

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Large granular lymphocytes (LGL) in primary Sjögren syndrome (pSS): immunophenotype and review on the pathological role of T cells in pSS

TO THE EDITOR: Primary Sjögren syndrome (pSS) is a chronic autoimmune systemic disease that mainly affects the exocrine glands, causing severe inflammation with accompanying destruction of the gland. It is also characterized by systemic symptoms and laboratory findings of polyclonal B-lymphocyte activation, hypergammaglobulinemia, and positive autoantibodies. Its pathophysiology is not yet fully understood; genetic factors seem to play a relatively minor role, while environmental ones, most likely infections, contribute to disease onset and progression by activation of the innate and adaptive immune systems. Although the role of B cells in the disease is better characterized, little is known about the involvement of T cells in pSS [1-3].

We report a case of a pSS patient who was followed up at our hematology unit for monoclonal CD8+ T lymphocytosis. We have discussed the immunophenotype of CD8+ T lymphocytes and reviewed the involvement of pathological CD8+ T lymphocytes in pSS.

Case report

In September 2012, a 39-year-old woman was referred to our outpatient service because of unexplained lymphocytosis, mild anemia, and thrombocytopenia. Together with the lymphocytosis, the patient developed xerophthalmia and xerostomia with anti-nuclear, extractable nuclear antigen, and Ro-SSA antibody positivity. A diagnosis of pSS was made following salivary gland biopsy. Clinical evaluation showed slight dryness of the mouth and eyes with no alterations to the spleen, liver, and lymph nodes. The tests performed on September 22, 2012 were significant for $9.61 \times 10^9/L$ leukocytes, $8.19 \times 10^9/L$ lymphocytes, $106 \times 10^9/L$ platelets, and 11.8 g/dL hemoglobin; normal liver and kidney function values were seen with a slight polyclonal rise in the immunoglobulin dosage. In addition, hepatitis markers (A, B, and C serology) and parasitological stool assays were negative.

Therefore, to investigate a possible lymphoproliferative disorder, bone marrow and imaging studies were carried out. Bone marrow biopsy showed an interstitial and often intra-sinusoidal infiltration by small-medium sized CD8+ T lymphocytes, which had partial CD5 expression. However, no other sites appeared to be involved since a total body CT examination showed no adenopathies or liver or spleen enlargement.

Flow cytometric analyses were performed in the peripheral blood and bone marrow samples using a FACS Canto II cytometer (BD Biosciences, Franklin Lakes, NJ, USA) equipped with three lasers (405, 488, 633 nm). A total of

100,000 events/tube were acquired, and fluorochrome-conjugated antibodies were used to investigate different lymphoid antigens (CD3, CD4, CD5, CD8, CD7, TCR $\alpha\beta$, TCR $\gamma\delta$, CD45RA, CD45RO, CD57, CD2, CD16-56, CD19, CD20, CD22, CD10, CCR7, CD27, CD28, and κ and λ light chains). The analysis of the peripheral blood confirmed lymphocytosis ($7.15 \times 10^9/L$ lymphocytes) determined by an increase in CD8⁺ T lymphocytes ($6.15 \times 10^9/L$) which had normal expression of CD3, CD2, and CD7 markers, but weak CD5 expression and partial (50%) expression of CD57. Further, CD8⁺ lymphocytes were positive for CD45RA, but they did not express CCR7 (Fig. 1A), which are features found in terminal effector memory T lymphocytes (T_{EMRA}) [4].

The bone marrow analysis revealed the presence of a very similar population, which accounted for 88% of all lymphocytes. Furthermore, they all appeared to present the $\alpha\beta$ T-cell receptor (TCR- $\alpha\beta$), and polymerase-chain reaction analysis of the TCR genes confirmed a clonal rearrangement of TCR β , while the TCR δ gene showed a polyclonal rearrangement (Fig. 1B).

This clinical and immunophenotypic condition is well identified as CD8⁺ T cell large granular lymphocytic (LGL) leukemia [5].

