Toxicity and Immunomodulatory Effects of Interleukin-2 After Autologous Bone Marrow Transplantation for Hematologic Malignancies

By Carl M. Higuchi, John A. Thompson, Finn B. Petersen, C. Dean Buckner, and Alexander Fefer

Autologous bone marrow transplantation (ABMT) for advanced hematologic malignancies is associated with high relapse rates. Interleukin-2 (IL-2) and lymphokine-activated killer (LAK) cells represent a potentially non-cross-resistant therapeutic modality that might prevent or delay relapses if used early after ABMT at a time when the tumor burden is minimal. However, high-dose chemoradiotherapy and ABMT might increase patients' susceptibility to IL-2 toxicity, and might interfere with immunologic responses to IL-2 in vivo. Therefore, to determine safety, tolerance, and immunomodulatory effects of IL-2 therapy early after ABMT, IL-2 was administered by continuous intravenous infusion to 16 patients 14 to 91 days (median, 33) after ABMT for acute leukemia, lymphoma, or multiple myeloma. Patients were sequentially assigned to escalating IL-2 “induction” doses (0.3 to 4.5 x 10^6 U/m^2/d, days 1 to 5), and all patients received a nonescalating IL-2 “maintenance” dose (0.3 x 10^6 U/m^2/d, days 12 to 21). Most patients exhibited mild to moderate fever, nausea, diarrhea, and/or skin rash with IL-2 infusions. The maximum tolerated “induction” dose was 3.0 x 10^6 U/m^2/d; dose-limiting toxicities were hypotension and thrombocytopenia. All toxicities reversed on stopping the IL-2 infusions, and all patients completed “maintenance.” Postinfusion lymphocytosis was exhibited by patients at all IL-2 dose levels. With the higher IL-2 doses, increased percentages of patients’ PBMC expressed CD16 and CD56, with augmented lysis of K562 and Daudi, reflecting the induction of natural killer and circulating LAK effector activities. Increased LAK precursor activity was exhibited by patients at all IL-2 dose levels. Thus, the IL-2 therapy regimen was safely tolerated after ABMT, and pronounced immunomodulatory effects were observed with the higher IL-2 doses. These studies support the planned use of IL-2 and LAK cells after ABMT in an attempt to reduce relapses of advanced hematologic malignancies.

