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Research Article

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Differential Effects of the Absence of Interferon-γ and IL-4 in Acute Graft-Versus-Host Disease after Allogeneic Bone Marrow Transplantation in Mice

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Abstract

Graft-versus-host disease (GVHD), in which immunocompetent donor cells attack the host, remains a major cause of morbidity after allogeneic bone marrow transplantation (BMT). To understand the role of cytokines in the pathobiology of GVHD, we used cytokine knockout (KO) mice as a source of donor T cells. Two different MHC-disparate strain combinations were examined: BALB/c ($H2^d$) donors into lethally irradiated C57BL/6 ($H2^b$) recipients or C57BL/6 ($H2^b$) donors into B10.BR ($H2^k$) recipients. Donor cells were from mice in which either the interferon-γ (IFN-γ) or the IL-4 gene was selectively disrupted to understand the role of these cytokines in acute GVHD. In both strain combinations the same pattern was noted with regard to GVHD onset and morbidity. All mice exhibited the classic signs of acute GVHD: weight loss with skin, gut, and liver pathology resulting in morbidity and mortality. Surprisingly, donor cells obtained from mice lacking IFN-γ gave rise to accelerated morbidity from GVHD when compared with cells from wild-type control donors. Similar results were obtained using normal donors when neutralizing antibodies to IFN-γ were administered immediately after the BMT. These results suggest that IFN-γ plays a role in protection from acute GVHD. In marked contrast, cells obtained from IL-4 KO mice resulted in protection from GVHD compared with control donors. Splenocytes from IFN KO mice stimulated with a mitogen proliferated to a significantly greater extent and produced more IL-2 compared with splenocytes obtained from IL-4 KO or control mice. Additionally, there was increased IL-2 production in the spleens of mice undergoing GVHD using IFN-γ KO donors.

These results therefore indicate, with regard to the TH1/TH2 cytokine paradigm, the absence of a TH1-type cytokine can be deleterious in acute GVHD, whereas absence of a TH2 cytokine can be protective. (*J. Clin. Invest.* 1998. 102: 1742–1748.) Key words: graft-versus-host disease • bone marrow transplant • IFN • IL-4 • cytokine knockout

Introduction

Graft-versus-host disease (GVHD)[1] remains a significant cause of morbidity after allogeneic bone marrow transplantation (BMT), thereby limiting the use and efficacy of BMT. GVHD occurs when donor alloreactive T cells become sensitized to the host, expand, and mediate destruction of host tissues (1, 2). This latter effector phase is thought to be amplified with the release of inflammatory cytokines (1, 2, 3). Cytokines clearly play a pivotal role in immune functions and GVHD is no exception. Unfortunately, there have been conflicting reports as to the role of cytokines in GVHD, particularly with regard to cytokines of TH1 versus TH2 cell types. It has been postulated that cytokines produced by TH1 cells (i.e., IL-2 and IFN-γ), being primarily associated with cell-mediated immune responses, would augment the T-cell responses against the host, thereby leading to greater GVHD in the recipients (3). Clinical and preclinical data would tend to support this, as TH1 cytokines such as IL-2 and IL-12 have been associated with increased GVHD (4, 5, 6). However, some preclinical models have demonstrated protective effects of IL-2 and IL-12 in GVHD, although the timing of administration appears critical (7, 8).

Conversely, cytokines from TH2 cells (i.e., IL-4 and IL-10) were considered immunosuppressive and, therefore, capable of inhibiting GVHD (3, 9). Transfer of TH2 cells after allogeneic BMT resulted in protection from GVHD (10). However, as with the TH1 cytokines, there have been conflicting reports indicating that TH2 cytokines can worsen the outcome of GVHD (11). Thus, with regard to the TH1/TH2 paradigm, it is not completely clear whether a particular pathway is protective or deleterious for GVHD. Many of these studies involved either the use of systemic administration of a particular cytokine or the use of neutralizing antibodies to block the cytokine activity in question. The use of neutralizing antibodies has been hampered by potential carrier effects of the antibodies,

Animal care was provided in accordance with the procedures outlined in the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication No. 86-23. 1985).

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1. *Abbreviations used in this paper:* BMT, bone marrow transplant; GVHD, graft-versus-host disease; KO, knockout; LN, lymph node; TDL, thoracic duct lymphocyte.

potential agonistic effects, and by the difficulty in determining the completeness of the neutralization at the tissue level. To more completely understand the role of cytokines in acute GVHD, we used cytokine knockout (KO) mice that were deficient in either IFN-γ or IL-4 as a source of donor T cells. We report here that donor cells obtained from IFN-γ KO mice resulted in accelerated morbidity from GVHD, whereas use of donor cells from IL-4 KO mice resulted in protection from GVHD. Thus, IFN-γ appears to be protective, whereas IL-4 potentiates acute GVHD involving MHC-disparate BMT in mice.