In November 2012, the patient started SS therapy with Hydroxychloroquine (Plaquenil) 200 mg once daily and prednisone 4 mg once daily, and in the following months, the number of lymphocytes returned closer to normal ($6.65 \times 10^9/L$ in February 2013) and the mild thrombocytopenia initially noted remained stable. Therefore, close monitoring, done with periodic testing and annual flow cytometric analysis of the peripheral blood, was done. The number of lymphocytes normalized within one year and has since remained constant (about $2 \times 10^9/L$ lymphocytes), but CD8⁺ cells have continued to remain higher than normal, representing 80 to 89% of the total T lymphocyte population, and have also continued to represent an important proportion of the total number of lymphocytes (54% of all lymphocytes in 2015, 42% in 2016 and 2017, 52% and then 57% in 2018, and 61% in 2019; see Fig. 2 for a graphical representation). Moreover, throughout the years, CD57 continued to be expressed by about 50% of these cells.

During the last visit in November 2019, a more in-depth analysis was performed, including CD27 and CD28 detection: naïve and central memory cells and over 70% of effector memory cells were found to express CD27 but not CD28, while 93.4% of T_{EMRA} cells did not express either

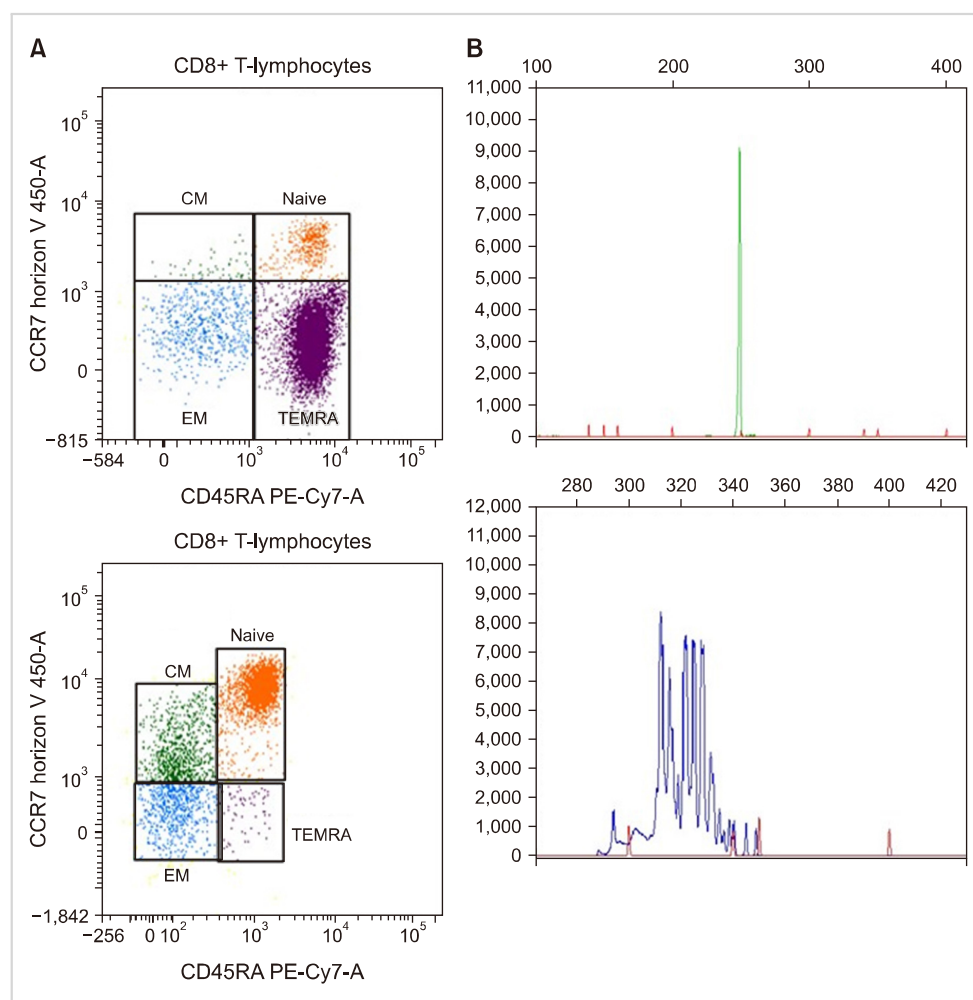


Fig. 1. (A) Immunophenotyping of circulating lymphocytes at the last observation (above) compared with immunophenotyping of circulating lymphocytes in a healthy donor (below). (B) TCR β and δ receptor rearrangements. TCR β (above) shows a monoclonal rearrangement, while TCR δ (below) is polyclonal. Abbreviations: CM, central memory T CD8⁺ cells; EM, effector memory T CD8⁺ cells; naïve, naïve T CD8⁺ cells; TEMRA, terminal effector memory T CD8⁺ cells.

