CONCISE REPORT

Autologous Transplantation of Blood-Derived Hemopoietic Stem Cells After Myeloblastic Therapy in a Patient With Burkitt's Lymphoma

By Martin Körling, Bernd Dörken, Anthony D. Ho, Antonio Pezzutto, Werner Hunstein, and Theodor M. Fiedler

A patient with Burkitt's lymphoma in complete remission received myeloblastic consolidation treatment with superfractionated total body irradiation (1,320 rad) and cyclophosphamide (200 mg/kg) followed by autologous transplantation of previously harvested and cryopreserved blood-derived hemopoietic stem cells. Seven successive leukaphereses were performed to yield a total of $5.2 \times 10^8$ mononuclear cells (MNC) comprising $15.1 \times 10^6$ CFU-GM or $4.34 \times 10^6$ CFU-GEMM. Following autologous blood stem cell transplantation (ABSCT), reconstitution of all cell lines occurred very rapidly, ie, 1,000 leucocytes per $\mu$L were reached after nine days, 500 granulocytes and 50,000 platelets per $\mu$L after ten days. B cells reached normal values around day 35 post-transplantation. CFU-GM first appeared in the circulating blood exhibiting an enormous overshoot. Some days later CFU-GM also appeared in the marrow. The kinetics and pattern of hemopoietic reconstitution after myeloblastic treatment and ABSCT provide clear evidence that blood-derived hemopoietic stem cells are capable of completely restoring hemopoietic function in man. A possible reconstitutive advantage of blood over marrow-derived stem cells is discussed.

Evidence in rodents, canines, and nonhuman primates indicates that hemopoietic progenitor cells capable of repopulating an aplastic marrow and maintaining lymphohemopoiesis circulate in the peripheral blood. Goodman and Hodgson first showed that lethally irradiated mice were fully reconstituted after transplantation of blood-derived leukocytes from closely related donor mice with identification of donor-type cells in the recipient. This was also the case in the lymphopoietic system, as was further demonstrated in the canine model.

At present, there is evidence that hemopoietic engraftment can be achieved with human peripheral blood leukocytes, although those clinical findings are controversial. From mice studies, some data suggest that circulating hemopoietic stem cells have a limited potential for self-renewal and therefore cannot maintain hemopoietic function as do marrow-derived stem cells. If this applies to the human situation, the concept of replacing marrow by blood-derived stem cells would not be clinically applicable. To define the reconstitutive potential of circulating human stem cells, we describe in the following the successful lymphohemopoietic reconstitution of a patient with Burkitt's lymphoma after myeloblastic treatment and autologous transplantation of blood-derived hemopoietic stem cells.

CASE REPORT

A 38-year-old male was admitted to the Heidelberg University Hospital with a fist-size tumor on the right side of the neck. From the biopsy, the immunohistological diagnosis of B-lymphoblastic non-Hodgkin's lymphoma (Burkitt's type) was made. The clinical staging (ultrasound, computerized axial tomography [CAT] scan, gallium-67 scans) subsequently performed revealed further abdominal tumor manifestation resulting in a stage III disease (St Jude classification). The induction treatment regimen consisted of two cycles of cyclophosphamide (1,000 mg/m$^2$ intravenously [IV]), vincristine (1.4 mg/m$^2$ IV), methotrexate (12.5 mg/m$^2$ IV), and prednisolone (1,000 mg/m$^2$ IV) together with two intrathecal administrations of methotrexate (12.5 mg/m$^2$) per course (COMP). Autologous marrow transplantation was then considered as intensive consolidation treatment. Since a regular multiple marrow aspiration from the pelvic site was not possible because of hyperostosis with a variable degree, we decided to use blood-derived, rather than marrow-derived, stem cells for engraftment. Approval for this experimental treatment was obtained from the hospital human investigational review board and appropriate informed written consent was given by the patient. Bone marrow biopsy and examination of the CSF did not show any sign of tumor cell involvement. There was no histological evidence for myelofibrosis and no clinical evidence for extramedullary hemopoiesis. The patient had received no prior radiation.

MATERIALS AND METHODS

Stem cell harvest. Sixteen days after completion of COMP treatment, blood stem cell collections were started. A total of seven consecutive leukaphereses were performed using a continuous-flow blood cell separator (FENWAL CS 3000, Fenwal Laboratories, Deerfield, III). The total blood volume processed per run was 10 liters at a flow rate of 70 mL/min. The centrifuge speed was adjusted to 1,000 rpm. Hydroxyethylstarch (HES) was used as RBC sedimentation enhancer at a total of 500 mL per run. The interval between stem cell harvests was between one and three days.

Freezing of leukapheresis-derived stem cells. The 200 mL harvested cell suspension per run was concentrated and reduced in volume to 100 mL, and mixed with the same volume of Spinner-minimum essential medium (S-MEM) supplemented with 20% dimethyl sulfoxide (DMSO). The final 200 mL cell suspension was distributed over two polyeylene bags (DELMED, Canton, Mass) and frozen in a computerized freezer (CRYOSON BV-6, Cryoson Deutschland GmbH, Schöllkrippen, West Germany) to $-100^\circ{\text{C}}$. The frozen cells were stored in the liquid phase of nitrogen until use.

