Interleukin-11 Promotes T Cell Polarization and Prevents Acute Graft-Versus-Host Disease after Allogeneic Bone Marrow Transplantation

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Abstract

Administration of IL-11 prevented lethal graft-versus-host disease (GVHD) in a murine bone marrow transplant (BMT) model (B6 → B6D2F1) across MHC and minor H antigen barriers (survival at day 50: 90 vs 20%, $P < 0.001$). Surpisingly, IL-11 administration polarized the donor T cell cytokine responses to host antigen after BMT with a 50% reduction in IFNγ and IL-2 secretion and a 10-fold increase in IL-4. This polarization of T cell responses was associated with reduced IFNγ serum levels and decreased IL-12 production in mixed lymphocyte cultures (MLC). In addition, IL-11 prevented small bowel damage and reduced serum endotoxin levels by 80%. Treatment with IL-11 also reduced TNFα serum levels and suppressed TNFα secretion by macrophages to LPS stimulation in vitro. IL-11 thus decreased GVHD morbidity and mortality by three mechanisms: (*a*) polarization of donor T cells; (*b*) protection of the small bowel; and (*c*) suppression of inflammatory cytokines such as TNFα. We conclude that brief treatment with IL-11 may represent a novel strategy to prevent T cell–mediated inflammatory processes such as GVHD. (*J. Clin. Invest.* 1998. 102:115–123.) Key words: total body irradiation • gastrointestinal tract • endotoxin • inflammatory cytokines • T cells

Introduction

IL-11 is a member of the IL-6 cytokine family that is produced by a variety of tissues including the central nervous system, thymus, lung, bone, skin, and connective tissue and has pleotropic effects (1). IL-11 stimulates megakaryopoesis and accelerates neutrophil recovery after myelosuppressive therapy (2–4). IL-11 also possesses potent antiinflammatory effects by virtue of its ability to inhibit nuclear translocation of nuclear factor-κB (NF-κB)[1] (5, 6). Preclinical studies have demonstrated the efficacy of IL-11 in treating inflammatory disorders including radiation-induced lung damage (7), inflammatory bowel disease (8), and sepsis (9). In addition, IL-11 downregulates IL-12 production by macrophages (10), which suggests that IL-11 may also modulate T cell–mediated inflammation.

Acute GVHD is the most common complication of allogeneic BMT and offers a unique perspective from which to study the effects of IL-11 on both inflammatory and T cell effector functions in vivo. During acute GVHD, cytokine dysregulation occurs as a consequence of synergistic interactions between cells of both myeloid and lymphoid lineages (11). T cells present in the donor inoculum encounter alloantigen and, in the presence of IL-12, secrete the Th1 cytokines IFNγ and IL-2 (12). IFNγ primes monocytes and macrophages to secrete large quantities of inflammatory cytokines after stimulation by LPS (13). Together with NK and T cells, these cytokines mediate GVHD target organ damage (14).

Given its antiinflammatory properties, we investigated the use of IL-11 in a well-characterized mouse model of GVHD directed against MHC and minor histocompatibility antigens. Our results show that IL-11 reduces small bowel injury and systemic inflammatory cytokine levels, which results in dramatically improved survival. Unexpectedly, IL-11 also polarizes T cell responses toward type 2 cytokine secretion (e.g., increased IL-4) and suppresses host macrophage secretion of IL-12. These findings demonstrate that IL-11 modulates the cytokine cascade of GVHD at several steps and suggests that it may have important uses in bone marrow and solid organ transplantation.

Methods

Mice. Female C57BL/6 (B6, H-2^b, Ly-5.2^+) and B6D2F1 (H-$2^{b/d}$, Ly-5.2^+)(15) mice were purchased from the Jackson Laboratories (Bar Harbor, ME). B6 Ly-5^a (H-2^b, Ly-5.1^+) (15) mice were purchased from Frederick Cancer Research Facility (Frederick, MD). The age of mice used as bone marrow transplantation (BMT) recipients ranged between 11 and 15 wk. Mice were housed in sterilized microisolator cages and received filtered water and normal chow, or autoclaved hyper-chlorinated drinking water for the first two weeks after BMT.

Bone marrow transplantation. Mice were transplanted according to a standard protocol as has been described previously (16). Briefly, on day 0 mice received 1,300 cGy or 1,550 cGy total body irradiation (^{137}Cs source), split into two doses separated by 3 h to minimize gastrointestinal toxicity. 5×10^6 bone marrow cells and 0.5×10^6 or 2×10^6 nylon wool purified splenic donor T cells were resuspended in 0.25 ml of Leibovitz's L-15 media, (Gibco BRL, Gaithersburg, MD)

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1. *Abbreviations used in this paper:* APC, antigen-presenting cell; BMT, bone marrow transplantation; CTL, cytotoxic T lymphocyte; GVHD, graft-versus-host disease; GVL, graft-versus-leukemia; MLC, mixed lymphocyte culture; TBI, total body irradiation; Tc, T cytotoxic; Th, T helper.

and injected intravenously into recipients after 1,300 cGy or 1,550 cGy of total body irradiation (TBI), respectively. Studies of T cell function and IL-12 production (see Figs. 2 and 4) were performed after 1,300 cGy of TBI to ensure sufficient numbers of living control mice. In some experiments, Ly-5^a (H-2^b, Ly-5.1$^+$) animals were used as donors (see below). Survival was monitored daily, recipient's body weights and GVHD clinical score were measured weekly. Donor cell engraftment was determined by examining percentage of Ly-5.1$^+$ cells in peripheral blood at day 28 after transplantation.

IL-11 treatment. Recombinant human IL-11 was supplied by Genetics Institute (Cambridge, MA) and had a specific activity of 1.6–2.1 $\times$ 10^6 U/mg as determined by T10 proliferation assay (17). IL-11 was diluted in 0.1% BSA/PBS before injection. Mice were injected subcutaneously with IL-11 (250 μg/kg per dose) twice daily from day −2 to +14 after BMT. Mice from the control groups received injection of diluent only. In some experiments mice received IL-11 only until day +7 after BMT.

Assessment of GVHD. The degree of systemic GVHD was assessed by a scoring system which sums changes in five clinical parameters: weight loss, posture (hunching), activity, fur texture, and skin integrity (maximum index = 10) (18). Individual mice were ear tagged and graded weekly from 0 to 2 for each criterion without knowledge of treatment group.

