Large Granular Lymphocyte Expansion after Allogeneic Hematopoietic Stem Cell Transplant Is Associated with a Cytomegalovirus Reactivation and Shows an Indolent Outcome

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Expansions of CD3+ large granular lymphocytes (LGLs) after allogeneic hematopoietic stem cell transplantation (HSCT) have been described. We sought to evaluate incidence, characteristics, and clinical significance of persistent T cell (T-)LGL after HSCT. Fourteen of 215 recipients (7%) were diagnosed with LGL expansions. Thirteen showed a CD3+/CD8+ immunophenotype, 5 of them with clonal TCR-γ rearrangement. The lymphocytes appeared at a median of 16 months (range, 3-58 months) after HSCT and lasted for a median time of 31 months (range, 2-179 months). Cytomegalovirus (CMV) reactivation ($P = .001$) and acute graft-versus-host disease (aGVHD) were associated with LGL expansion ($P = .02$). In the multivariate analysis, only CMV reactivation showed a significant association with T-LGL expansion (relative risk [RR]: 5.063; 95% confidence interval [CI]: 1.586-16.160; $P = .006$). The observed posttransplantation LGL expansions, even if monoclonal, showed a chronic, indolent course. Our data indicate that such expansions may be considered as an expression of chronic stimulation, triggered by CMV reactivation rather than a malignant transformation.

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INTRODUCTION

Large granular lymphocytes (LGLs) are immunophenotypically heterogeneous lymphocytes. Immunophenotypically, they can be either CD3-negative natural killer cells (NK-LGL) or activated CD3-positive cytotoxic T cells (T-LGL), the latter usually accounting for less than 5% of all lymphocytes in a normal peripheral blood [1]. Reactive expansions of T-LGL can occur within different situations such as autoimmune diseases, underlying malignant neoplasms [2], viral infections [3], especially cytomegalovirus (CMV) infection [4,5], or after therapy with tyrosine kinase inhibitors in patients with chronic myeloid leukemia [6]. Reactive T-LGL expansions are usually polyclonal, transient, and asymptomatic. Neoplastic clonal proliferation of T-LGL is classified as T cell large granular lymphocytic leukemia in the World Health Organization classification [7,8]. T-LGL leukemia is characterized by a persistent increase of the number of peripheral blood LGL, usually between 2 to 20 $\times$ 10^9/L without a clearly identified cause [8]. However, it is now recognized that a lower count (range, 0.4-2 $\times$ 10^9/L) may be compatible with the diagnosis [9]. After allogeneic hematopoietic stem cell transplant (HSCT), T-LGL expansions have been described [10,11]; however, data about characteristics and clinical outcome of patients with LGL populations after allogeneic HSCT are still sparse. Therefore, we aimed to evaluate frequency, clinical presentation, laboratory features, outcome, and risk factors of patients with a persistent T-LGL population after allogeneic HSCT.

METHODS

This was a single-center, cross-sectional study performed at the Hematology Division of the University of Basel, Switzerland. After allogeneic HSCT, all recipients were regularly controlled at 1, 3, 6, and 12 months, and thereafter at yearly intervals or more often when indicated. These consultations included assessment of medical history, physical examination, and laboratory tests, such as complete blood count including morphological analysis of peripheral blood cells, lymphocyte subpopulations, peripheral blood chimerism, and blood chemistry. Bone marrow

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(BM) investigation was performed regularly at 3 and 6 months, at 1, 2, and 5 years, and every 5 years thereafter. During the first 90 days after HSCT, additional monitoring of immunosuppression and CMV replication using pp65 antigenemia or real-time PCR was done as described in a previous study [12].

In this study, all postallogeneic HSCT recipients who came to regular controls between January 1, 2008, and December 31, 2009, were consecutively included. On control day, in accordance with our standards, previous blood counts were reviewed: if patients showed persistent lymphocytes (>3 G/L for more than 3 months) and abnormal CD4/CD8 ratios in the lymphocyte subpopulations, defined as CD4/CD8 <1.0 or >1.5, and extensive immunophenotyping of the peripheral blood cells was assessed. Flow cytometry analyses were performed using the flow cytometry system FACSCalibur (BD Biosciences, San Jose, CA). The immunophenotyping of the lymphocytes included the following antibody panel: CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD25, CD30, CD56, CD57, HLA-DR, TCRαβ, and TCRγδ. T-LGL expansion was defined as an abnormal T cell population type CD3+, CD8+, or CD4+, with expression of at least 1 of the NK markers (CD16, CD57, or CD56), and with presence of LGLs in peripheral blood films [13]. In all cases with an abnormal expansion of T-LGL cells, a TCR gene rearrangement from peripheral blood or BM was performed (4 color multiplex PCR assay and automated high-resolution fragment analysis on ABI 310 Genetic Analyzer; Applied Biosystems, Foster City, CA).

