

The regulation and activity of interleukin-12

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1. ABSTRACT

Interleukin-12 (IL-12) is a key cytokine in the development of T helper type 1 (Th1) cell polarization, and its production of IL-12 is redundantly regulated. An important pro-inflammatory cytokine, IL-12 has been shown to have potent immunomodulatory, antitumor, and anti-infection activity *in vitro* and *in vivo*. Therefore, following a series of promising results from preclinical animal models experiments, researchers have begun to explore the clinical use of recombinant human IL-12 (rhIL-12) for treating a variety of diseases. In a series of phase I and phase II clinical trials related to cancer, viral infections, and hematopoietic stem cell transplants (HSCT), various strategies of rhIL-12 administration have been used with promising preliminary clinical results associated with tolerable toxicities.

2. INTRODUCTION

Interleukin-12 (IL-12) is a cytokine that plays a key role in the regulation of innate and adaptive immune responses (1). IL-12 is secreted by B cells, macrophages/monocytes, dendritic cells (DC), as well as other antigen-presenting cells (APC) (2). IL-12 stimulates polarization of naïve T helper (Th) cells into Th1 cells while inhibiting the activation of Th2 cells. IL-12 induces Th1 associated responses by stimulating T cells and natural killer (NK) cells to produce interferon- γ (IFN- γ), and enhances the cytolytic activity of macrophages, T cells, and NK cells (3-7). IL-12 displays potent immuno-modulatory and antitumor activity in preclinical murine models (8-11). After a series of preclinical evaluations, recombinant human IL-12 (rhIL-12) is being tested in early phase clinical trials to evaluate its safety and efficacy in treating a variety of conditions.

IL-12 is a member of the Th1 cytokine family, which consists of IL-12p70, IL-23, IL-12p80, IL-27 and IL-35, all of which are further classified as part of the type 1 cytokine superfamily. Yet, IL-12 is distinguished from other type 1 cytokines because it is a heterodimeric complex composed of α and β subunits. IL-12 appears to bridge the gap between APC and effector cells and thus has great control over immune regulation. Homeostasis of the immune system and the overall balance between Th1 and Th2 polarization is critically dependent upon the activity of IL-12. A relative excess of IL-12 induced Th1 responses are largely responsible for pathological inflammatory responses, including autoimmune diseases and graft rejection. Thus, a more complete understanding of IL-12-related immune physiology may offer insights into the prevention and treatment of a variety of diseases.

3. THE REGULATION IN THE PRODUCTION OF IL-12

The major source for IL-12 is APC such as dendritic cells, macrophages, and monocytes in response to bacteria, bacterial products, or intracellular parasites (12). IL-12 activates both the innate and acquired immune systems by stimulating NK cells and T cells, and inducing their production of IFN- γ (13). By inducing IFN- γ production, IL-12 not only patterns T cells towards a Th1 immune response and causes rapid T cell proliferation, but also down-regulates IL-4 secretion, thus blocking propagation of Th2 immune responses (12, 14). Notably, both IL-12 and IFN- γ are needed for a Th1 immune effect to take place: secretion of one without the other is insufficient to initiate a Th1 immune response. Thus, IL-12 is necessary, though not sufficient, to initiate a Th1 immunity in T cells (12). The regulation of Th1 immunity appears to rely, in part, on the requirement for both IFN- γ and IL-12 stimulation of naïve T cells. IFN- γ secreting T cells are largely limited to those that co-express CD30, an activation antigen, suggesting that IL-12 may function physiologically through preferential interaction with CD30 $^{+}$ T cells (14).

IL-12 production is complex and regulated by both positive and negative feedback mechanisms (1). Positive feedback regulation of IL-12 is observed through its interaction with IFN- γ , also a Th1 cytokine, which enhances the production of IL-12 by phagocytic cells (15, 16). Furthermore, IL-12 is a potent inducer of IFN- γ production by T and NK cells (4, 17). Observing *in vitro* cultures or following *in vivo* infections with microorganisms, IFN- γ production appears to precede and to be required for IL-12 production (18). Thus, IL-12-induced IFN- γ acts as a potent positive feedback mechanism in inflammation by enhancing IL-12 production, which may represent a mechanism by which Th1 responses are maintained *in vivo*. Takenaka *et al* examined the inhibitory effects of Th2 cytokines IL-10, IL-6, and IL-4 on the regulation of IL-12 production induced through T-cell-dependent and -independent pathways by using murine macrophage/DC (19). Both IL-6 and IL-4 inhibited T-cell independent IL-12 production, however, IL-4 also potentiated T-cell-dependent production of IL-12.

IL-10 inhibited IL-12 production induced through both T-cell-dependent and -independent pathways. IL-10, which is usually produced by Th2 polarized T cells, limits macrophage/DC activity and down-regulates the Th1 immune response. Following IL-12-induced STAT4 transcription factor activation, IL-10 can also be produced by Th1 T cells (20). Thus, IL-12 is also able to down-regulate Th1 immunity through a negative feedback loop (12).

4. ROLE OF IL-12 IN ALLOGENEIC BONE MARROW TRANSPLANTATION

Allogeneic bone marrow transplantation (BMT) involved in complicated immune responses, as a key cytokine for Th1 polarization, IL-12 is important during the process of Graft-versus-Leukemia (GvL) and Graft-versus-Host disease (GvHD). Polarization of host or donor T cells to Th1 or Th2 immune responses can result in widely different consequences that may be either desirable or undesirable in subjects with cancer and those undergoing BMT. In cancer patients, Th1 T cell polarization can be desirable when it activates the cellular arm of the immune system capable of increased cytotoxicity against the tumor. Studies show that there is a fine balance between Th1 and Th2 immune responses that must be achieved in order for the full clinical benefit of allogeneic BMT to be reached. It was previously thought that Th1 immune responses are predominate after BMT and that Th1 immunity was responsible for increased GvL and GvHD effects. In contrast, Th2 immune responses were thought to be required to limit overstimulation of the immune system. Recent studies have shown that this model of Th1 versus Th2 immune responses is an over-simplification of transplant immunology. In one study, researchers found that both Th1 and Th2 immune responses could contribute to GvHD (21), while other studies demonstrate that administration of Th1 cytokines can inhibit GvHD (11,13) and that administration of anti-IL-12 antibodies could prevent the onset of acute GvHD (22). These conflicting results show that there are other factors contributing to the net effect of Th1 cytokines in GvHD onset, such as when and how the cytokines are administered.

In allogeneic hematopoietic stem cell transplantation (HSCT), Yang and Sykes *et al* (11,13) showed using a murine BMT model that recipients of 4,900 IU of IL-12 on the day of the transplant had noticeably less acute GvHD compared with mice not receiving IL-12. Significantly, the researchers did not see a reduction of GvL activity in irradiated mice receiving IL-12, a trade-off typical of many other GvHD treatments. Furthermore, the observed GvL effect was largely due to CD8-dependent alloreactivity, as removal of CD8 cells from the spleen diminished the effect (23). Also, the effect of IL-12 on GvL and GvHD were not seen in syngenic transplant recipients. These authors proposed that the protection from CD4-mediated GvHD and increased CD8-mediated GvL activity of IL-12 works through IFN- γ production (13, 24). IL-12-induced GvHD protection was associated with a marked reduction of GvHD-associated donor T-cell activation and expansion, which may be in part due to Fas-mediated

apoptosis of activated donor T cells (25). However, more recent studies have shown that another interleukin, IL-23, also a member of the IL-12 cytokine family, mediates inflammation and graft rejection while IL-12 delays graft rejection (26). A significant remaining question of importance to clinical translation is whether IL-12 administration or the transplantation of donor cells that secrete IL-12 can enhance GvL without increasing GvHD.

