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Cell size variations of large granular lymphocyte leukemia: Implication of a small cell subtype of granular lymphocyte leukemia with STAT3 mutations

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ABSTRACT

Large granular lymphocyte leukemia (LGL-L) has been morphologically defined as a group of lymphoproliferative disorders, including T-cell large granular lymphocytic leukemia (T-LGL-L), chronic lymphoproliferative disorders of NK cells (CLPD-NK) and aggressive NK cell leukemia. We investigated the morphological features of LGL leukemic cells in 26 LGL-L patients in order to elucidate relationships with current classifications and molecular backgrounds. LGL-L cells were mostly indistinguishable from normal LGL. Patients with STAT3 SH2 domain mutations showed significantly smaller cells compared with patients without STAT3 mutations. Four patients with T-LGL-L showed smaller granular lymphocytes with a median diameter of less than 13 μm, which were rarely seen in normal subjects. This small subtype of T-LGL-L was recognized among rather young patients and was associated with D661Y mutations in the STAT3 gene SH2 domain. In addition, all of them showed anemia including two cases with pure red cell aplasia. These results suggest the heterogeneity of T-LGL-L and a specific subtype with small variants of T-LGL-L.

1. Introduction

Normal large granular lymphocytes (LGL) have certain morphological characteristics, including larger size, low nuclear:cytoplasmic ratio, and azurophilic granules in the cytoplasm, among lymphocytes in the peripheral blood [1,2]. LGL lymphocytosis develops in various conditions, including those of a reactive or neoplastic nature [3]. Large granular lymphocyte leukemia (LGL-L) is a group of proliferative disorders of cytotoxic T cells or NK cells frequently complicated with cytopenia and autoimmune phenomena [4,5]. In the current WHO classification, T-cell large granular lymphocytic leukemia (T-LGL-L) and chronic lymphoproliferative disorders of NK cells (CLPD-NK) are included in the indolent types [6,7] and aggressive NK cell leukemia (ANKL) in the aggressive types [8]. The clinical and epidemiological characteristics of LGL-L differ among ethnic groups [9,10], together with ANKL occurring almost exclusively among East Asian populations [11]. Recent identification of activating mutations in Signal transducer and transactivation (STAT) 3 in T-LGL-and CLPD-NK and their association with clinical characteristics gave further insights into the pathophysiology of indolent LGL-L [12,13]. Mutations in several other genes and outside the SH2 domain of STAT3 gene have been also identified [14–17]. Among these mutations, mutations in STAT3 SH2 domain were most frequent.

One of the hallmark features of LGL leukemia is their morphology, which had been investigated in the earlier investigated era. However, integrated analyses with morphological, immunophenotypical and molecular findings on LGL leukemia are lacking, despite the advent of progress on epidemiological, cellular and molecular

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insights of LGL leukemia as well as the new WHO classification. In this study, we re-analyzed morphological features of LGL leukemic cells in the relation with these parameters.

2. Materials and methods

2.1. Patients

Patients diagnosed with LGL-L: T-LGL-L, CLPD-NK or ANKL, at Shinshu University Hospital, whose peripheral blood smears at initial presentation or before therapeutic interventions for LGL-L were available, were selected. The criteria for the diagnosis of each disease were based on the WHO classifications of 2008 [6–8]. T-LGL-L was diagnosed as follows: LGL morphology with typical surface phenotypes with CD2, CD3, T-cell receptor (TCR) αβ or γδ, CD8 and CD16/56, TCR γ-chain monoclonal rearrangement and an LGL count over 2000/μL. In some cases with LGL of less than 2000/μL, characteristic features other than the cell counts and persistent clinical features for more than 6 months were recognized using revised criteria. CLPD-NK was characterized by an LGL count over 700/μL with a phenotype of CD2 + CD3-CD56+/CD16 + TCR- for more than 6 months' duration. EBV was uniformly negative in cells with T-LGL-L and CLPD-NK. ANKL had the cellular characteristics of EBV-positive LGL with CD2 + CD3-CD56+/CD16 + TCR-, with the main involved sites being bone marrow, peripheral blood, liver and/or spleen with a diffuse pattern, as well as frequent associations with liver dysfunction, hemophagocytosis and a rapidly deteriorating clinical course. Since ANKL cells showed significant morphological differences from LGL to highly atypical types [11], cases with ANKL cells showing such pleomorphic morphology were excluded from this study and patients with LGL types were included. Normal subjects were recruited as healthy controls. The study protocol was approved by the Institutional Review Board of Shinshu University School of Medicine and performed in accordance with the Declaration of Helsinki.

