BIOLOGY

Lack of Antidonor Alloantibody Does Not Indicate Lack of Immune Sensitization: Studies of Graft Loss in a Haploidentical Hematopoietic Cell Transplantation Swine Model

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Loss of chimerism is an undesirable outcome of allogeneic hematopoietic cell transplantation (HCT) after reduced-intensity conditioning. Understanding the nature of cellular and humoral immune responses to HCT after graft loss could lead to improved retransplantation strategies. We investigated the immunologic responses after graft loss in miniature swine recipients of haploidentical HCT that received reduced-intensity conditioning. After the loss of peripheral blood chimerism, antidonor cellular responses were present without detectable antidonor antibody. Reexposure to donor hematopoietic cells after graft loss induced a sensitized antidonor cellular response. No induced antidonor antibody response could be detected despite evidence of cellular sensitization to donor cells. In contrast, unconditioned animals exposed repeatedly to similar doses of haploidentical donor cells developed antidonor antibody responses. These results could have important implications for the design of treatment strategies to overcome antidonor responses in HCT and improve the outcome of retransplantation after graft loss.

Biol Blood Marrow Transplant 18: 1629-1637 (2012) © *2012 American Society for Blood and Marrow Transplantation*

KEY WORD: Hematopoietic cell transplantation

INTRODUCTION

Reduced-intensity conditioning (RIC) regimens have facilitated hematopoietic cell transplantation (HCT) in patients who previously would not have been considered suitable candidates, owing to the toxicity of myeloablative preparatory regimens [1]. Although the risk of complications related to conditioning is decreased with RIC, the risk of graft loss is increased. Graft loss, whether secondary to rejection, the inability of the stem cells to engraft due to the lack of "space" [2], or poor graft quality [3], can have different but important immunologic consequences in the host. Rejection of the donor graft implies an active immunologic process in which donor cells sensitize the host (through cellular and/or humoral mechanisms). Conversely, if the loss of donor cells is not immunologic but rather related to a deficiency in stem cell "fitness" or quality [3], there may be no immunologic consequences (eg, sensitization). Factors related to graft loss include donor–recipient MHC mismatch, degree of host myeloablation, level of immunosuppression post-HCT, degree of host immunocompetence related to immediate preparatory regimens, level of T cell depletion of the donor graft, and presensitization to donor antigens, as is seen in patients with aplastic anemia [4-6]. To date, few clinical studies have assessed immune responses of patients after graft loss and reexposure of donor antigen.

In this study, we investigated the immunologic responses after graft loss in the Massachusetts General Hospital MHC-defined miniature swine, a clinically relevant large-animal model of HCT [7]. Recipients underwent RIC and received cytokine-mobilized peripheral blood mononuclear cells (PBMCs) haplomismatched at both MHC I and MHC II. The RIC regimen consisted of CD3 immunotoxin, 100 cGy of total body irradiation (TBI), and 45 days of cyclosporine A (referred to as the "ITC" regimen hereinafter).

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Financial disclosure: See Acknowledgments on page 1636.

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Received March 22, 2012; accepted August 2, 2012

1083-8791/$36.00
http://dx.doi.org/10.1016/j.bbmt.2012.08.004

Seventeen ITC-conditioned haplo-HCT recipients were engrafted with donor stem cells and maintained moderate to high (30%-70%) donor-derived chimerism in all hematopoietic lineages [8,9]. Four ITC-conditioned haplo-HCT recipients that did not engraft and lost peripheral blood chimerism are discussed in this report. Cellular and humoral antidonor MHC responses were studied before and after reexposure to donor antigen. Antidonor immune responses were compared in HCT recipients and naïve animals exposed to donor antigen. Our findings provide insight into the immunologic responses after graft loss and may serve as a guide for modifying preparatory regimens when subsequent retransplantation of immune-sensitized hosts is considered.

