Hematologic and Immunologic Effects of the Systemic Administration of Recombinant Interleukin-2 After Autologous Bone Marrow Transplantation


T cells from allogeneic bone marrow grafts are responsible for a graft versus leukemia effect. Use of recombinant Interleukin-2 (rIL-2) after autologous bone marrow transplantation (BMT) may enhance immune function and hopefully reproduce the allogeneic reaction. We report here the hematologic and immunologic changes observed in the first 10 patients of a phase 1 trial studying the infusion of IL-2 after autologous BMT. All patients had high-risk malignancies and received 6 days of a constant infusion of IL-2 (Eurocetus, Amsterdam, The Netherlands) at dose of 3 x 10^6 Cetus Units/m^2/d, 79 ± 12 days after autologous BMT. Clinical toxicities involving cutaneous, cholestatic, gastrointestinal, and hemodynamic effects occurred during IL-2 treatment but reversed in all cases. Completion of treatment was 91% of the scheduled dose of IL-2. Hematologic toxicity was moderate and transient with no graft failure. Increases in eosinophil and lymphocyte counts were significant (P < .05). Stimulation of the immune system was intense and prolonged, manifested by increase numbers of CD3+, CD3+ DR+, CD3+ CD25+ lymphocytes, and natural killer (NK) cells (all P < .01), and increase of Lymphokine-activated killers (LAK) and NK activities (P < .01 and P < .05). This study establishes the feasibility of a 6-day administration of rIL-2 after autologous BMT leading to a major immune activation 2.5 months after BMT.

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THE CURATIVE role of allogeneic bone marrow transplantation (BMT) in hematologic malignancies is now well established. Curability of the malignant disease is dependent on ablution of the tumor by an intensive conditioning regimen as well as from an "adoptive immunotherapy" for which donor cells are responsible and defined as the graft versus leukemia (GVL) reaction. If present, this reaction is far less significant after syngeneic or autologous BMT, leading to a high relapse rate under these conditions when compared with similar patients after allogeneic BMT. Furthermore, in the allogeneic setting the depletion of T cells from the donor's marrow abolishes the graft versus host disease (GVHD) but greatly increases the risk of relapse. This is probably due to the impairment of the GVL effect as T cells are considered major effectors of this reaction.

Interleukin-2 (IL-2) is a major growth factor of T lymphocytes and, following pharmacologic doses, has been shown to induce the regression of established cancers in animal models and in some patients with metastasis. Use of high doses of IL-2 after autologous BMT may activate T-cell reconstitution, known to be impaired in this setting, and hopefully induce a graft versus tumor reaction. We report here the hematologic and immunologic changes observed after a 6-day infusion of IL-2 2 months after autologous BMT, showing the feasibility of this approach and resulting in a major immune activation in vivo.

PATIENTS AND METHODS

Patients and transplant procedures. Ten consecutive patients with high-risk malignancies, eligible for our current autologous transplantation programs, were considered for this trial. All patients had normal brain computed tomographic (CT) scans, normal cardiac stress tests, and no hypoxemia at rest. Eastern Cooperative Oncologic Group (ECOG) performance status, assessed before BMT and IL-2 therapy, had to be between 0 and 1 in both cases.

Bone marrow was harvested at a time of complete marrow remission and was frozen after an in vitro purging in two patients (unique patient no. [UPN] = 26; UPN = 35) and without any purging in eight patients. One third of the marrow was kept as backup. Two thirds of the marrow was reinfused after various conditioning regimens: Cy-TBI consisting of cyclophosphamide (120 mg/kg) and fractioned total body irradiation (four patients); Cy-LPAM: cyclophosphamide (120 mg/kg) and L-phenylalanine mustard (melphalan) (140 mg/m^2) (three patients); BEAM: busulfan (16 mg/kg in 4 days) followed by melphalan (800 mg/m^2 in 4 days), and melphalan (140 mg/m^2) (two patients); BU-LPAM: busulfan (16 mg/kg in 4 days) followed by melphalan (140 mg/m^2). All patients had a double-lumen catheter (Quinton Instrument Company, Seattle, WA) inserted before BMT. No chemotherapy or corticosteroids were administered between BMT and IL-2 therapy.

