T/NK Large Granular Lymphocyte Leukemia and Coexisting Monoclonal B-Cell Lymphocytosis-like Proliferations

An Unrecognized and Frequent Association

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Abstract

T-cell large granular lymphocyte leukemia (T-LGLL) is a T-cell lymphoproliferative disorder that has recently been associated with B-cell dyscrasias on a spectrum ranging from dysgammaglobulinemia to lymphoma. To investigate the relationship between clonal B-cell and LGLL lymphoproliferations, we systematically studied lymphocytes in 57 patients with T-LGLL or NK lymphocytosis using flow cytometric methods sensitive to low-level B-cell populations. We identified 16 patients (28%) with abnormal B-cell populations; 9 (16%) of the patients had no known history of a B-cell lymphoproliferative disorder. We characterized these abnormal B-cell populations as monoclonal B-cell lymphocytosis and report a high frequency of monoclonal B-cell lymphocytosis in T/NK LGLL. Our findings suggest that certain pathologic factors may operate in patients with T/NK LGLL to drive low-level clonal B-cell proliferations.

As sensitive flow cytometric techniques have become more commonly used in clinical practice, there has been an increase in the detection of subtle disease states. Patients can routinely be identified who have abnormal flow cytometric findings in the absence of clinically appreciable disease.1,2 Monoclonal B-cell lymphocytosis (MBL) has recently been recognized in first-degree relatives of patients with chronic lymphocytic leukemia (CLL) and also in healthy people and healthy blood donors.3 The importance of MBL identification lies in the established increased risk of progression to CLL for CLL-phenotype MBL.1 Detailed criteria for diagnosis have been suggested for MBL.4 These include detection of a monotypic B-cell population in the peripheral blood with an overall κ/λ ratio of more than 3 or less than 0.3; greater than 25% of B cells lacking or expressing low-level surface immunoglobulin; or a disease-specific immunophenotype. Exclusion criteria include lymphadenopathy and organomegaly; associated autoimmune or infectious disease; a B-cell count of more than 5 × 10⁹/L; and any other feature diagnostic of a B-lymphoproliferative disorder, except the presence of a paraprotein.

Recently, Viny et al5 described B-cell dyscrasias in patients with T-cell large granular lymphocyte leukemia (T-LGLL), including CLL, monoclonal gammopathy of undetermined significance (MGUS), polyclonal hypergammaglobulinemia, or hypogammaglobulinemia. We applied a high-sensitivity flow cytometric technique using a CD19 live gate to maximize sensitivity and establish MBL as a frequent occurrence in patients with T-LGLL and chronic natural killer (NK)-cell lymphocytosis.
Materials and Methods

Cases of T-LGLL or NK lymphocytosis (referred to herein as T/NK LGLL) treated at Cleveland Clinic, Cleveland, OH, between April 28, 2000, and June 5, 2009, were retrospectively identified. A subset of the patients was previously described in a clinical study that did not address flow cytometric findings.6 The diagnosis of T-LGLL was established by using modified criteria by Semenzato et al.7 In addition to large granular lymphocyte (LGL) count, aberrant flow cytometric LGL population, and T-cell receptor (TCR)-γ rearrangement, the presence of an expanded clonal CD3 population by Vβ flow cytometry was added. Briefly, the presence of 3 of the 4 following criteria was consistent with a diagnosis of T-LGLL: (1) peripheral LGL count of more than 2,000/μL, (2) the presence of an expanded T-lymphocyte population by flow cytometry (most typically CD3+, CD16+, CD57+), (3) the presence of monoclonal TCR gene rearrangement shown by polymerase chain reaction, and (4) abnormal skewing of the TCR Vβ family (see below). For NK lymphocytosis, persistent expanded NK cell population, increased LGLs and cytopenias were required.8,9

Flow cytometric immunophenotyping was performed at the Cleveland Clinic as part of the diagnostic workup on a FACSCalibur (before July 2007) or a FACS Canto instrument (after July 2007; BD Biosciences, San Jose, CA). Each of these flow cytometric evaluations had been performed using a standard battery of fluorescently labeled monoclonal antibodies, including CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD23, CD16/CD56, CD45, CD57, and surface immunoglobulin (sIg) on a 4- or 6-color flow cytometer. Staining protocols were standard lyse/wash protocols as previously described.5,10 All antibodies were directly conjugated to fluororescent isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein complex (PerCP), PerCP-cyanine-5.5 (PerCP-Cy5.5), PE-cyanine 7 (Cy7), allophycocyanin (APC), or APC-Cy7 (all from BD Biosciences). Control experiments were performed using an autofluorescence tube on each case, with daily control experiments performed for specific antibodies.

