Bone marrow transplantation (BMT) can produce prolonged clinical remissions and cure in many patients with hematologic malignancies. Unfortunately, a number of individuals still fail despite BMT. In patients undergoing autologous or T-cell–depleted allogeneic transplantation, the primary cause for failure is relapse of the underlying malignancy. Presumably, disease relapse results from the proliferation of residual neoplastic clones not eliminated by the transplant ablative regimen. Efforts to reduce the incidence of relapse by intensifying the chemoradiotherapeutic conditioning regimen have generally been unsuccessful and have been associated with increased nonhematologic toxicities.

Immunologic factors appear to play a role in preventing disease recurrence post-BMT. It has been well established that recipients of allogeneic marrow have a lower incidence of relapse than recipients of genotypically identical syngeneic grafts. Additionally, in some series, it has been shown that the development of graft-versus-host disease (GVHD) is associated with a reduced frequency of leukemia recurrence post-BMT. The antineoplastic effect of allogeneic donor marrow on the recipient’s residual disease has been termed the graft-versus-leukemic (GVL) phenomenon. The cellular and humoral immune mechanisms underlying the GVL phenomenon have yet to be precisely characterized. Although GVHD appears to be associated with GVL, recent evidence would suggest that they may also be separable entities. A clinical study in which transplant recipients were administered an abbreviated course of immunosuppression for GVHD prophylaxis with the intention of increasing the frequency of GVHD resulted in greater morbidity and mortality from GVHD without a decrease in the rate of leukemic relapse. Consequently, it seems logical to direct current efforts toward strategies designed to stimulate GVL activity without necessarily inducing GVHD.

Recent laboratory studies have indicated that the addition of interleukin-2 (IL-2) to peripheral blood lymphocytes from both allogeneic and autologous transplant marrow recipients markedly augments the cytotoxic capacity of these cells against cultured tumor targets. Moreover, IL-2–activated lymphocytes from patients transplanted for chronic myelogenous leukemia (CML) have been shown to possess cytolytic activity against cryopreserved leukemic cells obtained before transplant. In murine transplant systems, the infusion of IL-2 early post-BMT has resulted in a reduction in tumor recurrence without the development of GVHD. These and similar reports have prompted us to investigate the clinical and immunologic effects of the administration of recombinant IL-2 (rIL-2) to patients after both T-cell–depleted allogeneic and autologous BMT. Considerable clinical data on the effects of IL-2 in patients with metastatic cancer have been accumulated from a number of studies over the past 7 years. At the
relatively high doses of IL-2 used in most of these trials, significant antitumor responses have occasionally been noted, but often at the expense of considerable toxicity. Fever, hypotension, jaundice, and azotemia have been frequent complications, often necessitating admission to intensive care units. Patients recovering from a recent BMT are likely to be in too fragile a condition to withstand this degree of toxicity. Moreover, the side effects associated with high-dose IL-2 preclude prolonged administration and thus make it an impractical long-term immunomodulating agent.

Anticipating the difficulties associated with the administration of conventional doses of IL-2 to transplant recipients for extended periods, we have constructed a clinical trial in which we administer rIL-2 in very low doses (2.0 × 10^9 U/d) by continuous infusion for 3 consecutive months post-BMT. Our intention is to develop an immunologically active regimen that can be administered for an extended period without excessive toxicities and without inducing GVHD. Based on recent laboratory studies and recent clinical trials, we reasoned that low doses of rIL-2 in vivo might selectively expand the number of activated killer cells in the circulation without inducing significant toxicity. Our initial experience suggests that prolonged infusion of rIL-2 at low doses is safe in both autologous and T-cell-depleted allogeneic marrow recipients and results in selective immunologic changes in vivo, specifically a marked increase in natural killer (NK) cells and NK cell activity.