According to protocol, patients involved in this trial were not treated before day 60. After day 60, if hematologic recovery was achieved, defined as granulocytes superior to 1 x 10^9/L and platelets superior to 100 x 10^9/L, patients were readmitted to receive IL-2 in a conventional ward.

IL-2 therapy. rIL-2 (Proleukin R, generously provided by Eurocetus, Amsterdam, The Netherlands) was delivered as a 24-hour constant infusion at 3 x 10^6 Cetus Units/m^2/d (corresponding to 18 x 10^6 IU/m^2/d) for 6 consecutive days. Patients were medicated, if required, to improve symptoms during recombinant IL-2 (rIL-2) therapy; fever was prevented with acetaminophen and indomethacin. Rigors were treated with meperidine, nausea with chlorpromazine, diarrhea with loperamide, and pruritis with dexochloperx. All patients received prophylactic antibiotics: pefloxacin and G penicillin; no systemic extra fluid was administered and furosemide was given, if needed, to maintain fluid balance. Vital signs were checked every 4 hours. Toxicity was graded according to the World Health Organization (WHO) scale. Weight toxicity was graded as...
follows: grade 1, 0% to 2.5% of weight increase; grade 2, 2.6% to 5%; grade 3, 5.1% to 10%; grade 4, above 10%. Proleukin R was interrupted when a grade 3 or several simultaneous grade 2 toxicities occurred, until completely resolved. IL-2 could be started again with a 50% dose decrease until it was clear that toxicity did not recur. Patients were discharged when all toxicities had been removed.

Protocol was reviewed and accepted by the Ethical Committee of the University of Medicine of Aix, Marseille, France. The whole treatment was explained in detail and informed consent was obtained from all patients or legal guardians. Analysis was conducted on October 1, 1989.

Biological evaluations. Patients had standard blood chemical and hematologic counts checked on a daily basis from the day before to the day after the infusion. During the following weeks, labs were done twice a week.

Immunologic monitoring was performed the first day before rIL-2 administration (day 0), then repeated on days 8, 14, and 28, consisting of two analyses.

1. Cell surface phenotyping was done by direct two color immunofluorescence analysis (ACT 3000, Odam, Brucker, Wissembourg, France) using several antibodies: IOT3 (CD3), IOT29 (monomorphic anti-HLA class II), IOT4 (CD4), IOT8 (CD8), IOT149 (CD25) were all from ImmunoTech, Marseille, France; NKH1 (anti-CD56, from Coulter, Hialeh, FL). Natural Killer (NK) cells were defined as CD3- and NKH1+ cells.

2. NK activity was assayed using a chromium 51 (CR51) release assay using the NK-sensitive cell line K562 as target. Lymphokine-activated killers (LAK) activity was similarly measured using the NK-resistant, LAK-sensitive Daupl cell line. Specific cytolytic values are expressed for an effector/target (E/T) cell ratio of 50:1.

Statistical evaluation. Biological values were compared with pre-rIL-2 values using Wilcoxon's matched pairs signed rank test.

RESULTS

Only two patients with residual disease could be evaluated for antitumoral response to rIL-2. Both had stable disease during and at least 1 month after IL-2. Outcome is displayed in Fig 1.

Clinical toxicity. On average rIL-2 was started on day 79 (± 12 days) after autologous BMT. Usual side effects occurred: consistent toxicities (grade ≥ 2) are listed in Table 1. Skin maculopopular erythema was constant and was extensive enough to mimic acute GVHD in five cases. Face, trunk, and legs were predominantly involved with occasional extension to flexural creases and ears. Puritis and cutaneous desquamation occurred in all cases. In two cases severe oral mucositis and conjunctivitis were seen. Skin biopsy was performed in only two cases and results were compatible with the pathologic diagnosis of acute GVHD (one grade I, one grade II). The first biopsy showed an epidermal basal cell vacuolization and a slight mononuclear cell infiltrate in the epidermis and around the dermal venules. The second one was characterized by a more intensive vacuolization with necrotic cells in the basal and suprabasal layers of the epidermis. The inflammatory infiltrate was more pronounced than in the usual GVHD in both dermis and epidermis where lymphocytes formed aggregates around necrotic cells. More dyskeratotic cells were found in the second sample than in the first one. No eosinophils were found in the inflammatory infiltrate of both cases.

