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Review Article

The antitumor potential of interleukin-12

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CONTENTS

Summary

Interleukin-12 (IL-12) is a multipotential cytokine produced by antigen-presenting cells. This cytokine induces interferon-γ (IFN-γ) production, stimulates growth of T and natural killer (NK) cells and promotes Th1-type helper T cell responses. Several studies have reported that IL-12 exerts potent antitumor and antimetastatic activity against different murine tumor models following its systemic or local administration. IL-12 not only inhibits tumor growth but it also induces regression of some well established solid malignancies. In addition, it may induce a specific memory immune response against the tumor. At the doses and regimens that induce these antitumor effects in animals, IL-12 therapy appears to have few associated toxic side effects. Gene therapy approaches using various vectors have elicited complete tumor regression and reduced associated toxicity. The antitumor activity of IL-12 is mostly dependent on T cells and IFN-γ production. Based on the potent IL-12 antitumor effects in animal models, feasibility and safety of systemic therapy with this cytokine in human cancer treatment has been evaluated in phase I clinical trials.

Introduction

Cytokines are considered as integral components of the complex intercellular communication network required to initiate and control an immune response. Studies in animal tumor models have indicated that treatment with several cytokines generally results in a dramatic alteration of tumor cell growth (1-4). However, severe toxicities associated with systemic administration of some of these cytokines are responsible for failure in transferring the therapy to human malignancies (5, 6). Recently, a new cytokine, interleukin-12 (IL-12), was reported to have several interesting *in vitro* and *in vivo* biological properties (7). Its central role in the biology of immune response suggests the possibility of its therapeutic use in various diseases (infection, allergy, tumors, immunodeficiency) and as an adjuvant in vaccination. Conversely, a defect in IL-12 production has been suggested to be a factor contributing to immune depression (8, 9). Many murine tumor models have demonstrated the potent antitumor and antimetastatic activities of IL-12. The present review will focus on the potential therapeutic effects of this cytokine in the treatment of malignancies.

Biological activity

IL-12 plays a pivotal role in the regulation of cell-mediated cytotoxicity and in the modulation of the cytokine network, and has potent antitumor activity (8, 10, 11). This heterodimeric cytokine is produced by phagocytic cells, B cells and other antigen-presenting cell (APC) types during early antigenic stimulation (12). The biological activity of IL-12 is mediated by its binding to membrane receptors on activated T or NK cells (13). Recently, a human IL-12 receptor component that acts as a high-affinity converter was isolated (14), in addition to the reported low-affinity subunit (15). IL-12 acts on T and NK cells by inducing proliferation and production of cytokines, primarily IFN-γ, and enhances the proliferation and activity of cytotoxic T lymphocytes (CTL) and NK cells (16-20) (Fig. 1). Thus, IL-12 represents a bridge between innate resistance and adaptive immune response (8). This cytokine is also required for optimal generation of T helper 1 (Th1) $CD4^+$ cells (21) and CTL (22). The equilibrium between IL-12 and IL-4 during the early immune response determines the evolution of the response to either Th1 or T helper 2 (Th2) phenotype (21). However, IL-12 does not appear to be an absolute requirement for the maintenance of an established Th1 response (23).