Methods

Mice. B10.BR/SgSnJ ($H2^k$), B6.C.$H2b^{m12}$ (bm12), and C57BL/6 IFN-γ (IFN-γ) KO mice were purchased from the Jackson Laboratory (Bar Harbor, ME). BALB/c ($H2^d$) and C57BL/6 (B6, $H2^b$) mice were purchased from the Animal Production Area (NCI-FCRDC, Frederick, MD). Breeding pairs of BALB/c IFN-γ KO and BALB/c IL-4 KO mice, originally purchased from the Jackson Laboratory (Bar Harbor, ME) were maintained as a colony in our facility. (B6 × 129Sv)F1 IL-4 KO mice were backcrossed six generations onto a B6 background. Donors and recipients were females, and were 8–10 wk of age at the time of BMT.

Induction of GVHD. For BMT involving the B6 into B10.BR strain combination, B10.BR recipients were irradiated with 800 cGy total body irradiation by X-ray at a dose rate of 0.41 cGy/min, as described (10). Recipients were given 10^7 splenocytes along with 8×10^6 BMC treated with anti-Thy 1.2 (antibody 30-H-12, rat IgG2b, provided by Dr. David Sachs, Charlestown, MA) and complement (Nieffenegger Company, Woodland, CA), as described previously (10). As compared to wild-type controls, cells from IFN-γ KO mice had a comparable $CD4^+$ and $CD8^+$ T cell content (21% versus 18% and 12% versus 13%, respectively) as determined by flow cytometry; spleen cells from IL-4 KO mice had a $CD4^+$ and $CD8^+$ T cell content identical to wild-type controls (21% and 12%, respectively). The BMC plus splenocytes were injected intravenously in 0.5 ml volume. For experiments involving the BALB/c into B6 strain combination, recipient B6 mice received lethal irradiation to 1,000 cGy by a ^{137}Cesium source. The mice then received 10^7 BMC and 2×10^7 splenocytes from donor mice. Mice were then monitored and weighed weekly. All moribund mice were killed. Survival data were plotted by the Kaplan-Meier method and analyzed by the Log-Rank test. A P value of less than 0.05 was considered significant. Mice in some groups received antibodies to IFN-γ (clone R4-6A2) injected at 500 μg/0.5 ml PBS intraperitoneally on days 0, 1, 2, and 3 after BMT. For the studies examining the in vivo expansion capability of IL-4 KO donor T cells, we used a B6 into bm12 model. Bm12 recipients were irradiated with 9.0 Gy TBI delivered by X-ray. Recipients were given 5×10^6 B6 bone marrow cells treated with anti-Thy1.2 mAb and complement. Purified B6 $CD4^+$ T cells (2×10^6) from B6 wild-type or IL-4 KO donors were added to T cell–depleted bone marrow cells. To purify $CD4^+$ lymph node (LN) T cells, single cell suspensions of axillary, mesenteric, and inguinal LN cells were obtained (as a source of GVHD-causing effector cells) by passing minced LN through a wire mesh and collecting them into RPMI 1640. Cell preparations were depleted of natural killer (NK) cells and $CD8^+$ cells (hybridoma 2.43, rat IgG2b, provided by Dr. David Sachs) by mAb coating and passaged through a goat anti–mouse and goat anti–rat Ig coated column (Biotex, Edmonton, Canada). The final composition of T cells in the donor graft was determined by flow cytometry and was 94% T cells. The bone marrow plus $CD4^+$ T cell inoculum was administered via caudal vein in 0.5 ml volume.

Thoracic duct cannulation. For thoracic duct lymphocyte (TDL) isolation, cannulae were inserted in the thoracic duct of recipients on day 6 post-BMT, and TDLs were collected over a period of 18 h (12). The effluent is collected at 1 ml per hour. Six recipients of wild-type and six recipients of IL-4 KO T cells were cannulated as well as four non-BMT controls. The effluent was pooled for quantification and FACS™.

Flow cytometry. The T cell, B cell, and granulocyte/macrophage constituency of TDL cells was measured using CD4 or CD8, CD45R/B220, and CD11a mAbs, respectively, obtained from PharMingen (San Diego, CA). TDLs were also assessed for the expression of activation antigens (IL2R alpha chain [CD25]; CD40 ligand [CD40L: gp39] CD69; CD80; CD86) or antigens associated with a memory cell phenotype (CD44; CD45Rb; ICAM-1 [CD54]; L-selectin [CD62L]) by 2 or 3 color flow cytometry using fluorescein isothiocyanate, phycoerythrin, or biotin- (along with SA-PerCP) conjugated mAb purchased from PharMingen or Becton-Dickinson (Mountain View, CA). Irrelevant mAb control values were subtracted from values obtained with relevant mAbs. All results were obtained using a FACScan™ (Becton-Dickinson). Forward and side scatter settings were gated to exclude red cells and debris. 7,000–10,000 cells were analyzed for each determination.