Mean weight gain was 4% ± 2% despite fluid restriction and frequent use of diuretics. Drop of blood pressure (BP) under 85 mm Hg, which did not respond to albumin infusion, occurred in three cases and needed use of 3.5 to 5 γ/kg of dopamine; for two of these patients BP remained unstable during 24 hours and they did not receive more IL-2. One gram-positive bacteriemia was documented in one patient (UPN = 46). No major cardiac or neurologic toxicities occurred. Toxicities were generally consistent during the last 2 days of the cycle, and always reversed promptly after discontinuation of rIL-2. No toxic deaths occurred during or after rIL-2 treatment.

Hematologic and immunologic changes. Hematologic recovery was achieved for all patients before the onset of IL-2 and all patients were independent from platelet transfusions for at least 3 weeks (Table 2).

Variation of hematologic parameters was significant during IL-2 therapy compared with pretreatment values. In some cases a decrease in counts was even major: two patients had less than 1.5 × 109/L granulocytes (0.7 and 1.5 × 109/L) and four patients had less than 50 × 109/L platelets (29, 36, 43, 48 × 109/L). In no case was a decrease in granulocytes or a decrease in platelets a cause of bacteriemia or bleeding. Furthermore, no patient was platelet transfused. For all patients, hemoglobin, platelet, and neutrophil counts stopped decreasing as soon as rIL-2 was discontinued. Maximum levels of eosinophilia occurred between days 8 and 14 (mean 1.9 × 109/L; data not shown in Table 2). For all patients, blood counts returned to pre–rIL-2 levels before day 28, lymphocyte and leukocyte counts being the last to normalize.

Lymphocyte subsets were abnormal before rIL-2 therapy in most patients with a decrease of CD4/CD8 ratio. Occasional high levels of NK cells and spontaneous NK and LAK activities following autologous BMT were noted as previously described by many investigators.17,22,28 After rIL-2 therapy several changes occurred in the lymphocyte subsets. The increase in CD3+ lymphocyte (P < .01) was related to activated T lymphocytes expressing HLA class II molecules (P < .01) or the IL-2 receptor (P < .01). CD4/CD8 ratio was further decreased (P = NS) during rIL-2 therapy followed by a progressive return to pretherapy values. Increase in NK cells (P < .01) remained longer than for other subsets such as CD25-positive cells. NK and LAK activities were both increased (P < .05 and P < .01), but the increase was more prolonged for NK activity while LAK activity decreased soon after therapy. Table 2 shows the range of immunologic response and individual data are presented in Fig 1. In all patients CD3+ lymphocytes and NK cells increased by at least four times. Identically NK and LAK activities were increased in most cases. However, in two cases (UPN 27, UPN 28) LAK and NK activities were undetectable both before and after IL-2 therapy.

Finally, all hematologic and immunologic changes returned to baseline values 3 weeks after IL-2.

DISCUSSION

First, this study established the clinical feasibility of a 6-day administration of high doses of rIL-2 80 days after autologous BMT. According to protocol no rIL-2 was begun
Fig 1. Individual immunologic variations. Data on days 0 (before therapy), 8, 14, and 28 are presented for nine patients. Data from one patient (UPN 35) are not available.
before day 60 to avoid major clinical and hematologic toxicity. All patients experienced usual moderate rIL-2–related side effects, all of them reversing rapidly after the end of treatment. So, the succession of two intensive therapies, like autologous BMT and rIL-2 did not lead to increased morbidity or mortality in these patients.

Second, it shows the absence of marked toxicity of IL-2 on the graft: serious concerns about hematologic toxicity were possible since IL-2–mediated suppression of hematopoiesis has been well documented. In this trial, changes in hemoglobin, platelets, and neutrophil counts were transient and moderate. No patients developed any bleeding during rIL-2 therapy and one patient had a bacteriemia: granulocytes in this patient never decreased under $2.8 \times 10^9/L$. As no dramatic clinical or hematologic toxicity occurred in this trial, earlier administration of rIL-2 (ie, between days 30 or 60) may be possible and is actually under investigation.

The ultimate goal of this approach was to stimulate the immune system after autologous BMT to induce a "graft versus-host like" reaction and hopefully a graft versus tumor effect. Clinical symptoms reminiscent of GVHD were obvious. Lymphocytes were dramatically increased, secondarily to an increase in activated T cells and NK populations. Immune activation lasted for longer periods for NK cells than for activated T lymphocytes. These changes are usual related side effect, all of them reversing rapidly after the end of treatment.