MATERIALS AND METHODS

Patient eligibility. Patients were eligible to receive IL-2 if they had undergone a BMT and were felt to have a moderate-high probability of disease recurrence post-BMT. Patients were considered candidates for protocol entry if they had been transplanted for relapsed or refractory acute leukemia, relapsed or refractory lymphoma, CML in accelerated phase or blast crisis, or solid tumor with metastases. Treatment with IL-2 was begun no sooner than 6 weeks and no later than 16 weeks after marrow infusion. Pretreatment evaluation in all patients included a physical exam, chest x-ray, electrocardiogram (ECG), complete blood count, serum chemistry (including liver enzymes), x-ray, electrocardiogram (ECG), complete blood count, serum chemistry, urinalysis, pulmonary function tests, arterial blood gas, thyroid function tests, and quantitative serum Igs. To fulfill criteria for study entry, patients were required to have a good performance status (ECOG 0-1) and normal or near normal laboratory parameters of hepatic, renal, and pulmonary function. Patients were required to have a hematocrit of 26%, an absolute neutrophil count of 0.5 × 10^9 cells/L, and a platelet count of 30,000/μL independent of transfusion. All patients were required to be free of active infection at protocol entry. Patients could receive concurrent prophylactic antibiotic therapy to prevent Herpes zoster (acyclovir) and Pneumocystis carinii (trimethoprim-sulfa or aerosolized pentamidine) infection. Patients with evidence of active GVHD or those receiving any immunosuppressive medications for GVHD prophylaxis were excluded from this study. Written informed consent was obtained in all cases. The treatment protocol was approved by the Dana-Farber Cancer Institute’s Scientific Review and Human Subjects Protection Committees.

IL-2 treatment. Patients were scheduled to receive rIL-2 (Hoffman-LaRoche, Inc, Nutley, NJ) at a dose of 2 × 10^9 U/m²/d via by uninterrupted continuous infusion for a period of 3 months. Each patient had an indwelling central line (either Hickman or Portacath) through which rIL-2 was administered. Delivery was accomplished with a portable computerized ambulatory delivery pump (Pharmacia/Deltex Model 5100 HF; Pharmacia/Deltex, St Paul, MN) worn by the patients. The rIL-2 was 95% pure (specific activity, 1.5 × 10^5 U/mg protein) and was supplied as a lyophilized powder that was reconstituted in 80 cc of normal saline. The supply of rIL-2 was renewed every 7 days by the outpatient pharmacist. No pump failures occurred. The treatment was completely performed on an outpatient basis with one hospital visit each week during the infusion period. The patients did not receive any prophylactic antipyretic or anti-inflammatory agents while on study. None were administered diuretics prophyllactically to prevent weight gain. During each weekly visit, clinical assessments were made by history, physical exam, and laboratory monitoring of hematologic, hepatic, and renal function. Thyroid function tests and Ig electrophoresis were performed at the beginning, middle, and end of the treatment course. If patients were unable to tolerate the initial treatment dose, rIL-2 was temporarily discontinued and then restarted at one-half the original daily dose (1 × 10^9 U/m²/d) when side effects abated. If this adjustment was tolerated, the dose was escalated back to 2 × 10^9 U/m²/d within 3 weeks.

Immunologic studies. Heparinized peripheral blood (60 cc) was drawn from each patient weekly for immunologic studies. Peripheral blood mononuclear cells (PBMC) were removed by Ficoll-Hypaque density centrifugation. Immune/phenotypic analysis of lymphoid subsets was performed on aliquots of cells by simultaneously staining with phycoerythrin (PE) and fluorescein isothiocyanate (FITC) conjugates of different monoclonal antibodies (MoAbs; Coulter Immunology, Hialeah, FL) and analyzing the degree of fluorescence on an EPICS ELITE (Coulter Electronics, Hialeah, FL). Cryopreserved mononuclear cells that had been obtained from patients or normal controls were thawed and evaluated for their ability to lyse NK-sensitive (K562) and NK-resistant (COLO) tumor cell targets. After thawing the cells and before the actual assays, effectors were incubated overnight in either media alone (RPMI 1640 with 10% human AB serum plus 1% penicillin-streptomycin, 2% L-glutamine, 1% sodium pyruvate) or media plus additional IL-2 (500 U/ml). Incubation was performed at 37°C in 24-well round-bottom plates at a concentration of 1 to 2 × 10^6 cells/ml. The effectors were then washed three times and set up against 51Cr-labeled targets at E:T ratios of 40:1, 20:1, and 10:1. Four-hour chromium release cytotoxicity assays were then performed as previously described. Individual patient samples obtained at different intervals before, during, and after treatment were tested simultaneously to obtain an accurate assessment of changes in cytotoxicity over time.