Pretransplant conditioning regimen. The pretransplant conditioning regimen consisted of a myeloblastic dose of total body irradiation superfractionated over four days at 120 rad single doses up to a total of 1,320 rad (lungs, 900 rad). A linear accelerator was used as the radiation source. Three single doses were administered per day at 8 AM, noon, and 4 PM. Following irradiation, cyclophosphamide was given intravenously; subsequently, total-body electrocardiogram was performed to ensure normal sinus rhythm. No event indicating serious morbidity was observed.

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Table 1. Number of Mononuclear Cells, Granulocyte-Macrophage Committed Stem Cells, and Pluripotent Stem Cells Collected by Continuous-Flow Separation, Cryopreserved, and Eventually Transfused

<table>
<thead>
<tr>
<th>Run No.</th>
<th>MNC x 10^6</th>
<th>CFU-GM x 10^6</th>
<th>CFU-GEMM x 10^6</th>
<th>MNC x 10^6</th>
<th>CFU-GM x 10^6</th>
<th>CFU-GEMM x 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.8</td>
<td>2.0</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
<td>0.9</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6.4</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.0</td>
<td>2.6</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15.0</td>
<td>3.9</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7.5</td>
<td>3.8</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.0</td>
<td>1.9</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55.2</td>
<td>15.1</td>
<td>4.34</td>
<td>51.9</td>
<td>18.8</td>
<td>9.3</td>
</tr>
<tr>
<td>Total per kg of body weight</td>
<td>0.77</td>
<td>0.21</td>
<td>0.06</td>
<td>0.72</td>
<td>0.26</td>
<td>0.13</td>
</tr>
</tbody>
</table>

ND, not determined because of technical problems.

Phamid (50 mg/kg) was given on each of four consecutive days (total dose 200 mg/kg).

In vitro stem cell assay. The presence and concentration of hemopoietic progenitor cells was examined in each harvested cell suspension, in the peripheral blood, as well as in the marrow before (only blood stem cells) and after AB SCT. We used the human multilineage in vitro assay in methylcellulose (CFU-GEMM) recently described by Fauser and Messner and modified according to Ash et al.8

Immunologic studies. The reconstitution of the B and T cell line after myeloablative treatment and AB SCT was followed by using specific T cell (OKT series, Ortho Diagnostic Systems, Raritan NJ) and B cell (HD 37) monoclonal antibodies. The latter antibody defines a B cell specific antigen that represents the broadest available marker for the B lineage (Cluster CD 19).

RESULTS

Cell harvest, freezing and autotransfusion. Prior to initial chemotherapy and AB SCT stem cell concentration in the peripheral blood was 26.7 CFU-GM per mL on the average (11.0 to 57.0 CFU-GM per mL; n = 3). CFU-GEMM were not detectable. Prior to each leukapheresis, the mean blood stem cell concentration was 375.3 CFU-GM per mL (154.6 to 406.0 CFU-GM per mL) and 146.4 CFU-GEMM per mL (4.7 to 601.6 CFU-GEMM per mL). As shown in Table 1, mononuclear cells including colony forming units (CFU) were collected by seven successive leukaphereses. The total number of MNC harvested so far was 55.2 x 10^6; that of CFU-GM, 15.1 x 10^6 or 4.34 x 10^6 CFU-GEMM. After a mean cryopreservation time of 74 days (63 to 93 days), the total number of MNC recovered was 51.9 x 10^6, that of CFU-GM 18.8 x 10^6 or 9.3 x 10^6 CFU-GEMM entailing an overall MNC recovery of 94% and a CFU recovery of > 100%. The number of MNC and of CFU-GM and CFU-GEMM transfused per kilogram of body weight is given in Table 1.

Reconstitution of leukocytes, platelets, and red blood precursor cells after AB SCT. Blood cell reconstitution after AB SCT occurred very rapidly and reached 1,000 leukocytes/μL on day 9, 500 polymorphonuclear cells/μL, and 50,000 platelets/μL on day 10 (Fig 1). Reticulocytes were first noted on day 11 (0.8 percent) increasing to 460/00 on day 18 (normal range, 0.5 to 1.5 percent of red blood cells). Platelets were substituted only twice. All three cell lines (reticulocytes not shown in Fig 1) exhibited a cellular

overshoot in the early regeneration phase. At the first examination on day 8 after AB SCT, peripheral blood T cells were already within the normal range. However, the circulating B cell concentration showed a steady increase, eventually reaching normal values at day 34 (Fig 2).

Reconstitution of hemopoietic precursor cells after AB SCT. As shown in Fig 3, blood CFU-GM reappeared on day 5 preceded by an initial peak on day 1, presumably due to the transfusion of precursor cells with the stem cell graft. The curve peaked on day 14, exhibiting an enormous overshoot of about 70 times the pretreatment value. Marrow-derived CFU-GM reappeared later on day 7 after AB SCT without showing an overshoot phenomenon. CFU-GEMM showed up briefly in the peripheral blood, but reached a plateau in the marrow at a lower normal range from day 40 on.