The effect of IL-12 on GvL has been partially addressed by a clinical study conducted in 2005 that enrolled 134 HSCT patients. Circulating IL-12 levels post-transplant were correlated with higher relapse free survival without an increase in observed incidence of GvHD. Furthermore, patients who started out with higher plasma levels of IL-12 had larger subsequent increases in IL-12 levels post-transplant. In patients with high levels of IL-12, 23% relapsed within 500 days post-transplant. In contrast, in patients with medium and low plasma IL-12, 40% and 49% of patients relapsed, respectively, within that timeframe. These data suggest that plasma IL-12 levels before HSCT may be associated with establishment of donor cell immunity, particularly GvL activity (27). As the primary cell that secretes IL-12, manipulation of DC subsets has been explored as a way of regulating IL-12 production *in vivo*.

The synthesis of IL-12 by distinct subsets of DC has been studied in mice and in humans. While myeloid DC (mDC) are known for their antigen presentation abilities, the function of plasmacytoid DC (pDC) is not as well characterized. DCs regulate immune responses through their production and secretion of various cytokines. In humans, mature mDC secrete IL-12 which induce Th1/Tc1 polarization (28), while immature pDC promote immune tolerance by secreting the Th2 polarizing cytokines IL-4 and IL-10 (29). pDC are able to regulate both the innate and the acquired immune system by the secretion of cytokines, and may even directly play a role in antigen presentation (30). However, the clinical significance of the content of donor DC in allogeneic HSCT remains controversial. A striking effect of enhanced relapse-free survival was seen among leukemia patients who received fewer than 0.9×10^6 pDC/kg compared to patients who received more than 0.9×10^6 pDC/kg as part of their transplant (31). Patients receiving fewer immature pDC showed less relapse and more chronic GvHD compared with patients who received larger numbers, with no correlation of pre-pDC content with the incidence or severity of acute GvHD. Predominance of pDC post-transplant and Th2 polarization is associated with a slower reconstitution of cellular immunity (32). Exploration of these intriguing clinical observations in pre-clinical models has been hampered by the differences between murine and human DC subsets. Murine DC subsets have a functional correspondence but incomplete phenotypic identity with human DC subsets. While both human pDC and murine pDC are important in innate immunity and synthesize large amounts of IFN- α in response to viral infection or CpG sequences bound to TLR9 (33,34), their effects on immune polarization of T-cells are quite different. Mature murine BM-derived pDC produce significant amounts of IL-12 and

polarize T-cells toward Th1 immune responses *in vitro* (34-37). Li *et al* showed that the net result of adding murine donor CD11b⁺ pre-pDC to murine stem cell grafts was to enhance Th1/Tc1 polarization and GvL activity of donor T cells with limited GvHD in MHC mis-matched and MHC matched, MiHA-mismatched murine allo-HSCT models (40,41). The human DC functional analog of murine pDC (with respect to Th1 polarization) are thought to be CD11c⁺ myeloid DC, which do produce large amounts of IL-12 and generate Th1 immune responses in effector T cells following indirect alloantigen presentation (38,39). Translation of the interesting results from graft engineering murine BMT to optimize early post-transplant IL12 production and Th1/TC1 donor T-cell polarization in humans will require early phase clinical trials to test the immunological consequences of selective enrichment and/or depletion of defined donor DC subsets in allogeneic transplantation.

5. ANTITUMOR ACTIVITY OF IL-12

In murine models, IL-12 has been shown to induce regression of established primary tumors, inhibit the formation of tumor metastases, and prolong the survival of tumor-bearing mice. Tumor regression after IL-12 administration in mice has been correlated with IFN- γ production and increased T-cell and NK-cell infiltration into tumors. There have been a number of suggested mechanisms by which IL-12 can induce tumor regression. These include activated T-cells and NK-cells directly killing tumor cells, alterations in the tumor microcirculation, and the IFN- γ -dependent inhibition of tumor angiogenesis (42). IL-12 may also enhance T cell survival by suppressing the anti-apoptotic signaling molecule Akt that normally induces the pro-apoptotic mediators Fas/FasL, TNF receptor, and TRAIL (43). In addition, the antitumor effects of IL-12 may have an anti-angiogenic component, which can be abrogated by the addition of IFN- γ -neutralizing antibodies (44-47). Besides an increase in intratumoral T-cells and NK-cells, a very high number of peritumoral B cells around the primary tumor has been described in IL-12-treated patients (48). After intratumoral rhIL-12 administration in head and neck squamous cell carcinoma patients, van Herpen *et al* detected an increased in B cell activation as determined by IgG subclass switch and an increase in IFN- γ mRNA expression. The broader outer region of the mantle zone could be seen in the lymph nodes of patients treated with IL-12, and these patients had a higher number of interfollicular B-blasts, which is indicative of B cell activation. Moreover, peritumoral B cell infiltration was a positive prognostic sign in head and neck squamous cell carcinoma patients (49).

5.1. The combination of IL-12 and other costimulatory cytokines

On basis of the provocative results of IL-12 administration in murine cancer models, several Phase I and Phase II studies in various cancer types (50-59) have been performed to apply these findings to clinical practice, but with modest clinical results. The immunomodulatory activity of IL-12 is considerably dependent on the presence of other costimulatory cytokines. Based on the data from

the animal models, a variety of clinical trials have been conducted using rhIL-12 combined with another cytokines.

Another potent antitumor cytokine, IL-2, can interact synergistically with IL-12 to enhance T and NK cell proliferation, IFN- γ production, and cytolytic activity, meanwhile augmenting the antitumor activity (60-63). Post-transplant peripheral blood mononuclear cells produced IFN- γ *in vitro* when stimulated by IL-12 plus IL-2 (64). In a murine study by Siapati *et al* (65), the combination of IL-12 and IL-2 had the greatest antitumor effect and eradicated or inhibited tumors in 91% of mice vaccinated with the neuroblastoma cell line compared with only 63% of mice vaccinated the cell line and treated with IL-12 alone. As IL-2 alone had little effect on tumor growth, the authors concluded that IL-2 potentiates the effect of IL-12 on stimulating the expansion of the T-cells. When the ability of IL-12 to activate unmanipulated peripheral blood NK cells and CD8+ T cells in humans was examined, it was found that these lymphocyte subsets only responded to IL-12 when co-stimulated with IL-2 (66). Recombinant human IL-2 has been shown to synergistically enhance NK cell activity when added to rhIL-12-treated lymphocyte cultures of normal controls. Moreover, IL-2 appears to potently up-regulate IL-12 receptor expression on lymphocytes in short-term cultures (67). Gollob *et al* also found that the down-regulation of IFN- γ induction during chronic therapy with twice-weekly intravenous rhIL-12 was associated with a diminished capacity for IL-12 to stimulate lymphocyte IFN- γ production *in vitro*; however, this acquired defect could be overcome if lymphocytes from rhIL-12-treated patients were stimulated *in vitro* with both IL-2 and IL-12 (50). Following these *in vitro* experiments, a phase I clinical trial was initiated to test whether the addition of IL-2 to rhIL-12 could reverse the *in vivo* down-regulation of IFN- γ induction and thereby increase tumor response rates (68). Treatment with rhIL-12 plus low-dose IL-2 resulted in increased IFN- γ plasma levels, and the up-regulation of IFN- γ -inducible protein-10, dramatically enhanced NK cell expansion, and modestly augmented the synthesis of tumor necrosis factor- α (TNF- α) and IL-10. The net result was a significant antitumor effect observed in some patients receiving rhIL-12 plus IL-2.

