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Low Counts of B Cells, Natural Killer Cells, Monocytes, Dendritic Cells, Basophils, and Eosinophils are Associated with Postengraftment Infections after Allogeneic Hematopoietic Cell Transplantation

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A B S T R A C T

Hematopoietic cell transplant (HCT) recipients are immunocompromised and thus predisposed to infections. We set out to determine the deficiency of which immune cell subset(s) may predispose to postengraftment infections. We determined day 28, 56, 84, and 180 blood counts of multiple immune cell subsets in 219 allogeneic transplant recipients conditioned with busulfan, fludarabine, and Thymoglobulin. Deficiency of a subset was considered to be associated with infections if the low subset count was significantly associated with subsequent high infection rate per multivariate analysis in both discovery and validation cohorts. Low counts of monocytes (total and inflammatory) and basophils, and low IgA levels were associated with viral infections. Low plasmacytoid dendritic cell (PDC) counts were associated with bacterial infections. Low inflammatory monocyte counts were associated with fungal infections. Low counts of total and naive B cells, total and $CD56^{high}$ natural killer (NK) cells, total and inflammatory monocytes, myeloid dendritic cells (MDCs), PDCs, basophils and eosinophils, and low levels of IgA were associated with any infections (due to any pathogen or presumed). In conclusion, deficiencies of B cells, NK cells, monocytes, MDCs, PDCs, basophils, eosinophils, and/or IgA plasma cells appear to predispose to postengraftment infections.

INTRODUCTION

Hematologic malignancies and benign disorders of hematopoiesis are often cured with allogeneic hematopoietic cell transplantation (HCT). However, HCT recipients are immunocompromised because of multiple factors such as a relatively small number of immune cells transferred with the graft, limited differentiation of the grafted hematopoietic stem cells into some immune cell subsets like T cells, and pharmacologic immunosuppression. Therefore, HCT recipients are at a substantial risk of infections, causing morbidity and mortality. This immunocompromised state lasts for at least 1 year [1-5]. Known predictive factors for high infection rates include an unrelated or HLA mismatched related donor (compared with a matched sib donor), cord blood or marrow graft (compared to mobilized blood stem cell graft), T cell–depleted graft, and the presence of significant graft-versus-host disease (GVHD) [5-7]. However, these known predictive factors cannot reliably distinguish between patients who will or will not develop infections. Thus, the discovery of new predictive markers is of interest.

Low count of an immune cell subset could be a useful marker for subsequent infections. More important, association between a low immune cell subset count and subsequent infections may help us understand the mechanism by which patients are rendered susceptible to infections. In addition, if it was known which immune cell subset deficiency predisposes to infections, the risk of infections

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could theoretically be minimized by the infusion of that subset from the donor, except if a high count of the subset could predispose to GVHD (eg, T cells) [8]. Whereas neutropenia appears to be the cardinal predisposing factor for early (pre-engraftment) infections, little is known about the deficiency of which immune cell subset(s) predisposes to postengraftment infections [9,10]. This knowledge is completely lacking for the setting of in vivo T cell depletion with polyclonal rabbit antithymocyte globulin, which has been increasingly used to prevent GVHD [11-15]. We set out to determine, in the setting of antithymocyte globulin–conditioned HCT, the low counts of which immune cell subsets on days 28, 56, 84, or 180 are associated with high rates of subsequent infections.

METHODS

Patients and Transplantation

Heparinized blood was collected from 231 patients who had undergone first allogeneic marrow or mobilized blood stem cell transplantation in Calgary between December 1, 2004 and August 31, 2010 and agreed to participate in this Research Ethics Board–approved study. Twelve patients were excluded because of graft failure, death, or relapse before day 30. The remaining 219 patients were divided into a discovery cohort (transplant date between December 1, 2004 and August 31, 2008) and a validation cohort (transplant date between September 1, 2008 and August 31, 2010) (Table 1).

Patients received myeloablative conditioning, typically fludarabine (250 mg/m^2 i.v.), busulfan (approximately 12.8 mg/kg i.v.), and antithymocyte globulin (4.5 mg/kg i.v.), and additional GVHD prophylaxis with methotrexate (days 1, 3, 6, and 11) and cyclosporine from day −1 until 3 to 6 months post-transplant or longer in the case of chronic GVHD [16]. Conditioning of some patients included total body irradiation (TBI) (4 Gy) [17].

Clinically significant GVHD (grades II to IV acute GVHD or chronic GVHD needing systemic therapy) was treated with a corticosteroid ± other immunosuppressive drug(s). Supportive care was similar for all patients. No antibacterial or antifungal prophylaxis was given routinely (except for trimethoprim-sulfamethoxazole for *Pneumocystis* prophylaxis). *Pneumocystis* prophylaxis was given until 6 months post-transplant or longer (in the case of chronic GVHD needing systemic therapy). Acyclovir was used until 6 to 12 months post-transplant or longer (in the case of chronic GVHD needing systemic therapy).