Histology. Tissues from the mice were placed in 10% formalin, imbedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Mitogen assay. Unfractionated spleen cells from either control mice or mice 4 and 8 d after BMT were plated in triplicate 96-well plates at the indicated concentrations with 10 ng/ml Con A (Sigma Chemical Co., St. Louis, MO) and 0.5 IU/ml recombinant human interleukin-2 (Hoffman La Roche Inc., Nutley, NJ) obtained from the NCI-FCRDC Repository (Frederick, MD). Cells were incubated for 4 d at 37°C with 5% CO_2 in a humidified incubator. On day 4, the cells were pulsed with 1μCi/well of ^{3}[H]thymidine (6.7 Ci/mmole sp. ac., New England Nuclear, Boston, MA) or the MTT (3-[4,5 dimethly-thiazol-2-yl] 2,5 diphenyl tetrazolium bromide (Boehringer Mannheim, Mannheim, Germany) dye, and incubated for an additional 24 h. The cells were harvested onto filters and incorporated radioactivity (cpm) was determined by scintillation counting or by spectrophotometric absorbance at 580 nm (OD) for MTT.

Lymphokine production. Unfractionated spleen cells (2×10^6 cells/well) were cultured in 24-well plates with 10 ng/ml Con A or PHA (Sigma Chemical Co.). Cells were incubated for 4 d at 37°C with 5% CO_2 in a humidified incubator. The cell-free supernatants were collected and frozen at −20°C. Frozen supernatants were thawed and assayed for cytokine production according to manufacturer's specifications. ELISA kits were purchased from Genzyme (Cambridge, MA) for determination of mouse IL-2 and R&D Systems (Minneapolis, MN) for determination of mouse IFN-γ. Mouse IL-4 ELISA was performed by the Lymphokine Testing Services (SAIC-Frederick, NCI-FCRDC).

Results

An absence of IFN-γ production by donor T cells results in accelerated morbidity from GVHD. We first assessed the role of IFN-γ on acute GVHD. IFN-γ KO mice (C57BL/6, $H2^d$) were used as donors for BMT with MHC-disparate B10, BR ($H2^k$) recipients. When IFN-γ KO mice were used as donors, the recipients succumbed rapidly to acute GVHD at a rate significantly ($P < 0.01$) greater than that of recipients of cells from control donors (Fig. 1 *A*). Similar results were obtained using another strain combination BALB/c ($H2^d$) into C57BL/6 ($H2^b$) (Fig. 1 *B*). Neutralizing antibodies to IFN-γ were also used to rule out the possibility that the lymphocytes from the KO mice were somehow altered in their development. The antibody was administered after BMT on days 0, 1, 2, and 3. In agreement with the data using the KO mice, the results demonstrate that recipients of T cells from normal MHC-disparate donors receiving the neutralizing antibodies to IFN-γ succumbed earlier to GVHD when compared with control recipi-

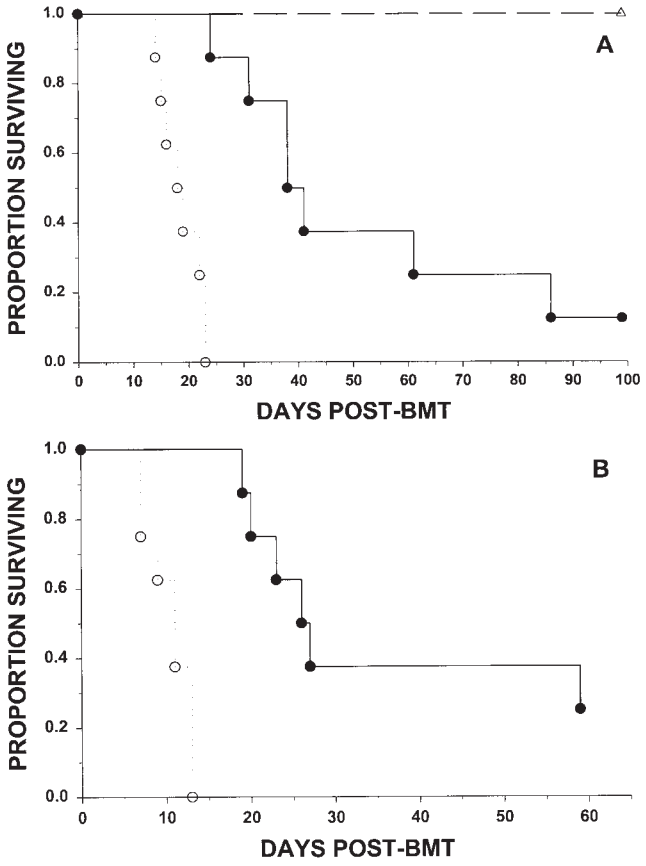

Figure 1. Effects of IFN-γ KO versus control donor cells on survival from GVHD. (*A*) B6 (●) and B6 IFN-γ KO (○) were used as donors into lethally irradiated B10.BR recipients. (△) B10.BR recipients receiving BALB/c BMC alone. (*B*) BALB/c (●) and BALB/c IFN-γ KO (○) were used as donors into lethally irradiated B6 recipients. All experiments had 10 mice per group. BMT performed as described in Methods. IFN-γ KO recipients had significantly ($P < 0.01$) decreased survival.