FACS analysis. FITC-conjugated mAb to mouse Ly 5.1 and Ly 5.2 antigens, FITC-conjugated CD4 and phycoerythrin (PE)-conjugated CD8 and B220 were purchased from PharMingen (San Diego, CA). F4/80 antibody, specific for murine macrophages was purchased from Caltag Laboratories (San Francisco, CA). Cells were first incubated with mAb 2.4G2 for 15 min at 4°C, then with the relevant FITC or PE conjugated mAb for 30 min at 4°C. Finally, cells were washed twice with PBS/0.2% BSA, fixed with PBS/1% paraformaldehyde and analyzed by FACScan® (Becton Dickinson, San Jose, CA).

Cell cultures. All culture media, incubation conditions, and studies of TNFα secretion were as previously described (19). Splenocytes were removed from animals 14 d after transplant and three to six spleens combined from each group. These cells were then layered over Ficoll-paque (Pharmacia LKB Biotechnology, Piscataway, NJ) and centrifuged at 800 *g* for 15 min. Cells were then collected from the interface and washed twice before suspension in supplemented 10% FCS/RPMI-1640. The percentage of CD4$^+$ and CD8$^+$ cells in the spleen of the IL-11 and control-treated populations were within 10% of each other without appreciable differences in CD4:CD8 ratios. These cells were plated in 96-flat bottomed plates (Falcon Labware, Lincoln Park, NJ) at a concentration of 2 $\times$ 10^5 T cells (CD4$^+$ plus CD8$^+$)/well with 10^5 irradiated (2,000 Rad) peritoneal macrophages lavaged from naive B6D2F1 (allogeneic) or B6 (syngeneic) animals. At 40 h, cultures were pulsed with ^{3}[H]thymidine (1 μCi per well) and proliferation was determined 20 h later on a 1205 Betaplate reader (Wallac, Turku, Finland). In separation experiments, CD4$^+$ cells were positively selected from splenocyte populations using the miniMACS system (Miltenyi Biotech, Bergisch Gladbach, Germany). After selection, positive and negative fractions were FACS stained and each fraction had < 1% contamination of opposing CD4$^+$ or CD8$^+$ cells. CD4$^+$ or CD8$^+$ populations were then plated with allogeneic macrophages and analyzed as above. T cell number was similarly determined in splenocytes from animals after syngeneic BMT and splenocytes containing 5 $\times$ 10^4 T cells were stimulated with 2.5 μg/ml of Con A and analyzed as above.

Cytokine ELISAs. The antibodies used in the TNFα assays were purchased from Genzyme Corp. (Cambridge, MA). Antibodies used in the IFNγ, IL-2, IL-4, and IL-12 p40 assays were purchased from PharMingen (San Diego, CA). All assays were performed according to the manufacturer's protocol. Briefly, samples were diluted 1:3 to 1:24 and TNFα, IFNγ, IL-2, IL-4, and IL-12 p40 proteins were captured by the specific primary mAb, and detected by horseradish peroxidase (TNFα) or biotin-labeled (IFNγ, IL-2, IL-4, and IL-12 p40) secondary mAbs. The biotin-labeled assays were developed with strepavidin and substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD). Plates were read at 450 nm using a microplate reader (Bio-Rad Labs, Hercules, CA). Recombinant murine TNFα (Genzyme Corp.) and IFNγ, IL-2, IL-4, and IL-12 p40 (PharMingen) were used as standards for ELISA assays. Samples and standards were run in duplicate and the sensitivity of the assays was 16–20 pg/ml for TNFα and IL-12 p40, 0.063 U/ml for IFNγ, and < 0.13 U/ml for IL-2 and IL-4. Supernatants were collected after 4 h of culture for TNFα, 24 h for IL-2, and 48 h for IFNγ, IL-12 p40 and IL-4 analysis. For TNFα and IL-12 p40 production by macrophages, results were expressed as units per 10^5 F4/80 positive cells (units/fraction of F4/80+ peritoneal cells).

^{51}Cr release assays. 2 $\times$ 10^6 P815 (H-2^d) or EL4 (H-2^b) tumor targets were labeled with 100 μCi of ^{51}Cr for 2 h. After washing three times, labeled targets were plated at 10^4 cells per well in U bottom plates (Costar, Cambridge, MA). Splenocytes from allogeneic BMT recipients (prepared as described above) were added to quadruplicate wells at varying effector to target ratios and incubated for 5 h. Maximal and background release was determined by the addition of Triton-X (Sigma Chemical Co., St Louis, MO) or media alone to targets, respectively. ^{51}Cr activity in supernatants taken 5 h later were determined in an auto-gamma counter (Cobra, Meriden, CT) and lysis was expressed as a percentage of maximum.

Serum LPS estimation. For determination of endotoxin concentration in serum, the Limulus Amebocyte Lysate (LAL) assay (Bio Whittaker, Walkersville, MD) was performed according to the manufacturer's protocol. Briefly, serum samples were collected and analyzed using pyrogen free materials, diluted 10-fold (vol/vol) in LAL reagent water and heated to 70°C for 5 min to remove any nonspecific inhibition to the assay. Samples were then incubated with equal volumes of LAL for 10 min at 37°C and developed with equal volumes of substrate solution for 6 min. The absorbance of the assay plate was read at 405 nm using the same microplate reader used in cytokine assays. Samples and standards were run in duplicate and the lower limit of detection was 0.15 U/ml. All units expressed are relative to the U.S. reference standard EC-6.

Histology. Formalin-preserved liver, distal small and transverse large bowel were embedded in paraffin, and 5-μm thick sections were stained with haematoxylin and eosin for histologic examination. Slides were coded and examined in a blinded fashion by one individual (J.M. Crawford), using a semi-quantitative scoring system for abnormalities known to be associated with GVHD (19, 20). Specifically, seven parameters each were scored for small bowel (villous blunting, crypt regeneration, crypt epithelial cell apoptosis, crypt loss, luminal sloughing of cellular debris, lamina propria inflammatory cell infiltrate, and mucosal ulceration), large bowel (crypt regeneration, crypt epithelial cell apoptosis, crypt loss, surface colonocyte vacuolization, surface colonocyte attenuation, lamina propria inflammatory cell infiltrate, and mucosal ulceration), and 10 parameters for liver (portal tract expansion by an inflammatory cell infiltrate, lymphocytic infiltrate of bile ducts, bile duct epithelial cell apoptosis, bile duct epithelial cell sloughing, vascular endothelialitis, parenchymal apoptosis, parenchymal microabscesses, parenchymal mitotic figures, hepatocellular cholestasis, and hepatocellular steatosis). The scoring system for each parameter denoted 0 as normal; 0.5 as focal and rare; 1 as focal and mild; 2 as diffuse and mild; 3 as diffuse and moderate; and 4 as diffuse and severe, as previously published in human (21, 22) and experimental (19, 20) GVHD histology. Scores were added to provide a total score for each organ; the maximum score for small and large bowel was thus 28 each, and for liver 40.