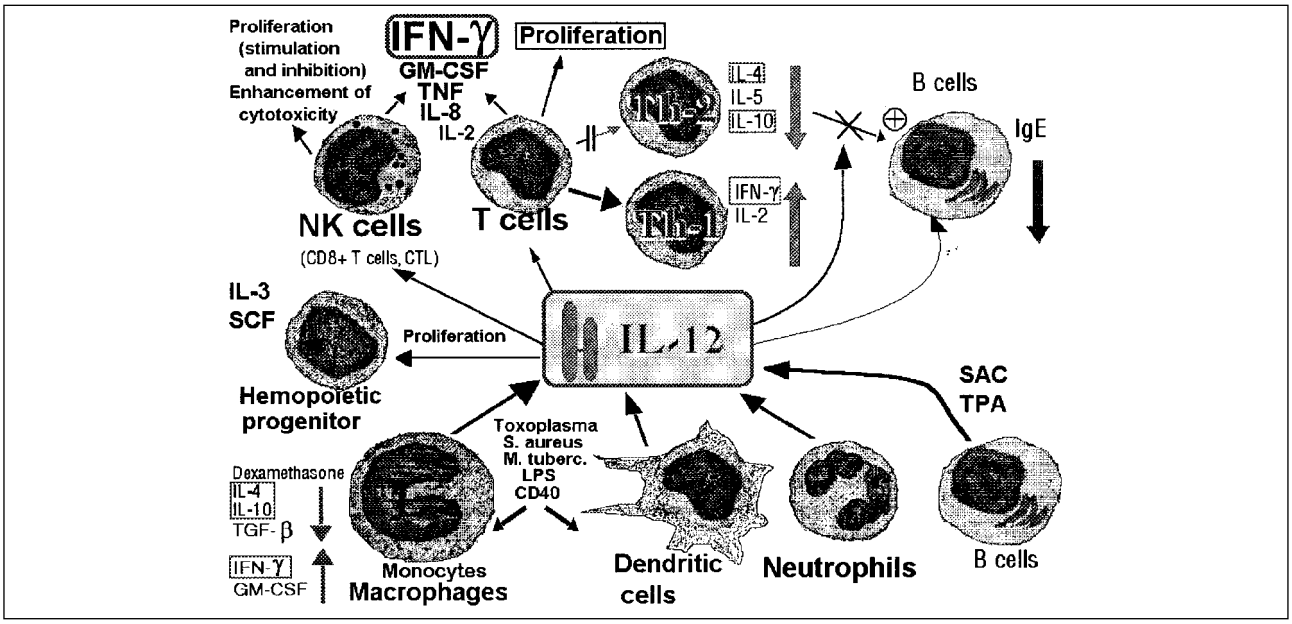

Fig. 1. IL-12 producing cells and functions. In addition to its ability to induce the production of IFN-γ by T and NK cells *in vitro* and *in vivo*, IL-12 is a major component of the cytokine cascade, controlling the production of the cytokines IL-2, GM-CSF, TNF and IL-8.

For the most part, the activities of IL-12 first described in *in vitro* studies can also be demonstrated *in vivo*. Systemic administration of murine IL-12 to normal mice induces serum IFN-γ secretion and increases NK and specific allogeneic CTL cytotoxic activity in the spleen, liver, lungs and peritoneal cavity (24). However, the peak of the enhancement of lytic activity differs between NK (day 2) and allogeneic T cell (day 5) responses. IL-12 treatment of normal mice causes multifocal infiltrates of mononuclear cells, including $CD8^+$ T cells, NK cells and monocytes, in the liver parenchyma and dose-dependent splenomegaly, largely resulting from enhanced extramedullary hematopoiesis (24). In contrast, toxic effects such as bone marrow suppression, anemia and hepatotoxicity are observed after IL-12 treatment. While mice tolerated systemic administration of IL-12 at doses up to 1 μg/day, in cynomolgus monkeys mortality has been observed at comparable doses (1-50 μg/kg/day) (25). Overall, *in vivo* IL-12 administration to mice enhances Th1-type cytokine responses and promotes cell-mediated immunity while inhibiting Th2-type cytokine responses (26).

In vitro activity

In vitro, IL-12 was shown to enhance the non-MHC restricted cytotoxicity mediated by NK cells from healthy donors against colon carcinoma and neuroblastoma cell lines (27, 28) and to enhance NK cell functions in most hairy cell leukemia patients (29). It also stimulates proliferation and cytotoxicity against autologous tumor cells mediated by lymphocytes infiltrating different types of tumors (30, 31). Using an allogeneic MLTC against P815 murine mastocytoma, Gajewsky *et al.* demonstrated that although murine IL-12 had little effect on the net proliferation of developing effector T cells, it increased the specific lytic activity of these cells (32). In this model, IL-12 was also found to act synergistically with B7-1 in inducing IFN-γ production during primary stimulation, and resulted in a shift toward a Th1 cytokine profile following secondary stimulation.