Both IL-15 and IL-18 are also key costimulatory cytokines, which, when combined with IL-12, induce IFN- γ production by T and NK cells (69, 70). In a study by Iinuma *et al*, IL-12 was combined with IL-18 via a DC/neuroblastoma cell fusion vaccine (71). The vaccine alone had a modest effect on increasing IFN- γ production, but the cotransfection of IL-12 and IL-18 led to a robust IFN- γ response. The authors also showed that both NK cells and CD8+ T-cells are activated by the fusion vaccine. IL-18 potentiated the impact of IL-12 and increased the survival of mice injected with neuroblastoma tumor cells. None of the mice injected with both IL-18 and IL-12 exhibited liver metastases, whereas 50% of mice injected with IL-12 alone had metastases. Mice inoculated with the combination vaccine also had significantly increased survival. In another study of mice treated with IL-12, the neutralization of endogenous IL-18 significantly blunted IFN- γ

production (72), further emphasizing the model that the biological activity of IL-12 *in vivo* is dependent on the presence and/or induction of endogenous costimulatory cytokines. In a Phase I trial of twice-weekly intravenous rhIL-12 administration in patients with metastatic renal cell cancer or malignant melanoma, rhIL-12 induced both IL-15 and IL-18 production *in vivo* (50). Since IL-15 and IL-18 are synthesized by activated monocytes and DC (73,74), it is possible that the direct activation of these antigen-presenting cells by rhIL-12 (75) *in vivo* is responsible for the induction of these costimulatory cytokines.

Lesinski *et al* showed that IFN- γ produced in response to IL-12 can up-regulate the level of JAK-STAT signaling intermediates in both tumor and immune effector cells. Importantly, the up-regulation of these critical signaling intermediates sensitized host immune cells to lower doses of IFN- α , leading to significantly enhanced survival of tumor-bearing mice (76). In a phase I clinical trial, a regimen of IL-12 plus IFN- α was well tolerated in patients with advanced malignancy, and IL-12 pretreatment effectively modulated IFN- α -induced Jak-STAT signal transduction in peripheral-blood mononuclear cells (77).

5.2. The combination of IL-12 and other treatments

Besides its use in combination with other cytokines, IL-12 has been used as an adjunct to other cancer therapies. Pelloso *et al* examined the efficacy of rhIL-12 administration after autologous stem cell transplantation. rhIL-12, when given after a median of 66 days post-transplant, resulted in a marked dose-dependent expansion of major lymphocyte subsets including CD4 T cells, B cells and NK cells *in vivo* during rhIL-12 treatment (78). Furthermore, significantly increased levels of serum IFN- γ were observed following both a single dose (64) and multiple doses (78) of IL-12.

Previously, it was shown that co-administration of IL-12 with peripheral blood mononuclear cells loaded with tumor antigen peptides induced specific cytolytic T-lymphocyte responses and tumor protection in mice, circumventing the need to generate DC (79). On that basis, Gajewski *et al*. conducted a Phase I clinical study to determine the optimal dose of rhIL-12 necessary to induce T-cell responses in combination with antigen-loaded peripheral blood mononuclear cells (80). In a follow-up phase II clinical study of immunization with Melan-A/MART-1 (81) peptide-pulsed autologous peripheral blood mononuclear cells and rhIL-12 in HLA-A2-positive patients with advanced melanoma, researchers observed a significant increase in Melan-A-specific IFN- γ -producing CD8+ T cells after immunization. In this trial, 10% of patients had a complete response, 20% of patients had a mixed response and 20% of patients achieved a stable disease state, with a median overall survival of 12 months. Furthermore, there was a statistical association between clinical response in individual patients and the increase in numbers of antigen-specific T-cells in their blood (82).

In a mouse brain tumor model, systemic administration of rhIL-12 enhanced the antitumor response to a fusion cell vaccine (83). The results of a Phase I

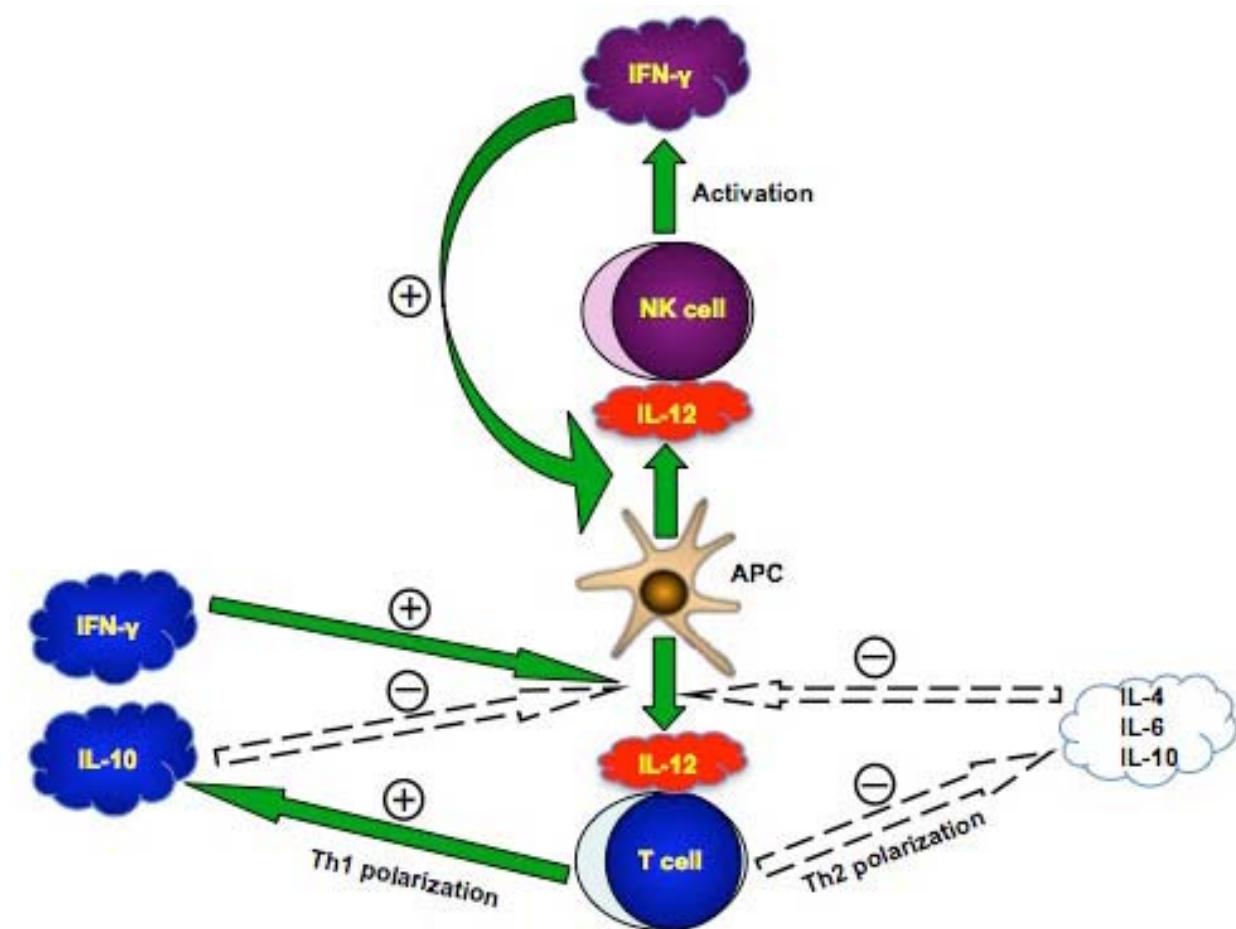


Figure 1. The positive and negative feedback in the production of IL-12. IL-12 secreted by APC activate NK cells and induce Th1 polarization to produce IFN- γ which enhance the production of IL-12 by a positive feedback mechanism. On the other hand, IL-12 secreted by APC block Th2 polarization to down-regulate Th2 cytokines which inhibit IL-12 production by a negative feedback mechanism. Th1 cells are not only involved in the positive feedback, but also the negative feedback because Th1 cells can produce IL-10 which is usually produced by Th2 cells.

clinical trial of fusion cells prepared with DCs and cultured autologous glioma cells indicate that this treatment safely induces anti-tumor immune responses (84). Based on the data from the phase I trial and an animal model, a phase II clinical trial of vaccine therapy using fusion cells and rhIL-12 has been performed (85). The efficacy of the combination of rhIL-12 administration with the fusion cell vaccine was greater than that of fusion cells alone, and administration of fusion cells and rhIL-12 safely induced partial antitumor responses in 4/15 patients with malignant glioma.