Electron microscopy. Small intestinal tissue was obtained at necropsy and fixed in Modified McDowell-Trumps fixative and then processed for electron microscopy. Briefly, the specimens were postfixed with 1% osmium tetroxide and then embedded in Polybd 812 resin (Polysciences Inc). Thin sections were cut and stained with lead citrate and uranyl acetate before study with a Phillips transmission electron microscope operating at 60 kV. Multiple sections and grids were examined without knowledge of treatment group.

Statistical analysis. Survival curves were plotted using Kaplan-Meier estimates. The Mann-Whitney U test was used for the statisti-

cal analysis of cytokine data, LPS levels, clinical scores, weight loss, and histology while the Mantel-Cox log rank-test was used to analyze survival data. $P < 0.05$ was considered statistically significant.

Results

IL-11 treatment reduces GVHD mortality and morbidity. We first studied the effect of IL-11 administration on GVHD morbidity and mortality following allogeneic BMT using a high TBI dose (1,550 cGy). IL-11 was given from day −2 to day +14 at a dose of 250 μg/kg subcutaneously twice daily. GVHD was severe, with mortality beginning on day +4 and continuing so that < 20% of control animals treated with diluent alone survived at day 45 (Fig. 1 *A*). By contrast, 90% of IL-11–treated allogeneic animals survived this period. Survival in syngeneic BMT recipients treated with diluent was 88% at day 45 (Fig. 1 *A*). After syngeneic BMT in 9–11-wk-old mice, IL-11 increased long term survival from 60 to 100% ($P < 0.02$), consistent with its demonstrated protection of the GI tract after TBI (23, 24). Clinical GVHD was also quantified using a clinical scoring system as described in Methods (18, 19). As shown in Fig. 1 *B*, control animals developed severe GVHD (clinical scores > 5) which progressed over the observation period whereas animals treated with IL-11 had only moderate GVHD (clinical scores between 3 and 5) that remained stable over the 45 d. As expected, the clinical scores of syngeneic animals gradually returned to baseline over this time. In separate experiments, IL-11 treatment after a BMT regimen using a lower dose TBI (1,300 cGy) and higher T cell dose (2×10^6) reduced GVHD mortality from 75 to 35% at day 70 ($P < 0.01$); the severity of clinical GVHD was also significantly reduced throughout the entire posttransplant period (data not shown). Donor engraftment as determined by Ly 5 phenotype at day 28 (see Methods) was 99.7±0.2% in the peripheral blood of IL-11–treated animals compared to 98.1±1.9% in control-treated animals, excluding mixed donor/host chimerism as a cause of tolerance to host tissues and reduced GVHD.

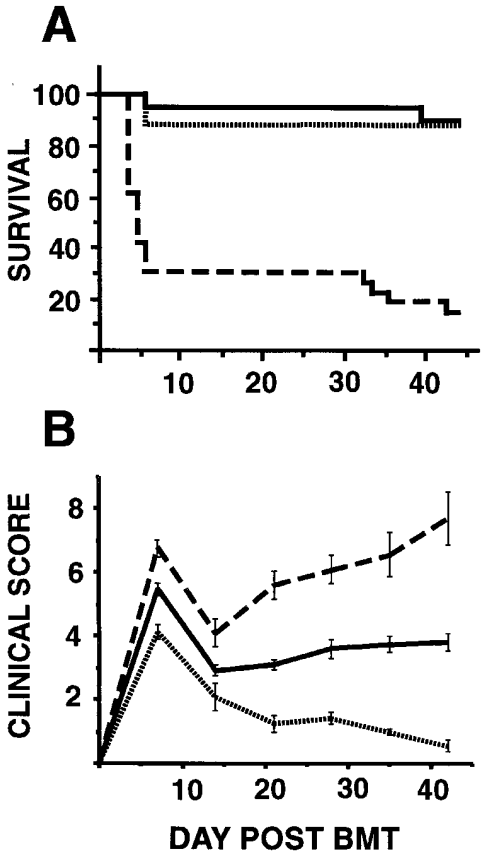

Figure 1. IL-11 reduces the mortality and morbidity of GVHD. B6D2F1 mice were transplanted with 5×10^6 bone marrow cells and 0.5×10^6 T cells from B6 mice after 1,550 cGy of TBI. IL-11 or control diluent was given subcutaneously from day −2 to +7. (*A*) Survival in control treated (dashed line, $n = 20$) and IL-11 treated (solid line, $n = 20$) animals after allogeneic BMT ($P < 0.001$) and syngeneic BMT (serrated line, $n = 9$). (*B*) GVHD clinical score. Animals were scored for clinical GVHD as described in Methods. GVHD is significantly less in IL-11–treated animals compared to controls at all time points ($P < 0.05$) and significantly higher than in syngeneic BMT recipients at all time points ($P < 0.01$). Data represent results mean±SE from two similar experiments.

IL-11 promotes a Th2/Tc2 cytokine phenotype after BMT but preserves CTL activity. Induction of GVHD fundamentally depends on the donor T cell response to host alloantigens (25). To evaluate the effects of IL-11 on donor T cell responses to host tissues, we studied T cell proliferation, cytokine production and CTL activity 14 d after allogeneic BMT. IL-11 treatment reduced proliferation, IFNγ, and IL-2 production to host alloantigens by > 50% in mixed lymphocyte culture (MLC) (Fig. 2 *A*). The stimulation index was reduced from 11.5 in control animals to 2.9 in IL-11–treated animals. IL-4 production by splenocytes from IL-11–treated animals was increased 10-fold. Over 85% of this IL-4 (7.2 U/ml) was derived from $CD4^+$ compared to $CD8^+$ cells (0.95 U/ml). Control $CD8^+$ T cells produced < 0.13 U/ml of IL-4, and thus IL-11 polarized both T cell subsets (Th2 and Tc2). Splenocytes from IL-11 treated recipients after syngeneic BMT also showed increased IL-4 and decreases in IL-2, IL-12, and IFNγ after stimulation with Con A, demonstrating that this effect of IL-11 was not limited to the responses of T cells to alloantigens (Table I). In contrast to the MLC findings, cytotoxic T lymphocyte (CTL) activity to host antigens was similar in control and IL-11–treated recipients (Fig. 2 *B*).

