Conditioning and Not Stem-Cell Source Determines the Kinetics of Graft-Versus-Host Disease Onset

The majority of HSCT recipients below 20 years receive BM as the stem-cell source whereas 75% of patients above 20 years of age receive mobilized blood (CIBMTR). The kinetics of GVHD incidence varies in patients receiving MAC and there is a tendency for patients receiving mobilized stem cells to have more chronic than acute GVHD as compared with patients receiving BM stem cells. In initial reports of delayed onset GVHD in RIC HSCT recipients (1) 85% of patients received G-CSF mobilized peripheral blood stem cells and 15% of patients received BM. Mohty et al. (2) investigated GVHD onset in a group of RIC HSCT recipients that had received BM-derived HSC and again GVHD onset was delayed. Experiments were therefore designed to determine whether the conditioning or the stem-cell source was responsible for the delayed onset GVHD seen in RIC HSCT recipients.

Mice

Female BALB/c (H-2^d) recipient mice were obtained from the Animal Resources Centre (WA, Australia). Breeding pairs of UBI-GFP/BL6 (H-2^b) mice were obtained from Dr. David Curtis and Prof. Alex Bobik (Baker Institute, VIC, Australia). UBI-GFP/BL6 mice express GFP under the control of the ubiquitin C promoter in all hematopoietic tissues and were used as HSC donors to enable evaluation of chimerism.

SUPPLEMENTAL FIGURE 1. Chimeric and histological confirmation of GVHD. (A) Mixed T-cell (CD4 and CD8) chimerism in splenocytes taken from RIC MHC matched, miHA mismatched HSCT recipients killed due to delayed onset GVHD. (B) Donor T-cell chimerism of RIC and MAC MHC matched, miHA mismatched HSCT recipients (n=3-6 mice per time point). Results from MHC mismatched HSCT recipients were directly comparable. (C) Goblet cell and crypt morphology in small and large intestine of (1) RIC UBI-GFP/BL6→BALB/c, (2) MAC UBI-GFP/BL6→BALB/c, (3) RIC UBI-GFP/BL6→BALB.B, and (4) MAC UBI-GFP/BL6→BALB.B mice killed for GVHD.
Mice were housed under specific pathogen-free conditions and allowed to acclimatize for one week before experimental work. The University of Queensland Animal Ethics Committee approved all animal work.

**Conditioning Regimens**

The RIC regimen consisted of 200 mg/kg per day of fludarabine (Schering AG, Berlin, Germany) injected IP on d-8 to d-4, 60 mg/kg per day of CY (Baxter Healthcare, Deerfield, IL) injected IP on d-3 to d-2, and 500 cGy of TBI on d-1. The MAC regimen consisted of 60 mg/kg per day of CY injected IP on d-3 to d-2 and 1000 cGy of TBI on d-1. Mice were irradiated with a dual-source $^{137}$Cs irradiator (Gammacell40).

**Granulocyte-Colony-Stimulating Factor Mobilization**

UBI-GFP/BL6 mice received 10 mg pegylated G-CSF/20 g mouse or saline (subcutaneous injection) on d-4 and d-2.

**Cell Preparation and Transplantation**

G-CSF mobilized and saline treated mice were anesthetized with isoflurane, and a cardiac puncture was performed; the blood was collected into 30 U/mL heparin and kept on ice. The mononuclear cells (MNC) fraction was collected after density centrifugation. Flow cytometric analysis was used to determine the T-cell (CD3) content of the BM (2.6%), non-mobilized spleen (30.2%), and G-CSF mobilized MNC (3.8%). G-CSF mobilized MNC (10$^7$/mouse) and nonmobilized spleen cell (10$^7$/mouse) suspensions were combined and injected IV into conditioned mice on d0.

**Graft-Versus-Host Disease Monitoring**

Mice were monitored for the onset and severity of GVHD using a scoring system modified from that of Hill et al. (25). Mice received a score from 0 to 2 for posture, activity, skin integrity, eye integrity, fur texture, and diarrhea. Zero indicates normal condition and a score of 2 indicates poor condition. Weight loss was also monitored and the maximum score given (>30% weight loss) was 2.5. Any animal that scored 2.0 for activity or diarrhea, 2.5 for weight loss, or achieved a cumulative score of 8.0 was considered to have severe GVHD and was killed, the BM and spleen collected for analysis.

**RESULTS**

We have developed murine models of RIC, which result in delayed onset GVHD. One of the concerns is that the delay in GVHD onset is caused by the stem-cell source rather than the conditioning. We designed experiments to compare GVHD onset in RIC or MAC mice that had received pegylated G-CSF mobilized blood to determine whether the stem-cell source results in delayed onset GVHD. Mice that received MAC and G-CSF mobilized MNC and nonmobilized spleen cells survived for 13.8 ± 3.1 days. Mice that received RIC and G-CSF mobilized MNC and nonmobilized spleen cells survived significantly longer (41 ± 12; P=0.05; supplemental Fig. 2). Delayed onset GVHD developed after RIC regardless of the HSC used.

**DISCUSSION**

Our murine HSCT results mirror the clinical observations with regard to later mean GVHD onset in MAC G-CSF HSCT recipients. The significant observation, however, is that there is a significant difference in GVHD onset between HSCT recipients that received mobilized blood stem cells after RIC or MAC. This clearly demonstrates that it is the type conditioning, and not the stem-cell source, that plays a major role in the timing of GVHD onset and validates the use of BM as the stem-cell source to determine why RIC is associated with delayed onset GVHD.

**REFERENCES**
