Interleukin 18 preserves a perforin-dependent graft-versus-leukemia effect after allogeneic bone marrow transplantation

Pavan Reddy, Takanori Teshima, Gerhard Hildebrandt, Ulrich Duffner, Yoshinobu Maeda, Kenneth R. Cooke, and James L. M. Ferrara

We have recently shown that early administration of interleukin 18 (IL-18) after bone marrow transplantation (BMT) attenuates acute graft-versus-host disease (GVHD) in a lethally irradiated parent into F1 (B6→B6D2F1) BMT model. In this study, we investigated whether IL-18 can maintain graft-versus-leukemia (GVL) effect in this context. B6D2F1 mice received transplants of T-cell-depleted (TCD) bone marrow (BM) and splenic T cells from either syngeneic (H2^b/d) or allogeneic B6 (H2^b) donors. Recipient mice were treated with recombinant murine IL-18 or the control diluent. Initial studies demonstrated that IL-18 treatment did not affect the proliferative responses or the cytolytic effector functions of T cells after BMT. In subsequent experiments, animals also received host-type P815 mastocytoma cells at the time of BMT. All syngeneic BM transplant recipients died from leukemia by day 18. The allogeneic BM transplant recipients effectively rejected their leukemia regardless of treatment and IL-18 significantly reduced GVHD-related mortality. Examination of the cytotoxic mechanisms with perforin-deficient donor T cells demonstrated that perforin is critical for the GVL effect. Taken together these data demonstrate that IL-18 can attenuate acute GVHD without impairing the in vitro cytolytic function or the in vivo GVL activity after allogeneic BMT.

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with the BM transplant inoculum. Survival was monitored daily. P815-induced leukemic death was defined by the occurrence of either macroscopic tumor nodules in liver or spleen or hindleg paralysis.\textsuperscript{15} GVHD death was defined by the absence of leukemia and the presence of clinical signs of GVHD.\textsuperscript{13,14} Animals surviving beyond day 50 after BMT were killed and the liver and spleen were harvested for flow cytometric evaluation (has sensitivity of 0.5\textsuperscript{15}).

**Fluorescence-activated cell sorting analysis**

Fluorescein isothiocyanate–conjugated monoclonal antibodies to mouse CD45.1\textsuperscript{1}, CD45.2\textsuperscript{1}, and H-2\textsuperscript{k,b} were purchased from Pharmingen (San Diego, CA). Cells were analyzed by 2-color flow cytometry on a FACScan cytometer (Becton Dickinson Immunocytochemistry Systems, San Jose, CA).\textsuperscript{13,15}

**Cell cultures**

Briefly, splenocytes were harvested from animals 14 days after transplantation and 3 spleens were combined from each group. Donor (CD45.1\textsuperscript{1}/CD3\textsuperscript{+}) T cells in the spleens were determined and normalized between groups. Donor T-cell engraftment was 94\% ± 4\% in the controls and 86\% ± 8\% in IL-18 recipients (P = NS) on day +14 after BMT. These cells were then plated in 96-well flat-bottomed plates (Falcon, Lincoln Park, NJ) at a concentration of 2 x 10\(^5\) T cells (CD45.1\textsuperscript{1}/CD3\textsuperscript{+})/well with 2 x 10\(^5\) irradiated (2000 rad) splenocytes harvested from naive B6D2F1 (alloge neic) or B6 (syngeneic) animals. At 48 hours, supernatants were collected for cytokine analysis and the cultures were pulsed with [\(^{3}H\)]-thymidine (1 \(\mu Ci\)/well; 0.037 MBq) and proliferation was determined 24 hours later on a 1205 Betaplate reader (Packard, Downers Grove, IL).

**Cytokine ELISAs**

Antibodies used in the interferon \(\gamma\) (IFN-\(\gamma\)) and IL-2 assays were purchased from Pharmingen. All assays were performed according to the manufacturer’s protocol in a 1:5 dilution. Plates were read at 450 nm using a microplate reader (Bio-Rad Labs, Hercules, CA). Recombinant murine IFN-\(\gamma\) and IL-2 (Pharmingen) were used as standards for enzyme-linked immunosorbent assays (ELISAs). Samples and standards were run in duplicate and the sensitivity of the assays was 0.063 U/mL for IFN, and less than 0.13 U/mL for IL-2.

**\(^{51}\)Cr release assays**

Briefly, splenocytes were removed from B6D2F1 recipients 14 days after BMT, and 3 spleens were combined from each group. Donor CD8\textsuperscript{+} cells in each group were determined and the counts were normalized. They were added at varying effector to target ratios and incubated for 4 hours with either allogeneic P815 (H-2\textsuperscript{b}) or syngeneic EL-4 (H-2\textsuperscript{k}) targets (2 x 10\(^6\) cells), labeled with 100 \(\mu Ci\) (3.7 MBq)\textsuperscript{51}Cr. \(^{51}\)Cr activity in supernatants was determined in an autogamma counter (Packard Instrument, Meriden, CT). The percentage of specific lysis was calculated as follows: 100 x (sample count – background count)/(maximal count – background count).\textsuperscript{15}

**Statistical analysis**

Survival curves were plotted using Kaplan-Meier estimates. The Mantel-Cox log-rank test was used to analyze survival data. Statistical significance was set at P < .05.

