Prolonged Impairment of Hematopoiesis After High-Dose Therapy Followed by Autologous Bone Marrow Transplantation

By Jorge Domenech, Claude Linassier, Emmanuel Gihana, Alain Dayan, Danielle Truglio, Myrtille Bout, Charlotte Petitdidier, Martine Delain, André Petit, Jean-Louis Brémond, Isabelle Desbois, Jean-Pierre Lamagnère, Philippe Colombat, and Christian Binet

Hematopoietic reconstitution has been studied in 180 patients after autologous bone marrow transplantation based on peripheral blood cell (PBC) recovery time and marrow progenitor counts sequentially tested for up to 4 years. Several factors that could influence hematopoietic reconstitution have been analyzed including sex, age, diagnosis, disease status, conditioning regimen, graft progenitor content, graft in vitro purging, and postgraft administration of growth factors. Before transplantation, marrow progenitor values were normal only for colony-forming unit granulocyte macophagocyte (CFU-GM) in contrast to colony-forming unit-erythroid (CFU-E), burst-forming unit-erythroid (BFU-E), and colony-forming unit-megakaryocyte (CFU-Meg). After transplantation, as described with allogenic grafts, these values remained low for several years, although PBC counts were nearly normalized within a few weeks. Pregraft values were reached after 2 years for CFU-GM and BFU-E, and after 4 years for CFU-E, while CFU-Meg failed to reach pregraft values after this time. Normal levels were reached after 4 years only by CFU-GM. On univariate and multivariate analysis, the following factors appeared to delay both PBC and marrow progenitor reconstitution: underlying disease (particularly acute myeloid leukemias), graft characteristics such as low stem cell content and in vitro purging, conditioning regimens with total body irradiation or busulfan, and lack of postgraft administration of growth factors. In conclusion, high-dose therapy followed by bone marrow transplantation induces a deep and prolonged impairment of hematopoiesis irrespective of any alloimmune reaction or postgraft immunosuppressive therapy. Besides an obvious stem cell quantitative defect, a persistent qualitative defect within the hematopoietic system may be involved, particularly with respect to stem cell self-renewal and commitment towards erythroid and megakaryocytic lineages.

Bone Marrow Transplantation (BMT) has opened new therapeutic avenues in onco-hematology allowing the use of intensive myeloablative regimens at supralethal doses. The rescue by allogeneic or autologous stem cells results in apparent complete hematopoietic reconstitution as shown by quite normal peripheral blood cell (PBC) counts1,2 and by normal marrow cellularity,2,3 which are reached within 2 months. More detailed analysis of hematopoietic reconstitution has been supplied by studies on short-term recovery of circulating4,5 or marrow,5,2,6,7 progenitors that appeared to predict engraftment after BMT.1,6,7 In addition, some studies of postgraft long-term marrow progenitor recovery have pointed to a deep and prolonged impairment of hematopoiesis with counts remaining at low levels up to 4 years.5,9,10 All these studies have been performed in patients transplanted particularly with allogeneic marrows. However, to analyze the effect of high-dose therapy on the hematopoietic system, autologous BMT (ABMT) provides an ideal model without the interference of alloimmune reactions between donor and recipient cells such as graft-versus-host disease (GVHD) or the need of immunosuppressive therapy for its prevention. Some factors influencing hematopoietic reconstitution have also been evaluated, but in most of these reports, only PBC recovery was considered, regardless of marrow progenitor recovery.14,15

We present here a long-term study of marrow progenitor reconstitution in a wide cohort of 180 patients transplanted with autologous marrows from a single institution, which has allowed us to test several factors that could affect peripheral and marrow hematopoietic reconstitution. These factors included parameters related to patient, graft, conditioning regimen, and postgraft administration of growth factors.

Materials and Methods

Patients. Reconstitution of hematopoiesis has been assessed between April 1984 and September 1992 on 180 patients transplanted with cryopreserved autologous marrows for malignancies including 59 non-Hodgkin’s lymphomas (NHL), 33 acute lymphoblastic leukemias (ALL), 30 acute myeloid leukemias (AML), 21 Hodgkin’s diseases (HD), 5 multiple myelomas (MM), and 32 solid tumors (ST) (11 testis carcinomas, 6 neuroblastomas, 4 breast carcinomas, 2 ovarian carcinomas, and 9 other various tumors). There were 108 males and 72 females with a median age (range) of 34 years (2 to 62 years); 34 patients were children under 15 years. Ninety-one patients were autografted in first remission, 86 in second remission or more, and 3 in progressive disease.