To study the physiologic correlates of this T cell polarization, we analyzed serum IFNγ levels 4 d after allogeneic BMT, when levels are maximal during GVHD (26, 27). Confirming the in vitro findings above, IL-11 treatment significantly reduced serum IFNγ levels (Fig. 3 *A*), which was also seen after allogeneic BMT using 1,300 cGy TBI (22.3±2.3 vs 13.1±2.5

Table I. IL-11 Effects on Syngeneic T Cell Responses

IL-11 treatment	−	+
Proliferation	49399±1683	43883±1543
IL-12 (pg/ml)	255±19	115*±19
IFNγ (U/ml)	134.7±9.6	73.9*±6.9
IL-2 (U/ml)	13.2±2.6	5.4*±0.3
IL-4 (U/ml)	10.7±0.8	21.7*±1.7

Responders were taken at day +14 from syngeneic BMT recipients treated with control diluent or IL-11 and stimulated with Con A. Results represent mean±SD of quadruplicate wells. T cell cytokine production in the absence of Con A was < 1 U/ml. *$P < 0.05$.

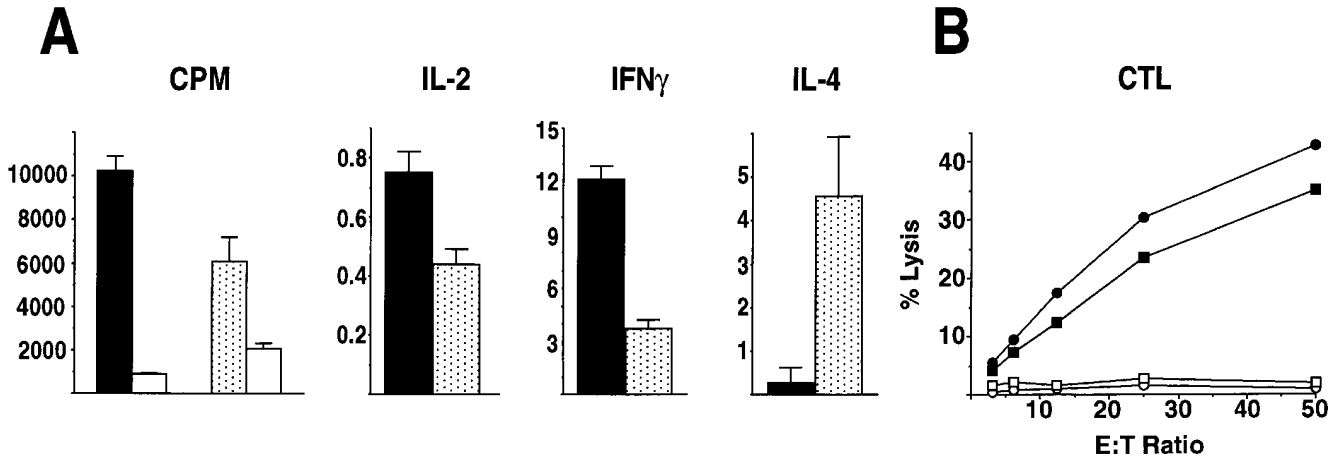

Figure 2. IL-11 administration reduces proliferation and cytokine production of donor cells to host allo-antigens in vitro but does not affect CTL activity. (*A*) Splenocytes were pooled from IL-11 or control-treated allogeneic animals (n = 3–6) 14 d after BMT and cultured together with irradiated host macrophages. (*Solid bar*) Splenocytes from control-treated animals. (*Dotted bar*) Splenocytes from IL-11–treated animals. (*Open bar*) Splenocyte responses to donor (B6) peritoneal macrophages. Proliferation was determined by ^{3}[H]thymidine incorporation. The Stimulation Index (expressed as the ratio of proliferation to host stimulators/proliferation to syngeneic stimulators) was 11.5 in controls and 2.9 in IL-11–treated animals. Proliferation of naive B6 splenic T cells to B6D2F1 stimulators was 25,000 cpm. Production of IL-2, IFNγ, and IL-4 by T cells from BMT recipients in response to host stimulators in culture supernatants was determined by ELISA. Solid bar vs dotted bar $P < 0.05$. Data represent mean±SD of quadruplicate wells and results shown are from one of three similar experiments in which the range of reductions were as follows: proliferation, 60–75%; IFNγ, 40–80%; IL-2, 40–60%. Increases in IL-4 ranged from eight- to 20-fold. (*B*) Splenocytes were pooled and used immediately in a standard 5-h ^{51}Cr release assay. Targets were p815 (H-2^{d}) or EL4 (H-2^{b}) tumor cell lines. (●) Splenocytes from controls against p815. (○) Splenocytes from controls against EL4. (■) Splenocytes from IL-11–treated animals against p815. (□) Splenocytes from IL-11–treated animals against EL4. Results were similar in three separate experiments in which the percentage of IL-11/control at maximum lysis ranged from 80 to 120%.

U/ml, $P < 0.05$). Considered together, these data demonstrate that IL-11 induces a partial Th2/Tc2 cytokine response without affecting cytotoxic T lymphocyte function.

IL-11 treatment reduces the production of IL-12 during acute GVHD. IL-12 is known to be a critical cytokine in the induction of Th1 responses (11) and blockade of IL-12 during BMT is sufficient to induce a Type 2 cytokine profile and reduce GVHD (28). We hypothesized that IL-11 might polarize T cell cytokine responses by the down-regulation of IL-12 from antigen presenting cells (APCs). We therefore studied the effect of IL-11 treatment on the production of IL-12 p40 in response to LPS from macrophages 7 d after BMT. As shown in Fig. 4 *A*, the maximum IL-12 p40 production was reduced by over 60% in IL-11–treated animals. IL-12 production in MLC 14 d after BMT was also reduced by 60% (Fig. 4 *B*), which may account for the polarization of donor T cell responses.

