

*Advances in Brief***Immunological Effects of Interleukin 12 Administered by Bolus Intravenous Injection to Patients with Cancer<sup>1</sup>**

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**Abstract**

The immunological effects of recombinant human interleukin 12 (rhIL-12) administration were examined during the conduct of a Phase I clinical trial. Forty patients with advanced cancer received bolus i.v. injections of rhIL-12 in doses ranging between 3 and 1000 ng/kg. Dose-dependent increases in serum IFN- $\gamma$  levels were seen during rhIL-12 therapy. Significant lymphopenia was observed 24 h after single i.v. injections of rhIL-12 at each dose level. The degree of lymphopenia was dose dependent, and a plateau effect was seen with rhIL-12 doses of 100 ng/kg and higher. Lymphocyte counts reached nadir levels at approximately 10 h after rhIL-12 injection and returned to baseline within 14 days postinjection. Rebound lymphocytosis, as seen after interleukin 2 therapy, was not observed after recovery from rhIL-12-induced lymphopenia. rhIL-12-induced lymphopenia involved all major lymphocyte subsets, although natural killer (NK) cell numbers were the most profoundly affected, and CD4 T-cell numbers were the least affected. CD2, LFA-1, and CD56 were transiently up-regulated on the surface of NK cells exposed to rhIL-12 *in vivo*. Peripheral blood mononuclear cells obtained from cancer patients before rhIL-12 therapy exhibited defective NK cell cytotoxicity and T-cell-proliferative responses. Peripheral blood mononuclear cells obtained after lymphocyte recovery following the administration of a single 500 ng/kg dose of rhIL-12 displayed augmented NK cell cytolytic activity in four of four

patients tested and enhanced T-cell proliferation in three of four patients tested. These studies confirm that doses of rhIL-12 resulting in significant immunological activity can be administered with acceptable toxicity to cancer patients. Furthermore, rhIL-12 therapy can reverse defects in NK cell and T-cell function that are associated with advanced cancer in humans.

**Introduction**

IL-12,<sup>3</sup> which was previously known as NK cell stimulatory factor or cytotoxic lymphocyte maturation factor, is a heterodimeric cytokine that regulates both innate and adaptive immune responses (1, 2). IL-12 supports the proliferation of activated T cells and promotes the selective maturation of activated CD4 T cells into helper effector cells of the Th1 phenotype and of activated CD8 T cells into CTLs (3–6). IL-12 also stimulates the proliferation and function of fully differentiated CTLs. Production of IFN- $\gamma$  by T cells and NK cells is potently induced by IL-12 (7, 8). Moreover, IL-12 augments NK cell cytotoxicity against tumor cells, virus-infected cells, and antibody-coated target cells (3, 9–12). By virtue of its effects on CD4 T cells, CD8 T cells, and NK cells, IL-12 seems to be a key cytokine that regulates cell-mediated immune responses. There is currently substantial interest in the potential therapeutic applications of IL-12 in human infectious, atopic, and malignant diseases (13–15).

IL-12 administration has potent antitumor effects in several murine models (16–19). IL-12 also stimulates the antitumor activity of PBMCs from patients with advanced solid tumors and patients with hematological malignancies who are undergoing allogeneic bone marrow transplantation (20, 21). Moreover, IL-12 enhances the killing of autologous tumor cells by tumor-infiltrating lymphocytes from patients with melanoma and ovarian cancer (22, 23). IL-12 can augment graft-versus-leukemia activity without promoting graft-versus-host disease after allogeneic bone marrow transplantation (24, 25). Furthermore, the injection of tumor cells transduced with the IL-12 genes can induce potent, specific, and durable antitumor immune responses (26–28). Thus, preclinical investigation has provided ample justification for clinical trials of IL-12 in malignant disease. The present studies were undertaken in conjunction with the first published Phase I trial of rhIL-12 in patients with cancer (29). rhIL-12 was found to have substantial biological effects when administered in doses that were generally tolerable in an outpatient setting.

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<sup>3</sup> The abbreviations used are: IL, interleukin; rhIL, recombinant human IL; NK, natural killer; PBMC, peripheral blood mononuclear cell; MoAb, monoclonal antibody; PBL, peripheral blood lymphocyte; TNF, tumor necrosis factor; MFI, mean fluorescence intensity.

## Materials and Methods

**Patients and Clinical Protocol.** Forty patients with advanced solid tumors (20 patients with renal cell cancer, 12 patients with melanoma, 5 patients with colon cancer, and 3 patients with other cancers) received i.v. injections of rhIL-12 (Genetics Institute, Cambridge, MA) during a Phase I study conducted at four institutions (29). Cohorts of four to six patients received escalating doses (from 3–1000 ng/kg/day) of rhIL-12 by bolus injection into a rapidly flowing i.v. line once and then, after a 2-week hiatus, daily for 5 days every 3 weeks. Patients who did not experience disease progression or dose-limiting toxicity could receive up to six 5-day multiple-dose treatment cycles. The clinical protocol was approved by the Institutional Review Board at each of the participating institutions, and written informed consent was obtained from each patient before treatment. Whole blood samples anticoagulated in heparin and EDTA were obtained at various time points and sent by express courier to a central reference laboratory (Corning-Nichols, San Juan Capistrano, CA) for total and differential leukocyte counts and immunophenotypic analysis. Serum was obtained from blood samples collected at various time points, frozen, and shipped periodically to the central laboratory for a determination of serum cytokine levels. Additional immunophenotypic and functional studies were performed using samples of blood obtained from four patients (patients 025, 027, 034, and 038) treated at the 500 ng/kg dose level at the Dana-Farber Cancer Institute. PBMCs were isolated on a Ficoll-diatrizoate gradient from heparinized venous blood obtained from these patients before, during, and after rhIL-12 therapy. Control PBMCs were isolated from blood samples obtained from healthy volunteer donors. Freshly isolated PBMCs were stained for immunofluorescence studies. Aliquots of PBMCs were cryopreserved in liquid nitrogen for later use in proliferation and cytotoxicity assays.

**MoAbs, Cytokines, and Medium.** Fluorochrome-conjugated murine MoAbs obtained from Coulter Immunology (Hialeah, FL) included T11 (CD2), IL-2R<sub>1</sub> (CD25), NKH1 (CD56), and isotype-matched control MoAb. FITC-conjugated anti-intercellular adhesion molecule 1 (CD54) was purchased from AMAC (Westbrook, ME). OKT3 (CD3; IgG2a) was used as a dilution of ascites. IL-2 (specific activity,  $1.05 \times 10^7$  units/mg) was kindly provided by Amgen (Thousand Oaks, CA), and IL-12 (specific activity,  $5.2 \times 10^6$  units/mg) was kindly provided by Genetics Institute. Culture medium for functional assays was formulated as described previously (30), except that 15% human AB serum or 15% FCS was used.

**Immunofluorescence Studies.** Samples of PBMCs were stained directly with fluorochrome-conjugated MoAbs, washed, fixed in 1% formaldehyde, and analyzed by flow cytometry as described previously (30) or using an EPICS Elite instrument from Coulter Immunology.