In vivo activity

Recombinant IL-12

The antimetastatic and antitumor effects of murine IL-12 have been examined using systemic administration or peritumoral injection of recombinant IL-12 protein against both experimentally induced and spontaneous models of murine pulmonary and hepatic metastases. IL-12 has shown antitumor activity in many subcutaneous murine tumor models, including malignant melanoma (B16F10) (33), reticulum cell carcinoma (M5076) (33), renal cell adenocarcinoma (Renca) (33), colon adenocarcinoma (MC38/colon 38, C26) (34, 35), methylcholanthrene-induced sarcoma (MCA-105 and MCA-207) (34), KA31 sarcoma (Brunda *et al.*, unpublished observation), B cell lymphoma (X5563) (36) and Lewis lung carcinoma (35).

Systemic and intraperitoneal daily treatment with IL-12 (5 days/week) efficiently inhibited the growth of subcutaneously injected B16 melanoma tumor cells and enhanced the survival time in a dose-dependent manner, even when treatment was initiated 2 weeks after tumor inoculation (33). Similar results were obtained in the

Renca tumor model after either intratumoral or systemic administration of IL-12 to 14-day, well-established subcutaneous tumor bearing mice (33). Therapeutic intervention with systemic administration of IL-12 initiated as late as day 28 after injection of tumor cells (M5076) resulted in suppression of tumor growth, as well as a reduction in the number of metastases and a >2-fold increase in survival time of inoculated animals (33, 34). IL-12 was also found to significantly reduce the growth of metastasis induced by intravenous injection of B16 cells (33) and spontaneous metastasis of Lewis lung carcinoma (35).

Systemic administration of IL-12 has been shown to suppress tumor growth for a prolonged period of time. However, the tumor did not completely regress but showed a delayed growth, resulting in death of the recipient animal. Conversely, peritumoral injection of IL-12 induced complete regression in some tumor models such as Renca or MCA-105 (33, 37). Seven days of peritumoral injection of IL-12 initiated at day 14 after subcutaneous inoculation of MCA-105 dose-dependently suppressed tumor growth. The rates of complete tumor eradication were 16, 40 and 50% in MCA-105, MC38 and MCA-207, respectively (37). In addition, mice “cured” of their tumors (complete remission) following IL-12 treatment showed a delayed growth of the same but not other tumors, following subsequent rechallenge. This suggests that IL-12 may induce a memory immune response against the tumor (33, 34). At doses of IL-12 resulting in tumor regression, the toxicities observed were similar to those observed in normal mice and included mild anemia, slight leukopenia and a small increase in hepatic transaminases.

Gene transfer

Although IL-12 has demonstrated potent antitumor effects when injected systemically, adverse effects have been observed in some mouse strains and in primates. Moreover, results obtained with peritumoral injections of IL-12 (complete regression of the tumor and induction of protective immunity against tumor rechallenge) suggested that local delivery of IL-12 by gene transfer may more efficiently enhance proliferation and differentiation of effector cells, so that they eradicate tumor cells. Local secretion of IL-12 should also limit its toxic effects. Recent studies suggest that IL-12 plays a critical costimulatory role with B7 expression on APCs, in inducing proliferation and IFN-γ production from Th1 clones (32, 38). In this context, IL-12 may be the ideal cytokine for inducing tumor-specific immunity when administered at the tumor site. Several types of vehicles can be used to transfer genes into tumor cells for the purpose of gene therapy. Disabled viruses such as retroviruses, adenoviruses, adeno-associated viruses and herpes viruses can be used (39-43), as well as nonviral approaches such as the injection of naked DNA, liposomes containing cDNA or DNA-coated particles.