IL-11 treatment reduces small bowel damage and serum LPS levels. Serum LPS levels are increased during GVHD and they correlate with damage to the GI tract (an important target organ of GVHD) (19). IL-11 reduces the damage of chemoradiotherapy to small bowel crypts (23, 24, 29). We therefore measured serum LPS levels and evaluated small bowel pathology in transplanted animals using a semiquantitative index in which a number of histological parameters were each scored on a scale of 0 to 4 and then summed (see Methods). As shown in Fig. 3 *B*, the serum level of LPS at day 5 in animals receiving IL-11 after allogeneic BMT was reduced to that seen after syngeneic BMT. IL-11 treatment also reduced the GI damage in allogeneic BMT recipients to that seen after syngeneic BMT (Fig. 3 *C*); apoptosis, lymphocytic infiltrate, brush border loss, and sloughing into the lumen were all reduced. Electron microscopy of the small bowel (Fig. 5) revealed that IL-11 dramatically protected the small bowel from severe disruptions in villous integrity characterized by destruction of microvilli, disruption of intercellular tight junctions, and cellular necrosis, helping to explain the profound effects of IL-11 on GVHD mortality.

IL-11 treatment reduces TNFα production in vivo and in vitro. TNFα is an inflammatory cytokine that acts in several phases of GVHD pathophysiology (11, 13, 19, 30). Because TBI alone can potentiate TNFα production by macrophages in response to LPS (19), we evaluated macrophages taken from animals immediately after TBI. IL-11 pretreatment for 2 d significantly suppressed TNFα secretion to LPS after TBI (464±36 vs 223±12 pg, $P < 0.05$), reducing it to that secreted by unirradiated macrophages (197±16 pg). Similar reductions were also seen in the presence of IFNγ priming (data not shown), confirming the potency of IL-11 in abrogating the proinflammatory effects of TBI. Polarization of T cells before BMT also reduces TNFα levels and prevents mortality (31). Given the findings above, we hypothesized that IL-11 could also decrease TNFα during acute GVHD both because T cells were polarized and less able to prime mononuclear cells, and because LPS was contained within an intact GI tract and was unable to translocate to the systemic circulation. Serum TNFα levels were therefore measured in BMT recipients at day +5, the time of peak mortality. As shown in Fig. 3 *D*, IL-11 treat-

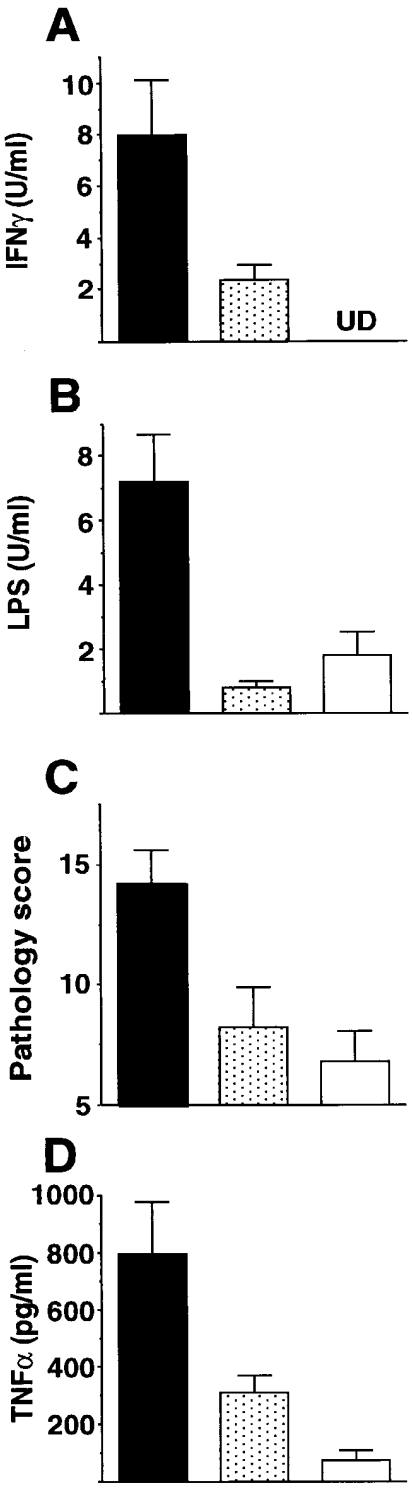

Figure 3. (*A*) IL-11 reduces early systemic IFNγ levels after allogeneic BMT. IFNγ levels were determined in sera from animals 4 d after allogeneic BMT. (*Solid bar*) Controls, ($n = 10$); (*dotted bar*) IL-11–treated animals ($n = 10$). Levels after syngeneic BMT were under the limit of detection (UD), ($n = 4$). Dotted bar vs solid bar and dotted bar vs UD, $P < 0.01$. (*B*) IL-11 reduces systemic LPS serum levels 5 d after allogeneic BMT. (*Solid bar*) Controls ($n = 14$). (*Dotted bar*) IL-11–treated animals ($n = 12$). (*Open bar*) Syngeneic BMT recipients, ($n = 10$). Solid bar vs dotted bar and solid bar vs open bar, $P < 0.01$. (*C*) IL-11 reduces small bowel damage after allogeneic BMT. Randomized coded slides were scored semi-quantitatively, as described in Methods. The total scores are shown for specimens from each animal group. (*Solid bar*) Controls, ($n = 20$) (*dotted bar*) IL-11–treated animals ($n = 10$); and (*open bar*) syngeneic BMT recipients ($n = 9$). Solid bar vs dotted bar and solid bar vs open bar, $P < 0.01$. (*D*) IL-11 reduces TNFα production in vivo. TNFα levels were determined in the sera of animals 5 d after BMT. (*Solid bar*) Controls ($n = 14$); (*dotted bar*) IL-11–treated animals, ($n = 10$) and (*open bar*) syngeneic BMT recipients ($n = 9$). Solid bar vs dotted bar, $P < 0.05$, dotted bar vs open bar, $P < 0.01$. All results represent mean±SE.

ment significantly reduced serum TNFα levels in allogeneic BMT recipients, albeit to levels that remained higher than after syngeneic BMT.

Brief IL-11 treatment provides long-term protection of GVHD target organs. Previous studies have shown that Th2 polarization alone does not always prevent GVHD target tissue damage despite improved survival (32). We therefore studied the long-term effects of IL-11 on two GVHD target organs, the GI tract and liver (33). Samples were taken from animals 45 d after transplantation and processed and scored as described in Methods. Table II shows that IL-11 treatment protected the small bowel to the greatest extent, although there is also a trend towards less damage in the large bowel and liver. Combined histopathologic scores for small bowel, large bowel, and liver demonstrated a significant overall protection. In addition, the profound immunosuppression of GVHD, reflected in delayed T and B cell reconstitution (34, 35), was significantly reduced by IL-11 (Table II).

