood smears had shown no abnormal circulating cells and a bone marrow biopsy had shown no histologically detectable lymphoid infiltration. Thrombocytopenia recurred 6 months later, associated with an increase in liver size and abnormal liver function tests. Bone marrow and liver confirmed initial findings. The patient was then treated with an anthracycline-containing regimen. A complete response was documented after three monthly courses on the basis of histologic examination of the liver and bone marrow. The patient was subsequently treated by high-dose cyclophosphamide and total body irradiation followed by autologous bone marrow transplantation. A hepatic relapse was histologically documented 8 months later, associated for the first time with a minimal lymphoid infiltration of the bone marrow. Despite chemotherapy, the patient died 24 months after diagnosis.

In patient 2, the spleen weighed 6,500 g. Before splenectomy, a bone marrow biopsy had shown a minimal lymphoid infiltration and atypical lymphoid cells had been recognized in blood smears. After splenectomy, the patient received four courses of the same chemotherapy regimen as patient 1, followed by maintenance therapy with high-dose cyclophosphamide. A complete remission was documented by rebiopsy of liver and marrow performed at 1 and 2 years after diagnosis. Relapse occurred at 3 years and was shown by the recurrence of thrombocytopenia, increase in liver size, and alteration of liver function tests. Liver and marrow biopsies documented a tumor infiltration. White blood cell counts were normal, but examination of smears showed that half of the lymphocytes were atypical medium-sized and large cells. Despite therapy, the patient died 42 months after diagnosis.
MATERIALS AND METHODS

Cell specimen preparation. Peripheral blood mononuclear cells (PBMN) were obtained by blood sedimentation on a Ficoll-Hypaque (FH) gradient (Pharmacia, Uppsala, Sweden; density 1.077 kg/L), stored frozen in liquid nitrogen, and thawed when necessary. In addition to the processing for routine histologic analysis, frozen tissue specimens were obtained for immunohistologic and genomic studies. Part of the spleen of patient 1 was teased through a sieve to make a single-cell suspension that was enriched on an FH gradient and stored frozen. Part of the spleen of patient 2, and liver and bone marrow biopsies of both patients were snap-frozen in liquid nitrogen after incubation in a gum-sucrose solution.

MoAbs. The MoAbs used in this study are listed in Table 1. TCR-α/β cells were identified by MoAbs WT31 and βF1.WT31 detects an epitope of the TCR-β chain both in its free form and in the mature α/β heterodimer, and thus can be used for staining frozen tissue sections and cytospin after incubation in a gum-sucrose solution. TCR γ/δ cells were identified by several MoAbs. Anti-TCRγ1,δ specific for an invariant epitope of the δ chain, identifies all γ/δ expressing cells and is thus operationally a pan-TCR γ/δ cell marker; anti-δTCS1 reacts with a variable Vδ1/Jδ1-encoded epitope of the δ chain, thus identifying only a subpopulation of normal γ/δ cells. MoAb anti-TiyA identifies a predominant population of cells expressing a Vγ9-encoded γ chain. These MoAbs were used on live cell suspensions, cytospin preparations, and frozen tissue sections.

Table 1. MoAbs Used in This Study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen</th>
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<tr>
<td>Pan-B cell</td>
<td></td>
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<tr>
<td>B1*</td>
<td>CD20</td>
</tr>
<tr>
<td>B4*</td>
<td>CD19</td>
</tr>
<tr>
<td>Pan-T cell</td>
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<tr>
<td>Leu-1†</td>
<td>CD5</td>
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<tr>
<td>Leu-4†</td>
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<td>CD8</td>
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<td>CD4</td>
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<td>Leu-6†</td>
<td>CD1</td>
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<td>T-cell receptor</td>
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</tr>
<tr>
<td>WT31†</td>
<td>αβ heterodimer</td>
</tr>
<tr>
<td>βF1§</td>
<td>γδ heterodimer</td>
</tr>
<tr>
<td>δTCR1‖</td>
<td>Vδ1,Jδ1</td>
</tr>
<tr>
<td>δTCR5‖</td>
<td>Vγ9</td>
</tr>
<tr>
<td>TiyA§</td>
<td></td>
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<tr>
<td>Tac#</td>
<td>CD25</td>
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<td>NK</td>
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<tr>
<td>Leu-11b†</td>
<td>CD16</td>
</tr>
<tr>
<td>NKH1*</td>
<td>CD56</td>
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* Coulter Immunology, Hialeah, FL.
† Beckton-Dickinson, Mountain View, CA.
‡ Sanbio bv, Uden, The Netherlands.
§ Gift from M. Brenner.
‖ T-cell Sciences, Cambridge, MA.
¶ Gift from Th. Hercend.
# Gift from Th. Waldmann.