MATERIALS AND METHODS

Patient selection. Sixteen patients, ages 15 to 59 (median, 33) who had undergone ABMT for histologically or cytologically documented acute non-lymphoblastic leukemia (ANL; n = 8), non-Hodgkin’s lymphoma (NHL; n = 3), Hodgkin’s disease (HD: n = 4), or multiple myeloma (MM; n = 1) entered study at a median of 33 days (range, 14 to 91) after ABMT (Table 1). Patients had had a variety of pretransplant conditioning and marrow purging regimens, and all had recovered from ABMT-associated acute toxicities with Karnofsky performance scores of ≥70. They were not receiving corticosteroids or other immunosuppressive therapy, and had trilineage marrow engraftment documented by marrow aspiration. Eligibility requirements included: adequate BM function (neutrophils, ≥1,000/mL; platelets >20,000/mL with or without transfusion support); adequate renal function (creati-
Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>UPN</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>IL-2 Onset (post-ABMT day)</th>
<th>Karnofsky Score</th>
<th>Conditioning Protocol*</th>
<th>Marrow Purging</th>
<th>ABMT Outcome (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>U2746</td>
<td>44/F</td>
<td>ANL, 1st rel</td>
<td>91</td>
<td>90</td>
<td>4</td>
<td>4HC</td>
<td>Remission (23+)</td>
</tr>
<tr>
<td></td>
<td>F4726</td>
<td>26/M</td>
<td>ANL, 1st rel</td>
<td>58</td>
<td>80</td>
<td>3</td>
<td>aB</td>
<td>Relapsed (10)</td>
</tr>
<tr>
<td></td>
<td>U8692</td>
<td>39/M</td>
<td>NHL, 2nd rel</td>
<td>35</td>
<td>90</td>
<td>1</td>
<td>aB</td>
<td>Relapsed (10)</td>
</tr>
<tr>
<td></td>
<td>F5248</td>
<td>20/F</td>
<td>ANL, 2nd rem</td>
<td>31</td>
<td>80</td>
<td>4</td>
<td>—</td>
<td>Remission (12+)</td>
</tr>
<tr>
<td></td>
<td>F4169</td>
<td>35/M</td>
<td>MM, refractory</td>
<td>21</td>
<td>80</td>
<td>3</td>
<td>aB</td>
<td>Remission (10+)</td>
</tr>
<tr>
<td>II</td>
<td>U8644</td>
<td>24/F</td>
<td>NHL, refractory</td>
<td>49</td>
<td>80</td>
<td>5</td>
<td>aB</td>
<td>Relapsed (4)</td>
</tr>
<tr>
<td></td>
<td>F5188</td>
<td>59/F</td>
<td>ANL, 2nd rel</td>
<td>66</td>
<td>70</td>
<td>2</td>
<td>—</td>
<td>Relapsed, died (4)</td>
</tr>
<tr>
<td></td>
<td>F5196</td>
<td>40/F</td>
<td>HD, 1st rel</td>
<td>58</td>
<td>90</td>
<td>1</td>
<td>—</td>
<td>Remission (11+)</td>
</tr>
<tr>
<td></td>
<td>F3663</td>
<td>40/F</td>
<td>HD, 1st rel</td>
<td>19</td>
<td>80</td>
<td>1</td>
<td>—</td>
<td>Remission (9+)</td>
</tr>
<tr>
<td>III</td>
<td>F4144</td>
<td>34/F</td>
<td>ANL, 1st rel</td>
<td>23</td>
<td>70</td>
<td>4</td>
<td>4HC</td>
<td>Remission (8+)</td>
</tr>
<tr>
<td></td>
<td>U1377</td>
<td>32/M</td>
<td>NHL, refractory</td>
<td>17</td>
<td>80</td>
<td>6</td>
<td>—</td>
<td>Relapsed (3)</td>
</tr>
<tr>
<td></td>
<td>F4989</td>
<td>15/F</td>
<td>ANL, 1st rem</td>
<td>60</td>
<td>80</td>
<td>4</td>
<td>—</td>
<td>Remission (9+)</td>
</tr>
<tr>
<td></td>
<td>F5301</td>
<td>38/M</td>
<td>ANL, 1st rel</td>
<td>54</td>
<td>80</td>
<td>3</td>
<td>4HC</td>
<td>Remission (8+)</td>
</tr>
<tr>
<td></td>
<td>F4810</td>
<td>30/M</td>
<td>ANL, 1st rel</td>
<td>21</td>
<td>80</td>
<td>2</td>
<td>4HC</td>
<td>Remission (7+)</td>
</tr>
<tr>
<td>IV</td>
<td>F5251</td>
<td>30/F</td>
<td>HD, 4th rel</td>
<td>27</td>
<td>70</td>
<td>6</td>
<td>—</td>
<td>Relapsed (5)</td>
</tr>
<tr>
<td></td>
<td>F4888</td>
<td>33/F</td>
<td>HD, 2nd rel</td>
<td>14</td>
<td>90</td>
<td>5</td>
<td>—</td>
<td>Relapsed (3)</td>
</tr>
</tbody>
</table>

Abbreviations: UPN, unique patient number; ANL, acute nonlymphoblastic leukemia (n = 8); NHL, non-Hodgkin’s lymphoma (n = 4); MM, multiple myeloma (n = 1); HD, Hodgkin’s disease (n = 4); rel, relapse; rem, remission; aB, monoclonal anti-P-cell antibody and complement; 4HC, 4-hydroxy-cyclophosphamide.

*Protocol 1, cyclophosphamide (60 mg/kg/d) x 2d, TBI (200 cGy/d) x 6d; protocol 2, busulfan (4 mg/kg/d) x 4d, cyclophosphamide (60 mg/kg/d) x 2d, protocol 3, busulfan (4 mg/kg/d) x 4d, cyclophosphamide (60 mg/kg/d) x 2d, protocol 4, busulfan (2 mg/kg/d) x 4d, cyclophosphamide (30 mg/kg/d) x 2d, TBI (200 cGy/d) x 6d; protocol 5, etoposide (2400 mg/m²/d) + bischloroethylnitrosourea, TBI (200 cGy/d) x 6d; protocol 6, etoposide (45 mg/kg/d) x 4d, cyclophosphamide (25 mg/kg/d) x 2d, TBI (200 cGy/d) x 6d.

