Antitumor and Antimetastatic Activity of Interleukin 12 against Murine Tumors

By Michael J. Brunda,* Leopoldo Luistro,* Rajeev R. Warrier,‡ Rosemary B. Wright,* Brian R. Hubbard,§ Molly Murphy,§ Stanley F. Wolf,§ and Maurice K. Gately‡

*From the Departments of *Oncology and ‡Inflammation/Autoimmune Diseases, Hoffmann-La Roche Inc., Nutley, New Jersey 07110; and §Genetics Institute Inc., Cambridge, Massachusetts 02140*

Summary

It has recently been demonstrated that in vivo administration of murine interleukin 12 (IL-12) to mice results in augmentation of cytotoxic natural killer (NK)/lymphocyte-activated killer cell activity, enhancement of cytolytic T cell generation, and induction of interferon γ secretion. In this study, the in vivo activity of murine IL-12 against a number of murine tumors has been evaluated. Experimental pulmonary metastases or subcutaneous growth of the B16F10 melanoma were markedly reduced in mice treated intraperitoneally with IL-12, resulting in an increase in survival time. The therapeutic effectiveness of IL-12 was dose dependent and treatment of subcutaneous tumors could be initiated up to 14 d after injection of tumor cells. Likewise, established experimental hepatic metastases and established subcutaneous M5076 reticulum cell sarcoma and Renca renal cell adenocarcinoma tumors were effectively treated by IL-12 at doses which resulted in no gross toxicity. Local peritumoral injection of IL-12 into established subcutaneous Renca tumors resulted in regression and complete disappearance of these tumors. IL-12 was as effective in NK cell–deficient beige mice or in mice depleted of NK cell activity by treatment with antiasialo GM1, suggesting that NK cells are not the primary cell type mediating the antitumor effects of this cytokine. However, the efficacy of IL-12 was greatly reduced in nude mice suggesting the involvement of T cells. Furthermore, depletion of $CD8^+$ but not $CD4^+$ T cells significantly reduced the efficacy of IL-12. These results demonstrate that IL-12 has potent in vivo antitumor and antimetastatic effects against murine tumors and demonstrate as well the critical role of $CD8^+$ T cells in mediating the antitumor effects against subcutaneous tumors.

Human IL-12 is a disulfide-bonded heterodimeric cytokine consisting of a 40- and a 35-kD subunit (1, 2). The genes for this cytokine have been cloned (3, 4) and purified recombinant protein has been produced. A number of biological properties of human IL-12 have been evaluated in vitro. Among its properties are the ability to act as a NK cell and T cell growth factor (5–7), to enhance NK/LAK cell cytolytic activity (1, 7–9), to augment cytolytic T cell responses (9), and to induce secretion of cytokines, particularly IFN-γ, from T and NK cells (1, 10). Since both T and NK cells have been implicated as antitumor effector cells (11) and IFN-γ has been shown to have antitumor activity in animals (12, 13), IL-12 has the potential to be used as an immunomodulatory cytokine in the therapy of malignancies.

The ability to test the in vivo activities of IL-12 have been limited since human IL-12 is inactive on murine cells, but recently the genes for murine IL-12 have been cloned (14). Injection of mice with recombinant murine IL-12 augments NK activity, enhances allogeneic cytolytic T cell responses, and induces secretion of IFN-γ, thus confirming the previously described in vitro activities of IL-12 (Gately, M. K., R. R. Warrier, S. Honasoge, D. A. Faherty, S. E. Connaughton, T. D. Anderson, U. Sarmiento, B. R. Hubbard, and M. Murphy, manuscript submitted for publication). Based on these in vivo results, the antitumor and antimetastatic activities of murine IL-12 against a number of murine malignancies have been evaluated. We demonstrate in this study that systemic administration of IL-12 can inhibit the growth of both established subcutaneous tumors and experimental pulmonary or hepatic metastases, and that local peritumoral injections of IL-12 can result in regression of established subcutaneous tumors. Based on results obtained using mice deficient in lymphocyte subsets and antibody depletion experiments, the antitumor efficacy of IL-12 is mediated primarily through $CD8^+$ T cells.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/93/10/1223/08 $2.00
Volume 178 October 1993 1223–1230

Materials and Methods

Mice. C57BL/6, C57BL/6 *bg/bg* beige, C57BL/6 *bg/+* heterozygous beige control and BALB/c mice, 6–8 weeks of age, were purchased from The Jackson Laboratories, (Bar Harbor, ME). BALB/c nude mice were obtained from Harlan-Sprague Dawley, Inc. (Madison, WI). Mice were routinely screened and found to be free of mycoplasma and selected murine viruses.

Tumor Cell Lines. B16F10 malignant melanoma cells (15) and Renca renal cell adenocarcinoma cells (16), obtained from Dr. R. Wiltrout (Biological Response Modifiers Program, Frederick, MD), were maintained in vitro in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 25 mM $NaHCO_3$, and 60 μg/ml L-glutamine. M5076, a reticulum cell sarcoma (12, 17), was maintained as a stationary suspension culture in RPMI 1640 supplemented with 17% equine serum (Hyclone Laboratories, Inc., Logan, UT), 1 mM sodium pyruvate, 50 μg/ml gentamicin, glutamine, penicillin, and streptomycin. All reagents for cell culture were purchased from GIBCO BRL (Gaithersburg, MD) unless otherwise designated. All cell lines were found to be free of mycoplasma and viruses as above.