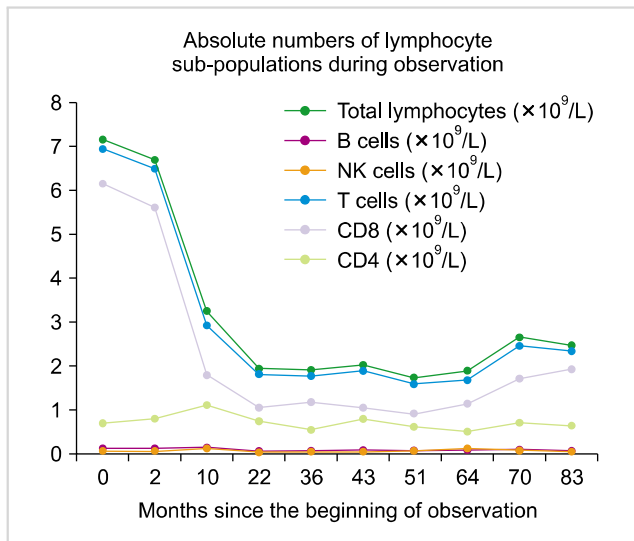


Fig. 2. Graph detailing variations in lymphocyte numbers during observation.

CD27 or CD28 (data not shown).

Discussion

LGL leukemia is a rare condition accounting for 2-3% of all mature lymphoid leukemias [6]. The finding of LGL in the context of autoimmune disorders is common, but it often occurs in rheumatoid arthritis or in the presence of less specific autoimmune features (such as positivity to autoantibodies, e.g., antinuclear antibodies); in pSS, less than 15 cases have been described [7-9].

LGL disorders are characterized by proliferation of LGL cytotoxic lymphocytes of either T-cell (mainly CD3+ TCRαβ+CD8+CD57+CD56+/- CD16+/-, rarely CD4+CD8+/-) or, less frequently, NK-cell (CD3-CD2+CD16+CD56+CD57+/-) origin [10].

In our case, the LGL immunophenotype was characterized as CD3+, CD8+, TCRαβ+, CD57+, CD45RA+, CD62L-, CD5dim, CD27, and CD28, ascribable to a sub-population of T_{EMRA} lymphocytes [4]. This physiological population arises from central memory cells in a context of homeostatic proliferation in the absence of an antigen, appearing for example after the acute phase of a viral infection [11]; similarly, LGL cells are thought to originate from chronic inflammation, in which prolonged antigen stimulation act as the triggering event [10]. However, while physiological T_{EMRA} cells are characterized by a low proliferative capacity and a high rate of cell death [11, 12], LGL cells have prolonged survival and activity mainly through pathological activation of the STAT pathway [10].

The relationship between pSS and LGL is not at all clear. One analysis of circulating T cell subpopulations in pSS had identified no difference in numbers of circulating CD57+ cells between pSS patients and healthy controls and had only found a decrease in CD8bright, CD27+ and CD57+ cells (which could correspond to a T_{EMRA} sub-population)

in patients with anti-SSA/SSB antibodies compared to those without antibodies [2]. On the other hand, a recent multi-disciplinary study has associated T_{EMRA} cells with pSS-specific patterns of gene transcription and protein dysregulation—meaning that this cell population presents modifications in gene transcription and protein processing which are specific to disease (although the relationship between these cells and transcriptome and proteomic changes are likely more subtle and in need of further study) [13].

The fact that the LGL in this case, as well as in all others reported [7-9], was determined by CD8+ T cells could represent a further argument that CD8+ cells undergo important modifications in pSS, which should be taken in consideration. Indeed, in our case, the diagnosis of LGL was made together with that of pSS, suggesting that the two diseases share at least part of the pathogenesis, if not a common etiology, as has been suggested in a previous report [7]. With regards to the former, one hypothesis which has already been proposed [10] is that the continuous immune stimulation enabled a mutated clone to develop and lose its typical high turnover rate. In line with this hypothesis, therapy aimed at immune system control would lead to lymphocyte count normalization [7, 8]; indeed, our patient was managed with immunosuppressive therapy and is still in good health, with a good lymphocytosis control even years later.

