Florid splenic gamma/delta T-cell proliferation in patients with splenomegaly and cytopenias: A "high stakes" diagnostic challenge

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Abstract

Objectives: Splenic gamma/delta T-cell proliferation is rare and correct diagnosis is critical for adequate clinical management. **Methods:** Two splenectomy cases from patients with splenomegaly and cytopenias were studied by morphological evaluation, extensive immunophenotyping, FISH and molecular studies. The clinicopathologic findings were compared with splenic T gamma/delta neoplasia, notably hepatosplenic T-cell lymphoma (HSTL) and T-cell large granular lymphocytic leukemia (TLGL) of the variety T gamma/delta. **Results:** The enlarged spleens showed expanded red pulp with markedly increased gamma/delta T cells, which share significant to complete overlapping morphology and immunophenotype with the neoplastic gamma/delta T cells in HSTL and gamma/delta TLGL. However, they were polyclonal by molecular study and showed no evidence of isochromosome 7q. Splenectomy alone led to long-term clinical remission in both patients. **Conclusions:** Two florid reactive splenic gamma/delta T-cell proliferations mimicking gamma/delta T-cell neoplasia were reported for the first time in English literature. Recognition of this exceedingly rare phenomenon is critical in prevention of misdiagnosis with potentially catastrophic consequences.

Keywords: gamma/delta T cells; hepatosplenic T-cell lymphoma; primary cutaneous gamma/delta T-cell lymphoma; gamma/delta T-cell large granular lymphocytic leukemia

Introduction

Gamma/delta T cells ($T_{\gamma\delta}$) are mainly distributed in mucosal surfaces, such as intestine and skin, but also represent a minor subset of lymphocytes in peripheral blood, spleen, liver, etc. [1-3]. In the spleen, $T_{\gamma\delta}$ constitutes approximately 2% and 5% of total lymphocytes in normal and reactive conditions, respectively [4]. Neoplastic proliferations of $T_{\gamma\delta}$, including hepatosplenic T-cell lymphoma (HSTL), primary cutaneous γ/δ T-cell lymphoma and T-cell large granular lymphocytic leukemia (T-LGL) of the variety $T_{\gamma\delta}$ have been well described, the former two often associated with splenomegaly and cytopenias [5-7]. In most cases, it is not difficult to separate benign reactive vs. neoplastic $T_{\gamma\delta}$ proliferations based on comprehensive clinical and pathological evaluation. Marked reactive expansions of $T_{\gamma\delta}$ may occur in the blood [3]. Florid reactive proliferation of $\gamma\delta$ T cells in spleen mimicking neoplasia has not been reported in English literature and represent a pitfall with potentially catastrophic consequences. Here we report two female patients with splenomegaly and cytopenias associated with marked $T_{\gamma\delta}$ proliferation in the spleen. Detailed clinicopathologic features from these 2 patients are described and compared with $T_{\gamma\delta}$ variants of HSTL and T-LGL.

Clinical Findings

Case 1

A 37-year-old female presented with approximately two years of persistent neutropenia and thrombocytopenia with neutrophils around 1.0 k/cumm and platelet around 100.0 k/cumm. Her hemoglobin (Hb) and hematocrit (HCT) were always normal. Physical examination and computed tomography (CT) imaging showed splenomegaly. There was no hepatomegaly or lymphadenopathy. Her other complaints included sporadic joint pain and occasional episodes of sinusitis and bronchitis. Laboratory workup revealed positive hepatitis C (HCV) antibody but it was negative for HCV viral load by polymerase chain reaction (PCR). Serum liver functions, antinuclear antibody, rheumatoid factor, erythrocyte sedimentation rate, C-reactive protein, and human immunodeficiency virus (HIV) studies were all normal or negative. Bone marrow examination performed eleven months before the splenectomy revealed hypercellular marrow with maturing trilineage hematopoiesis. Flow cytometric analysis of the bone marrow aspirate revealed immunophenotypically unremarkable T cells and polyclonal B cells. Her neutropenia showed good but transient response to intravenous immunoglobulin (IVIG) therapy. A laparoscopic splenectomy was performed. There was no other treatment. Seven days' post-splenectomy her neutrophil count was 8.0 k/cumm and platelet at 745 k/cumm. Her three-month post-splenectomy neutrophil count was 8.9 k/cumm and platelet at 536 k/cumm. She was symptom free at 18 months from her splenectomy.

