Immunological Consequences of Interleukin 12 Administration after Autologous Stem Cell Transplantation

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ABSTRACT

Purpose: The purpose is to determine the immunological effects of recombinant human interleukin (rhIL)-12 therapy after autologous stem cell transplantation.

Experimental Design: Twelve patients (8 non-Hodgkin’s lymphoma, 2 Hodgkin’s disease, and 2 plasma cell myeloma) were treated with rhIL-12 by bolus i.v. injection in doses of 30, 100, or 250 ng/kg starting at a median of 66 days post-transplant. Immunological assays were performed using serum and peripheral blood mononuclear cell (PBMC) samples obtained on study.

Results: Dose-dependent increases in the total lymphocyte count occurred during rhIL-12 therapy. The absolute number of peripheral blood CD4 T cells increased up to 16.3-fold, CD8 T cells up to 20.5-fold, B cells up to 11-fold, and natural killer (NK) cells up to 12.3-fold during rhIL-12 administration and returned to pretreatment baseline levels after discontinuation of rhIL-12. CD56bright NK cells expanded dramatically in the blood of a patient with baseline lymphopenia before rhIL-12 therapy. In vitro proliferation of patient PBMCs in response to IL-12 was indistinguishable from that of PBMCs obtained from healthy control subjects. Moreover, spontaneous in vitro proliferation of patient PBMCs increased significantly during rhIL-12 therapy. Increased levels of IFN-γ and IL-18 were detected in the serum of patients treated in the 100 and 250 ng/kg dose cohorts during the first multiple dose cycle.

Conclusions: Expansion of T, B, and NK cells occurs in vivo during rhIL-12 therapy after autologous stem cell transplantation for hematological malignancies. In contrast to their striking defect in IL-12-induced IFN-γ production, post-transplant patient PBMCs exhibit normal proliferative responses to IL-12 in vitro. Additional investigation of rhIL-12 for posttransplantation immunotherapy is warranted.

INTRODUCTION

Interleukin (IL)-12 is a cytokine that plays an important role in the regulation of innate and adaptive immune responses (1, 2). IL-12 supports the proliferation of activated T cells and promotes the differentiation of CD4 T cells into helper effector cells of Th1 phenotype and of CD8 T cells into cytotoxic T lymphocytes, respectively (3–6). Moreover, IL-12 augments the cytolytic activity of natural killer (NK) cells (3, 7, 8) and stimulates IFN-γ production by both NK cells and T cells (9, 10). In preclinical tumor models, IL-12 therapy induces regression of established primary tumors, inhibits the formation of distant metastases, and prolongs the survival of tumor-bearing mice (11–14). In several animal models, production of IFN-γ in vivo has been shown to be necessary but not sufficient for the antitumor effects of IL-12 (12, 13, 15, 16).

Objective tumor responses have been seen during recombinant human (rh)IL-12 treatment of patients with advanced solid tumors (17–19) and refractory lymphoma (20, 21). However, the efficacy of rhIL-12 has been limited in patients with disseminated, bulky tumors. Preclinical models suggest that IL-12-based immunotherapy is most effective in the setting of a small tumor burden (11, 12, 22). Patients with hematological malignancies often achieve a state of minimal residual disease after high-dose chemotherapy and autologous stem cell transplantation. This may therefore be a particularly favorable setting in which to test the antitumor efficacy of rhIL-12. We have conducted a Phase I study to determine whether rhIL-12 could be safely administered after autologous transplantation (23). In the context of that clinical trial, ancillary laboratory research studies were undertaken to evaluate the immunological effects of rhIL-12 after autologous transplantation. Our results provide the first demonstration that rhIL-12 administration can induce the in vivo expansion of major lymphocyte subsets in humans.

