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

Role of Interferon-γ

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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 cystokine 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.

ABBREVIATION

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

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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-γR) requires 2 subunits, IFN-γRα chain (IFN-γR1 or CD119) and IFN-γRβ chain (IFN-γR2). Although IFN-γR2 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-γR2 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

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-$2L^{d+}$) 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 → nonirradiated F1, C57BL/6 → (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/6xDBA2)F1 mice.[23] A recent study consistently demonstrated that in a C57BL/6 → 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→B10[27] and CBD2F1→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 cells[27] (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-$\gamma^{+/+}$ 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] FN-γ has also been demonstrated to facilitate the induction of allograft tolerance[35-37] 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 apoptosis[40-42] 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→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 $IFN\text{-}\gamma^{+/+}$ 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 properties 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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References

1. Firestein GS, Roeder WD, Laxer JA, Townsend KS, Weaver CT, Hom JT, et al. A new murine CD4+ T cell subset with an unrestricted cytokine profile. **J Immunol 1989;143:518-25**.

2. Paliard X, de Waal Malefijt R, Yssel H, Blanchard D, Chretien I, Abrams J, et al. Simultaneous production of IL-2, IL-4, and IFN-gamma by activated human CD4+ and CD8+ T cell clones. **J Immunol 1988;141:849-55**.
3. Sad S, Mosmann TR. Single IL-2-secreting precursor CD4 T cell can develop into either Th1 or Th2 cytokine secretion phenotype. **J Immunol 1994;153:3514-22**.
4. Pernis A, Gupta S, Gollob KJ, Garfein E, Coffman RL, Schindler C, et al. Lack of interferon gamma receptor beta chain and the prevention of interferon gamma signaling in TH1 cells. **Science 1995;269:245-7**.
5. Bach EA, Szabo SJ, Dighe AS, Ashkenazi A, Aguet M, Murphy KM, Schreiber RD. Ligand-induced autoregulation of IFN-gamma receptor β chain expression in T helper cell subsets. **Science 1995;270:1215-8**.
6. Hayes MP, Wang J, Norcross MA. Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-gamma of lipopolysaccharide-inducible p35 and p40 genes. **Blood 1995;86:646-50**.
7. Hochrein H, O'Keeffe M, Luft T, Vandenabeele S, Grumont RJ, Maraskovsky E, et al. Interleukin (IL)-4 is a major regulatory cytokine governing bioactive IL-12 production by mouse and human dendritic cells. **J Exp Med 2000;192:823-33**.
8. Ebner S, Ratzinger G, Krosbacher B, Schmuth M, Weiss A, Reider D, et al. Production of IL-12 by human monocyte-derived dendritic cells is optimal when the stimulus is given at the onset of maturation, and is further enhanced by IL-4. **J Immunol 2001;166:633-41**.
9. Biedermann T, Zimmermann S, Himmelrich H, Gumy A, Egeter O, Sakrauski AK, et al. IL-4 instructs TH1 responses and resistance to Leishmania major in susceptible BALB/c mice. **Nat Immunol 2001;2:1054-60**.
10. Sad S, Marcotte R, Mosmann TR. Cytokine-induced differentiation of precursor mouse CD8+ T cells into cytotoxic CD8+ T cells. **Immunity 1995;2:271-9**.
11. Fowler DH, Breglio J, Nagel G, Eckhaus MA, Gress RE. Allospecific CD8+ Tc1 and Tc2 populations in graft-versus-leukemia effect and graft-versus-host disease. **J Immunol 1996;157:4811-21**.
12. Perussia B. Lymphokine-activated killer cells, natural killer cells and cytokines. **Curr Opin Immunol 1991;3:49-55**.
13. Godfrey DI, Hammond KJ, Poulton LD, Smyth MJ, Baxter AG. NKT cells: facts, functions and fallacies. **Immunol Today 2000;11:573-83**.
14. Krenger W, Hill GR, Ferrara JLM. Cytokine cascades in acute graft-versus-host disease. **Transplantation 1997;64:553-8**.
15. Murphy WJ, Blazar BR. New strategies for preventing graft-versus-host-disease. **Curr Opin Immunol 1999;11:509-15**.
16. Yang YG. The role of interleukin-12 and interferon-γ in GVHD and GVL. **Cytokines Cell Mol Ther 2000;6:41-6**.
17. Yang YG, Dey BR, Sergio JJ, Pearson DA, Sykes M. Donor-derived interferon γ is required for inhibition of acute graft-versus-host disease by interleukin 12. **J Clin Invest 1998;102:2126-35**.
18. Murphy WJ, Welniak LA, Taub DD, Wiltrout RH, Taylor PA, Vallera DA, et al. Differential effects of the absence of interferon-γ and IL-4 in acute graft-versus-host disease after allogeneic bone marrow transplantation in mice. **J Clin Invest 1998;102:1742-8**.
