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Case Report

Persistent non-neoplastic γδ-T cells in cerebrospinal fluid of a patient with hepatosplenic (γδ) T cell lymphoma: a case report with 6 years of flow cytometry follow-up

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Abstract: Hepatosplenic (γδ) T-cell lymphoma (HSTCL) is an uncommon T-cell lymphoma with an aggressive clinical course and poor prognosis. Bone marrow and peripheral blood are frequently involved, with central nervous system involvement less common. We describe a case of a 31-year old man diagnosed with a γδ HSTCL in 2003, successfully treated with chemotherapy and allogeneic stem cell transplantation, and followed from 2003 to present. Four-color flow cytometry (FC) was performed on a BD FACSCalibur and data analyzed with CellQuest Pro and FCS Express software. For cerebrospinal fluid (CSF), all cells were acquired due to limited material. Cytological correlation was available on all specimens. Molecular studies for T-cell gene rearrangement were non-contributory. By FC, the diagnostic HSTCL immunophenotype was CD3 (+), CD7 (+), CD2 (+), CD5 (-), CD4 (-), CD8 (-), TCR γδ (+). Subsequent CSF FC analysis revealed a distinct population of γδ T-cells in all specimens, ranging from <1% to 13% of lymphocytes. Consistently, the γδ T-cells exhibited a different immunophenotypic profile from the reported diagnostic immunophenotype; they expressed CD5, and exhibited a heterogeneous pattern of CD8 expression. Comparison to in-house cases from patients with hairy cell leukemia and concomitant increases in non-neoplastic γδ T-cells was performed. The persistent γδ T-cells from the CSF of the patient with HSTCL were immunophenotypically consistent with non-neoplastic γδ T-cells. We describe an unusual case of persistent γδ T-cells in the CSF of a patient during 6 years of flow cytometric follow-up after treatment for γδ HSTCL. By cytology, non-neoplastic and malignant γδ T-cells are often difficult to distinguish. FC analysis helps to make this distinction, even with a limited panel. By FC, the γδ-T cells in the CSF of this patient are immunophenotypically consistent with non-neoplastic γδ T-cells. Remarkably, this finding is underscored by the patient's unusual clinical picture; he remains well and disease free.

Key words: Gamma-delta T cells, hepatosplenic T cell lymphoma, cerebrospinal fluid, flow cytometry, T-cell gene rearrangement

Introduction

T cells can be classified into two major subsets based on surface expression of T-cell receptor (TCR) αβ or γδ heterodimers [1, 2]. γδ T-cells represent a minor subset (1-5%) of circulating T-cells, and may be increased in various infections, inflammatory and autoimmune processes, and even malignancy [3-5]. Hepatosplenic T-cell lymphoma (HSTCL) is a rare entity, usually of γδ TCR type, accounting for a very minor subset of mature T-cell lymphomas in adults [6]. It mainly occurs in young adult males and has a poor prognosis [7-9]. The neoplastic T cells are derived from non-activated cytotoxic γδ T-cells, particularly from the V-δ1 subset [7-12]. The immunophenotype of the malignant γδ T-cells has been explored by both flow cytometry and immunohistochemical studies, and are typically CD2(+), CD3(+), CD4(-), CD5(-), CD7(+), CD8 variably (+), CD16(+), CD56(+), and CD57(-) [8-10, 13]. Recently, neoplastic γδ T cells have been reported to exhibit lower expression of the TCR γδ /CD3 complex, a useful FC feature to rapidly differentiate malignant cells from their non-neoplastic counterparts [14].

Central nervous system (CNS) involvement by non-Hodgkin lymphomas is a poor prognostic feature. Although the benefit of prophylactic intrathecal or intraventricular chemotherapy is uncertain, this treatment is often recommended in these high risk patients [15]. FC analysis has shown increased sensitivity in detecting abnormal lymphocytes within the cerebrospinal fluid (CSF), when compared to conventional cytological examination [16-18].

HSTCL frequently involves the bone marrow and, in later stages, peripheral blood [6]. Involvement of the CNS is rare, but has been reported [19]. Histologically, the tumor cells typically exhibit sinusoidal infiltration in the spleen, liver, and bone marrow [6, 20]. Immunophenotypically, the tumor cells are characteristically positive for CD2, CD3, CD7, γδ TCR and negative for CD5, CD4, and CD8 [7, 21]. Treatment of HSTCL usually requires intensive chemotherapy and bone marrow transplant. CSF involvement by HSTCL is extremely rare, a poor prognostic feature, and impetus for intrathecal or intracranial chemotherapy.