We compared patients with T-LGL expansion to the cohort of patients without LGL expansion, using the chi-square test for categorical data and the Mann-Whitney *U* test for continuous variables. Variables included into the analysis were age, sex, diagnosis (malignant versus nonmalignant diseases), conditioning regimen (myeloablative versus reduced intensity conditioning), total body irradiation (TBI), type of donor, graft source, acute graft-versus-host disease (aGVHD) or chronic GVHD (cGVHD), remission status at control, and CMV reactivation. To identify independent prognostic risk factors, a multivariate stepwise linear regression analysis was performed. Differences between the results of comparative tests were considered significant if the 2-sided *P* value was <.05. Statistical analysis was performed using SPSS statistical software (SPSS for Windows, Release 17, SPSS, Inc., Chicago, IL).

Patients provided a written informed consent to have their data on disease, treatment, and outcome including late complications reported in an anonymous way to the registry. Clinical surveillance of HSCT recipients was approved by local institutional review boards. Patient characteristics, HSCT conditioning regimens, and clinical outcome data were collected prospectively and stored in the local institution database registry.

RESULTS AND DISCUSSION

Within 215 post-HSCT evaluated patients, 14 (7%) had an LGL expansion. Patients' characteristics with and without LGL expansion are summarized in Table 1. Primary disease, remission status, and HSCT characteristics of patients with LGL expansion are detailed in Table 2. The median time between HSCT and the beginning of lymphocytes, and between HSCT to study time was 16 months (range, 3-58 months) and 56 months (range, 3-159 months), respectively. The median lymphocyte count was 4.24 G/L (range, 3.0-26.5 G/L), and the median count of the T-LGL population was 2.10 G/L (range, 1.25-11.52 G/L) (Table 3). The median duration of the lymphocytes was 31 months (range, 2-179 months). The immunophenotyping in all these patients showed a T-LGL proliferation. In 13 of 14 patients, the following phenotype was found: CD3+, CD8+, CD4-, CD2+, CD5+, CD7+, and TCRαβ+. From these 13 patients, 7 expressed CD16+ CD57+; 5 expressed CD57+; and 1 expressed CD16+, CD56+ and, CD57+. In 1 of the 14 patients, the population was CD3+, CD4+, CD8-, additionally expressing CD2+, CD5+, CD7+, CD56+, CD57+, and TCRαβ+. The TCR-γ gene rearrangement examination was performed in all 14 patients (from peripheral blood in 8 of 14 patients [57%]; and from BM in 6 of 14 patients [43%]). In 5 patients, clonality was found (1 patient showed biclonality). Detailed descriptions of patients' characteristics with LGL expansion are summarized in Table 3.

Neutrophil counts were normal in all patients with LGL expansion. Eight of 14 patients (57%) showed a mild hyporegenerative anemia. Thrombocytopenia was present in 2 of 14 patients (14%) (1 associated with graft rejection, and 1 associated with CMV infection). Five of 14 recipients showed elevated antinuclear antibodies, 4 of them with a slight increase and 1 with a moderate increase. One patient had elevated rheumatoid factors, 2 patients had mild hypergammaglobulinemia, 3 patients had minimal M-gradient, and 1 patient had a mild positive polyspecific antiglobulin test for IgG without clinical evidence of hemolysis, as well as a monoclonal IgG λ of 7 g/L.

At the time of diagnosis of LGL expansion, 1 patient had persistent disease (primary myelofibrosis) and the chimerism was recipient type. This patient never engrafted after umbilical cord blood transplantation. The other 13 patients had a complete hematological remission with 100% donor type chimerism in peripheral blood. One of these 13 patients had a BCR-ABL1 molecular relapse of a B lymphoblastic leukemia at study time. Three patients were