Table 2. Study Design: IL-2 Dosage Schedule

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients</th>
<th>IL-2 &quot;Induction&quot; (d1-5)</th>
<th>IL-2 &quot;Maintenance&quot; (d12-21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1-5</td>
<td>0.3 x 10⁶ U/m²/d</td>
<td>0.3 x 10⁶ U/m²/d</td>
</tr>
<tr>
<td>II</td>
<td>6-9</td>
<td>1.0 x 10⁶ U/m²/d</td>
<td>0.3 x 10⁶ U/m²/d</td>
</tr>
<tr>
<td>III</td>
<td>10-14</td>
<td>3.0 x 10⁶ U/m²/d</td>
<td>0.3 x 10⁶ U/m²/d</td>
</tr>
<tr>
<td>IV</td>
<td>15-16</td>
<td>4.5 x 10⁶ U/m²/d</td>
<td>0.3 x 10⁶ U/m²/d</td>
</tr>
</tbody>
</table>

Recombinant IL-2. Recombinant human IL-2 (provided by Hoffmann-LaRoche, Inc, Nutley, NJ) has a specific activity of 1.2 to 1.5 x 10⁶ BRMP U/mg of protein. IL-2 was supplied as a lyophilized powder containing 100 mg of IL-2, with 25 mg of human serum albumin/10⁶ units of IL-2, and 5 mg of mannitol/10⁶ units of IL-2. The powder was reconstituted in sterile saline for injection.

Immunologic monitoring. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by fractionation on a Ficoll-Hypaque gradient (Pharmacia, Piscataway, NJ). Immunologic monitoring, performed on all patients, consisted of the flow cytometric analysis of a patient’s lymphocyte phenotype using a panel of monoclonal antibodies (MoAbs), and the assessment of natural killer (NK) and LAK cytotoxicity. Patients were studied before IL-2 therapy, 24 hours after the 5-day induction course of IL-2 (usually Day 7), and 24 hours after the maintenance course of IL-2 (usually Day 23).

Lymphocyte phenotype determination. PBMC were incubated with fluoro-chrome-conjugated MoAbs for 30 minutes at 4°C. After being washed twice, the stained cells were analyzed using the Ortho Cytofluorograf-50 HH (Orthodiagnostic Systems, Westwood, MA). Lymphocyte gates were set based on forward and horizontal light scatter, and the analysis was performed using 488-nm blue light from a Model 164-05 argon laser (Spectra Physics, Mountain View, CA). MoAbs were purchased commercially (Becton Dickinson, Mountain View, CA) and included: anti-Leu-4 (anti-CD3) for human T cells; anti-Leu-3a (anti-CD4) for helper/inducer T cells; anti-Leu-2a (anti-CD8) for suppressor/cytotoxic T cells; anti-IL-2 receptor (anti-CD25); anti-Leu-11a (anti-CD16) for Fe IgG receptor on NK cells and neutrophils; and anti-Leu-19 (anti-CD56) for NK cells and cytotoxic T-cell subsets.

NK cytotoxicity. Freshly isolated PBMC from patients were tested for cytotoxicity against NK-sensitive K562 targets with a 4-hour ⁵¹Cr-release assay, using various E:T ratios (0.8 to 100:1). The target cells were labeled with 0.2 mCi ⁵¹Cr/10⁶ cells (sodium chromate; New England Nuclear, Boston, MA) for 1 hour at 37°C. Effector cells and labeled target cells (10⁴/well) were cocultured in
triplcate, at a final volume of 200 mL in U-bottomed microtiter wells for 4 hours at 37°C in a humidified 5% CO₂ atmosphere. Percentage lysis was determined according to the formula:

\[
\% \text{ of lysis} = \frac{\text{Experimental release} - \text{spontaneous release}}{\text{Maximal release} - \text{spontaneous release}} \times 100\%
\]

Spontaneous release was less than 10%, and the maximum release was determined by resuspension of 10⁵ target cells into the assay supernatant. Data are presented in LU per 10⁵ effector cells; 1 LU was defined as the number of effector cells required to lyse 33% of the target cells.