RESULTS

Patient characteristics. Thirteen patients who underwent BMT at Dana-Farber Cancer Institute were treated with IL-2 between January 1990 and January 1991. Patient characteristics are outlined in Table 1. There were nine males and four females. Median age was 37 years (range, 24 to 49). Diseases for which BMT was performed included acute lymphoblastic leukemia (ALL) (n = 3), non-Hodgkin’s lymphoma (NHL) (n = 3), acute myelogenous leukemia (AML) (n = 2), breast cancer (n = 2), CML (n = 1), myelodysplastic syndrome (MDS) (n = 1), and ovarian cancer (n = 1). Twelve patients had a history of relapsed or refractory tumor before transplantation. One patient with ALL was in his first complete remission (CR), but had required three chemotherapy regimens to achieve CR and was considered an induction failure. All patients were thus
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with a prior history of Hodgkin’s disease and mantle NHL), autologous marrow had been purged with multiple carboplatinum 266 mg/m²/d for 4 days, carboplatinum 400 mg/m²/d for 6 days. The three patients with solid tumors had been treated with ifosfamide 3 g/m²/d for 4 days, thiotepa 166 mg/m²/d for 2 days, and etoposide 400 mg/m²/d for 4 days. Seven individuals had received autologous marrow grafts. In four (three ALL and one NHL), autologous marrow had been purged with multiple anti-B-cell antibodies and complement. The remaining six patients had received HLA-identical allogeneic marrow that had been purged of mature T cells with anti-CD6 antibody and complement as previously described. None of the patients had evidence of GVHD and none were taking any immunosuppressive agents. rIL-2 was begun at a dose of 5 x 10⁵ U/m²/d after a delay of 1 and 4 weeks, respectively. At this dose level, both patients tolerated treatment without difficulty. When rIL-2 was escalated back to the original starting dose (2.0 x 10⁵ U/m²/d) after 3 weeks of therapy, no adverse reactions were noted. The remaining 11 patients tolerated initiation of rIL-2 without incident.

Other side effects were generally not noted until later in the course of treatment, after at least 4 to 5 weeks on rIL-2. These adverse effects were mild and did not result in discontinuation of rIL-2 infusion. Fatigue was the most common complaint and occurred in six patients, but was mild and did not interfere with daily activities. A 5% to 7% gain in body weight was documented in 5 of 13 individuals, all of whom had peripheral edema. Five patients developed a nonproductive cough; however, only one (with preexisting emphysema) complained of subjective dyspnea on exertion. Cough abated within 1 week of completing treatment in all patients. Localized rashes with differing clinical presentations occurred in six patients, but were transient and resolved before discontinuing rIL-2. It is unclear if the development of these rashes was in any way related to treatment. Thyroid function test abnormalities were noted in five individuals. Four patients experienced asymptomatic increases (2) or decreases (2) in thyroid-stimulating hormone (TSH) levels without changes in serum (T4) thyroxine levels. TSH normalized after withdrawal of IL-2 in these patients. One patient (UPN 605), who was not conditioned with TBI, developed clinical hypothyroidism with a T4 of 1.0 μg/dL and TSH of 98.2 pg/dL after 9 weeks on rIL-2. Antimicrosomal and antithyroglobulin antibodies were present at high titres. Administration of levothyroxine of therapy. None of the allogeneic or autologous marrow recipients developed any clinical signs of GVHD while receiving rIL-2.

The toxicities encountered in our 13 subjects are listed in Table 2. They can be separated into early and late consequences of treatment. Two individuals, both recipients of CD6-depleted allogeneic marrow, developed fever (38.5°C), nausea, and vomiting within 72 hours of initiation of rIL-2. When therapy was interrupted, symptoms resolved within 3 days. rIL-2 was restarted in both patients at a lower dose (1 x 10⁵ U/m²/d) after a delay of 1 and 4 weeks, respectively. At this dose level, both patients tolerated treatment without difficulty. When rIL-2 was escalated back to the original starting dose (2.0 x 10⁵ U/m²/d) after 3 weeks of therapy, no adverse reactions were noted. The remaining 11 patients tolerated initiation of rIL-2 without incident.