ents (Table I, Exp. 1). In all of the experiments, the recipients exhibited classical signs of acute GVHD: weight loss with significant pathology in the liver correlating with morbidity (Figs. 2 and 3). Thus, the absence of IFN-γ results in accelerated morbidity from GVHD after allogeneic BMT.

An absence of IL-4 production by donor T cells results in protection from GVHD. IL-4 is considered antagonistic to IFN-γ with regard to the generation of TH1 versus TH2 helper activity (13). IL-4 KO mice have been reported to exhibit higher levels of IFN-γ production in response to antigens (14). Therefore, IL-4 KO mice were used as donors to ascertain their effect on GVHD. The results indicate that recipients of cells from IL-4 KO mice had significantly greater survival when compared with mice receiving cells from control donors in both allogeneic strain combinations (BALB/c into B6 or B6 into B10.BR) (Fig. 4, *A* and *B*). However, the extent of the protection appeared modest compared with the acceleration of GVHD seen using the IFN-γ KO donors (Table I, Exp. 2). Therefore, the absence of IL-4 resulted in significant protection from GVHD.

To assess the effects of the IL-4 KO on T cell engraftment and in vivo expansion, we performed experiments involving

Table I. Role of IFN-γ on Survival from Acute GVHD

Experiment	Donor	Recipient	Treatment*	Day of death Mean±SD
1	BALB/c	B6	5% rat serum‡	45±8
	BALB/c	B6	Anti–IFN-γ‡	16±7§§
2	B6	BALB/c	None	10±2.1
	B6 IL-4 KO	BALB/c	None	> 25**

*BMT was performed as described in Methods. **All mice were alive after 25 d. ‡Mice received 5% rat serum or anti–IFN-γ (500 mg) in 0.2 ml PBS/animal/d by intraperitoneal injection on days 0 through 3 after bone marrow transplant. §§ $P \leq 0.002$ (ranks or ANOVA on ranks test as appropriate) significantly different from control.

thoracic duct cannulation of the recipients in which cells are removed 6 d after BMT. The results (Table II) demonstrate that comparable numbers of donor $CD4^+$ T cells could be detected 6 d after BMT, using either B6 or B6 IL-4 KO donors (0.7×10^6 cells/ml with 98% donor $CD4^+$ phenotype using control donors versus 1.1×10^6 cells/ml with a 99% donor $CD4^+$ T cell phenotype using IL-4 KO donors). Wild-type cells produced 16.8×10^6 per day and the IL-4 KO produced 26.4×10^6 per day (98–99% of which were $CD4^+$ in the two groups), representing a massive expansion considering only 2×10^6 $CD4^+$ T cells were infused on day 0 and not all T cells will circulate in the thoracic duct lymphatics in a 24-h time period. This data indicates that the mechanism by which the use of IL-4 KO donor cells results in delay of GVHD is not due to impairment of expansion of donor T cells. These results are the converse of the data obtained with the IFN-γ KO donors suggesting that the loss of IFN-γ is deleterious and loss of IL-4 protective in acute GVHD using MHC-disparate combinations.

Proliferative effects and cytokine production by mitogen-stimulated splenocytes. We then assessed the responsiveness of splenic T cells from the different cytokine KO mice to the mitogen, Con A. Splenocytes from BALB/c IFN-γ KO exhibited greater proliferative responses to the mitogen than splenocytes from BALB/c IL-4 KO or control BALB/c splenocytes (Table III). When supernatants from these cells were examined for cytokine levels, it was found that splenocytes from IFN-γ KO failed to produce IFN-γ, as expected (Table IV). Surprisingly, though, IL-2 levels were significantly higher

Table II. Characterization of Cells Recovered by Thoracic Duct Cannulation after BMT

	Untreated B6		Group 1 B6→bm12		Group 2 B6 IL-4 KO	
	4.4×10^6/ml		0.7×10^6/ml		1.1×10^6/ml	
#TDL/ml lymph % positive MFI	%	MFI	%	MFI	%	MFI
Donor CD4	29	518	98	877	99	876
$CD4^+$ $CD40L^+$	0.5	—	16	58	12	109
$CD4^+$ $IL\text{-}2R^+$	3	151	61	178	49	137
$CD4^+$ $B7\text{-}1^+$	1	61	3	43	2.5	68
$CD4^+$ $B7\text{-}2^+$	3	33	10	34	11	66

[a]Cells were recovered by TDL 6 d after BMT. [b]BMT and cannulation were performed as described in Methods.