Recombinant Murine IL-12. Murine rIL-12 was expressed in CHO cells that had been stably transfected with the IL-12 p40 and p35 cDNAs and was purified as previously described (Gately, M. K., et al., manuscript submitted for publication). SDS-PAGE analysis of the purified IL-12 indicated it to be ≥95% pure with a small amount of contaminating IL-12 p40 monomer. Monomeric IL-12 p40 does not bind to the IL-12 receptor (18) and lacks biological activity (3, 4). Contamination by endotoxin, as assessed by the *Limulus* amebocyte assay, was <5 EU/mg IL-12. The sp act of purified murine rIL-12, as determined by its ability to cause proliferation of human PHA blasts (18), was $\sim 7 \times 10^6$ U/mg protein. For administration to mice, rIL-12 was diluted in PBS containing 100 μg/ml mouse serum albumin (Miles Scientific, Naperville, IL or Sigma Chemical Co., St. Louis, MO).

Tumor Experiments. Exponentially growing tumor cells were harvested by brief trypsinization (B16F10 and Renca), washed, and injected subcutaneously or intravenously into groups of 10 mice on day 0, and intraperitoneal treatment with various doses of IL-12 once per day five times per week was initiated between days 1 and 28, depending on the experiment. Specific protocols are presented in the legends of individual experiments. The diameters of subcutaneous tumors were measured twice weekly with calipers, and volume was calculated by the formula: Vol = (longest diameter) × (shortest diameter)^2 (Gately, M. K., et al., manuscript submitted for publication). For metastasis experiments, approximately 3 wk after intravenous injection of tumor cells, mice were killed and the number of metastases enumerated as previously described (15). Statistical evaluation of the data was performed using the nonparametric one-tailed Mann-Whitney U test (17). Experiments were repeated two to eight times, and data from representative experiments are presented.

Antibody Depletion Experiments. Antiasialo GM1 (anti-ASGM1) was purchased from Biochemical Diagnostics, Inc. (Edgewood, NY). Mice were injected intraperitoneally with 0.4 ml of 1:5 dilution of anti-ASGM1 or normal rabbit serum on the days indicated. Under these conditions of treatment with antiasialo GM1 splenic NK activity, is reduced to undetectable levels (17).

Anti-CD4 (clone GK1.5, rat IgG2b) (19) and anti-CD8 (clone 2.43, rat IgG2b) (20) hybridomas were purchased from the American Type Culture Collection (Rockville, MD). Ascites were prepared in pristane-treated nude mice and Ig purified by the procedure of Reik et al. (21). Mice were injected intraperitoneally with 1 mg of Ig on the days indicated. Under these conditions, antibody treatment depleted >95% of the appropriate cell population in the spleen.

Results

We initially evaluated the effect of IL-12 on experimental pulmonary metastases of the B16F10 melanoma. After intravenous injection with tumor cells on day 0, C57BL/6 mice were treated intraperitoneally beginning on day 1 with varying doses of IL-12 five times per week for 3 wk. At the end of the treatment period, there was a dose-dependent inhibition of experimental metastases in the IL-12–treated mice (Fig. 1 *A*). In the group receiving 1 μg per injection, the median number of metastases was reduced to 41 compared with 200 in the diluent-treated mice ($p < 0.005$). To determine if treatment could be initiated at a time when metastases were al-

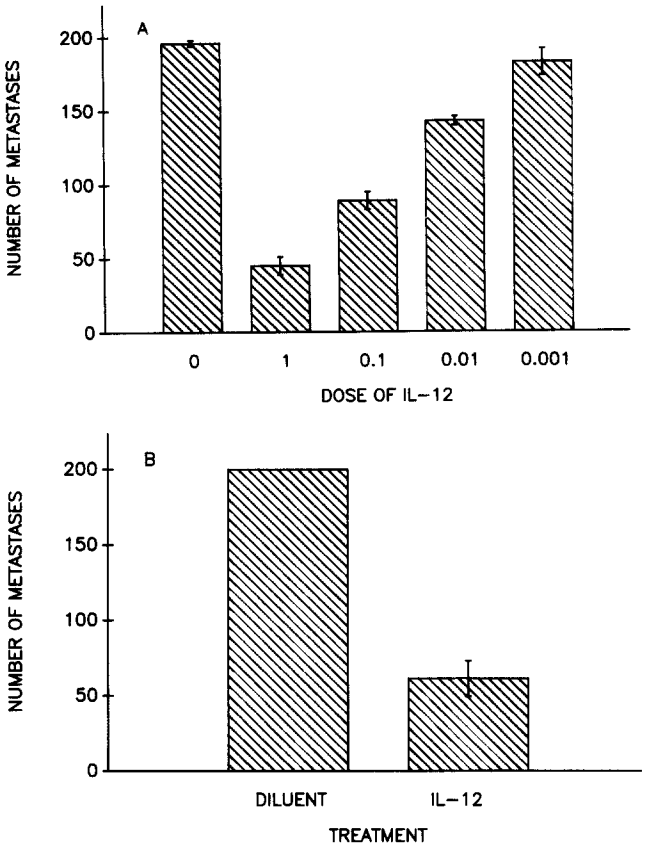

Figure 1. Inhibition of B16F10 metastases by IL-12. (*A*) Mice were injected intravenously with 2 × 10^5 B16F10 cells on day 0 and treatment with diluent or varying doses of IL-12 per injection was initiated intraperitoneally on day 1. Mice were treated five times per week until animals were killed on day 22. (*B*) Treatment with 1 μg per injection of IL-12 five times per week was initiated on day 7 and mice were killed on day 22. Data are represented as mean ± SE of 10 mice per group.

ready firmly established, mice were similarly injected intravenously with tumor cells on day 0, but the initiation of treatment was delayed until day 7. As shown in Fig. 1 *B*, under these experimental conditions, treatment with 1 μg of IL-12 also significantly reduced the number of experimental metastases ($p < 0.0005$). It is clear from these results that IL-12 has potent antitumor activity in this experimental metastasis model and that treatment of animals with IL-12 can be initiated after metastases have already been established.

