Dichotomous Role of Interferon-γ in Allogeneic Bone Marrow Transplant

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Abstract
Interferon (IFN)-γ is a pleiotropic cytokine with a central role in innate and adaptive immunity. As a potent pro-inflammatory and anti-tumor cytokine, IFN-γ is conventionally thought to be responsible for driving cellular immune response. On the other hand, accumulating evidence suggests that IFN-γ also has immuno-suppressive activity. An important role for IFN-γ in inhibiting graft-versus-host disease (GVHD) has been demonstrated in murine models, in spite of IFN-γ being one of the key factors amplifying T cell activation during the process of acute GVHD, the major complication and cause of post-transplant mortality in allogeneic bone marrow transplantation (BMT). At the same time, IFN-γ facilitates graft-versus-leukemia (GVL) activity. Dissociation of GVL effects from GVHD has been the ultimate goal of allogeneic BMT in the treatment of hematological malignancies. This paradoxical role of IFN-γ makes modulating its activity a promising strategy to maximize GVL while minimizing GVHD and improve clinical outcomes in BMT. In this review, the effects of IFN-γ on GVHD and GVL are discussed with consideration of the mechanism of IFN-γ action.

Keywords
IFN-γ; Graft-versus-host disease; Graft-versus-leukemia; Allogeneic bone marrow transplant

INTRODUCTION
The interferons were originally discovered as anti-viral agents and were classified into type I and type II according to receptor specificity and sequence homology [1,2]. IFN-γ is the sole type II IFN and is synthesized by CD4+ Th1 lymphocytes, CD8+ cytotoxic lymphocytes, NK cells, B cells, NK cells and antigen-presenting cells (APCs) [3–7]. The regulation of T-cell IFN-γ production includes induction by IL-12 and IL-18 and down-regulation by IL-4, IL-10, transforming growth factor-β and glucocorticoids [8–13]. Although the immune system appears to develop normally in the absence of pathogen, IFN-γ knock-out mice showed decreased ability in their resistance to bacterial, parasitic and viral infections, suggesting a pivotal role for IFN-γ in the induction of cellular immune response [14]. The current model for the activation of donor T-cells in pathogenesis of graft-versus-host disease (GVHD) implicates IFN-γ as a central regulatory cytokine in the initiation and maintenance of allo-reactivity in allogeneic hematopoietic progenitor cell transplantation (HPCT) [15,16].

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During the development of GVHD, IFN-γ mediates multiple effects including priming of macrophages to produce pro-inflammatory cytokines and nitric oxide (NO) in response to lipopolysaccharide (LPS), up-regulating expression of adhesion molecules, chemokines, major histocompatibility (MHC) II antigen and FAS on APC, resulting in enhanced antigen presentation and recruitment of effector cells [15–17]. Increased serum levels of IFN-γ were associated with the severity of acute GVHD in mice and anti-IFN-γ antibodies prevented gastrointestinal GVHD in a CBA→F1 mice model [18–21]. While all these data demonstrated that IFN-γ can amplify GVHD, IFN-γ has been shown to also limit GVHD, organ transplantation rejection and autoimmunity [18,22–26]. This paradoxical effects of IFN-γ on GVHD can be partially explained by the observation that IFN-γ can prevent T cell activation directly by inducing T cell growth arrest and apoptosis or indirectly by altering the function of dendritic cells (DC), the most efficient APC population [27–30]. The effect of IFN-γ on the immunological microenvironment is also mediated in part by IFN-γ-inducible genes that serve counter-regulatory roles in immune activation, including indoleamine-2, 3-dioxygenase (IDO), inducible nitric oxide synthase (iNOS) and heme oxygenase (HO)-1 that can limit activation of effector T-cells [30–32]. This review summarizes current knowledge of the role of IFN-γ on GVHD and GVL based primarily on results from murine model systems, but with attention to potential clinical translation of these findings.

Role of IFN-γ in GVHD and GVL

**Effects of exogenous administration of IFN-γ**—Based on the immuno-suppressive effects of IFN-γ, investigators have shown that administration of exogenous IFN-γ can prevent GVHD in murine BMT model [24], suggesting the clinical administration of exogenous IFN-γ as a novel strategy to prevent GVHD in allogeneic HPCT [33]. The effect of exogenous IFN-γ administration in murine BMT model systems varies by strain, timing of administration, and intensity of conditioning, which suggest in part the reasons for the conflicting results of the effect of this cytokine on acute GVHD [24,34]. For example, Brok et al. found that high levels of IFN-γ immediately after BMT is crucial for the prevention of GVHD. Treatment with 50,000 U IFN-γ twice weekly for a period of 5 weeks, starting at the day of BMT, was an optimal treatment protocol to prevent GVHD in a fully H-2-mismatched BMT model. A shorter treatment course (one week with a higher dose of IFN-γ), did not result in significantly improved survival compared to untreated control animals [24]. The timing of IFN-γ administration was a critical factor, since a delay of 3 days from the time of BMT resulted in substantial GVHD-induced mortality [24]. Similarly, as shown by Szebeni et al., administration of IFN-γ from day 2 onwards had no effect on GVHD [35].

