

## T-cell receptor $\gamma\delta$ -large granular lymphocytic leukemia associated with an aberrant phenotype and TCR-V $\beta$ 20 clonality

Large granular lymphocytic (LGL) leukemia is a rare heterogeneous disorder of mature lymphocytes with a characteristic morphology, multiple autoimmune disorders and indolent clinical course. Most cases exhibit a T-cell phenotype of CD3, CD8 and CD57 positivity, while the minority exhibit a CD2, CD56, and CD16 positive NK-cell phenotype. We report a case of a 71-year-old female suffering from a TCR $\gamma\delta$  positive T-cell leukemia with a morphology compatible to LGL leukemia. She referred to the hospital for investigation of mild anemia, lymphocytosis, neutropenia and hyperglobulinemia. Peripheral blood and bone marrow were occupied by mature large granular lymphocytes with abundant azurophilic granules. The immunophenotype was CD3+, CD2+, CD5+, CD7+, CD4-, CD8-, CD16-, CD56-, CD57- and the V $\beta$  repertoire analysis showed clonal reactivity with V $\beta$ 20 mAb. The patient was diagnosed as having T-LGL and was treated with G-CSF. So far, she experiences an indolent clinical course. To our knowledge, this is a rare case of TCR $\gamma\delta$  positive T-LGL leukemia with the aberrant immunophenotype of CD3+, CD4-, CD8-, CD16-, CD56-, CD57-.

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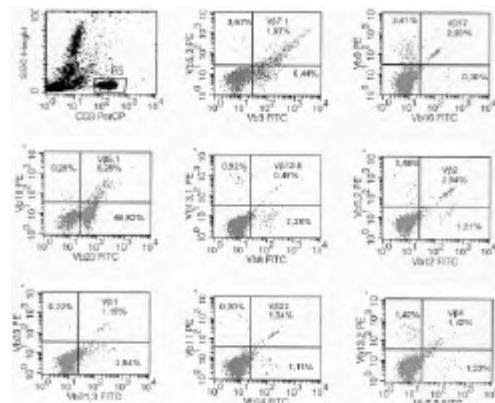
Large granular lymphocytic (LGL) leukemia is a rare heterogeneous disorder, but it is clinically, morphologically, and immunologically distinct. LGL leukemia cells have characteristic morphologic features of large lymphocytes with abundant cytoplasm and fine or coarse azurophilic granules. The updated criterion for the diagnosis of LGL leukemia is the demonstration of clonal expansion of a population of granular lymphocytes.<sup>1,2</sup> Two phenotypes are commonly described. A T-cell phenotype (T-LGL) exhibits CD3, CD8, and some NK cell markers such as CD16 and CD57 with T-cell receptor gene rearrangements. This type is associated with chronic neutropenia, rheumatoid arthritis, splenomegaly, and humoral immune abnormalities. A less common phenotype is a more classical NK cell (NK-LGL) with CD2, CD56, and CD16. These cases do not exhibit TCR gene rearrangement and have distinct clinical features with more significant anemia, thrombocytopenia, and hepatosplenomegaly. The clinical course is indolent in most cases.<sup>3,4,5</sup>

We report a case of T-cell leukemia with an indolent clinical course, who presented with mild neutropenia, lymphocytosis, autoimmune hemolytic anemia, hyperglobulinemia and antinuclear antibodies. The T-cells were TCR $\gamma\delta$  positive with the aberrant immunophenotype of CD3+, CD2+, CD5+, CD7+, CD4-, CD8-, CD16-, CD56-, CD57-. The lymphocyte morphology was compatible to T-LGL leukemia.

### Case report

A 71-year-old female was referred to the Department of Hematology of the University Hospital of Ioannina in October 2002 for investigation of mild anemia, lymphocytosis, neutropenia and hyperglobulinemia detected in a routine blood analysis. She had no previous medical history. Physical examination revealed neither hepatosplenomegaly nor lymphadenopathy.

**Figure 1.** Morphological features in the peripheral blood smear: the majority of the lymphocytes were mature large lymphocytes, with a high nuclear to cytoplasmic ratio, beaded chromatin and a basophilic cytoplasm containing a few abundant azurophilic granules.