A series of experiments was then performed to determine if IL-12 also has activity against subcutaneously growing B16F10 tumors. Initially, mice were injected subcutaneously with 10^6 tumor cells on day 0, and treatment with varying amounts of IL-12 was initiated on day 7 when small tumors were present in the animals. As previously demonstrated with experimental metastases, there was a dose-dependent inhibition of tumor growth with maximal effects observed at a dose of 1 μg per injection (Fig. 2 *A*). At all doses tested, no gross toxicity was evident and all animals survived the therapy, although at the 5 μg dose some lethargy was observed. To determine if IL-12 could increase the survival of B16F10 tumor-bearing mice and to test if the effect of prolonged IL-12 treatment on tumor growth, tumor-bearing mice were treated with 1 μg per injection of IL-12 five times per week until day 50. As seen in Fig. 2 *B*, there was a large increase in survival time of IL-12–treated mice compared with

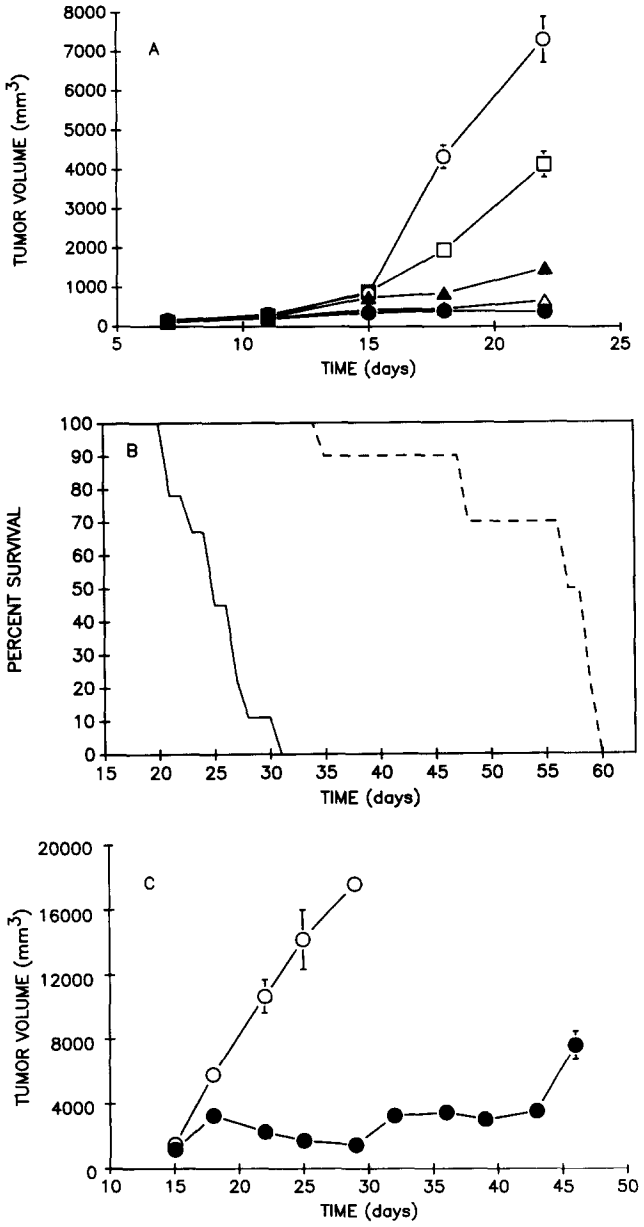

Figure 2. Inhibition of subcutaneous growth of B16F10 tumors. (*A*) Mice were injected subcutaneously with 10^6 B16F10 cells on day 0 and intraperitoneal treatment with diluent (○—○) or 5 μg (●—●), 1 μg (△—△), 0.1 μg (▲—▲), or 0.01 μg (□—□) of IL-12 was initiated on day 7. Mice were treated five times per week until animals were killed on day 22. (*B*) Survival of mice treated intraperitoneally with diluent (*solid line*) or 1 μg of IL-12 (*dashed line*) per injection five times per week through day 50. (*C*) Treatment of mice was initiated on day 14. Mice were treated intraperitoneally with diluent (○—○) or 1 μg of IL-12 (●—●) per injection five times per week for 3 wk. Data are represented as mean ± SE of 10 mice per group.

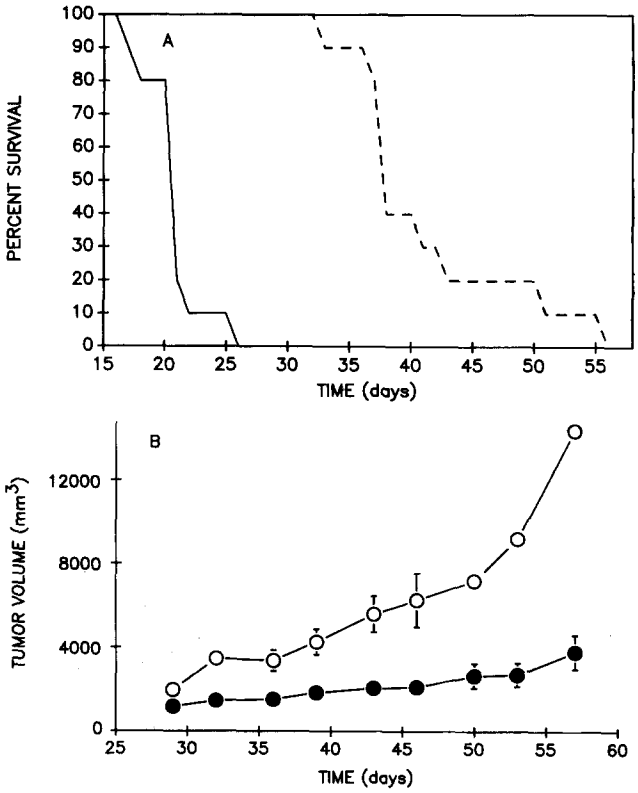

Figure 3. Inhibition of M5076 experimental hepatic metastases and subcutaneous tumor growth by IL-12. (*A*) Survival of mice injected intravenously on day 0 with 10^5 M5076 tumor cell and treated intraperitoneally with diluent (*solid line*) or 1 μg of IL-12 (*dashed line*) per injection five times per week for 3 wk was initiated on day 1. (*B*) Mice were injected subcutaneously with 10^6 M5076 tumor cells on day 0 and intraperitoneal treatment with diluent (○—○) or 1 μg of IL-12 (●—●) per injection was initiated on day 28. Animals were treated five times per week for 4 wk. Data represented as mean ± SE of 10 mice per group.