2.2. Morphological evaluation of LGL

Peripheral blood smears with Wright-Giemsa staining were digitally imaged using NanoZoomer (Hamamatsu Photonics, Hamamatsu, Japan) and the long diameter of each lymphocyte was measured with image software, NDP.view 2 (Hamamatsu Photonics) consecutively, except for deformed cells. Cytoplasmic azurophilic granules were evaluated with a light microscope (Olympus, Tokyo, Japan) using a ×100 objective lens. Three azurophilic granules in the cytoplasm were minimally required for defining a granular lymphocyte [18,19].

2.3. Identification of activating mutations in SH2 domain in the STAT3 gene

STAT3 gene mutations were identified as previously reported using Sanger sequencing methods, allele-specific PCR and allele-specific quantitative PCR [13,20] in 24 patients except for a T-LGL-L and a CLPD-NK cases whose mononuclear cells were unavailable.

2.4. Statistical analysis

Comparison between groups was carried out using Student's *t*-test, Mann-Whitney *t*-test or chi-squared test as appropriate. These analyses were performed with EZR ver. 1.27 [21].

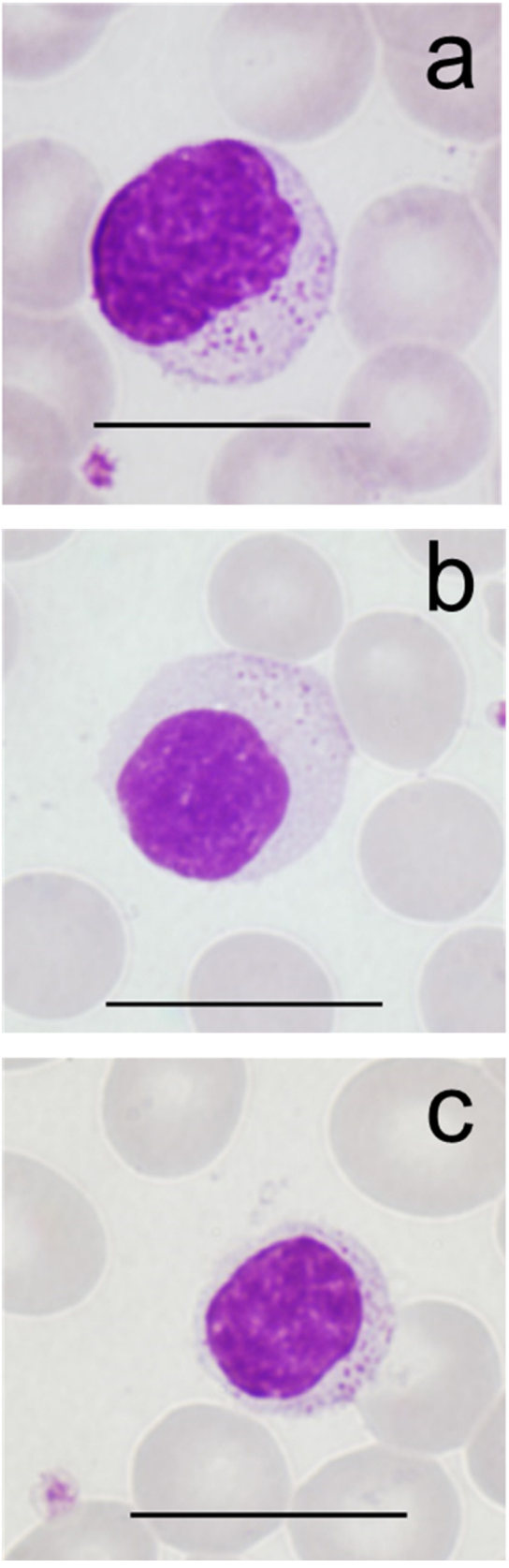

Fig. 1. Morphology of large granular lymphocytes (LGL).
a: Typical LGL in a normal subject, b: representative LGL in an LGL leukemic patient (UPN17) and c: "small" LGL-L cell (UPN8). Each scale bar indicates 15 μm.