We report on a patient with γδ HSTCL diagnosed in 2003, who consistently exhibited increased γδ T-cells in the CSF. As CSF may contain non-neoplastic γδ T-cells, the challenge, in this case, was to determine whether the increased γδ T-cells detected by FC represented CNS involvement. Ultimately, the FC immunophenotype of the γδ T-cells, even with limited cellularity of the CSF samples, consistently differed from the diagnostic HSTCL immunophenotype, and was highly suggestive of non-neoplastic γδ T-cells.

Clinical history

A 31-year old male patient was diagnosed with (γδ) HSTCL in 2003 on a liver biopsy. Outside flow cytometry analysis on the bone marrow aspiration revealed that the malignant γδ T-cells were CD2(+), CD3(+), CD7(+), CD5(-), CD4(-) and CD8(-). Upon admission to our hospital, increased γδ T-cells were detected by flow cytometry in the bone marrow aspirate. Initial flow cytometry evaluation of cerebrospinal fluid (CSF) revealed increased γδ T-cells (11% of lymphocytes). He initially was treated with 4 cycles of EPOCH; then 2 cycles of EPOCH with fludarabine. Because of the high suspicion of CNS involvement, prophylactic chemotherapy through lumbar puncture was also administered. He experienced a partial remission and was treated with reduced-intensity allogeneic peripheral blood stem cell transplant (2004), after conditioning chemotherapy with fludarabine and Cytoxan. No evidence of HSTCL was detected in a liver biopsy by FC immunophenotyping 5 months after the peripheral blood stem cell transplant. He has been closely followed with bone marrow and CSF studies by FC and to the present day. Molecular studies for T-cell gene rearrangement were non-contributory.

Materials and method

Clinical data was obtained through chart review. Morphologic and cytologic evaluation was performed. Four-color flow cytometry was utilized for immunophenotyping. For FC, all the cells in CSF specimens were acquired for evaluation (due to limited material). The samples of CSF were processed and acquired without red blood cell lysis. CSF specimens were stained for 30 minutes within 24 hour of collection with a panel of limited antibodies due to low cellularity in CSF, including CD3-PerCP, CD5-APC, CD5-FITC, CD45-PerCP, CD8-PerCP, CD8-APC, TCRαβ–FITC, and TCRγδ-PE. The antibodies were used in the following combinations: Tube 1) TCRαβ/TCRγδ/CD3/CD5; Tube 2) TCRαβ/TCRγδ/CD45/CD8. Rare exceptions included 2 CSF cases where different combinations were utilized: CD5/TCRγδ/CD3/CD8; TCRαβ/TCRγδ/CD8/CD5. All cells were fixed in 1.0% paraformaldehyde and stored at 4°C for up to 12 h before acquisition. Normal cells within specimens served as internal controls. Samples were acquired on a BD Biosciences FACSCalibur flow cytometer. The sensitivity of fluorescent detectors was set and monitored using Calibrite beads (BD Biosciences) according to the manufacturer's recommendations. Data (collected in list mode) was analyzed with CellQuest Pro software (BD Bioscience) and FCS Express (De Novo Software). Gating strategy: For tube 1, T-cells were identified by gating on CD3(+) cells fulfilling FSC and SSC properties characteristic of lymphocytes. Further gating was performed on γδ T-cells to examine expression of CD5. For tube 2, CD45(+) cells were identified, fulfilling FSC and SSC properties characteristic of lymphocytes.

Further gating was performed on γδ T-cells to examine expression of CD8.