PBMN and on spleen cell suspensions by indirect immunofluorescence and cytofluorometric analysis (Odam ATC 3000, Wissembourg, France) using fluorescein isothiocyanate (FITC)-labeled goat anti-mouse F(ab′)2 fragments (Cappel, Downingtown, PA). Cell phenotyping was also performed on cryostat sections of spleen, liver, and bone marrow samples, and on cytospins prepared from PBMN. The immunofluokinase phosphatase (APAAP) method10 was used. Rabbit anti-mouse immunoglobulins (Igs) and alkaline phosphatase-antiphosphatase complexes were obtained from DAKO (Dakopatts, Denmark).

Cell genotyping. DNA analysis was performed using standard methods detailed elsewhere.11 DNA was extracted from spleen, marrow, and PBMN samples. Ten micrograms of DNA was digested with each restriction endonuclease, subjected to electrophoresis in 0.8% agarose gel, transferred onto a nitrocellulose filter by the method of Southern, and hybridized to 32P-labeled DNA probes. Rearrangement of TCR β genes was analyzed using a probe19 specific for the constant regions (Cβ1 and Cβ2) after DNA digestion with EcoR1, BamHI, and HindIII endonucleases. Rearrangement of α gene rearrangement was performed using a probe specific for the heavy-chain joining region after DNA digestion with EcoR1, BamHI, and HindIII endonucleases. Analysis of Ig gene rearrangement was performed using a probe specific for the heavy-chain joining region after BamHI and HindIII digestion (all of the above probes were kindly provided by Th. Rabbits).

RESULTS

Histopathologic findings. In both patients, the spleen was greatly enlarged but cut surfaces were free of nodules. Tumor cells diffusely infiltrated the cords and sinuses of the red pulp (Fig 1). This infiltration was associated with atrophy of the white pulp. Tumor cells were medium-sized lymphocytes with a regular or slightly indented nucleus and a large pale cytoplasm. No erythrophagocytosis by tumor cells was noted. In both patients the main feature of liver involvement was a sinusoidal infiltration by lymphoid cells with the same morphologic features. This infiltration was responsible for a mild sinusoidal dilatation that was associated with occasional pseudopeliotic lesions and perisinusoidal fibrosis in patient 1. Portal infiltration was moderate in patient 1 and absent in patient 2. Bone marrow specimens were hypercellular in both patients. A minimal sinusoidal lymphoid infiltration causing a mild sinus dilatation was observed in patient 2.

Phenotypic findings. In both patients, spleen and liver contained a predominant population of cells with a CD3+CD2+CD4−CD8−βF1/WT31− phenotype. This unusual phenotype was observed both in cell suspension and frozen sections. In addition, the malignant cells in the spleen and liver expressed the TCR-γδ as shown by anti-TCRδ1 and δTCS1 MoAbs (Figs 2 and 3; Tables 2 and 3). Large numbers of circulating lymphoma cells were detected by phenotyping the PBMN in patient 2. By contrast, the PBMN in patient 1 contained normal numbers of CD3+CD4+ and CD3+CD8+ cells.

The expression of the TCR γ chain by lymphoma cells could not be assessed on tissue sections, due to the background staining observed with the anti-TiyA MoAb. However, cells expressing γ-chain were identified in cell suspensions from spleen (patient 1) and PBMN (patient 2); their number paralleled that of δTCS1+ cells.

Examination of bone marrow biopsies confirmed in both
patients the presence of a discrete lymphoid infiltration (morphologically undetectable in patient 1) displaying a sinusal topography and an identical TCRγδ* phenotype. It should be noted that the identification of CD4- lymphoma cells in liver and marrow sections was made difficult by the presence of CD4+ macrophages.

Finally, lymphoma cells identified by both their sinusal/sinusoidal topography and their βF1/WT31-γδ* phenotype were shown to lack CD5 and CD7 pan-T antigens as well as the natural killer (NK)-associated markers CD16 and CD56.

Genotypic findings. In both patients, hybridization of spleen cell DNA with the Jδ probe showed two nongermline fragments resulting from the rearrangement of the TCR-δ locus involving the two alleles; a rearrangement was found in PBMN from patient 2 identical to that in the spleen, whereas PBMN from patient 1 displayed a germline configuration (Fig 4). Hybridization with the Cβ probe showed a germline pattern in patient 1, whereas spleen cell DNA and PBMN from patient 2 contained a clonal rearrangement involving the Cβ2 segment of the TCR-Cβ gene (Fig 5). Ig heavy chain genes displayed a germline configuration in both patients.