All blood products were from cytomegalovirus- (CMV) seronegative donors and were leukocyte depleted. CMV DNAemia was monitored; preemptive therapy was used [18]. Monitoring of Epstein-Barr virus DNAemia was not done, neither was monitoring for any other virus apart from CMV. Follow-up for infections ended at the time of death, graft failure, relapse, second malignancy, last contact, or day 730, whichever occurred first. Median follow-up for infections was 730 days (range, 34 to 730 days).

Immune Cell Subset Enumeration

The blood for enumeration of immune cell subsets was drawn on approximately days 28, 56, 84, and 180 after HCT (Table 1). The counts of most subsets were determined by flow cytometry as described [8] and defined as shown in Supplementary Table 1. Total WBC, eosinophil, and neutrophil counts were determined by a clinical hematology laboratory (Calgary Laboratory Services, Calgary, Alberta, Canada or other accredited laboratory). As a surrogate of total body plasma cell count and function, IgM, IgG, and IgA serum levels were measured by a clinical laboratory on days 84 and 180 post-transplant (typically using automated immunoturbidimetry analyzer, Integra 800; Roche Diagnostics, Mannheim, Germany). Patients who received IgG supplementation within 2 months before a time point were excluded from analysis of the association between IgG level on that time point and subsequent infections. The median counts of the cell subsets at the studied time points have been published [19] and for convenience are also given in Table 2.

Definitions of Infections

Definite infection was defined as an illness with symptoms and signs consistent with an infection and microbiologic documentation of a pathogen. For zoster virus, clinical diagnosis was considered sufficient. Microbiologic documentation included isolation of the pathogen by culture from a sterile site or a nonsterile site (if from a nonsterile site, the organism had to be clinically judged as pathogenic) or histologic/immunohistologic evidence. Culture-documented bacteremia or fungemia was counted even in the absence of symptoms or signs of infection, unless the organism was clinically judged as contaminant. CMV reactivation was counted as a viral infection only if treated (preemptively or because of presumed or documented CMV disease) [18]. Post-transplant lymphoproliferative disorder was counted as a viral infection and was defined as an illness with signs or imaging results consistent with post-transplant lymphoproliferative disorder (eg, fever not due to other causes, lymphadenopathy, splenomegaly, or a mass) with Epstein-Barr virus DNA above 400 copies/μg leukocyte DNA or in situ hybridization/immunohistologic evidence of post-transplant lymphoproliferative disorder.

Presumed (clinical) infection was defined as illness with symptoms and signs consistent with an infection and no microbiologic documentation of a pathogen. However, presumed oral, gastrointestinal, conjunctival, and respiratory tract infections were discounted because they could not be reliably distinguished from GVHD or allergy. Fever without other symptoms/signs was also discounted because it could not be reliably attributed to an infection. Hemorrhagic cystitis was discounted because it could not be differentiated from conditioning regimen–induced cystitis. Sinusitis and pneumonia were counted only if radiologically documented.

A recurrent infection was counted as multiple infections if the episodes were separated by more than a 4-week asymptomatic period. A chronic infection (with asymptomatic periods lasting ≤4 weeks) was counted as 1 infection. A polymicrobial infection of 1 organ or several adjacent organs was counted as 1 infection. An infection in ≥2 nonadjacent organs because of the same microorganism was counted as 1 infection (disseminated).

Severe infection was defined as infection resulting in hospitalization. For infections occurring during hospitalization, severe infection was defined as infection that would result in hospitalization if the patient was an outpatient.

Infection rate was defined as the number of infections per 365 days at risk within a post-transplant time interval. Infection rates were assessed for any infections (definite or presumed), any severe infections (severe definite or severe presumed), severe definite infections, viral infections, bacterial infections, and fungal infections.

STATISTICS

Spearman rank correlation was used to evaluate correlations between the counts of each immune cell subset at predefined time points and the rate of infections in subsequent time intervals (eg, for day 84, intervals of days 84 to 179, 84 to 364, and 84 to 730 were considered; Supplementary Figure 2). For each immune cell subset for which the count appeared to be significantly associated with an infection rate in a subsequent time interval ($P < .05$), a multivariate analysis (MVA) was performed as Poisson regression, comparing infection rates in patients with high versus low subset counts. Cutoff between high and low counts of an immune cell subset was determined using receiver-operator characteristic curves as the point with the highest sum of specificity and sensitivity for developing at least 1 infection in the interval. In cases where the point of the highest sum of specificity and sensitivity fell outside of the 10th to 90th percentile, the point of the highest sum of specificity and sensitivity within the 10th to 90th percentile was used. The MVA was done to adjust for factors that could predispose to infections (other than the cell subset count). The covariates we included were significant GVHD (grades II to IV acute GVHD or chronic GVHD needing systemic therapy) in that interval (yes or no), donor type (HLA-matched sibling versus other), and stem cell source (marrow versus blood stem cells).

In the Poisson regression, days at risk were used as the offset. In the MVA, $P < .01$ was considered significant. This was an arbitrary correction for multiple comparisons; the correction was far less strict than Bonferroni correction because we assumed that counts of the various immune cell subsets were partly related to each other. For every association significant in the discovery cohort, the association was analyzed by MVA in the validation cohort. To be considered validated, the association must have been significant at $P < .05$ in the validation cohort. Additionally, the median percentile of the rate of infections in the low subset count group had to be equal or higher than that in the high subset