**Cytotoxicity Assays.** Cryopreserved PBMCs were thawed, incubated overnight at 37°C in medium alone or in medium containing cytokines, and plated as effector cells in standard 4-h chromium release assays as described previously (9). The percentage of specific cytotoxicity was calculated from raw data from triplicate wells using a published formula (31).

K562 (a NK-sensitive human myeloid leukemia cell line)

and COLO205 (a NK-resistant human colon adenocarcinoma cell line) were obtained from the American Type Culture Collection (Rockville, MD) and maintained in continuous suspension cultures as described previously (30).

**Proliferation Assays.** Cryopreserved PBMCs were thawed and plated at 30,000 cells/well in 96-well U-bottomed microtiter plates in medium alone or in medium containing a 1:200 final dilution of OKT3 ascites. After 3 days of culture at 37°C, 1  $\mu$ Ci of tritiated thymidine was added to each well; assays were harvested 18 h later, and tritiated thymidine incorporation was determined using a liquid scintillation counter as described previously (9).

**Statistical Analysis.** Calculations of the means, SDs, SEs, and *P*s from the paired Student's *t* test were performed on a Macintosh Duo Dock computer (Apple, Cupertino, CA) using the StatView software program (Brainpower, Calabasas, CA) according to the manufacturer's instructions.

## Results

**Effect of rhIL-12 Therapy on PBL Subsets.** Before rhIL-12 therapy, most patients with advanced cancer enrolled on study exhibited normal absolute numbers of lymphocytes in the peripheral blood (Table 1). Significant reductions in absolute PBL counts were seen 24 h after single i.v. bolus injections of rhIL-12. Absolute lymphocyte counts declined by 17–21% in patients treated with 3 and 10 ng/kg rhIL-12, by 50% in patients treated with 30 ng/kg, and by 78–80% in patients treated with rhIL-12 doses of 100 ng/kg and higher. For all rhIL-12 dose cohorts, the absolute lymphocyte counts returned to baseline within 14 days of treatment; there was no evidence of significant rebound lymphocytosis after rhIL-12 bolus injections (data not shown).

rhIL-12-induced lymphopenia involved all of the major lymphocyte subsets, including CD4 T cells, CD8 T cells, B cells, and NK cells. The effects of rhIL-12 on PBL subsets in 13 patients treated at the 500 ng/kg dose level are summarized in Table 2. Significant reductions in all lymphocyte subsets are evident 24 h after a single i.v. bolus injection of rhIL-12, with recovery to baseline levels occurring by day 14 postinjection. Significant differences in the magnitude of the rhIL-12 effect on specific lymphocyte subsets were seen. In patients treated at the four highest dose levels (100, 250, 500, and 1000 ng/kg), the total lymphocyte counts 24 h postinjection were  $21 \pm 1\%$  (mean  $\pm$  SE) of baseline counts. However, the absolute number of NK cells declined to  $6 \pm 3\%$  of baseline ( $P < 0.005$  compared to the decrease in total lymphocytes), whereas the absolute number of CD4 T cells declined to  $29 \pm 5\%$  of baseline ( $P < 0.025$  compared to the decrease in total lymphocytes). The degree of decline in CD8 T cells ( $15 \pm 6\%$  of baseline) and B cells ( $16 \pm 2\%$  of baseline) was intermediate between that of NK cells and CD4 T cells.

The kinetics of lymphocyte disappearance and recovery after single bolus injections of rhIL-12 were examined in detail in four patients receiving a dose of 500 ng/kg. Lymphocyte counts fell slightly but consistently 30 min after a bolus i.v. injection of rhIL-12, followed by a return toward baseline 2 h after rhIL-12 administration (Fig. 1). A sharp decline in the lymphocyte count then occurred at ~6–10 h after rhIL-12

**Table 1** Effect of rhIL-12 therapy on absolute lymphocyte counts of cancer patients

rhIL-12 dose cohort (n)	Total absolute lymphocyte count (cells/ $\mu$ l) <sup>a</sup>		P <sup>b</sup>
	Before rhIL-12	After rhIL-12	
3 ng/kg (3)	1533 $\pm$ 451	1267 $\pm$ 306	$\leq$ 0.05
10 ng/kg (4)	950 $\pm$ 545	750 $\pm$ 507	$\leq$ 0.05
30 ng/kg (4)	2725 $\pm$ 1037	1350 $\pm$ 968	$\leq$ 0.05
100 ng/kg (3)	1400 $\pm$ 755	300 $\pm$ 100	$\leq$ 0.05
250 ng/kg (6)	1900 $\pm$ 857	380 $\pm$ 130	$\leq$ 0.001
500 ng/kg (13)	1731 $\pm$ 992	338 $\pm$ 229	$\leq$ 0.0005
1000 ng/kg (3)	1533 $\pm$ 306	333 $\pm$ 231	$\leq$ 0.025

<sup>a</sup> Values are the mean  $\pm$  SD of the absolute number of lymphocytes just before and approximately 24 h after a single i.v. injection of rhIL-12 at the indicated dose. The normal range for the absolute lymphocyte count in the reference laboratory used is 800–4800 cells/ $\mu$ l.

<sup>b</sup> P are from the paired Student's *t* test.

**Table 2** Effect of rhIL-12 therapy on PBL subsets of cancer patients

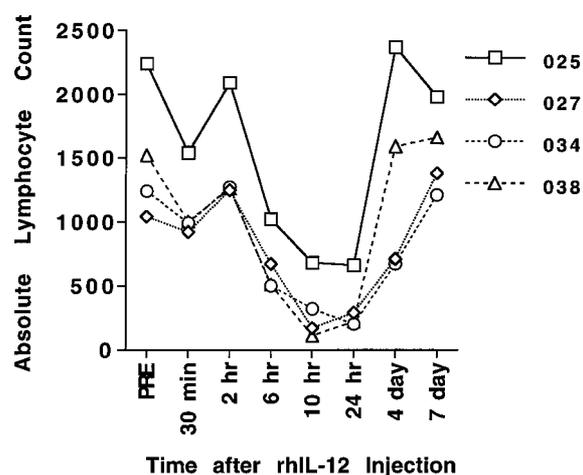
Values are the mean  $\pm$  SD of the absolute number (cells/ $\mu$ l) of total lymphocytes or lymphocytes expressing CD3 (total T cells), both CD3 and CD4 (CD4 T cells), both CD3 and CD8 (CD8 T cells), CD20 (B cells), or CD16 and/or CD56 in the absence of CD3 (NK cells) in peripheral blood drawn just before, 24 h after, or 14 days after a single i.v. bolus injection of rhIL-12 at a dose of 500 ng/kg.

