Hematopathology / T-LGL Leukemia After Allogeneic BMT for B-Cell Lymphoma

T-Cell Large Granular Lymphocytic Leukemia of Donor Origin Occurring After Allogeneic Bone Marrow Transplantation for B-Cell Lymphoproliferative Disorders

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Abstract

T-cell lymphoproliferative disorders are uncommon occurrences after bone marrow transplantation (BMT). We describe 2 patients in whom a monoclonal T-cell large granular lymphocytosis (T-LGL) developed after allogeneic BMT for B-cell lymphoproliferative disorders. Both patients showed a persistent expansion of CD3+, CD8+, and CD57+ large granular lymphocytes of donor origin with clonally rearranged T-cell receptor \( \gamma \) genes and no evidence of Epstein-Barr virus–related infection. The manifestations were consistent with T-LGL leukemia as defined by the World Health Organization criteria. In both patients, graft-vs-host disease developed, and 1 had recurrent episodes of cytomegalovirus viremia. The other patient had received a graft from a hepatitis C antibody–positive donor without developing any signs of hepatitis C infection. Both patients remain in complete remission from their B-cell lymphoproliferative disorders and do not have symptoms related to T-LGL leukemia. These data show that T-LGL leukemia should be included as one of the types of posttransplantation lymphoproliferative disorders that can occur after allogeneic BMT for B-cell neoplasms.

Large granular lymphocytes (LGLs) are morphologically distinct but immunophenotypically heterogeneous lymphocytes of activated CD3+ cytotoxic T cells (T-LGLs) or CD3–natural killer cells that mediate non–major histocompatibility complex–restricted cytotoxicity. CD3+CD8+ T-LGLs normally constitute fewer than 5% of peripheral blood lymphocytes. They may increase in response to viral infections, autoimmune diseases, or underlying malignant neoplasms.1 The increase of LGLs on a reactive basis usually is asymptomatic, transient, and polyclonal.

The neoplastic clonal proliferation of T-LGLs is categorized as T-cell large granular lymphocytic leukemia in the World Health Organization classification.2 This entity is rare and characterized by persistent lymphocytosis (>6 months) with a typical immunophenotype (CD3+CD8+CD56+CD57+) and T-cell receptor (TCR) gene rearrangement. The clinical course usually is indolent, but rapidly progressing cases have been reported.3,4

A polyclonal or oligoclonal expansion of T-LGLs after allogeneic bone marrow transplantation (BMT) has been described, and a relationship with viral infections was proposed in some cases.3,6 Because of their nonclonal nature, these T-LGLs were nonprogressive and self-limiting; they might be associated with a lower rate of disease relapse after BMT.5,6 We report 2 cases of clonal T-LGL of donor origin after allogeneic BMT for B-cell lymphomas.

Materials and Methods

Case Histories

Patient 1

A 47-year-old man was given a diagnosis of B-cell chronic lymphocytic leukemia (CLL) in December 2001, had a partial...
response to chemotherapy, and underwent an allogeneic BMT from his HLA-matched, cytomegalovirus (CMV)-negative brother in June 2003. The patient and donor had negative results for Epstein-Barr virus (EBV); however, the donor was positive for hepatitis C antibody. In contrast, the recipient was negative for hepatitis C antibody at transplantation and during his last follow-up. A skin rash, lymphocytosis, and mild eosinophilia developed within 1 month after transplantation and were consistent with graft-vs-host disease (GVHD). Five months after transplantation, he had lymphocytosis characterized by LGLs. Flow cytometric studies confirmed an immunophenotype of CD3+/CD8+/CD57+. The diagnosis of a clonal T-LGL population was confirmed by TCRγ gene rearrangement. A bone marrow sample was negative for CLL but had an interstitial and focal infiltrate of T-LGL cells. The patient had persistent T-cell large granular lymphocytosis but remained free of CLL at 6 months of follow-up.