6. ANTIVIRAL ACTIVITY OF IL-12

A Th1 coordinated immune response is favorable for host control of infection with intracellular organisms, such as viruses and intracellular parasites, as well as one of the central actions of IL-12 is to shift the immune system from a Th2 response to a Th1 response, and its prime function is to promote cytotoxic functions of T and NK cells and to stimulate Th1-type of effector mechanisms to

combat microbial infections (1). Several investigators have suggested that one potential reason the immune system is unable to eradicate some viruses is that there is a shift of the human immune system away from a predominant Th1 phenotypic response to a Th2 response (86-89). On basis of these properties, IL-12 has been tested in several mouse models for human infectious diseases in which induction of Th1-type responses is critical for protective immunity. Recombinant murine IL-12 (rmIL-12) demonstrates potent activity against a variety of chronic viral infection, including herpesvirus, hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), lymphocytic choriomeningitis virus, encephalomyocarditis virus, vesicular stomatitis virus, influenza and murine acquired immunodeficiency syndrome. In animal models, administration of rmIL-12 reduced viral titers or improved survival (90-97). Based off the results in these animal models, rhIL-12 has been administrated in clinical trials in different patients with a variety of viral infections. *In vitro* studies showed rhIL-12 can increase anti-human immunodeficiency virus (HIV)-specific cytolytic activity of

peripheral blood mononuclear cells of HIV-infected patients (98), with less potential than does rhIL-2 for upregulating HIV replication (99). In contrast to IL-2, IL-12 inhibited programmed cell death via apoptosis of Th1 CD4+ T cells which is a phenomenon implicated in the progressive loss of CD4+ T cells in advanced HIV disease (100). Jacobson *et al* conducted a phase I trial of a single dose of rhIL-12 in HIV-infected patients with 100-500 CD4 cells/ μ l, and found increases in NK and CD8+ T cells without observed changes in the absolute CD4+ T cell counts or plasma HIV viral loads (101). After single dose rhIL-12 administration showed biologic activity, they used multi-dose rhIL-12 administration and observed strikingly less increase in serum IFN- γ compared with the IFN- γ response to single dose IL-12 (102), but increases in serum neopterin possibly represented increases of IFN- γ because neopterin was produced by macrophages under control of IFN- γ (103). Moreover, no significant changes in plasma HIV viral load, lymphocyte subsets, or lymphocyte proliferative were observed. The difference between results of multi-dose rhIL-12 and single dose rhIL-12 may be due to tolerance to rhIL-12 that developed following repeated rhIL-12 dosing. Although multi-dose rhIL-12 administration did not show significant effect on patients with HIV infection, Van der Meide *et al* found that IL-12 enhanced not only cellular but also humoral immune responses to HIV-1 subunit vaccine protein gp120 in non-human primates when coadministered as a potent adjuvant (104). They suggested that lower doses of various subunit vaccines administered in conjunction with IL-12 may provide a much broader level of protection than in the absence of this cytokine. Therefore IL12 administration as an adjuvant may be a promising strategy for improving antibody and T cell immune responses to a CMV vaccine. Jacobson *et al* showed coadministration adjuvant rhIL-12 with Towne CMV vaccine resulted in rhIL-12 dose-related increases in peak anti-CMV gB IgG titers and CMV viral lysate-specific CD4+ T cell proliferation responses induced by vaccine alone. Furthermore, more patients with rhIL-12 administration developed a positive CD8+ T cell IFN- γ response to pp65 peptide after Towne vaccination compared with the placebo group (105). In contrast to the effects on HIV viral load, rhIL-12 can suppress HCV plasma viral load to undetectable levels, although relapse occurred when treatment was stopped (106).

7. TOXICITY OF rhIL-12

Toxicities associated with intravenous rhIL-12 use are similar to those of other cytokines and frequently include fever, chills, headache, nausea, fatigue, liver transaminitis, and myelotoxicity. After rhIL-12 administration, serum levels of IFN- γ are markedly elevated because of NK and T cell activation. IFN- γ powerfully enhances the ability of phagocytic cells to produce IL-12 (107). This strong positive feedback mechanism likely promotes positive activity but may exacerbate the development of toxicity with exogenous administration. To test the role of IFN- γ in the toxicology

of IL-12, IFN- γ receptor-deficient (IFN- γ R-/-) 129/Sv and wild-type 129/Sv mice were dosed with rmIL-12 for 4 days. In contrast to wild-type 129/sv mice, hematopoietic toxicity was not observed in IFN- γ R-/- mice. Higher levels of committed bone marrow hematopoietic progenitor cells were present in IFN- γ R-/- mice compared with IL-12 treated wild-type mice, suggesting that the hematopoietic toxicity of IL-12 in mice results directly from IFN- γ (108,109).

8. CONCLUSION

IL-12 is a unique heterodimeric cytokine with important immunoregulatory activities in both innate and adaptive immunity, antitumor activity and antiviral activity. The production of IL-12 is regulated by both positive and negative feedback mechanisms. A variety of Phase I and Phase II clinical trials have examined the use of rhIL-12 in patients with viral infections or cancer. rhIL-12 administration was generally safe and well tolerated, and rhIL-12 induced clinical antitumor effects in some tumor patients. rhIL-12 may also be a promising adjuvant to some virus vaccines. As a potent immunostimulatory cytokine, IL-12 paradoxically limited GvHD and promoted the GvL effects of allogeneic BMT in murine BMT models. Thus, the use of IL-12 to modulate early post-transplant immune polarization of donor T cells may enhance the GvL activity of the graft while limiting GvHD for patients undergoing BMT. However, given the clear differences in transplant immunology between mice and human, preclinical studies using the most relevant large animal model to test the safety of IL-12 administration in allogeneic HSCT should be undertaken prior to testing IL-12 in a human phase I clinical trial of patients undergoing allogeneic HSCT.