Results

In July 2003, a diagnosis of HSTCL was rendered on the liver biopsy, which demonstrated extensive sinusoidal infiltration by malignant lymphoid cells (**Figure 1**). Bone marrow involvement was also documented at that time. Reportedly, in the liver biopsy, the neoplastic lymphoid cells were CD45(+), UCHL-1(+), CD5(-), CD4(-) and CD8(-), by immunohistochemistry. This was consistent with results of FC immunophenotyping, which reportedly identified CD2(+), CD3(+), CD7(+), CD5(-), CD4(-) and CD8(-) cells in the bone marrow. From 2003 to 2009, a total of 18 CSF samples were submitted for FC analysis (**Table 1**). Thirteen of 18 (72%) CSF samples demonstrated a significant population of γδ T-cells, ranging from 1-13% of the lymphocytes and 1-13% of the T-cells. Five of 18 (28%) CSF samples had rare γδ T-cells, comprising < 1% of the lymphocytes. The initial CSF specimen exhibited a marked increase in γδ T-cells (11% of T-cells) and was highly worrisome for involvement. However, in all subsequent CSF specimens, the γδ T-cells consistently showed a different expression pattern from the reported diagnostic immunophenotype; specifically, the cells exhibited CD5 expression and distinctly heterogeneous expression of CD8 (**Figure 2**). Occasionally, dim CD8 expression was noted (**Table 1**). Based on this subtle difference in the expression pattern of CD5 and CD8, the possibility of non-neoplastic γδ T-cells in the CSF was considered (**Figure 2, A, B, C**). For comparison, the CSF immunophenotype was examined alongside representative cases of peripheral blood with increased non-neoplastic γδ T-cells, from patients with hairy cell leukemia and no history of HSTCL (**Figure 2, D, E, F**). Based on the similarity of the expression patterns of CD5 and CD8, the γδ T-cells of the CSF were interpreted as non-neoplastic. In contrast, malignant populations of γδ T-cells exhibited a more homogeneous quality in their antigen expression (**Figure 2, G, H, I**, representative example). Immunophenotypically, the findings correlated with cytology (**Table 1**). All 18 CSF specimens were submitted for cytology examination; although rare atypical cells were noted in 5/18 samples, all CSF specimens were ultimately interpreted as negative for malignant cells. Molecular studies were non-contributory. Although all TCR gene rearrangement studies (with the exception of one non-diagnostic study) were negative on 8/18 CSF cases evaluated, no documentation of a diagnostic clonal TCR gene rearrangement was available for comparison. These findings are compatible with non-neoplastic γδ T-cells in the CSF of this patient with a history of HSTCL.

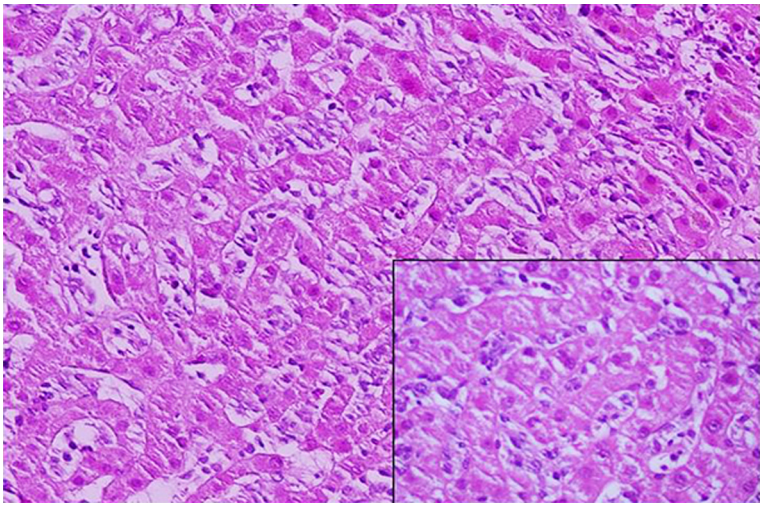

Figure 1. Hepatosplenic (γδ) T-cell lymphoma in a liver biopsy. Note the striking sinusoidal infiltration of neoplastic γδ T-cells (H&E 200X). Inset: (H&E 400X).

Table 1. Flow cytometric (FC) immunophenotyping and cytologic correlation on 18 CSF specimens from 2003-2009 from a patient with hepatosplenic (γδ) T-cell lymphoma

Date of CSF evaluation	gd T-cells as (%) of lymphocytes by FC	gd T-cells as (%) of T-cells by FC	CD5 on gd T-cells by FC	CD8 on gd T-cells by FC	Atypical cells on cytology	Malignant cells on cytology
7/17/2003	11%	11%	NP	Predominantly (-)	Present	(-)
8/27/2003	5%	8%	NP	Heterogeneous	(-)	(-)
9/18/2003	10%	12%	(+)	Heterogeneous	(-)	(-)
10/6/2003	13%	13%	NP	Heterogeneous	(-)	(-)
11/6/2003	1%	7%	NP	Heterogeneous	(-)	(-)
12/1/2003	4%	5%	NP	Heterogeneous	(-)	(-)
1/5/2004	4%	4%	(+)	Heterogeneous	Rare	(-)
4/23/2004	1%	1%	(+)	Heterogeneous	Rare	(-)
8/24/2004	2%	2%	(+)	Heterogeneous	(-)	(-)
12/1/2004	1%	1%	(+)	Heterogeneous	(-)	(-)
6/9/2005	1%	1%	NP	Heterogeneous	Rare	(-)
12/21/2005	<1%	1%	NP	Heterogeneous	(-)	(-)
6/14/2006	<1%	1%	NP	Heterogeneous	(-)	(-)
1/4/2007	<1%	1%	(+)	Dim	(-)	(-)
2/8/2007	3%	3%	(+)	Heterogeneous	Rare	(-)
8/20/2007	1%	1%	(+)	Dim	(-)	(-)
1/23/2008	<1%	2%	(+)	Heterogeneous	(-)	(-)
4/14/09	<1%	1%	(+)	Heterogeneous	(-)	(-)

