The γδ variant of T cell large granular lymphocyte leukemia is very similar to the common αβ type: report of two cases

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Received: 25 April 2008 / Accepted: 28 July 2008 / Published online: 23 August 2008
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Abstract The vast majority of cases of T cell large granular lymphocyte (T-LGL) leukemia have a CD3+, CD4−, CD8+ phenotype and express the αβ T cell receptor. Whether the rare γδ variant should be included in the same diagnostic category is currently unclear. Two well-characterized cases of γδ T-LGL leukemia were identified by our laboratory in 2007. These two cases and other reports of γδ T-LGL leukemia were compared with the common αβ variant. Other than more often being negative for both CD4 and CD8 (in about 35% to 40% of cases), the γδ variant of T-LGL leukemia is similar to the common αβ type in virtually all respects and should be included in the general category of T-LGL leukemia. However, it is important to exclude other more aggressive γδ T cell lymphoproliferative disorders.

Keywords Large granular lymphocytes • γδ lymphocytes • T cell leukemia

T cell large granular lymphocyte (T-LGL) leukemia is a clonal lymphoproliferative disorder of cytotoxic T cells often associated with cytopenias but having a relatively indolent course [1, 2]. The vast majority of cases have a CD3+, CD4−, CD8+ phenotype and express the αβ T cell receptor (TCR). However, there are rare variants including cases that express the γδ TCR. The CD4 and CD8 expression pattern of these cases is not well-established; some may be CD4− and CD8− like most normal γδ lymphocytes [3]. Since many types of γδ leukemias or lymphomas have a more aggressive clinical course, there is some controversy whether such cases should be included in the general T-LGL leukemia category [4]. Herein are described two cases of γδ T-LGL leukemia that presented in an indolent fashion very similar to the common αβ variant.

Report of two cases

Clinical histories

Case 1 An 88-year-old woman presented with a history of frequent urinary tract infections along with relatively stable leukopenia and mild anemia that had been present for over 10 years. During the previous 6 years, the platelet count had become borderline low. At the time of this evaluation in June 2007, the white blood count (WBC) was 2,500/μL with 95% lymphocytes and 5% monocytes, hemoglobin 10.7 g/dL, mean corpuscular volume (MCV) 90.8 fL, and platelets 161,000/μL. On physical exam, there was no palpable lymphadenopathy or hepatosplenomegaly. The patient was treated with methotrexate (10 mg orally every week) and the absolute neutrophil count (ANC) had risen to 1,300/μL 7 months later. She did have a Klebsiella pneumoniae urinary tract infection in April 2008 when the ANC was 1,000/μL.

Case 2 A 69-year-old man with a 30-year history of rheumatoid arthritis was first noted to have neutropenia 2 years before this evaluation. The patient had no recurrent sinopulmonary infections, but for the previous 6 months he had a persistent staphylococcal infection of his left foot. At presentation in April 2007, the WBC was 2,000/μL with 3% bands, 12% segmented neutrophils, 1% eosinophils, 68% lymphocytes, and 16% monocytes. Hemoglobin was
16.4 g/dL, MCV 87.1 fl, and platelets 204,000/μL. There was no palpable organomegaly or lymphadenopathy, but ultrasound showed mild to moderate hepatosplenomegaly. The patient was treated with cyclophosphamide (50 mg orally every day, decreased to 25 mg after 2 months) and the ANC had risen to 2,300/μL 3 months later. He was hospitalized for a right upper lobe pneumonia at that time. Subsequent ANCs for the next 6 months were highly variable ranging between 160 and 900/μL.

Microscopic, immunophenotypic, and genetic findings

Case 1 The peripheral blood smear showed a majority of large granular lymphocytes. The bone marrow aspirate showed nearly 80% erythroid precursors and only rare myeloid cells. The biopsy had 50% cellularity with occasional small lymphoid aggregates and a subtle interstitial increase in small lymphocytes. Immunohistochemical staining showed a significant increase in small CD3+, CD8+ T cells mainly in an interstitial pattern. In the few small lymphoid aggregates, CD3 and CD20 each marked about half the lymphocytes with CD4+ cells exceeding CD8+ cells by roughly three to one. There were only rare interstitial B cells. A G&S stain showed some areas with a slight increase in reticulin fibrosis. Flow cytometry performed on the bone marrow aspirate showed 64% lymphocytes with over 90% T cells expressing CD2, CD3, CD5, and CD7 without aberrancy. The CD4:8 ratio was 1.1. Nearly half the T cells were CD4−, CD8− and the majority expressed TCR γδ along with CD57 (Fig. 1); CD16 and CD56 were negative. TCR gene rearrangement studies by polymerase chain reaction (PCR) were performed on a subsequent peripheral blood sample and showed clonal γ and β patterns. Cytogenetics were normal.