Marrow graft processing. A volume of 600 to 1,000 mL of BM was collected by iliac crest and in a few cases by sternal puncture. Grafts were concentrated with a semicontinuous flow cell separator Haemonetics 30 S (Haemonetics Corp, Braintree, MA) and contained a median (range) of 1.69 (0.57 to 6.30) × 10^8 nucleated cells per kg of body weight. Concentrated grafts were purged in vitro in 76 cases (30 ALL, 20 NHL, 19 AML and 7 ST, including 6 neuroblastomas) using mafosfamide9 or a combination of mafosfamide and doxorubicin9 at doses individually adjusted. Marrows were immediately frozen in bags using a Nicool ST 316 programmed freezing unit (CFPO, Paris, France) and stored in liquid nitrogen at −196°C. Median number (range) of CFU-GM was 10.57 (1.0 to 249.10) × 10^3/kg after graft concentration and 0.15 (0 to 40.77) × 10^3/kg after graft purging.

From the Laboratory of Hematology, Department of Medical Oncology and Blood Diseases and the Regional Blood Bank, University Hospital of Tours, Tours, France.

Submitted May 19, 1994; accepted January 11, 1995.

Supported by Grant No. 6283 from ARC Villejuif and from Comité d’Indre et Loire de la Ligue Nationale contre le Cancer, France.

Address reprint requests to Jorge Domenech, MD, PhD, Laboratoire d’Hematologie, Hôpital Bretonneau, 2 Bédonnellé, 37044 Tours Cedex, France.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1995 by The American Society of Hematology.

0006-4971/95/8511-0033$3.00/0

Blood, Vol 85, No 11 (June 1), 1995: pp 3320-3327
**Pregraft conditioning regimens.** ALL patients received fractionated total body irradiation at 10 Gy for 4 days followed by cyclophosphamide (Cy) 50 mg/kg/d for 2 days (Bsf/Cy). AML patients received busulphan (Bs) 4 mg/kg/d for 4 days followed by Cy 50 mg/kg/d for 4 days (Bs/Cy), but TBI/Cy in six cases. NHL patients received 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) 300 mg/m² for 1 day, etoposide (VP-16) 200 mg/m²/d with cytosine arabinoside (Ara-C) 200 mg/m²/d both for 4 days followed by high-dose melphalan (HDM) 140 mg/m² for 1 day (BEAM) but TBI/Cy in 19 cases. HD patients received either BEAM in 12 cases or BCNU 300 mg/m² for 1 day with Cy 35 mg/kg/d and VP-16 200 mg/m²/d for 4 days (BCV) in 9 cases. MM patients received HDM associated with TBI in 1 case. Testis carcinoma patients received VP-16 350 mg/m²/d with cisplatin 40 mg/m²/d for 5 days and Cy 1.6 g/m²/d for 4 days. The other ST patients received various regimens including HDM, Bs/Cy, BCNU, VP-16, vincristine, mitoxanthrone, or TBI. Thus, among the 180 patients studied, 61 received TBI (33 ALL, 19 NHL, 6 AML, 2 ST, and 1 MM) and 21 received Bs (18 AML, 3 ST).

**BMT.** At the time of transplantation (day 0) marrow bags were quickly thawed in a 37°C waterbath at the bedside and were immediately infused intravenously. After transplantation, 29 patients received recombinant human hematopoietic growth factors by intravenous route at 5 µg/kg/d from day 0 and until neutrophil counts exceeded 1 x 10⁹/L for 3 consecutive days. GM-CSF (Schering-Pough, Madison, NJ) was infused in 14 NHL, 3 HD, and 2 AML and G-CSF (Amgen, Thousand Oaks, CA) in 7 ST, 2 HD, and 1 NHL.

**Postgraft hematopoietic reconstitution.** PBC recovery was assessed by the time in days necessary to reach counts of leukocytes >1 x 10⁹/L, neutrophils >0.5 x 10⁹/L, and platelets >50 x 10⁹/L. Marrow progenitor recovery was sequentially evaluated by sternal puncture on day 30 (n = 76), day 90 (n = 104), day 180 (n = 54), day 365 (n = 75), after 2 years (n = 34) and after 4 years (n = 17). In each sample CFU-GM, CFU-E, BFU-E, and CFU-Meg counts per 2 x 10⁶ mononuclear cells (MNC) were compared with pregraft values (at graft collection) and with normal values obtained from 101 patients undergoing cardiac surgery without any hematologic disorder after informed consent.