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Abbreviations: PR, partial remission; AP, accelerated phase; RAEB-T, refractory anemia with excess blasts.
reversed the signs and symptoms of hypothyroidism. After cessation of rIL-2, thyroid function returned to normal. After L-thyroxine was withdrawn, the patient remained euthyroid.

Abnormalities of hepatic and renal function were infrequent and were not associated with any symptoms. No patient experienced an increase in serum creatinine secondary to rIL-2 therapy. A twofold to threefold elevation in serum alkaline phosphatase was observed in four patients. The laboratory abnormality developed over a period of 4 to 6 weeks, peaked, and then returned toward normal levels while the patients were still receiving rIL-2. No concurrent elevations in serum transaminases or serum bilirubin were noted in these four patients; one individual (UPN 1377) developed a transient episode of gallstone pancreatitis after 8 weeks of IL-2 but completed therapy after a brief interruption of treatment.

Infections developed in two patients receiving rIL-2. Bacteremia associated with indwelling Hickman lines was documented at 4 and 10 weeks of study, respectively. Enterobacter agglomerans and Rhodococcus were the organisms isolated in these cases. Both infections were easily eradicated with intravenous antibiotics and line removal. No metastatic foci of infection developed in these patients.

In all, 9 of 13 patients (69%) completed the full 3-month course of continuous intravenous rIL-2 therapy. One patient (UPN 1342) terminated treatment after 10 weeks because of the Hickman line infection mentioned above. UPN 606, who had evidence of residual breast cancer at the initiation of rIL-2, elected to stop therapy after 7 weeks when clinical evaluation revealed no significant shrinkage of her tumor. Treatment was stopped in two patients at 6 weeks because of disease relapse. Both patients had been transplanted for relapsed acute leukemia. Currently, 5 of 10 patients who began rIL-2 in CR are alive and free of disease with a median follow-up of 19 months post-BMT (range, 12 to 22 months).

Hematologic effects of low-dose continuous IL-2 after BMT. In previous clinical trials in patients with metastatic cancer, treatment with high doses of IL-2 has been found to exert a profound effect on the absolute number of lymphocytes present in the circulation. In those studies, the infusion of IL-2 resulted in significant lymphocytopenia, while discontinuation of treatment was reported to induce a rebound lymphocytosis. In our current study in BMT recipients receiving low-dose IL-2 (2 x 10^6 U/m²/d) by continuous infusion, we observed a decrease in the absolute lymphocyte count in 9 of 13 patients after 1 week of IL-2 therapy. However, with continued IL-2 infusion, a steady increase in the lymphocyte count occurred in all patients. After 2 weeks of therapy, the majority of patients had a higher number of circulating lymphocytes than at the initiation of treatment. Over the entire course of treatment, 12 of 13 patients (92%) experienced at least a twofold increase in circulating lymphocytes. In the 10 patients completing 3 months of IL-2, the median lymphocyte count increased from 880 x 10^6 cells/μL initially to 2,420 x 10^6 cells/μL (Fig 1). This increase in lymphocyte count was substantially greater than that observed in a cohort of 81 autologous and allogeneic marrow recipients transplanted in 1989 and 1990 who did not receive rIL-2 and whose counts were followed-up between 3 and 6 months post-BMT (Fig 1). In all but two of our patients, withdrawal of IL-2 was associated with a decrease in circulating lymphocytes, not with a rebound lymphocytosis.

In addition to stimulating an increase in lymphocytes, treatment with IL-2 was associated with an increase in the eosinophil count. Eosinophilia was observed in all 13 patients in our series. However, in contrast to the kinetics of its effects on the lymphocyte count, IL-2 induced a rapid increase in circulating eosinophils that peaked after 3 weeks of treatment and was followed by a gradual decline to near baseline levels over the 3-month course (Fig 2). Other white blood cell components did not appear to be affected by IL-2. Specifically, low-dose IL-2 infusion was not associated with any significant change in the number of circulating neutrophils or monocytes.

The platelet count decreased by more than 20% (21% to 40%) in 10 of 13 patients in our series. This decrease was usually observed within the first 2 weeks on IL-2. Continued treatment was not associated with a further decrease in platelets. Figure 2 shows the change in the median platelet count over the course of therapy. None of our patients experienced greater than a 40% decrease in platelets. No patient had any bleeding episodes and none required platelet transfusion, including the four subjects whose platelet counts were between 60,000 and 80,000 when beginning IL-2.