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10. REFERENCES

1. G Trinchieri: Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigenspecific adaptive immunity. *Annu Rev Immunol* 13, 251-276 (1995)
2. A D'Andrea, M Rengaraju, NM Valiante, J Chehimi, M Kubin, M Aste, SH Chan, M Kobayashi, D Young, E Nickbarg, R Chizzonite, SF Wolf & G Trinchieri: Production of natural killer cell stimulatory factor interleukin 12 by peripheral blood mononuclear cells. *J Exp Med* 176, 1387-1398 (1992)
3. M Kobayashi, L Fitz, M Ryan, RM Hewick, SC Clark, S Chan, R Loudon, F Sherman, B Perussia & G Trinchieri: Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J Exp Med* 170, 827-845 (1989)
4. R Manetti, P Parronchi, MG Giudizi, MP Piccinni, E Maggi, G Trinchieri & S Romagnani: Natural killer cell stimulatory

factor (interleukin 12 (IL-12)) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 177, 1199-1204 (1993)

5. S Chouaib, J Chehimi, L Bani, N Genetet, T Tursz, F Gay, G Trinchieri & F Mami-Chouaib: Interleukin-12 induces the differentiation of major histocompatibility complex class I-primed cytotoxic T-lymphocyte precursors into allospecific cytotoxic effectors. *Proc Natl Acad Sci USA* 91, 12659-12663 (1994)

6. RA Seder, R Gazzinelli, A Sher & WE Paul: Interleukin-12 acts directly on CD4+ T cells to enhance priming for interferon-gamma production and diminishes interleukin-4 inhibition of such priming. *Proc Natl Acad Sci USA* 90, 10188-10192 (1993)

7. MJ Brunda: Interleukin-12. *J Leukoc Biol* 55, 280-288 (1994)

8. MJ Brunda, L Luistro, L Rumennik, RB Wright, M Dvorozniak, A Aglione, JM Wigginton, RH Wiltrout, JA Hendrzak & AV Palleroni: Antitumor activity of interleukin 12 in preclinical models. *Cancer Chemother Pharmacol* 38 (suppl.), S16-S21 (1996)

9. CL Nastala, HD Edington, TG McKinney, H Tahara, MA Nalesnik, MJ Brunda, MK Gately, SF Wolf, RD Schreiber, WJ Storkus & MT Lotze: Recombinant IL-12 administration induces tumor regression in association with IFN- γ production. *J Immunol* 153, 1697-1706 (1994)

10. L Romani, P Puccetti & F Bistoni: Interleukin-12 in infectious diseases. *Clin Microbiol* 10, 611-636 (1997)

11. M Sykes, GL Szot, PL Nguyen & DA Pearson: Interleukin-12 inhibits murine Graft-versus-Host disease. *Blood* 86 (6), 2429-2438 (1995)

12. A Stern, J Magram & D Presky: Interleukin 12 an Integral Cytokine in the Immune Response. *Life Sciences* 58 (8), 639-654 (1996)

13. YG Yang, JJ Sergio, DA Pearson, GL Szot, A Shimizu & M Sykes: Interleukin-12 Preserves the Graft-Versus-Leukemia Effect of Allogeneic CD8 T Cells While Inhibiting CD4-Dependent Graft-Versus-Host Disease in Mice. *Blood* 90 (11), 4651-4660 (1997)

14. M Alzona, HM Jäck, PE Simms & TM Ellis: Interleukin-12 Activates Interferon- γ Production by Targeted Activation of CD30 $^{+}$ T cells. *Ann NY Acad Sci* 795, 127-136 (2006)

15. M Kubin, JM Chow & G Trinchieri: Differential regulation of interleukin-12 (IL-12), tumor necrosis factor alpha, and IL-1 beta production in human myeloid leukemia cell lines and peripheral blood mononuclear cells. *Blood* 83 (7), 1847-1855 (1994)

16. MA Cassatella, L Meda, S Gasperini, A D'Andrea, X Ma & G Trinchieri: Interleukin-12 production by human polymorphonuclear leukocytes. *Eur J Immunol* 25 (1), 1-5 (1995)

17. SH Chan, B Perussia, JW Gupta, M Kobayashi, M Pospisil, HA Young, SF Wolf, D Young, SC Clark & G Trinchieri: Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. *J Exp Med* 173, 869-879 (1991)

18. IE Flesch, JH Hess, S Huang, M Aguet, J Rothe, H Bluethmann & SH Kaufmann: Early interleukin 12 production by macrophages in response to mycobacterial infection depends on interferon gamma and tumor necrosis factor alpha. *J Exp Med* 181, 1615-1621 (1995)

19. H Takenaka, S Maruo, N Yamamoto, M Wysocka, S Ono, M Kobayashi, H Yagita, K Okumura, T Hamaoka, G Trinchieri & H Fujiwara: Regulation of T cell-dependent and -independent IL-12 production by the three Th2-type cytokines IL-10, IL-6, and IL-4. *J Leukoc Biol* 61 (1), 80-87 (1997)

20. JR Saraiva Christensen, M Veldhoen, TL Murphy, KM Murphy & A O'Garra: Interleukin-10 production by Th1 cells requires interleukin-12- induced STAT4 transcription factor and ERK MAP kinase activation by high antigen dosage. *Immunity* 31 (2), 209-219 (2009)

21. T Yi, Y Chen, L Wang, G Du, D Huang, D Zhao, H Johnston, J Young, I Todorov, DT Umetsu, L Chen, Y Iwakura, F Kandee, S Forman & D Zeng: Reciprocal differentiation and tissue-specific pathogenesis of Th1, Th2, and Th17 cells in graft-versus-host disease. *Blood* 114 (14), 3101-3112 (2009)

22. E Williamson, P Garside, JA Bradley, IA More & AM Mowat: Neutralizing IL-12 during induction of murine acute graft-versus-host disease polarizes the cytokine profile toward a Th2-type alloimmune response and confers long term protection from disease. *J Immunol* 159, 1208-1215 (1997)

23. YG Yang & M Sykes: The Role of Interleukin-12 in Preserving the Graft-Versus-Leukemia Effect of Allogeneic CD8 T Cells Independently of GvHD. *Leukemia and Lymphoma* 33 (5-6), 409-420 (1999)

24. BR Dey, YG Yang, GL Szot, DA Pearson & M Sykes: Interleukin-12 inhibits Graft-versus-Host Disease through an Fas-mediated mechanism associated with alterations in donor T-cell activation and expansion. *Blood* 91 (9), 3315-3322 (1998)

25. YG Yang, BR Dey, JJ Sergio, DA Pearson & M Sykes: Donor-derived interferon γ is required for inhibition of acute Graft-versus-Host Disease by interleukin 12. *J Clin Invest* 102, 2126-2135 (1998)

26. S Goriely & M Goldman: Interleukin-12 family members and the balance between rejection and tolerance. *Current Opinion in Organ Transplantation* 13, 4-9 (2008)

27. V Reddy, AG Winer, E Eksioglu, HU Meier-Kriesche, JD Schold & JR Wingard: Interleukin 12 is Associated with Reduced Relapse without Increased Incidence of Graft-versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplant. *Biol Blood Marrow Transplant* 11 (12), 1014-1021 (2005)

28. M Mohamadzadeh, S Olson, WV Kalina, G Ruthel, GL Demmin, KL Warfield, S Bavari & TR Klaenhammer: Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. *Proc Natl Acad Sci USA* 102, 2880-2885 (2005)

29. M Rissoan, V Soumelis, N Kadokami, G Grouard, F Briere, R de Waal Malefyt & YJ Liu: Reciprocal control of T-helper cell and dendritic cell differentiation. *Science* 283 (5405), 1183-1186 (1999)

30. J Villadangos & L Young: Antigen-Presentation Properties of Plasmacytoid Dendritic Cells. *Immunity* 29 (3), 352-361 (2008)

31. EK Waller, H Rosenthal, TW Jones, J Peel, S Lonial, A Langston, I Redei, I Jurickova & MW Boyer: Larger numbers of CD4 (bright) dendritic cells in donor bone marrow are associated with increased relapse after allogeneic bone marrow transplantation. *Blood* 97 (10), 2948-2956 (2001)

32. I Volpi, K Perruccio, A Tosti, M Capanni, L Ruggeri, S Posati, F Aversa, A Tabilio, L Romani, MF Martelli & A Velardi: Postgrafting administration of granulocyte colony-stimulating factor impairs functional immune recovery in recipients of human leukocyte antigen haplotype-mismatched hematopoietic transplants. *Blood* 97 (8), 2514-2521 (2001)