Lymphocyte subset	Before rhIL-12	24 h after rhIL-12	14 days after rhIL-12
Total lymphocytes	1731 $\pm$ 992	338 $\pm$ 229	1677 $\pm$ 891
Total T cells	1259 $\pm$ 636	274 $\pm$ 182	1283 $\pm$ 702
CD4 T cells	932 $\pm$ 584	236 $\pm$ 176	878 $\pm$ 653
CD8 T cells	299 $\pm$ 166	33 $\pm$ 22	347 $\pm$ 169
B cells	229 $\pm$ 234	31 $\pm$ 34	179 $\pm$ 169
NK cells	166 $\pm$ 121	7 $\pm$ 6	180 $\pm$ 110

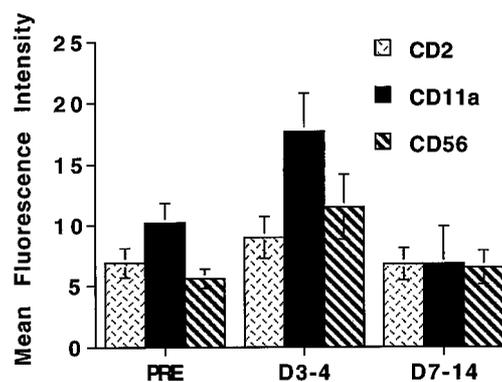
injection, and lymphocyte counts remained near the nadir level 24 h after injection. Lymphocyte numbers returned to baseline 3–5 days after a single injection of rhIL-12, with no evidence of a significant rebound lymphocytosis.

Of interest, during the 5-day multiple-dose treatment cycles, the nadir lymphocyte count generally occurred 24 h after the first dose of rhIL-12; the lymphocyte count tended to rise during the last day or two of the cycle, despite the continued administration of rhIL-12 (data not shown). Like serum neopterin production (29), but unlike IFN- $\gamma$  secretion (29, 32), lymphopenia did not seem to be attenuated in patients receiving multiple 5-day cycles of rhIL-12 (data not shown).

**Up-Regulation of NK Cell Adhesion Molecules *in Vivo* during IL-12 Therapy.** IL-12 has been shown to up-regulate several cell surface adhesion molecules on human NK cells *in vitro*, including CD2, CD11a/CD18 (LFA-1), CD54 (intercellular adhesion molecule 1), and CD56 (NCAM; Refs. 9, 10, and 33). The expression of these adhesion molecules by NK cells during rhIL-12 therapy was examined in four patients treated with 500 ng/kg rhIL-12. Peripheral blood NK cells obtained 3–4 days after single i.v. injections of rhIL-12 at 500 ng/kg exhibited 1.4  $\pm$  0.1-fold increases in the surface density of CD2 ( $P \leq 0.05$ ), 1.8  $\pm$  0.1-fold increases in CD11a ( $P \leq 0.005$ ), and 2.6  $\pm$  0.5-fold increases in CD56 ( $P \leq 0.025$ ) compared to NK cells



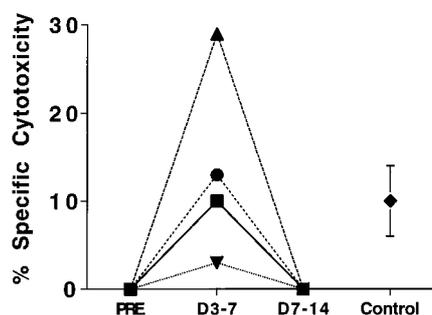
**Fig. 1** Kinetics of lymphopenia and lymphocyte recovery in peripheral blood after the administration of rhIL-12. Absolute PBL counts (total cells/microliter) were determined at the indicated times after i.v. bolus injections of rhIL-12 at a dose of 500 ng/kg. The results shown are the absolute PBL counts of four different patients (patients 025, 027, 034, and 038). Note that a nonlinear time scale has been used to clearly display the changes that occur during the first 24 h after IL-12 dosing.



**Fig. 2** Up-regulation of NK cell adhesion molecules by rhIL-12 therapy. The MFI of staining with CD2, CD11a, and CD56 MoAbs was determined by flow cytometry for PBMCs obtained from four patients before (PRE), 3–4 days after (D3–4), and 7–14 days after (D7–14) a single i.v. bolus injection of 500 ng/kg rhIL-12. The MFI for CD2 and CD11a staining was performed by an electronic gating on CD56<sup>+</sup> cells. Values are the mean  $\pm$  SE of the MFI (in arbitrary units).

obtained before rhIL-12 therapy (Fig. 2). It should be noted that the up-regulation of NK cell adhesion molecules during rhIL-12 treatment was transient: CD2, CD11a, and CD56 levels on PBMCs obtained 7–14 days after rhIL-12 injections were not significantly different ( $P \geq 0.05$ ) from those seen before rhIL-12 therapy. Significant up-regulation of CD25 (IL-2R $\alpha$ ) or CD54 on NK cells obtained from patients treated with rhIL-12 was not detected in these studies (data not shown).

One of these four patients (patient 027) received only the single-dose cycle before going off study due to the discovery of brain metastases; the other three patients went on to receive multiple-dose cycles of rhIL-12. CD56<sup>+</sup> NK cells obtained from

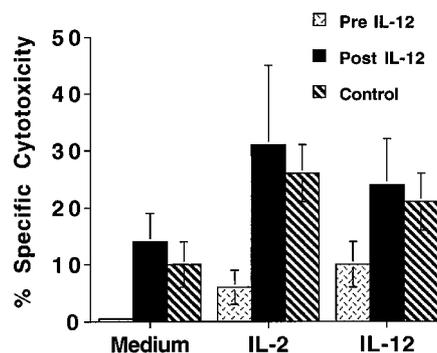


**Fig. 3** Augmentation of NK cell cytolytic activity by rhIL-12 therapy. PBMCs obtained from four patients before (*PRE*), 3–7 days after (*D3–7*), or 7–14 days after (*D7–14*) a single i.v. bolus injection of 500 ng/kg rhIL-12 were tested for cytotoxicity against radiolabeled K562 target cells. Values are the mean (from triplicate wells) percentage of specific cytotoxicity at an E:T ratio of 10:1. The mean  $\pm$  SE of the results from eight healthy volunteer donors is shown for comparison (*Control*).

two of the latter patients 3–4 days after the fifth rhIL-12 injection in the second multiple-dose cycle exhibited  $1.6 \pm 0.1$ -fold increased levels of CD2,  $2.0 \pm 0.1$ -fold increased levels of CD11a, and  $2.2 \pm 0.1$ -fold increased levels of CD56 compared to NK cells obtained before the first rhIL-12 dose in that cycle; these differences were statistically significant ( $P \leq 0.025$  for all three comparisons).

Preincubation of IL-12-activated normal NK cells with CD2 or CD11a MoAb partially inhibited the lysis of K562 cells; a combination of CD2 and CD11a MoAbs strongly inhibited K562 lysis (data not shown). In contrast, CD56 MoAb had no significant effect on NK cell cytolytic activity. These data are very similar to the results of MoAb blocking studies using IL-2-activated NK cells (30) and suggest that up-regulation of CD2 and LFA-1 on NK cells *in vivo* during IL-12 therapy may well have functional consequences.