### Table 1. Patients' Characteristics, Toxicities, and Outcome

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Conditioning Regimen</th>
<th>Tumoral Status Before BMT</th>
<th>IL-2</th>
<th>Day of Onset</th>
<th>% Scheduled Doses</th>
<th>Grade ≥ 2 Toxieties</th>
<th>Outcome (mo post BMT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>44/F</td>
<td>All</td>
<td>Cy-TBI</td>
<td>Second CR</td>
<td>CR</td>
<td>78</td>
<td>100</td>
<td>GI, fever, * BP</td>
<td>Relapse (6), dead (10)</td>
</tr>
<tr>
<td>26</td>
<td>7/M</td>
<td>All</td>
<td>Cy-TBI</td>
<td>Second CR</td>
<td>CR</td>
<td>86</td>
<td>100</td>
<td>Fever</td>
<td>Relapse (7), dead (9)</td>
</tr>
<tr>
<td>27</td>
<td>24/F</td>
<td>Breast carcinoma</td>
<td>Cy-LPAM</td>
<td>Metastatic Stable disease</td>
<td>CR</td>
<td>98</td>
<td>100</td>
<td>Skin, fever, weight, pulmonary</td>
<td>Progression, alive (12)</td>
</tr>
<tr>
<td>28</td>
<td>55/F</td>
<td>Ovarian carcinoma</td>
<td>Cy-LPAM</td>
<td>CR, CR</td>
<td></td>
<td>86</td>
<td>100</td>
<td>Skin, fever, liver, weight</td>
<td>CCR, alive (10)</td>
</tr>
<tr>
<td>35</td>
<td>30/F</td>
<td>All</td>
<td>Cy-TBI</td>
<td>Second CR</td>
<td>CR</td>
<td>82</td>
<td>95</td>
<td>Skin, GI, fever, BP</td>
<td>CCR, alive (9)</td>
</tr>
<tr>
<td>54</td>
<td>8/M</td>
<td>AML</td>
<td>Misulban-LPAM</td>
<td>1st CR</td>
<td>CR</td>
<td>74</td>
<td>100</td>
<td>Fever, * liver</td>
<td>CCR, alive (9)</td>
</tr>
<tr>
<td>46</td>
<td>38/M</td>
<td>Malignant thymoma</td>
<td>Cy-LPAM</td>
<td>Metastatic PR</td>
<td></td>
<td>70</td>
<td>100</td>
<td>Fever</td>
<td>Progression, alive (9)</td>
</tr>
<tr>
<td>66</td>
<td>25/F</td>
<td>NHL</td>
<td>BEAM</td>
<td>PR, PR</td>
<td>CR</td>
<td>71</td>
<td>100</td>
<td>Fever, GI</td>
<td>CCR, alive (8)</td>
</tr>
<tr>
<td>63</td>
<td>39/M</td>
<td>NHL</td>
<td>BEAM</td>
<td>PR, CR</td>
<td></td>
<td>90</td>
<td>100</td>
<td>Skin</td>
<td>CCR, alive (8)</td>
</tr>
<tr>
<td>72</td>
<td>49/F</td>
<td>NHL</td>
<td>Cy-TBI</td>
<td>PR, PR</td>
<td></td>
<td>52</td>
<td>75</td>
<td>Skin, liver, BP</td>
<td>Relapse (3), alive (6)</td>
</tr>
</tbody>
</table>

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; NHL, non-Hodgkin lymphoma; CR, complete remission; PR, partial remission; CCR, continuous complete remission; GI, gastrointestinal.

*Grade 3 toxicity.