diluent-treated control mice. Although tumor growth was suppressed for a prolonged time by IL-12 treatment, no "cures" were obtained, and tumors eventually grew, resulting in death of the animals. To evaluate the effect of IL-12 against larger B16F10 tumors, treatment with 1 μg of IL-12 per injection was delayed until day 14. As seen in Fig. 2 *C*, tumors grew progressively in control animals through day 29, at which point all animals had died. In contrast, the growth of tumors in mice treated with IL-12 was greatly diminished. These results demonstrate that IL-12 has marked antitumor activity against subcutaneous B16F10 tumors.

Since antitumor activity of IL-12 had only been demonstrated against the B16F10 melanoma, the antitumor efficacy of IL-12 was next evaluated against additional tumors. After intravenous injection of M5076 reticulum cell sarcoma, experimental hepatic metastases are formed (17). As seen in Fig. 3 *A*, diluent-treated mice have a median survival of 21 d with all animals dead by day 26, but treatment of mice with IL-12 results in an increase in median survival time to 38 d. After subcutaneous injection of M5076 tumor cells, tumors develop much more slowly than in mice injected with B16F10 melanoma cells. Thus, intraperitoneal treatment of mice with IL-12 was begun 4 wk after implantation of tumor cells. Under these conditions, IL-12 markedly reduced tumor growth (Fig. 3 *B*). In these IL-12–treated animals, spontaneous hepatic metastases are also greatly reduced (Brunda, M. J., and T. D. Anderson, unpublished observation). Therefore, the antitumor activity of IL-12 is evident against both subcutaneous tumor growth and experimental hepatic metastases of the M5076 reticulum cell sarcoma.

To evaluate further the antitumor activity of IL-12, mice were injected subcutaneously with the Renca renal cell adenocarcinoma and treated with IL-12. The Renca renal cell adenocarcinoma is a murine tumor of spontaneous origin in BALB/c mice that mimics histologically renal cell carcinoma in humans (16) and is sensitive to various cytokines in vivo (16, 22). Beginning on day 14, intraperitoneal treatment of mice with IL-12 resulted in a dramatic inhibition of tumor growth (Fig. 4 *A*), although complete regression of tumors was not observed. Since it has previously been reported that regression of the Renca tumor occurs after transfection of IL-4 into this tumor cell (22), mice bearing day 14 subcutaneous Renca tumors were injected peritumorally with 1 μg of IL-12. As seen in Fig. 4 *B*, tumors were markedly inhibited and, in fact, tumor regression occurred with no tumor detectable in 70% of treated mice after 60 d. Upon rechallenge with Renca cells alone, tumor growth is greatly inhibited in these survivors (data not shown). Thus, IL-12 has pronounced antitumor activity against the Renca renal cell adenocarcinoma and can, under certain conditions, result in regression of established tumors.

Since IL-12 has been demonstrated to have effects on both NK and T cells (1–10, 14, and Gately, M. K., et al., manuscript submitted for publication), the antitumor activity of IL-12 might be mediated through either of these cell types. To determine if the antitumor efficacy of IL-12 was primarily mediated through NK cells, its activity was evaluated against

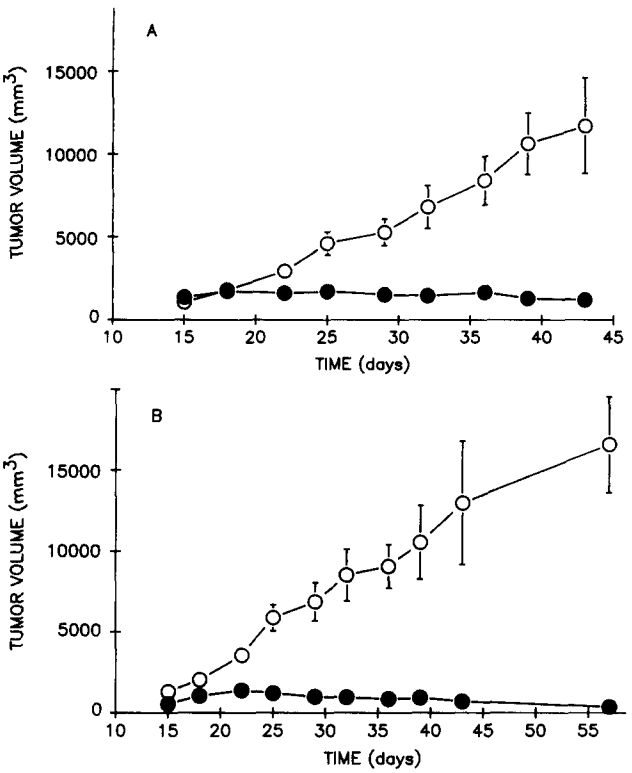

Figure 4. Inhibition of Renca tumors by IL-12. Mice were injected subcutaneously on day 0 with 10^6 Renca tumor cells and treatment with diluent (○—○) or 1 μg of IL-12 (●—●) per injection five times per week for 4 wk was initiated on day 14. (*A*) Treatment by intraperitoneal route. (*B*) Treatment by peritumoral route. Data represented as mean ± SE of 10 mice per group.

