

Graft-versus-Host Disease and Graft-versus-Leukemic Effect in Allogeneic Bone Marrow Transplantation

Role of Interferon- γ

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ABBREVIATION

GVHD	Graft-versus-host disease
BMT	Bone marrow transplant
GVL	Graft-versus-leukemia

Interferon- γ (IFN- γ), a cytokine produced by activated type 1 T cells (Th1 and Tc1), NK, and NKT cells, is a potent stimulator of cell-mediated immunity. IFN- γ activates macrophages and stimulates the activity of NK cells and cytotoxic CD8 T cells and has been considered a pivotal pathogenic factor in acute graft-versus-host disease (GVHD). However, increasing evidence indicates that this cytokine is not required for, and can inhibit the development of, acute GVHD. Importantly, IFN- γ has been demonstrated to facilitate the graft-versus-leukemic (GVL) effect of allogeneic bone marrow transplantation. Although the mechanisms are incompletely understood at this point, current information on the role of IFN- γ in regulating the alloreactivity of donor T cells, and in the induction of GVHD and GVL effects, is reviewed in this article.

Introduction

T helper (Th) lymphocytes can be divided into 2 distinct subsets of effector cells based on their functional capabilities and the profile of cytokines they produce. The generation of Th1 or Th2 cells from Th precursor cells normally reflects the outcome of naive T cell activation. Th1 cells are defined by their production of Interferon- γ (IFN- γ) and tumor necrosis factor- β (lymphotoxin), and Th2 cells produce IL-4, IL-5, IL-6, and IL-13. Th0 cells, a common precursor cell for both Th1 and Th2 subsets, produce IL-2 and a mixture of the 2 cytokine patterns.¹⁻³ It has been shown that Th1 and Th2 cells exhibit differential responsiveness to IFN- γ . A functional IFN- γ receptor (IFN- γ R) requires 2 subunits, IFN- γ R α chain (IFN- γ R1 or CD119) and IFN- γ R β chain (IFN- γ R2). Although IFN- γ R2 plays only a minor role in ligand binding, it is necessary for IFN- γ signaling.^{4,5} Unlike Th precursors

and Th2 cells, Th1 cells, which are capable of producing IFN- γ after activation, do not respond to IFN- γ , due to the lack of IFN- γ R2 expression.^{4,5} IFN- γ has been shown to enhance IL-12 production, which thereby stimulates Th1 cell differentiation.^{6,7} However, signaling via IL-4, or with less potency, granulocyte-macrophage colony-stimulating factor, is required for IFN- γ to stimulate the production of the bioactive form (p70) of IL-12 by antigen-presenting cells, and stimulation with IFN- γ alone leads to secretion of the nonbioactive p40 homodimer form, antagonist IL-12.⁷ It is striking that the production of the bioactive p70 form of IL-12 is primarily controlled by IL-4, a Th2 cytokine.⁷⁻⁹

Cytolytic CD8⁺ effector T cells can also be classified into 2 subtypes based on their cytokine-producing profiles. Type 1 CD8⁺ T (Tc1) cells secrete IL-2 and IFN- γ , whereas type 2 CD8⁺ T (Tc2) cells produce IL-4, IL-5, and IL-13.^{10,11} In addition to the Th1

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and Tc1 cells, activated NK and NKT cells are also potent producers of IFN- γ .^{12,13} There has been increasing debate regarding the role of type 1 and type 2 T cell cytokines in the pathogenesis of acute graft-versus-host disease (GVHD) (for a review, see refs. 14-16). In this article, we have highlighted some of the murine studies on the role of IFN- γ in the development of GVHD and in the induction of graft-versus-leukemia (GVL) effects.