**Effect of rhIL-12 Therapy on the Cytolytic Activity of NK Cells.** The absolute number of NK cells in the peripheral blood of most patients before rhIL-12 treatment was normal. Nevertheless, PBMCs obtained before rhIL-12 therapy exhibited very poor cytolytic activity toward the NK-sensitive cell line K562 (Fig. 3). The level of K562 lysis mediated by patient PBMCs was significantly less ( $P \leq 0.05$ ) than that mediated by control PBMCs from eight healthy donors. Despite relatively normal numbers of peripheral blood NK cells, defective NK cytolytic activity is commonly seen in patients with advanced cancer (20, 34, 35). As expected, NK cytolytic activity could be augmented by the *in vitro* incubation of patient PBMCs with nanomolar concentrations of IL-2 or picomolar concentrations of IL-12 (Fig. 4). Moreover, PBMCs obtained 3–7 days after single injections of rhIL-12 at 500 ng/kg demonstrated enhanced cytotoxicity toward K562 (Figs. 3 and 4). NK cytotoxicity mediated by PBMCs obtained after rhIL-12 administration was significantly greater ( $P \leq 0.05$ ) than that mediated by PBMCs collected before rhIL-12 therapy and was indistinguishable ( $P > 0.1$ ) from that mediated by unstimulated PBMCs from healthy donors (Fig. 4). As was seen with the up-regulation of NK cell adhesion molecules, augmented NK cytotoxicity was evident



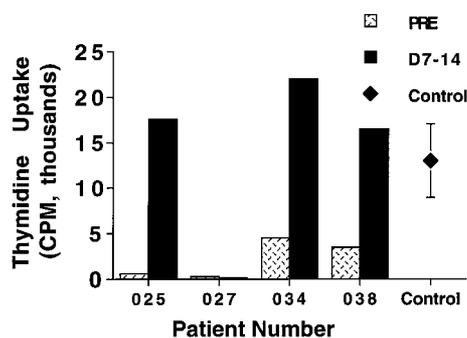
**Fig. 4** Effects of exogenous cytokines on NK cell cytolytic activity before and after rhIL-12 therapy. PBMCs obtained from four patients before (*stippled bars*) or 3–7 days after (*■*) a single i.v. bolus injection of 500 ng/kg rhIL-12 or from eight healthy volunteer donors (*▨*) were incubated overnight in medium alone, 500 units/ml (3 nM) IL-2, or 10 units/ml (30 pM) IL-12 as indicated and tested for cytotoxicity against radiolabeled K562 target cells. Values are the mean  $\pm$  SE of the percentage of specific cytotoxicity at an E:T ratio of 10:1. The cytotoxicity mediated by patient PBMCs obtained after IL-12 therapy is not significantly different from that mediated by control PBMCs ( $P > 0.1$  for all three comparisons).

during the early recovery phase after rhIL-12-induced lymphopenia (about 3–5 days after rhIL-12 injection) and had returned to baseline by day 14 postinjection (Fig. 3). At the relatively low E:T ratios used in these studies (due to the limited number of PBMCs available), significant killing of NK-resistant COLO205 targets was not detected before or after rhIL-12 therapy (data not shown).

After incubation *in vitro* with nanomolar concentrations of IL-2 or picomolar concentrations of IL-12, PBMCs obtained 3–7 days after rhIL-12 injections mediated higher levels of K562 killing (Fig. 4). The response of PBMCs obtained after IL-12 therapy was not significantly different than that of resting PBMCs obtained from healthy donors. Thus, NK cells exposed to rhIL-12 *in vivo* can remain responsive to further stimulation with IL-2 and IL-12.

**Effects of rhIL-12 Therapy on the Proliferation of PBMCs in Response to CD3 Ligation.** Incubation with IL-12 *in vitro* enhances the proliferation of activated normal T lymphocytes (9, 36, 37). PBMCs obtained from patients before rhIL-12 therapy exhibited poor proliferation in response to CD3 triggering (Fig. 5). In 3 of 4 patients tested, however, PBMCs obtained after a single injection of rhIL-12 at 500 ng/kg demonstrated markedly higher proliferative responses to CD3 MoAb (Fig. 5). When data from all four patients were analyzed, the increase in CD3-induced PBMC proliferation seen after rhIL-12 administration remained statistically significant ( $P \leq 0.05$ ). In contrast to the NK cell responses described above, enhanced T-cell proliferation was not consistently seen during the early recovery phase after rhIL-12-induced lymphopenia (data not shown) but was observed ~7–14 days after rhIL-12 injection (Fig. 5).

**Production of Cytokines *in Vivo* during rhIL-12 Therapy.** It has been reported previously that systemic administration of rhIL-12 can induce the production of IFN- $\gamma$  in patients with advanced cancer (29, 32). Increased serum IFN- $\gamma$  levels were readily detected 24 h after single i.v. bolus injections of



**Fig. 5** Augmentation of PBMC proliferation by rhIL-12 therapy. PBMCs obtained from four patients (025, 027, 034, and 038) before (PRE, stippled bars) or 7–14 days after (D7–14, ■) a single i.v. bolus injection of 500 ng/kg rhIL-12 were tested in 4-day proliferation assays as described in “Materials and Methods.” Values are the mean cpm from triplicate wells of PBMCs incubated with CD3 MoAb. The cpm of PBMCs incubated in medium alone was  $1972 \pm 2576$ . The mean  $\pm$  SE of results from eight healthy volunteer donors is shown for comparison (Control).

rhIL-12 at doses of 30 ng/kg and higher (Table 3). The magnitude of the serum IFN- $\gamma$  levels achieved during rhIL-12 therapy seemed to be rhIL-12 dose dependent. As reported previously (29), peak IFN- $\gamma$  levels in the serum tended to diminish with repetitive 5-day multiple-dose cycles of rhIL-12. Moreover, during the 5-day treatment cycles, IFN- $\gamma$  levels tended to peak on day 3 of therapy and began to decline on days 4 and 5, despite the continued administration of rhIL-12 (data not shown). At the time points assayed in this study, TNF was not detected in the serum of any patient treated with rhIL-12 (data not shown).

## Discussion

IL-12 has profound effects on innate and adaptive immune responses in murine models of infectious disease, autoimmunity, and cancer. Development of effective rhIL-12-based immunotherapy for human diseases will be facilitated by an elucidation of the effects of rhIL-12 on the human immune system *in vivo*. The present report describes the immunological consequences of systemic rhIL-12 treatment in patients with cancer.

Biological effects, including fever and mild lymphopenia, were seen in some patients treated with the lowest doses (3 and 10 ng/kg) of rhIL-12, although IL-12 was not detectable in the serum of these patients (29). Transient lymphopenia has also been observed in cancer patients receiving rhIL-12 by s.c. injection (38). Substantial lymphopenia and serum IFN- $\gamma$  levels in excess of 300 pg/ml were seen in patients treated with rhIL-12 doses of 30 ng/kg and higher. Such doses resulted in peak serum IL-12 concentrations between  $\sim 744$  pg/ml or  $\sim 11$  pM (in the 30 ng/kg cohort) and 19,575 pg/ml or  $\sim 280$  pM (in the 1,000 ng/kg cohort) (29). These results are in good agreement with preclinical data indicating that IL-12 concentrations of  $\sim 10$ – $100$  pM can induce potent *in vitro* activation of human lymphocytes (7, 9, 37).