**Clonogenic assays.** BM cells were separated through a Ficoll-sodium-metrizoate gradient (d = 1.077, Lymphoprep, Nycomed SA, Oslo, Norway). The MNC fraction was washed twice and plated in a semisolid culture system. CFU-GM were cultured as previously described with modifications in 0.3% agar with McCoy’s 5A medium (Boehringer-Mannheim, Meylan, France) supplemented with 15% fetal bovine serum (FBS) (GIBCO, Cergy-Pointoise, France) and 15% human placenta-conditioned medium (HPCM) as a source of growth factor, except during the first 2 years of the study where colony-stimulating activity was supplied by an underlayer of mononuclear peripheral blood cells obtained from normal blood donors. CFU-E, BFU-E, and CFU-Meg were cultured according to the technique described by Messner in 0.9% methylcellulose (Sigma, St Quentin-en-Yvelines, France) with Iscove’s medium (Boehringer-Mannheim) supplemented with 30% human plasma (from a pool of 10 normal blood donors) and 5 x 10⁻³ mol/L 2-mercaptopethanol (Sigma). Growth factors were supplied by 5% phytomenadione-stimulated leukocyte-conditioned medium (PHA-PCM) and 1 U/mL human purified (CNTS, Paris, France) or recombinant (Boehringer-Mannheim) erythropoietin. Cells were plated in triplicate 1 mL cultures at 2 x 10⁶/mL into Petri dishes (Nunc, Denmark) and incubated at 37°C in a humidified 5% CO₂ atmosphere. The culture plates were scored by inverted microscopy on day 10 for CFU-GM (aggregates >40 cells), on day 7 for CFU-E (aggregates >20 hemoglobinized cells), on day 14 for BFU-E (aggregates >200 hemoglobinized cells consisting of three or more clusters) and CFU-Meg (aggregates >3 bright translucent cells).

**Statistical analysis.** For comparison of value distributions, the following nonparametric tests were used: Mann-Whitney’s U test for unpaired series and Wilcoxon’s T test for paired series. Analysis of the factors influencing hematopoietic reconstitution was assessed by univariate analysis using Mann-Whitney’s U test or least-squares method and by multivariate analysis using multiple stepwise regression method. Several factors related to the patient and to the transplantation procedure were studied: patient age, sex (male or female), disease status at transplantation time (in first remission or not), progenitor content in the graft (log number of CFU-GM/kg after graft concentration), marrow purging (yes or no), belonging to a diagnosis category (AML, ALL, NHL, HD, MM, ST) and to a conditioning regimen category (TBI, Bs/Cy, other), and postgraft administration of growth factor (yes or no). To better evaluate parameters such as conditioning regimen or marrow purging irrespective of diagnosis, multivariate analysis was also performed in the subgroup of NHL patients, because some of these procedures have been specifically applied in acute leukemias and not in NHL.

**RESULTS**

**PBC recoveries.** For all the patients median days (range) to reach leukocytes >1 x 10⁹/L, neutrophils >0.5 x 10⁹/L, and platelets >50 x 10⁹/L were 18 days (7 to 82 days), 17 days (7 to 82 days), and 28 days (7 to 390 days), respectively. For the patients who did not receive any growth factor after ABMT, these values were 19 days (7 to 82 days), 18 days (8 to 82 days), and 30 days (7 to 390 days) whereas, for those who did, PBC recoveries were 13 days (7 to 19 days), 13 days (7 to 19 days), and 19.5 days (8 to 252 days).