The Role of IFN- γ in the Induction of Lethal Acute GVHD

IFN- γ gene knockout mice provide a powerful tool for evaluating the role of IFN- γ in the pathogenesis of GVHD. By using these mice, 2 independent groups demonstrated in 1998 that IFN- γ production by the donor cells is not required for the development of lethal acute GVHD in irradiated mice after infusion of splenocytes and marrow cells from major histocompatibility complex (MHC)-mismatched allogeneic donors.^{17,18} Host-derived IFN- γ is also redundant in the induction of lethal acute GVHD, as the disease can be induced in the complete absence of this cytokine, that is, in IFN- γ -deficient mice after infusion of allogeneic cells from IFN- γ -deficient donors.¹⁷ In some situations, IFN- γ can even be protective for allogeneic bone marrow transplantation (BMT) recipients. It has been observed that T cells from IFN- γ -deficient donors induce more severe GVHD compared to that induced by IL-4 (a Th2 cytokine)-deficient donors.¹⁸ Marked down-modulation of donor CD4 T cell-mediated GVHD by IFN- γ has recently been demonstrated in a single MHC class II-mismatched murine BMT model.¹⁹ Administration of CD4⁺ T cells, along with marrow cells from IFN- γ -deficient C57BL/6 mice to lethally irradiated B6.C-H2^{bm12} (bm12) mice (disparate at class II), led to 100% death by 20 days, whereas all bm12 mice receiving a similar cell inoculum from IFN- γ wild-type C57BL/6 mice survived long-term.¹⁹ Similar to its effect on CD4 T cells, IFN- γ has also been demonstrated to inhibit GVHD induced by donor CD8 T cells. Although BALB/c (H-2L^{d4}) mice did not develop lethal GVHD after infusion of bone marrow and CD8 T cells from 2C mice that are transgenic for a host class I (H-2L^d)-specific T cell receptor,²⁰ administration of similar numbers of bone marrow

and CD8 T cells from IFN- γ -deficient 2C donors caused severe acute GVHD with 100% mortality.⁶⁸ It has been reported that administration of exogenous IFN- γ prevents GVHD in allogeneic BMT recipients.^{21,22}

In contrast to these results, it has also been reported that IFN- γ is critical for early GVHD lethality in a parent \rightarrow nonirradiated F1, C57BL/6 \rightarrow (C57BL/6xDBA/2)F1, model.²³ In this study, the recipient mice were transplanted with donor lymph node cells and splenocytes without hematopoietic stem cells. It therefore seems likely that hematopoietic failure, due to destruction of recipient hematopoietic cells, is a primary cause of the early mortality in this model. Indeed, despite the delayed mortality, administration of IFN- γ -deficient C57BL/6 splenocytes and lymph node cells resulted in greater weight loss and more severe destruction of parenchymal GVHD target tissues in (C57BL/6xDBA/2)F1 mice.²³ A recent study consistently demonstrated that in a C57BL/6 \rightarrow bm12 (class II only-mismatched) combination, IFN- γ is protective in lethally irradiated recipients of allogeneic donor marrow and T cells but deleterious in sublethally irradiated mice receiving allogeneic T cells only.¹⁹ This role of IFN- γ in eliminating recipient hematopoietic cells can actively be exploited to benefit in the setting of hematologic malignancies. It has been shown that lymphohematopoietic GVH reactions that selectively eliminate host lymphohematopoietic cells, including lymphoma cells, can be induced without severe systemic GVHD in allogeneic BMT recipients.²⁴⁻²⁶ IFN- γ is required for the induction of optimal anti-lymphohematopoietic GVH reactions while inhibiting GVHD in mice.⁶⁸ Such lymphohematopoietic GVH reactions might be beneficial in leukemic patients receiving allogeneic BMT if they predominantly eliminate host lymphohematopoietic cells.

Role of IFN- γ in IL-12-Mediated GVHD Protection

It has been previously demonstrated that a single injection of IL-12 at the time of BMT markedly inhibits the development of acute GVHD in mice.²⁷⁻²⁹ In murine acute GVHD models, serum levels of IFN- γ are increased in association with the activation of allogeneic donor T cells.^{30,31} However,

