Review

Role of Interferon-γ in GVHD and GVL

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Interferon (IFN)-γ, a potent proinflammatory cytokine produced by multiple types of cells (e.g., activated T, NK and NKT cells), plays important and complex roles in both innate and adaptive immune responses. There may be a correlation between the IFN-γ level and GVHD severity in patients receiving allogeneic hematopoietic cell transplantation. However, such a correlation may just reflect the presence of large numbers of activated T cells, and does not necessarily imply a harmful role of IFN-γ in the pathogenesis of GVHD. There has been increasing experimental evidence that IFN-γ is not required and may even inhibit GVHD. Paradoxically, IFN-γ facilitates graft-versus-leukemic (GVL) effects. Thus, IFN-γ blockade is likely deleterious in patients after allogeneic hematopoietic cell transplantation, and not beneficial as previously suggested. *Cellular & Molecular Immunology*. 2005;2(5):323-329.

Key Words: GVHD, IFN-γ, leukemia, transplantation, T cell

Introduction

IFN-γ is a pleiotropic cytokine signaling through the IFN-γ receptor (IFN-γR), which is expressed on most cell types (1, 2). A functional IFN-γR consists of two subunits, IFN-γRα chain (IFN-γR1 or CD119) and IFN-γRβ chain (IFN-γR2). IFN-γR2 plays only a minor role in ligand binding, but it mediates IFN-γ signaling (3, 4). T helper (Th) lymphocytes can be divided into Th1 and Th2 subsets of effector cells and the generation of these Th subsets from Th precursor cells normally reflects the outcome of T cell activation. The ability to make IFN-γ is an important criterion to differentiate the two Th subsets: Th1 cells produce IFN-γ along with TNF-β (lymphotoxin); and Th2 cells produce IL-4, IL-5, IL-6, and IL-13. Th subsets also exhibit differential responsiveness to IFN-γ. Th1 cells, which are capable of producing IFN-γ, do not respond to IFN-γ due to the lack of IFN-γR2 expression (3, 4).

The terminology of Th1/Th2 subsets of CD4 T cells has been extended to other immune cell populations, such as cytolytic CD8 effector T cells and NK cells. Similar to Th subsets, type 1, but not type 2, CD8 cytotoxic T (5, 6) and NK (7) cells secrete IFN-γ. In addition to type 1 T and NK cells, antigen presenting cells also produce IFN-γ after being activated (8-10). However, different cell lineages may use distinct transcriptional mechanisms for regulating IFN-γ production. For instance, T-bet is required for control of IFN-γ production in CD4 and NK, but not in CD8 cells (11).

It has been suggested that type 1, but not type 2, cells are terminally differentiated cells, and that type 2 cells may further differentiate into fully matured type 1 cells (12). Although this argues with the current dogma of type 1 and type 2 cell differentiation (i.e., type 0-to-1 or 2), both type 1 and type 2 T cells are nevertheless activated T cells and mediate distinct immune responses. There has been increasing debate regarding the role of type 1 and type 2 T cells and cytokines in the pathogenesis of graft-versus-host disease (GVHD) (13-15). This review discusses the role of IFN-γ in the induction of GVHD and graft-versus-leukemia (GVL) effects by allogeneic hematopoietic cell transplantation (allo-HCT), with a focus on experimental studies in mice.

Immune regulation by IFN-γ

IFN-γ is vital for both innate and adaptive immunity. Of note, this cytokine is essential for the induction and regulation of anti-microbial and anti-tumor immune responses. IFN-γ- and IFN-γR-deficient mice demonstrate compromised immunity to multiple intracellular pathogens, indicating an important role for IFN-γ in the induction of cellular immune responses (16, 17). Deficiency in IFN-γ also increases the host susceptibility to the development of spontaneous and

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Received Oct 1, 2005. Accepted Oct 21, 2005.

inducible tumors (18). The mechanisms by which IFN-γ enhances anti-microbial and tumor immunity are multiple. In one such mechanism, for example, IFN-γ may improve antigen presentation and act directly on CD8 T cells to stimulate the development of CTLs (19-22).