Mean IFN- $\gamma$  levels in the serum of patients treated with rhIL-12 doses of 250 ng/kg and higher were similar to the serum IFN- $\gamma$  levels observed in normal or tumor-bearing mice receiv-

**Table 3** Effect of rhIL-12 therapy on serum IFN- $\gamma$  levels

Values are the mean  $\pm$  SD of the IFN- $\gamma$  levels in the serum of patients treated at the indicated dose levels of rhIL-12. Serum was isolated from blood drawn just before, 24 h after, or 7 days after single i.v. bolus injections of rhIL-12 as indicated. The lower limit of detection for the assay was 10 pg/ml; a value of 10 pg/ml was used for samples with undetectable levels of IFN- $\gamma$ .

rhIL-12 dose cohort (n)	Serum IFN- $\gamma$ level (pg/ml)		
	Before rhIL-12	24 h after rhIL-12	7 days after rhIL-12
3 ng/kg (4)	10 $\pm$ 0	17 $\pm$ 15	10 $\pm$ 0
10 ng/kg (4)	10 $\pm$ 0	26 $\pm$ 14	10 $\pm$ 0
30 ng/kg (4)	10 $\pm$ 0	338 $\pm$ 450	10 $\pm$ 0
100 ng/kg (4)	10 $\pm$ 0	679 $\pm$ 388	10 $\pm$ 0
250 ng/kg (6)	11 $\pm$ 3	2096 $\pm$ 645	12 $\pm$ 3 <sup>a</sup>
500 ng/kg (14)	44 $\pm$ 126	5443 $\pm$ 7150	10 $\pm$ 1
1000 ng/kg (4)	10 $\pm$ 0	9119 $\pm$ 3952	16 $\pm$ 11

<sup>a</sup> Results were available for five patients only.

ing IL-12 doses that are associated with substantial antitumor efficacy (16, 17). In several murine tumor models, it has been shown that the induction of IFN- $\gamma$  production is necessary but not sufficient for the antitumor activity of IL-12 (17, 18, 39). TNF was not detected in the serum of patients treated with rhIL-12 in this study. IL-12 by itself has been shown to be a poor inducer of TNF production by human lymphocytes *in vitro* (10, 40). Nevertheless, we cannot exclude the possibility that rhIL-12 injections induced a transient production of TNF *in vivo*. It is noteworthy that fever and chills generally occurred 8–12 h after rhIL-12 injections, and that serum samples for cytokine levels were not routinely obtained during this time period.

The lymphopenia observed during rhIL-12 therapy is likely to reflect the *in vivo* activation of lymphocytes, with their subsequent margination and/or extravasation into tissues. The tissue compartments into which activated PBLs putatively redistribute after rhIL-12 administration to humans are currently unknown. Increased numbers of mononuclear cells were found in the spleen, liver, and lungs of normal mice given multiple IL-12 injections i.p. (4). Although new splenomegaly or hepatomegaly were not noted during the frequent physical examinations of patients in this study, it is possible that the liver function test abnormalities commonly found after IL-12 treatment (29) were due in part to hepatic infiltration by activated lymphocytes. Further study will be required to determine whether the presence of activated lymphocytes correlates with the observed rhIL-12-induced effects on the liver, oral mucosa, or gut. Moreover, because tumor biopsies were not obtained during rhIL-12 therapy in this study, it is not known whether rhIL-12 causes the infiltration of activated lymphocytes into human tumors.

rhIL-12-induced lymphopenia involved all of the major lymphocyte subsets, although NK cells seemed to be the most profoundly affected. NK cells were virtually absent from the peripheral blood 24 h after single injections of rhIL-12 at doses exceeding 100 ng/kg. CD4 T cells declined the least after rhIL-12 injections, and the decline in CD8 T cells and B cells was intermediate between that of NK cells and CD4 T cells. Our results are in agreement with those of Bajetta *et al.* (38), who

observed a relative sparing of CD4 T cells in the lymphopenia induced by s.c. injections of rhIL-12. These consistent differences in the effects of IL-12 therapy on particular lymphocyte subsets could be due to differential redistribution of the latter from peripheral blood to extravascular tissues. Administration of IL-12 to normal mice resulted in significantly increased numbers of NK cells and CD8 T cells in the liver and NK cells and B cells in the spleen, whereas the number of CD4 T cells in these sites remained essentially unchanged (4).

It is currently not clear whether this selective redistribution of lymphocyte subsets is due to differences in expression of IL-12 receptor subunits, signaling events after the interaction of IL-12 with its receptor, responses to secondary cytokines induced by IL-12 administration, or other factors.

There are several notable differences between the lymphopenia associated with high-dose bolus IL-2 therapy and the lymphopenia observed after rhIL-12 injections in this study. After high-dose i.v. bolus injections of IL-2, marked lymphopenia occurs within 1 h, nadir lymphocyte counts occur at ~4 h, partial recovery is evident by 24 h, and striking rebound lymphocytosis occurs 1–2 days postinjection (41). In contrast, lymphocyte counts after rhIL-12 injections did not reach their nadir until ~10 h, remained at nadir levels at 24 h, and did not recover to baseline until several days postinjection. Furthermore, rebound lymphocytosis during recovery from rhIL-12-induced lymphopenia was not observed. The latter was anticipated, based on preclinical *in vitro* studies; IL-2 supports the proliferation of resting human NK cells and some T-cell subsets (42, 43), whereas IL-12 does not induce the proliferation of resting T cells and is a very poor mitogen for most human NK cells (7, 9, 37). Pharmacokinetics may explain the differences in duration of lymphopenia caused by the two cytokines. The half-life of IL-2 after i.v. bolus injection is less than 10 min, and >95% of the cytokine is predicted to be cleared within 30 min of injection (41). In contrast, the half-life of rhIL-12 after i.v. bolus injection is 5–10 h, and significant IL-12 levels can be detected in the serum more than 24 h after a single 500 ng/kg rhIL-12 dose (29). The reason for the delayed onset of lymphopenia after rhIL-12 bolus injections is less clear. It is possible that the lymphopenia is not due to a direct stimulation of lymphocytes by rhIL-12 itself but is rather the result of secondary cytokines produced *in vivo* in response to rhIL-12. Alternatively, the kinetics of lymphocyte activation may differ after the administration of IL-2 as compared to rhIL-12.

Up-regulation of several cell surface molecules has been described after stimulating human NK cells with IL-2 or IL-12 *in vitro* (9, 10, 33, 44). Up-regulation of CD2, CD11a, and CD56 in NK cells was also seen in patients treated with rhIL-12. It is important to note that such up-regulation was detected only during the early recovery phase, ~3–4 days after IL-12-induced lymphopenia; the expression of these adhesion molecules in NK cells had returned to baseline levels by 7–14 days postinjection. It is therefore possible that even higher levels of some adhesion molecules were expressed in NK cells during the nadir phase after rhIL-12 injection. Nevertheless, the relatively modest (~1.5–2.5-fold) increases in adhesion molecules seen in recovery phase NK cells are likely to be functionally important (45). As has been described previously for IL-2-activated NK cells (9, 44), we found that adhesive and/or signaling interactions medi-

ated through CD2 and LFA-1 contributed to the lysis of malignant target cells by IL-12-activated normal NK cells.<sup>4</sup> Although CD56 is also up-regulated in NK cells stimulated *in vitro* or *in vivo* with IL-12, the role of CD56 in NK cell function remains unclear. In accordance with the results of most other studies (46), we have not detected any effect of CD56 MoAb on the NK cell lysis of target cells (30). Preliminary data suggest that CD56 may contribute to the adhesion of lymphocytes to subendothelial matrix proteins (47). Thus, up-regulation of CD56 in NK cells could participate in NK cell extravasation and/or trafficking in tissues during rhIL-12 therapy.