Case 2

A 41-year-old female with history of hypothyroidism presented with shortness of breath on exertion and left upper quadrant pain due to cold antibody hemolytic anemia, Hb 5.2 g/dL and hematocrit 15.2%, massive splenomegaly and mild hepatomegaly. She reported no fever, weight loss or night sweats. Absolute neutrophil and platelet counts were normal. Physical examination and CT imaging revealed splenomegaly and hepatomegaly but no lymphadenopathy. A bone marrow aspirate and biopsy performed 25 days before splenectomy was hypercellular due to marked erythroid hyperplasia and mild granulocytic and megakaryocytic hyperplasia without atypical lymphoid infiltrate. Immunohistochemistry and flow cytometry of this specimen did not detect any aberrant T-cell population. The hemolysis was only partially controlled with IVIG and rituximab therapies. She ultimately required transfusion of greater than 40 units of packed red blood cells. A splenectomy was performed two months after her initial presentation. Hemolysis and liver dysfunction resolved with no additional therapies. Subsequently, serum varicella zoster virus IgM titers were found to be mildly elevated but she never had a rash. All other infectious and autoimmune work up was negative. She remains without evidence of infection, lymphoproliferative disorder or hemolysis 5 years and 8 months following splenectomy.

Morphology:

Spleens from both patients were enlarged, weighing 902 grams in case 1 (in aggregate) and 1030 grams in case 2, and without gross focal lesion. Histologic sections revealed red pulp expansions in both cases. In case 1, the white pulp architecture was clearly preserved. Small to medium-sized lymphoid cells with slightly irregular nuclear contours and inconspicuous nucleoli were increased in the red pulp (Fig.1A and 1B). Rare, small non-necrotizing granulomata were identified.

In case 2 the white pulp was atrophic consistent with prior immunosuppressive therapies. The red pulp was massively expanded by a mixture of extramedullary hematopoiesis -- mostly erythroid with minor components of megakaryocytic and granulocytic lineages -- with a cordial and sinusoidal lymphoid infiltrate of mostly small mature lymphocytes without overt cytological atypia. Erythrophagocytosis was easily identified on air-dried Wright-Giemsa stained touch preparation (Fig. 1C). A concurrent liver biopsy showed a mild infiltrate of small mature lymphocytes with a portal and sinusoidal distribution.

Immunophenotype:

Flow cytometric analysis revealed approximately 25% $\gamma\delta$ T cells in the lymphoid population, (Fig. 2) in both cases. These cells expressed the following: CD2, sCD3 (slightly brighter than the $\alpha\beta$ T cells), CD7 (partial to positive), and T cell receptor (TCR) $\gamma\delta$. These cells were negative for CD4, CD8, and CD16. Immunophenotypically unremarkable $\alpha\beta$ T cells and polyclonal B cells (data not shown) were also detected. Immunohistochemical stains (IHC) (Fig. 3A-D) in case 1 showed the infiltrating cells within the spleen red pulp were T cells positive for CD2 and CD3 with partial expression of CD7 and absence of CD5. These T cells were also positive for TCR gamma chain, TIA1 and are negative for TCR beta chain, CD4, CD8, granzyme B, perforin, CD30 and ALK-1. CD20 stain highlights B cells enriched in the white pulp. Immunohistochemistry in case 2 highlighted an intrasinusoidal and intracordal infiltrate of T cell positive for CD3 and TIA1 (data not shown). In situ hybridization for Epstein-Barr virus RNA was negative in case 2. Table 1 summarizes the immunophenotype for these 2 cases.

The bone marrow examination from case 2 performed 25 days prior to splenectomy revealed no overt increase in T cells, no clusters and no intravascular arrays as have been associated with T-LGL [8]. In contrast to the spleen, the marrow shows only very rare CD56+ lymphocytes (Fig 3 E-G).

Molecular and Genetics:

In case 1, interphase fluorescence in situ hybridization (FISH) using dual color probes to 7q11.23 and 7q31 showed no evidence of deletion or extra signal of chromosome 7q. No FISH was performed for case 2. Molecular studies by PCR show no clonal TCR gamma gene rearrangements in either case and no clonal TCR beta gene rearrangement in case 2.