**Marrow progenitor recoveries.** Evolution of marrow progenitor counts from pregraft period up to 4 years after ABMT is shown on Fig 1A-D. Before ABMT, CFU-GM values did not differ from control values with 85% of patients having normal values (above the 10th percentile) while CFU-E, BFU-E, and CFU-Meg values appeared to be decreased (P < .0001) with 60%, 50%, and 58% of patients having normal values, respectively. No difference was seen in pregraft values of all progenitor types with respect to sex, age, diagnosis, disease status, marrow purging, or postgraft administration of growth factors. However, when conditioning regimen was taken in account in all the patients, BFU-E values were higher in patients having not received Bs or TBI (P < .0174) and were lower in patients who received Bs (P < .0430), whereas these results were not found in NHL patients. After ABMT, CFU-GM reached pregraft values after 2 years and normal values after 4 years. The lowest proportion of patients with normal CFU-GM values was found on day 90 and represented 35% of the patients, while they were 55% after 1 year. CFU-E and BFU-E reached pregraft values after 4 years and 2 years, respectively, however, both progenitors still failed to return to normal values after 4 years. The lowest proportion of patients with normal CFU-E and BFU-E values was found between day 30 and day 180 representing 24% to 32% and 14% to 17% of the patients, respectively. After 1 year they were 48% and 23%, respectively. CFU-Meg levels remained low for 4 years as neither pregraft values nor normal values were reached during this period of time where the proportion of patients with normal values represented 15% to 26% of the patients.
Effect of patient factors on hematopoietic reconstitution. The influence of patient diagnosis is summarized in Table 1. AML patients displayed, after ABMT, delayed short-term recovery of PBC (especially for platelets), CFU-GM, CFU-E, and BFU-E (on day 30) as well as delayed long-term recovery of erythroid progenitors (at 1 and 4 years). ALL patients, despite delayed short-term recovery of CFU-GM (on day 30) as seen in AML patients, had long-term recovery of CFU-GM, BFU-E and CFU-Meg (at 1 year) shorter than other patients. In contrast to patients with acute leukemias, NHL patients showed shorter short-term recovery of leukocytes (especially for neutrophils) and of CFU-GM (on day 30). ST patients, despite delayed neutrophil recovery, displayed faster BFU-E recovery particularly at short-term (on day 30) but also at middle-term (on day 90). HD and MM, as well as other patient factors including sex, age, and disease status (first remission or not), did not appear to influence hematopoietic recovery. Nevertheless in the NHL group, multivariate analysis showed that patients receiving transplants in first remission displayed faster leukocyte ($P < .001$) and neutrophil ($P < .001$) recoveries.

Effect of graft characteristics on hematopoietic reconstitution. Log CFU-GM number per kilo of body weight in the concentrated graft correlated negatively with PBC recovery, especially for neutrophils but also for total leukocytes and platelets. Likewise, CFU-GM content correlated positively with short-term (on day 30) and middle-term (on day 90) marrow CFU-GM recovery in univariate and multivariate analysis (Table 2). Among patients with purged marrows, these correlations were not found, taking in account CFU-GM values after purging. On the other hand, marrow in vitro purging resulted in a slower short-term recovery of PBC and CFU-GM (on day 30), but also in a slower long-term recovery of CFU-Meg (at 2 years) as assessed by univariate and multivariate analysis (Table 2). These data were confirmed by multivariate analysis in the NHL patient group for recoveries of leukocytes ($P < .001$) and neutrophils ($P < .001$), as well as for recoveries of CFU-Meg at 1 year ($P < .05$) and at 2 years ($P = .05$).

Effect of conditioning regimen on hematopoietic reconstitution. TBI and Bsf in pregraft high-dose therapy appeared also to delay hematopoietic reconstitution (Table 2). Thus,
when either of these conditioning regimens was used, slower short-term recovery of platelets and CFU-GM (on day 30) were found by univariate and multivariate analysis, as well as slower middle-term recovery of erythroid and megakaryocyte progenitors (on day 90), as shown by univariate analysis. However, platelet recovery appeared slower with Bsf than with TBI (P = .043) whereas CFU-GM recovery on day 30 was not different. In addition, Bsf resulted in a slower long-term recovery of erythroid progenitors (at 1 and 4 years) and CFU-Meg (at 1 year). In NHL patients, TBI was only associated by univariate analysis with delayed platelet recovery (P = .007) and day 90 CFU-Meg (P = .038) recoveries and by multivariate analysis with delayed platelet recovery (P < .01).

**Effect of postgraft administration of growth factors on hematopoietic reconstitution.** Postgraft administration of GM-CSF or G-CSF (Table 2) shortened PBC recoveries (especially for leukocytes and neutrophils) and was associated with higher CFU-GM counts after 2 years (including only patients having received GM-CSF).

