Asymptomatic T-cell large granular lymphocyte leukemia with an unusual immunophenotype

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Abstract

T-cell large granular lymphocyte (T-LGL) leukemia represents a clonal proliferation of cytotoxic T-cells which etiology has not been entirely elucidated. However, $CD4^+$, $CD4^-$, $CD8^-$, $CD4^+$, $CD8^+$ cases have been described. The disease is usually characterized by cytopenias and a modest lymphocytosis. The majority of patients with T-LGL leukemia remains asymptomatic for a long period and will require treatment later during the course of their disease. Hereby we describe a case of T-LGL leukemia diagnosed by flow cytometry, which presented indolent course and required no treatment so far.

Introduction

T-cell large granular lymphocyte (T-LGL) leukemia is a disease characterized by clonal proliferation of cytotoxic T cells ($CD8^+$ cells). The etiology of T-cell LGL leukemia has not been entirely elucidated; however, chronic antigenic stimulation with exogenous antigens such as human T-cell lymphotrophic virus or putative endogenous autoantigens may be responsible for inducing the activation and clonal expansion of effector $CD8^+$ LGLs.[1] T-LGL leukemia appears more frequently in the elderly, with median age of diagnosis at 60 years and median survival of more than 10 years.[2,3] The distribution of the disease is equal among males and females.

The diagnosis of LGL leukemia should be suspected in all patients with unexplained cytopenias and high numbers of LGL cells by morphology and established by flow cytometry. TLGL leukemia usually appears with an aberrant immunopnenotype and clonal cells.

T-LGL malignant cells are characterized by the following immunophenotype: cytoplasmic and membrane $CD3^+$, $CD4^-$, $CD8^+$, $CD2^+$, $CD5^+$, $CD7^+$, $CD57^+$, $CD16^+$, $CD56^-$, $TCRab^+$.[3,4] However, case reports have described patients with $CD4^+$, $CD8^-$ LGL leukemia, dual positive $CD4^+$, $CD8^+$ and dual negative $CD4^-$, $CD8^-$ cases.[5,6]

Briefly three of the four following criteria is consistent with a diagnosis of T-LGL leukemia: i) peripheral LGL count of more than 2000/μL; ii) the presence of the expanded T-lymphocyte population by flow cytometry; iii) the presence of monoclonal TCR gene rearrangement shown by shown by polymerase chain reaction; iv) abnormal skewing of the TCR Vb family.[7,8]

In this report, we describe a case of T-LGL leukemia which appears with no clinical findings and a consistent lympocytosis.

Case Report

A 50-year-old female patient was admitted to our hospital due to an increase of the absolute lymphocyte count, in routine hematological tests. The absolute white blood cell count was 6.02×10^9/L, while the absolute lymphocyte count was 5.14×10^9/L. All the other biochemical, hematological and microbiological parameters revealed no findings. Computed tomography scan was performed in order to eliminate haematological or solid organ malignancy. Examination of peripheral blood smear showed the presence of large granular cells (Figure 1) and flow cytometry was performed. A subpopulation of T cells was detected expressing the following immunophenotype: $CD3^+$, $CD4^+$, $CD8^-$, $CD2^+$, $CD5^+$, $CD7^+$, $CD56^+$, $CD57^+$ and $TCRab^+$. These lymphocytes were monoclonal $CD4^+$ cells and the TCR Vb3 clone was expressed in 70% of these cells (Figure 2).

Two years after diagnosis the patient presented with malignant pleural mesothelioma. Flow cytometry was performed and the cells detected at the pleural fluid were $CD3^+$ at percentage of 80% but revealed no clonality by TCR Vb receptor analysis.

Flow cytometry

Flow cytometric immunophenotyping was performed on a FACSClibur instrument (BD Biosciences, San José, CA, USA) using the following antibodies panel; CD3, CD4 , CD8, CD2, CD5, CD7, CD56, CD57 (all from BD Biosciences) and IOTest® Beta Mark TCR Vβ Repertoire Kit (Beckman Coulter, Marseille, France). Immunophenotypical analysis was performed on CellQuest software (BD Biosciences).

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Key words: large granular lymphocytes, T-LGL leukemia, T cell receptor, flow cytometry.