Discussion:

We report for the first time two cases of marked reactive $\gamma\delta$ T-cell proliferation in the red pulp of massively enlarged spleens from two female patients who presented with thrombocytopenia in case 1 and refractory hemolytic anemia in case 2. As summarized in Table 1, the morphology and immunophenotype of these $\gamma\delta$ T cells show significant to complete overlap with the neoplastic cells seen in $\gamma\delta$ T-cell neoplasm associated with splenomegaly, notably HSTL and T-LGL, such as loss or downregulation of CD5, CD4 and CD8 double-negatives, expression of CD56, altered CD3 intensity compared to $\alpha\beta$ T cells, etc. [5, 9-13]. Correct and prompt recognition of these rare and phenotypically unusual, but benign reactive $\gamma\delta$ T cell proliferations versus the latter two neoplastic conditions is critical as the latter two diseases require adequate chemotherapy.

HSTL is an aggressive T-cell neoplasm most frequently associated with a neoplastic proliferation of $\gamma\delta$ T-cells involving spleen, liver and bone marrow. It is seen most commonly in young males with marked thrombocytopenia, accompanied often by anemia and leukocytopenia. There is marked hepatosplenomegaly but no lymphadenopathy. Peripheral blood is usually not involved at the early phase of disease. Morphologically the neoplastic T cells in the spleen, liver and bone marrow typically show an intrasinusoidal infiltration pattern with formation of clusters and aggregates. The neoplastic cells are clonal by T-cell receptor gamma gene rearrangement study. Isochromosome 7q is detectable by FISH in most HSTL cases [5]. Our patients were both young females with marked splenomegaly; and also hepatomegaly in case 2. However, there was no evidence of clonal T-cell receptor gamma gene rearrangement in either of these 2 cases. No isochromosome 7q was detected by FISH in case 1 though no FISH study was performed in case 2. Most importantly, both patients exhibited spontaneous resolution and were symptom free after splenectomy.

T-LGL is a clonal T-cell proliferation with persistent increase in clonal large granular lymphocytes in peripheral blood with no clearly identifiable cause. Most T-LGLs are TCR α/β type with a small subset exhibiting TCR γ/δ type [6, 14]. γ/δ T-LGLs, unlike its normal counterpart γ/δ T cells, are mostly CD4-, CD8+. CD4- and CD8- γ/δ T-LGL has been rarely reported and is typically CD2+, CD3+, CD7+, CD5- or dim +, TIA1+, granzyme B+/- . There is usually no increase of LGL cells in peripheral blood. CD4-/CD8- γ/δ T-LGL tends to affect elderly people and associates with neutropenia, anemia, and splenomegaly. Though T-LGL is an overall indolent process, CD4-/CD8- γ/δ T-LGL appears to be somewhat more aggressive than typical $\alpha\beta$ T-LGL, requiring treatment more often [9, 11-13]. Both of our patients were relatively young (37 and 41 years old, respectively) with cytopenias and marked splenomegaly. Resected spleens showed increased γ/δ T cells with an immunophenotype essentially very similar to those for γ/δ T-LGLs, but quite different than typical α/β T cells that are most frequently evaluated in the clinical setting. Recognition of the normal patterns of antigen expression on reactive γ/δ T-cells is crucial to avoid overdiagnosis of immunophenotypic aberrancy and perhaps malignancy [3]. In both of our cases, molecular studies for T-cell receptor gene rearrangements clearly indicated a polyclonal T-cell process, in contrast to T-LGL which is by definition clonal.

In summary we reported two unique cases of atypical γ/δ T-cell proliferation extensively involving the splenic red pulp. There are overlapping clinicopathological features with γ/δ T-cell neoplasms, i.e. HSTL $_{\gamma/\delta}$ and γ/δ T-LGLs (Table 2). The expression of CD56 in case 2 may raise concern for a neoplastic process. However, CD56 expression is seen in close to 40% of benign γ/δ T-cell hyperplasia [3]. In the end, our cases represent clearly benign processes exhibiting no evidence of clonality and spontaneous resolution following splenectomy. Awareness of this reactive condition is critically necessary to guide clinical and pathological evaluation of such patients in order to prevent making a wrong diagnosis of a malignant disease such as HSTL $_{\gamma/\delta}$ and γ/δ T-LGL in a patient with a potentially benign, spontaneously resolving disease. A comprehensive study including detailed clinical evaluation, careful morphological examination, immunophenotyping by flow cytometric analysis and/or IHC together with specific molecular genetic studies is necessary to arrive at a correct diagnosis to guide appropriate management. Additional study of similar cases is needed in order to establish the spectrum of florid γ/δ T-cell hyperplasias of the spleen and establish more definitive criterion to separate benign from malignant processes.