In an attempt to clarify the respective role of GM-CSF (predominantly used in NHL) and G-CSF (predominantly used in ST), hematopoietic reconstitution of NHL patients who received GM-CSF and of ST patients who received G-CSF was compared with patients who did not receive any growth factor in each group of patients. GM-CSF reduced recovery time (median values) for leukocytes >1 × 10^9/L (13 v 17.5 days; P = .0020) and for neutrophils >0.5 × 10^9/L (13 v 17 days; P = .0232) but not for platelets >50 × 10^9/L and increased marrow BFU-E counts after 1 year as shown by univariate analysis (53 v 12; P = .0561), as well as by multivariate analysis (P < .025). In contrast, G-CSF reduced recovery times not only for leukocyte >1 × 10^9/L (15 v 21 days; P = .0127) and for neutrophils >0.5 × 10^9/L (15 v 21 days; P = .0014) but also for platelets >50 × 10^9/L (17 v 23 days; P = .0056). In addition, G-CSF increased marrow counts of CFU-E (48 v 20; P = .0472) and of BFU-E (128 v 4; P = .0441) on day 30.

**DISCUSSION**

This long-term study of hematopoietic reconstitution in a wide cohort of patients transplanted with autologous marrow showed delayed recovery of marrow progenitors up to 4 years despite a trend to normalize PBC counts within a few weeks. Our findings are in agreement with those reported following allogenic BMT, as well as after ABMT, but in a few patients. A decrease of circulating progenitors has been described as well. Nevertheless, ABMT differ from allogenic BMT with respect to three main factors that can affect hematopoietic reconstitution: (1) use of grafts from patients previously treated with myelotoxic agents, (2) use of grafts usually cryopreserved, and (3) lack of GVHD in the absence of graft T-cell depletion and postgraft immunosuppressive therapy. This latter point makes it difficult to interpret after allogenic BMT the true effect of high-dose therapy on hematopoiesis because it has been demonstrated that GVHD, as well as immunosuppressive therapy, particu-
Particularly with methotrexate,12 influence PBC and progenitor recoveries.11,12,24

In the present study, PBC recoveries were comparable to those reported elsewhere after ABMT.14,16 They appear not to be different from those following allogeneic BMT,3,18 except for lymphocyte recovery that has been found to be faster after ABMT. Considering marrow progenitor recovery, whereas CFU-GM and BFU-E reached pregraft levels after 2 years, only CFU-GM counts became normal after 4 years, while CFU-Meg still remained below pregraft levels. It has to be noted that CFU-GM were the only progenitors found at normal levels before transplantation. Similar prolonged marrow progenitor counts below normal values have been described following allogeneic BMT, not only for CFU-GM,5,8-11 but also for BFU-E, CFU-Meg, and CFU-GEMM.5,11,12 However, CFU-GM and BFU-E seem to recover faster after allogeneic BMT with postgraft cyclosporin A treatment than after ABMT, whereas an opposite observation was reported for T-cell progenitors.13 This discrepancy between peripheral and marrow recoveries can be explained by compensatory mechanisms leading to adequate PBC counts produced by reduced numbers of marrow stem cell clones. This hypothesis is supported by studies showing increased proportion of cycling progenitors after allogeneic BMT not only for CFU-GM,13 but also for more immature ones such as CFU-GEMM.11 An amplification of the differentiated compartment beyond progenitor stage or a developmental block of hematopoietic stem cells due to conditioning regimens that transplantation of limited cell numbers cannot restore, a prolonged qualitative defect has been shown in CFU-GM proliferative capacity or in stem cell self-renewal.14 These cells would predominantly be triggered toward differentiation at the expense of their self-renewal capacity. However, differentiation could also be impaired qualitatively, as suggested by our study. Thus, the prolonged low levels of erythroid and megakaryocyte progenitors that we found after ABMT may probably