PATIENTS AND METHODS

Patients and Clinical Trial Design. Twelve patients who had undergone high-dose chemotherapy and autologous peripheral blood stem cell transplantation for hematological malignancies (8 non-Hodgkin’s lymphoma, 2 Hodgkin’s disease, and 2 plasma cell myeloma) were treated with rhIL-12 on a National Cancer Institute-sponsored, Phase I clinical trial (T97-0027) as reported previously (23). The clinical protocol was approved by the Institutional Review Board at Indiana University Medical Center, and written informed consent was obtained from each patient before treatment on study. rhIL-12 therapy was initiated at a median of 66 days (range, 26–210 days) after autologous transplantation. rhIL-12 was administered in doses of 30, 100, or 250 ng/kg by rapid bolus i.v. injection once and then, after a 2-week hiatus, daily for 5 days...
every 3 weeks. All patients received the single test dose (cycle 0) and at least one multiple-dose treatment cycle; continuation of treatment beyond cycle 1 was optional. Some patients had one or more of rhIL-12 doses reduced or held because of toxicity, as described previously (23).

Blood samples were obtained before and at various time points after the initiation of rhIL-12 therapy. Peripheral blood mononuclear cells (PBMCs) were isolated on a Ficoll-diatrizoate gradient from venous blood samples. Control PBMCs were obtained from healthy volunteer donors. Freshly isolated PBMCs were used for immunofluorescence studies. Aliquots of PBMCs were cryopreserved in liquid nitrogen for subsequent in vitro studies. Blood samples were also obtained from 2 patients with relapsed lymphoma treated in an National Cancer Institute-sponsored, Institutional Review Board-approved Phase II study of rhIL-12 (T97-0050; Ref. 21). These patients received rhIL-12 500 ng/kg by s.c. injection twice a week indefinitely in the absence of disease progression or unacceptable toxicity.

Reagents. Fluorochrome-conjugated monoclonal antibodies specific for CD2, CD3, CD4, CD8, CD16, and CD20 were purchased from Beckman-Coulter (Brea, CA) and for CD56, CD94, CD158a, CD158b, CD158e (NK1b), CD159a (NK2-A), CD161 and the β1 subunit of the IL-12 receptor (IL12R β1) from BD PharMingen (San Diego, CA). ELISA kits specific for human IL-18 and IFN-γ were purchased from R&D Systems (Minneapolis, MN). Culture medium was prepared as previously described (24), except that 15% human AB serum was substituted for 15% human AB serum.

Immunophenotypic Analysis. PBMCs were stained directly with fluorochrome-conjugated monoclonal antibodies, washed, fixed in 1% formaldehyde, and analyzed by flow cytometry as previously described (24) using a FACScan or FACScalibur instrument from Becton Dickinson (San Diego, CA). During analysis, forward and side scattering properties were used to create a lymphocyte gate. Thresholds for discriminating levels of staining above background were established by analysis of PBMCs stained with FITC- and phycoerythrin-conjugated control monoclonal antibodies. The absolute number of various lymphocyte subsets was calculated by multiplying the total lymphocyte count (derived from a routine complete blood count performed at the same time a blood sample was obtained for flow cytometry studies) by the percent of cells in a sample expressing the relevant phenotype (derived from flow cytometric analysis).

Proliferation Assays. Cryopreserved PBMCs were thawed and plated at 50,000 cells/well in 96-well U-bottomed microtiter plates in medium alone or medium containing IL-12 as indicated. After 3–4 days of culture at 37°C, 1 μCi 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 (7). The cpm from triplicate wells was calculated for each experimental condition for each patient or control sample.

Serum Cytokine Levels. Serum was obtained from whole blood samples collected at various time points on study and stored in aliquots at −70°C. Serum samples were subsequently thawed, and levels of human IL-18 and IFN-γ were measured using specific ELISA kits according to the manufacturer’s instructions.