Despite the extent and frequency of blood sampling required on this study, treatment with low-dose IL-2 did not appear to have a significant effect on hemoglobin levels and only 3 of 13 patients required red blood cell transfusion. However, two of these patients were still red blood cell
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A. Neutrophil Count

B. Platelet Count

C. Eosinophil Count

Fig 2. Effects of rIL-2 infusion on hematologic parameters. The absolute neutrophil (A), platelet (B), and eosinophil (C) counts in patients treated continuously with rIL-2. Data points represent the median values in the study population at each time.

transfusion dependent when starting IL-2. In particular, no significant change in reticulocyte count was noted in patients while on study.

Immunophenotypic effects. Immunophenotypic analysis of PBMC was performed routinely while patients received rIL-2. The fraction of lymphocytes expressing the NK cell-associated antigen NKH1 (CD56) increased steadily over time, rising from a median of 15% initially to 70% within 8 weeks of initiating IL-2 therapy (Fig 3A). There was a concomitant increase in the absolute number of NK cells as well (Fig 3B). NK cell number increased from a median of 126 cells/μL to greater than 2,000 cells/μL over the 3-month course of treatment. In contrast, in 61 marrow transplant patients from 1989 to 1990 who did not receive rIL-2, the median NK cell count remained 140 to 175 NK cells/μL between 3 and 6 months post-BMT (Fig 4). The absolute number of NK cells increased in all 13 patients treated with rIL-2. Examination of peripheral smears from patients showed a significant proportion of cells with large granular lymphocyte (LGL) morphology, thus corroborating our immunophenotypic findings.

Dual-color immunofluorescence studies provided further information on the effects of IL-2 on NK cells in vivo. The density of membrane expression of the NK cell surface antigen, CD56, increased in all patients receiving rIL-2. These NKH1high cells were almost exclusively CD2- and CD3-. rIL-2 appeared to stimulate both CD16+ and CD16- NK cell subsets equally so that the majority of NK cells (>75%) became simultaneously CD56high and CD16+ (Fig 5). Despite the positive effect rIL-2 exerted on NK cell number, there was no change in the relative expression of CD25, the p55 chain of the IL-2 receptor, on CD56+ cells.

Fig 3. Effects of prolonged infusion of low-dose rIL-2 on peripheral blood T cells and NK cells. (A) The percentages of (— — —) CD56+ NK cells and (— — —) CD3+ T cells were determined by direct immunofluorescence analysis of PBMC at biweekly intervals during rIL-2 infusion and after completion of treatment. Results represent median values for all patients receiving rIL-2 at each interval. (B) The absolute numbers of circulating (— — —) CD56+ NK cells and (— — —) CD3+ T cells were calculated based on the combined results of immunofluorescence analysis and determination of the absolute number of peripheral blood lymphocytes in each patient. Results represent median values for all patients receiving rIL-2 at each interval.
In contrast to the proliferation of NK cells induced by IL-2, no significant change in the number of circulating T lymphocytes was noted in the majority of our patients (Fig 3). Only 4 of 13 individuals (31%) experienced a twofold or greater increase in T-cell number. In those four individuals, the increase in T cells appeared to be confined to the CD4+ subset. While treatment with rIL-2 did not appear to affect the expression of activation antigens such as Ia on the T-cell surface, the relative expression of CD25 did increase in 6 of the 13 patients evaluated. All four patients who responded to rIL-2 with an increase in T-lymphocyte number were noted to have an increase in the relative expression of CD25 on their T cells. The minimal change in T-cell number we observed in our 13 subjects receiving rIL-2 over this 3-month period was similar to that noted in transplant recipients not treated with rIL-2 over an equivalent period (Fig 4).

Treatment with rIL-2 had an inconsistent effect on B-lymphocyte recovery post-BMT. In the majority of individuals, no significant change in B-cell number was noted. Quantitative serum Ig levels were available on 10 patients, none of whom had been receiving exogenous γ-globulin. Four experienced at least a 25% decrease in IgG during rIL-2 therapy, while three patients were observed to have a 25% increase in IgG levels. No patients developed serious hypogammaglobulinemia (IgG < 500 μg/μL) while on study. There was no significant change in the median IgG level in the study population over the course of treatment.