<table>
<thead>
<tr>
<th>Factors</th>
<th>Parameters of Reconstitution (median values)</th>
<th>Factor</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coefficient r</td>
<td>P Value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log CFU-GM/kg in the graft after concentration</td>
<td>PBC</td>
<td>L&gt;1 × 10^9/L</td>
<td>r = -0.228</td>
<td>.0024</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N&gt;0.5 × 10^9/L</td>
<td>r = -0.291</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P&gt;50 × 10^9/L</td>
<td>r = -0.154</td>
<td>.0454</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d30 CFU-GM</td>
<td>r = -0.268</td>
<td>.0210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d90 CFU-GM</td>
<td>r = -0.298</td>
<td>.0025</td>
</tr>
<tr>
<td>Purged marrow</td>
<td>PBC</td>
<td>L&gt;1 × 10^9/L</td>
<td>19</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N&gt;0.5 × 10^9/L</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P&gt;50 × 10^9/L</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d30 CFU-GM</td>
<td>11</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 yr CFU-Meg</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td>PBC</td>
<td>P&gt;50 × 10^9/L</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>with TBI or Bsf</td>
<td>d30 CFU-GM</td>
<td>40</td>
<td>20.5</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d90 CFU-GM</td>
<td>8.5</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>d30 CFU-GM</td>
<td>0</td>
<td>0.5</td>
<td>0.0287</td>
</tr>
<tr>
<td>with TBI</td>
<td>d30 CFU-GM</td>
<td>36</td>
<td>22</td>
<td>0.0444</td>
</tr>
<tr>
<td>with Bsf</td>
<td>d30 CFU-GM</td>
<td>8</td>
<td>45</td>
<td>0.0141</td>
</tr>
<tr>
<td></td>
<td>d90 CFU-GM</td>
<td>67</td>
<td>24</td>
<td>0.0022</td>
</tr>
<tr>
<td></td>
<td>d90 CFU-E</td>
<td>7</td>
<td>44.5</td>
<td>0.0109</td>
</tr>
<tr>
<td></td>
<td>d90 BFU-E</td>
<td>0</td>
<td>25</td>
<td>0.0099</td>
</tr>
<tr>
<td></td>
<td>d90 CFU-Meg</td>
<td>0.5</td>
<td>8</td>
<td>0.201</td>
</tr>
<tr>
<td></td>
<td>1 yr BFU-E</td>
<td>4</td>
<td>16</td>
<td>0.0141</td>
</tr>
<tr>
<td></td>
<td>1 yr CFU-Meg</td>
<td>0</td>
<td>0.5</td>
<td>0.0273</td>
</tr>
<tr>
<td></td>
<td>4 yr BFU-E</td>
<td>34</td>
<td>194</td>
<td>0.0370</td>
</tr>
<tr>
<td></td>
<td>4 yr CFU-E</td>
<td>11</td>
<td>79</td>
<td>0.0693</td>
</tr>
<tr>
<td>Growth factor after ABMT</td>
<td>PBC</td>
<td>N&gt;0.5 × 10^9/L</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>P&gt;50 × 10^9/L</td>
<td>15</td>
<td>18</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>P&gt;50 × 10^9/L</td>
<td>19.5</td>
<td>30</td>
<td>0.0238</td>
</tr>
<tr>
<td></td>
<td>2 yr CFU-GM</td>
<td>244*</td>
<td>89</td>
<td>0.0494</td>
</tr>
</tbody>
</table>

Values are medians from patients presenting the factor studied versus those from patients not presenting this factor. Recoveries are expressed for peripheral cells as the number of days required to recover the indicated blood concentrations and for marrow progenitors as counts per 2 × 10^9 mononuclear cells plated. Univariate analysis was performed by linear regression method for log CFU-GM/kg and Mann-Whitney's U test for the other factors and multivariate analysis by multiple stepwise regression method.

Abbreviations: TBI, total body irradiation; Bsf, busulfan; d, day; yr, year; PBC, peripheral blood cells; L, leukocytes; N, neutrophils; P, platelets.* All patients have received GM-CSF.
be attributed to a trouble of commitment toward these two lineages rather than to the quantitative deficiency of these progenitors present at graft collection. This postgraft differentiation impairment may be explained by several mechanisms not exclusive of each other: (1) decreased production of some hematopoietic growth factors by regulatory cells as reported for IL-3, GM-CSF, and stem cell factor, (2) defect of the microenvironment, and (3) intrinsic abnormality of stem cells. However, we also noted low levels of erythroid and megakaryocyte progenitors in recipients before transplantation. This could reflect toxicity of previous chemotherapies that influence postgraft reconstitution, as shown in experimental models for specific drugs. In humans, low pregraft levels of platelets, of CFU-Meg, or of CFUGEMM have been associated with delayed hematopoietic reconstitution, while marrow grafts from previously untreated patients lead to significantly faster PBC recoveries. Nevertheless, we did not find in these patients any influence of the length of induction-consolidation therapy (reflected by the number of remissions) on hematopoietic reconstitution. These data support those we have previously reported on the number of chemotherapy courses in patients autografted with mafosfamide-purged marrows. However, in the present study considering NHL patients, we found that transplantation after a short history of the disease (in first remission) resulted in faster leukocyte and neutrophil recoveries, as shown in other reports.

