The Effect of Interleukin 12 Desensitization on the Antitumor Efficacy of Recombinant Interleukin 12

Christina M. Coughlin, Maria Wysocka, Giorgio Trinchieri, and William M. F. Lee


ABSTRACT

Use of the cytokine interleukin 12 (IL-12) has been shown to enhance the rejection of a variety of murine tumors, but preclinical and clinical studies have revealed that recombinant IL-12 (rIL-12) can produce severe toxicity. In an effort to improve the tolerance and therapeutic effectiveness of this cytokine, we investigated the influence of giving a single dose of recombinant murine IL-12 (rmIL-12) a week prior to daily cytokine administration (predosing) on its toxic and antitumor effects. These studies were performed in C3H/HeN mice, in which a course of rmIL-12 at standard doses without predosing induced rejection of syngeneic K1735 melanomas in 33%, and in A/J mice, in which treatment induced rejection of syngeneic B7-1+ SCK (SCK.B7-1) mammary carcinomas in 63%. Administration of a predose of rmIL-12 markedly reduced cytokine toxicity in a dose-dependent manner and allowed safe administration of up to 8-fold higher doses of daily rmIL-12 in C3H/HeN mice and 4-fold higher doses of rmIL-12 in A/J mice. Predosing followed by either standard or high daily doses of rmIL-12 did not significantly alter most end points of rmIL-12 treatment of K1735 or SCK.B7-1 tumors (survival, death from tumor, development of protective immunity, and so on), but they appeared to attenuate early control of tumorigenesis by rmIL-12. Evidence for the latter comes from a shortening of the characteristic rmIL-12-induced delay in tumor appearance and in the frequent appearance of tumors that subsequently regress. However, higher doses appear to produce better therapeutic results than standard doses of rmIL-12 after predosing. Predosing severely blunted induction of serum IFN-γ levels by rmIL-12, which probably accounts for many of the effects of predosing on rmIL-12 toxicity and efficacy. Thus, predosing desensitizes mice to the toxic effects of rIL-12 and allows much higher doses to be given but, despite this, it does not improve and, by some criteria, it attenuates rIL-12 therapeutic outcome. Our results do not support the use of predosing as a way to enhance the effectiveness of rIL-12 in cancer clinical trials.

INTRODUCTION

IL-123 is a cytokine normally released by professional antigen-presenting cells in the early stages of an immune response that promotes cell-mediated immunity (1). It induces naïve CD4 T cells to differentiate into TH1 cells, which produce IL-2 and IFN-γ and, thereby, shift the response in favor of cell-mediated immune mechanisms by providing help for CD8 T-cell expansion (2) and by inducing B cells to produce opsonizing antibodies (3, 4). Together with its ability to enhance the cytotoxicity of natural killer and CD8 T cells (5, 6), these effects of IL-12 are expected to increase immune destruction of tumor cells. Additionally, IFN-γ is produced by IL-12-stimulated natural killer and T cells. IFN-γ, besides enhancing cell-mediated immune mechanisms (reviewed in Ref. 7), can retard tumor growth by inhibiting tumor angiogenesis (8) and enhancing immune recognition of tumor cells through up-regulated MHC expression (7, 9, 10). These direct and indirect effects of IL-12 lead one to predict that this cytokine should have significant antitumor effects.

IL-12 has demonstrated therapeutic efficacy against many murine tumors. Parenteral administration of rmIL-12 has been shown to induce regression of Renca adenocarcinomas (11) and MC105 sarcomas (12) and delay growth of B16F10 melanomas (11). In large part, this effectiveness is due to rmIL-12’s relatively long half-life of 2–3 h (1), which permits it to exert sustained effects when administered on a daily basis. Using bicistronic retroviral vectors, cells have been engineered to express both the p35 and p40 subunits of this heterodimeric cytokine and secrete bioactive IL-12 (13, 14). rmIL-12 secretion by MCA207 and MCA102 sarcomas prevents tumor development and induces regression of previously established MCA207 sarcomas (13). In addition, C-26 tumor cells engineered to express rmIL-12 develop tumors that subsequently regress (15, 16). The antitumor effectiveness of administered rmIL-12 and rmIL-12 local secretion by tumor cells in mice suggests that this cytokine may be an effective antitumor agent against human cancers.