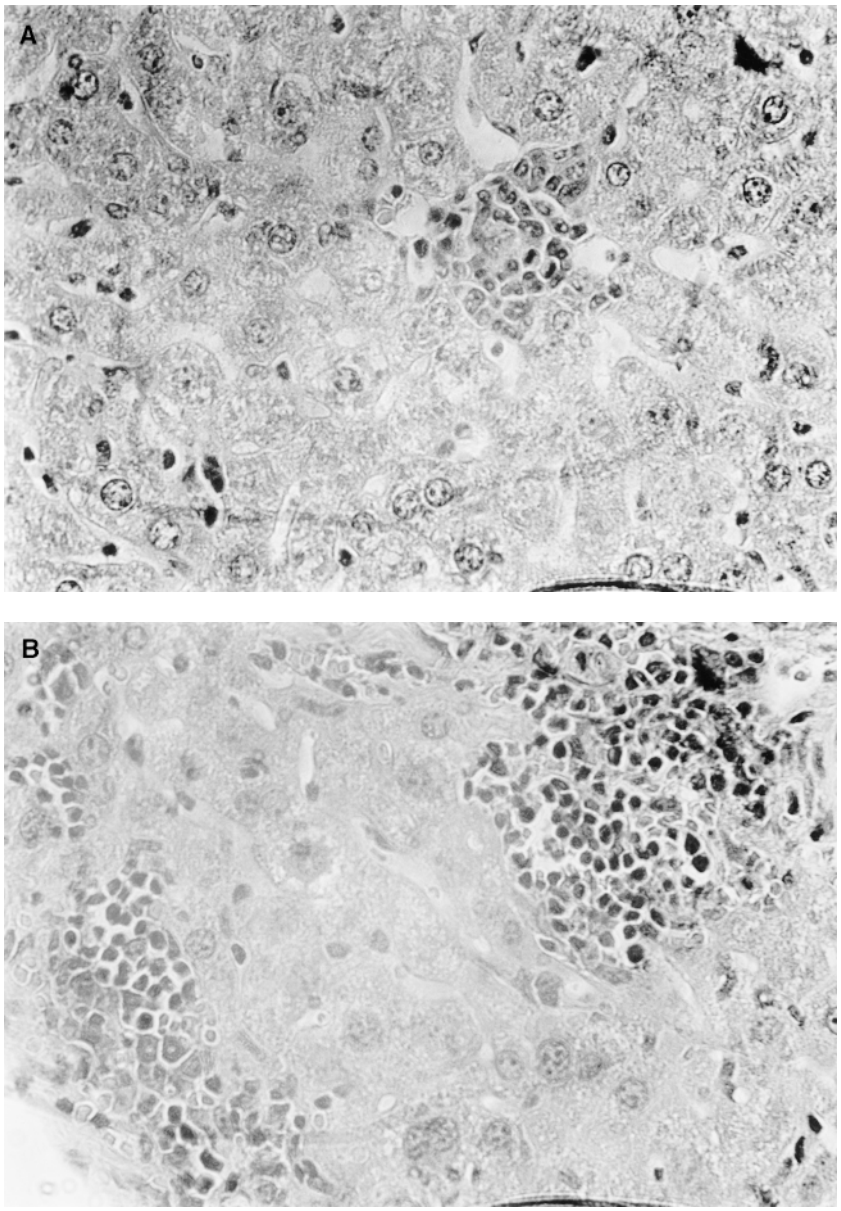

Figure 2. Histological evaluation of mice receiving allogeneic BMT and cells from IFN-γ KO mice. Livers from B6 mice receiving BALB/c IFN-γ KO versus wild-type control donor cells were examined 12 d after BMT. (*A*) A representative liver section from a recipient receiving control splenocytes. (*B*) A representative liver section from a recipient receiving IFN-γ KO cells. In both livers, particularly with the IFN-γ KO donor, extensive mononuclear cell infiltration can be seen. Magnification 400×.

in this group compared with control and IL-4 KO splenocytes (Table IV). Conversely, no detectable IL-4 was present in the supernatants from mitogen-activated IL-4 KO splenocytes. IL-2 and IFN-γ levels were comparable to control splenocytes. These results indicated that, rather than detecting a deficit of TH1-type cytokines from IFN-γ KO mice and a deficit of TH2-type cytokines from IL-4 KO splenocytes, mitogen-activated cells from IFN-γ KO T cells produce greater amounts of IL-2. This can at least partially explain the in vivo effects on GVHD using the IFN-γ KO donors.