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Figure legends:

Figure 1. Morphological features of case 1 (A and B) and case 2 (C). A. Spleen with preserved white pulp and expansion of the red pulp by a lymphoid infiltrate (H&E, 100X). B. Cytology of the red pulp infiltrate with mostly small cells, many with clear cytoplasm (H&E, 500X). C. Touch imprint of spleen showing extramedullary hematopoiesis and erythrophagocytosis (arrow) (Wright-Giemsa, 1000X).

Figure 2. Flow cytometric analysis showing approximately 25% $\gamma\delta$ T cells in the gated lymphoid population. Case 1 (A, B); Case 2 (C, D).

Figure 3. Immunohistochemical stains showing abundant CD3⁺/CD5⁻/TIA1⁺ $\gamma\delta$ T cells in the splenic red pulp in Case 1 (A-D, 200X), and few CD3⁺/CD8⁺/CD56⁻ T cells in the bone marrow from case 2 (E-F, 200X). A-CD3, B-CD5, C-TIA-1, D-Tg; E-CD3, F-CD8, G-CD56.

Table 1. Summary of immunophenotype by flow cytometry and immunohistochemistry.

Table 2. Comparison of our cases with CD4⁻/CD8⁻ $\gamma\delta$ T-LGL and $\gamma\delta$ HSTCL.

Highlights

- Two cases of florid splenic gamma/delta T-cell proliferation first reported here.
- Differential diagnosis with gamma/delta T-cell neoplasms is challenging and critical.
- Key differences between reactive gamma/delta T-cell proliferation versus gamma/delta T-cell neoplasms described.

Fig. 1

Fig. 2

Fig. 3

Table 1. Summary of immunophenotype by flow cytometry and immunohistochemistry

Antigen	TCR $\gamma\delta$	TCR $\alpha\beta$	CD 2	CD 3	CD 4	CD 5	CD 7	CD 8	CD 16	CD 56	CD 57	CD 94	TI A- 1	Granzyme B	Perforin
Case 1	+	-	+	+	-	-	P	-	-	-	N/A	N/A	+	-	-
Case 2	+	-	+	+	-	P	+	-	-	+	-	-	+	N/A	N/A

Abbreviations: N/A, not available; P, partially positive

Table 2. Comparison of our cases with CD4-/CD8- $\gamma\delta$ T-LGL and $\gamma\delta$ HSTCL

Clinical features	CD4-/CD8- $\gamma\delta$ T-LGL Variant	Our cases	$\gamma\delta$ HSTCL
Incidence	More than rare	N/A	Uncommon
Age / sex	Elderly (39-88 yr)	37 and 41 yr / F	Young adult male
B symptoms	Uncommon	None	Common
Hepatosplenomegaly	Moderate splenomegaly	Splenomegaly in both, hepatomegaly in case 2	Marked
Lymphadenopathy	Rare	None	Rare
Neutropenia/anemia	Common, often severe	Mild	Variable
Thrombocytopenia	Uncommon, often mild	Mild	Common, often marked
Autoimmune association	Common	Yes	Uncommon
Elevated LDH	Uncommon	N/A	Common
Clinical course	Indolent	Indolent	Aggressive
Morphologic features			
Neoplastic cells	Mature-looking large granular lymphocytes	small to medium-sized lymphocytic proliferation	Immature-looking medium-sized cells, variable morphology
Bone marrow	Mostly interstitial infiltration (single-cell layer)	N/A	Predominantly intrasinusoidal infiltration
Spleen	Predominantly cordal infiltration in spleen	Predominantly cordal infiltration in spleen	Predominantly intrasinusoidal infiltration
Immunophenotype			
Markers	CD3+/CD4-/CD8-/CD7+/CD5-/CD56-	CD3+/CD4-/CD8-/CD7+ (partial)/CD5-/CD56+/-	CD3+/CD4-/CD8-/CD7+/CD5-/CD56+
T-cell receptor	$\gamma\delta$	$\gamma\delta$	$\gamma\delta$
TIA-1/granzyme B/perforin	+/(+/-)/-	+/-/-	+/-/- (inactive cytotoxic profile)
Isochromosome 7q	Negative	Negative	Majority + (70%)
TCR rearrangement	Clonal	Polyclonal	Clonal

Abbreviations: T-LGL, T-cell large granular lymphocytic leukemia; HSTCL, hepatosplenic T-cell lymphoma; N/A, non-applicable or not available; LDH = lactate dehydrogenase;