Use of IL-12 in humans has been complicated by toxicity. Phase I toxicity studies of rhIL-12 established a maximum tolerated dose for i.v. administration. However, in subsequent Phase II efficacy and toxicity studies involving patients with renal cell carcinomas, administration of the same daily dose of rhIL-12 produced severe toxicity and death in two patients (17). The notable difference between the studies was that patients in the Phase I study received a dose of rhIL-12 prior to the initiation of daily dosing (a predose), whereas patients in the Phase II study were started immediately on daily injections of rhIL-12. Murine studies subsequently confirmed that giving a predose of rmIL-12 ameliorated the toxicity of daily cytokine administration (17).4 These results suggest that a predose of rIL-12 would improve tolerance of a given dose of rIL-12 and allow higher doses of rIL-12 to be administered safely. The studies described below examine the effects of rmIL-12 predosing on its antitumor efficacy.

MATERIALS AND METHODS

Mice. Female A/J mice, 6–8 weeks old, were purchased from The Jackson Laboratory (Bar Harbor, ME). Female C3H/HeN mice of a similar age were purchased from Harlan Sprague Dawley (Indianapolis, IN). All animals were maintained in microisolator cages and handled under aseptic conditions.

Cell Lines. The SCK mammary carcinoma cell line was derived from a spontaneous mammary tumor that arose in a female A/J mouse (H-2b) and was maintained in RPMI supplemented with 10% FCS and penicillin/streptomycin (18, 19). Ectopic expression of B7-1 by SCK cells was accomplished by infection with the retroviral vector MV6.mB7-1, as described previously (20). The K1735 melanoma cell line was derived from a tumor induced by croton oil treatment and UV irradiation in a C3H mouse (H-2b) and was maintained in DMEM supplemented with 10% FCS and penicillin/streptomycin (21).

IL-12 Administration. rmIL-12 was provided by Genetics Institute (Andover, MA). Our experience indicates that different production lots of rmIL-12 may be used.

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3 The abbreviations used are: IL-12, interleukin 12; rmIL-12, recombinant murine IL-12; rhIL-12, recombinant human IL-12; rIL-12, recombinant IL-12; DLT, dose-limiting toxicity.


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Toxicity
grade 3: death
grade 2: lethargy
grade 1: ruffled fur, normal activity

Fig. 1. Toxicity in mice receiving rmIL-12 with and without predosing. A. groups of A/J mice were predosed or not with 1.0 μg of rmIL-12 on the days indicated, and daily injections, for 10 doses given over 12 days (see "Materials and Methods"), were begun on day 0. Groups of C3H/HeN (B) and A/J (C) mice were predosed with PBS (vehicle) or 0.5 μg or 1.0 μg of rmIL-12, and 7 days later, they were begun on daily injections of IL-12 at various daily doses on our 10 dose/12 day schedule. IL-12 toxicity was monitored in individual mice and scored according to the following scale: grade 1, mice have ruffled fur and normal activity level; grade 2, mice have ruffled fur, hunched habitus and lethargy, but respond to physical stimulation; grade 3, mice are withdrawn and unresponsive to stimulus. All mice that reached grade 3 toxicity were dead within 24—48 h. The level of predose and daily doses are indicated. Data points, individual mice in a given experiment; the highest level of toxicity observed over the course of the injections is indicated. Shading, grades of toxicity, as indicated on the Y-axis. The data presented in B and C are a compilation of observations from several experiments.

RESULTS

Administering a dose of rmIL-12 a week prior to initiating daily dosing (predosing) reduces cytokine toxicity (17). We studied the effect of predosing in C3H/HeN and A/J mice, which are the syngeneic hosts for K1735 melanomas and SCK mammary carcinomas, respectively. These mice are susceptible to rmIL-12 toxicity at doses near those used to partially inhibit the growth of their tumors and, thus, are suitable for studying the effects of predosing on rmIL-12 tolerance and antitumor effectiveness. In the experiments that follow, predosed mice were given a single i.p. injection of rmIL-12 7 days before daily i.p. injections of rmIL-12 (10 injections over 12 days) were started. Controls were given the same daily injections of rmIL-12 without a predose or with a PBS (vehicle) predose. In tumor experiments, mice were injected s.c. with K1735 or SCK.B7-1 cells at the time of their first daily rmIL-12 injection and scored daily for toxicity (see scale in "Materials and Methods") and the presence and size of tumors.