Statistical Analysis. The lower limit of detection for the IFN-γ ELISA is 8 pg/ml, and the upper limit of linearity is 1000 pg/ml. For data analysis, samples with levels < 8 pg/ml were assigned a value of 8 pg/ml, and samples with levels > 1000 pg/ml were assigned a value of 1000 pg/ml. The lower limit of detection for the IL-18 ELISA is 12.5 pg/ml, and the upper limit of linearity is 1000 pg/ml. For data analysis, samples with levels < 12.5 pg/ml were assigned a value of 12.5 pg/ml, and samples with levels > 1000 pg/ml were assigned a value of 1000 pg/ml. Means, SE, and t test comparisons were calculated using Statview software (Abacus Concepts, Piscataway, NJ).

RESULTS
Effect of rhIL-12 Therapy Posttransplant on Peripheral Blood Lymphocyte (PBL) Subsets. The mean absolute lymphocyte count of the entire study group (n = 12) was within the normal range at the time that rhIL-12 therapy was initiated, although 3 patients had lymphocyte counts ≤ 500 cells/μl (23). The distribution of lymphocyte subsets in the blood of study patients was as expected for patients after autologous stem cell transplantation (25, 26). After the initial injection of rhIL-12 in cycle 0, all patients developed transient, profound lymphopenia involving all of the major lymphocyte subsets (23). In contrast, during multiple dose treatment cycles, patients had significant increases in their PBL counts (Fig. 1). The absolute PBL count tended to increase 2–3 days after treatment during the first multiple dose cycle, decline over the next 2 weeks (while patients were off rhIL-12), and then increase again during the second multiple dose treatment cycle. This was a dose-dependent phenomenon. During rhIL-12 therapy, the absolute lymphocyte count increased by 1.2 ± 0.1-fold (mean ± SE) in the 30 ng/kg cohort, 1.7 ± 0.3-fold in the 100 ng/kg cohort, and 2.7 ± 0.6-fold in the 250 ng/kg cohort (P < 0.05 for comparison of 250 ng/kg to either 30 or 100 ng/kg cohort; P > 0.1 for
Table 1  Expansion of peripheral blood lymphocyte (PBL) subsets during recombinant human interleukin 12 (rhIL-12) therapy after transplantation

| Lymphocyte subset | Before rhIL-12 | During rhIL-12 | After rhIL-12 | P<sup>b</sup><br>  
|-------------------|---------------|----------------|---------------|----------------
| Total PBL         | 1233 ± 180    | 2067 ± 370     | 1132 ± 242    | ≥0.01          |
| Total T cells     | 792 ± 169     | 1276 ± 308     | 823 ± 237     | ≤0.05          |
| CD4 T cells       | 132 ± 29      | 218 ± 46       | 123 ± 34      | ≤0.005         |
| CD8 T cells       | 577 ± 141     | 904 ± 275      | 607 ± 202     | >0.05          |
| B cells           | 78 ± 14       | 131 ± 24       | 129 ± 36      | ≤0.025         |
| Natural killer cells | 202 ± 54  | 360 ± 57       | 146 ± 22      | ≤0.01          |

<sup>a</sup> Values are mean ± SE (n = 12) of the absolute number of total PBL or of PBL expressing CD3 (total T cells), CD4 (CD4 T cells), CD8 (CD8 T cells), CD20 (B cells), or CD56 in the absence of CD3 (natural killer cells). Samples were obtained just before the first dose of rhIL-12, during rhIL-12 therapy, and at the final study visit (23 ± 3 days after the last dose of rhIL-12). The peak value observed for each patient during rhIL-12 therapy has been included in the analysis. Peak values were observed at the rest period visit after cycle 1 or 2 in all but 3 patients.

<sup>b</sup> Ps (from paired Student’s t test) for comparison of results from samples obtained before and during rhIL-12 therapy.

comparison of 30 and 100 ng/kg cohort). Overall, the total lymphocyte count increased by 1.7-fold, total T cells by 1.6-fold, CD4 T cells by 1.7-fold, CD8 T cells by 1.6-fold, B cells by 1.7-fold, and NK cells by 2.2-fold during rhIL-12 therapy (Table 1). These increases were statistically significant for all lymphocyte subsets, except CD8 T cells. In contrast, at the time of the off-study visit, the numbers of total lymphocytes and of all lymphocyte subsets were not significantly different from pretreatment baseline levels (P > 0.05–0.4).