**Factors that regulate T-cell production of IFN-γ**—The central role of donor T-cells in both GVHD and GVL effects has been demonstrated by the markedly reduced frequency and severity of GVHD but increased leukemia relapse in patients given allografts stringently depleted of T-cells [36]. Different methods targeting donor T-cells have been tested in efforts to limit GVHD while preserving GVL [15,37–42]. The administration of IL-12, a potent inducer of IFN-γ production, at the time of allo-BMT had a significant protective effect against GVHD which can be eliminated by anti-IFN-γ antibody or using IFN-γ-deficient T-cells [43–45]. Similar results were obtained from administration of IL-18, another potent inducer of IFN-γ production [46,47].

Of note, growing data has revealed the importance of both host-type and donor-type APCs in regulating T-cell activity in allogeneic BMT model [48–51]. Our group initially reported that large numbers of plasmacytoid DC precursors in donor bone marrow are associated with increased relapse after allogeneic BMT [52]. Recently, using an allogeneic murine BMT model (C57BL/6→B10.BR), the addition of donor IL-12-producing CD11b−APC to a graft composed of purified hematopoietic stem cells (HSC) and T-cells was shown to remarkably
improve long-term leukemia-free survival without increasing GVHD. Intriguingly, higher number of IFN-γ-producing donor T-cells was seen among recipients of CD11b− APC and all of the beneficial effects of donor CD11b− APC on post-transplant survival were abrogated when IFN-γ knock-out mice were used as T-cell donors (Table 1) [53–55]. These data are consistent with other murine models in which IFN-γ production enhances the GVL activity of donor T-cells while limiting GVHD mortality. In addition, IFN-γ can increase the sensitivity of tumor cells to CTL activity via up-regulation of FAS and MHC expression [22]. More recently, Wang et al. reported that IFN-γ could promote lymphohematopoietic graft-versus-host reactions (LGVHR) and GVL with limited GVHD effects [56].

**IFN-γ expression by NK and NKT cells**—Natural killer (NK) cells were initially described as radio-resistant host cells that mediated graft rejection in lethally irradiated F1 mice transplanted with parental type T-cell depleted bone marrow [57,58]. Recent studies have shown that NK cells have dual ability to mediate both bone marrow rejection (host NK cells) and to promote engraftment and GVL activity (donor NK cells). Asai et al. found that mice injected with activated NK cells of donor type survived longer with less GVHD. Furthermore, administration of activated NK cells resulted in significant GVL effects as evidenced by increased survival and fewer lung metastases in mice bearing a colon adenocarcinoma. The GVL effects were associated with high levels of IFN-γ from donor NK and could be partially abrogated by anti-IFN-γ antibody [7]. In addition, activated NK cells are able to prime DC to secrete IL-12 and to induce CD8 T cell memory response through an IFN-γ-dependent pathway [59,60]. However, other experiments demonstrated that DC activation by NK cells was mediated through direct cell to cell interaction [61]. Thus donor NK cells secreted IFN-γ can augment the anti-tumor activity of the allograft in direct and indirect mechanisms.

NKT cells have been demonstrated to regulate suppressive responses through local IFN-γ production in transplantation rejection, autoimmune diseases and GVHD [62]. In vitro expansion and transplantation of cytolytic CD8+ NKT cells reduced GVHD as compared to unfractionated donor splenocytes [6]. Type II NKT cells that lack Vα14Jα18 expression and are CD1d-restricted, protect against GVHD in an IFN-γ-dependent way compared to type I Vα14Jα18 TCR+ NKT cells [63,64].

**IFN-γ expression by CD25+CD4+ regulatory T cells**—IFN-γ is one of the major mediators of the immunosuppressive role of CD25+CD4+ regulatory T (T reg) cells [65,66]. Stimulation of ex vivo expanded T reg cells with alloantigen can induce rapid and transient IFN-γ production which causes immune suppression by multiple mechanisms. IFN-γ can directly inhibit T cell activation by inducing apoptosis or retarding T-cell proliferation. IFN-γ also acts on APC in close proximity to T reg limiting their ability to activate T-cells. IFN-γ enhances expression of iNOS and IDO, the release of which will subsequently prevent T cell proliferation and activation [31]. Several reports have shown that depletion of T reg cells accelerates GVHD, and addition of T reg cells reduces GVHD. In a murine BMT model, Negrin et al. showed that inoculation of donor T reg into tumor-bearing recipients inhibited expansion of alloreactive T-cells and GVHD. However, the percentage of IFN-γ-producing conventional T-cells and serum IFN-γ in mice that received grafts containing T reg cells were also decreased [67]. The differential effect of IFN-γ secreted by T reg in these models can be explained by the various roles of IFN-γ in different stages of GVHD. Very early expression of IFN-γ by T reg during the phase of T cell activation could lead to the initiation of counter-regulatory effects that limit the extent of T-cell alloactivity.