IL-12 can augment the cytolytic activity of human NK cells *in vitro* and murine NK cells *in vivo* (3, 4, 7, 9, 10). In the present study, we have demonstrated that the systemic administration of IL-12 can also stimulate the cytolytic activity of human NK cells *in vivo*. Single i.v. injections of rhIL-12 at 500 ng/kg can augment the lysis of NK-sensitive targets (natural killing); the lysis of NK-resistant targets (lymphokine-activated killer activity) was not detected during rhIL-12 therapy. However, the number of PBMCs available for these studies was quite limited, and the use of relatively low E:T ratios may have precluded our ability to detect lymphokine-activated killer activity. Moreover, informative cytotoxicity assays could not be performed using PBMCs obtained within the first few days after rhIL-12 injections due to the absence of NK cells in the peripheral blood. Thus, the results of assays using PBMCs obtained several days after rhIL-12 administration may underestimate the level of NK cell cytotoxicity achieved *in vivo* during rhIL-12 therapy. Paucity of NK cells in the peripheral blood due to rhIL-12-induced lymphopenia may account for the diminished natural killing and antibody-dependent cellular cytotoxicity exhibited by PBMCs obtained from HIV-infected patients after s.c. injections of rhIL-12 (48).

Exposure to nanomolar concentrations of IL-2 *in vitro* further enhanced the NK cytolytic activity of PBMCs obtained from patients on rhIL-12 therapy. Similarly, picomolar concentrations of IL-12 strongly augment the NK cytotoxicity of PBMCs from cancer patients receiving low-dose continuous infusions of IL-2 (20). Moreover, in murine models of primary and metastatic renal cell carcinoma, combined therapy with IL-2 and IL-12 was more efficacious than treatment with either cytokine alone (49). Thus, it is rational to consider using IL-2 and rhIL-12 together during cancer immunotherapy in attempts to optimize tumor killing.

PBLs and tumor-infiltrating lymphocytes obtained from patients with advanced cancer often exhibit subnormal proliferation in response to T-cell mitogens (50–52). Defective T-cell function in cancer patients seems to be due in part to a diminished expression of signal-transducing  $\zeta$  chains in the T-cell receptor complex (53, 54). It is therefore not surprising that PBMCs obtained from cancer patients before IL-12 therapy demonstrated poor proliferation in response to CD3 triggering. Nevertheless, PBMCs from three of four patients tested after rhIL-12 treatment exhibited substantially higher proliferative responses to CD3 MoAb. These data suggest that exposure to

<sup>4</sup> Unpublished data.

rhIL-12 *in vivo* can reverse defects in T-cell as well as NK cell function that are associated with advanced cancer in humans. We did not examine whether rhIL-12 therapy altered the expression of  $\zeta$  chains in the T cells of these patients. Future studies should also investigate the effects of rhIL-12 treatment on the differentiation of Th1 and Th2 helper effector cells and on the function of CTLs.

Due to pragmatic considerations, NK cell and T-cell functional assays were performed only with PBMCs obtained from patients treated with the maximum tolerated dose (500 ng/kg) of rhIL-12 in this clinical study. Therefore, the minimum dose of rhIL-12 that can be administered to achieve *in vivo* activation of human T cells and NK cells has not been defined. Significant lymphopenia and increases in serum IFN- $\gamma$  levels were seen in patients treated with 30 or 100 ng/kg rhIL-12, doses that were associated with only minimal toxicity (29). Our studies have thus established that the administration of relatively low, non-toxic doses of rhIL-12 can produce significant biological effects in humans. This is relevant for the potential use of rhIL-12 in the treatment of nonmalignant conditions, for which even moderately toxic therapies may be considered unacceptable.

The schedule and route of rhIL-12 administration that will produce optimal biological effects are currently unknown. The toxicity of rhIL-12 has been shown to be highly dependent on its schedule of administration. Pretreatment with a single dose of IL-12 can protect mice, nonhuman primates, and humans from severe toxicities associated with multiple daily doses of the cytokine (32, 55). The precise mechanisms of this protective effect have not been determined, nor has it been established whether the desirable biological effects of rhIL-12 are comparably schedule dependent. Further investigation is required to elucidate the dose, route, and schedule of rhIL-12 administration that are optimal for the treatment of human cancer and nonmalignant diseases.