B16F10 tumors in NK cell deficient *bg/bg* beige (23). Treatment of either *bg/bg* beige or phenotypically normal *bg/+* heterozygotes with 1 μg per injection of IL-12 resulted in a comparable degree of tumor inhibition (Fig. 5). To confirm and extend these results, Renca tumor–bearing BALB/c mice were depleted of NK cells using anti-ASGM1 and treated with IL-12. No loss of IL-12–induced antitumor efficacy was observed in these NK cell–depleted mice (Fig. 6). Taken together, these results strongly suggest that the antitumor efficacy of IL-12 is not mediated through NK cells.

To evaluate the potential role of T cells on IL-12–induced antitumor efficacy, the antitumor effect of IL-12 was tested against B16F10 tumors in nude mice (Fig. 7 *A*). Although there was a slight delay in tumor growth, the effect of IL-12 was markedly diminished compared with that observed in euthymic mice. The greatly reduced activity of IL-12 was also obtained in BALB/c nude mice bearing syngeneic Renca tumors (Fig. 7 *B*). To evaluate further the requirement for T cells in mediating IL-12–induced antitumor efficacy and to determine the role of T cell subsets, Renca tumor–bearing mice were treated with anti-CD4 or anti-CD8 antibodies. As seen in Fig. 8, the activity of IL-12 is not affected in mice depleted of $CD4^+$ T cells but substantially, although not

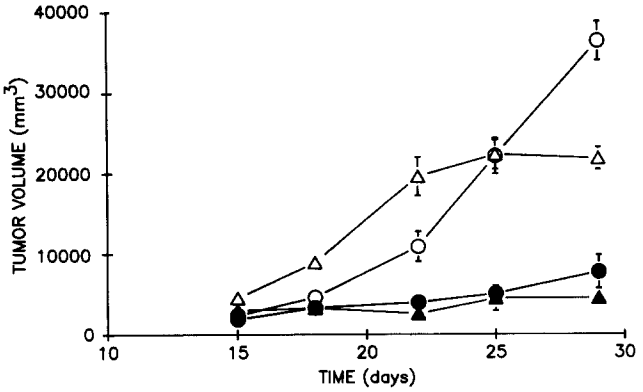

Figure 5. Antitumor effects of IL-12 in NK cell–deficient beige mice. Animals were injected subcutaneously with 10^6 B16F10 tumor cells on day 0 and intraperitoneal treatment with diluent (*open symbols*) or 1 μg of IL-12 (*filled symbols*) per injection five times per week until the end of the experiment was initiated on day 14 in *bg*/+ heterozygous mice (○/●) or *bg*/*bg* beige mice (△/▲). Data are represented as mean ± SE of 10 mice per group.

completely, lost in mice treated with anti-CD8. These results demonstrate that $CD8^+$ T cells are critical for mediating the antitumor effects of IL-12.

Discussion

Using purified recombinant murine IL-12, we have demonstrated that this cytokine has potent antitumor and antimetastatic activity against a number of murine tumors of various histological types. Therapeutic intervention by systemic administration of IL-12 can be initiated when tumors or metastases are well established, up to day 28 after injec-

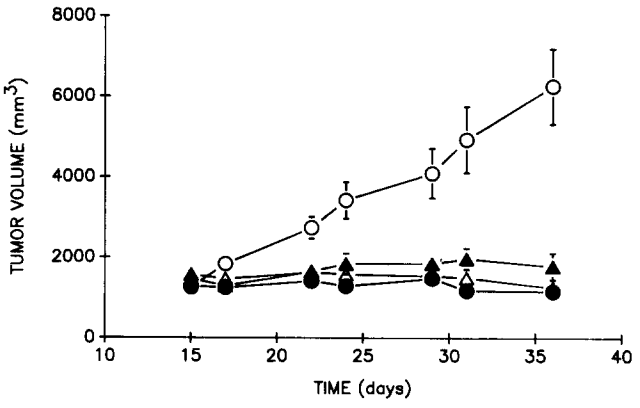

Figure 6. Antitumor effects of IL-12 in mice treated with anti-ASGM1. Animals were injected subcutaneously with 10^6 Renca tumor cells on day 0 and intraperitoneal treatment with diluent (○—○) or IL-12 (●—●) was initiated on day 14 and continued five times per week for 4 wk. Other groups were treated with IL-12 beginning on day 14 and were injected intraperitoneally with either NRS (△—△) or anti-ASGM1 (▲—▲) on days 15, 17, 21, 24, 28, and 31. Data are represented as mean ± SE of 10 mice per group.

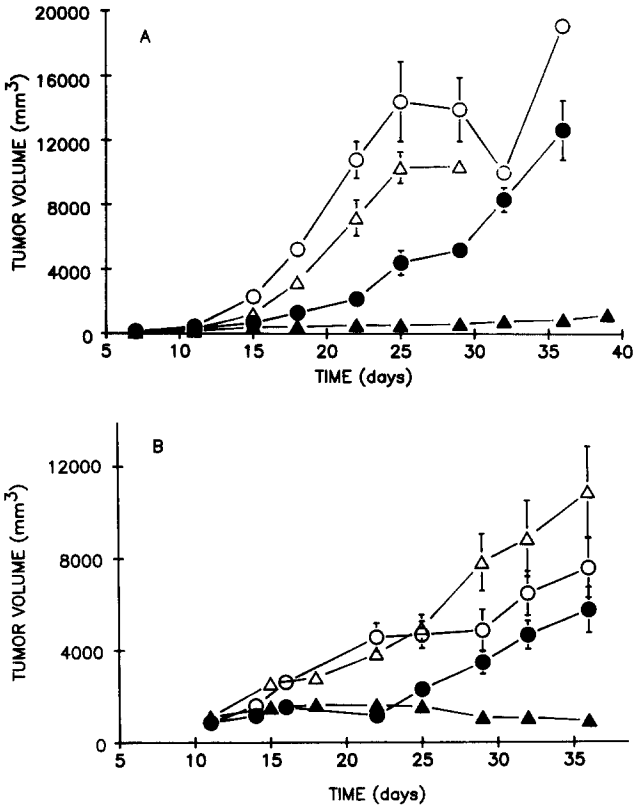

Figure 7. Reduced efficacy of IL-12 in tumor-bearing nude mice. Mice were treated intraperitoneally five times per week for 4 wk with diluent (*open symbols*) or 1 μg IL-12 (*closed symbols*) beginning on day 7. Data are represented as mean ± SE of 10 mice per group. (*A*) B6 (△/▲) or BALB/c nude mice (○/●) were injected subcutaneously with 10^6 B16F10 tumor cells on day 0. (*B*) BALB/c (△/▲) or BALB/c nude mice (○/●) were injected subcutaneously with 10^6 Renca tumor cells on day 0.