The effect of paracrine secretion of IL-12 on tumor establishment and vaccination models was first examined by Tahara *et al.* using a retroviral system (44). Coinjection of BL-6 murine melanoma cells and allogeneic fibroblasts (NIH3T3) transfected with cDNAs encoding both chains of IL-12 (3T3-IL-12) (100-240 U/10^6 cells/48 h of IL-12) significantly delayed tumor growth in mice in a dose-dependent manner. Moreover, immunization with irradiated tumor cells and 3T3-IL-12 followed by a subsequent tumor challenge resulted in delay of tumor appearance, with a relatively small amount of IL-12 secretion (2.4 U/10^6 cells/48 h of IL-12). These results support the possibility of using IL-12 in the preparation of cancer vaccines and further suggest that local delivery of IL-12 may lead to the development of an effective antitumor immune response. Transfection of MCA207 murine sarcoma cells with a polycistronic retroviral vector, which can express both IL-12 subunits, resulted in a complete loss of tumorigenicity of MCA207 cells and long-term immunity (43). Injection of MCA207-IL-12 cells also caused regression of up to 3 days-established contralateral nontransfected MCA207 tumors. The C26 murine colon carcinoma induces tumors that are minimally sensitive to *in vivo* systemic treatment with IL-12. Transduction into C26 cells of both IL-12 genes using a polycistronic retroviral vector resulted in cells that produced low levels of IL-12 and that were significantly delayed in inducing tumor formation *in vivo* (45).

Recently, constructions of adenovirus expressing murine IL-12 (Ad-IL-12) have been reported (46, 47). Intratumoral injection of Ad-IL-12 induced substantial or complete hepatic tumor regression of the poorly immunogenic MCA-26 colon carcinoma and significantly prolonged survival of tumor bearing mice. A combination of Ad-IL-12 and Ad-IL-2, which express human IL-2, induced a complete regression in 68% of mice and an overall response rate of 95%, compared to 38% and 86%, respectively, with Ad-IL-12 alone. Both treatments resulted in a protective immunity following rechallenge with fresh tumor cells (47). Intratumoral administration of Ad-IL-12 alone into mice bearing polyoma middle T-induced mammary carcinomas also resulted in potent antitumor effect (48).

A new suicidal vector system based on Semliki Forest virus (SFV) and characterized by high expression levels and broad host range was reported (49). We have undertaken studies to analyze the possibility of using an SFV vector as a new method of expressing human IL-12 (50). Inoculation of tumor cells genetically engineered to secrete cytokines is followed by the development of antigen-specific immunity. Studies in a variety of models suggested that in addition to transgenes, the first-generation adenoviral vector expressed viral proteins may activate CTL leading to destruction of virus-infected cells. One of the disadvantages of gene therapy with recombinant adenoviruses was that cytokine gene expression could not be detected readily after a second administration of the virus, which was reported to be associated with the development of antiviral neutralizing antibodies (51).

Interestingly, the SFV vector is less immunogenic since no structural proteins are produced in the host cells (52). The use of the SFV expression system for gene transfer therapy may also avoid possible oncogene activation by integration of the retroviral vector. Another advantage of using the SFV expression system for gene transfer in tumor therapy is that cells infected with SFV are certain to die after infection and cytokine secretion. It is then tempting to speculate that, *in vivo*, dead cells would provide the tumor infiltrating lymphocytes with tumor antigens released at the tumor site, together with high levels of IL-12, therefore increasing CTL expansion, recruitment of helper T cells and NK cells and enhancement of the host APC function, while minimizing systemic toxicity. The SFV vector may prove to be a valuable means of enhancing a protective local and systemic immune response against a small tumor burden. Experiments aimed at further developing IL-12 delivery *in vivo* are under way in our laboratory.

Mechanistic effects

Role of T cells

The antitumor effects of IL-12 can be obtained independently of NK cells since comparable activity is observed in NK cell deficient beige mice or in mice depleted of NK cell activity by treatment with antiasiallo GM_1 (33). In contrast, antitumor activity of IL-12 is substantially reduced in nude mice (33), suggesting a critical role for T cells. In the Renca tumor model, depletion of $CD8^+$ T cells, but not $CD4^+$ T cells, significantly reduced IL-12 antitumor effects (33), demonstrating a critical role for $CD8^+$ T cells. On the other hand, it has been shown that the depletion of both $CD4^+$ and $CD8^+$ T cells, but not the depletion of either one alone, completely abrogated the IL-12 antitumor effect (34). However, using the MCA-207 tumor model, IL-12 had no reduced antitumor effect in mice treated with anti-CD8 or anti-CD4 antibodies (34).