Predosing Ameliorates rmIL-12 Toxicity. To study the effect of rmIL-12 predosing on cytokine tolerance, we gave A/J and C3H/HeN mice increasing amounts of rmIL-12 as a predose and determined their tolerance to various daily doses of rmIL-12. In these studies, grade 1 toxicity (ruffled fur, normal behavior) was considered acceptable because signs were mild and always resolved if rmIL-12 injections were stopped. Toxicity at or above grade 2 (ruffled fur and lethargy) was considered DLT because some mice developing these signs progressed and died, even when rmIL-12 injections were stopped. To determine the optimal interval between the predose and the beginning of daily dosing for protection from rmIL-12 toxicity and death, cohorts of A/J mice were predosed or not with a single injection of 1.0 μg of rmIL-12 on days -14, -10, -7, -3, or -1, and started
on daily 1.0 μg/day dosing, beginning on day 0. Toxicity at grade 1 or worse was observed in all mice except those receiving their predose on day −7, (Fig. 1A) which, therefore, became our standard day for predosing in all subsequent experiments. In C3H/HeN mice not given a predose or given a predose of PBS (vehicle), DLT was reached at 0.25 μg daily, but it was not seen with a 0.125 μg daily dose. Mice predosed with 0.5 μg tolerated daily doses up to 0.5 μg, and mice predosed with 1.0 μg tolerated daily doses up to 1.0 μg (Fig. 1B). Higher daily doses of rmIL-12 were not tested in 1.0 μg predosed C3H/HeN mice, because DLT was seen with higher doses in similarly predosed A/J mice (Fig. 1C) and because C3H/HeN mice have a lower native tolerance to rmIL-12 than A/J mice. In A/J mice predosed with PBS, DLT was observed with a 0.5 μg daily dose, but not with 0.25 μg. However, a 0.5 μg rmIL-12 daily dose was tolerated in mice receiving a 0.5 μg predose (Fig. 1C). These results show that predosing ameliorates rmIL-12 toxicity in both strains of mice, that mice given a predose tolerate significantly higher daily doses of rmIL-12, and that tolerance increases with the size of the predose.

Effect of Predosing on rmIL-12 Inhibition of K1735 Tumorigenesis. Systemic administration of rmIL-12 began at the time of K1735 tumor cell injection induces tumor rejection in a significant fraction of C3H/HeN mice (20), allowing us to examine what effect, if any, predosing has on rmIL-12 effectiveness against K1735 tumors. We examined whether rmIL-12 predosing at various levels affected the efficacy of rmIL-12 given at the standard daily dose (0.125 μg) or at the highest tolerated daily dose for the predose. Mice were predosed with 0.25, 0.5, or 1.0 μg of rmIL-12 and, 7 days later, injected with 10⁶ K1735 cells and begun on 0.125 μg of daily rmIL-12. As seen from the data in Table 1, outcome was not significantly affected by the level of the predose used. Considered as a group, the 27% survival of predosed mice was similar to the 34% survival of mice concurrently treated with 0.125 μg of rmIL-12/day without a predose (P > 0.1; Fisher two-tailed t test) and significantly better than the 10% survival of untreated mice (P < 0.1). Interestingly, the median times to tumor appearance were 24–29 days in predosed mice, compared to 32 days in mice treated without a predose and 21–22 days in untreated mice, reflecting a reduction in the characteristic delay in K1735 tumor appearance engendered by rmIL-12 therapy (Table 1 and Fig. 2A). Parameters of tumor progression after their appearance, such as rate of tumor growth and time between tumor appearance and death, were not affected by rmIL-12 therapy or predosing (data not shown). Thus, predosing did not significantly change most parameters of effectiveness of a standard course of rmIL-12 against K1735 tumors, but it did reduce rmIL-12-induced delay in K1735 tumor development.