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## References

- Trinchieri, G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.*, *13*: 251–276, 1995.
- Hendrzak, J. A., and Brunda, M. J. Interleukin-12: biologic activity, therapeutic utility, and role in disease. *Lab. Invest.*, *72*: 619–637, 1995.
- Gately, M. K., Wolitzky, A. G., Quinn, P. M., and Chizzonite, R. Regulation of human cytolytic lymphocyte responses by interleukin-12. *Cell. Immunol.*, *143*: 127–142, 1992.
- Gately, M. K., Warriar, R. R., Honasoge, S., Carvajal, D. M., Faherty, D. A., Connaughton, S. E., Anderson, T. D., Sarmiento, U., Hubbard, B. R., and Murphy, M. Administration of recombinant IL-12 to normal mice enhances cytolytic lymphocyte activity and induces production of IFN- $\gamma$  *in vivo*. *Int. Immunol.*, *6*: 157–167, 1994.
- Mehrotra, P. T., Wu, D., Crim, J. A., Mostowski, H. S., and Siegel, J. P. Effects of IL-12 on the generation of cytotoxic activity in human CD8<sup>+</sup> T lymphocytes. *J. Immunol.*, *151*: 2444–2452, 1993.
- Trinchieri, G. Interleukin-12 and its role in the generation of TH 1 cells. *Immunol. Today*, *14*: 335–338, 1993.
- Kobayashi, M., Fitz, L., Ryan, M., Hewick, R. M., Clark, S. C., Chan, S., Loudon, R., Sherman, F., Perussia, B., and Trinchieri, G. 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.
- Chan, S. H., Perussia, B., Gupta, J. W., Kobayashi, M., Pospisil, M., Young, H. A., Wolf, S. F., Young, D., Clark, S. C., and Trinchieri, G. Induction of interferon  $\gamma$  production by natural killer cell stimulatory factor: characterization of responder cells and synergy with other inducers. *J. Exp. Med.*, *173*: 869–879, 1991.
- Robertson, M. J., Soiffer, R. J., Wolf, S. F., Manley, T. J., Donahue, C., Young, D., Herrmann, S. H., and Ritz, J. Response of human natural killer (NK) cells to NK cell stimulatory factor (NKSF): cytolytic activity and proliferation of NK cells is differentially regulated by NKSF. *J. Exp. Med.*, *175*: 779–788, 1992.
- Naume, B., Gately, M., and Espevik, T. A comparative study of IL-12 (cytotoxic lymphocyte maturation factor)-, IL-2-, and IL-7-induced effects on immunomagnetically purified CD56<sup>+</sup> NK cells. *J. Immunol.*, *148*: 2429–2436, 1992.
- Chehimi, J., Valiante, N. M., D'Andrea, A., Rengaraju, M., Rosado, Z., Kobayashi, M., Perussia, B., Wolf, S. F., Starr, S. E., and Trinchieri, G. Enhancing effect of natural killer cell stimulatory factor (NKSF/interleukin-12) on cell-mediated cytotoxicity against tumor-derived and virus-infected cells. *Eur. J. Immunol.*, *23*: 1826–1830, 1993.
- Lieberman, M. D., Sigal, R. K., Williams, N. N., II, and Daly, J. M. Natural killer cell stimulatory factor (NKSF) augments natural killer cell and antibody-dependent tumoricidal responses against colon carcinoma cell lines. *J. Surg. Res.*, *50*: 410–415, 1991.
- Hall, S. S. IL-12 at the crossroads. *Science (Washington DC)*, *268*: 1432–1434, 1995.
- Brunda, M. J., and Gately, M. K. Interleukin-12: potential role in cancer therapy. *In*: V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), *Important Advances in Oncology 1995*, pp. 3–18. Philadelphia: J. B. Lippincott, 1995.
- Robertson, M. J., and Ritz, J. Interleukin 12: basic biology and potential applications in cancer treatment. *The Oncologist*, *1*: 93–102, 1996.
- Brunda, M. J., Luistro, L., Warriar, R. R., Wright, R. B., Hubbard, B. R., Murphy, M., Wolf, S. F., and Gately, M. K. Antitumor and antimetastatic activity of interleukin 12 against murine tumors. *J. Exp. Med.*, *178*: 1223–1230, 1993.
- Nastala, C. L., Edington, H. D., McKinney, T. G., Tahara, H., Nalesnik, M. A., Brunda, M. J., Gately, M. K., Wolf, S. F., Schreiber, R. D., Storkus, W. J., and Lotze, M. T. Recombinant IL-12 administration induces tumor regression in association with IFN- $\gamma$  production. *J. Immunol.*, *153*: 1697–1706, 1994.
- Zou, J-P., Yamamoto, N., Fujii, T., Takenaka, H., Kobayashi, M., Herrmann, S. H., Wolf, S. F., Fujiwara, H., and Hamaoka, T. Systemic administration of rIL-12 induces complete tumor regression and protective immunity: response is correlated with a striking reversal of suppressed IFN- $\gamma$  production by anti-tumor T cells. *Int. Immunol.*, *7*: 1135–1145, 1995.
- Mu, J., Zou, J-P., Yamamoto, N., Tsutsui, T., Tai, X-G., Kobayashi, M., Herrmann, S., Fujiwara, H., and Hamaoka, T. Administration of recombinant interleukin 12 prevents outgrowth of tumor cells metastasizing spontaneously to lung and lymph nodes. *Cancer Res.*, *55*: 4404–4408, 1995.
- Soiffer, R. J., Robertson, M. J., Murray, C., Cochran, K., and Ritz, J. Interleukin-12 augments cytolytic activity of peripheral blood lymphocytes from patients with hematologic and solid malignancies. *Blood*, *82*: 2790–2796, 1993.
- Rossi, A. R., Pericle, F., Rashleigh, S., Janiec, J., and Djeu, J. Y. Lysis of neuroblastoma cell lines by human natural killer cells activated by interleukin-2 and interleukin-12. *Blood*, *83*: 1323–1328, 1994.
- Andrews, J. V. R., Schoof, D. K., Bertagnolli, M. M., Peoples, G. E., Goedegebuure, P. S., and Eberlein, T. J. Immunomodulatory effects of interleukin-12 on human tumor-infiltrating lymphocytes. *J. Immunother.*, *14*: 1–10, 1993.