Discussion

We have shown that IL-11 substantially reduces GVHD morbidity and mortality in an experimental BMT model. Short-term administration of IL-11 not only improved recipient survival, but provided long-term protection of GVHD target organs, especially the small intestine. Our data demonstrate that IL-11 achieves this effect at three points within the cytokine cascade of acute GVHD (Fig. 6). First, during recipient conditioning, IL-11 minimizes GVHD damage to the small bowel and prevents the sensitization of host macrophages to the effects of TBI which reduces LPS levels. During phase 2 (donor T cell activation), IL-11 induces polarization of donor T cells by downregulating IL-12, subsequently reducing the production of IFNγ both in vivo and in vitro. Finally, IL-11 directly suppresses the production of inflammatory cytokines by monocytes and macrophages in response to LPS during phase 3, thereby further suppressing the secretion of TNFα that contributes to target cell apoptosis.

The ability of IL-11 to reduce small bowel injury (Fig. 3 *C*) is critical to its prevention of systemic GVHD. At least two potential mechanisms may be involved. First, IL-11 has direct protective effects on the GI tract epithelium in models of chemotherapy and radiation induced injury (23, 24, 29, 36, 37). Small bowel crypt recovery is improved via protection of clonogenic crypt cells (24), reductions in apoptosis (29), and increased cellular mitotic index after chemoradiotherapy (23). These studies, which served as the basis for the IL-11 treatment schedule used in our experiments, showed that maximum crypt protection occurred if IL-11 treatment began before irradiation and then continued for a further 3 d (24). The ability of IL-11 to enhance the structural integrity of the gastrointestinal

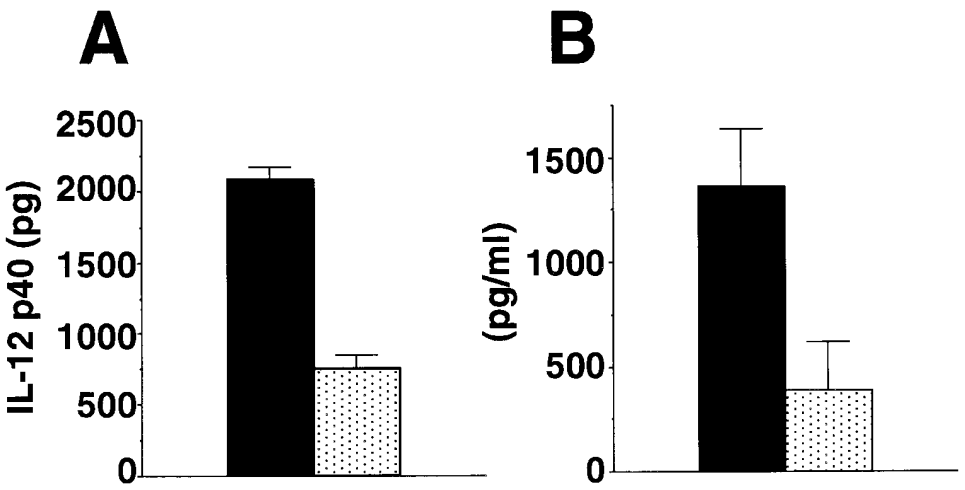

Figure 4. IL-11 reduces IL-12 p40 production. (*A*) Macrophages were pooled from four allogeneic recipients 7 d after BMT in each treatment group and cultured in the presence of LPS (0.01 μg/ml). Supernatants were collected at 48 h and analyzed by ELISA. Data represents mean±SD of quadruplicate wells, expressed as units per 10^5 F4/80+ cells. Macrophages from syngeneic BMT recipients produced 203±24 pg. Dotted bar vs solid bar, $P < 0.05$. (*B*) Supernatants taken 48 h after initiation of MLC as in Fig. 2 were analyzed by ELISA. Data represent combined mean±SD of three experiments. Dotted bar vs solid bar, $P < 0.01$.

mucosal barrier (Fig. 5) has also been noted in experimental models of colitis (38). The requirement that IL-11 treatment be commenced before conditioning may reflect its ability to decrease epithelial proliferation (and hence susceptibility to damage) due to prolongation of the G1-S phase of the cell cycle (39). Furthermore, the trophic effect of IL-11 on already damaged small bowel epithelium (23, 40) implies a second mechanism that operates independently of the cell cycle.

A second pathway by which IL-11 may protect the GI tract is by direct suppression of inflammatory cytokines. TNFα causes necrosis in the GI tract (41) and IFNγ increases mucosal permeability by altering tight junction integrity (42, 43). Individual blockade of each of these cytokines during GVHD reduces GI tract injury (41, 44) and additive effects might therefore be expected by IL-11 treatment. The maintenance of tight junction integrity and preservation of the GI epithelium (observed in IL-11–treated recipients) is consistent with reduced levels of both these cytokines. The normal CTL responses to alloantigens in IL-11–treated animals suggest that CTL effectors alone are not responsible for the small bowel injury during GVHD. Although surprising, this conclusion is consistent with the findings of Baker et al. who demonstrated variability among GVHD target organ susceptibility to CTL damage (45). Such variations were confirmed in a recent study where FasL (but not TNFα) mediated GVHD hepatic damage and TNFα, (but not FasL) was an important effector of GI tract damage (46). Both the FasL and TNFα pathway caused GVHD skin pathology. The GI tract seems particularly susceptible to the inflammatory cytokines generated during GVHD and IL-11 may reduce this susceptibility directly by maintaining epithelial cellular structure and indirectly by reducing MHC antigen expression through decreases in IFNγ. The individual contributions of each of these potential mechanisms is currently under investigation.

The polarization of T cells by IL-11 during T cell activation appears to be mediated by the suppression of IL-12, although IL-11 may have additional direct effects on T cells which are known to bear IL-11 receptors (Trepicchio, W., personal communication). IL-11 has been shown to block IL-12 p70 production from human peripheral blood mononuclear cells in vitro (10). Our data are the first to confirm a physiological consequence of this effect, because IL-12 p40 levels correlate closely with biologically active IL-12 p70 (12). The inhibition of IL-12 is important, since blockade of IL-12 results in Th2 polarization (28), the default T cell pathway in the absence of IFNγ-producing cells (47). The ability of IL-11 to polarize T cell cytokine production is consistent with the obligatory role of IL-12 in the generation of Th1/Tc1 cells (48) although polarization by IL-4 does not effect CTL generation and the cytolytic capacity of Tc2 cells is not impaired (49). The unaffected CTL response in IL-11–treated animals, which is mediated by mature $CD8^+$ T cells in this model (50), is therefore not surprising. Indeed, preservation of the CTL response may have important implications for maintaining a graft-versus-leukemia (GVL) effect, a therapeutic benefit of allogeneic BMT in malignant diseases. The separation of GVHD and GVL through inhibition of $CD4^+$ cells has been demonstrated in other experimental BMT models (51, 52).