Contributions: PKP, AK, AP, GV, experimental design, experiments execution; PKP, AK, AP, GV, LB, LD, NK, AG, hypotheses conception, data analysis, and paper preparation.

Conflict of interests: the authors declare no potential conflict of interests.

Received for publication: 21 October 2011.
Revision received: 29 May 2012.
Accepted for publication: 7 June 2012.

Licensee PAGEPress, Italy
Clinics and Practice 2012; 2:e68
doi:10.4081/cp.2012.e68

Discussion

T-LGL leukemia, a subtype of chronic lymphocytic leukemia, is characterized by the clonal expansion of a $CD3^+$ subpopulation. These cells are characterized as large granular lymphocytes and appear with a distinct morphology. LGLs are large cells (2x red blood cells) characterized by a condensed nucleus, abundant pale basophilic cytoplasm and small azurophilic granules. T-LGL expansion are not only detected in malignancies but they can also be reactive and can be seen in several clinical conditions, including post-splenectomy patients, patients with HIV infections[9] and in patients after allogeneic bone marrow or solid organ transplantation.[10] However in T-LGL leukemia, cells usually appear with an aberrant immunopnenotype and appear to be clonal. In the case presented above, the initial suspicion of T-LGL leukemia was presented based on the morphology and the detection of LGLs at the peripheral blood smear. Furthermore, the immunophenotype of these cells was studied by flow cytometry.

Besides the aberrant immunophenotype of LGLs the clonality is necessary to establish the diagnosis. Clonal T cells have the same sequence of TCR genes in contrast to normal individuals where *TCR* repertoire is stable and polyclonal. One of the key questions in large granular lympocytosis is whether these cells represent reactive or neoplastic proliferation.

A clonal population is indicative of malignancy, however differential diagnosis should be made from non malignant conditions as mentioned above (HIV-Epstein-Barr virus). The methods used to identify clonal T cells include polymerase chain reaction (PCR), Southern blotting and flow cytometry. PCR technique compared to Southern blotting appears to be quicker to perform and more effective if using poor quality DNA, and it has largely replaced it. Flow cytometry is a relatively quick and inexpensive method that offers quantitative evaluation of the expression of different V genes within different T cells.[11] According to a study by Feng *et al*., flow cytometric analysis of TCR-V beta showed 100% concordance with *TCR* gene rearrangement, suggesting that there is no need for further confirmation of flow cytometric findings.[12]

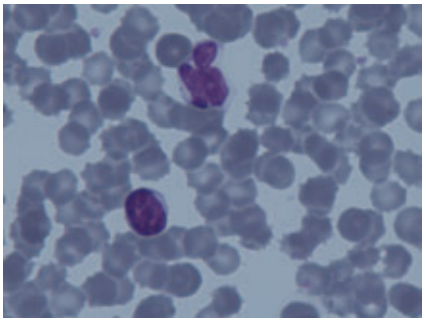

Figure 1. Peripheral blood smear demonstrating circulating abnormal lymphocytes.

The majority of patients with T-LGL leukemia will have an indolent clinical course. T-LGL leukemia is characterized by neutropenia, anemia and/ or thrombocytopenia, and a modest lymphocytosis. Due to neutropenia, which appears at some time during the disease, T-LGL leukemia clinically appears with bacterial infections, which typically include cellulitis and respiratory infections. Other symptoms include fatigue (due to anemia) and constitutional symptoms (fever, night sweats, weight loss). Physical examination reveals modest splenomegaly (in about two thirds of the patients), hepatomegaly and rarely enlarged lymph nodes. Despite this impressive array of symptoms, up to one third of patients are diagnosed based on the detection of asymptomatic cytopenia.[4,13,14] Rarely T-LGL leukemia appear in younger individuals with a most aggressive clinical course, which is associated with NK cell leukemia and T-LGL expressing CD3$^+$, CD8$^+$, CD56$^+$ markers.[15]

A unique feature of T-LGL leukemia is that it is often associated with other clinical conditions. Autoimmune disorders, especially rheumatoid arthritis[16] (serological or clinical), coexist in up to one third of patients with T-LGL leukemia. Besides, several studies report association with other hematologic disorders, including pure red cell aplasia, B cells diseases and other malignancies.[17]