a single injection of IL-12 on the day of BMT completely alters the kinetics of IFN- γ production. Studies in the A/J \rightarrow B10²⁷ and CBD2F1 \rightarrow B6D2F1 (Yang et al., unpublished data) strain combinations showed that serum IFN- γ levels are markedly increased in IL-12-treated allogeneic BMT recipients on days 2 and 3 post-BMT, when IFN- γ is undetectable in untreated GVHD control mice. In contrast, by day 4, when high levels of IFN- γ are detected in sera of GVHD controls not treated with IL-12, IFN- γ becomes almost undetectable in sera of IL-12-treated mice. Unlike the untreated allogeneic BMT recipients, in which IFN- γ is primarily produced by activated T cells, the early IFN- γ in IL-12-treated mice is produced mainly by NK or NKT cells²⁷ (Dey, Yang, and Sykes, unpublished data). Further studies have demonstrated that this early IFN- γ production in response to IL-12 is required for inhibition of GVHD by IL-12. Administration of anti-IFN- γ mAb on day 1 post-BMT completely eliminated the protective effect of IL-12 against GVHD,³² and IL-12 was unable to inhibit GVHD induced by IFN- γ -deficient allogeneic T cells.¹⁷ IL-12 cannot prevent donor T cell activation and GVHD mortality in C57BL/6 mice transplanted with IFN- γ -deficient BALB/c T cells, whereas it does so effectively in C57BL/6 mice receiving IFN- γ ^{+/+} allogeneic donor cells.¹⁷

It has been reported that an environment with a high concentration of IFN- γ in the absence of IL-4 favors production of antagonistic IL-12.⁷ Thus, it is possible that the early IFN- γ production by NK cells in response to IL-12, before donor T cells have been activated, may inhibit the subsequent development of GVHD effector cells. On the other hand, Fas-mediated donor T cell apoptosis has been shown to be one of the likely mechanisms for inhibition of donor T cell activation and expansion in IL-12-treated allogeneic BMT recipients,²⁸ suggesting that IFN- γ may, directly or indirectly, regulate Fas expression or sensitivity to death through the Fas pathway, thereby inducing apoptosis of GVH effector cells. Consistent with this possibility, it has been reported that IFN- γ plays an important role in regulating Fas-mediated death of activated T lymphocytes.^{33,34} IFN- γ has also been demonstrated to facilitate the induction of allograft tolerance³⁵⁻³⁷ through mechanisms involving an

apoptosis-independent down-regulation of T cell proliferation.^{35,36} Such direct anti-proliferative function of IFN- γ may also contribute to the inhibition of donor T cell activation and expansion in allogeneic BMT recipients treated with a single dose of IL-12. In addition, inducible nitric oxide (iNO) has been shown to play an important role in IFN- γ -induced immunosuppression by inhibiting antigen-driven proliferation^{38,39} or inducing apoptosis⁴⁰⁻⁴² of antigen-specific T cells. It remains unknown whether or not iNO is also involved in the IFN- γ -dependent GVHD inhibition induced by a single injection of IL-12 at the time of BMT.

IFN- γ Is Required for Optimal Donor CD8 T Cell-Mediated GVL Effects in Allogeneic BMT Recipients

Potent GVL effects are an important benefit of allogeneic BMT in humans. To be of maximal clinical benefit, however, these must be achieved without severe GVHD. Studies using an EL4 (H-2^b) leukemia/lymphoma model showed that irradiated C57BL/6 recipient mice inoculated with EL4 leukemia and allogeneic A/J bone marrow and spleen cells can be simultaneously protected from both GVHD- and leukemia-induced mortality when IL-12 is given.³² Like the protective effect against GVHD, the GVL effect in IL-12-treated mice is also dependent on IFN- γ . Treatment with neutralizing mAb against IFN- γ on day 1 post-BMT attenuates the anti-tumor activity of allogeneic CD8 T cells in IL-12-treated allogeneic BMT recipients.³² Acute GVHD has proved to be largely CD4 T cell-dependent in most fully MHC plus multiple minor antigen-mismatched strain combinations in mice.⁴³⁻⁴⁸ In the A/J \rightarrow C57BL/6 combination, depletion of donor CD8 T cells by mAb does not prevent the development of acute GVHD. In contrast, substantial numbers of CD4-depleted donor spleen cells do not induce acute GVHD. However, GVL effects against EL4 leukemia are dependent on donor CD8⁺ cells and independent of CD4 T cells.^{32,43} Together, these studies indicate that IFN- γ is required for optimal CD8-mediated GVL effects and inhibition of CD4-induced GVHD in allogeneic BMT recipients treated with a single injection of IL-12 on the day of transplantation.