The ability of Th2 cytokine polarization of donor T cells to reduce the severity of acute GVHD has been shown in a number of different experimental models, although the methods used to induce polarization have differed in each instance. First, the administration of IL-4 to donor mice induces a Th2 phenotype and when these cells are used in the donor inoculum, GVHD is reduced (31, 53). Second, the addition of IL-4 to primary MLC of donor T cells and host stimulator cells results in T cell polarization and a reduction in acute GVHD when these cells are used as the donor T cell inoculum (16). However, GVHD target organs are not protected long-term by this approach (32). Third, the use of G-CSF for the mobilization of donor cells also induces Th2 polarization and reduces GVHD (54), which may help to explain the lower incidence of severe acute GVHD observed after transplantation using G-CSF mobilized stem cells (55). Indeed, G-CSF mobilized donor cells contain up to 10-fold more T cells than in conventional BMT, but their use is not associated with the predicted increase in acute GVHD (56), although chronic GVHD may be increased (57). All these approaches interrupt the cascade that leads to secretion of inflammatory cytokines such as TNFα through reductions in IFNγ priming of monocytes (16, 28, 54).

The suppression of inflammatory cytokines by IL-11 has

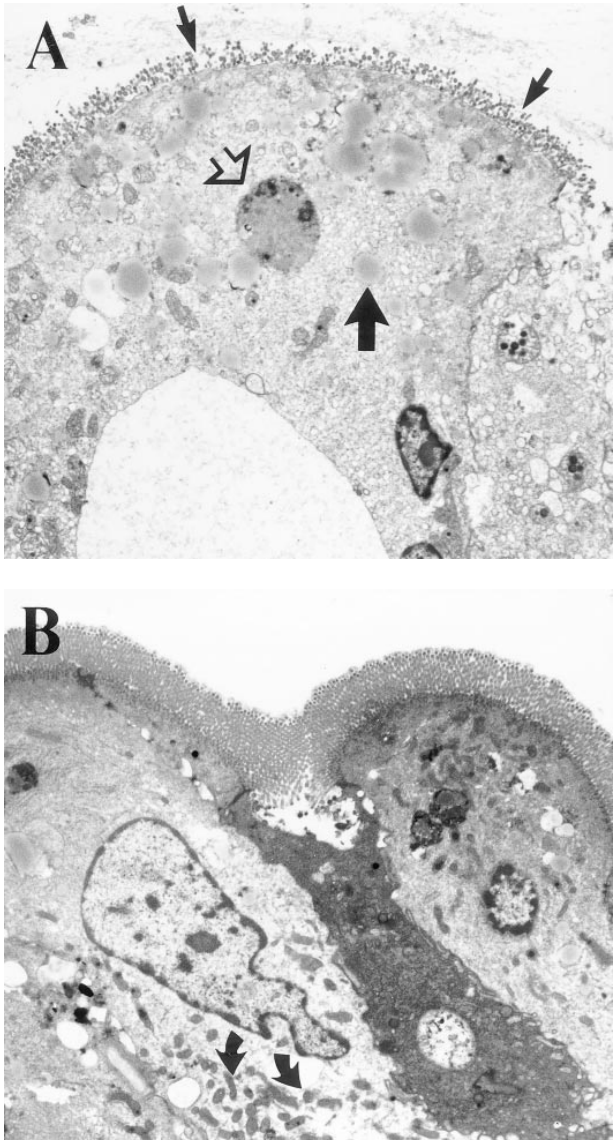

Figure 5. IL-11 preserves the integrity of the small bowel during acute GVHD. Transmission electron microscopy viewed at 3,300× of epithelium of the small intestine 5 d after allogeneic BMT using 1,550 cGy of TBI as conditioning. (*A*) diluent and (*B*) IL-11–treated animals. Note the loss of surface microvilli (*thin solid arrows*) and the apoptotic apical cell with pyknotic nuclear changes (*open arrow*), and lipid dissolution (*thick solid arrows*) (*A*). Intact intercellular junctions, viable cytoplasmic organelles (mitochondria, curved arrows), and normal microvilli are evident in (*B*).

recently been the focus of intensive research (5, 7, 10). In a mouse model of endotoxemia, pretreatment with IL-11 prevented increases in TNFα, IL-1β, and IFNγ production after LPS stimulation in vitro and in vivo (5) and is thought to be the result of NF-κB suppression (6). In preclinical studies, IL-11 has been shown to protect the lung from radiation-induced injury through nearly complete inhibition of TNFα production (7). The ability of IL-11 to improve inflammatory bowel disease also shows therapeutic promise (8). The mechanism for this improvement has not yet been elucidated, but the correlation with Th1 dominance of inflammatory bowel disease is intriguing (58, 59), particularly as IL-11 tends to polarize T cell function toward Th2 cytokines. Taken together, our data suggest that IL-11 suppresses TNFα generation during GVHD by: (*a*) a direct suppression of TNFα secretion by monocytes; (*b*) a reduction in IFNγ (which sensitizes macrophages to LPS); (*c*) reduced systemic LPS levels that could stimulate production of TNFα.

Table II. Day 45 Target Organ GVHD Pathology and Immune Reconstitution

	ALLO + control (n = 4)	ALLO + IL-11 (n = 5–9)	Syngeneic (n = 3)
GI histology score			
Small bowel	10.8±1.5	3.8±0.8*	6.0±1.2‖
Large bowel	5.3±2.3	3.7±0.4	2.0±0.0§
Liver	11.3±2.8	8.7±1.2	6.0±1.5§
Total GVHD score	27.0±3.2	16.1±1.5‡	13.3±2.0§
Immune reconstitution			
CD4+	0.61±0.23	2.61±0.41‡	12.0±0.51§
CD8+	0.50±0.13	1.45±0.14‡	5.50±0.46§
B220+	0.83±0.56	6.92±1.40§	55.89±10.86§

Scores (0–4) for each parameter below were assessed on coded slides and summed as described in Methods. Small bowel: villous blunting, crypt regeneration, loss of enterocyte brush border, luminal sloughing of cellular debris, crypt cell apoptosis, outright crypt destruction, and lamina propria lymphocytic infiltrate. Large bowel: crypt regeneration, surface coloncytes, coloncyte vacuolization, surface colonocyte attenuation, crypt cell apoptosis, outright crypt destruction, and lamina propria lymphocytic infiltrate. Liver: portal tract expansion and infiltrate, bile duct infiltrate, nuclear multilayering, pyknotic duct cells, intraepithelial cells, vascular endothelialitis, hepatocellular pan-lobular necrosis, acidophil bodies, microabscesses, mitotic figures, and steatosis. Total GVHD score: mean±SE of the sum of scores for small bowel, large bowel, and liver from individual animals in each group. Immune reconstitution: mean±SE of $CD4^+$, $CD8^+$, and $B220^+$ cells (× 10^6 per spleen). *$P <$ 0.01 vs allo + control, ‡$P < 0.02$ vs allo + control, §$P < 0.05$ vs allo + control, ‖$P = 0.08$ vs allo + control.