**Cytolytic activity.** At regular intervals, we evaluated the capacity of mononuclear cells from patients receiving rIL-2 to destroy NK-sensitive (K562) and NK-resistant (COLO) tumor cell lines. Before treatment with rIL-2, cryopreserved cells from our patients exhibited low levels of cytotoxicity against these radiolabeled targets. The degree of cytotoxicity was similar to that observed in cryopreserved cells from normal controls and from transplant recipients not administered rIL-2. During treatment with rIL-2, cytotoxicity against K562 increased substantially (Fig 6A). Two weeks after discontinuation of rIL-2, cytotoxic capacity decreased, but remained above pretreatment levels. A similar pattern, but to a lesser degree, was observed in experiments with the NK-resistant cell line COLO (Fig 6B). In contrast, there was no increase in the degree of cytotoxicity observed in PBMC obtained from patients between 3 and 6 months post-BMT who were not treated with rIL-2.

We also observed that cells obtained from patients on rIL-2 could be manipulated further in vitro to enhance their cytolytic activity.
adjustable degree of lymphocytosis was observed in the allogeneic and autologous patients. No difference in the effects on platelet and neutrophil counts was noted in the two groups. While allogeneic recipients generally had higher numbers of NK cells than autologous recipients when beginning rIL-2, both groups of patients showed a significant and comparable increase in NK cells in response to IL-2 (Fig 2). In both autologous and allogeneic patients not receiving IL-2, NK cell recovery is usually complete within 2 months post-BMT, the approximate time at which our study patients began rIL-2 therapy. It is interesting that three of six allogeneic but only one of seven autologous patients experienced a twofold increase in the number of CD4+ T lymphocytes while on therapy. This finding may reflect the normal recovery of T cells post-CD6-depleted allogeneic BMT and may not constitute a direct effect of rIL-2. Lastly, no significant differences were noted in the cytotoxic capacity of mononuclear cells from allogeneic and autologous patients against either K562 or COLO cell lines.

DISCUSSION

The rationale for the use of IL-2 after marrow transplantation is based on several experimental observations. First, freshly isolated leukemia and lymphoma cells have previously been shown to be sensitive to lysis by IL-2 induced lymphocyte-activated killer (LAK) cells in vitro. Moreover, IL-2-responsive LAK precursors have been identified in the peripheral blood of patients as early as 3 weeks after high-dose chemoradiotherapy and marrow infusion. After allogeneic BMT, isolated mononuclear cells stimulated by IL-2 in vitro have been shown to be cytotoxic for recipient leukemia cells in chromium release assays. More importantly, these LAK cells, when incubated with CML cells in vitro, have been shown to mediate cytotoxicity. Overnight incubation with additional rIL-2 (500 U/mL) in vitro had profound effects on the killing of both K562 and COLO targets (Fig 6). The longer the duration of in vivo rIL-2 treatment, the greater the sensitivity to additional rIL-2 in vitro. After discontinuation of rIL-2 therapy, this sensitivity to further in vitro stimulation began to decline.

Comparison of the effects of rIL-2 on allogeneic and autologous marrow recipients. We compared the clinical and immunologic effects of rIL-2 administration in the seven autologous and six CD6-depleted allogeneic marrow recipients who received treatment. Therapy was generally well tolerated by both groups, although the two individuals who developed fever and constitutional symptoms and who required a temporary reduction in IL-2 dose were both allogeneic BMT patients. There was no difference in the incidence of rash, cough, or liver function test (LFT) elevations in the two groups, and no patient developed GVHD. Likewise, there was no difference in the effect of rIL-2 on hematologic parameters. A comparable degree of lymphocytosis was observed in the allogeneic and autologous patients. No difference in the effects on platelet and neutrophil counts was noted in the two groups. While allogeneic recipients generally had higher numbers of NK cells than autologous recipients when beginning rIL-2, both groups of patients showed a significant and comparable increase in NK cells in response to IL-2 (Fig 2). In both autologous and allogeneic patients not receiving IL-2, NK cell recovery is usually complete within 2 months post-BMT, the approximate time at which our study patients began rIL-2 therapy. It is interesting that three of six allogeneic but only one of seven autologous patients experienced a twofold increase in the number of CD4+ T lymphocytes while on therapy. This finding may reflect the normal recovery of T cells post-CD6-depleted allogeneic BMT and may not constitute a direct effect of rIL-2. Lastly, no significant differences were noted in the cytotoxic capacity of mononuclear cells from allogeneic and autologous patients against either K562 or COLO cell lines.