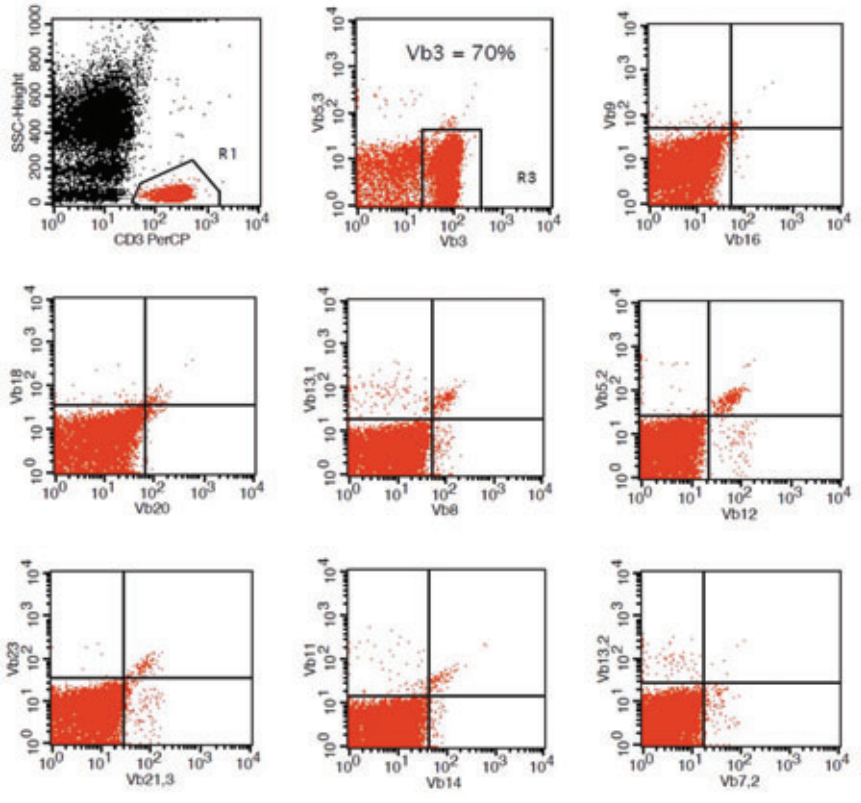

Figure 2. TCRvb repertoire clonality analysis demonstrating positive TCR Vb3.

The majority of patients with T-LGL leukemia require treatment at some point in their disease. The therapeutic options include granulocyte colony-stimulating factor, steroids, low-dose of methotrexate, cyclophosphamide, cyclosporine A, purine analogs and alemtuzumab.[18] However some patients with mild cytopenias remain asymptomatic for a long period without requiring therapy.[19]

In our case T-LGLs appear with a rare CD4$^+$ clonal immunophenotype. CD4$^+$ large granular lymphocytic leukemia has got different clinical and haemotogic features than the ones described above. These rare cases appear with lack of neutropenia, splenomegaly and anemia, and there is no apparent association with rheumatoid arthritis or other autoimmune disease. However CD4$^+$ LGLs are very often associated with malignancies (27-29%) and in most cases described T cell proliferation is diagnosed after the associated tumor is discovered. Flow cytometry is an important diagnostic tool, as the aberrant immunophenotype in conjunction with demonstration of clonality, is apparent in all cases described. The immunophenotype described includes CD3$^+$, CD4$^+$, CD8 (-/DIM), TCRab$^+$, CD56$^+$ LGL cells. The clinical course of the patient described is benign, in contrast to CD8 T-LGL leukemia, which in most cases at some point requires therapy.[20]

The patient described in this case report has an immunnophenotype and a clinical course characteristic of CD4$^+$ T-LGL leukemia. The clonal subpopulation was detected two years before the diagnosis of the associated malignancy and according to the follow up the treatment required is consistent with the malignancy.

In conclusion, the case presented above adds to the small number of previously reported cases. Slight clinical differences from the cases described in the literature can be detected at the herein described case. Flow cytometry is indicated as an important diagnostic tool in investigating lymphocytosis and revealing immunophenotypic aberrancies and demonstrating clonality.

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