There has been increasing evidence that IFN-γ also down-regulates or restricts immune responses. IFN-γ plays an important role in the maintenance of T cell homeostasis and eliminates activated CD4 (23-27) and CD8 (27-29) T cells by inducing apoptosis. IFN-γ promotes Fas-mediated activation-induced cell death (AICD) of CD4 T cells (30, 31). IFN-γ has also been found to restrain CD4 T cell activation *in vitro* by promoting caspase-8-dependent apoptosis of T cells through the transcriptional activity of Stat1, the major transcription factor activated by IFN-γR (23). *In vivo*, absence of IFN-γ leads to enhanced expansion and reduced death of antigen-specific $CD8^+$ T cells in a *Listeria* infection model (32). IFN-γ also plays an inhibitory role in memory T cell generation. IFN-γ producing effector Th1 cells are short-lived; in contrast, activated Th1 cells that do not produce IFN-γ can efficiently develop into CD4 memory T cells and are responsible for sustaining immune responses (27, 33). A recent study showed that IFN-γ controls the rate of memory CD8 T cell generation. In this study, inflammation prevented DC vaccine-induced acceleration of CD8 memory T cell generation in wild-type mice, but not in IFN-γR-deficient mice (34). Although the mechanisms remain largely unknown, these studies indicate that IFN-γ has a complex role in the regulation of immune responses.

Role of IFN-γ in GVHD and GVL

Allo-HCT is an effective therapeutic approach for the treatment of many otherwise fatal hematologic malignancies. Although reduced leukemic relapse rates resulting from GVL effects have been observed in patients receiving HLA-mismatched marrow compared to HLA-identical transplants, the high incidence of intractable GVHD presents an enormous obstacle to HLA-mismatched allo-HCT in humans (35, 36). T cells are the major effector cells of GVHD in allo-HCT recipients. The high incidence of leukemia relapse associated with T cell depletion indicates that efficient GVL effects are also largely dependent on donor T cells. Thus, methods that can selectively inhibit the GVHD-promoting activity of allogeneic T cells while preserving allogeneic T cell-mediated GVL effects would be ideal for the use of allo-HCT in the treatment of hematologic malignancies.

The role of IFN-γ in GVHD in lethally irradiated recipients

IFN-γ gene knockout mice provide a powerful tool for evaluating the role of IFN-γ in the pathogenesis of GVHD. Using IFN-γ knockout mice, two independent groups demonstrated in 1998 that IFN-γ production by the donor cells is not required for the development of lethal acute GVHD in MHC-mismatched allo-HCT experiments (37, 38). Host-derived IFN-γ is also not required, as GVHD can be induced in the complete absence of this cytokine, i.e., in a situation where both the recipient and donor are IFN-γ-deficient (37). Unexpectedly, IFN-γ can be protective for allo-HCT recipients. In a single MHC class II-mismatched murine allo-HCT model, IFN-γ significantly inhibits the induction of GVHD by donor CD4 T cells (39). Administration of CD4 T cells along with bone marrow 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. In contrast, all bm12 mice receiving a similar cell inoculum from IFN-γ wild-type C57BL/6 mice survived long-term (39). Similar to its effect on CD4 T cells, IFN-γ also inhibits GVHD induced by donor CD8 T cells. It has been shown in various murine allo-HCT models that infusion of IFN-γ-deficient allogeneic bone marrow and CD4-depleted spleen cells induces severe lethal GVHD, while the recipients of similar allo-HCT from wild-type donors develop only mild GVHD (40, 41). The protective effect of IFN-γ on CD8 GVHD was further demonstrated in a 2C TCR-transgenic mouse model, in which CD8 T cells from IFN-γ-deficient 2C mice transgenic for a host class I ($H\text{-}2L^d$)-specific T cell receptor (42) induced severe lethal GVHD in irradiated BALB/c ($H\text{-}2L^{d+}$) mice, whereas the recipients of CD8 T cells from IFN-γ wild-type 2C mice survived long term (40).