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The rationale for the use of IL-2 after marrow transplantation is based on several experimental observations. First, freshly isolated leukemia and lymphoma cells have previously been shown to be sensitive to lysis by IL-2 induced lymphocyte-activated killer (LAK) cells in vitro. Moreover, IL-2-responsive LAK precursors have been identified in the peripheral blood of patients as early as 3 weeks after high-dose chemoradiotherapy and marrow infusion. After allogeneic BMT, isolated mononuclear cells stimulated by IL-2 in vitro have been shown to be cytotoxic for recipient leukemia cells in chromium release assays. More importantly, these LAK cells, when incubated with CML cells in vitro, have been shown to mediate cytotoxicity. Overnight incubation with additional rIL-2 (500 U/mL) in vitro had profound effects on the killing of both K562 and COLO targets (Fig 6). The longer the duration of in vivo rIL-2 treatment, the greater the sensitivity to additional rIL-2 in vitro. After discontinuation of rIL-2 therapy, this sensitivity to further in vitro stimulation began to decline.

Comparison of the effects of rIL-2 on allogeneic and autologous marrow recipients. We compared the clinical and immunologic effects of rIL-2 administration in the seven autologous and six CD6-depleted allogeneic marrow recipients who received treatment. Therapy was generally well tolerated by both groups, although the two individuals who developed fever and constitutional symptoms and who
liquid culture, can inhibit leukemia colony growth in colony-forming unit granulocyte-macrophage (CFU-GM) assays. In contrast, these LAK cells do not inhibit colony growth of normal donor marrow CFU-GM. Depletion studies have suggested that the majority of LAK and antileukemic activity generated by IL-2 under these circumstances is derived from cells with immunophenotypic characteristics of NK cells. Indeed, NK clones specifically cytotoxic for cryopreserved recipient leukemic cells can be generated from the peripheral blood of patients shortly after allogeneic BMT.

In this report, we show that low doses of rIL-2 can be continuously administered for prolonged periods to patients who have recently undergone autologous or T-cell-depleted allogeneic BMT. In addition, we have found that such treatment has profound immunologic consequences without producing significant toxicity. All patients treated with rIL-2 at 2 × 10^5 U/m^2/d showed a steady time-dependent increase in the number of NK cells in their peripheral blood. The absolute number of circulating NK cells in these individuals increased 15-fold on average during the 90-day course of treatment. In contrast, no significant change in T-lymphocyte number was observed in the majority of patients during this time. Functionally, we noted an increase in the capacity of mononuclear cells from these patients to destroy NK-sensitive and NK-resistant tumor targets in chromium release assays. Moreover, the sensitivity of these cells to further activation by rIL-2 in vitro was markedly enhanced while patients were receiving rIL-2 in vivo.

The selective expansion of NK cells in response to low-dose rIL-2 is consistent with what is known of the distribution of the different forms of the IL-2 receptor on lymphoid cells. The IL-2 receptor consists of two distinct components, a 55-Kd chain and a 75-Kd chain. Each component can exist independently on the cell surface. The two chains can also interact, forming a p55-p75 heterodimer. This structure binds IL-2 with high affinity and can transmit a proliferative signal to the cell. This heterodimer has been identified on both activated NK cells and activated T cells. Resting T lymphocytes do not express the high-affinity receptor. In contrast, a subset of resting NK cells has been found to express this heterodimer. Indeed, it has been shown that resting NK cells can proliferate in the presence of far lower concentrations of rIL-2 in vitro than can isolated unactivated T cells. It may be that the presence of this high-affinity heterodimer on NK cells accounts for the preferential response to low-dose rIL-2 that we observed in our patients.