Case 2 Increased large granular lymphocytes were noted on the peripheral smear. The bone marrow aspirate showed 40% erythroid precursors with a low G/E ratio of 1.0:1.2 along with 20.0% lymphs and 3.4% mature plasma cells. The biopsy had 50% cellularity with an interstitial and nodular infiltrate of small lymphocytes. The interstitial population was highlighted with CD3. The large majority of these expressed CD8 and not CD4 (Fig. 2). Rare scattered B cells marked with CD20. In the lymphoid aggregates, the majority of cells were CD3+, but CD4+ cells exceeded CD8+ cells by roughly five to one with only occasional CD20+ B cells. A G&S stain showed some areas with a slight increase in reticulin fibrosis. Flow cytometry on the aspirate showed about 40% of marrow nucleated cells in the lymphocyte region with about 95% T cells. The CD4:8 ratio was 0.6. About half the T cells expressed CD57. CD16 and CD56 were negative while roughly a fourth of the T cells had loss of CD5. About two-thirds of the lymphocytes lacked both CD4 and CD8. A similar proportion of the lymphocytes (presumably the same population) expressed TCR γδ. PCR studies showed clonal rearrangements of both TCR γ and β (Fig. 3). Cytogenetics were normal.

Comment

TCR γδ T cells normally comprise only 0.5% to 5% of peripheral blood lymphocytes [5]. However, they are highly prevalent in certain peripheral tissues such as the skin, intestinal tract, and mammary ducts where they play a central role in the early response to infections. Expansions of γδ T cells have been described with Epstein–Barr virus infection, human immunodeficiency virus infection, and congenital immunodeficiency syndromes [6].
Similar to their representation in the peripheral blood, the \( \gamma\delta \) variant of T-LGL leukemia probably accounts for less than 5% of T-LGL leukemia cases [7]. Most investigators report that the vast majority of circulating and bone marrow \( \gamma\delta \) lymphocytes are CD4\(^-\) and CD8\(^-\), but at least one study found that nearly half are CD4\(^-\), CD8\(^+\) [8]. The immunophenotype of \( \gamma\delta \) T-LGL leukemia is variable. Both patients in this report were CD4\(^-\), CD8\(^-\), CD57\(^+\), CD16\(^-\), and CD56\(^-\). About 35% to 40% of \( \gamma\delta \) T-LGL leukemia cases are CD4\(^-\), CD8\(^-\) [7, 9]. The remaining cases are CD4\(^-\), CD8\(^+\) with CD8 expression often dim. Note, however, that the CD4\(^-\),CD8\(^-\) immunophenotype is not entirely specific for \( \gamma\delta \) lymphoid proliferations. Rare cases of \( \alpha\beta \) T-LGL leukemia being CD4\(^-\), CD8\(^-\) have been reported [10, 11]. Similar to \( \alpha\beta \) T-LGL leukemia where over 95% of cases express CD57, the vast majority of \( \gamma\delta \) cases express CD57 (86% in the series by Sandberg [7]). Also, like the common \( \alpha\beta \) variant, CD16 and CD56 expression is more variable. Based on Sandberg’s series and other case reports, CD16 and CD56 are each expressed in nearly half of the \( \gamma\delta \) T-LGL leukemia cases. Perhaps 20% and 60% of \( \alpha\beta \) T-LGL leukemia cases express CD56 and CD16, respectively; rarely are all three NK markers coexpressed [2].

The bone marrow findings in our two cases conformed to the general pattern described by Osuji et al. for the common \( \alpha\beta \) variant [12]. That group described the bone marrow histology in 38 cases of T-LGL leukemia. Ninety-seven percent of cases had an interstitial infiltration of CD3\(^+\) cells that were CD8\(^+\) in all but one instance. Nonparatrabecular lymphoid nodules were seen in 55% of cases and were predominantly CD4\(^+\) T cells surrounding CD20\(^+\) collections of B cells. A reliable distinction between reactive lymphoid infiltrates and T-LGL leukemia in bone marrow biopsies is difficult. Interstitial clusters of six or more T cells expressing CD8, TIA-1, or granzyme B reportedly favor an LGL leukemia (either T cell or NK cell) [13].