To determine if this increase in IL-2 production could be at least partially responsible for the increased morbidity from GVHD using IFN-γ KO donors, we assessed IL-2 production from spleen cells of the recipient mice undergoing GVHD. In these experiments spleen cells were taken from mice 4 d after BMT. After mitogen stimulation with Con A, the supernatants were assessed for IL-2 levels. The results (Fig. 5) demonstrate that spleen cells from mice receiving IFN-γ KO cells had greater amounts of IL-2 upon further activation. Thus, the increased IL-2 production by IFN-γ KO mice can be correlated in vivo with greater GVHD in the recipients.

Discussion

Cytokines play a pivotal role in immune interactions, including pathogenic interactions as in GVHD, in which the alloreactive donor cells recognize and attack the immunocompromised host. There has been increasing debate concerning the role of TH1/TH2 cytokines in the genesis and pathology of acute GVHD. Acute GVHD is primarily cell mediated so the hypothesis that TH1-type cytokines (i.e., IFN-γ and IL-2), which promote cell-mediated immune responses would also augment GVHD was well founded (3). However, a number of murine studies have demonstrated that high dose IL-2 given immediately after an allogeneic transplant can be protective (6, 15). Additionally, IL-12, a potent inducer of IFN-γ, has also been shown to be protective in GVHD (8). Our results support

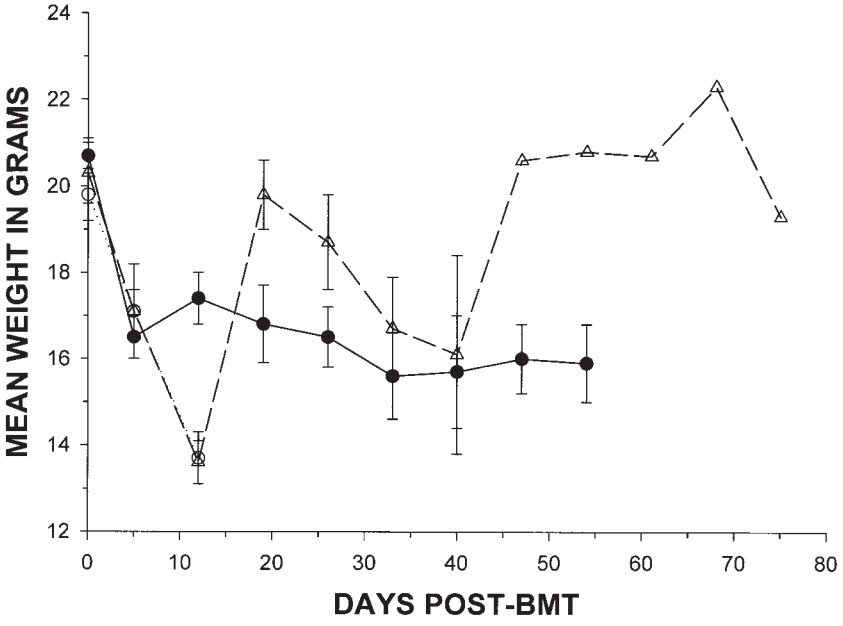

Figure 3. Weights from mice receiving allogeneic BMT. BALB/c (●), BALB/c IFN-γ KO (○), and BALB/c IL-4 KO (△) were used as donors into B6 recipients. BMT performed as described in Methods. This representative experiment had 10 mice per group. IFN-γ KO recipients had significantly ($P < 0.05$) lower weights versus control recipients. Conversely, IL-4 KO recipients had significantly greater ($P < 0.05$) weights at various times.