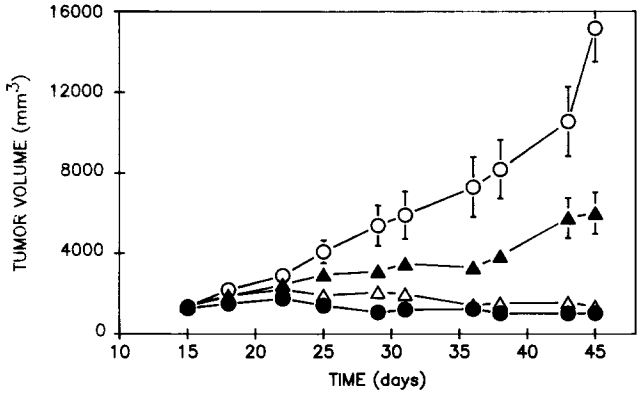

Figure 8. Effect of depletion of $CD4^+$ or $CD8^+$ T cells on antitumor efficacy of IL-12. Mice were injected subcutaneously with 10^6 Renca tumor cells on day 0 and intraperitoneal treatment five times per week until the end of the experiment with diluent (○—○) or 1 μg IL-12 (●—●) was initiated on day 14. Other groups of mice were injected with IL-12 and either anti-CD4 (△—△) or anti-CD8 (▲—▲) on days 14, 21, and 28. Data are represented as mean ± SE of 10 mice per group.

tion of tumor cells in the M5076 model, resulting in inhibition of tumor growth, reduction in the number of metastases and an increase in survival time. Furthermore, after local peritumoral injections of IL-12, established subcutaneous tumors regress, resulting in animals that are tumor free.

The mechanism through which IL-12 exerts its antitumor activity is at present unknown, but based on its established in vivo activities several potential hypotheses can be suggested. IL-12 may have direct growth inhibitory effects on tumor cells. This is unlikely since addition of IL-12 in vitro to the murine tumor cells used in our studies resulted in no inhibition of tumor cell proliferation (Brunda, M. J., and L. Luistro, unpublished observation) and there is reduced activity of this cytokine against tumors in nude mice (Fig. 7). However, IL-12 has been shown to induce production and secretion of IFN-γ (1, 10, and Gately, M. K., et al., manuscript submitted for publication), which might then in turn inhibit proliferation of tumor cells (24). Such a proposed mechanism would be consistent with the lack of direct in vitro antiproliferative activity on tumor cells and the observed in vivo efficacy of IL-12. IFN-γ has been previously shown to have activity in vivo against murine tumors, although not to the extent observed with IL-12 (12, 13). Both T and NK cells can produce IFN-γ in response to IL-12 *in vitro* (1, 10), but if this hypothesis is correct, it would appear that T cell production of IFN-γ is critical to the antitumor activity of IL-12 *in vivo* because the efficacy of IL-12 is greatly reduced in athymic nude mice (Fig. 7). It is unlikely that the antitumor efficacy of IL-12 is directly an effect of the level of IFN-γ induced since IL-12 induces an approximately fivefold higher level of IFN-γ in nude mice than in euthymic mice (Hendrzak, J., and M. Brunda, unpublished observation) but IL-12 has a substantially reduced antitumor effect in nude mice (Fig. 7). IL-12 retains some activity in nude mice, which may reflect the induction of IFN-γ or other cytokines. Treatment of mice with antibodies to IFN-γ may resolve some of these issues but in other studies (25, 26) this approach yielded conflicting results. Recently, IFN-γ knock out mice have been developed (27) and the evaluation of the antitumor activity of IL-12 in these animals could prove interesting.

In addition to secretion of IFN-γ, stimulation of human NK or T cells with human IL-12 results in secretion of tumor necrosis factor (6), which can have antiproliferative effects on tumor cells (28). Recent data using cells from SCID mice demonstrate that incubation of these cells with murine IL-12 resulted in no secretion of TNF in vitro using conditions that induced IFN-γ (29). Likewise, in preliminary experiments, no TNF was induced from normal murine spleen cells stimulated by murine IL-12 in vitro (Hendrzak, J., and M. Brunda, unpublished observations). Thus, it appears that IL-12 is a relatively poor inducer of TNF in mice. However, the contribution of TNF or potentially other cytokines in mediating the antitumor effects of IL-12 in vivo needs to be addressed in future experiments.