To determine whether the higher doses of rmIL-12 allowed by predosing affects therapeutic efficacy, predosed mice were given daily rmIL-12 at the highest doses tolerated following the predose. Survival of predosed mice treated with daily doses of 0.25, 0.5, and 1.0 μg of rmIL-12 was similar, and as a group, their 40% survival was similar to the 34% survival of mice treated with 0.125 μg/day without predosing (P > 0.1; Table 1). No difference was noted between treatment groups in the rate of tumor growth and length of time mice lived with tumors. Again noted was a reduction in the rmIL-12-induced delay in tumor appearance, with medians of 20–28 days in predosed mice treated with maximal doses of rmIL-12 compared to 32 days in conventionally treated mice and 21–22 days in untreated mice (Table 1 and Fig. 2B). Thus, giving a predose and high doses of rmIL-12 did not significantly affect most outcomes of rmIL-12 treatment, but it did reduce the rmIL-12-induced delay in K1735 tumor appearance, demonstrating that high doses of rmIL-12 did not overcome this effect of predosing. At each predose level, the result of high-dose rmIL-12 was the same or marginally better than those of standard dose treatment. Combined, 27% of predosed mice given standard rmIL-12 survived, compared to 40% of predosed mice given high-dose rmIL-12 (P > 0.1; Table 1). The kinetics of tumor appearance were similar for the two groups (Fig. 2C).

Effect of Predosing on SCK.B7–1 Tumor Development. rmIL-12 also has antitumor effects against SCK mammary carcinomas in A/J mice, but by itself, daily rmIL-12 merely delays and does not prevent SCK tumorigenesis. However, rmIL-12 induces SCK tumor rejection and protective immunity in a majority of mice when the cells have been engineered to express B7–1 T-cell stimulatory molecules (20). Thus, we studied the effects of predosing when rmIL-12 acts synergistically with B7–1 to induce SCK tumor rejection. When A/J mice were given a predose of 0.5 or 1.0 μg of rmIL-12 and, 7 days later, 2.5 × 10⁶ SCK.B7–1 cells and daily rmIL-12 at 0.25 μg/day, 70, 40, and 30% of mice predosed with 0.5 μg of rmIL-12 survived in three separate experiments, and 40% of mice predosed with 1.0 μg of rmIL-12 survived (Table 2). Survival in the first 0.5-μg predosed group was atypically good, given the results of repeat experiments, and must be due to experimental variation. Altogether, the 45% survival of predosed mice was not significantly different from the 63% survival in mice treated without a predose (P > 0.1).

Table 1 Effect of rmIL-12 predosing on rmIL-12 efficacy against K1735 tumors

<table>
<thead>
<tr>
<th>Therapy group</th>
<th>Predose</th>
<th>Dose</th>
<th>Experiment</th>
<th>Tumors</th>
<th>Survival</th>
<th>Therapy group</th>
<th>Time to tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>No rmIL-12 therapy</td>
<td>None</td>
<td>None</td>
<td>1</td>
<td>9/10 (0)</td>
<td>1/10</td>
<td>10%</td>
<td>22</td>
</tr>
<tr>
<td>No predose + standard rmIL-12 therapy</td>
<td>None</td>
<td>0.125 μg</td>
<td>1</td>
<td>7/12 (0)</td>
<td>5/12</td>
<td>34%</td>
<td>32</td>
</tr>
<tr>
<td>Predose + standard rmIL-12 therapy</td>
<td>0.25 μg</td>
<td>0.125 μg</td>
<td>1</td>
<td>7/10 (0)</td>
<td>3/10</td>
<td>27%</td>
<td>32</td>
</tr>
<tr>
<td>Predose + high-dose rmIL-12 therapy</td>
<td>0.25 μg</td>
<td>0.25 μg</td>
<td>1</td>
<td>7/10 (0)</td>
<td>3/10</td>
<td>27%</td>
<td>32</td>
</tr>
</tbody>
</table>

a Standard dose therapy in C3H/HeN mice is the highest tolerated dose without predosing, 0.125 μg/day. Higher doses of rmIL-12 can only be administered with a predose, due to DLT.

b This table summarizes the results from three separate experiments.

c The tumors column indicates the number of mice developing tumors, including those that subsequently regressed. The number of regressors in a group is indicated in parentheses.

d Survival is noted as the number of survivors/number of mice in that group.

e Therapy group survival is the total of the three groups from the survival column and is expressed as a percent.