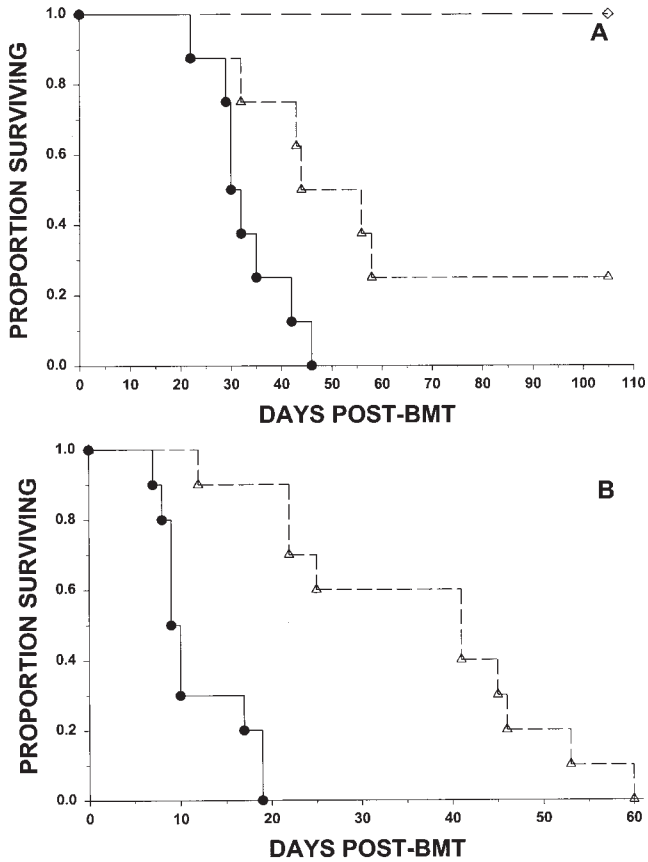

Figure 4. Effects of IL-4 KO versus control donor cells on survival from GVHD. (*A*) B6 (●) and B6 IL-4 KO (△) were used as donors into lethally irradiated B10.BR recipients. (◇) B10.BR recipients received B6 BMC alone. (*B*) BALB/c (●) and BALB/c IL-4 KO (△) were used as donors into lethally irradiated B6 recipients. All experiments had 10 mice per group. BMT performed as described in Methods. IL-4 KO recipients had significant ($P < 0.01$) increases in survival.

these data and suggest that, at least in the early stages of GVHD, TH1-type cytokines such as IFN-γ can play a significant role in ameliorating GVHD. The demonstration of delayed GVHD when cells from the IL-4 KO mouse were used also suggest that TH2-type cytokines can play a role in accelerating acute GVHD. Our in vivo data are also in agreement with the initial report characterizing the IFN-γ KO mouse in which increased responsiveness of the lymphocytes to alloantigens in vitro was observed (16). IFN-γ has been well documented to suppress T cell functions in vitro (17). This is now confirmed by us with regard to alloantigen reactions in vivo. Previous studies have found that systemic administration of IFN-γ can prevent GVHD, although the model used in those studies was more delayed with regard to onset than ours (18, 19). Additionally, in those reports it was postulated that exogenous IFN-γ administration worked by lowering endogenous production, suggesting that IFN-γ is actually deleterious in GVHD (19). The data shown here would argue for a more direct role for the suppressive activities of IFN-γ, as there is no

Table III. Mitogen Responsiveness of Splenocytes from IFN-γ KO and IL-4 KO Mice

Spleen cells	Cells/well ($\times 10^5$)	OD±SEM		CPM±SEM	
BALB/c	5.0	0.405	(0.030)	21321	(3976)
	2.5	0.142	(0.019)	7247	(1441)
	1.25			1563	(99)
BALB/c IL-4 KO	5.0	0.425	(0.285)	ND	
	2.5	0.088	(0.060)	ND	
BALB/c IFN-γ KO	5.0	0.766‡	(0.050)	41118‡	(3554)
	2.5	0.133	(0.054)	9883‡	(349)
	1.25			3108	(1086)

‡ $P = 0.003$ (Student's *t* test) significantly different from BALB/c control at the same level. ND, not done. Splenocytes were incubated with Con A at 10 ng/ml and after 72 h effects on proliferation were assessed by ^{3}[H]thymidine (CPM) or MTT (OD) incorporation.

Table IV. Lymphokine Analysis of Con A–stimulated Spleen Cells from IFN-γ KO and IL-4 KO Mice

	IL-2 (pg/ml)	IFN-γ (pg/ml)	IL-4 (pg/ml)
BALB/c	352±27	570±378	126±99
BALB/c IL-4 KO	1124±676	590±380	< 6±0
BALB/c IFN-γ KO	2357±862‡	< 2±0	144±109
B6	698±66	1071±403	54±19
B6 IFN-γ KO	2374±370‡	< 2±0	73±17

Splenocytes were incubated with Con A at 10 μg/ml and, after 4 d, the supernatants were collected and cytokine levels were determined. The results are the mean±SEM of three independent experiments. ‡$P <$ 0.05.

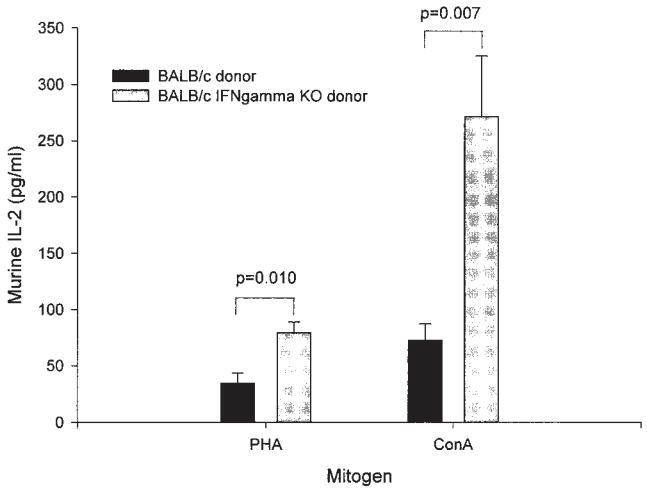

Figure 5. Mitogen-stimulated IL-2 production from day 14 BMT splenocytes. One and one quarter million splenocytes from B6 recipients of BALB/c or BALB/c IFN-γ KO donor cells were incubated for 96 h in 10 μg/ml Con A or 5 μg/ml PHA after which the supernatants were collected and murine IL-2 levels were determined. The results from five mice per group are expressed as the mean±SEM.