Among other patient factors, influence of diagnosis appears also obvious as acute leukemias displayed slower short-term recoveries (for PBC and day 30 CFU-GM), as compared with NHL, a fact consistent with a clonal abnormality present at the stem cell level. The worst recoveries found in AML for PBC (particularly for platelets) and for erythroid progenitors at short and long-term provide evidence for a deeper stem cell defect at earlier stages than in ALL. These delayed recoveries in AML have been described as well by others for PBC and for marrow progenitors, especially for CFU-Meg. ST patients had slower neutrophil recovery but faster BFU-E recovery at short-term (on day 30) and at middle-term (on day 90) probably because, despite heavier conditioning regimens, their stem cell reserve was less damaged by less intensive induction-consolidation therapies. In contrast to diagnosis, no influence was found for sex or patient age, as reported by others in allogeneic BMT.

Graft characteristics, such as progenitor content and marrow purging, are critical factors too. Influence of graft CFU-GM content on PBC recovery has been previously reported by investigators who found a linear correlation or only a threshold of \(1 \times 10^3\) CFU-GM/kg below which recovery appears to be delayed. In our study, although all the patients were grafted with CFU-GM counts above this threshold, a linear relationship was still found between the logarithm of CFU-GM content in the graft and the time for leukocyte, neutrophil, and in a lesser extent, platelet recoveries. These correlations were found only when CFU-GM content was calculated before purging, but not after purging or after freezing in contrast with data reported by Rowley et al. However, these investigators showed that demonstration of such correlations after purging is dependent on technical conditions. The use of assays in soft agar with HPCM, as in our study, seems inadequate, as opposed to more sensitive assays in methylcellulose with PHA-LCM, which probably reflects better the pluripotent stem cell content. In addition, purging with mafosfamide doses individually adjusted could have hidden these correlations. Very few studies have been published about the role of graft progenitor content on marrow progenitor recovery and did not show any correlation. Lazarus et al only found a correlation between pregraft marrow BFU-E concentrations and postgraft marrow CFU-GM counts on day 60. The present study, in a larger series of patients, has pointed to predictive value for log CFU-GM/kg in the graft on marrow CFU-GM recovery at days 30 and 90, suggesting that evaluation of graft CFU-GM content can reflect its capacity of engraftment, at least at short and middle term. Delay in PBC recovery after transplantation with purged marrows, as shown here, has already been well documented by others for leukocytes and especially for platelets. However, we have shown the effect of purging on platelet recovery only by univariate and not by multivariate analysis, in contrast to other factors more strongly associated with delayed platelet recoveries, such as AML or conditioning regimens with TBI or Bsf. In a murine experimental model, this delay has been attributed to marrow purging and/or stromal cell damage by previous chemotherapies for platelets and specifically to marrow purging for leukocytes.

Purging effect on long-term marrow progenitor recovery, which has not yet been reported in humans to the best of our knowledge, included not only lower short-term CFU-GM counts, but also lower long-term CFU-Meg counts. These findings suggest a toxic effect on late and also early stem cells of in vitro mafosfamide-purging, perhaps in synergy with in vivo treatment as reported in mice. Graft freezing could have enhanced these modifications because this additional factor, which was not tested here, has been associated with slower PBC recoveries.