LAK effector (LAKe) and LAK precursor (LAKp) activities. LAKe was defined by the ability of the patient’s freshly isolated PBMC to lyse NK-resistant Daudi targets in a 4-hour ⁵¹Cr-release assay as described above. LAKp was defined by the ability of the patient’s PBMC to lyse Daudi targets after 5-day incubation with 1,000 U/mL IL-2.

Statistical analysis. Paired two-tailed Student’s t-test was used to compare mean cell counts and mean percentages of lymphocytes expressing particular phenotypic determinants. Median lytic activities (LU/10⁷) were compared using the Mann-Whitney U-test.

RESULTS

Clinical toxicity. All 16 patients entered on study were evaluable for toxicity. One of five patients in group I withdrew from study for personal reasons after completing IL-2 “induction,” and did not receive the IL-2 “maintenance.” All other patients completed treatment; however, one patient in group III and both patients in group IV required interruption of IL-2 “induction” after 3 to 4 days due to grade III toxicity. Two patients missed 1 to 2 days of IL-2 “maintenance” due to the malfunction of the IL-2 infusion pump or logistic problems.

The clinical toxicity is summarized in Table 3. The most frequent toxicities were fever, nausea, diarrhea, and skin rash. High-grade fever (>39°C) occurred universally at the higher IL-2 doses, generally beginning within 24 hours of initiating IL-2 therapy and subsiding within 24 hours after terminating the IL-2 infusions. Nausea and diarrhea occurred at all dose levels, but was usually relieved with anti-emetic or anti-diarrheal therapy. The skin rash usually consisted of a bright erythema beginning on the second or third day of IL-2 therapy, which was sometimes followed by a dry desquamation. Oliguria or azotemia (creatinine >2 times normal) did not occur, although mild weight gain (<10% of the pretreatment body weight) was exhibited by two patients. Three patients experienced subjective dyspnea without associated hypoxemia, and two patients developed documented infections during treatment—one with fever and bacteremia and the other with bacteriemia; both resolved with appropriate antibiotic therapy. All toxicities were fully reversible after termination of the IL-2, and no hepatic or cardiac dysfunction was noted.

Dose-limiting toxicities included thrombocytopenia and hypotension. One patient in group III and one in group IV exhibited severe thrombocytopenia during IL-2 “induction” that required termination of the infusions. The first patient experienced prompt resolution of the thrombocytopenia, but the second patient experienced gastrointestinal bleeding requiring 5 units of transfused blood. Two of two patients in group IV exhibited significant hypotension (40 to 60 mm Hg below baseline systolic) requiring discontinuation of the IL-2 “induction” after 3.5 to 4 days. The hypotension promptly resolved after termination of the IL-2 infusions. The patient in group IV who experienced both severe thrombocytopenia and hypotension also exhibited persistent fevers and culture-negative bilateral interstitial pulmonary infiltrates despite the termination of IL-2, suggesting an infectious etiology. The fevers and infiltrates slowly resolved over 1 week with empiric antibiotic therapy.

The IL-2 “maintenance” dose was well tolerated in all patients, even among patients who had experienced dose-limiting toxicity requiring interruption of the IL-2 “induction.” Toxicities were limited to mild to moderate nausea, diarrhea, fever, and skin rash. Seven patients have relapsed 3 to 10 months after ABMT, and nine patients remain alive and in remission 7 to 23+ months after ABMT (Table 1).

Hematologic effects. The majority of patients required blood product transfusions before, during, and after IL-2 therapy. Among patients requiring platelet transfusions, the frequency of transfusions increased during IL-2 administration, but all patients became independent of platelet transfusions after termination of the IL-2 therapy. Significant increases in neutrophils were noted in group III, from a pretreatment mean of 1,140/mL to 2,010/mL on d7 (P < .05) and to 3,070/mL on d23 (P < .05). The effects of IL-2 therapy on the neutrophil counts are summarized in Fig 1. Clinically significant neutropenia (<500/mL) was not induced in any patient at any time. Indeed, mean neutrophil counts tended to increase throughout the course of IL-2 therapy. Significant increases in neutrophils were noted in group III, from a pretreatment mean of 1,140/mL to 2,010/mL on d7 (P < .05) and to 3,070/mL on d23 (P < .05).