19. Welniak LA, Blazar BR, Anver MR, Wiltrout RH, Murphy WJ. Opposing roles of interferon-gamma on CD4+ T cell-mediated graft-versus-host disease: effects of conditioning. **Biol Blood Marrow Transplant 2000;6:604-12**.
20. Dey B, Yang YG, Preffer F, Shimizu A, Swenson K, Dombkowski D, et al. The fate of donor T-cell receptor transgenic T cells with known host antigen specificity in a graft-versus-host disease model. **Transplantation 1999;68:141-9**.
21. Brok HP, Heidt PJ, van der Meide PH, Zurcher C, Vossen JM. Interferon-gamma prevents graft-versus-host disease after allogeneic bone marrow transplantation in mice. **J Immunol 1993;151:6451-9**.
22. Brok HP, Vossen JM, Heidt PJ. Interferon-gamma-mediated prevention of graft-versus-host disease: development of immune competent and allotolerant T cells in chimeric mice. **Bone Marrow Transplant 1997;19:601-6**.
23. Ellison CA, Fischer JM, HayGlass KT, Gartner JG. Murine graft-versus-host disease in an F1-hybrid model using IFN-γ gene knockout donors. **J Immunol 1998;161:631-40**.
24. Sykes M, Sheard MA, Sachs DH. Graft-versus-host-related immunosuppression is induced in mixed chimeras by alloresponses against either host or donor lymphohematopoietic cells. **J Exp Med 1988;168:2391-6**.
25. Pelot MR, Pearson DA, Swenson K, Zhao G, Sachs J, Yang YG, et al. Lymphohematopoietic graft-vs.-host reactions can be induced without graft-vs.-host disease in murine mixed chimeras established with a cyclophosphamide-based nonmyeloablative conditioning regimen. **Biol Blood Marrow Transplant 1999;5:133-43**.
26. Spitzer TR, McAfee S, Sackstein R, Colby C, Toh HC, Multani P, et al. Intentional induction of mixed chimerism and achievement of antitumor responses after nonmyeloablative conditioning therapy and HLA-matched donor bone marrow transplantation for refractory hematologic malignancies. **Biol Blood Marrow Transplant 2000;6:309-20**.
27. Sykes M, Szot GL, Nguyen PL, Pearson DA. Interleukin-12 inhibits murine graft-versus-host disease. **Blood 1995;86:2429-38**.
28. Dey BR, Yang YG, Szot GL, Pearson DA, Sykes M. Interleukin-12 inhibits graft-versus-host disease through an Fas-mediated mechanism associated with alterations in donor T-cell activation and expansion. **Blood 1998;91:3315-22**.
29. Yang YG, Dey B, Sergio JJ, Sykes M. Interleukin-12 prevents severe acute graft-versus-host disease (GVHD) and GVHD-associated immune dysfunction in a fully major histocompatibility complex haplotype-mismatched murine bone marrow transplantation model. **Transplantation 1997;64:1343-52**.
30. Szebeni J, Wang MG, Pearson DA, Szot GL, Sykes M. IL-2 inhibits early increases in serum gamma interferon levels associated with graft-versus-host-disease. **Transplantation 1994;58:1385-93**.
31. Wang MG, Szebeni J, Pearson DA, Szot GL, Sykes M. Inhibition of graft-versus-host disease by interleukin-2 treatment is associated with altered cytokine production by expanded graft-versus-host-reactive CD4+ helper cells. **Transplantation 1995;60:481-90**.
32. Yang YG, Sergio JJ, Pearson DA, Szot GL, Shimizu A, Sykes M. Interleukin-12 preserves the graft-versus-leukemia effect of allogeneic CD8 T cells while inhibiting CD4-dependent graft-versus-host disease in mice. **Blood 1997;90:4651-60**.
33. Novelli F, D'Elios MM, Bernabei P, Ozmen L, Rigamonti L, Almerigogna F, et al. Expression and role in apoptosis of the alpha- and beta-chains of the IFN-gamma receptor on human Th1 and Th2 clones. **J Immunol 1997;159:206-13**.
34. Oyaizu N, McCloskey TW, Than S, Hu R, Kalyanaraman VS, Pahwa S. Cross-linking of CD4 molecules upregulates Fas antigen expression in lymphocytes by inducing interferon-γ and tumor necrosis factor-α secretion. **Blood 1994;84:2622-31**.
35. Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. **Science 1993;259:1739-42**.
36. Hassan AT, Dai Z, Konieczny BT, Ring GH, Baddoura FK, Abou-Dahab LH, et al. Regulation of alloantigen-mediated T-cell proliferation by endogenous interferon-gamma: implications for long-term allograft acceptance. **Transplantation 1999;68:124-9**.
37. Markees TG, Phillips NE, Gordon EJ, Noelle RJ, Shultz LD, Mordes JP, et al. Long-term survival of skin allografts induced by donor splenocytes and anti-CD154 antibody in thymectomized mice requires CD4(+) T cells, interferon-γ, and CTLA4. **J Clin Invest 1998;101:2446-55**.
38. Willenborg DO, Fordham SA, Staykova MA, Ramshaw IA, Cowden WB. IFN-gamma is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: a possible role for nitric oxide. **J Immunol 1999;163:5278-86**.
39. Koblish HK, Hunter CA, Wysocka M, Trinchieri G, Lee WM. Immune suppression by recombinant interleukin (rIL)-12 involves interferon gamma induction of nitric oxide synthase 2 (iNOS) activity: inhibitors of NO generation reveal the extent of rIL-12 vaccine adjuvant effect. **J Exp Med 1998;188:1603-10**.
40. Tarrant TK, Silver PB, Wahlsten JL, Rizzo LV, Chan CC, Wiggert B, et al. Interleukin 12 protects from a T helper type 1-mediated autoimmune disease,