We have recently evaluated the role of IFN- γ in regulating the GVHD-inducing activity and GVL effects of CD8 T cells in mice not receiving IL-12 treatment. In these studies, C57BL/6 mice were lethally irradiated and transplanted with CD4-depleted (or purified CD8⁺) spleen cells and marrow cells from wild-type or IFN- γ -deficient BALB/c mice with or without host-type EL4 lymphoma cells.⁶⁸ Remarkably, the results demonstrate that the GVHD-inducing activity and GVL effects of allogeneic CD8 T cells can be separated by a single cytokine, IFN- γ . Compared to the IFN- γ wild-type CD8 T cells, IFN- γ -deficient donor CD8 T cells induce more severe systemic GVHD but weaker GVL effects against host-type lymphoma cells in allogeneic BMT recipients. IFN- γ has been shown to mediate anti-tumor effects by directly inhibiting tumor cell growth and inducing T cell-mediated anti-tumor responses.⁴⁹⁻⁵⁴ However, EL4 cells are not susceptible to an IFN- γ -mediated anti-proliferative effect in vitro and are highly sensitive to alloreactive CTLs from both IFN- γ ^{+/+} and IFN- γ -deficient BALB/c mice. Treatment with IFN- γ in vitro up-regulates the expression of Fas and MHC class I on EL4 cells, but only moderately increased the susceptibility of EL4 cells to the cytotoxicity of allogeneic CD8 T cells, consistent with the high expression of class I and Fas and the sensitivity to alloreactive CTLs of EL4 cells without exposure to IFN- γ . Therefore, the reduced GVL effect in mice transplanted with IFN- γ -deficient donor CD8 T cells is unlikely due to a lack of IFN- γ -mediated direct inhibition of leukemic cell growth, or to reduced killing of donor CTLs against EL4 cells.

It has been shown that IFN- γ plays an important role in regulating chemokine production and thereby directing the tissue infiltration of activated, including alloantigen-primed, T cells.⁵⁵⁻⁵⁹ Studies using an immunogenic tumor model demonstrated that the failure of cytolytic effectors ("tumor-antigen"-specific CD8 T cells) to remain at the site of the tumor is a major limitation in the ability of CD8 T cell responses to control tumor growth.⁶⁰ Contact-dependent lysis is also critical for alloreactive CTLs to mediate GVL effects in allo-BMT recipients. It has been reported that Tc1 cells are more efficient than Tc2 cells in migrating into the draining lymph nodes in vivo, and that the in vivo homing proper-

ties of IFN- γ -competent Tc1 cells differ from those of IFN- γ -deficient Tc1 cells.⁶¹ Moreover, it has been suggested that IFN- γ contributes to alloreactive donor T cell infiltrates in lymphoid tissues and lymphoid hypoplasia associated with GVHD,^{62,63} suggesting that IFN- γ may direct alloresponses toward the lymphohematopoietic system rather than the parenchymal GVHD target tissues. Thus, it is possible that the reduction of GVL effects in mice receiving IFN- γ -deficient donor cells reflects a lack of sufficient contact between donor CD8 T cells and the leukemic cells within the lymphohematopoietic system, and that the increased GVHD is due to increased T cell migration into the parenchymal GVHD target tissues.

Concluding Remarks

IFN- γ is primarily produced by activated T cells. Therefore, the level of IFN- γ in patients receiving allogeneic BMT may reflect ongoing alloresponses.⁶⁴⁻⁶⁷ However, the correlation between high levels of IFN- γ and severe GVHD does not necessarily reflect a harmful role of this cytokine in the pathogenesis of GVHD. Although controversies remain, it is clear that lethal acute GVHD can be induced in the absence of IFN- γ and, at least in some situations, that IFN- γ may inhibit the development of GVHD. Moreover, this cytokine is required for the induction of optimal GVL effects. Thus, global suppression of IFN- γ production should be avoided as an approach to preventing or treating GVHD in leukemic patients. Greater understanding of the mechanisms by which IFN- γ regulates the alloreactivity of donor T cells would facilitate the development of approaches to dissociating GVL effects from GVHD, and ultimately refine clinical protocols for the performance of HLA-mismatched allogeneic BMT in leukemic patients.

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