**Expansion of Total and CD56<sup>bright</sup> NK Cells in a Patient Receiving rhIL-12 Posttransplant.** Patient 10 underwent autologous stem cell transplantation followed by consolidative radiotherapy for primary refractory Hodgkin’s disease. At the time of initiation of rhIL-12 treatment (6.9 months posttransplant), he was profoundly lymphopenic, with a total T-cell count of 27, CD4 T-cell count of 6, CD8 T-cell count of 15, and NK cell count of 51 cells/μl. During rhIL-12 therapy, his total absolute lymphocyte count increased to 1100 (Fig. 1), and there was a 16.3-fold increase in the CD4 cell count, 20.5-fold increase in the CD8 cell count, 11-fold increase in the B-cell count, and 12.3-fold increase in the NK cell count (data not shown). Before rhIL-12 treatment, NK cells constituted 17% of this patient’s PBL, and 17% of his NK cells (2% of total PBL) exhibited the CD56<sup>bright</sup> phenotype. Total and CD56<sup>bright</sup> NK cells expanded dramatically in the blood of this patient during rhIL-12 therapy (Fig. 2). By the second multiple dose treatment cycle, the absolute number of total peripheral blood NK cells had increased to 627 cells/μl (60% of PBL) and CD56<sup>bright</sup> NK cells had increased to 154 cells/μl (18% of PBL). The expanded CD56<sup>bright</sup> NK cells in this patient resembled normal CD56<sup>bright</sup> NK cells in the blood of healthy control subjects (27–31); they expressed high levels of CD2, CD94, and NKG2-A (CD159a), but little or no CD16, p58.1 (CD158a), p58.2 (CD158b), or NKB1 (CD158e; data not shown).

At the time of his off-study visit (17 days after the last dose of rhIL-12), the patient’s absolute lymphocyte count had declined to pretreatment baseline levels (384 cells/μl). Nevertheless, the fraction of total NK cells (55%) and CD56<sup>bright</sup> NK cells (25%) remained elevated (Fig. 2C). This is all of the more remarkable in that the dose of rhIL-12 had been reduced to 30 ng/kg during the last three multiple dose treatment cycles (cycles 4–6) for this patient because of recurrent grade 3 liver function test abnormalities requiring sequential dose reductions from 250 ng/kg (cycles 0–2) and 100 ng/kg (cycle 3).

Fig. 2  Expansion of peripheral blood natural killer (NK) cells during recombinant human interleukin 12 therapy. Peripheral blood mononuclear cells were obtained from patient 10 before recombinant human interleukin 12 treatment (A), on day 36 (cycle 2, day 1) of study (B), and on day 141 (the off-study visit) of study (C) and analyzed by flow cytometry as described in “Patients and Methods.” Logarithm of red fluorescence (CD56-PE) is displayed on the abscissa and logarithm of green fluorescence (CD3-FITC) on the ordinate. The percentage of CD56<sup>+</sup> CD3-negative NK cells among total gated lymphocytes was 17 (A), 60 (B), and 55 (C), respectively. The percentage of CD56<sup>bright</sup> CD3-negative NK cells (region R2) was 2 (A), 18 (B), and 25 (C), respectively.
Effect of rhIL-12 Therapy on Lymphocyte-Proliferative Responses. Expansion of PBL subsets in vivo during rhIL-12 therapy suggests that posttransplant patient lymphocytes or their precursors may proliferate in response to IL-12. To test this hypothesis directly, we obtained PBMCs from posttransplant patients before rhIL-12 therapy and stimulated them in vitro with IL-12. Patient PBMCs exhibited significant proliferation in response to IL-12 alone, which was comparable with that of normal control PBMCs (Table 2). Baseline proliferation of patient PBMCs in medium alone did not differ significantly from that of normal control PBMCs. However, proliferation in medium alone of PBMCs obtained during rhIL-12 therapy; this increase in proliferation in the absence of deliberate in vitro stimulation was statistically significantly ($P \leq 0.025$). Nevertheless, in vitro proliferation in response to exogenous IL-12 was not significantly increased comparing PBMCs obtained during rhIL-12 therapy to PBMCs obtained before rhIL-12 therapy (data not shown).