Injection of recombinant IL-12 (1 μg/day) or transduction of C26 cells with IL-12 cDNAs (low secretion of IL-12: 30-80 pg/ml) resulted in delayed tumor onset, due to NK cells and, in part, to $CD8^+$ cells (45, 53). The *in vivo* growing tumors were characterized by an extremely poor lymphocytic infiltrate. However, *in vivo* depletion of $CD4^+$ cells resulted in a significant increase in tumor infiltration with $CD8^+$ and NK cells and in complete remission of the tumor in approximately half of the animals (45). Transduction of C26 cells with a polycistronic retrovirus (high secretion of IL-12: 5 ng/ml) resulted in complete tumor regression associated with infiltration of NK and $CD8^+$ cells. These results suggest that the amount of IL-12 available at the tumor site may determine both the type and the number of infiltrating lymphocytes (53). As observed in other experimental tumor systems (54, 55), IL-12 may activate $CD4^+$ cells that have an inhibitory effect on the tumor infiltration and antitumor activity of $CD8^+$ cells.

Although some discrepancies exist regarding the results of T cell depletion studies between tumor systems, the involvement of T cells appears to be required for mediating IL-12 antitumor effects. Recently, Hendrzak *et al.* have demonstrated that IL-12 can also activate cytolytic macrophages (J. Hendrzak *et al.*, unpublished observation) and that tumors undergoing IL-12-mediated regression have large infiltrates of $Mac1^+$ macrophages (T. Anderson *et al.*, unpublished observation). Thus, macrophages may also be involved in the antitumor effects of IL-12.

Role of IFN-γ

Since IL-12 is the most potent single inducer of IFN-γ, involvement of this cytokine has been tested by several groups. High levels of IFN-γ are found in the sera of both normal and tumor-bearing mice treated with IL-12 (24, 54). Antibodies to IFN-γ (IFN-γ depletion) partially, but not entirely, inhibited the antitumor effects of IL-12 against B16F16 (54), Renca (54) and MC38 (34) tumors, indicating the critical role of IFN-γ in IL-12 antitumor activity. In contrast, antibody to tumor necrosis factor had no inhibitory effect (34). However, data showing that IL-12 induces an approximately 5-fold higher level of serum IFN-γ in nude mice compared to euthymic mice, but has greatly reduced antitumor activity in nude mice, suggest the absence of a direct correlation between the induction of IFN-γ by IL-12 and antitumor efficacy (33, 54). Thus, IFN-γ may be necessary but not sufficient to mediate the antitumor effects of IL-12. IL-12 induces the secretion of IFN-γ from either T or NK cells, but IFN-γ induced from T cells is sufficient to promote antitumor efficacy since there is no loss of IL-12 activity in NK cell deficient/depleted mice (33).

Recently, Fallarino *et al.* demonstrated that endogenous IL-12 may be necessary for tumor rejection *in vivo* (56). In this murine model where a variant of P815, P1.HTR, was found to be rejected in the hind footpads by one-third of syngeneic DBA/2 mice, they demonstrated that neutralization of IL-12 *in vivo* by injection of a rabbit anti-IL-12 antiserum prevented rejection of the tumors and inhibited the high IFN-γ response to tumor cell challenge observed in nontreated mice.

Antiangiogenic activity

The observation that the antitumor activity of IL-12 is diminished, but not completely abrogated, in SCID, nude and $CD8^+$-depleted euthymic mice suggest that an additional mechanism may play a role in the antitumor activity of IL-12. Angiogenesis is a critical step for tumor growth and metastasis. Recently, Voest *et al.*, using a mouse model of corneal neovascularization, demonstrated that IL-12 was a potent inhibitor of angiogenesis (57). The IL-12-induced inhibition of neovascularization was prevented by administration of anti-IFN-γ antibodies. The

antiangiogenic activity of IFN-γ is controversial and is mainly based on *in vitro* observations (58). However, experimental evidence has been provided indicating that IFN-γ is efficient in inducing the production of interferon-inducible protein-10 (IP-10) (59), a -C-X-C- chemokine that exhibits potent antiangiogenic activity both *in vitro* and *in vivo* (60). Tumor cells engineered to produce IP-10 are rejected in a T cell-dependent fashion (60). IFN-γ also inhibits metalloproteinase production, which is required for the breakdown of the extracellular matrix to allow new capillaries to sprout (61). Therefore, inhibition of metalloproteinase may affect angiogenesis.