experimental autoimmune uveitis, through a mechanism involving interferon-γ, nitric oxide, and apoptosis. **J Exp Med 1999;189:219-30**.

41. Lasarte JJ, Corrales FJ, Casares N, Lopez-Diaz de Cerio A, Qian C, Xie X, et al. Different doses of adenoviral vector expressing IL-12 enhance or depress the immune response to a coadministered antigen: the role of nitric oxide. **J Immunol 1999;162:5270-7**.
42. Whitcup SM, Rizzo LV, Lai JC, Hayashi S, Gazzinelli R, Chan CC. IL-12 inhibits endotoxin-induced inflammation in the eye. **Eur J Immunol 1996;26:995-9**.
43. Sykes M, Abraham VS, Harty MW, Pearson DA. IL-2 reduces graft-versus-host disease and preserves a graft-versus-leukemia effect by selectively inhibiting CD4+ T cell activity. **J Immunol 1993;150:197-205**.
44. Korngold R, Sprent J. Surface markers of T cells causing lethal graft-vs-host disease to class I vs class II H-2 differences. **J Immunol 1985;135:3004-10**.
45. Vallera DA, Soderling CC, Kersey JH. Bone marrow transplantation across major histocompatibility barriers in mice: III. Treatment of donor grafts with monoclonal antibodies directed against Lyt determinants. **J Immunol 1982;128:871-5**.
46. Uenaka A, Mieno M, Kuribayashi K, Shiku H, Nakayama E. Effector cells of lethal graft-versus-host disease (GVHD) in nude mice. **Transplant Proc 1989;21:3031-2**.
47. Thiele DL, Charley MR, Calomeni JA, Lipsky PE. Lethal graft-vs-host disease across major histocompatibility barriers: requirement for leucyl-leucine methyl ester sensitive cytotoxic T cells. **J Immunol 1987;138:51-7**.
48. Palathumpat V, Dejbachsh-Jones S, Strober S. The role of purified CD8+ T cells in graft-versus-leukemia activity and engraftment after allogeneic bone marrow transplantation. **Transplantation 1995;60:355-61**.
49. Nastala CL, Edington HD, McKinney TG, Tahara H, Nalesnik MA, Brunda MJ, et al. Recombinant IL-12 administration induces tumor regression in association with IFN-γ production. **J Immunol 1994;153:1697-706**.
50. Brunda MJ, Sulich V, Bellantoni D. The anti-tumor effect of recombinant interferon alpha and gamma is influenced by tumor location. **Int J Cancer 1987;40:807-10**.
51. Sayers TJ, Wiltrout TA, McCormick K, Husted C, Wiltrout RH. Antitumor effects of alpha-interferon and gamma-interferon on a murine renal cancer (Renca) in vitro and in vivo. **Cancer Res 1990;50:5414-20**.
52. Brunda MJ, Luistro L, Hendrzak JA, Fountoulakis M, Garotta G, Gately MK. Role of interferon-γ in mediating the antitumor efficacy of interleukin-12. **J Immunother Emphasis Tumor Immunol 1995;17:71-7**.
53. Tannenbaum CS, Wicker N, Armstrong D, Tubbs R, Finke J, Bukowski RM, et al. Cytokine and chemokine expression in tumors of mice receiving systemic therapy with IL-12. **J Immunol 1996;156:693-9**.
54. Tan J, Crucian BE, Chang AE, Aruga E, Aruga A, Dovhey SE, et al. Interferon-gamma-inducing factor elicits antitumor immunity in association with interferon-gamma production. **J Immunother 1998;21:48-55**.