Differential Effects of rhIL-12 on Lymphocyte Subsets of Patients Receiving rhIL-12 by s.c. Injection Posttransplant. In contrast to the results observed for our posttransplant patients, increases in the absolute numbers of major PBL subsets have not been reported for advanced solid tumor patients receiving rhIL-12 by repeated bolus i.v. injection or chronic s.c. injection (17, 20, 32, 33). This could be due to differences in underlying diagnosis (solid tumors versus hematological malignancies), treatment status (posttransplant versus nontransplant), and/or the doses and schedules of rhIL-12 used. To begin to address this issue, we studied two patients with aggressive lymphoma that relapsed 24 and 12 months, respectively, after autologous transplantation; they began rhIL-12 therapy 27 and 13 months after stem cell infusion. These patients received rhIL-12 (500 ng/kg twice a week) by s.c. injection continuously 13 months after stem cell infusion (27). Serum IFN-$\gamma$ levels were also measured before daily rhIL-12 injections during the first multiple dose treatment cycle. Serum IFN-$\gamma$ levels were undetectable at all time points tested during cycle 1 for the 3 patients treated in the 30 ng/kg cohort (data not shown). However, strikingly increased percentages of T cells expressing CD161 and the IL-12R$\beta_1$ receptor were observed (Fig. 3). The fraction of CD8 T cells that expressed high levels of the LFA-1 adhesion molecule was also increased during chronic rhIL-12 therapy (Fig. 3). In contrast, percentage of NK cells expressing CD161 during rhIL-12 therapy did not differ significantly from that of control PBMCs.

Serum IFN-$\gamma$ Levels during Multiple-Dose Treatment Cycles. We have previously reported that IFN-$\gamma$ can be detected in the serum ~6 h and reaches peak levels ~12 h after a single bolus i.v. injection of rhIL-12 posttransplant (23). Serum IFN-$\gamma$ levels were also measured before daily rhIL-12 injections during the first multiple dose treatment cycle. Serum IFN-$\gamma$ levels were undetectable at all time points tested during cycle 1 for the 3 patients treated in the 30 ng/kg cohort (data not shown). In contrast, ~24 h after the first rhIL-12 injection in cycle 1, IFN-$\gamma$ was detected in the serum of 5 of 6 patients treated in the 100 ng/kg cohort and all 3 patients treated in the 250 ng/kg cohort (Fig. 4). Serum IFN-$\gamma$ levels were detectable throughout the 5 days of rhIL-12 dosing for patients in the 250 ng/kg cohort, although they fell markedly between days 4 and 5. Patients in the 100 ng/kg cohort had detectable serum IFN-$\gamma$ levels only on days 2 and 3 of the cycle. Administration of a single test dose of rhIL-12 (as was done in cycle 0 of this study) has been shown to lead to

![Figure 3](image-url)
diminished IFN-γ production during subsequent multiple dose treatment cycles (34). Serum IFN-γ levels (41 ± 13 pg/ml for the 100 ng/kg cohort, 118 ± 7 pg/ml for the 250 ng/kg cohort) 24 h after the first injection of rhIL-12 in cycle 1 did appear to be lower than serum IFN-γ levels (104 ± 70 pg/ml for the 100 ng/kg cohort, 267 ± 69 pg/ml for the 250 ng/kg cohort) 24 h after the single injection of rhIL-12 in cycle 0. However, these differences were not statistically significant (P > 0.05).