IFN-γ also mediates the protection against GVHD in IL-12-treated allo-HCT recipients. Studies using murine allo-HCT models have shown that a single injection of IL-12, a potent inducer of IFN-γ production, at the time of allo-HCT inhibits GVHD in lethally irradiated recipients (43-46). The effect of IL-12 treatment is IFN-γ-dependent because: 1) administration of neutralizing anti-IFN-γ mAb on day 1 post-HCT completely eliminates the protective effect of IL-12 against GVHD (44), and 2) IL-12 fails to inhibit GVHD induced by IFN-γ-deficient allogeneic T cells (37). Fas-mediated donor CD4 T cell apoptosis is one of the mechanisms involved in the inhibition of GVHD by IL-12 (45). Thus, IFN-γ may play an important role in IL-12-induced Fas expression and apoptosis of host-reactive donor T cells during GVHD induction.

IL-18, another potent inducer of IFN-γ production**,** has also been found to inhibit GVHD in lethally irradiated allo-HCT recipients (47, 48). Similar to the GVHD-inhibiting effect of IL-12 discussed above, the effect of IL-18 is also IFN-γ-dependent and requires Fas expression on donor T cells (47).

The role of IFN-γ in GVHD in non-myeloablative recipients

GVHD pathogenesis differs depending on the recipient conditioning. It has been reported that IFN-γ is critical for induction of early GVHD lethality in a parent → non-conditioned F1 (C57BL/6 → B6D2 (C57BL/6 × DBA/2)F1) model (49). In this study, the recipient mice were transplanted with donor lymph node and spleen cells without bone marrow cells, so that the inoculum contained minimal numbers of 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. Consistent with this possibility, the recipients of allo-HCT from IFN-γ-deficient donors showed greater weight loss and more severe destruction of parenchymal GVHD target tissues than those receiving allo-HCT from wild-type donors (49).

Similar results were also obtained from studies in a C57BL/6 → bm12 (class II only-mismatched) combination, in which IFN-γ was protective in lethally-irradiated recipients of allogeneic donor marrow and T cells, but deleterious in sublethally-irradiated mice receiving only allogeneic T cells (39). Recent studies have shown that IFN-γ facilitates GVH reactions that selectively eliminate the recipient lympho-hematopoietic cells while inhibiting GVHD in mice (40) (Wang et al., IFN-γ promotes lymphohematopoietic GVH reactions while attenuating GVHD in murine allogeneic hematopoietic cell transplantation models, Abstract presented at FOCIS 2005). These data indicate that IFN-γ is also protective against GVHD in allo-HCT recipients after non-myeloablative conditioning. The role of IFN-γ in selectively eliminating recipient hematopoietic cells can be exploited to benefit allo-HCT recipients with hematologic malignancies (see discussion below).

The role of IFN-γ in GVL effects

Potent GVL effects are an important benefit of allo-HCT in humans. In order to be of maximal clinical benefit, however, these effects must be achieved without severe GVHD. Studies using an EL4 (H-2^b) leukemia/lymphoma model showed that irradiated C57BL/6 mice that received EL4 leukemia and allo-HCT from A/J (H-2^a) donors can be simultaneously protected from both GVHD- and leukemia-induced mortality when IL-12 is given (44). Like the protective effect against GVHD, the GVL effect in IL-12-treated mice also depends on IFN-γ. Treatment with IFN-γ-neutralizing mAb attenuates the anti-tumor activity of allogeneic T cells in IL-12-treated allo-HCT recipients (44). Acute GVHD has been proven to be largely CD4 T cell-dependent in most fully MHC plus multiple minor antigen-mismatched strain combinations in mice (50-55). In the A/J → 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 (44, 50). Thus, the above described studies indicate that IFN-γ is required for optimal CD8-mediated GVL effects and inhibition of CD4-induced GVHD in IL-12-treated allo-HCT recipients.