Effects on circulating lymphocyte subsets. The effects of IL-2 therapy on the total lymphocyte counts are summarized in Fig 2. A pronounced lymphopenia was exhibited by all three treatment groups within 48 hours of initiating IL-2 therapy: lymphocyte counts decreased from a pretreatment mean of 1,010/mL to 360/mL (P < .05), from 860/mL to 480/mL (P < .05), and from 620/mL to 190/mL (P < .01)
on d3 of treatment in groups I, II, and III, respectively. All groups exhibited a "rebound" lymphocytosis 24 hours after termination of IL-2 "induction" (d7). Group I exhibited a small, but significant increase in mean lymphocyte counts on d7 (1,790/mL, \(P < .05\)), whereas the lymphocytosis on d7 was more dramatic in group II (6,880/mL, \(P < .01\)) and in group III (5,630/mL, \(P < .05\)). Mean lymphocyte counts subsequently decreased, but remained elevated above baseline pretreatment values throughout IL-2 "maintenance" in all groups.

The lymphocytes were analyzed for surface phenotype immediately before IL-2 treatment (d0), and at 24 hours after IL-2 "induction" (d7) to coincide with the time of maximal lymphocytosis. Table 4 summarizes the effects of IL-2 therapy on circulating T-lymphocyte subsets. Increases in CD3+ T cells were noted in all treatment groups, with the most prominent increases noted in CD8+ lymphocytes in groups II and III. Increases in lymphocytes expressing CD4 and CD25 (IL-2 receptor) were also noted.

Figure 3 illustrates the effect of IL-2 "induction" on percentage of circulating lymphocytes expressing CD16 and CD56, determinants associated with NK and LAK activities. Whereas the mean percentage of cells expressing CD16 (Fig 3, upper panel) was unchanged in groups I and II, group III exhibited a significant increase in CD16+ cells (9% on d0 v 36% on d7, \(P < .05\)). Group III also exhibited a significant increase in mean percentage of cells expressing CD56 (Fig 3, lower panel, 26% on d0 v 54% on d7, \(P < .05\)). Group I, receiving the lowest IL-2 dose, exhibited a small, but significant increase in CD56+ cells (14% on d0 v 28% on d7, \(P < .05\)).

Phenotypic analyses performed immediately before IL-2 "maintenance" (d12), and 24 hours after completion of IL-2 "maintenance" (d23) did not show further significant alterations in the phenotypic profiles of circulating lymphocyte subsets. No statistically significant differences were noted between patient groups.

DISCUSSION

Major approaches to decreasing the relapse rate after ABMT for advanced hematologic malignancies involve modifications of pretransplant chemotherapy or chemoradiotherapy conditioning regimens, and the development of techniques for purging the autologous marrow of tumor cells. Limitations to the former approach include the inability of patients to withstand the more intensive therapy due to non-marrow toxicity and the persistence of chemoradiotherapy-resistant malignant cells. Recent evidence that tumor cell lines that are resistant to chemotherapeutic agents in vitro remain susceptible to LAK-mediated lysis support the hypothesis of non-cross-resistance between chemoradiotherapy and IL-2 and LAK therapy. Thus, IL-2 and LAK therapy could potentially eradicate the residual malignancy after ABMT, and might obviate the need for...
IL-2 AFTER AUTOLOGOUS BMT

Table 4. Effect of IL-2 “Induction” on Circulating T-Lymphocyte Subsets

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-IL-2 (d0)</th>
<th>24 h post-IL-2 (d7)</th>
<th>24 h post-IL-2 (d7)</th>
<th>24 h post-IL-2 (d7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.60 ± 0.17</td>
<td>0.99 ± 0.25*</td>
<td>4.98 ± 1.73*</td>
<td>4.40 ± 0.10</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td>24 h post-IL-2 (d7)</td>
<td>0.57 ± 0.28</td>
<td>2.45 ± 1.69</td>
</tr>
<tr>
<td>Group II</td>
<td>0.25 ± 0.05</td>
<td>0.41 ± 0.04*</td>
<td>1.51 ± 0.71</td>
<td>0.96 ± 0.80</td>
</tr>
<tr>
<td>Group III</td>
<td>0.32 ± 0.12</td>
<td>0.65 ± 0.26</td>
<td>3.86 ± 1.16*</td>
<td>2.94 ± 1.00*</td>
</tr>
<tr>
<td>(n = 4)</td>
<td></td>
<td></td>
<td>0.01 ± 0.01</td>
<td>0.31 ± 0.11*</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significantly greater than pre-IL-2 value, P < .05.