**Patient 2**

A 57-year-old man was given a diagnosis of Waldenström macroglobulinemia (WM) in July 1999. He responded initially to treatment with cladribine, but warm autoimmune hemolytic anemia developed, and he was treated with prednisone and splenectomy. He underwent a fully HLA-matched allogeneic BMT from his CMV-seropositive brother in May 2002. His posttransplantation course was complicated by GVHD and recurrent episodes of CMV viremia treated with steroids and ganciclovir, respectively. Four months after transplantation, mild lymphocytosis developed. A bone marrow sample was negative for WM, although the IgM level at that time was slightly elevated to 291 mg/dL (2.91 g/L). The bone marrow revealed a lymphocytic infiltrate of T-cell origin by immunohistochemical analysis. The lymphocytosis persisted, and 1 year later, flow cytometric analysis showed a population of CD3+/CD8+/CD57+ cells consistent with T-LGL. The diagnosis was confirmed by the presence of a clonal TCRγ gene rearrangement by polymerase chain reaction (PCR). The patient remained in complete remission from WM at 18 months of follow-up.

**Morphologic and Immunophenotypic Analyses**

Bone marrow aspirates and trephine biopsy specimens were examined morphologically, and immunophenotyping was performed with a panel of monoclonal antibodies on B–5–fixed, paraffin-embedded sections. For flow cytometric analysis, a 100-µL aliquot of cell suspension was added to an appropriate monoclonal antibody combination. A whole-blood lysis method using Optilyse C Lysing Solution (Immunotech, Marseille, France) was used as a fixative to eliminate RBCs. The phenotype of lymphocytes was determined by using 3-color direct immunofluorescence and the Beckman/Coulter FC-500 (Coulter, Miami, FL). Lymphocytes were gated using a forward vs side-scatter histogram. The following antibodies were used in relevant combinations and conjugated with fluorescein isothiocyanate, phycoerythrin, or phycoerythrin–cyanin 5: CD3, CD4, CD5, CD2, CD7, CD8, CD16, CD56, CD57, CD25, TCRαβ, TCRγδ, CD19, CD20, CD23, FMC7 (Coulter), and κ and λ light chains (DAKO, Carpinteria, CA).

**Molecular Studies**

DNA was extracted from the peripheral blood and bone marrow samples from the 2 patients and assessed for the presence of TCRγ gene rearrangements by PCR using 2 pairs of Vγ and Jγ primers. PCR analysis of B-cell clonality was performed with primers to the framework region III and joining region of the immunoglobulin heavy chain (IgH) gene essentially as described by Aubin et al. The presence of EBV was assessed by PCR using primers specific for the BanW1 region of the virus. Appropriate positive, negative, and internal control samples were run with each specimen. In this study, PCR analysis for TCRγ rearrangements also was performed on the peripheral blood mononuclear cells from the respective donors for the 2 cases. The donor-recipient chimerism study was performed by quantitative PCR with fluorochrome-labeled primers for polymorphic microsatellite markers containing short tandem repeat sequences.

**Results**

**Morphologic and Immunophenotyping Findings**

The clinicopathologic features are summarized in Table 1 for both patients. Serial peripheral blood samples demonstrated persistent lymphocytosis of medium-sized lymphoid cells with round nuclei, coarse chromatin, inconspicuous nucleoli, and azurophilic cytoplasmic granules. Immunophenotyping by flow cytometry confirmed that these cells were CD2+, CD3+, CD7+, CD8+, CD57+, TCRαβ+, TCRγδ–, and CD56–, consistent with T-LGLs. No clonal B-cell populations were detected. Immunohistologic examination of serial bone marrow biopsy specimens from both patients showed multiple lymphoid aggregates in the intertrabecular spaces that were CD3+, CD57+, and CD20–. The remainder of the bone marrow showed a good hematopoietic reserve.

**Molecular Analysis**

In a serial follow-up after BMT, PCR for the TCRγ gene showed a distinct clonal band in specimens from both patients. A clonal B-cell population was not detected by PCR. Samples from both patients were negative for EBV by PCR. Chimerism studies showed 100% donor cell engraftment for patient 1 and 99% donor cell engraftment for patient 2.
Discussion

We report a post–allogeneic BMT lymphoproliferative disorder of large granular T lymphocytes in 2 patients with B-cell lymphoproliferative disorders. The persistent lymphocytosis, morphologic appearance, immunophenotype, and clonal rearrangement of the TCRγ gene are consistent with the WHO criteria for T-LGL leukemia. Both cases had T-LGL of donor origin as determined by chimerism studies.