**IFN-γ expression by mesenchymal stem cells (MSCs)**—Accumulating data have showed that CD34+ fibroblast-like MSCs can be potently immunosuppressive and inhibit GVHD in murine BMT models. More intriguingly, MSCs expanded ex vivo have been used to successfully treat ongoing, acute and steroid-refractory GVHD in clinical trials [68,69].
Mechanisms of suppression of GVHD by MSCs involve their effects on other immune cells including T-cells, APCs, NK cells, and B cells [70,71]. A recent study from Zhang et al. showed that MSCs could not only drive immature DC or mature DC to escape from apoptosis, but also induce mature DC into a distinct regulatory DC population capable of inhibiting T-cell proliferation, activity and IFN-γ production through a Jagged-2-dependent mechanism [72]. Blockade of IFN-γ pathway, using IFN-γ knock out T cells or IFN-γ receptor deficient MSCs abolished the immunosuppressive effect of MSCs [71,73]. IFN-γ can also stimulate the production of IDO by MSCs, which in turn inhibited the proliferation of activated T or NK cells [74]. However, Shi et al. reported that IFN-γ is necessary but not sufficient for the immunosuppressive function of MSCs. IFN-γ must be present along with any one of three other proinflammatory cytokines, TNF-α, IL-1α, or IL-1β to induce immunosuppression by MSCs [73]. More recently, Polchert et al. found that MSCs, pre-treated with IFN-γ, were activated and could suppress GVHD more efficiently than MSCs that were not activated. The MSC activation was dependent on the magnitude of IFN-γ exposure, with increased IFN-γ exposure leading to increased MSC suppression of GVHD. These IFN-γ “activated MSC” present a new strategy for preventing GVHD using fewer MSC. Taken together, local production of IFN-γ by T reg, NK cells or activated conventional T-cells may induce host-type MSC to become activated and more immunosuppressive, thereby limiting GVHD [75]. The possibility that MSC may also limit GVL activity of donor T-cells is of some concern, a recent clinical trial of the pre-captive administration of MSC was characterized by an excess of early leukemia relapse [76] (Figure 1).

Molecular and cellular effects of IFN-γ

**IFN-γ limits expansion of allo-reactive T-cells**—IFN-γ can suppress GVHD by inhibiting activation and expansion of both donor CD4+ and CD8+ T-cells by inducing apoptosis and cell cycle arrest [22,77]. The mechanism of alloreactive T cell apoptosis induced by IFN-γ in transplant model is not completely clear. In vitro studies have indicated that upon IFN-γ treatment, FAS is upregulated and activated to induce apoptosis in a series of cell lines [78,79]. In vivo studies have consistently shown that IFN-γ regulates expression of both FAS/FASL on alloreactive donor T-cells. In addition, many other IFN-γ-regulated genes, including IDO on APC were also found to be involved in IFN-γ-induced apoptosis and will be discussed below.

Global gene expression profiles of livers after experimental allogeneic and syngeneic BMT using oligonucleotide microarray showed up-regulation of IFN-γ-inducible genes, including MHC class II molecules, and genes related to leukocyte trafficking at day 7 prior to the development of GVHD. At day 35 after allogeneic BMT, when hepatic GVHD was histologically evident, genes related to cellular effectors and acute-phase proteins were up-regulated, whereas genes largely related to metabolism and endocrine function were down-regulated [80]. These data suggested that the temporal sequence of increased expression of genes associated with the attraction and activation of donor T-cells induced by IFN-γ early after BMT is important in the initiation of GVHD.

