Interferon (IFN)-γ is a pleiotropic cytokine with a central role in innate and adaptive immunity. As a potent pro-inflammatory and antitumor 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 immunosuppressive activity. An important role for IFN-γ in inhibiting graft-versus-host disease (GVHD) has been demonstrated in murine models, despite IFN-γ being one of the key factors amplifying T cell activation during the process of acute GVHD (aGVHD), 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 hematologic malignancies. This paradoxic 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.

**INTRODUCTION**

The interferons (IFNs) were originally discovered as antiviral 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, natural killer (NK) cells, B cells, NKT cells, and antigen-presenting cells (APCs) [3-7]. The regulation of T cell IFN-γ production includes induction by interleukin (IL)-12 and IL-18 and downregulation by IL-4, IL-10, transforming growth factor (TGF)-β and glucocorticoids [8-13]. Although the immune system appears to develop normally in the absence of pathogens, IFN-γ knockout 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].

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), upregulating 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 (aGVHD) in mice and anti-IFN-γ antibodies prevented gastrointestinal (GI) GVHD in a CBA → F1 mice model [18-21]. Although all these data demonstrated that IFN-γ can amplify GVHD, IFN-γ has been shown to also limit GVHD, organ transplantation rejection, and autoimmune [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 immunologic microenvironment is also mediated in part by IFN-γ-inducible genes that serve counterregulatory 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 immunosuppressive effects of IFN-γ, investigators have shown that administration of exogenous IFN-γ can prevent GVHD in a murine bone marrow transplantation (BMT) model [24], suggesting the clinical administration of exogenous IFN-γ as a novel strategy to prevent GVHD in allogeneic hematopoietic progenitor cell transplantation (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 aGVHD [24,34]. For example, Brok et al. [24] 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 days, starting at the day of BMT, was an optimal treatment protocol to prevent GVHD in allogeneic hematopoietic progenitor cell transplantation (HPCT) [33]. 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 BM 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 (LFS) 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 posttransplant survival were abrogated when IFN-γ knockout 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, whereas it limits GVHD mortality. In addition, IFN-γ can increase the sensitivity of tumor cells to CTL activity via upregulation of FAS and major histocompatibility complex (MHC) expression [22]. More recently, Wang et al. [56] reported that IFN-γ could promote lymphohematopoietic graft-versus-host reactions (LGVHR) and GVL with limited GVHD effects.

**IFN-γ expression by NK and NKT cells**

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 BM [57,58]. Recent studies have shown that NK cells have the dual ability to mediate both BM rejection (host NK cells) and to promote engraftment and GVL activity (donor NK cells). Asai et al. [7] 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

<table>
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<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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<tr>
<td>BM CD11b+ APC</td>
<td>No change</td>
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↑/↓ indicates increase/decrease compared to recipients transplanted with HSC, T cells, and no APC; HSC, hematopoietic stem cell; GVL, graft-versus-leukemia; APC, antigen-presenting cells; GVHD, graft-versus-host disease; IFN, interferon; BM, bone marrow.
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. 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 antitumor 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 compared to unfractionated donor splenocytes [6]. Type II NKT cells that lack Vα14 Jα18 expression and are CD1d-restricted, protect against GVHD in an IFN-γ-dependent way compared to type I Vα14 Jα18 TCR+ NKT cells [63,64] (Figure 2).

**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 that the addition of T reg cells reduces GVHD. In a murine BMT model, Negrin et al. [67] showed that inoculation of donor T reg into tumor-bearing recipients inhibited expansion of allogeneic 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. 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 counterregulatory effects that limit the extent of T cell alloactivity (Figure 1).

**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 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. [72] showed that MSCs could not only drive immature DCs or mature DCs to escape from apoptosis, but also induce mature DCs into a distinct regulatory DC population capable of inhibiting T cell proliferation, activity, and IFN-γ production through a Jagged-2-dependent mechanism. Blockade of the 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. [73] 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. More recently, Polchert et al. [75] found that MSCs, pretreated 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. The possibility that MSC may also limit GVL activity of donor T cells is of some concern, a recent clinical trial of the preemptive administration of MSCs 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 models 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 shortly.

Global gene expression profiles of livers after experimental allogeneic and syngeneic BMT using oligonucleotide microarray showed upregulation 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 upregulated, whereas genes largely related to metabolism and endocrine function were downregulated [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 upregulated 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 immunosuppressive effects. 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 reg 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 counterregulatory effects including cell death [84]. Based on these interesting findings, use of a small molecule inhibitor of IDO D1-MT to reverse the immunosuppression, has entered a phase I clinical trial.

By using IDO knockout mice as recipients, Jasper-son and colleagues [85] 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. Intriguingly, a recent clinical study showed that DCs and monocytes from HPCT patients developing aGVHD were less able to upregulate 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 upregulation of IDO in DCs, suggesting that time and tissue-dependent IFN-γ synthesis by donor T cells may initiate counterregulatory immune mechanisms that limit overall GVHD activity, whereas it permits selective GVL effects (Figures 2 and 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 transplants. 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, because 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.

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