Acute GVHD remains the major obstacle to the wider application of allogeneic BMT. Clinical BMT requires intensive conditioning regimens which increase the severity of GVHD by amplifying the inflammatory cytokine cascade (19). Current immunosuppressive drugs such as cyclosporine A and prednisone offer significant but incomplete protection against GVHD, particularly in the setting of multiple donor–host histocompatibility antigen disparities that pertain to unrelated donor BMT. Interestingly, cyclosporine A is a poor inhibitor of IFNγ secretion induced by IL-12 (60) and IL-11 may therefore offer an attractive noncross-reactive strategy to suppress both the IL-12 driven T cell response and the inflammatory response mediated by LPS. The ability of IL-11 to alter such cytokine profiles may also hold therapeutic potential for other diseases where Th1 responses play a prominent pathogenic role, such as autoimmune diseases (61) and rejection of solid organ transplants where Th2 cytokines appear to reduce the barrier to tolerance despite efficient CTL generation (62).

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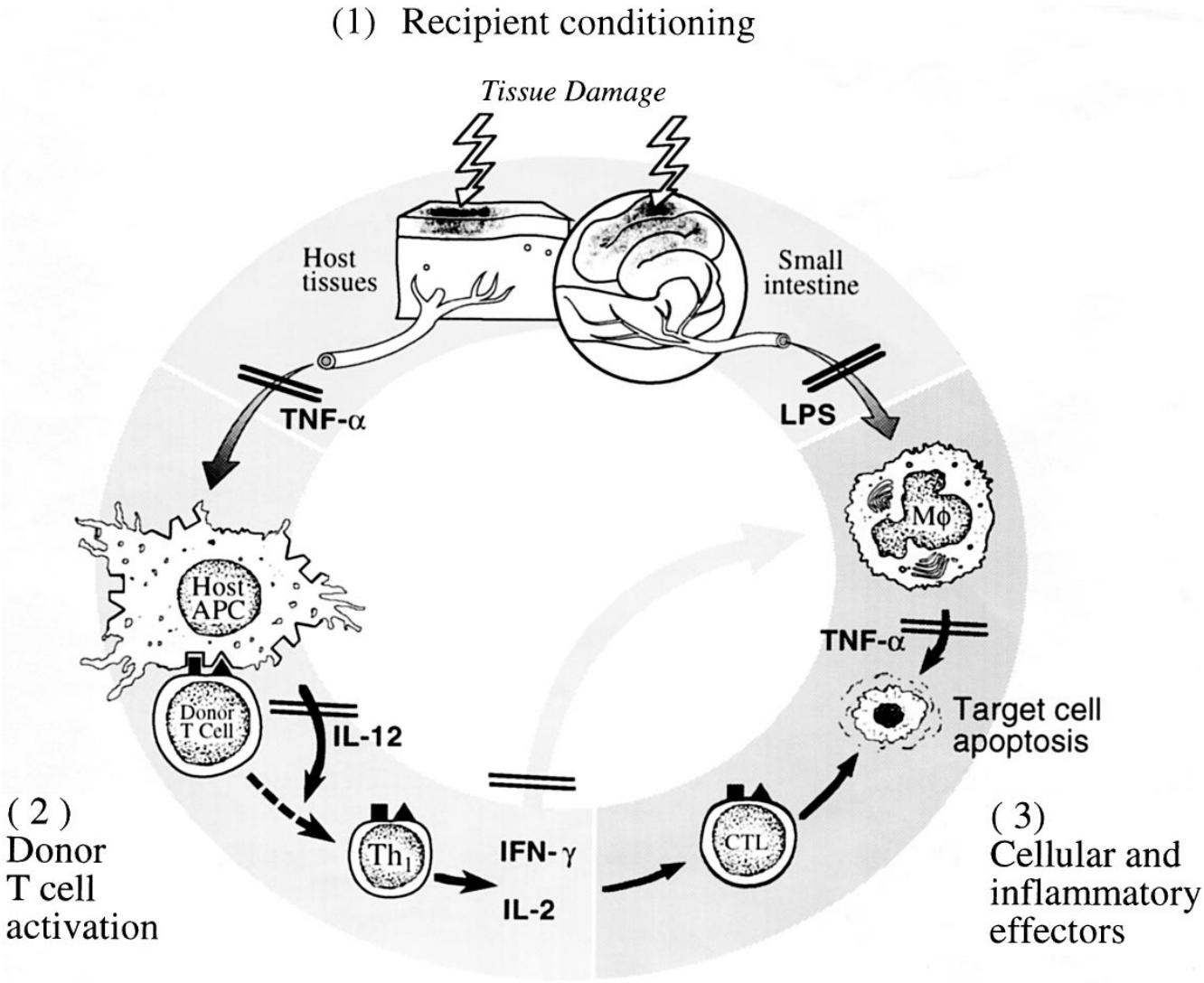

Figure 6. The effects of IL-11 on the cytokine cascades of GVHD. The development of acute GVHD is proposed to develop in a three-phase process where mononuclear phagocytes and other accessory cells are responsible for both initiation of a GVH reaction and for the subsequent injury to host tissues after complex interactions with cytokines. In phase 1, IL-11 reduces damage from the conditioning regimen in the intestinal mucosa, which prevents translocation of LPS from the intestinal lumen into the circulation. IL-11 also reduces the sensitization of host macrophages by TBI to secrete TNFα and it diminishes IL-12 production by host APCs. As a consequence, IL-11 prevents IFNγ secretion by donor T cells (although CTL function remains unaffected) and systemic TNFα is lowered, culminating in reduced target organ damage and improved survival.

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