Use of Bsf or TBI in conditioning regimens appeared to delay short-term and long-term hematopoietic recoveries, especially for erythroid progenitors, up to 4 years. These data are consistent with those reported by Testa et al. Thus, studies in mice have shown that TBI induces long-term damage in a dose-dependent manner, for more than 1 year, on both CFU-S and stromal cells, the latter being more resistant. Chemotherapy including Bsf, BCNU, or Cy in conjunction with TBI have also been found to cause persistant damage on CFU-S in contrast to 5-fluorouracil or Cy alone. In addition, Bsf treatment permanently reduced stromal cell population. Impairment of hematopoietic recovery found in the present study, was more obvious for platelets and longer for marrow progenitors (including erythroid progenitors) with Bsf than with TBI. These findings could be related to a persistant damage of the microenvironment or to a toxicity on primitive stem cells involved in a possible endogeneous reconstitution. However, the role of Bsf on platelet and erythroid progenitor recoveries could not be distinguished from the diagnosis of AML, which was closely linked to this conditioning regimen, and was associated particularly with delayed recoveries of the same hematologic parameters. In contrast, TBI was found to specifically delay platelet and day 90 CFU-Meg recoveries.
In vivo administration of growth factors (GM-CSF or G-CSF) after transplantation shortens recovery time for leukocytes and neutrophils in agreement with previous reports for GM-CSF, G-CSF. More surprising is the role on platelet recovery, which appears to be played by G-CSF, when analyzed in the ST patient group only. This was not found by Sheridan et al in patients transplanted predominantly for hemopathies, although this growth factor seemed to reduce the duration of platelet transfusion support. However, these investigators showed a clear reduction of platelet recovery time when marrow was combined with G-CSF-mobilized peripheral stem cells. Indirect influence of G-CSF on erythropoiesis cannot be excluded, as we found an increase in marrow erythroid progenitor counts on day 30, as reported for circulating BFU-E, as well as for circulating CFU-GM during its infusion. With GM-CSF, Lazarus et al also described a parallel increase of CFU-CM and BFU-E counts both in the marrow and blood, but a decrease of these progenitors after discontinuation of infusions. Nevertheless they did not study long-term progenitor reconstitution, as in our study where an increase of BFU-E after 1 year and of CFU-GM after 2 years was noted. These findings are of particular interest because they suggest an influence, not only on early, but also on late engraftment carried by postgraft administration of this growth factor, which was supposed to enhance homing of stem cells and then grafting efficiency. In contrast, a previous report showed that GM-CSF or G-CSF, when infused during the recovery phase of repeated high-dose Cy therapy, decreases the engraftment capacity of marrow cells collected at this time. These data, together with our results, suggest differential effects of GM-CSF on the quality of stem cells according to subsequent use of chemotherapy. Primitive stem cells exposed to this growth factor can be triggered into cycle but then can be killed by cycle-dependent chemical agents.

Finally, analysis of marrow progenitor recovery after high-dose therapy followed by ABMT showed a deep and prolonged impairment of hematopoiesis irrespective of any alloimmune reaction or any postgraft immunosuppressive therapy, comparable to that described after allogenic BMT. This long-term damage may involve serious consequences for the patient, such as secondary hemopathies or poor tolerance to subsequent myelotoxic treatments in case of relapse. The present study showed that hematopoietic reconstitution depends on a combination of several factors including underlying disease and/or its induction-consolidation treatment, graft characteristics (as stem cell content or purging), conditioning regimens, and perhaps postgraft administration of growth factors. Further studies are in progress at our institution to evaluate long-term marrow progenitor reconstitution following transplantation with peripheral blood stem cells mobilized by chemotherapy and growth factors such as GM-CSF or G-CSF. In addition, we are using long-term marrow cultures to better understand the actual impact of intensive therapies on the different marrow components of the hematopoietic system.

ACKNOWLEDGMENT

We thank Prof James O. Armitage and Dr Pierre Goube de Lafor est for their helpful comments and suggestions. We are particularly grateful to Prof Michel Marchand for providing us with the control bone marrow samples, to Dominique Dessay and Anne-Françoise Nicolas for their technical assistance, and to Marie-Thérèse Vasseur for preparing the manuscript.

REFERENCES

15. Stewart FM, Kaiser DL, Ishitani KP, Pirsch GW, Niskanen E: Progenitor cell numbers (CFU-GM, CFU-D, and CFU-Mix) and
hematopoietic recovery following autologous bone marrow transplantation. Exp Hematol 17:974, 1989


17. Rowley SD, Piantadosi S, Marcellus DC, Jones RJ, Davidson NE, Davis JM, Kennedy J, Wiley JM, Wingard JR, Yeager AM, Santos GW: Analysis of factors predicting speed of hematologic recovery after transplantation with 4-hydroperoxycyclophosphamide-purged autologous bone marrow grafts. Bone Marrow Transplant 7:183, 1991

18. To LB, Roberts MM, Haylock DN, Dyson PG, Branford AL, Thorp D, Ho JQK, Dart GW, Horvath N, Davy MLJ, Otway CLM, Abdi E, Juttner CA: Comparison of haematological recovery times and supportive care requirements of autologous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and allogeneic bone marrow transplants. Bone Marrow Transplant 9:277, 1992


Prolonged impairment of hematopoiesis after high-dose therapy followed by autologous bone marrow transplantation

J Domenech, C Linassier, E Gihana, A Dayan, D Truglio, M Bout, C Petitdidier, M Delain, A Petit and JL Bremond