f Time to tumor is determined as the number of days between the injection of tumor cells and appearance of detectable tumors. This value is the median of all mice that developed tumors in the group.
Fig. 2. The effect of predosing on antitumor efficacy of rmIL-12 against K1735 melanomas in C3H/HeN mice. Cohorts of C3H/HeN mice were predosed with 0.25, 0.5, or 1.0 μg by i.p. injection 7 days prior to challenge with 10⁶ K1735 cells. The same day as tumor cells were injected, mice began receiving daily injections of rmIL-12 on our 10 dose/12 day schedule (see "Materials and Methods"). In each experiment, a cohort of mice received K1735 cells and no rmIL-12 (---), no predose prior to daily rmIL-12 (—), and a predose prior to daily rmIL-12 (solid gray lines). A, mice treated with the standard dose of rmIL-12 (0.125 μg) with and without a predose; B, mice treated with the highest tolerated dose with predosing (see Table I and Fig. 1A) and the standard dose without predosing; C, a comparison of predosed mice receiving the standard dose (---) and the higher doses (—). The data are plotted by treatment groups as the percentage of mice developing tumors. Arrows, days on which tumors were observed to completely regress.

Parameters of tumor growth, such as growth after appearance or the length of time mice lived with tumors, were not affected by predosing (data not shown), but predosing did reduce the rmIL-12-induced delay in SCK.B7—1 tumor development (Table 2 and Fig. 3A). Notably, among the predosed mice that survived, four initially developed palpable tumors that subsequently regressed. This is an unusually high incidence, given that we had previously seen only three cases of regression in more than 89 mice bearing SCK.B7—1 tumors.

Higher daily doses (0.5 or 1.0 μg) of rmIL-12 following a predose of 0.5 or 1.0 μg produced a cumulative 70% survival, which was similar to the 63% survival seen after conventional dosing (Table 2 and Fig. 3B). Parameters of tumor growth and length of time mice lived with tumors were also similar between groups, and notably, the adverse effect of predosing on rmIL-12-induced tumorigenesis delay was not evident in mice receiving the 1.0-μg predose and dose. One survivor in the predosed group had regression of a palpable tumor. When these results are compared to those in mice given 0.25 μg of rmIL-12 after predosing (Table 2 and Fig. 3C), the survival difference is significant (0.05 < P < 0.1) and indicates that predosed mice challenged with SCK.B7—1 cells benefit from the higher doses of rmIL-12 that are tolerated.
Predosing Does Not Inhibit the Development of Protective Antitumor Immunity. Mice that survive K1735 or SCK.B7–1 tumor cells are protected at some level from a subsequent rechallenge of the respective wild-type tumor cells. When C3H/HeN survivors were rechallenged with 10⁴ K1735 cells about 60 days later, 4 of 8 survivors of conventional rmIL-12 treatment rejected their rechallenge (50%), whereas 5 of 14 predosed survivors rejected their rechallenge (36%). When A/J survivors were rechallenged with 10⁵ SCK cells about 60 days later, 10 of 13 non-predosed, rmIL-12-treated survivors rejected their rechallenge (78%), whereas 24 of 28 predosed survivors rejected their rechallenge (86%). These percentages are comparable to previous results (20, 23) and demonstrate that predosing does not appear to affect the development of protective antitumor immunity following rejection of K1735 or SCK.B7–1 tumors.