LGLs were identified by their expression of CD3, CD4, CD8, CD16/CD56, and/or CD57 in a CD45-side scatter lymphocyte gate. Vβ flow cytometry was performed as previously described.6 B cells were identified through their expression of CD19, bright CD45, and appropriate side-scatter properties. The percentage of lymphocytes expressing B-cell markers was identified, along with the percentage of B cells coexpressing CD5, CD10, CD23, and sIg.

Two antibody combinations were primarily used in each case. One tube containing CD45-PerCP or PE-Cy7, CD19-APC, CD5-PE, and CD23-FITC was used, as was one tube containing CD45-PerCP or PE-Cy7, CD19-APC, κ-FITC, and λ-PE. The tube containing anti-immunoglobulin light chains was acquired by using a CD19+ “live gate” to maximize analytic sensitivity, and this was used to assess relative κ and λ expression characteristics. At least 20,000 CD19+ events or, if fewer B cells were present, the entire sample (250 μL of whole blood) was acquired for this CD19 live-gated tube. Retrospectively, forward-scatter, side-scatter, CD20 (in 6-color flow cytometric cases), and CD45 characteristics and Boolean gating were used to ensure the analyzed population was B cells. Briefly, CD19 bright/low side-scatter primary live gate data were viewed using a CD45/low-side-scatter (or CD20 in 6-color analysis) plot. A CD45/low side-scatter gate was set, and those data were then further successively viewed in a forward-side-scatter gate to exclude nonlymphocyte events. These data were used for κ/λ analysis. A population was defined as a cluster of more than 50 events. Data were analyzed by using FCSExpress, version 3.0 (De Novo, Los Angeles, CA).

Results

We retrospectively identified 57 cases of T/NK LGLL with flow cytometric data. The mean age was 63.4 years, and 21 patients were female (37%). The mean absolute lymphocyte count (ALC) for all patients was 4,373/μL (4.373 × 109/L), with 21 patients (37%) having an ALC of more than 5,000 (5.0 × 109/L). The mean absolute B-lymphocyte count (BLC) for all patients was 0.131 × 109/L, and no patients had a BLC of more than 5.0 × 109/L. In 6 patients (11%), there was a history of rheumatoid arthritis, and 3 had other autoimmune diseases (Crohn disease, myositis, and autoimmune hepatitis). In 4 patients (7%), lymphadenopathy was present, and 24 patients (42%) had splenomegaly, as would be expected in patients with T/NK LGLL.

The number of B cells on which the κ/λ analysis was based ranged from 72 to 19,602, with a median of 913 B cells analyzed. Of 57 patients, 16 (28%) were retrospectively identified as having a monotypic B-cell population as defined by a κ/λ ratio of more than 3 or less than 0.3 or a significant (>25%) population of slg– B cells. The findings were subtle given the small number of B cells present in these cases and were most easily found when CD19+ live gaging was used to increase the number of events available for review. In 7 patients (12%), the κ/λ ratio was more than 3 or less than 0.3, and 10 (18%) had a population of CD19+ cells with a slg– phenotype constituting more than 25% of all B cells. CD5 was coexpressed in 4 patients, including 3 with previously diagnosed CLL. Examples of cases are shown in Image I.

In all, 16 patients (28%) were found to have an MBL-like B-cell population. Retrospective chart review demonstrated a history (before documentation of the MBL-like population) of treated CLL in 2, previously treated plasma cell myeloma in 1, Waldenström macroglobulinemia in 1,
and IgM \( \lambda \) MGUS in 1. Two patients were found to have \( \gamma \)-heavy chain disease (HCD) by serum immunofixation during subsequent clinical workup (within 1 month of flow cytometric evaluation). One of these patients was later found to have marrow involvement by a low-grade B-cell lymphoproliferative disorder (2 months after demonstration of the MBL population), and the other patient had evidence of disease by serum immunofixation only. In both of these patients, a substantial proportion of B cells in peripheral blood lacked sIg by flow cytometry.

Because of the association of rheumatoid arthritis with T-LGLL (present in 6 patients overall; 2 with abnormal B-cell flow cytometric results and 4 without), we looked for an association between rheumatoid arthritis and the presence of an MBL-like proliferation but found none (\( P = 1.0; \) Fisher exact test). Similarly, the use of methotrexate (associated with iatrogenic B-cell lymphomas) was not associated with the presence of an MBL-like proliferation (4 patients in the group with abnormal B-cell flow cytometric findings and 9 of the remaining patients, \( P = 1.0; \) Fisher exact test).