Production of IL-18 in Vivo during rhIL-12 Therapy Posttransplant. IL-18 is detectable in the serum of patients with advanced solid tumors during treatment with rhIL-12 (19), and IL-18 can act synergistically with IL-12 to promote IFN-γ production (35–37). Production of IL-18 in vivo could augment IFN-γ production during rhIL-12 therapy. We therefore measured IL-18 levels in the serum of patients who received rhIL-12 therapy posttransplant. IL-18 was detectable in the serum of all patients before rhIL-12 treatment. Pretreatment serum IL-18 levels in these patients were 479 ± 78 pg/ml (mean ± SE; n = 12), which appears to be higher than the levels (126 ± 44 pg/ml; n = 46) detected in the serum of healthy volunteer subjects (data provided by manufacturer of the ELISA). Peak serum IL-18 levels during rhIL-12 therapy were 804 ± 63 pg/ml, which represents a significant increase (P ≤ 0.005) compared with pretreatment baseline levels. Overall, serum IL-18 levels increased by 2.2 ± 0.4-fold during rhIL-12 therapy. Serum IL-18 levels increased by 1.2 ± 0.3-fold in the 30 ng/kg cohort, 1.9 ± 0.3-fold in the 100 ng/kg cohort, and 3.9 ± 0.3-fold in the 250 ng/kg cohort (P ≤ 0.005 for comparison of 250 ng/kg to either 30 ng/kg or 100 ng/kg cohort; P > 0.1 for comparison of 30 ng/kg and 100 ng/kg cohort). Serum IL-18 tended to reach peak levels on day 4 or 5 of the first multiple dose treatment cycle (Fig. 5). At the rest period visit (4–5 days after the last injection of rhIL-12), serum IL-18 levels (Fig. 5), unlike serum IFN-γ levels (Fig. 4), remained higher than pretreatment baseline levels (P ≤ 0.005).

DISCUSSION

To our knowledge, this study provides the first demonstration that administration of rhIL-12 alone can induce the expansion of major human lymphocyte subsets in vivo. Dose-dependent significant increases in the absolute numbers of peripheral blood CD4 T cells, B cells, and NK cells were observed during rhIL-12 therapy. These lymphocyte subsets declined to pretreatment baseline levels shortly after the discontinuation of rhIL-12, eliminating the unlikely possibility that some of the changes were because of delayed engraftment posttransplant rather than the effects of rhIL-12 therapy. Expansion of all major subsets of immunocompetent cells during rhIL-12 therapy might not only contribute to antitumor activity posttransplant but could ameliorate the immunodeficiency known to occur after autologous stem cell transplantation for cancer (38).

Previous studies of nontransplant cancer patients have shown that injections of rhIL-12 can cause transient, profound lymphopenia involving all of the major lymphocyte subsets (32, 33). It is likely that this lymphopenia reflects the in vivo activation of PBL, followed by their extravasation into tissue spaces (4). Recovery from rhIL-12-induced lymphopenia, unlike rhIL-2-induced lymphopenia (39), is not associated with a rebound lymphocytosis; the numbers of PBL subsets recover to pre-IL-12 baseline levels (17, 32, 33). We have observed a very similar occurrence of profound lymphopenia followed by recovery to baseline levels after single bolus i.v. injections of rhIL-12 during cycle 0 of therapy posttransplant (23). However, the expansion of major PBL subsets during subsequent multiple
dramatic expansion of total and CD56bright NK cells occurred in 1 patient treated with rhIL-12 posttransplant. Similar expansion of CD56bright NK cells has been observed in both posttransplant and nontransplant cancer patients during prolonged administration of rhIL-2 in relatively low doses (44–46). CD56bright NK cells, which comprise ~10% of normal human peripheral blood NK cells, express high levels of CD2, CD94, and NKG2-A but express little or no CD16 or killer cell Ig-like receptors on the cell surface (27–31). Compared with CD56dim NK cells, CD56bright NK cells exhibit less potent cytolytic activity but greater proliferative responses and production of IFN-γ in response to several stimuli (27). It has been hypothesized that CD56dim and CD56bright NK cells belong to distinct NK cell subsets that subserve different effector functions in vivo. It is not clear why dramatic expansion of CD56bright NK cells was observed in patient 10 but not in other patients receiving rhIL-12 posttransplant (data not shown). However, patient 10 also experienced the greatest increase over baseline levels in the absolute numbers of CD4 T cells, CD8 T cells, and B cells during rhIL-12 therapy. Thus, it seems likely that his lymphocytes were peculiarly sensitive to the proliferative signals provided by rhIL-12.