(+) Positive; (-) Negative; (NP) Not performed due to limited cellularity.

Discussion

This case is unique for a number of reasons: 1) the patient is alive and disease free 6 years after the diagnosis of γδ HSTCL (typically, median survival is less than 2 years [11]); 2) the results of the diagnostic CSF work-up, which identified increased γδ T-cells, were initially highly suspicious for involvement by HSTCL, an extremely rare finding and portending a very poor prognosis; 3) the patient was successfully treated with systemic and prophylactic intrathecal chemotherapy and peripheral blood stem cell transplant; 4) the γδ T-cells were consistently detected in the CSF during the 6 years of follow up after treatment; 5) the immunophenotypic pattern of the CSF γδ T-cells were consistent with that of reactive/non-neoplastic gamma-delta T-cells, rather than with the patient's diagnostic HSTCL immunophenotype.

Increased γδ T-cells may be associated with a variety of infections, inflammatory and autoimmune diseases, and lymphoproliferative disorders. The increase may be observed, in both peripheral blood [3-5] and CNS [22, 23]. In our clinical service, we have observed many cases of hairy cell leukemia with a concomitant increase in γδ T-cells, and skewed T-cell repertoires are well documented in this disease [24]. Therefore, we utilized the γδ T-cells expression patterns from these cases for comparison to our index case. Based on the pattern of CD5 and CD8, it is noted that the patient's persistently increased γδ T-cells in the CSF are immunophenotypically compatible with reactive/non-neoplastic γδ T-cells.

Reactive γδ T-cells are often difficult to distinguish from malignant γδ T-cells because they are cytologically similar on a cytospin preparation.