**Results and discussion**

We have previously demonstrated that administration of IL-18 early in the time course of allogeneic BMT attenuated early in vivo donor T-cell proliferation by enhancing activation induced Fas-mediated apoptosis and resulted in reduced GVHD.\textsuperscript{10} We now determined whether the effects of IL-18 on acute GVHD severity were also associated with a decrease in donor responses to host antigens after BMT. B6D2F1 mice received 13 Gy of TBI followed by infusion of BM and T cells from either allogeneic B6 Ly5.2 or syngeneic donors as described in “Study design.” Donor splenic T cells were harvested from allogeneic BM transplant recipients on day +14 and then restimulated in vitro with B6D2F1 stimulators in standard mixed lymphocyte reaction (MLR) cultures. As shown in Figure 1A, splenic T cells from both IL-18–treated or control allogeneic recipients displayed similar proliferative responses to host antigens. There was also no difference in secretion of either IFN-\(\gamma\) or IL-2 between T cells from IL-18–treated or control mice (Figure 1B,C). We next tested the cytotoxic capability of donor CD8\textsuperscript{+} T cells harvested from recipient spleens on day +14 against host type P815 (H-2\textsuperscript{b}) tumor targets, which was also equivalent between the 2 groups (Figure 1D). Taken together with our previous study,\textsuperscript{10} these data demonstrate that administration of IL-18 to BM transplant recipients attenuates donor T-cell proliferation by enhancing Fas-mediated increasing activation induced cell death early after BMT but does not alter donor T-cell responses to host antigens measured 2 weeks after BMT.

The preservation host-specific responses in donor cells suggested that GVL effects might be preserved. We next determined the ability of IL-18 treatment to promote leukemia-free survival after allogeneic BMT in a well-established mouse GVL model described in “Study design.”\textsuperscript{15,16} As expected all recipients of syngeneic BM transplants receiving P815 tumor cells died with evidence of massive hepatosplenomegaly. Although IL-18 by itself has been shown to possess antitumor effects,\textsuperscript{8,17} all IL-18–treated syngeneic mice that received P815 cells also died from leukemia by day 18, thus ruling out any direct antitumor effect of IL-18 in this system (Figure 2A). All allogeneic BM transplant recipients treated with control died by day 40. By contrast, 50\% of IL-18–treated allogeneic animals survived the entire observation period (P < .03). Clinical GVHD scores were also more severe in controls than in IL-18–treated animals (5.6 ± 0.7 versus 3.5 ± 0.4, P < .05) consistent with our previous observation.\textsuperscript{10} In each case, allogeneic recipients effectively rejected their leukemia with no evidence of tumor at autopsy. In additional experiments with a lower dose (1 x 10\(^6\)) of allogeneic T cells, 25\% of the control compared to 70\% of IL-18–treated recipients survived (P < .04) and had less clinical GVHD. No animals showed morphologic evidence of leukemia at the end of the observation period, demonstrating that IL-18 preserved GVL effect at both higher and lower T-cell doses. We tested for minimal residual leukemia by killing all the surviving animals on day 50 and analyzing peripheral blood and splenocytes

Figure 1. Donor T-cell cytokine and cytolytic functions after BMT. B6D2F1 animals were injected with control diluent (solid bar) or IL-18 (dotted bar) from day –2 to +2, received 13 Gy TBI, and received transplants of 5 x 10\(^5\) TCD BM and 2 x 10\(^6\) splenic T cells from B6 Ly5.2 (CD45.1\textsuperscript{+}) donor mice. Splenocytes from the recipients (n = 3/group) were harvested on day 14 after BMT, combined and normalized for donor T cells (CD45.1\textsuperscript{+} and CD3\textsuperscript{+}); and restimulated in quadruplicate with irradiated naive host (B6D2F1):splenocytes in MLR cultures. Supernatants were collected after 48 hours of culture and proliferation was determined by pulsing with [\(^{3}H\)]-thymidine (1 \(\mu Ci\)/well; 0.037 MBq) for an additional 20 hours. T-cell proliferation (A), IFN-\(\gamma\) secretion (B), and IL-2 production (C) were all similar (solid bar versus dotted bar, P = NS). Results from 1 of 3 similar experiments are shown. (D) Splenocytes harvested from allogeneic animals on day 14 after BMT were pooled (n = 3/group), and normalized for donor CD8\textsuperscript{+} cells and used in a \(^{51}\)Cr release assay. CTL activity against allogeneic P815 in control (A) and IL-18 (●) groups was similar; there was no significant lysis of syngeneic targets by either group (IL-18, □; control, □).
Figure 2. IL-18 retains a perforin-dependent GVL effect. (A) B6D2F1 mice were injected with IL-18 or diluent, given 13 Gy TBI, and received transplant of allogeneic or syngeneic BM and T cells as in Figure 1. All animals were also injected intravenously with 2000 P815 tumor cells on day 0. (Allogeneic: control treated, ○, n = 8 and IL-18 treated, □, n = 8 or syngeneic: control treated, ◦, n = 8 and IL-18 treated, □, n = 8 donors) *P < .03 for ○, allo IL-18 versus ○, allo control. Data from 1 of 2 similar experiments are shown. (B) Lethally irradiated (13 Gy) B6D2F1 mice received transplants of 5 × 10^6 TCD BM from wild-type B6 (H2b) and 2 × 10^6 splenic T cells from allogeneic pfp^-/ B6 (H2d) donors. Survival in control (○, n = 8) and IL-18-treated (□, n = 8) recipients of T cells from pfp^-/ donors was significantly different. *P < .01, ○ versus □. (C) B6D2F1 mice underwent transplantations as above and injected intravenously with 2000 P815 cells on day 0. All syngeneic (■, n = 10), allogeneic control (●, n = 10), allogeneic IL-18-treated pfp^-/ T cells (□, n = 10) and allogeneic IL-18-treated wild-type T cells (○, n = 10) survived. P = NS, ○ versus ■, and P < .04, ○ versus □.

References

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