In an attempt to determine cytokine and chemokine expression in tumors of mice receiving systemic therapy with IL-12, Tannenbaum *et al.* suggested that IL-12 initiates a cytokine-chemokine network in which IL-12 responsive cells within the tumor express IFN-γ, which in a paracrine fashion is able to induce tumor cells to synthesize IP-10 (62). This factor may contribute to the recruitment of macrophages and NK effector cells. Recently, Sgadari *et al.* demonstrated that murine IL-12 profoundly inhibits basic fibroblast growth factor (bFGF)-induced matrigel neovascularization *in vivo*, and that this IL-12 effect was neutralized by systemic administration of antibodies to either murine IFN-γ or IP-10 (63). This suggests that IP-10 was an important mediator of angiogenesis inhibition by IL-12. Experiments are in progress in our laboratory to investigate the antiangiogenic activity of IL-12 in human tumors.

Clinical studies

The potential use of IL-12 in human tumor therapy has been strongly supported by the above reported properties and based on preclinical models. Major adverse effects, limiting systemic administration of the T cell growth factors IL-2 and IL-4, have occurred in the course of cancer immunotherapy, mainly involving vascular leak syndrome. Since such prominent vascular effects of IL-12 were not observed in murine models using several strains of mice and only in a relatively small proportion of primates (25), it is expected that humans will tolerate therapeutic doses of IL-12. However, IL-12 might be associated with other toxic side effects. In particular, systemic administration of therapeutic doses of IL-12 induced death in one strain of mice (C3H) (25).

Encouraged by the potent antitumor effects of IL-12, clinical trials were initiated in July 1994 by several groups. A phase I trial was conducted by Genetic Institute (Cambridge, Massachusetts) to determine the safety and efficacy of intravenously administered, genetically engineered IL-12 in the treatment of cancer (64). In the course of this multicenter trial, treatment with IL-12 was toxic in most of the 17 renal cell carcinoma patients participating in the trial. The toxicities affected multiple organ systems, and 2 of the patients died. These complications appeared to be due to the dosing regimen. Indeed, the patients in this study began treatment with multiple doses of IL-12, whereas in earlier studies, patients were first given various single doses of IL-12 to determine the maximum tolerated dose, followed a few weeks later by multiple doses of the drug. Studies performed in mice and monkeys have shown a similar pattern: treatment with a single dose of IL-12, followed by a rest period, and then multiple doses, was not harmful. Multiple high doses of IL-12 are highly toxic if they are given without an initial single dose, whereas multiple low doses, even without an initial single dose, appear to be safe.

Nevertheless, because of the biological and possibly clinical relevance of IL-12, it is important that its biological activity, production and, in particular, functional interaction with other cytokines continue to be elucidated. Secretion of immunosuppressive cytokines such as TGF-β is one of several mechanisms by which tumors have evolved to escape an immune response (65, 66). It is conceivable that local concentration of TGF-β could attain levels that suppress cytokine responses *in vivo*. Recently, we provided evidence that TGF-β mediates immunosuppression of human alloreactive response by inducing a dramatic inhibition of IL-12 production during primary allostimulation and by interfering with IL-12 stimulatory pathway (67). The understanding of the molecular basis of immunosuppressive cytokine inducing alteration of effector cell responsiveness to IL-12 may be important to the rationale for appropriate strategies in IL-12-based immune intervention.

At present, tumor cells engineered with cytokine genes represent an attractive clinical prospect and promising results have been obtained in animal models. Nevertheless, it has not been determined if expression of immunoregulatory cytokines by human tumors can overcome immunosuppression and elicit tumor rejection.

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