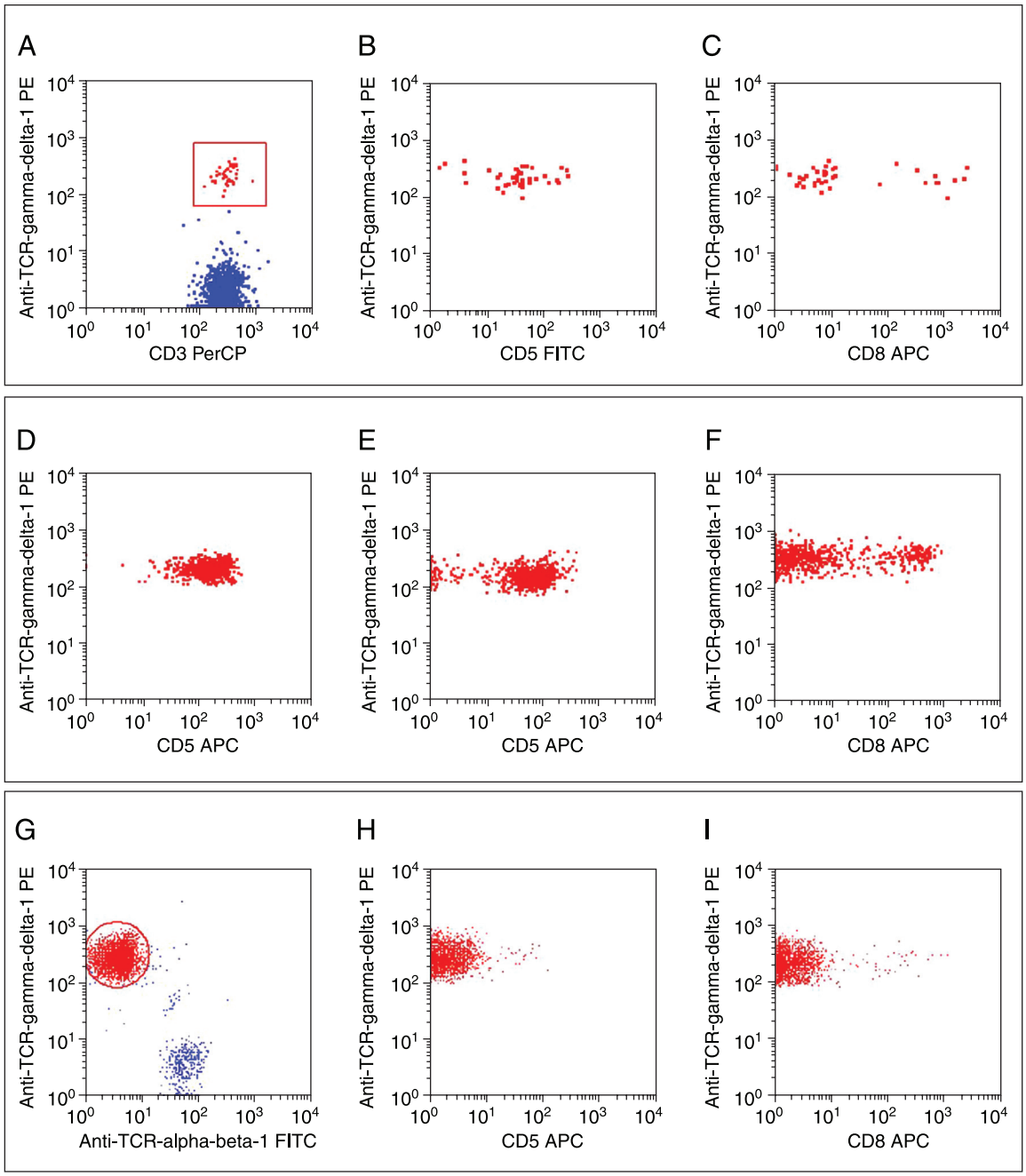

Figure 2. Persistent γδ T-cells in CSF after treatment for hepatosplenic (γδ) T-cell lymphoma. γδ T-cells (red) were identified on CD3(+) cells or CD45 bright(+) cells fulfilling FSC and SSC properties characteristic of lymphocytes. A representative FC immunophenotyping of the patient's CSF is shown (A, B, C). The patient's γδ T-cells (A) demonstrate predominantly positive expression of CD5 (B), with few CD5 dim to negative events, and heterogeneous expression of CD8 (C). The pattern of CD5 and CD8 expression is consistent with the immunophenotype of non-neoplastic γδ T-cells that are often observed in cases of hairy cell leukemia (representative examples: D, E, F). In contrast, a representative immunophenotype from an additional patient with hepatosplenic γδ T-cell lymphoma involving blood (G, H, I), demonstrates a homogeneous antigen expression pattern on the malignant γδ T-cells. Based on the pattern and heterogeneity of CD5 and CD8 expression, the persistent γδ T-cells in CSF (A, B, C) are consistent with non-neoplastic γδ T-cells.

Due to the often paucicellular nature of CSF, only a limited FC antibody panel can be applied, which poses a challenge to pathologists trying to distinguish malignant cells from non-neoplastic γδ T-cells. Additionally, non-neoplastic γδ T-cells may exhibit diminished expression of CD5 and CD7, and also partial or complete loss of CD5 expression [5]. As T-cell antigen loss is a distinguishing feature of many T-cell lymphomas, it is important to bear this in mind when specifically examining gamma-delta T-cell populations.

To the best of our knowledge, this report documenting persistent non-neoplastic CSF γδ T-cells in a patient treated for γδ HSTCL is unique to the medical literature. Although much remains to be elucidated regarding the specific functions of γδ T-cells, their capacity to recognize antigens independently of major histocompatibility complex class I distinguishes them from αβ T-cells. They appear to serve an additional role in immune surveillance and are attractive potential agents in cancer immunotherapy [25]. It is unclear what role the persistent γδ T-cells played in the remarkable resiliency and longevity of this particular patient who was diagnosed with a disease that is typically very aggressive. In the transplant setting, donor derived γδ T-cells persist and can been detected even 5 years after allogeneic transplant [26]. γδ T-cells can recognize antigens expressed on host cells, and specific γδ T-cell subtypes function in tumour surveillance [3]. This self-reactive quality, though not well understood, may have contributed to the unusually good outcome experienced by this patient.

Ultimately, the nature of this patient's CSF γδ T-cells is underscored by the unusual clinical picture; he remains well and disease free.

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