marrow purging by eradicate reinfused clonogenic malignant cells. This study was initiated to determine the tolerance to, safety of, and immunomodulatory effects of IL-2 when administered after ABMT. The IL-2 was administered as early as possible after documentation of trilineage marrow engraftment to provide the consolidative therapy in a setting of minimal residual disease.

The design of the IL-2 regimen was based on the anticipated design of future regimens involving IL-2 plus LAK cells. Immunologic responses to IL-2 and LAK therapy are related to the IL-2 dose and to the mode of IL-2 administration.2n.2' Highest degrees of rebound lymphocytosis and the greatest augmentation of NK, LAKc, and LAKp activities are observed with higher IL-2 doses administered by CIV.20 LAK cell yields are maximized by timing the leukapheresis to coincide with the peak of rebound lymphocytosis, which usually occurs 24 hours after the completion of IL-2 “induction.” Therefore, in this trial the IL-2 “induction” dose was escalated between patient groups to define the maximum tolerated dose and the optimum dose for inducing immunomodulatory effects. The 6-day interval between IL-2 “induction” and “maintenance” was provided to allow time for recovery from toxicities of the IL-2 “induction,” and to provide the necessary time interval for potential leukapheresis for exogenous LAK cell generation. The IL-2 “maintenance” dose was not escalated on the presumption that lower doses of IL-2 would be sufficient for maintaining the function of infused and/or endogenously activated cytotoxic cells, and to ensure that patients could safely tolerate the prolonged treatment regimen.

The clinical toxicities observed among the ABMT recipients paralleled the spectrum of toxicities commonly observed in untransplanted solid tumor patients.8-10,23-25 Fever, nausea, diarrhea, and rash were common, but were not dose-limiting. Although IL-2-induced fluid retention and...
weight gain were not a significant problems among the ABMT recipients, hypotension was dose-limiting. Because the clinical features of IL-2-associated capillary-leak syndrome overlap with those of venoocclusive disease of the liver, dose-limiting hemodynamic toxicities were not unexpected. Dose-limiting hypotension has been observed by Gottlieb et al and by Blaise et al, who have treated other groups of patients with different ABMT and IL-2 regimens.

Hematologic effects of IL-2 were of particular concern among the ABMT recipients because IL-2 can suppress growth of BM progenitors in vitro, and because anemia and thrombocytopenia are induced among solid tumor patients receiving IL-2 therapy. Although thrombocytopenia did prove to be dose-limiting, the IL-2 did not significantly exacerbate anemia or induce clinically significant neutropenia. Most patients were safely supported through the IL-2, particularly at higher IL-2 dose levels. Although altered neutrophil function and an increased incidence of infections have been associated with IL-2 and LAK therapy, only two patients in this trial developed documented bacterial infections. Most significantly, all toxicities, including dose-limiting hypotension and thrombocytopenia, reversed completely with the termination of the IL-2 infusions. Thus, despite potentially additive or cumulative toxicity, the combination of high-dose chemoradiotherapy and ABMT followed by moderately high doses of systemic IL-2 was safely tolerated by patients with advanced hematologic malignancies.

Immunologic competence after ABMT, particularly with respect to IL-2 responsiveness, is likely to be a critical factor in determining the efficacy of posttransplant IL-2 therapy. Although abnormalities of immune function may persist for years after ABMT, NK cells and IL-2-responsive LAK precursor cells are rapidly reconstituted, and can be isolated from the peripheral circulation within the first few weeks after ABMT. The current study demonstrates that IL-2 therapy can result in significant immunomodulation during the period of early immunologic reconstitution after ABMT in a fashion comparable with that observed among untransplanted solid tumor patients. The IL-2 “induction” resulted in a rapid, transient lymphopenia within the first 48 hours of therapy, followed by a rebound lymphocytosis 24 hours after completing the 5-day infusion. This effect was particularly prominent at the higher IL-2 doses, with the most dramatic increases noted in the CD8+ T-cell subset.