**Figure 2.** Three-colour Flow Cytometric analysis of blood mononuclear cells: Performed on lysed whole blood with gate on CD3+ PerCP T-cells and TCR-V $\beta$  repertoire Kit, Beckman Coulter. Acquisition analysis on a Becton Dickinson FACScan Flow Cytometer and CellQuest program.

**Figure 3.** Clonogram representation, TCR-V $\beta$  repertoire gated on CD3+ T-cells, which demonstrates a clonality on V $\beta$ 20 69% (min 0%-max .%)

Peripheral blood counts at presentation were: white blood cells (WBC)  $6.4 \times 10^9/L$ , neutrophils  $0.99 \times 10^9/L$ , lymphocytes  $4.79 \times 10^9/L$ , platelets (PLT)  $250 \times 10^9/L$  and hemoglobin (Hb) 11.9 gr/dL. In the peripheral blood smear, the majority of the lymphocytes were mature large granular lymphocytes with abundant azurophilic granules (Figure 1). Biochemical parameters were normal, including lactate dehydrogenase (420 IU/L) and beta2-microglobulin (1892  $\mu\text{g/L}$ ), except for polyclonal IgM hyperglobulinemia. Serological findings were antinuclear antibodies (1/1280), low levels of complement C3 and C4

(82 IU/mL and 2 IU/mL respectively), high levels of RF (82 IU/mL) and positive direct and indirect anti-human immunoglobulin Coomb's test and cryoglobulins. The patient was seronegative for EBV, CMV, HBV, HCV, HIV and HTLV-1.

Bone marrow aspiration and biopsy revealed a significant lymphocytosis (25-30% of the nucleated cells) with a morphology similar to that of the peripheral blood. The infiltration was diffused.

#### Flow Cytometric Analysis

Analyses of leukocytes in whole blood were performed using two different fluorochromes. In the two-color analyses (phycoerythrin - PE and fluorescein - FITC), 100  $\mu$ l of whole blood were incubated with 20  $\mu$ l of each antibody, which were the pan-leukocyte-labeling antibodies (CD45+/CD14+) specific for the respective leukocyte subpopulation. After incubation, red blood cells were lysed (FACS Lysing Solution; Becton Dickinson, CA), washed twice in PBS (600 g/10min) and finally resuspended in this buffer. Data acquisition and analysis of lymphocytes was performed within one hour of preparation, using the CELLQuest Software (Becton Dickinson). The results were expressed as a percentage of the antibody-positive cells in the overall cell population, that was defined as the number of cells with specific fluorescence higher than the isotype control and autofluorescent samples. The expression of the CD phenotype in the population was performed on mononuclear cells (PBMCs) with scatter gate on the lymphocyte fraction and determined as follows: CD3+ 94%, CD19+ 5%, CD16+56+ 4%, TCR $\alpha\beta$ + 21%, TCR $\gamma\delta$ + 70%, CD8+ 10%, CD4+ 15%, CD38+ 94%, CD5+ 91%, CD7+ 89%, CD2+ 92%, HLA-DR+ 92%, CD1a+ 0%, CD20+ 4%, CD22 +1%, CD23+ 2%, CD79b 4%, FMC-7+ 2%, sIgM+ 0%, ?+ 0%,  $\lambda$ + 0%, CD103-0%, CD10+ 0%, CD34+ 0%, CD25+ 3%.

In a further analysis using three different fluorochromes, the specific population was gated on CD3-PerCP fluorochrome and the V $\beta$  domain expression was studied using the FITC and PE mixtures of TCR V $\beta$  Repertoire Kit (Beckman Coulter). The results of immunophenotype suggested the existence of a clonality V $\beta$ 20mAb cells including: V $\beta$ 5.3+ 3,63%, V $\beta$ 7.1+ 1,97%, V $\beta$ 3+ 0,44%, V $\beta$ 9+ 3,41%, V $\beta$ 17+ 2,20%, V $\beta$ 16+ 0,20%, V $\beta$ 18+ 0,25%, V $\beta$ 5.1+ 5,25%, V $\beta$ 20+ 69,82%, V $\beta$ 13.1+ 0,92%, V $\beta$ 13.6+ 0,49%, V $\beta$ 8+ 2,28%, V $\beta$ 5.2+ 0,69%, V $\beta$ 2+ 2,94%, V $\beta$ 12+ 1,21%, V $\beta$ 23+ 0,22%, V $\beta$ 1+ 1,19%, V $\beta$ 21.3+ 2,54%, V $\beta$ 11+ 0,20%, V $\beta$ 22+ 1,34%, V $\beta$ 14+ 1,11%, V $\beta$ 13.2+ 1,42%, V $\beta$ 4+ 1,43%, and V $\beta$ 7.2+ 1,23% (figure 2).