Relapse material. Material was obtained from both patients. The sinusal/sinusoidal topography of the malignant infiltration in liver and bone marrow was identical to that found at presentation; it was of note that lymphoma cells had now become morphologically detectable in the bone marrow in patient 1. In patient 2, large cells were observed for the first time among the malignant population, this being confirmed in PBMN specimens.

In patient 1, immunohistologic analysis of liver sections showed that the sinusoidal lymphoma cells were still of CD3+C2*βF1- phenotype; however, only a minor population of cells had detectable TCR-δ chain. Material was not available for DNA analysis. In patient 2, βF1- tumoral cells infiltrating the liver and bone marrow had become TCR-δ- and CD3-; they expressed only CD2. The phenotypic analysis of a PBMN suspension gave results consistent with the presence of a population of CD3-WT31- cells that were also TCR-γδ-; this was further confirmed in cytopsin preparations in which the large lymphoid cells were shown to have...
this unexpected phenotype. However, DNA from marrow cells contained two Jb rearranged fragments identical to those found at presentation (Fig 4). Hybridization with the Cb probe showed a clonal rearrangement of the TCR-\(\delta\) chain gene similar to that found initially in the spleen, although only one allele was involved at that time (Fig 5).

**DISCUSSION**

The present findings add to the phenotypic heterogeneity of PTCL by showing that cases of malignant lymphomas occur as a result of a clonal proliferation of TCR-\(\gamma\delta\) cells.

PTCL encompass a clinically heterogeneous spectrum of malignant conditions with a frequent extra-nodal involvement.\(^{22,23}\) The diagnosis of PTCL is based on the histologic evaluation of tissue biopsies and on the demonstration of CD3\(^+\)CD4\(^-\) or CD3\(^+\)CD8\(^-\) phenotype, or less often a CD3\(^+\)CD4\(^-\)CD8\(^+\) or CD3\(^+\)CD4\(^-\)CD8\(^+\) phenotype. In addition, loss of expression of several pan-T differentiation antigens is frequently observed\(^{24}\) that correlates with the presence of a clonal process as established from genotypic studies.\(^{8}\) Therefore, the discordant expression of pan-T antigens results in various phenotypes within PTCL, including cases with loss of the CD3 molecule associated with the TCR.\(^{13,25}\) It is now established that normal T cells express two mutually exclusive types of CD3-associated TCR, namely \(\alpha\beta\) and \(\gamma\delta\). Several MoAbs have been developed that identify TCR-\(\gamma\delta\) expressing cells.\(^{8,26}\) Some MoAbs, such as the anti-Ti\(\gamma\)A and anti-\(\delta\)TCS1 used in this study, recognize variable gene encoded epitopes and thus delineate subsets of TCR-\(\gamma\delta\) cells. Others, such as the anti-TCR\(\delta\)1, are panreactive with all TCR-\(\gamma\delta\) cells, suggesting that they are directed against a C-encoded framework region. The availability of the MoAbs has recently allowed the phenotype and tissue distribution of TCR-\(\gamma\delta\) cells to be defined. In humans, a mean of 4% of CD3\(^+\) cells express the TCR-\(\gamma\delta\) in fetal and postnatal thymus, peripheral blood, and lymphoid organs.\(^{8,26}\) TCR-\(\gamma\delta\) cells have been generally referred to as double negative (CD4\(^-\)CD8\(^-\)), although a sizeable portion may express low levels of CD8, especially in peripheral lymphoid tissues.\(^{26}\) Finally, TCR-\(\gamma\delta\) cells can also be characterized by their lack of reactivity with MoAbs against the TCR-\(\alpha\beta\) heterodimer, such as WT3\(^{1}\) and \(\beta\)F1.\(^{1}\)

The two patients described here presented as hepatosplenic disease. The morphologic pattern of lymphoma was that of a sinusoidal/sinusoidal infiltration in spleen, liver, and marrow. These patients resemble those previously reported by others as "erythrophagocytic T\(\gamma\) lymphoma"\(^{14}\) with regard to clinical features and tissue distribution, although they differ by the absence of tumor-cell erythrophagocytosis and CD16 (Fcr1 receptor). The present data demonstrate unambiguously that the hepatosplenic lymphoma had both the genotypic and phenotypic characteristics of the TCR-\(\gamma\delta\) subpopulation. Thus, in the two patients reported here, the TCR-\(\delta\) clonal rearrangement was shown to be productive.

<table>
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<th>Table 2. Patient 1</th>
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<tr>
<td><strong>Phenotype</strong></td>
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<td><strong>Initial presentation</strong></td>
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<td><strong>Relapse</strong></td>
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Abbreviation: NE, staining not evaluable as a result of diffuse background on sections.