However, further research is needed to answer the remaining open questions about the origin of this population, its nature, and its actions.

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Atypical presentation of hairy cell leukemia: a report and comprehensive review

TO THE EDITOR: Pulmonary medicine consultation was sought for a 65-year-old man referred from an outside hospital with complaints of breathlessness, mediastinal lymphadenopathy, and intermittent low-grade fever accompanied by worsening anemia and low total leukocyte count. The patient had a history of one month of first-line anti-tuberculous therapy (ATT) based on a provisional diagnosis of pulmonary tuberculosis. His chest radiography and thoracic contrast-enhanced computerized tomography (CECT) scan

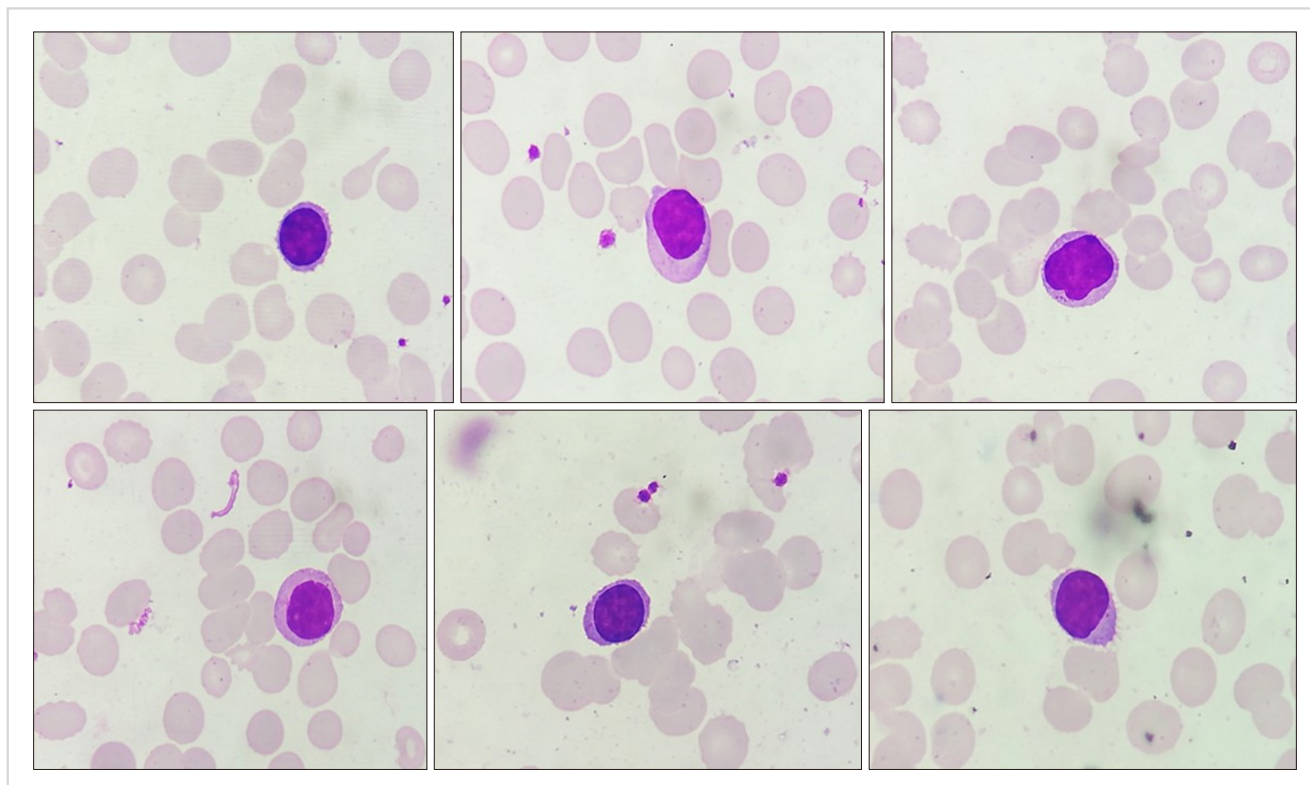


Fig. 1. Spectrum of circulating lymphoid cells in the peripheral blood in this case. Note the absence of characteristic circumferential hair-like projections in most cells (Leishman Giemsa stain, $\times 400$).