In conclusion, the lymphocytes were TCR $\gamma\delta$ -positive. The V $\beta$  repertoire analysis showed clonal reactivity with V $\beta$ 20 mAb of 69% (min 0%-max 9,73%) as shown in figure 3.

The patient was diagnosed as T-LGL leukemia with the aberrant phenotype TCR $\gamma\delta$ +, CD3+, CD2+, CD5+, CD7+, CD4-, CD8-, CD16-, CD56-, CD57-. She was treated with G-CSF. The neutropenia resolved but lymphocytosis remained stable. The patient so far, has shown an indolent course.

#### Discussion

T-cell large granular leukemia is a heterogeneous disorder with morphological features of large lymphocytes with cytoplasmic granules.<sup>1,2</sup> LGL leukemia cells have a mature T-cell immunophenotype. The majority of cases are TCR $\alpha\beta$ +, CD3+, CD4+, CD8+, while rare variants have been observed: TCR $\alpha\beta$ +, CD3+, CD4+, CD8- or TCR $\alpha\beta$ +, CD3+, CD4+, CD8+. CD57 is often expressed

in the common type. There is no unique karyotypic abnormality. For most cases the clinical course is indolent and non-progressive. Morbidity is associated with neutropenia. Although atypical immunophenotypes have been occasionally observed<sup>13,14,15,16</sup>, T-LGL leukemia with positive TCR $\gamma\delta$  and negativity to CD4, CD8, CD16/56 and CD57 has not yet been reported.<sup>3,4,5</sup>

The present case was compatible with T-LGL according to the clinical manifestation, the morphology of lymphocytes and the clinical course. However, the immunophenotype of TCR $\gamma\delta$ -positive, double-negative T-lymphocytes was quite unusual. The possibility of hepatosplenic, nasal or enteropathy-type  $\gamma\delta$  T-cell lymphoma is excluded, since in our case splenic, liver, nasal or gastrointestinal involvement was absent and the clinical course was indolent.<sup>7</sup> The possibility of T-lineage acute leukemia was also excluded, due to completely different cell morphology, clinical manifestation and clinical course.<sup>8</sup> T-prolymphocytic leukemia (T-PLL) with double-negative T-lymphocytes is a rare variant, but is usually TCR $\alpha\beta$ -positive.<sup>9</sup> Only one case of TCR $\gamma\delta$ -positive T-PLL has been reported.<sup>10</sup> However, T-PLL is characterized by morphological features of lymphocytes with prominent nucleous and cytoplasmic basophilia with no granules and an aggressive clinical course. Autoimmune lymphoproliferative syndrome is another entity that could be under consideration, since it exhibits common features with LGL. The syndrome is characterized by lymphocytosis due to defective lymphocyte apoptosis, hepatosplenomegaly, autoimmune disorders and an immunophenotype of *double negative* CD3+, CD4-, CD8-T cells but is usually TCR $\alpha\beta$ -positive. However, the disorder is nonmalignant and clonal expansion of the population of lymphocytes is not determined.<sup>11,12</sup>

Further accumulation of T-LGL cases is required to clarify whether T-LGL of this immunophenotype accounts for a rare variant of T-LGL, or this is an exceptional case.

G.Vartholomatos,<sup>1\*</sup> V. Alymara,<sup>2\*</sup> L. Dova,<sup>1</sup> N. Kolaitis,<sup>1</sup> K. L. Bourantas<sup>2</sup>

<sup>1</sup>Haematology Laboratory - Unit of Molecular Biology, University Hospital of Ioannina; <sup>2</sup>Department of Internal Medicine, Haematology Unit, University Hospital of Ioannina

\*equivalent authors

Correspondence: G.Vartholomatos PhD,  
Zygomalli 21, Ioannina, 45 332, GREECE  
Tel:+3 26510- 99726 Fax:+3 26510- 99418  
E-mail: gvarthol@cc.uoi.gr

