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Allogeneic Hematopoietic Stem Cell Transplantation Recipients Have Defects of Both Switched and IgM Memory B Cells

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Allogeneic hematopoietic stem cell transplant (HSCT) recipients were assessed to elucidate memory B cell defects underlying their increased susceptibility to infections, particularly by encapsulated bacteria. Circulating IgM memory B cells ($CD19^+$, $CD27^+$, IgM^+) and switched memory B cells ($CD19^+$, $CD27^+$, IgM^-) were enumerated in allogeneic HSCT recipients (n = 37) and healthy controls (n = 35). T lymphocyte subpopulations and serum levels of immunoglobulins, including IgG subclasses, and antibodies to pneumococcal polysaccharides were also assayed. Allogeneic HSCT recipients were deficient in both switched memory and IgM memory B cells compared to healthy controls (both $P < .0001$), irrespective of time post-HSCT. Switched memory B cell deficiency correlated with $CD4^+$ T cell deficiency, and both correlated with serum levels of IgG1 ($P < .0001$), possibly reflecting impaired B cell isotype switching in germinal centres. "Steady-state" serum levels of antibodies to pneumococcal polysaccharides did not correlate with circulating memory B cells. Graft-versus-host disease (GVHD) was associated with lower IgM memory B cell counts and lower serum levels of IgG2, IgG4, IgA, and pneumococcal antibodies. The increased susceptibility of allogeneic HSCT patients to infection may reflect a combination of memory B cell defects, which are most common in patients with a history of GVHD.

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INTRODUCTION

Individuals who have previously received a Hematopoietic stem cell transplant (HSCT) have an increased risk of acquiring infections by bacteria, particularly encapsulated bacteria. This is associated with a deficiency of circulating memory B lymphocytes (B cells) and serum immunoglobulins and impaired production of antibodies to polysaccharide antigens that may persist for many years. Affected individuals can therefore be considered to have an acquired antibody deficiency disorder [1-3]. Further elucidation of the abnormalities of B cell numbers and function underlying this disorder may result in improved diagnostic methods and treatments.

Streptococcus pneumoniae (pneumococcus) is the most frequent pathogen in allogeneic HSCT patients, and may cause severe and sometimes fatal invasive infections months or years following transplantation, with an incidence of up to 27% in long-term survivors and frequent recurrences [4-6]. Pneumococcal infections following allogeneic HSCT often occur relatively late and are 3 times more frequent than in autologous HSCT recipients [7]. Risk factors for invasive pneumococcal disease postallogeneic HSCT include graft-versus-host disease (GVHD), splenectomy, and the use of antithymocyte globulin (ATG) or total-body irradiation (TBI) for conditioning [2,4,6,7]. However, the immunologic defects associated with pneumococcal

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disease in these patients have not been completely defined.

Antibody responses to capsular polysaccharide antigens are the most impaired. For example, Nordoy et al. [8] found that only a minority of patients responded to vaccination with pneumococcal polysaccharides even 4-10 years post-HSCT, despite all patients maintaining "protective" serum levels of tetanus toxoid antibody [8]. Others have shown that "protective" levels of antibodies against pneumococcal antigens decrease during the first year after transplantation, regardless of serum immunoglobulin levels [3]. The opsonophagocytic activity of antibodies to pneumoccoal polysaccharides is also impaired [9].

In addition to pneumococci, other pathogens also cause disease with increased frequency or severity in HSCT recipients [10]. This has been associated with immune defects that include low serum levels of immunoglobulins, particularly IgG2, IgG4, and IgA, impaired antibody responses and hyposplenism, which are well-documented acquired immune defects in adult and pediatric allogeneic HSCT recipients [1,3,6,8,11,12].

Early studies revealed that the majority of circulating B cells 1 year after HSCT are naïve IgD^+ B cells [13], and that such B cells lack somatic mutations in the immunoglobulin heavy chain variable region, indicating a maturational arrest [14]. Human naïve B cells exported from the bone marrow, have a $CD19^+CD27^-IgM^{low}IgD^+$ immunophenotype, and pass through a transitional B cell immunophenotype ($CD38^+$) prior to becoming $CD27^+$ memory B cells [15]. The CD27 molecule has been recognized as a cell-surface marker that identifies somatically mutated memory B cells, which are critical for the production of protective antibody responses [16-18]. The human memory B cell compartment ($CD19^+$, $CD27^+$) contains 2 subpopulations; (1) IgM memory B cells (IgM^+), which possess a prediversified IgM antigen receptor and are capable of responding immediately to the antigens of encapsulated bacteria in a T cell-independent fashion [17-19]; and (2) switched memory B cells (IgM^-), which require T cell costimulation to produce high affinity IgG and other isotypes of antibody within germinal centres of lymphoid tissue [20]. Abnormal T cell-dependent B cell responses after allogeneic-HSCT might reflect impaired germinal center formation and B cell isotype switching [21], although information is incomplete.

HSCT has been associated with a deficiency of $CD27^+$ memory B cells, particularly in recipients with GVHD [22,23], although it has been recently shown that memory B cells transferred by allogeneic bone marrow transplantation can contribute to the antibody repertoire of the recipient [24]. Deficiency of IgM memory B cells in asplenic and aged individuals has been associated with an increased susceptibility to invasive pneumococcal disease [17]. Many other studies have demonstrated that abnormalities of circulating memory B cell numbers also exist in a variety of other disorders [25-28].

The aim of this study was to further characterize the nature of the memory B cell deficiency in allogeneic HSCT recipients. In particular, we sought to determine if such individuals have a deficiency of IgM or switched memory B cells that might account for their increased susceptibility to invasive pneumococcal disease, and to determine if serum levels of IgM, IgA, total IgG, and IgG subclasses and "steady-state" IgG and IgG2 antibodies to pneumococcal polysaccharides are related to circulating memory B cell numbers. We also investigated the relationship between numbers of circulating memory B cells and the presence of GVHD, previous use of ATG and TBI, time since transplantation, and the current circulating T cell counts.

MATERIAL AND METHODS

Patients and Controls

We undertook a cross-sectional study of 37 patients who had previously received an allogeneic HSCT and 35 healthy controls. Informed consent was obtained from all patients and controls. Allogeneic HSCT recipients were at least 6 months posttransplantation and in remission at the time of assessment. Patient information collected included time since transplantation, type of allogeneic transplantation (sibling or matched unrelated), previous autologous transplantation, history of acute and/or chronic GVHD (aGVHD, cGVHD), previous ATG or TBI, and splenectomy. All subjects were >18 years of age. Demographic characteristics of patients and controls are given in Table 1. Laboratory assessments were performed on a single occasion between July 2005 and August 2007.

Analysis of Memory B Cells by Flow Cytometry

Leukocytes were enriched from a 10-mL peripheral blood sample anticoagulated with ethylenediaminetetraacetic acid (EDTA) by collecting a phosphate-buffered saline (PBS)-washed buffy coat and resuspending it in approximately 1 mL of PBS Flow Buffer containing 2% fetal calf serum (FCS). The following antibodies coupled with fluoroscein isothiocyanate (FITC), R-phycoerythrin (PE), or the tandem dye R-phycoeythrin_cyanin 5.1 (PC5) were used for flow cytometry; anti-CD19-PC5 (clone J4.119 Immunotech, Marseille, France), anti-CD27-PE (clone IA4-CD27 Immunotech), anti-IgM-FITC (polyclonal fab′2 Dako, Glostrup, Denmark), isotype control-PE (clone 679.1Mc7 Immunotech), isotype control-FITC (fab′2 Dako). A total of 100 μL of diluted