### Table 2. Hematologic and Immunologic Evolution: Mean ± SD (range)

<table>
<thead>
<tr>
<th></th>
<th>Pre-IL-2</th>
<th>Nadir</th>
<th>Day 8</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes $\times 10^9/L$</td>
<td>5.2 ± 2</td>
<td>3.4 ± 1.1*</td>
<td>15.5 ± 11.1*</td>
<td>8.1 ± 4.4†</td>
<td>4.8 ± 1.5</td>
</tr>
<tr>
<td>Hemoglobin: g/100 mL</td>
<td>10.4 ± 1.1</td>
<td>9 ± 0.6*</td>
<td>10.1 ± 1.4</td>
<td>11 ± 1.2</td>
<td>11.5 ± 2</td>
</tr>
<tr>
<td>Platelets $\times 10^9/L$</td>
<td>175 ± 105</td>
<td>84 ± 50*</td>
<td>85 ± 52*</td>
<td>180 ± 141</td>
<td>143 ± 58</td>
</tr>
<tr>
<td>Neutrophils $\times 10^9/L$</td>
<td>3.3 ± 1.3</td>
<td>2.5 ± 0.1*</td>
<td>4.3 ± 3.5</td>
<td>3.6 ± 2.3</td>
<td>2.2 ± 1.7</td>
</tr>
<tr>
<td>Eosinophils $\times 10^9/L$</td>
<td>0.2 ± 0.3</td>
<td>0.5 ± 0.7†</td>
<td>1.1 ± 1.8*</td>
<td>0.9 ± 1.7</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Lymphocytes $\times 10^9/L$</td>
<td>1.3 ± 0.6</td>
<td>0.3 ± 0.11*</td>
<td>9.2 ± 7.7*</td>
<td>3.2 ± 2.2*</td>
<td>1.5 ± 1</td>
</tr>
<tr>
<td>CD3$^+$ $\times 10^9/L$</td>
<td>0.7 ± 0.7</td>
<td>—</td>
<td>4.5 ± 4.9*</td>
<td>1 ± 1</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>0.77 ± 0.5</td>
<td>—</td>
<td>0.28 ± 0.08</td>
<td>0.35 ± 0.14</td>
<td>0.73 ± 0.21</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>0.2, 1.5</td>
<td>—</td>
<td>0.16, 0.35</td>
<td>0.14, 0.5</td>
<td>0.55, 1</td>
</tr>
<tr>
<td>CD3$^+$/DR$^+$ $\times 10^9/L$</td>
<td>0.5 ± 0.5</td>
<td>—</td>
<td>3.3 ± 4*</td>
<td>0.6 ± 0.8</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>CD3$^+$/CD25$^+$ $\times 10^9/L$</td>
<td>0.1 ± 0.2</td>
<td>—</td>
<td>0.9 ± 1.2*</td>
<td>0.1 ± 0.05</td>
<td>0.05 ± 0.05</td>
</tr>
<tr>
<td>NKH1$^+$/CD3$^-$ $\times 10^9/L$</td>
<td>0.1 ± 0.05</td>
<td>—</td>
<td>2.2 ± 1.7*</td>
<td>2.7 ± 1.5†</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>%NK cytosis</td>
<td>4 ± 4.3</td>
<td>—</td>
<td>14.4 ± 11.9†</td>
<td>16.1 ± 14.2</td>
<td>19.8 ± 3</td>
</tr>
<tr>
<td>%LAK cytosis</td>
<td>0.36 ± 0.5</td>
<td>—</td>
<td>8.3 ± 10.8*</td>
<td>9.1 ± 13.6</td>
<td>3.7 ± 2.9</td>
</tr>
<tr>
<td>%LAK cytosis</td>
<td>0.86 ± 1.6</td>
<td>—</td>
<td>25.3 ± 34</td>
<td>35</td>
<td>13.7</td>
</tr>
</tbody>
</table>

*Significant differences from pre-IL-2 value with $P < .01$.
†Significant differences from pre-IL-2 value with $P < .05$.
administration can modulate immune reconstitution after autologous BMT in a period of defective IL-2 production. A longer period of stimulation may still increase and prolong these effects and is presently under investigation.

It is obviously impossible to evaluate the clinical impact of IL-2 on the outcome of autologous BMT in this small group of patients. Moreover, most of them had no detectable disease at the time of rIL-2. The effect of rIL-2 on the eradication of minimal residual disease after autologous BMT can only be appreciated on the relapse rate of larger cohorts of selected patients with longer follow-up. However, these results demonstrating the clinical feasibility of IL-2 after autologous BMT and showing the marked immune effects induced in vivo by this approach invite further studies.

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Hematologic and immunologic effects of the systemic administration of recombinant interleukin-2 after autologous bone marrow transplantation

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