55. Liao F, Rabin RL, Yannelli JR, Koniaris LG, Vanguri P, Farber JM. Human Mig chemokine: biochemical and functional characterization. **J Exp Med 1995;182:1301-14**.
56. Luster AD, Weinshank RL, Feinman R, Ravetch JV. Molecular and biochemical characterization of a novel gamma-interferon-inducible protein. **J Biol Chem 1988;263:12036-43**.
57. Luster AD, Ravetch JV. Biochemical characterization of a gamma interferon-inducible cytokine (IP-10). **J Exp Med 1987;166:1084-97**.
58. Tran EH, Prince EN, Owens T. IFN-γ shapes immune invasion of the central nervous system via regulation of chemokines. **J Immunol 2000;164:2759-68**.
59. Koga S, Auerbach MB, Engeman TM, Novick AC, Toma H, Fairchild RL. T cell infiltration into class II MHC-disparate allografts and acute rejection is dependent on the IFN-gamma-induced chemokine Mig. **J Immunol 1999;163:4878-85**.
60. Shrikant P, Mescher MF. Control of syngeneic tumor growth by activation of CD8+ T cells: efficacy is limited by migration away from the site of induction of nonresponsiveness. **J Immunol 1999;162:2858-66**.
61. Helmich BK, Dutton RW. The role of adoptively transferred CD8 T cells and host cells in the control of the growth of the EG7 thymoma: factors that determine the relative effectiveness and homing properties of Tc1 and Tc2 effectors. **J Immunol 2001;166:6500-8**.
62. Klimpel GR, Annable CR, Cleveland MG, Jerrells TR, Patterson JC. Immunosuppression and lymphoid hypoplasia associated with chronic graft versus host disease is dependent upon IFN-gamma production. **J Immunol 1990;144:84-93**.
63. Parfrey NA, El-Sheikh A, Monckton EA, Cockfield SM, Halloran PF, Linetsky E. Interferon-gamma gene expression during acute graft-versus-host disease: relationship to MHC induction and tissue injury. **J Pathol 1999;189:99-104**.
64. Niederwieser D, Herold M, Woloszczuk W, Aulitzky W, Meister B, Tilg H, et al. Endogenous IFN-gamma during human bone marrow transplantation. Analysis of serum levels of interferon and interferon-dependent secondary messages. **Transplantation 1990;50:620-5**.
65. Dickinson AM, Sviland L, Hamilton PJ, Usher P, Taylor P, Jackson G, et al. Cytokine involvement in predicting clinical graft-versus-host disease in allogeneic bone marrow transplant recipients. **Bone Marrow Transplant 1994;13:65-70**.
66. Tanaka J, Imamura M, Kasai M, Sakurada K, Miyazaki T. Cytokine gene expression after allogeneic bone marrow transplantation. **Leuk Lymphoma 1995;16:413-8**.
67. Das H, Imoto S, Murayama T, Mizuno I, Sugimoto T, Taniguchi R, et al. Kinetic analysis of cytokine gene expression in patients with GVHD after donor lymphocyte infusion. **Bone Marrow Transplant 2001;27:373-80.**
68. Yang YG, Qi J, Wang MG, Sykes M. Donor derived interferon-K separates graft-versus-leukemia effects and graft-versus-host disease induced by donor CD8 T cells. **Blood 2002; 99 (in press).**