Production of IFN-γ in vivo was observed during both single dose (23) and multiple dose (Fig. 4) treatment cycles of rhIL-12 after autologous transplantation. However, we have previously reported that serum IFN-γ levels seen during cycle 0 of rhIL-12 posttransplant were substantially lower than IFN-γ levels in serum of nontransplant cancer patients receiving the same doses of rhIL-12 (23). Moreover, serum IFN-γ levels measured during the first multiple dose treatment cycle were even lower than those observed after a single injection of rhIL-12 in cycle 0. The relatively low levels of IFN-γ produced in vivo during rhIL-12 therapy posttransplant may be of concern because IFN-γ production is necessary for the antitumor activity of rhIL-12 in several animal models (12, 13, 15, 16). Because IL-18 can act synergistically with IL-12 to stimulate IFN-γ secretion (35–37) and has been reported to be induced in vivo during rhIL-12 therapy (19), we speculated that failure to produce IL-18 might contribute to the relatively low IFN-γ levels seen during rhIL-12 therapy posttransplant. Unexpectedly, we detected higher than normal levels of IL-18 in the serum of patients before rhIL-12 treatment. Furthermore, serum IL-18 levels increased significantly during rhIL-12 therapy. Thus, defective IFN-γ production during rhIL-12 treatment posttransplant occurs despite the presence of substantial serum levels of IL-12 and IL-18. We currently have no data to explain this apparent paradox. It is possible that most, if not all, of the serum IL-18 that we detected was bound to IL-18 binding protein and hence biologically inactive. Some isoforms of IL-18 binding protein bind to IL-18 with high affinity and prevent IL-18 from engaging its cell surface receptor (47, 48). Availability of reagents that specifically measure free IL-18 as well as inhibitory isoforms of IL-18 binding protein will be needed to adequately address this issue.

The fundamental mechanisms of defective IFN-γ production during rhIL-12 administration posttransplant have not yet been determined. However, we have shown that PBMCs obtained from patients posttransplant and stimulated directly with rhIL-12 in vitro also exhibit defective IFN-γ production (23). Therefore, posttransplant patient PBMCs appear to be intrinsi-
ically deficient in their capacity to secrete IFN-γ in response to rHL-12. Additional laboratory studies are in progress to elucidate the mechanisms underlying this phenomenon. However, posttransplant patient PBMCs can produce IFN-γ in vitro after stimulation by IL-12 plus IL-2 (23) or IL-12 plus IL-18 (our unpublished data). Posttransplant IL-2 therapy has been shown to be feasible for patients with hematologic malignancies (45, 46). Moreover, combined rHL-2 and rHL-12 treatment is tolerable in patients with solid tumors and appears to reverse the attenuation of IFN-γ production that is seen during prolonged therapy with rHL-12 alone (49). rHL-18 can also be given safely in biologically active doses to patients with advanced cancer (50). Thus, combination cytokine therapy might circumvent defective IL-12-induced IFN-γ production posttransplant. It is hoped that such an approach will improve the efficacy of cytokine-based immunotherapy after autologous stem cell transplantation for cancer.

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