Interestingly, in both our cases, interstitial CD8\(^+\) T cells were slightly increased even though the neoplastic \( \gamma\delta \) T cells were CD8\(^-\) by flow cytometry on the marrow aspirate. Since \( \gamma\delta \) T cells often weakly express CD8, perhaps this observation reflects the different monoclonal antibodies used in flow cytometry (SK1\(^{17}\); BD Biosciences) versus immunohistochemistry (C8/144B; Dako) in our laboratory or different sensitivities by the two techniques. Alternatively, the interstitial CD8\(^+\) T cells could be an immune

![Fig. 2](image)

**Fig. 2** Case 2—immunohistochemistry performed on the bone marrow biopsy (×10). **a** Increased interstitial CD8\(^+\) T cells. **b** Predominantly CD4\(^+\) T cells in a lymphoid aggregate with relatively few interstitially.

**Fig. 3** Case 2—T cell receptor gamma PCR 6% polyacrylamide gel. Patient bone marrow sample in lane 3 (from left) shows a discrete clonal band. Lane 1 has marker bands. Lanes 1, 4, and 5 have polyclonal patterns. Lane 6 is a positive control. T cell receptor beta reaction was also clonal (not shown). PCR primers described by McCarthy et al. [18, 19]
epiphenomenon and not part of the neoplastic population as has been described by Morice for NK-LGL leukemia [14].

Both cases had severe neutropenia with the ANC 300/µL or less. Like the common αβ variant, neutropenia is commonly associated with γδ T-LGL leukemia with an ANC below 1,500/µL in 50% to 70% of cases [7, 9]. Pure red cell aplasia has been described in about 5% of cases similar to the common αβ T-LGL leukemia [5, 7, 9].

One of our two patients had rheumatoid arthritis. A history of rheumatoid arthritis has been described in about 20% of γδ T-LGL leukemia cases [7, 9, 10] compared with about 25% of αβ cases [1].

Whether the type of TCR (γδ versus αβ) should be a pivotal diagnostic criterion for T cell lymphoproliferative disorders is unclear. Hepatosplenic T cell lymphoma was originally restricted to γδ cases, but subsequent reports described an essentially indistinguishable αβ subtype. In contrast, the γδ subtype of subcutaneous panniculitis-like T cell lymphoma is purported to have a significantly worse prognosis than its αβ counterpart, thus generating a separate provisional entity [15]. Saito et al. reported an aggressive clinical course for 10 of 11 γδ T cell neoplasms, but interestingly the one indolent case was a T-LGL leukemia that was CD4−, CD8− and after 4 years of follow-up, the patient had no progression of disease without treatment [16].

Recent reviews are reluctant to place the γδ subtype of T-LGL leukemia in the general T-LGL leukemia diagnostic category largely based on concerns regarding the aggressive clinical behavior of many γδ T cell lymphoproliferative disorders [1, 3, 4]. However, our two cases and a review of the literature on this topic suggest that γδ T-LGL leukemia is usually indolent and rarely may even spontaneously regress [17].

Conclusion

In summary, it is proposed that the γδ variant of T-LGL leukemia be included in the general diagnostic category of T-LGL leukemia. Other than often being CD4 and CD8 “double negative” (in 35% to 40% of cases), it is essentially indistinguishable from the common αβ variant in terms of cytology, immunophenotype, bone marrow morphology, clinical presentation, and prognosis. As a cautionary note, however, when a γδ lymphoproliferative disorder is encountered, it is crucial to exclude more aggressive disorders (e.g., hepatosplenic T cell lymphoma and peripheral T cell lymphoma) and also transient reactive γδ expansions (e.g., infection-related). This requires full evaluation of the morphology, immunophenotype, genetic data, and clinical presentation. Further studies with more patients are needed to confirm these findings and recommendations.

Acknowledgement We thank Marshfield Clinic Research Foundation for its support through the assistance of Alice Stargardt and Marie Fleisner in the preparation of this manuscript.

Ethical standards All research involving human subjects conducted at Marshfield Clinic with the approval of the Marshfield Clinic Research Foundation Investigational Review Board (IRB) is performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The Marshfield Clinic Research Foundation’s IRB has determined that a case series involving only individuals who are or who have been under the care of the proposed author do not meet the research definition.

Conflict of interest The authors declare that they have no conflict of interest.

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