23. Zeh, H. J., III, Hurd, S., Storkus, W. J., and Lotze, M. T. Interleukin-12 promotes the proliferation and cytolytic activity of immune effectors: implications for the immunotherapy of cancer. *J. Immunother.*, *14*: 155–161, 1993.
24. Sykes, M., Szot, G. L., Nguyen, P. L., and Pearson, D. A. Interleukin-12 inhibits murine graft-versus-host disease. *Blood*, *86*: 2429–2438, 1995.
25. Yang, Y.-G., Sergio, J. J., Pearson, D. A., Szot, G. L., Shimizu, A., and Sykes, M. Interleukin-12 preserves the graft-versus-leukemia effect of allogeneic CD8 T cells while inhibiting CD4-dependent graft-versus-host disease in mice. *Blood*, *90*: 4651–4660, 1997.
26. Tahara, H., Zitvogel, L., Storkus, W. J., Zeh, H. J., III, McKinney, T. G., Schreiber, R. D., Gubler, U., Robbins, P. D., and Lotze, M. T. Effective eradication of established murine tumors with IL-12 gene therapy using a polycistronic retroviral vector. *J. Immunol.*, *154*: 6466–6474, 1995.
27. Meko, J. B., Yim, J. H., Tsung, K., and Norton, J. A. High cytokine production and effective antitumor activity of a recombinant vaccinia virus encoding murine interleukin 12. *Cancer Res.*, *55*: 4765–4770, 1995.
28. Colombo, M. P., Vaglini, M., Spreafico, F., Parenza, M., Chiodoni, C., Melani, C., and Stoppacciaro, A. Amount of interleukin-12 available at the tumor site is critical for tumor regression. *Cancer Res.*, *56*: 5231–5234, 1996.
29. Atkins, M. B., Robertson, M. J., Gordon, M., Lotze, M. T., DeCoste, M., DuBois, J. S., Ritz, J., Sandler, A. B., Edington, H. D., Garzone, P. D., Mier, J. W., Canning, C. M., Battiato, L., Tahara, H., and Sherman, M. L. Phase I evaluation of intravenous recombinant human interleukin 12 in patients with advanced malignancies. *Clin. Cancer Res.*, *3*: 409–417, 1997.
30. Robertson, M. J., Caligiuri, M. A., Manley, T. J., Levine, H., and Ritz, J. Human natural killer cell adhesion molecules: differential expression after activation and participation in cytotoxicity. *J. Immunol.*, *145*: 3194–3201, 1990.
31. Pross, H. F., Callewaert, D., and Rubin, P. Assays for NK cell cytotoxicity: their values and pitfalls. In: E. Lotzova and R. B. Herberman (eds.), *Immunobiology of Natural Killer Cells*, pp. 2–20. Boca Raton, FL: CRC Press, 1986.
32. Leonard, J. P., Sherman, M. L., Fisher, G. L., Buchanan, L. J., Larsen, G., Atkins, M. B., Sosman, J. A., Dutcher, J. P., Vogelzang, N. J., and Ryan, J. L. Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon- $\gamma$  production. *Blood*, *90*: 2541–2548, 1997.
33. Rabinowich, H., Herberman, R. B., and Whiteside, T. L. Differential effects of IL12 and IL2 on expression and function of cellular adhesion molecules on purified human natural killer cells. *Cell. Immunol.*, *152*: 481–498, 1993.
34. Robertson, M. J., and Ritz, J. Biology and clinical relevance of human natural killer cells. *Blood*, *76*: 2421–2438, 1990.
35. Baxevas, C. N., Reclos, G. J., Gritzapis, A. D., Dedousis, G. V. Z., Missitzis, I., and Papamichail, M. Elevated prostaglandin E2 production by monocytes is responsible for the depressed levels of natural killer and lymphokine-activated killer cell function in patients with breast cancer. *Cancer (Phila.)*, *72*: 491–501, 1993.
36. Gately, M. K., Desai, B. B., Wolitzky, A. G., Quinn, P. M., Dwyer, C. M., Podlaski, F. J., Familletti, P. C., Sinigaglia, F., Chizzonite, R., Gubler, U., and Stern, A. S. Regulation of human lymphocyte proliferation by a heterodimeric cytokine, IL-12 (cytotoxic lymphocyte maturation factor). *J. Immunol.*, *147*: 874–882, 1991.
37. Perussia, B., Chan, S. H., D'Andrea, A., Tsuji, K., Santoli, D., Pospisil, M., Young, D., Wolf, S. F., and Trinchieri, G. 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.
38. Bajetta, E., Vecchio, M. D., Mortarini, R., Nadeau, R., Rakhit, A., Rimassa, L., Fowst, C., Borri, A., Anichini, A., and Parmiani, G. Pilot study of subcutaneous recombinant human interleukin 12 in metastatic melanoma. *Clin. Cancer Res.*, *4*: 75–85, 1998.
39. Brunda, M. J., Luistro, L., Hendrzak, J. A., Fountoulakis, M., Garotta, G., and Gately, M. K. Role of interferon- $\gamma$  in mediating the antitumor efficacy of interleukin-12. *J. Immunother.*, *17*: 71–77, 1995.
40. Aste-Amezaga, M., D'Andrea, A., Kubin, M., and Trinchieri, G. Cooperation of natural killer cell stimulatory factor/interleukin-12 with other stimuli in the induction of cytokines and cytotoxic cell-associated molecules in human T and NK cells. *Cell. Immunol.*, *156*: 480–492, 1994.
41. Lotze, M. T., Matory, Y. L., Ettinghausen, S. E., Rayner, A. A., Sharrow, S. O., Seipp, C. A. Y., Custer, M. C., and Rosenberg, S. A. *In vivo* administration of purified human interleukin 2. II. Half-life, immunologic effects, and expansion of peripheral lymphoid cells *in vivo* with recombinant IL 2. *J. Immunol.*, *135*: 2865–2875, 1985.
42. Bich-Thuy, L. T., Dukovich, M., Peffer, N. J., Fauci, A. S., Kehrl, J. H., and Greene, W. C. Direct activation of human resting T cells by IL 2: the role of an IL 2 receptor distinct from the Tac protein. *J. Immunol.*, *139*: 1550–1556, 1987.
43. Trinchieri, G., Matsumoto-Kobayashi, M., Clark, S. C., Seehra, J., London, L., and Perussia, B. Response of resting human peripheral blood natural killer cells to interleukin 2. *J. Exp. Med.*, *160*: 1147–1169, 1984.
44. Savary, C. A., and Lotzova, E. Adhesion molecules on MHC-nonrestricted lymphocytes: high density expression and role in oncology. *Lymphokine Cytokine Res.*, *11*: 149–156, 1992.
45. Springer, T. A. Adhesion receptors of the immune system. *Nature (Lond.)*, *346*: 425–434, 1990.
46. Lanier, L. L., and Hemperly, J. J. CD56 and CD57 cluster workshop report. In: S. F. Schlossman, L. Boumsell, W. Gilks, J. M. Harlan, T. Kishimoto, C. Morimoto, J. Ritz, S. Shaw, R. Silverstein, T. Springer, T. F. Tedder, and R. F. Todd (eds.), *Leucocyte Typing V: White Cell Differentiation Antigens*, pp. 1398–1400. Oxford, United Kingdom: Oxford University Press, 1995.
47. Zocchi, M. R., and Poggi, A. Human  $\gamma\delta$  T lymphocytes use NCAM to interact with the subendothelial matrix. *J. Natl. Cancer Inst.*, *87*: 846–847, 1995.
48. Kohl, S., Sigaroudinia, M., Charlebois, E. D., and Jacobson, M. A. Interleukin-12 administered *in vivo* decreases human NK cell cytotoxicity and antibody-dependent cellular cytotoxicity to human immunodeficiency virus-infected cells. *J. Infect. Dis.*, *174*: 1105–1108, 1996.
49. Wigginton, J. M., Komschlies, K. L., Back, T. C., Franco, J. L., Brunda, M. J., and Wiltout, R. H. Administration of interleukin 12 with pulse interleukin 2 and the rapid and complete eradication of murine renal carcinoma. *J. Natl. Cancer Inst.*, *88*: 38–43, 1996.
50. Alexander, J. P., Kudoh, S., Melsop, K. A., Hamilton, T. A., Edinger, M. G., Tubbs, R. R., Sica, D., Tuason, L., Klein, E., Bukowski, R. M., and Finke, J. H. T-cells infiltrating renal cell carcinoma display a poor proliferative response even though they can produce interleukin 2 and express interleukin 2 receptors. *Cancer Res.*, *53*: 1380–1387, 1993.
51. Al-Sarraf, M., Sardesai, S., and Vaitkevicius, V. K. Clinical immunologic responsiveness in malignant disease. II. *In vitro* lymphocyte response to phytohemagglutinin and the effect of cytotoxic drugs. *Oncology (Basel)*, *26*: 357–368, 1972.
52. Whittaker, M. G., Rees, K., and Clark, C. G. Reduced lymphocyte transformation in breast cancer. *Lancet*, *1*: 892–893, 1971.
53. Finke, J. H., Zea, A. H., Stanley, J., Longo, D. L., Mizoguchi, H., Tubbs, R. R., Wiltout, R. H., O'Shea, J. J., Kudoh, S., Klein, E., Bukowski, R. M., and Ochoa, A. C. Loss of T-cell receptor  $\zeta$  chain and p56<sup>lck</sup> in T-cells infiltrating human renal cell carcinoma. *Cancer Res.*, *53*: 5613–5616, 1993.
54. Nakagomi, H., Petersson, M., Magnusson, I., Juhlin, C., Matsuda, M., Mellstedt, H., Taupin, J.-L., Vivier, E., Anderson, P., and Kiessling, R. Decreased expression of signal-transducing  $\zeta$  chains in tumor-infiltrating T-cells and NK cells of patients with colorectal carcinoma. *Cancer Res.*, *53*: 5610–5612, 1993.
55. Sacco, S., Heremans, H., Echtenacher, B., Buurman, W. A., Amraoui, Z., Goldman, M., and Ghezzi, P. Protective effect of single interleukin-12 (IL-12) predose against the toxicity of subsequent chronic IL-12 in mice: role of cytokines and glucocorticoids. *Blood*, *90*: 4473–4479, 1997.

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## Immunological Effects of Interleukin 12 Administered by Bolus Intravenous Injection to Patients with Cancer

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