DISCUSSION

Transplantation of circulating stem cells may have advantages over the use of marrow derived stem cells such as:

Fig 1. Kinetics of recovery of leukocytes, granulocytes, and platelets in the peripheral blood after AB SCT.
Fig 2. Kinetics of recovery of T and B cells in the peripheral blood after ABSCT (day of ABSCT = day 0).

(1) in patients at risk for general anaesthesia, continuous-flow apheresis offers an alternative and safe way for stem cell harvest;

(2) stem cell harvest is feasible in case of damage to the marrow collection site by previous radiotherapy or tumor involvement;

(3) hemopoietic reconstitution after myeloablative treatment seems to be more rapid for the WBC line, and therefore the aplasia-related risks in the early posttransplant period are lowered;

(4) the ratio between normal hemopoietic stem cells and clonogenic tumor cells in the peripheral blood of patients with malignant lymphohemopoietic disorders in remission might be in favor of the former, a hypothesis yet to be proven.

From the clinical point of view, blood stem cell transplantation to restore hemopoietic function after myeloablative treatment has to fulfill two major requirements: (1) hemopoietic reconstitution (including the lymphopoietic system) must be complete; and (2) the reconstituted hemopoietic function must be permanent.

In an extensive clinical study, Goldman et al were the first investigators to have clearly shown that blood-derived hemopoietic stem cells harvested in the chronic phase of patients with chronic myelogenous leukemia (CML) can re-establish hemopoietic function after myeloablative treatment for blast crisis.10 Unfortunately, most stem cells so far collected and eventually transfused originate from a Ph1-positive tumor cell clone, ie, their repopulating capability does not necessarily mean that "normal" stem cells act the same way. We attempted to make a step forward to "normality" by using stem cells collected in a transient chemotherapeutically induced Ph1-negative phase.11 Those normal Ph1-negative blood-derived stem cells completely restored hemopoietic function without reappearance of the Ph1 chromosome. Unfortunately, the observation time after ABSCT was too short to be able to draw definite conclusions about the character and quality of the transfused stem cell clones. One attempt has been made to use syngeneic blood-derived stem cells for marrow repopulation. In this case, hemopoietic function was incomplete and has therefore failed so far, because of insufficient CFU-GM numbers transfused.12 On the other hand, fatal graft v host disease following blood transfusions from normal donors into immunosuppressed patients has been reported, indicating allogeneic engraftment of accidentally transfused hemopoietic precursor cells.13 Furthermore, the rate of successful marrow engraftment in patients with aplastic anemia is probably increased by the additional transfusion of "buffy coat" cells in terms of stem cell support.14

The present case provides clear evidence that complete
hemopoietic reconstitution can be achieved after myeloabla-
tive treatment using normal blood-derived stem cells includ-
ing the lymphopoietic system. The minimal number of
CFU-GM per kilogram body weight (based on canine trans-
plantation data) needed for safe hemopoietic reconstitu-
tion was exceeded by a factor of two to five.\textsuperscript{15,16} The high yield of
leukapheresis-derived hemopoietic precursor cells reflects an
expansion of the blood stem cell pool at the time of stem cell
harvest. Since leukapheresis started just 2 weeks after com-
pletion of chemotherapy, stem cell harvest was probably
performed during a chemotherapy-induced blood stem cell
overshoot as described first by Richman et al.\textsuperscript{17} On the other
hand, blood stem cell concentration before initial chemother-
apy and ABSCT was found to be at relatively normal level.

It is noteworthy that hemopoietic reconstitution occurred
very rapidly, due to the high numbers of hemopoietic precursor
cells transfused. The question whether blood-derived human stem cells exhibit a restorative advantage over mar-
row-derived ones is still speculative. From animal studies some evidence for a difference in the proportion of
CFU-GM to pluripotent stem cells in cell suspensions
obtained from blood and bone marrow. In canine studies, Appelbaum\textsuperscript{18} calculated that for a successful engraftment,
2.4 times as many CFU-GM were required from autologous
bone marrow than from peripheral blood, indicating a rela-
tively higher pluripotent stem cell number per CFU-GM in
blood than in bone marrow.\textsuperscript{18} Evidence for a higher restor-
ative potential of blood-\textsuperscript{\textsubscript{\textgamma}} bone-marrow-derived precursor
cells was described in the canine model for lymphopoiesis\textsuperscript{18} as
well as for lymphopoiesis and granulocytopoiesis.\textsuperscript{19}

The potential of circulating stem cells to sustain hemo-
poiesis after myeloablative treatment and ABSCT is well
documented in dogs. Blood stem cell transfused dogs were
observed for as long as 3 years, after which they were killed
and found to have a well-functioning hemopoiesis.\textsuperscript{19} Our
patient's blood and marrow cellularity is in the normal range
for more than 7 months after ABSCT. Nevertheless, a
long-term follow-up is needed to judge the quality of the
blood-derived graft.

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