IL-12 can both enhance the growth (5–7) and augment the cytolytic activity (1, 7–9, and Gately, M. K., manuscript submitted for publication) of NK/LAK and T cells. Thus, the antitumor efficacy of IL-12 might be mediated through stimulation of one or both these lymphocyte populations. It is unlikely that NK cells are primarily involved since the antitumor activity of IL-12 is maintained in both NK cell–deficient beige mice (Fig. 5) and in mice depleted of NK cells with anti-ASGM1 (Fig. 6). However, T cells appear to be necessary to obtain maximal effects since the efficacy of IL-12 treatment is greatly diminished in nude mice (Fig. 7). Under other experimental conditions, the antitumor activity of a number of cytokines has been reported to be dependent upon T cells (22, 30, 31). It has been suggested that IL-12 promotes the development of Th1 cells (32, 33), and these in turn may positively regulate the expansion and/or activation of other lymphoid cell populations. After depletion of T cell subsets with mAbs, the efficacy of IL-12 was decreased in mice depleted of $CD8^+$ T cells but remained intact in mice depleted of $CD4^+$ T cells (Fig. 8). These results indicate that $CD8^+$ T cells are the critical cell type for mediating the antitumor efficacy of IL-12. The efficacy of IL-12 is not completely eliminated in $CD8^+$ T cell–depleted mice. This finding may reflect the effect of IL-12 on the few remaining $CD8^+$ T cells or on other cell types, such as macrophages. It appears that $CD4^+$ T cells are not necessary for mediating the antitumor effects of IL-12, which is puzzling in light of the effects of IL-12 on $CD4^+$ Th1 cells (32, 33). However, since IL-12 also stimulates cytokine secretion from NK cells (1, 10), the cytokine-secreting function of $CD4^+$ T cells may be replaced by NK cells under the conditions used in these tumor therapy experiments. In addition, exogenously administered IL-12 may at least partially replace any requirement for endogenously produced IL-2, since the biological activities of IL-12 substantially overlap those of IL-2.

A number of cytokines, including IFN-α and IL-2, have been demonstrated to be active in murine tumor models (11–13, 15, 30) and, subsequently, to be useful in the treatment of human malignancies (11, 34, 35). In contrast, with other cytokines, such as IFN-γ or TNF, the activity demonstrated in animal models (11–13, 31) has not translated to successful use of these proteins for therapy of human malignancies (11, 34). The data presented in this study clearly establish IL-12 as a cytokine that has potent antitumor and antimetastatic activities in several murine tumor models through an immune-mediated, T cell–dependent mechanism. Future clinical trials with this cytokine will determine if the activity demonstrated in animals can be translated into efficacy against human malignancies.

Address correspondence to Dr. Michael J. Brunda, Department of Oncology, Hoffmann-La Roche Inc., Nutley, NJ 07710.

Received for publication 7 April 1993 and in revised form 9 June 1993.