33. P Martin, GM Del Hoyo, F Anjuère, CF Arias, HH Vargas, A Fernández-L, V Parrillas & C Ardavín: Characterization of a new subpopulation of mouse CD8alpha⁺ B220⁺ dendritic cells endowed with type 1 interferon production capacity and tolerogenic potential. *Blood* 100 (2), 383-390 (2002)

34. M O'keeffe, H Hochrein, D Vremec, B Scott, P Hertzog, L Tatareczuch & K Shortman: Dendritic cell precursor populations of mouse blood: identification of the murine homologues of human blood plasmacytoid pre-DC2 and CD11c⁺ DC1 precursors. *Blood* 101 (4), 1453-1459 (2003)

35. B Pulendran, JL Smith, G Caspary, K Brasel, D Pettit, E Maraskovsky & CR Maliszewski: Distinct dendritic cell subsets differentially regulate the class of immune response *in vivo*. *Proc Natl Acad Sci U S A* 96 (3), 1036-1041 (1999)

36. M Gilliet & YJ Liu: Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J Exp Med* 195 (6), 695-704 (2002)

37. M Colonna, G Trinchieri & YJ Liu: Plasmacytoid dendritic cells in immunity. *Nat Immunol* 5 (12), 1219-1226 (2004)

38. YJ Liu: IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol* 23, 275-306 (2005)

39. S Lonial, C Torre, E David, W Harris, M Arellano & EK Waller: Regulation of alloimmune responses by dendritic cell subsets. *Exp Hematol* 36 (10), 1309-1317 (2008)

40. JM Li, LT Southerland, Y Lu, KA Darlak, CR Giver, DW McMillin, WA Harris, DL Jaye & EK Waller: Activation, immune polarization, and graft-versus-leukemia activity of donor T cells are regulated by specific subsets of donor bone marrow antigen-presenting cells in allogeneic hemopoietic stem cell transplantation. *J Immunol* 183 (12), 7799-7809 (2009)

41. JM Li & EK Waller: Donor antigen-presenting cells regulate T-cell expansion and antitumor activity after allogeneic bone marrow transplantation. *Biol Blood Marrow Transplant* 10 (8), 540-551 (2004)

42. F Cavallo, E Di Carlo, M Butera, R Verrua, MP Colombo, P Musiani & G Forni: Immune events associated with the cure of established tumors and spontaneous metastases by local and systemic interleukin 12. *Cancer Res* 59, 414-421 (1999)

43. T Khan, JA Hixon, JK Stauffer, E Lincoln, TC Back, J Brenner, S Lockett, K Nagashima, D Powell & JM Wigginton: Therapeutic modulation of Akt activity and antitumor efficacy of interleukin-12 against orthotopic murine neuroblastoma. *J Natl Cancer Inst* 98, 190-202 (2006)

44. EE Voest, BM Kenyon, MS O'Reilly, G Truitt, RJ D'Amato & J Folkman: Inhibition of angiogenesis *in vivo* by interleukin 12. *J Natl Cancer Inst* 87, 581-586 (1995)

45. C Sgadari, AL Angiolillo & G Tosato: Inhibition of angiogenesis by interleukin-12 is mediated by the interferon-inducible protein 10. *Blood* 87, 3877-3882 (1996)

46. CS Tannenbaum, N Wicker, D Armstrong, R Tubbs, J Finke, RM Bukowski & TA Hamilton: Cytokine and chemokine expression in tumors of mice receiving systemic therapy with IL-12. *J Immunol* 156, 693-699 (1996)

47. CM Coughlin, KE Salhany, M Wysocka, E Aruga, H Kurzawa, AE Chang, CA Hunter, JC Fox, G Trinchieri & WM Lee: Interleukin-12 and interleukin-18 synergistically induce murine tumor regression which involves inhibition of angiogenesis. *J Clin Invest* 101, 1441-1452 (1998)

48. CM van Herpen, JAWM van der Laak, IJ de Vries, JH van Krieken, PC de Wilde, GJ Adema & PH de Mulder: Intratumoral rhIL-12 administration in head and neck squamous cell carcinoma patients modifies the lymph node architecture and induces a NK cell infiltration in the primary tumor. *Clin Cancer Res*, 2005;11:1899-909.

49. CM van Herpen, R van der Voort, JA van der Laak, IS Klasen, AO de Graaf, LC van Kempen, IJ de Vries, TD Boer, H Dolstra, R Torensma, JH van Krieken, GJ Adema & PH de Mulder: Intratumoral rhIL-12 administration in

head and neck squamous cell carcinoma patients induces B cell activation. *Int J Cancer* 123 (10), 2354-2361 (2008)

50. JA Gollob, JW Mier, K Veenstra, DF McDermott, D Clancy, M Clancy & MB Atkins: Phase I trial of twice-weekly intravenous interleukin 12 in patients with metastatic renal cell cancer or malignant melanoma: ability to maintain IFN-gamma induction is associated with clinical response. *Clin Cancer Res* 6 (5), 1678-1692 (2000)

51. E Bajetta, M Del Vecchio, R Mortarini, R Nadeau, A Rakhit, L Rimassa, C Fowst, A Borri, A Anichini & G Parmiani: Pilot study of subcutaneous recombinant human interleukin 12 in metastatic melanoma. *Clin Cancer Res* 4, 75-85 (1998)

52. RJ Motzer, A Rakhit, LH Schwartz, T Olencki, TM Malone, K Sandstrom, R Nadeau, H Parmar & R Bukowski: Phase I trial of subcutaneous recombinant human interleukin-12 in patients with advanced renal cell carcinoma. *Clin Cancer Res* 4, 1183-1191 (1998)

53. MJ Robertson, C Cameron, MB Atkins, MS Gordon, MT Lotze, ML Sherman & J Ritz: Immunological effects of interleukin 12 administered by bolus intravenous injection to patients with cancer. *Clin Cancer Res* 5, 9-16 (1999)

54. JE Portielje, WH Kruit, M Schuler, J Beck, CH Lamers, G Stoter, C Huber, M de Boer-Dennert, A rakhit, RL Bolhuis & WE Aulitzky: Phase I study of subcutaneously administered recombinant human interleukin 12 in patients with advanced renal cell cancer. *Clin Cancer Res* 5, 3983-3989 (1999)

55. CM van Herpen, R Huijbens, M Looman, J de Vries, H Marres, J van de Ven, R Hermsen, G Adema & PH de Mulder: Pharmacokinetics and immunological aspects of a phase Ib study with intratumoral administration of recombinant human interleukin-12 in patients with head and neck squamous cell carcinoma: a decrease of T-bet in PBMCs. *Clin Cancer Res* 9, 2950-2956 (2003)

56. JP Leonard, ML Sherman, GL Fisher, LJ Buchanan, G Larsen, MB Atkins, JA Sosman, JP Dutcher, NJ Vogelzang & JL Ryan: Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon- γ production. *Blood* 90, 2541-2548 (1997)

57. RJ Motzer, A Rakhit, JA Thompson, J Nemunaitis, BA Murphy, J Ellerhorst, LJ Schwartz, WJ Berg & RM Bukowski: Randomized multicenter phase II trial of subcutaneous recombinant human interleukin-12 versus interferon-a2a for patients with advanced renal cell carcinoma. *J Interferon Cytokine Res* 21, 257-263 (2001)

58. JA Hurteau, JA Blessing, SL DeCesare & WT Creasman: Evaluation of recombinant human interleukin-12 in patients with recurrent or refractory ovarian cancer: a gynecologic oncology group study. *Gynecol Oncol* 82, 7-10 (2001)