IFN-γ is also required for the optimal induction of GVL effects of donor CD8 T cells in allo-HCT recipients not receiving IL-12 treatment (40). 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 (40). 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 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 recipients.

Possible mechanisms for separation of GVHD and GVL by IFN-γ

The role of IFN-γ in donor T cell apoptosis

Previous studies on GVHD inhibition by IL-12 and IL-18 revealed that IFN-γ may downregulate GVH responses by promoting Fas-mediated apoptosis of recipient antigen-activated donor T cells (37, 45, 47). This is supported by the observation that IFN-γ is required for IL-12-induced reduction of activated donor T cells in allo-HCT recipients (37). A recent study provided direct evidence demonstrating the role of IFN-γ in stimulating apoptosis of alloreactive CD8 T cells. In this study, donor CD8 T cell apoptosis was significantly inhibited/delayed in the recipients of allo-HCT from IFN-γ-deficient donors compared to those receiving allo-HCT from wild-type donors (41).

The role of IFN-γ in donor T cell proliferation

Like other cells, T cell proliferation (cell cycle traverse) is governed by the ordered activation of cyclin-dependent kinases (CDKs) (56). Studies using gene-targeted knockout mice and transgenic mice have demonstrated that the CDK inhibitor, $p27^{Kip1}$ plays a critical role in controlling T cell proliferation, and the lack of $p27^{Kip1}$ expression results in a significant increase in T cell numbers (57, 58). Naïve CD8 T cells have high expression of $P27^{Kip1}$ and low CDK6 and CDK2 kinase activity, whereas G0/G1 memory CD8 T cells have low expression of $P27^{Kip1}$ and high CDK6 kinase activity, the latter favoring rapid cell division (59). There is evidence that IFN-γ can prevent growth factor-induced down-regulation of $P27^{Kip1}$ and thereby inhibit cell proliferation (60). A recent study showed that blockade of B7-H1 and PD-1 markedly inhibits the expansion of anti-CD3-stimulated CD4 T cells by inhibiting cell cycle progression *via* an IFN-γ-dependent mechanism (61). These observations indicated a role for IFN-γ in regulation of T cell division, and raised a possibility that blockade of IFN-γ may result in over-proliferation of donor T cells in allogeneic recipients (37, 41).

The role of IFN-γ in regulatory T (T-reg) cell function

IFN-γ plays an important role in costimulatory blockade-induced allograft tolerance, in which T-reg cells are likely involved (62). Furthermore, IFN-γ production by alloantigen-reactive $CD4^+CD25^+$ T-reg cells is critical for their regulatory function *in vivo* (63). It was observed that the generation and function of $CD4^+CD25^+$ T-reg cells is markedly impaired in IFN-γ-deficient mice, and that *in vivo* neutralization of IFN-γ dramatically reduces the suppressive effect of alloantigen-specific T-reg cells (63). Thus, a reduction in the number

and/or function of T-reg cells might also contribute to the exacerbation of GVHD in allo-HCT recipients when IFN-γ is absent. However, it should be noted that IFN-γ has also been found to inhibit self-antigen-induced generation/activation of $CD4^+CD25^+$ T-reg cells (64). It is likely that IFN-γ may play a distinct role in regulation of T-reg cells, and its action might be controlled by factors such as the timing and quantity of IFN-γ production and the strength of antigen stimulation.