Predosing Reduces rmIL-12 Induction of Serum IFN-γ Levels. rmIL-12 administration increases endogenous production of IFN-γ (12, 22), which is responsible for many manifestations of rmIL-12 toxicity and efficacy. To determine whether altered rmIL-12-stimulated IFN-γ production could explain the effects of predosing, we monitored serum IFN-γ levels in representative mice from cohorts in the tumorigenesis experiments after their fourth rmIL-12 injection, a time when serum IFN-γ levels are elevated. C3H/HeN mice given 0.125 μg rmIL-12 injections with their K1735 tumor cells had higher serum IFN-γ levels (2739 ± 903 pg/ml; Fig. 4A) than naive mice (29 ± 16 pg/ml) or mice given tumor cells alone (65 ± 3 pg/ml; Table 1, experiment 3). Predosing with 1.0 μg of rmIL-12 blunted induction of serum IFN-γ levels by 0.125 μg of rmIL-12 given daily (277 ± 42 pg/ml). Mice given a 1.0-μg predose and daily dosing also had low serum IFN-γ levels (42 ± 7 pg/ml), but there is no comparison value for mice given 1.0 μg daily without a predose because of the rapid lethality of such treatment. Similar results were found in A/J mice receiving rmIL-12 (Fig. 4B) shows the results for mice in experiment 2 of Table 2). These results indicate that predosing attenuates the induction of IFN-γ levels by daily rmIL-12 administration and probably explain some of the effects of predosing on rmIL-12 tolerance and therapeutic efficacy. To prove that IFN-γ is important for rmIL-12-induced toxicity and death in our studies, we treated A/J mice with anti-IFN-γ or normal rat antibody on days −1, 0, 2, and 4, and started daily doses of rmIL-12 at 1.0 μg/day on day 0. All mice receiving NRA or no antibody developed severe toxicity and died by day 5, whereas those treated with anti-IFN-γ displayed no signs of toxicity. To determine whether blunted IFN-γ induction resulted from altered metabolism of administered rmIL-12 after predosing, we measured serum rmIL-12 levels. These levels were similar in both predosed and non-predosed mice after rmIL-12 administration but were sufficiently low and variable that we can only exclude major effects of predosing.

DISCUSSION

In these studies, we have sought to determine the effects of predosing on the toxicity and antitumor efficacy of rmIL-12. We show that predosing reduces cytokine toxicity and allows much higher daily doses to be safely administered. In syngeneic mice challenged with K1735 or SCK.B7–1 tumor cells, predosing did not significantly alter rmIL-12-induced tumor rejection or host survival compared to standard rmIL-12 treatment, even when higher doses of daily rmIL-12 was given. Other parameters of antitumor efficacy, such as duration of host survival with tumor, growth rate of tumors after detection, and development of protective immunity, also were not significantly affected. However, the characteristic delay in tumor appearance brought about by rmIL-12 treatment was generally shortened by predosing. Thus, predosing did not enhance the antitumor effectiveness of rmIL-12, and by one parameter, predosing diminished it.

Our studies found generally concordant results for the effect of predosing on rmIL-12 therapy of two murine tumors in different host strains using the cytokine either as a single agent or in synergistic combination with B7–1 costimulation, making us confident that the results fairly represent the effects of predosing in mice. Amelioration of rmIL-12 toxicity and improvement in dose tolerance were so consistent and striking that, reinforced by the results of human rmIL-12 trials (17), this effect of predosing is likely to be generally valid. On the other hand, the precise effect of predosing on the outcome of rmIL-12 tumor therapy may vary with the tumor target and model. In our models, attenuation of therapeutic efficacy by predosing was mild or transient, and it significantly affected only certain parameters of outcome. Thus, although we found that predosing attenuates aspects of rmIL-12 antitumor activity (discussed below), we could envision that in other tumor models, its effect may be different.

Although predosing did not alter the effectiveness of rmIL-12 treatment of K1735 and SCK.B7–1 tumors by most objective meas-
Fig. 3. The effect of predosing on antitumor efficacy of rmIL-12 against SCK.B7-1 mammary carcinomas in A/HeJ mice. Cohorts of A/HeJ mice were predosed with 0.5 or 1.0 µg by i.p. injection 7 days prior to challenge with 2.5 × 10⁶ SCK.B7-1 cells. The same day as tumor cells were injected, mice began receiving daily injections of rmIL-12 on our 10 dose/12 day schedule (see “Materials and Methods”). In each experiment, a cohort of mice received SCK.B7-1 cells and no rmIL-12 (---), no predose prior to daily rmIL-12 (— — —) and a predose prior to daily rmIL-12 (solid gray lines). A, mice treated with the standard dose of rmIL-12 (0.125 µg) with and without a predose; B, mice treated with the highest tolerated dose with predosing (see Table 1 and Fig. 1B) and the standard dose without predosing; C, a comparison of predosed mice receiving the standard dose (— — —) and the higher doses (——). The data are plotted as the percentage of mice developing tumors. Arrows, days on which tumors were observed to completely regress.
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Fig. 4. Serum IFN-γ levels in mice receiving tumor cells and rmIL-12 with and without predosing. Serum IFN-γ levels were determined for three representative mice for each group in the tumorigenesis experiments. Randomly selected mice were bled by retroorbital venipuncture 6 h after the fourth dose of rmIL-12. Each experiment includes three age-matched normal mice of that strain that were left untreated. IFN-γ was determined by RIA (see "Materials and Methods"). IFN-γ measurements are indicated on the Y-axis in pg/ml serum. The predose and daily doses are indicated (predose/daily dose) for each group. A, C3H/HeN mice injected with K1735 cells; B, A/J mice injected with SCK.B7-1 cells.