59. CM van Herpen, M Looman, M Zonneveld, N Scharenborg, PC de Wilde, L van de Locht, MA Merkx, GJ Adema & PH de Mulder: Intratumoral administration of recombinant human interleukin 12 in head and neck squamous cell carcinoma patients elicits a T-helper 1 profile in the locoregional lymph nodes. *Clin Cancer Res* 10, 2626-2635 (2004)

60. MK Gately, DE Wilson & HL Wong: Synergy between recombinant interleukin 2 (rIL 2) and IL 2-depleted lymphokine-containing supernatants in facilitating allogeneic human cytolytic T lymphocyte responses *in vitro*. *J Immunol* 136, 1274-1282 (1986)

61. SH Chan, B Perussia, JW Gupta, M Kobayashi, M Pospisil, HA Young, SF Wolf, D Young, SC Clark & G Trinchieri: Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. *J Exp Med* 173, 869-879 (1991)

62. B Perussia, SH Chan, A D'Andrea, K Tsuji, D Santoli, M Pospisil, D Young, SF Wolf & G Trinchieri: Natural killer (NK) cell stimulatory factor or IL-12 has differential effects on the proliferation of TCR-alpha beta+, TCR-gamma delta+ T lymphocytes, and NK cells. *J Immunol* 149, 3495-3502 (1992)

63. AR Rossi, F Pericle, S Rashleigh, J Janiec & JY Djeu: Lysis of neuroblastoma cell lines by human natural killer cells activated by interleukin-2 and interleukin-12. *Blood* 83, 1323-1328 (1994)

64. MJ Robertson, D Pelloso, R Abonour, RA Hromas, RP Jr Nelson, L Wood & K Cornetta: Interleukin-12 immunotherapy after autologous stem cell transplantation for hematologic malignancies. *Clin Cancer Res* 8, 3383-3393 (2002)

65. KE Siapati, S Barker, C Kinnon, A Michalski, R Anderson, P Brickell, AJ Thrasher & SL Hart: Improved antitumour immunity in murine neuroblastoma using a combination of IL-2 and IL-12. *Br J Cancer* 88, 1641-1648 (2003)

66. JA Gollob, CP Schnipper, E Orsini, E Murphy, JF Daley, SB Lazo, DA Frank, D Neuberg, & J Ritz: Characterization of a novel subset of CD8+ T cells that expands in patients receiving interleukin-12. *J Clin Invest* 102, 561-575 (1998)

67. KS Wang, DA Frank & J Ritz: Interleukin-2 enhances the response of natural killer cells to interleukin-12 through up-regulation of the interleukin-12 receptor and STAT4. *Blood* 95, 3183-3190 (2000)

68. JA Gollob, KG Veenstra, RA Parker, JW Mier, DF McDermott, D Clancy, L Tutin, H Koon & MB Atkins: Phase I trial of concurrent twice-weekly recombinant human interleukin-12 plus low-dose IL-2 in patients with melanoma or renal cell carcinoma. *J Clin Oncol* 21 (13), 2564-2573 (2003)

69. WE Carson, JG Giri, MJ Lindemann, ML Linett, M Ahdieh, R Paxton, D Anderson, J Eisenmann, K Grabstein

The regulation and activity of interleukin-12

& M Caliguri: Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J Exp Med* 180, 1395-1403 (1994)

70. MJ Micallef, H Ohtsuki, K Kohno, F Tanabe, T Ushio, M Namba, T Tanimoto, K Torigoe, M Fuji, M Ikeda, S Fukuda & M Kurimoto: Interferon- γ -inducing factor enhances T helper 1 cytokine production by stimulating human T cells: synergism with interleukin-12 for interferon- γ production. *Eur J Immunol* 26, 1647-1651 (1996)

71. H Iinuma, K Okinaga, R Fukushima, T Inaba, K Iwasaki, A Okinaga, I Takahashi & M Kaneko: Superior protective and therapeutic effects of IL-12 and IL-18 gene-transduced dendritic neuroblastoma fusion cells on liver metastasis of murine neuroblastoma. *J Immunol* 176, 3461-3469 (2006)

72. G Fantuzzi, DA Reed & CA Dinarello: IL-12-induced IFN- γ is dependent on caspase-1 processing of the IL-18 precursor. *J Clin Invest* 104, 761-767 (1999)

73. H Jonuleit, K Wiedemann, G Muller, J Degwert, U Hoppe, J Knop & AH Enk: Induction of IL-15 messenger RNA and protein in human blood-derived dendritic cells: a role for IL-15 in attraction of T cells. *J Immunol* 158, 2610-2615 (1997)

74. S Stoll, H Jonuleit, E Schmitt, G Muller, H Yamauchi, M Kurimoto, J Knop & AH Enk: Production of functional IL-18 by different subtypes of murine and human dendritic cells (DC): DC derived IL-18 enhances IL-12-dependent Th1 development. *Eur J Immunol* 28, 3231-3239 (1998)

75. U Grohmann, ML Belladonna, R Bianchi, C Orabona, E Airoldi, MC Fioretti & P Puccetti: IL-12 acts directly on DC to promote nuclear localization of NF- κ B and primes DC for IL-12 production. *Immunity* 9, 315-323 (1998)

76. GB Lesinski, B Badgwell, J Zimmerer, T Crespin, Y Hu, G Abood & WE 3rd Carson: IL-12 pretreatments enhance IFN-alpha induced Janus kinase-STAT signaling and potentiate the antitumor effects of IFN-alpha in a murine model of malignant melanoma. *J Immunol* 172, 7368-7376 (2004)

77. CF Eisenbeis, GB Lesinski, M Anghelina, R Parihar, D Valentino, J Liu, P Nadella, P Sundaram, DC Young, M Sznol, MJ Walker & WE 3rd Carson: Phase I study of the sequential combination of interleukin-12 and interferon alfa-2b in advanced cancer: evidence for modulation of interferon signaling pathways by interleukin-12. *J Clin Oncol* 23 (34), 8835-8844 (2005)

78. D Pelloso, K Cyran, L Timmons, BT Williams & MJ Robertson: Immunological consequences of interleukin 12 administration after autologous stem cell transplantation. *Clin Cancer Res* 10, 1935-1942 (2004)

79. F Fallarino, C Uyttenhove, T Boon & TF Gajewski: Improved efficacy of dendritic cell vaccines and successful immunization with tumor antigen peptide-pulsed peripheral blood mononuclear cells by coadministration of recombinant murine interleukin-12. *Int J Cancer* 80 (2), 324-333 (1999)

80. TF Gajewski, F Fallarino, A Ashikari & M Sherman: Immunization of HLA-A2 melanoma patients with MAGE-3 or MelanA peptide-pulsed autologous peripheral blood mononuclear cells plus recombinant human interleukin 12. *Clin Cancer Res* 7, 895s-901s (2001)

81. PG Coulie, V Brichard, A Van Pel, T Wölfel, J Schneider, C Traversari, S Mattei, E De Plae, C Lurquin, JP Szikora, JC Renaud & T Boon: A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med* 180, 35-42 (1994)

82. AC Peterson, H Harlin & TF Gajewski: Immunization with Melan-A peptide-pulsed peripheral blood mononuclear cells plus recombinant human interleukin-12 induces clinical activity and T-cell responses in advanced melanoma. *J Clin Oncol* 21 (12), 2342-2348 (2003)

83. Y Akasaki, T Kikuchi, S Homma, T Abe, D Kofe & T Ohno: Antitumor effect of immunizations with fusions of dendritic and glioma cells in a mouse brain tumor model. *J Immunother* 24, 106-113 (2001)