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#### References

1. Semenzato G, Zambello R, Starkebaum G, Oshimi K, Loughran TP: The lymphoproliferative disease of granular lymphocytes: Updated criteria for diagnosis. *Blood* 89:256, 1997
2. Dhodapkar MV, Li Chin-Yang, Lust JA, Tefferi A, Philylyk RL. Clinical Spectrum of Clonal Proliferations of T-Large Granular Lymphocytes: A T-cell Clonopathy of Undetermined Significance? *Blood* 84 :1620, 1994
3. Jennings CD, Foon KA. Recent Advances in Flow Cytometry: Application to the Diagnosis of Hematologic Malignancy. *Blood* 90: 2863, 1997
4. Langerak AW, Beemd R, Wolvers-Tettero ILM, Boor PPC, Lochem EG, Hooijkaas H, Dongen JJM. Molecular and flow cytometric analysis of the V $\beta$  repertoire for clonality assessment in mature TCR $\alpha\beta$  T-cell proliferations. *Blood* 98:165,

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5. Hoyer JD, Ross CW, Li C-Y, Witzig TE, Gascoyne RD, Dewald GW, Hanson CA True T-cell chronic lymphocytic leukemia: a morphologic and immunophenotypic study of 25 cases. *Blood* 86:1163, 1995
  6. Lang DF, Rosenfeld CS, Diamond HS, Shaddock RK, Zeigler ZR: Successful treatment of T-g lymphoproliferative disease with human recombinant granulocyte colony stimulating factor. *Am J Hematol* 40:66, 1992
  7. Wolf-Peeters C de, Achten R. ?? T-cell lymphomas: a homogeneous entity? *Histopa hology* 36:294, 2000
  8. Langerak AW, Wolvers-Tettero ILM, van den Beemd MWM, van Wering ER, Ludwig W-D, Hahlen K, Necker A, van Dongen JJM Immunophenotypic and immunogenotypic characteristics of TCR??+ T-cell acute lymphoblastic leukemia. *Leukemia* 13:206, 1999
  9. Matutes E, Brito-Babapulle V, Swansbury J, Ellis J, Morilla R, Dearden C, Sepere A, Catovsky D Clinical and laboratory features of 78 cases of T-prolymphocytic leukemia. *Blood* 78:3269, 1991
  10. Sugimoto T, Imoto S, Matsuo Y, Kojima K, Yasukawa M, Murayama T, Kohfuku J, Mizuno I, Yakushijin K, Sada A, Nishimura R, Koizumi T. T-cell receptor ?? T-cell leukemia with the morphology of T-cell prolymphocytic leukemia and a postthymic immunophenotype. *Ann Hematol* 80:749, 2001
  11. Deutsch M, Tsopanou E, Dourakis SP. The autoimmune lymphoproliferative syndrome (Canale-Smith) in adulthood. *Clin Rheumatol.* 23:43, 2004
  12. Bleesing JJ, Brown MR, Straus SE, Dale JK, Siegel RM, Johnson M, Lenardo MJ, Puck JM, Fleisher TA. Immunophenotypic profiles in families with autoimmune lymphoproliferative syndrome. *Blood* 98:2466, 2001
  13. Sun T, Cohen NS, Marino J, Koduru P, Cuomo J, Henshall J. CD3+, CD4-, CD8- large granular T-cell lymphoproliferative disorder. *Am. J. Hematol.* 1991;37:173-178
  14. Scott CS, Richards SJ, Sivakumatan M, Steed AJ, Norfolk DR, Milligan CW, Short M. Persistent clonal expansions of CD3+ TCR gamma delta+ and CD3+TCRalpha beta+ CD4- CD8- lymphocytes associated with neutropenia. *Leukemia Lymphoma* 1994;14:429-440
  15. Saito T, Togitani K, Murakami J, Watanabe T, Tanosaki R, Kobayashi Y, Matsuno Y, Tobinai K. Granular lymphocytic leukemia derived from gd T-cell expressing cytotoxic molecules. *Leukemia Research* 2001;25:259-261
  16. Makishima H, Ishida F, Saito H, Ichikawa N, Ozaki Y, Ito S, Ota M, Katsuyama Y, Kiyosawa K. Lymphoproliferative disease of granular lymphocytes with T-cell receptor gamma delta-positive phenotype : restricted usage of T-cell receptor gamma and delta subunit genes. *Eur J Haematol* 2003;70:212-218.