* Negative for pan-B, CD25, and CD1 antigens. Cells also lacked the CD16 and CD56 NK-associated antigens.
†Percent of positive cells in the suspension.
Lymphoma cells expressed the γδ heterodimer as shown by anti-TCRδ1 MoAb, lacked the αβ heterodimer as shown by WT31/βF1 MoAbs; they had a CD4- CD8- phenotype as do most normal human TCR-γδ cells. Clonal rearrangement of the TCR-δ locus has been described recently in cases of leukemias and lymphomas.17-28 However, the lineage specificity of clonal DNA rearrangement is not absolute and a conclusive lineage definition requires an immunophenotypic study demonstrating that the rearrangement is productive,6,29 as in the two cases presented. In patient 2 the finding of a simultaneous TCR-β rearranged fragment lends additional support to the clonal nature of the proliferation; in view of the WT31/βF1- phenotype, it can be interpreted as indicating either abnormal transcription or defective translation of a normal transcription product. TCR-δ rearrangements resulting in immature transcripts have been previously reported in TCR-γδ cell lines developed from human thymus.30

In the two patients, a striking feature was the coexpression by lymphoma cells of the TiγA and δTCS1 epitopes of the TCR-γδ as shown by study of cell suspensions. This phenotype was in concordance with the pattern of δ gene rearrangements observed (Fig 4), including a 12-kb band after DNA digestion by HindIII restriction enzyme. Such a rearrangement involves Vδ1/Jδ1 genes31 and is frequently encountered in acute lymphocytic leukemia.32,33 In addition, we could confirm this unique genotype/phenotype by showing that lymphoma cells in the patients did not react with a MoAb specific for the Vδ2 encoded product.8 Studies of gene assignment of the epitopes expressed by normal human clones has led to the delineation of several subsets within the normal TCR-γδ cell population, depending on the preferential pairing of a given V/J-encoded γ chain with a given V/J-encoded δ chain.8 Thus, the TiγA-δTCS1 phenotype, ie, Vγ9-Vδ1/Jδ1 genotype, corresponds to that of a very minor population of γδ T cells in normal peripheral blood lymphocytes.34

It will be of interest to determine whether preferential rearrangements correlate with surface receptors responsible for homing to distinct sites. Recent studies have focused on the localizations of TCR-γδ cells within peripheral tissues. For example, human TCR-γδ cells reside preferentially in the sinuses of the splenic red pulp.26,35 Our study reinforces the concept of a preferential homing to distinct histologic microenvironments by showing that malignant proliferations of TCR-γδ cells occur within the lumen of vascular sinuses in the splenic red pulp, as well as in liver and marrow.

In addition to CD3 antigen, normal thymus, peripheral blood, and spleen TCR-γδ lymphocytes express the CD2,
CD5, and CD7 pan-T antigens. In contrast, CD3+ lymphoma cells in our patients lacked CD5 and CD7. Therefore, the nonexpression of these pan-T antigens, a general feature of T-cell lymphomas, is also found in TCR-γδ lymphomas. Other phenotypic characteristics have been recently reported in T-cell lymphoma, such as discordant expression of CD3 and TCR-β chain. Thus, in tissue sections cases occur with a CD3+βF1 phenotype that might conceivably represent CDS, and CD7 pan-T antigens. In contrast, CD3+ lymphoma cells in our patients lacked CDS and CD7. Therefore, since we completed this study, we have identified two malignant expansions of TCR-γδ cells. However, in a recent study none of 41 PTCL studied cases (including seven cases with a CD3+βF1 phenotype) expressed the TCR-β1 antigen. Since we completed this study, we have identified two additional cases of hepatosplenic lymphoma with identical immunohistologic features; in our experience (to be published), TCR-γδ lymphomas remain much less frequent than their TCR-αβ counterpart.

In the two patients, the phenotype of the malignant cells at relapse differed from the initial one, although the recurrent lymphoma exhibited the same sinusoidal tropism. In patient 1, the expression of TCR-γδ became weaker with only a minor portion of cells being stained by the anti-TCRβ1 and anti-TCRδ1 antibody. In patient 2, the lymphoma cells did not express the δ-chain and the CD3 molecule. In this patient, the persistence of an identical biallelic rearrangement of the δ locus provides evidence that the malignant cells at the time of relapse arose from the same malignant precursor. Therefore, the phenotypic variations must have taken place within the clonal progeny. Phenotypic changes have been reported during the course of T-cell lymphomas leading to the concept that idiotype-negative cells emerging on tumor progression exist in the lymphoma cell population before treatment.

We conclude that a distinct entity can be delineated within PTCL that consists of prominent liver and spleen enlargement due to sinusoidal and red pulp infiltration by tumor cells expressing the TCR-γδ. The study of further cases is required to determine whether, as suggested by the outcome in our patients, this condition has a poor prognosis.

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REFERENCES


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