The effect of IFN-γ on tumor cells

Although the above described roles for IFN-γ in apoptosis, cell division and T-reg cell function may be involved in the inhibition of GVHD by this cytokine, they cannot explain the GVL-promoting effect of IFN-γ. It has been shown that IFN-γ can mediate anti-tumor effects by directly inhibiting tumor cell growth and inducing T cell-mediated anti-tumor responses (65-70). The potent anti-tumor effects of IFN-γ in IFN-γR-deficient mice inoculated with syngeneic melanoma cells demonstrated a direct *in vivo* cytotoxicity of IFN-γ on tumors (71). However, in the studies showing a role of IFN-γ in promoting GVL effects, the tumor cells (EL4) used are not susceptible to an IFN-γ-mediated anti-proliferative/cytotoxic effect (40). Similarly, the induction of anti-tumor effects by recipient leukocyte infusions (RLI) also requires IFN-γ, but does not involve a direct cytotoxicity of IFN-γ on tumor cells (72). These data indicate that IFN-γ may mediate anti-tumor effects in allo-HCT recipients through mechanisms independent of its direct cytotoxic activity on tumor cells. Among these, a mechanism involving direct interaction of IFN-γ with tumors is increasing the sensitivity of tumor cells to CTL activity *via* upregulation of Fas and MHC expression (40, 73, 74).

The role of IFN-γ in lymphohematopoietic GVH reactions

Studies both in human and in animal models have shown that a conversion from mixed to full allogeneic donor chimerism can occur without clinical GVHD in allogeneic recipients, demonstrating that lymphohematopoietic GVH reactions can be selectively preserved while the capability to mediate tissue GVHD is suppressed (75-77). Such GVH reactions directed against host lymphohematopoietic cells can eliminate host leukemic cells and lead to long-term remissions in recipients of allo-HCT who have lymphomas (77). IFN-γ has been shown to facilitate anti-lymphohematopoietic GVH reactions, while inhibiting GVHD in mice. In the absence of IFN-γ, donor T cells induce more severe lesions in parenchymal target tissues, but a decreased elimination of the recipient hematopoietic cells (40, 41). Thus, the distinct effect of IFN-γ on the induction of anti-lymphohematopoietic GVH reactions versus alloresponses causing parenchymal tissue GVHD is considered an important mechanism for IFN-γ-mediated separation of GVL from GVHD.

IFN-γ and anti-tumor immunity of NK and NKT cells

It has been reported that donor NK cells mediate GVL effects in recipients of allo-HCT (78-80). Experiments in murine models have shown that CD4- and CD8-double negative (DN) spleen cells, which do not mediate detectable anti-tumor activity when injected alone, are required for the optimal induction of GVL effects, which are donor CD8 T cell-dependent (40). The data imply that donor DN splenocytes act synergistically with donor CD8 T cells to augment the anti-leukemic alloreactivity, and such a role of DN splenocytes possibly reflects the anti-tumor effects of NK and/or NKT cells.

NK and NKT cells are essential effector cells of the innate immune system that mediate anti-tumor responses. Recent evidence demonstrates that NK and NKT cells also play an important role in the induction of adaptive T cell anti-tumor responses (81). It has been reported that dendritic cells (DCs) can elevate NK cell cytotoxic activity and IFN-γ production leading to enhanced innate anti-tumor immune responses (82). On the other hand, NK cells also activate DCs leading to improved T cell anti-tumor immunity and this process is largely dependent on NK cell production of IFN-γ (20-22). Taken together, these studies suggest that NK and NKT cells are likely involved in IFN-γ-mediated GVL effects in allo-HCT recipients.

Concluding remarks

IFN-γ plays critical and pleiotropic roles in regulation of GVH responses. Of note, this cytokine, at least in murine models, facilitates GVL effects while inhibiting GVHD in allo-HCT recipients. Much is unknown about mechanisms by which IFN-γ differentiates GVL- and GVHD-associated alloreactivity. It also remains to be determined whether the effect of IFN-γ on GVHD versus GVL depends on the cell types and timing of its production, and on the degree of donor-recipient MHC disparities. A better understanding of these questions should help develop novel strategies for patients with hematopoietic malignancies where maximal GVL effects will be harnessed with little or no clinical GVHD.

Acknowledgements

We thank Drs Jessica Sachs and Toshiki Saito for critical reading of the manuscript, and Luisa Raleza for expert assistance with the manuscript. Work from the authors' laboratory is supported by a research grant from American Cancer Society (RSG-03-227-01-LIB).

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