Several other clinical trials using IL-2 after BMT have recently been reported. Our study differs from those trials in several important respects. The daily dose of IL-2 that we administered to our patients was generally less than one-tenth that administered to patients in these previous reports. Presumably, as a consequence of this lower daily dose, the toxicities we encountered have been minimal. Specifically, no patients in our series developed hypotension, pulmonary capillary leak syndrome, or azotemia. Only two individuals required temporary interruption of therapy and all treatment could be administered in the outpatient setting. In most cases, treatment could have been continued for longer than 3 months. In contrast to our findings, the side effects noted in previous trials have been substantial and have often precluded prolonged treatment with IL-2. In fact, 3 of 10 patients treated by Blaise et al after BMT became hypotensive and required pressor support. By administering a lower daily dose of IL-2, we have been able to administer treatment for an extended period of time, thus resulting in a progressive increase in NK cell number and activity without significant toxicity. Interestingly, because of the 90-day duration of treatment in our study compared with the 5- to 7-day period in other trials, the total dose of IL-2 administered to our patients surpassed that given in these previous reports.

Another noteworthy feature of our trial is the inclusion of allogeneic transplant recipients in the study population. Previous studies of IL-2 post-BMT have largely involved recipients of autologous marrow only. Fear of inducing significant GVHD has made many investigators understandably wary of treating allogeneic marrow recipients with IL-2. Their concern is predicated on the assumption that IL-2 administration will induce the proliferation of T lymphocytes, the cells felt to be responsible for mediating GVHD. Indeed, Favrot et al reported the development of severe GVHD in an individual administered IL-2 after allogeneic BMT. The absence of GVHD in our series can be explained in part by the selective stimulatory effects of low-dose IL-2 on lymphoid cells. At the dose of IL-2 we used, the majority of patients experienced increases in the number of NK cells without a change in T-cell number. T-cell depletion of allogeneic marrow may also have significant impact with respect to the absence of IL-2-induced GVHD. It has been reported in animal studies that mice administered IL-2 after unmanipulated allogeneic BMT rapidly developed GVHD and died, whereas mice who had received IL-2 after T-depleted BMT survived. Selective T-cell depletion with T12 (CD6) antibody does not target donor NK cells, and NK cells are the first lymphoid population to recover post-BMT. Most patients receiving CD6-depleted marrow (86%) engraft without GVHD and do not require immune suppressive therapies. Whether IL-2 administered at the low dose we used in this trial would have similar immunologic effects and a similar toxicity profile in recipients of unpurged allogeneic marrow as in recipients of CD6-depleted allogeneic marrow remains unclear.

Although low-dose IL-2 did not exert a substantial effect on T lymphocytes in the majority of patients, there were 4 of 13 individuals whose T cells did increase in number as a result of treatment. This stimulatory effect was noted almost exclusively in the CD4 T-cell subset. A measured degree of T-cell activation that does not induce GVHD is likely to be beneficial after BMT. First, T cells, as well as NK cells, may play an important role in the GVL phenomenon. Also, defects in T-cell function have been well documented post-BMT. Specifically, there is a reduction
in the number of CD4⁺ lymphocytes, an impairment in proliferative response to mitogenic stimuli, and a defect in triggering through the CD3 pathway of activation. These functional abnormalities may lead to defects in cell-mediated immunity post-BMT and are likely responsible for the development of viral and other opportunistic infections in this setting. IL-2, by affecting activated T cells, might contribute to host antimicrobial defenses. Indeed, in several instances, rIL-2 administration has been shown to bolster host immunity. In uremic patients with deficient antibody response to hepatitis B vaccination, IL-2 treatment has resulted in an improvement in the immune response to inoculation. Also, patients with lepromatous leprosy administered intradermal rIL-2 have been documented to have regression of lesions. Thus, the benefits of rIL-2 post-BMT may extend beyond a reduction in disease relapse and may address another important problem, that of infectious complications after transplant.

In the present study, we have established that prolonged infusions of low-dose IL-2 are both immunologically active and safe after autologous and CD6 T-cell-depleted allogeneic BMT. Our laboratory data indicate that lymphocytes activated with low-dose IL-2 in vivo are sensitized to further activation with additional IL-2 in vitro. This observation may form the basis of future clinical trials in immune modulation post-BMT. We now plan to initiate further clinical studies to assay the efficacy of this approach in preventing disease relapse after transplantation.

REFERENCES


Clinical and immunologic effects of prolonged infusion of low-dose recombinant interleukin-2 after autologous and T-cell-depleted allogeneic bone marrow transplantation

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