A summary of the data for all 16 patients is given in Table 2. Age, sex, presence of lymphadenopathy or splenomegaly, methotrexate treatment, WBC count, ALC count, and BLC count did not correlate with finding an abnormal B-cell population (Table 1). Analysis of TCR \( V_\beta \) expression by flow cytometry had been performed in 13 of these 16 cases (Table 2). Although \( V_\beta \) family restriction was identified in all cases of T-LGLL, there was no family that was preferentially expressed.

### Discussion

MBL has been reported to occur in approximately 5% of non–hematology/oncology outpatients between the ages of 60 and 80 years with normal blood counts and no history of cancer and in 13% to 18% of patients with first-degree relatives with CLL. Because of the recent association of B-cell dyscrasias with T/NK LGLL, we undertook this study to more carefully examine the flow cytometric findings of B cells in T/NK LGLL.

Of 57 cases of T/NK LGLL in our study, 16 (28%) showed MBL-like B-cell populations. Although some patients had associated B-cell malignancy that was known or found during the workup for T/NK LGLL (eg, CLL, \( \gamma \)-HCD, or myeloma), many patients had no such association. These latter B-cell populations seemed similar to the recently described phenomenon of MBL. The definition of MBL is evolving, but current recommendations would exclude patients with splenomegaly, lymphadenopathy, autoimmune disease, a specific phenotype, or evidence of B-cell lymphoproliferative disorder other than paraprotein. In the patient population for this study, we would omit the presence of splenomegaly and autoimmune disease as exclusionary criteria because they are common manifestations in patients with T-LGLL. In this context, 9 (16%) of 57 patients with T/NK LGLL were found to have a bona fide MBL.

The clinical significance of MBL in patients with T/NK LGLL is unknown. We were unable to find any association between clinical or basic hematologic features and the presence of MBL. Analysis of TCR \( V_\beta \) expression by flow cytometry had been performed in 13 cases (Table 2). No preference for \( V_\beta \) family use was seen. Whether there are implications in response to therapy or survival is also unknown and will require further follow-up.

Of the 16 cases with abnormal B-cell populations, 8 had a concurrent paraprotein. When comparing surface immunoglobulin restriction on B cells with the paraprotein, we found analogous light chain restriction in 6 cases, but findings were discordant in 2 cases (cases 13 and 14). Case 14 had a history of \( \kappa \)-restricted CLL but lost surface immunoglobulin...
expression after therapy. Case 13 had an entirely different pattern of light chain restriction between paraprotein and MBL. This suggests that the MBL and source of the paraprotein are unrelated in some cases.

Cases 10 and 15 had MBL-like populations and \( \gamma \)-heavy chain only on immunofixation, a feature of \( \gamma \)-HCD. This latter association is intriguing owing to the rarity of this HCD. To our knowledge, these are the first reported cases of T/NK LGLL with \( \gamma \)-HCD. One case had low-level IgG heavy chain paraprotein without histologic evidence of B-cell leukemia/lymphoma and could also be considered as having features of an IgG heavy chain MGUS. Such cases have been rarely reported but seem to have a good prognosis compared with HCD. It is interesting that in both cases of \( \gamma \)-HCD, there...
were substantial sIg– B-cell populations. In γ-HCD, the B cells produce immunoglobulin heavy chains that do not associate with light chains due to an alteration in the C\(_{H1}\) region of the heavy chain.\(^1\) The finding of sIg– populations in patients with γ-HCD seems consistent with this lack of light chain association.

The underlying biologic mechanisms that allow for development of concurrent T/NK LGLL and MBL are also unknown; however, several intriguing possibilities exist. First, it is possible that the clonal T/NK LGLL disease in our patients is a direct reaction to the aberrant B-cell populations, not unlike the proposed role of other stimuli such as viral infection, allograft, and self-antigens.\(^8\) Because only 9 patients had underlying rheumatoid arthritis or other autoimmune disease, the recognition of MBL in patients with T/NK LGLL may provide another source of antigenic stimulation that provides a route to eventual development of an LGL proliferation. Second, because antigenic drive in patients with CLL or marginal zone lymphomas has been described, it is possible that these MBL-like populations are driven or dependent on the T/NK LGLL.\(^15,16\) Third, it is possible that both T/NK LGLL and MBL are independent clonal reactions to as yet undefined chronic antigenic stimulation.

A prior report has established concurrent B-cell dyscrasia in patients with T/NK LGLL.\(^5\) We extend the spectrum of B-cell disorders to include MBL, as well as HCD and multiple myeloma. The incidence of MBL proliferations (16%) seems much higher than in the general population and may reflect altered immune status in patients with T/NK LGLL.

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References