MATERIALS AND METHODS

Animals

Animals were selected from our herd of MGH MHC-defined miniature swine [10,11]. The donors ranged in age from 4 to 8 months, and the recipients were age 9-12 weeks and weighed 9-13 kg at the time of HCT. Donor and recipients were chosen to differ by 1 MHC haplotype at both MHC I and MHC II. Recipients expressed swine leukocyte antigen (SLA)^{ad}, and donors expressed SLA^{ac}, hereinafter referred to as AD and AC, respectively. To facilitate the detection of chimerism, the donors were positive for pig allelic antigen (PAA), a nonhistocompatibility cell surface antigen present on all differentiated hematopoietic cells in animals that express this gene allele [12]. The recipients were PAA-negative.

HCT Protocol

The HCT protocol consisted of a combination of irradiation, T cell depletion, and hematopoietic cell infusion from a single-haplotype MHC-mismatched donor, with 45 days of cyclosporine cover for the peri-infusion/postinfusion period. Serum cyclosporine (CyA) levels were maintained between 400 and 800 ng/uL. T cell depletion was achieved using pCD3 immunotoxin (CD3-IT) [13] for 4 days before HCT. This recombinant CD3 immunotoxin selectively binds porcine CD3 and contains a diphtheria toxin subunit, which results in protein synthesis inhibition and depletion of CD3$^+$ cells [14]. Animal 18862 did not receive CD3-IT.

Donor animals were cytokine-mobilized for 5-7 days with recombinant porcine IL-3 and porcine stem cell factor (Immerge Biotherapeutics, Cambridge, MA), each at a dose of 0.1 mg/kg for the first 30 kg of body weight and 0.05 mg/kg for each additional kg, as reported previously [8]. Peripheral blood mononuclear cells (PBMCs) were collected by leukapheresis (COBE BCT, Lakewood, CO) beginning on the 5th day of cytokine therapy and continuing until the target cell number was attained. After the initial leukapheresis, 5-12 $\times$ 10^9 PBMCs per kg (total of 5-12 $\times$ 10^{10} PBMCs, as animals averaged 10 kg in weight) were infused i.v. daily. Enteral CyA (Sandimmune) was administered via a gastrostomy tube starting at 1 day before mobilized PBMC infusion and continuing for 45 days. CyA whole blood levels were maintained at 400-800 ng/mL for the first 30 days before being tapered over the subsequent 15 days to 200 ng/mL, at which point CyA was discontinued.

Donor Antigen Exposure after HCT

Intravenous delivery of unselected PBMCs

Nonmobilized leukocytes were collected by leukapheresis from the original hematopoietic cell donor and then infused i.v. into the recipient at a normalized dose (CD3$^+$T cells) to include 5 $\times$ 10^7 donor T cells/kg of recipient body weight. Animals that underwent HCT received a donor leukocyte infusion (DLI) from the same HCT donor animal.

Subcutaneous PBMCs

Donor PBMCs were collected from donor whole blood. Eighty million donor PBMCs were washed with PBS and injected s.c. with a 19-gauge needle (in the inguinal region) with or without complete Freund's adjuvant. Cells were suspended in a total volume of 5 mL PBS before injection. The animals that underwent HCT were immunized with PBMCs from the same donor.

Skin grafting

A vascularized skin flap [9] was transplanted from the inguinal region of the donor and placed on the neck of the recipient. The skin graft was monitored daily for color, temperature, and texture change.

Assessment of Chimerism

PAA is expressed on hematopoietic cells from PAA$^+$ donor animals but not in PAA$^-$ recipients. Peripheral blood, bone marrow, and thymic donor chimerism were assessed by flow cytometry (FACScan; BD Biosciences, San Jose, CA), as described previously [8,12,15]. The following swine-specific antibodies were used: CD3ϵ (898H2-6-15; mouse IgGaK) [16], CD4 (74-12-4; mouse IgG2bK), CD8α (76-2-11; mouse IgG2aK), CD172 (74-22-15; mouse IgG1K) [17-20], CD5 [21], and PAA (1038H-10-9; IgMK), For assessment of chimerism, PAA staining was used to distinguish donor-origin and recipient-origin cells [20]. Monocyte and granulocyte chimerism was determined by gating on CD172$^+$ mononuclear cells and granulocytes, respectively.