References

1. Kobayashi, M., L. Fitz, M. Ryan, R.M. Hewick, S.C. Clark, S. Chan, R. Loudon, F. Sherman, B. Perussia, and G. Trinchieri. 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biological effects on human lymphocytes. *J. Exp. Med.* 170:827.
2. Stern, A.S., F.J. Podlaski, J.D. Hulmes, Y.E. Pan, P.M. Quinn, A.G. Wolitzky, P.C. Familletti, D.L. Stremlo, T. Truitt, R. Chizzonite, and M.K. Gately. 1990. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. *Proc. Natl. Acad. Sci. USA.* 87:6808.
3. Wolf, S.F., P.A. Temple, M. Kobayashi, D. Young, M. Dicig, L. Lowe, R. Dzialo, L. Fitz, D. Ferenz, R.M. Hewick, et al. 1991. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biological effects on T and natural killer cells. *J. Immunol.* 146:3074.
4. Gubler, U., A.O. Chua, D.S. Schoenhaut, C.M. Dwyer, W. McComas, R. Motyka, N. Nabavi, A.G. Wolitzky, P.M. Quinn, P.C. Familletti, and M.K. Gately. 1991. Coexpression of two distinct genes is required to generate secreted, bioactive cytotoxic lymphocyte maturation factor. *Proc. Natl. Acad. Sci. USA.* 88:4143.
5. Gately, M.K., B. Desai, A.G. Wolitzky, P.M. Quinn, C.M. Dwyer, F.J. Podlaski, P.C. Familletti, F. Sinigaglia, R. Chizzonite, U. Gubler, and A.S. Stern. 1991. Regulation of human lymphocyte proliferation by a heterodimeric cytokine, IL-12 (cytotoxic lymphocyte maturation factor). *J. Immunol.* 147:874.
6. Perussia, B., S.H. Chan, A. D'Andres, K. Tsuji, D. Santoli, M. Pospisil, D. Young, S.F. Wolf, and G. Trinchieri. 1992. 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.
7. Robertson, M.J., R.J. Soiffer, S.F. Wolf, T.J. Manley, C. Donahue, D. Young, S.H. Herrmann, and J. Ritz. 1992. Responses of human natural killer (NK) cells to NK cell stimulatory factor (NKSF): cytolytic activity and proliferation of NK cells are differentially regulated by NKSF. *J. Exp. Med.* 175:779.
8. Naume, B., M. Gately, and T. Espevik. 1992. A comparative study of IL-12 (cytotoxic lymphocyte maturation factor)-, IL-2-, and IL-7-induced effects on immunomagnetically purified CD56$^+$ NK cells. *J. Immunol.* 148:2429.
9. Gately, M.K., A.G. Wolitzky, P.M. Quinn, and R. Chizzonite. 1992. Regulation of human cytolytic lymphocyte responses by interleukin-12. *Cell. Immunol.* 143:127.
10. Chan, S.H., B. Perussia, J.W. Gupta, M. Kobayashi, M. Pospisil, H.A. Young, S.F. Wolf, D. Young, S.C. Clark, and G. Trinchieri. 1991. Induction of interferon γ production by natural killer cell stimulatory factor: characterization of the responding cells and synergy with other inducers. *J. Exp. Med.* 173:869.
11. Kedar, E., and E. Klein. 1992. Cancer immunotherapy: are the results discouraging? Can they be improved? *Adv. Cancer Res.* 59:245.
12. Brunda, M.J., V. Sulich, and D. Bellantoni. 1987. The antitumor effect of recombinant interferon alpha and gamma is influenced by tumor location. *Int. J. Cancer.* 40:807.
13. Truitt, G.A., J.M. Bontempo, L.L. Stern, V. Sulich, D. Bellantoni, P.W. Trown, and M.J. Brunda. 1989. Efficacy and toxicity elicited by recombinant interferons alpha and gamma when administered in combination to tumor-bearing mice. *Biotechnol. Ther.* 1:1.
14. Schoenhaut, D.D., A.O. Chua, A.G. Wolitzky, P.M. Quinn, C.M. Dwyer, W. McComas, P.C. Familletti, M.K. Gately, and U. Gubler. 1992. Cloning and expression of murine IL-12. *J. Immunol.* 148:3433.
15. Brunda, M.J., D. Rosenbaum, and L. Stern. 1984. Inhibition of experimentally-induced murine metastases by recombinant alpha interferon: correlation between the modulatory effect of interferon treatment on natural killer cell activity and inhibition of metastases. *Int. J. Cancer.* 34:421.
16. Salup, R.R., T.C. Back, and R.H. Wiltrout. 1987. Successful treatment of advanced murine renal cell cancer by bicompartmental adoptive chemoimmunotherapy. *J. Immunol.* 138:641.
17. Brunda, M.J., D. Bellantoni, and V. Sulich. 1987. *In vivo* antitumor activity of combinations of interferon alpha and interleukin-2 in a murine model. Correlation of efficacy with the induction of cytotoxic cells resembling natural killer cells. *Int. J. Cancer.* 40:365.
18. Gately, M.K., and R. Chizzonite. 1992. Measurement of human and mouse interleukin 12. *In* Current Protocols in Immunology, Vol. 1. J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, and W. Strober, editors. John Wiley & Sons Inc., New York. 6.16.1–18.
19. Wilde, D.B., P. Marrack, J. Kappler, D.P. Dialynas, and F.W. Fitch. 1983. Evidence implicating L3T4 in class II MHC antigen reactivity: monoclonal antibody GK1.5 (anti-L3T4) blocks class II MHC antigen-specific proliferation, release of lymphokines, and binding by cloned murine helper T lymphocyte lines. *J. Immunol.* 131:2178.
20. Sarmiento, M., A.L. Glasebrook, and F.W. Fitch. 1980. IgG or IgM monoclonal antibodies reactive with different determinants on the molecular complex bearing Lyt 2 antigen block T cell-mediated cytolysis in the absence of complement. *J. Immunol.* 125:2665.
21. Reik, L.M., S.L. Maines, D.R. Ryan, W. Levin, S. Bandiera, and P.L. Thomas. 1987. A simple, chromatographic purification procedure for monoclonal antibodies. Isolation of monoclonal antibodies against cytochrome P450 isozymes. *J. Immunol. Methods.* 100:123.
22. Golumbek, P.T., A.J. Lazenby, H.I. Levitsky, L.M. Jaffee, H. Karasuyama, M. Baker, and D.M. Pardoll. 1991. Treatment of established renal cancer by tumor cells engineered to secrete interleukin-4. *Science (Wash. DC).* 254:713.
23. Roder, J.C., M.-L. Matthes, W. Domzig, and H.J. Wigzell. 1979. The beige mutation in the mouse. II. Selectivity of the natural killer (NK) cell defect. *J. Immunol.* 123:2174.
24. Brunda, M.J., and R.B. Wright. 1986. Differential antiproliferative effects of combinations of recombinant interferons alpha and gamma on two murine tumor cell lines. *Int. J. Cancer.* 37:287.
25. Bucy, R.P., D.W. Hanto, E. Berens, and R.W. Schreiber. 1988. Lack of an obligate role for IFN-γ in the primary *in vitro* mixed lymphocyte response. *J. Immunol.* 140:1148.
26. Giovarelli, M., A. Santoni, C. Jemma, T. Musso, M.A. Giuffrida, G. Cavallo, S. Landolfo, and G. Forni. 1988. Obligatory role of IFN-γ in induction of lymphokine-activated and T lymphocyte killer activity, but not in boosting of natural cytotoxicity. *J. Immunol.* 141:2831.
27. Dalton, D.K., S. Pitts-Meek, S. Keshav, I.S. Figari, A. Bradley, and T.A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon-γ genes. *Science (Wash. DC).* 259:1739.
28. Vassalli, P. 1992. The pathophysiology of tumor necrosis factors. *Annu. Rev. Immunol.* 10:411.
29. Tripp, C.S., S.F. Wolf, and E.R. Unanue. 1993. Interleukin

12 and tumor necrosis factor α are costimulators of interferon γ production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. *Proc. Natl. Acad. Sci. USA.* 90:3725.

30. Mulé, J.J., J.C. Yang, R. Lafreniere, S. Shu, and S.A. Rosenberg. 1987. Identification of cellular mechanisms operational *in vivo* during the regression of established pulmonary metastases by systemic administration of high-dose recombinant interleukin-2. *J. Immunol.* 139:285.
31. Hock, H., M. Dorsch, T. Diamantstein, and T. Blankenstein. 1991. Interleukin 7 induces $CD4^+$ T cell–dependent tumor rejection. *J. Exp. Med.* 174:1291.
32. Hsieh, C.-S., S.E. Macatonia, C.S. Tripp, S.F. Wolf, A. O'Garra, and K.M. Murphy. 1993. Development of T_h1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science (Wash. DC).* 260:547.
33. Manetti, R., P. Parronchi, M.G. Giudizi, M.-P. Piccinni, E. Maggi, G. Trinchieri, and S. Romagnani. 1993. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)–specific immune response and inhibits the development of IL-4–producing Th cells. *J. Exp. Med.* 177:1199.
34. Baron, S., S.K. Tyring, W.R. Fleichmann, Jr., D.H. Coppenhaver, D.W. Niesel, G.R. Klimpel, G.J. Stanton, and T.K. Hughes. 1991. The interferons. Mechanisms of action and clinical application. *J. Am. Med. Assoc.* 266:1375.
35. Rosenberg, S.A., M.T. Lotze, L.M. Muul, A.E. Chang, F.P. Avis, S. Leitman, W.M. Linehan, C.N. Robertson, R.E. Lee, J.T. Rubin, et al. 1987. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. *N. Eng. J. Med.* 316:889.