Circulating cytotoxic effector activities were augmented by the higher IL-2 “induction” doses. The maximally tolerated IL-2 dose induced increases in both NK and LAKp activities that paralleled increases in the mean percentages of circulating lymphocytes bearing the NK-associated CD16 determinant and in the LAK-associated CD56 determinant. Such an effect was less apparent with lower IL-2 “induction” doses. Consistent with our previously reported observations, CD56 expression was uniformly high among the ABMT recipients. By contrast, CD16 expression was discordantly low, particularly among patients treated with the lower IL-2 doses, implying that CD16+CD56+ cells comprised a significant proportion of the circulating lymphocyte pool. Interestingly, patients treated with an intermediate IL-2 “induction” dose (group II) exhibited increased LAKp activity without increased NK activity and without significant increases of CD16 or CD56 expression. These observations suggest that the NK and LAKp activities are mediated by distinct cell populations with differential IL-2 sensitivities, and that CD16 expression is prerequisite to the augmentation of NK activity by IL-2.

Despite the augmentation of NK and LAKp activities with the higher IL-2 “induction” doses, the cytotoxicity diminished during the 6-day rest interval, and remained at baseline pretreatment levels throughout IL-2 “maintenance.” The failure to detect an immunomodulatory effect of the “maintenance” is probably due to the low IL-2 dose used, because the same daily dose did not result in augmented cytotoxic function when used as “induction.” It remains possible that low-dose IL-2 “maintenance” may be
sufficient to support the cytotoxic activities of LAK cells generated by ex vivo culture, and such an approach is now under investigation. However, a higher, more toxic “maintenance” dose may ultimately be necessary for effective post-transplant IL-2 and LAK cell therapy.

The ABMT recipients exhibited maximal lymphocytosis 24 hours after the termination of IL-2 “induction” and LAKp activity was found to be coincidentally increased in the majority of patients tested. Although LAKp activity did not differ between patient groups, leukapheresis and LAK cell yields can be expected to be greater with higher IL-2 “induction” doses. Although the role of LAK cells in human cancer therapy has not yet been established, murine studies suggest that LAK cell infusions increase the therapeutic potential of IL-2 therapy, and complete responses have been observed more frequently among renal cell carcinoma patients treated with IL-2 plus LAK than among those treated with IL-2 alone. The feasibility of high-dose IL-2 plus LAK cell generation and infusions after ABMT is therefore being presently explored.

Similar phase I trials of IL-2 using different IL-2 preparations administered by different schedules to different ABMT patient populations have been recently reported by two other groups. Gottlieb et al used escalating doses of IL-2 (Glaxo, Geneva, Switzerland) administered over 3 to 5 days by bolus infusion or by continuous infusion 1 to 4 weeks after ablative chemotherapy or chemotherapy and ABMT for acute leukemia or myeloma. Blaise et al used a single dose of IL-2 (EuroCetus, Amsterdam, The Netherlands) administered by 6-day continuous infusion 7 to 14 weeks after ABMT for various hematologic and non-hematologic malignancies. The present study differs in using escalating fixed doses of IL-2 (Hoffmann-LaRoche) administered 2 to 13 weeks after ABMT by 5-day continuous infusion, followed by a non-escalating low-dose maintenance infusion over 10 additional days. Each study has independently concluded that IL-2 can be administered safely after ABMT with demonstrable immunomodulatory effects; however, only small numbers of heterogenous patients have thus far been studied. Carefully designed larger scale studies are necessary to document the effects of disease status, prior treatment, ABMT regimen, marrow purging, and autologous LAK cell infusions on toxicity, immunomodulation, and the clinical response to IL-2 in this setting.

ACKNOWLEDGMENT

The authors thank B. Bolonesi, L. Benz, and K. Beach for their help with study coordination and data management, C. Lindgren, K. Kovacs, M.J. Schreifels, and J.R. Hill for their expert technical assistance, and Hoffmann-LaRoche, Inc for generously providing the IL-2.

REFERENCES


Toxicity and immunomodulatory effects of interleukin-2 after autologous bone marrow transplantation for hematologic malignancies

CM Higuchi, JA Thompson, FB Petersen, CD Buckner and A Fefer