those given standard dose rmIL-12 goes along with this trend to poorer outcome. Giving high-dose rmIL-12 does not restore the delay in tumorigenesis brought about by rmIL-12 therapy and antagonized by predosing. With the exception of A/J mice given a predose and daily dose of 1.0 μg of rmIL-12, mice treated with standard or high-dose rmIL-12 after predosing display similar kinetics of tumor development.

The ability of a predose of rmIL-12 to blunt the subsequent induction of serum IFN-γ levels by rmIL-12 is significant, given the importance of IFN-γ as a mediator of rmIL-12’s toxic (24, 25) and therapeutic effects (12, 20). The inhibition of IFN-γ induction by predosing correlates with reduction of rmIL-12 toxicity and improved rmIL-12 dose tolerance, and a pathogenic role for endogenously produced IFN-γ in rmIL-12 toxicity has been shown through IFN-γ antibody neutralization studies (26) and studies using IFN-γ receptor knockout mice (24). Correlation also exists between the effects of predosing on rmIL-12 induction of IFN-γ and delay in tumor appearance. A mechanistic link exists between the two inasmuch as our earlier studies showed that antibody neutralization of endogenously produced IFN-γ also abrogated rmIL-12-induced delay of SCK tumorigenesis (20). Association between reduced serum IFN-γ levels achieved by two very different means and reduced tumorigenesis delay suggests that the delay may be due to high IFN-γ levels. IFN-γ could directly retard early K1735 and SCK tumor development through inhibition of tumor angiogenesis (8), inhibition of tumor cell proliferation,5 stimulation of host cytotoxic mechanisms, augmentation of tumor cell MHC expression (7, 9, 10), or any combination of the above.

The blunting effect of predosing on IFN-γ induction by rmIL-12 contrasts with the lack of effect of predosing on many parameters of rmIL-12 antitumor efficacy. This dissociation suggests that high IFN-γ levels are not essential for rmIL-12 to exert most of its antitumor effects, which contrasts with studies showing that antibody neutralization of endogenous IFN-γ abrogates rmIL-12 antitumor effectiveness (12, 20). The apparent discrepancy could be explained if neutralizing antibodies reduce available levels of IFN-γ more completely than predosing and, therefore, inhibit rmIL-12 activities more effectively. An alternative and not mutually exclusive explanation could be that antibodies neutralize secreted IFN-γ whatever the source and location, whereas predosing may only inhibit IFN-γ production by certain subsets of cells. Cells without inhibited production of IFN-γ would be able to participate in or initiate critical cellular interactions that mediate many of rmIL-12’s antitumor effects.

In summary, our observations indicate that predosing unequivocally reduces the toxicity associated with rmIL-12 administration and allows higher doses to be given safely. Even with this, however, predosing produces no obvious therapeutic benefit and attenuates some of the effectiveness of subsequently administered rmIL-12. Optimal therapeutic results were obtained using rmIL-12 conventionally at doses that do not produce overt signs of rmIL-12 toxicity. Because of differences between rIL-12s and their use in mice and humans (different proteins, half-lives, route and schedule of administration, established tumors, and so on), we caution against extrapolating our results to humans, but our studies do not support using predosing to increase rhIL-12 dose tolerance in cancer clinical trials. In this context, it is worth noting that administration of maximally
tolerated doses of rIL-12 may produce unexpected or undesirable immunological outcome (27, 28). The paradigm of "more is likely to be better," which has been successfully used to develop cytotoxic therapies for cancer, cannot be applied uncritically to the development of biologicals for cancer therapy.

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REFERENCES


The Effect of Interleukin 12 Desensitization on the Antitumor Efficacy of Recombinant Interleukin 12

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