**IFN-γ induces IDO expression in APC and host epithelial cells**—IDO is an intracellular heme-containing enzyme that catalyzes the essential amino acid tryptophan. IDO gene can be prominently up-regulated by IFN-γ in a promoter-dependent way through STAT1 and IRF-1 binding sites (GAS and ISRE) or by activation of PI3K, JNK or NF-κB [31,81]. Broad evidence supports IDO as an important mediator of peripheral tolerance induced by IFN-γ. IDO-mediated immunosuppression includes depletion of tryptophan in the microenvironment and local secretion of the products of tryptophan metabolism that have direct immuno-suppressive effect. For instance, kynurenine and 3-hydroxykynurenine are cytotoxic to human T-cells, B and NK cells. Other metabolic products 3-hydroxyanthranilic acid and
quinolinic acid are able to induce Th1 cell apoptosis [31,82]. In addition, IDO can also help maintaining T regs in their normal suppressive phenotype [83] (Figure 2). Interestingly, a recent study showed that transgenic expression of IDO resulted in increased tubular epithelial cell apoptosis in a FAS/FASL-dependent, caspase-8-mediated mechanism, suggesting that integration of a network of IFN-γ-regulated pathways can result in counter-regulatory effects including cell death [84]. Based on these interesting findings, use of a small molecule inhibitor of IDO D1-MT to reverse the immuno-suppression, has entered a phase I clinical trial.

By using IDO knock-out mice as recipients, Jasperson and colleagues demonstrated that without host IDO expression, mice experienced markedly higher GVHD morbidity, suggesting that IDO can act at sites of GVHD to decrease T-cell proliferation. Thus modulation of IDO synthesis in GVHD target organs may represent an interesting strategy for limiting gut GVHD [85]. Intriguingly, a recent clinical study showed that DCs and monocytes from HPCT patients developing acute GVHD were less able to up-regulate IDO on exposure to IFN-γ than healthy volunteers or those with milder GVHD [86]. However, the mechanism of IDO expression in DC on the regulation of T-cell alloactivity during GVHD is not completely understood. Recently, using a murine transplant model, we found that donor T cell-derived IFN-γ causes up-regulation of IDO in DCs, suggesting that time and tissue-dependent IFN-γ synthesis by donor T-cells may initiate counter-regulatory immune mechanisms that limit overall GVHD activity while permitting selective GVL effects (Figure 2,3).

CONCLUSION

The dual effects of IFN-γ on GVHD and GVL make understanding the role of local production of IFN-γ during the initial interaction of donor T-cells and NK cells with host- and donor-type APC critical to achieve the goal of separating GVL from GVHD. The effects of IFN-γ are dependent upon local concentrations in the lymph node or epithelial microenvironments, the timing of synthesis or exogenous administration, and the stage of the development of the allo-immune response in transplant. However, much remains unknown about the precise pathways of IFN-γ action and the reciprocal actions of IFN-γ derived from different cell subsets. Another challenge is to understand the mechanisms by which IFN-γ signaling and cellular effects integrate with other cytokines, since cells in vivo are not exposed to a single stimulus in isolation. Better murine models and analysis of local effects of the cytokine milieu on T-cells at multiple time points and tissue sites in the first few hours and days after clinical allogeneic HPCT will help elucidate the role of IFN-γ in inducing GVL activities while limiting GVHD. Understanding of these questions should lead to the development of novel strategies for patients undergoing BMT to maximize GVL with limited GVHD.

Acknowledgments

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Figure 1. IFN-γ-producing cells show different effects on GVHD and GVL.
Θ increase; ⊙ inhibit; ? unknown
Figure 2. Role of IFN-γ during the development of GVHD and GVL
The conditioning regimen leads to damage of host tissues and allows the translocation of LPS and other pro-inflammatory cytokines into the circulation, leading to the activation of donor T-cell by host APC[17]. Interaction between donor IL-12-producing APC with donor T cells can increase the IFN-γ production by activated T-cells. High local levels of IFN-γ subsequently induce IDO expression on donor APC [Lu et al. 2009, in preparation]. IDO⁺ APCs help maintain the tolerant phenotype of T-reg which inhibits the Th1 and Th17 polarization of donor T-cells, resulting in down-modulation of T-cell allo-reactivity and GVHD effect[83].
Figure 3. IFN-γ-induced IDO expression on host epithelial cells inhibits GVHD

Donor T-cells induce GVHD effects that cause target epithelial cell apoptosis [17,22]. IDO expression on host epithelial cells induced by IFN-γ from donor T-cells inhibits GVHD [85]. In addition, IFN-γ enhances GVL effect through both direct and indirect mechanisms, resulting in apoptosis of tumor cells [18].
Impact of cotransplant different donor APC subsets with HSC and T cells on Th1/Th2 polarization based on intracellular cytokine staining and serum cytokines in tumor-bearing recipient mice[55].

<table>
<thead>
<tr>
<th>Donor APC subsets</th>
<th>GVHD</th>
<th>GVL</th>
<th>Survival</th>
<th>IFN-γ</th>
<th>IL-4</th>
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<td>BM CD11b− APC</td>
<td>No change</td>
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<td>BM CD11b+ APC</td>
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↑/↓: increase/decrease compared to recipients transplanted with HSC, T-cells and no APC.