There are several reports of nonclonal T-LGL expansions after allogeneic BMT, usually in response to CMV infections.5,6 A donor-derived T-LGL leukemia recently was reported after allogeneic BMT for chronic myeloid leukemia in the accelerated phase.12 The T-LGL leukemia arose 3 months after BMT, and progressive pancytopenia gradually developed that led to death 7 months later. In contrast, neither of our patients has developed pancytopenia and both remain in remission from their B-cell lymphoproliferative disorders and nonsymptomatic at 6 and 18 months, respectively, after the diagnosis of T-LGL leukemia. Long-term follow-up is required to address the outcome for these 2 patients.

The pathogenesis of post–allogeneic BMT T-LGL leukemia is unclear. Nonclonal T-LGL lymphocytosis following BMT may be related to chronic antigenic stimulation due to GVHD or viral infection. One may speculate that additional genetic changes in these expanded T-LGLs then might lead to T-LGL leukemia. In our cases, both patients had GVHD, and one of them had recurrent CMV infections after BMT. The other patient received a graft from a hepatitis C antibody–positive donor. The recipient has remained negative for hepatitis C antibody. It is conceivable that the clonal lymphocytosis of donor origin resulted from the previous hepatitis C

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
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<tbody>
<tr>
<td>Age (y)/sex</td>
<td>47/M</td>
</tr>
<tr>
<td>Initial diagnosis</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Graft-vs-host disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Cytomegalovirus viremia</td>
<td>No</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>Negative</td>
</tr>
<tr>
<td>T-LGL after bone marrow transplantation (mo)</td>
<td>5</td>
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<tr>
<td>CBC count at diagnosis of T-LGL</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dL (g/L)</td>
<td>11.7 (117)</td>
</tr>
<tr>
<td>WBC count, /µL (× 10⁹/L)</td>
<td>9,200 (9.2)</td>
</tr>
<tr>
<td>Platelet count, × 10¹²/µL (× 10⁹/L)</td>
<td>202 (202)</td>
</tr>
<tr>
<td>Large granular lymphocytes, × 10⁹/L</td>
<td>2.5</td>
</tr>
<tr>
<td>Time since T-LGL diagnosis (mo)</td>
<td>6</td>
</tr>
<tr>
<td>Outcome</td>
<td>Complete remission for chronic lymphocytic leukemia</td>
</tr>
</tbody>
</table>

T-LGL, T-cell large granular lymphocytosis.

Image II Peripheral blood smear showing medium-sized abnormal lymphocytes with condensed nuclear chromatin, abundant cytoplasm, and fine to coarse granules (Wright, ×1,000).

Image III Multiparameter flow cytometric analysis of peripheral blood showing that the abnormal lymphoid cells were CD3+, CD8+, and CD57+. FITC, fluorescein isothiocyanate; PC5, PE–cyanin 5; PE, phycoerythrin.
infection in the donor. EBV DNA was not detected in either of our cases; this is similar to the case of Wing et al\textsuperscript{12} and consistent with the view that unlike natural killer–type LGL,\textsuperscript{13} EBV infection might not have a role in T-LGL leukemia after bone marrow or organ transplantations.\textsuperscript{14} It is possible that a combination of chronic antigenic stimulation related to GVHD or viral infections and immunosuppression with decreased immunosurveillance led to clonal formation of a donor-derived T-cell population.

The donor origin of the T-LGLs raised the question whether neoplastic T-LGL clones were transferred from donors to recipients. The rare occurrence of passage of tumor cells from donors to recipients has been described after solid organ transplantation,\textsuperscript{15,16} and even healthy donors with normal-appearing CBC and differential counts can harbor malignant neoplasms.\textsuperscript{17} In our cases, this is unlikely because both donors were healthy with normal blood counts, and their bone marrow samples showed normal hematopoiesis with no evidence of a lymphocytic infiltrate. Furthermore, PCR for peripheral mononuclear cells in both donors was negative for TCR\γ rearrangements (data not shown).

Our study illustrates that T-LGL leukemia can occur as a posttransplantation lymphoproliferative disorder in patients who have undergone transplantation for B-cell lymphoma. This complication should be included in the differential diagnosis of an unexplained lymphocytosis after BMT. Chimerism studies to determine the origin of neoplasia developing after allogeneic BMT are important to assist in the management of these patients. Malignant neoplasms of recipient but not donor origin might be controllable by enhancing the putative graft-vs-malignancy effect through the reduction of immunosuppression, use of cytokines such as interleukin-2, or donor leukocyte infusions.

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References