endogenous IFN-γ made by the alloreactive T cells. Previous attempts to neutralize IFN-γ with antibodies have suggested, but not conclusively shown, that IFN-γ can suppress acute GVHD (20). Our data is also in agreement with a recent report demonstrating that IFN-γ KO mice had decreased cardiac allograft survival compared with control recipients (21). In that study, the IFN-γ KO mice were used as recipients, and decreased graft survival was correlated with heightened IL-2 transcripts in the mononuclear cell infiltrates (21). In addition, in other models of autoimmunity, the use of IFN-γ KO mice has been demonstrated to result in accelerated or increased disease pathology (22, 23, 24). Interferon has also been suggested to play a critical role in the induction of tolerance (25). Thus, the immunosuppressive properties of interferon appear substantial and relevant to a variety of disease states. It will, therefore, be of interest to assess the effects of other TH1 cytokine knockouts (i.e., IL-12) in the generation of GVHD. Our data using the neutralizing antibodies support the data with the KO mice and suggest that the effects of an absence of IFN-γ in the KO is similar with control wild-type effector cells whose IFN-γ is neutralized.

The role of TH2-type cytokines in GVHD is also under considerable debate. It has been reported that TH2 cells and cytokines can both suppress and augment GVHD (9, 10, 11, 26, 27). It is clear that the model used to assess effects on GVHD may be key as some strain combinations (i.e., BALB/c into BALB/c × C57BL/6 F1) can result in a GVHD with autoimmune-like sequelae and antibodies to IL-4 have been reported to be protective in this instance (28). We have been unsuccessful in using anti-IL-4 antibodies to mimic the effects seen with the IL-4 KO cells but, in those studies, very large amounts were given (28). Moreover, the model used here in which there was total MHC incompatibility represents a more classical acute GVHD model without the autoimmune pathology seen in the parent into F1 model. However, IL-4 may play a role in the inflammatory response which may account for the protection seen using cells from the KO. Additionally, it has been reported that skewing to a TH1 or TH2 response can be dependent on background genes (i.e., BALB/c producing predominantly TH2 responses versus C57BL/10 or C57BL/6 backgrounds that produce TH1 responses) (24). The results presented here demonstrate similar effects of the cytokine in question in acute GVHD, regardless of the strain combination. Thus, the demonstration that the absence of IL-4 prolonged survival and absence of IFN-γ accelerated pathology in both strain combinations indicates the pivotal role of these cytokines in GVHD.

It is of interest that there were increases in IL-2 from spleen cells of both IFN-γ KO mice and mice receiving these cells undergoing GVHD. An increase in IL-2 production by the donor cells from IFN-γ KO mice would increase GVHD pathology. Although in vivo neutralization studies with IL-2 could confirm this, the requirement of IL-2 in T cell proliferative responses makes these studies difficult to draw firm conclusions from the comparison of such neutralization effects with control recipients.

It is still not known what role the conditioning process (i.e., irradiation) is playing in the outcome. Conditioning of the recipient clearly has a pivotal role in determining the onset and pathology seen in GVHD (30). Preliminary data indicates that using IFN-γ KO donors in a sublethal model results in protection from GVHD (data not shown). Additionally, it has been recently reported in a non-lethal GVHD model that cells from IFN-γ KO mice are also protective (31). This would indicate that the conditioning procedure, and subsequent inflammation resulting from it, can significantly alter the role of IFN-γ in GVHD. The radioprotective properties of IFN-γ may therefore play a role in protecting the recipient from the damage of radiation and GVHD. More work needs to be performed to determine if IFN-γ is actively suppressing the immune response, or is merely downregulating IL-2 production by the TH1 cells. Conversely, it needs to be determined whether IL-4 is promoting immune/inflammatory responses. The similar decreases in weight early post-BMT and increase in weight later post-BMT, in mice using IL-4 KO cells versus IFN-γ KO cells, suggests that IL-4 may be involved in GVHD pathogenesis, but perhaps at a later phase of the response than IFN-γ.

We have recently found that activated NK cells of donor type can suppress the occurrence of GVHD after allogeneic BMT (32). Similar to the studies with IL-2, the timing also appeared critical for protection to be seen (32). In these studies, it was found that the TGF-β was at least partially responsible

for the protection afforded by the NK cells (32). As NK cells can produce significant amounts of IFN-γ, it will be of interest to ascertain if IFN-γ production is also contributing to their suppressive effects on GVHD.

These results, therefore, suggest that IFN-γ can be protective and IL-4, detrimental, in acute GVHD in MHC-disparate BMT, although the complex roles of these cytokines in this disease needs to be further studied.

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