84. T Kikuchi, Y Akasaki, M Irie, S Homma, T Abe & T Ohno: Results of a phase I clinical trial of vaccination of glioma patients with fusions of dendritic and glioma cells. *Cancer Immunol Immunother* 50, 337-344 (2001)

85. T Kikuchi, Y Akasaki, T Abe, T Fukuda, H Saotome, JL Ryan, DW Kufe & T Ohno: Vaccination of glioma patients with fusions of dendritic and glioma cells and recombinant human interleukin 12. *J Immunother* 27 (6), 452-459 (2004)

86. DR Lucey, GP Melcher, CW Hendrix, RA Zajac, DW Goetz, CA Butzin, M Clerici, RD Warner, S Abbadessa, K Hall, R Jaso, B Woolford, S Miller, NI Stocks, CM Salinas, WH Wolfe, GM Shearer & RN Boswell: Human immunodeficiency virus infection in the US Air Force: seroconversions, clinical staging, and assessment of a T helper cell functional assay to predict change in CD4+ T cell counts. *J Infect Dis* 164, 631-637 (1991)

87. K Kobayashi, M Ishii, T Igarashi, T Satoh, Y Miyazaki, Y Yajima, K Ukai, H Suzuki, A Kanno, Y Ueno, T Miura & T Toyota: Profiles of cytokines produced by CD4-positive T lymphocytes stimulated by anti-CD3 antibody in patients with chronic hepatitis C (see comments). *J Gastroenterol* 33, 500-507 (1998)

88. S Shapiro, V Gershtein, N Elias, E Zuckerman, N Salman & N Lahat: mRNA cytokine profile in peripheral blood cells from chronic hepatitis C virus (HCV)-infected patients: Effects of interferon-alpha (IFN-alpha) treatment. *Clin Exp Immunol* 114, 55-60 (1998)

89. JL Schultze, S Michalak, J Lowne, A Wong, MH Gilleece, JG Gribben & LM Nadler: Human non-germinal

The regulation and activity of interleukin-12

center B cell interleukin (IL)-12 production is primarily regulated by T cell signals CD40 ligand, interferon gamma, and IL-10: Role of B cells in the maintenance of T cell responses. *J Exp Med* 189 (1), 1-12 (1999)

90. JA Carr, J Rogerson, MJ Mulqueen, NA Roberts & RF Booth: Interleukin-12 exhibits potent antiviral activity in experimental herpes virus infections. *J Virol* 71, 7799-7803 (1997)

91. JS Orange & CA Biron: Characterization of early IL-12, IFN-alpha, and TNF effects on antiviral state and NK cell responses during murine cytomegalovirus infection. *J Immunol* 156, 4746-4756 (1996)

92. FA Ennis, M Tamura & J Cruz: Effect of recombinant murine interleukin 12 on influenza pneumonia in a murine model. *Genetics Institute Technical Report PB 009*, 93 (1993)

93. R Matsuo, M Kobayashi, DN Herndon, RB Pollard & F Suzuki: Interleukin-12 protects thermally injured mice from herpes simplex virus type 1 infection. *J Leukoc Biol* 59, 623-630 (1996)

94. JS Orange, TP Salazar-Mather, SM Opal, RL Spencer, AH Miller, BS McEwen & CA Biron: Mechanism of interleukin-12-mediated toxicities during experimental viral infections: role of tumor necrosis factor and glucocorticoids. *J Exp Med* 181 (3), 901-904 (1995)

95. JS Orange, SA Wolf & CA Biron: Effects of IL-12 on the response and susceptibility to experimental viral infections. *J Immunol* 152, 1253-1264 (1994)

96. L Ozmen, M Aguet, G Trinchieri & G Garotta: The *in vivo* activity of interleukin-12 is mediated by gamma interferon. *J Virol* 69, 8147-8150 (1995)

97. RT Gazzinelli, NA Giese & HC Morse: *In vivo* treatment with interleukin-12 protects mice from immune abnormalities observed during murine acquired immunodeficiency syndrome (MAIDS). *J Exp Med* 180, 2199-2208 (1994)

98. M Clerici, DR Lucey, JA Berzofsky, LA Pinto, TA Wynn, SP Blatt, MJ Dolan, CW Hendrix, SF Wolf & GM Shearer: Restoration of HIV-specific cellmediated immune responses by interleukin-12 *in vitro*. *Science* 262, 1721-1724 (1993)

99. A Foli, MW Saville, MW Baseler & R Yarchoan: Effects of the Th1 and Th2 stimulatory cytokines interleukin-12 and interleukin-4 on human immunodeficiency virus replication. *Blood* 85, 2114-2123 (1995)

100. J Estaquier, T Idziorek, W Zou, D Emilie, CM Farber, JM Bourez & JC Ameisen: T helper type 1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD95 (FAS/APO-1)-mediated apoptosis of CD41 T cells from human immunodeficiency virus-infected persons. *J Exp Med* 182, 1759-1767 (1995)

101. MA Jacobson, D Hardy, E Connick, J Watson & M DeBruin: Phase 1 trial of a single dose of recombinant human interleukin-12 in human immunodeficiency virus-infected patients with 100-500 CD4 cells/ μ l. *J Infect Dis* 182 (4), 1070-1076 (2000)

102. MA Jacobson, J Spritzler, A Landay, E Chan, D Katzenstein, B Schock, L Fox, J Roe, S Kundu, R Pollard & AACTG 325 Protocol Team: A phase I, placebo-controlled trial of multi-dose recombinant human interleukin-12 in patients with HIV infection. *AIDS* 16, 1147-1154 (2002)

103. C Huber, JR Batchelor, D Fuchs, A Hausen, A Lang, D Niederwieser, G Reibnegger, P Swetly, J Troppmair & H Wachter: Immune responseassociated production of neopterin: release from macrophages primarily under control of interferon-gamma. *J Exp Med* 160, 310-316 (1984)

104. PH van der Meide, F Villinger, AA Ansari, RJ Groenestein, MC de Labie, YJ van den Hout, WH Koornstra, WM Bogers & JL Heeney: Stimulation of both humoral and cellular immune responses to HIV-1 gp120 by interleukin-12 in Rhesus macaques. *Vaccine* 20 (17-18), 2296-2302 (2002)

105. MA Jacobson, E Sinclair, B Bredt, L Agrillo, D Black, CL Epling, A Carvidi, T Ho, R Bains, V Girling & SP Adler: Safety and immunogenicity of Towne cytomegalovirus vaccine with or without adjuvant recombinant interleukin-12. *Vaccine* 24 (25), 5311-5319 (2006)

106. CB O'Brien, DK Moonka, BS Henzel, M Caufield & MF DeBruin: A pilot trial of recombinant interleukin-12 in patients with chronic hepatitis C who previously failed treatment with interferon-alpha. *Am J Gastroenterol* 96 (8), 2473-2479 (2001)

107. MT Lotze, G Trinchieri, M Gately & S Wolf (eds): Interleukin 12: Cellular and molecular immunology of an important regulatory cytokine. *Ann Acad Sci NY* 795, 1-457 (1996)

108. BD Car, VM Eng, B Schnyder, A Shakhov, G Woerly, U Steckholzer, S Huang, M Aguet, TD Anderson & B Ryffel: IL-12 induced *in vivo* pathologic alterations are modified in interferon- γ receptor negative mice. *Am J Pathol* 147, 1693-1707 (1995)

109. VM Eng, BD Car, B Schnyder, M Lorenz, S Lugli, M Aguet, TD Anderson, B Ryffel & VFJ Quesniaux: The stimulatory effects of interleukin-12 on hematopoiesis are inhibited by IL-12 induced IFN- γ ; *in vivo*. *J Exp Med* 181, 1893-1898 (1995)

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