Result of Attempted Hematopoietic Reconstitution Using Isologous, Peripheral Blood Mononuclear Cells: A Case Report

By R. A. Abrams, D. Glaubiger, F. R. Appelbaum, and A. B. Deisseroth

In order to test the effect of peripheral blood mononuclear cell infusions on hematopoietic recovery in man we intensively leukopheresed a normal identical twin and obtained $9.8 \times 10^9$ peripheral blood mononuclear cells containing $4 \times 10^8$ CFU-C. These isologous cells were infused into his identical twin brother who had received 150 rad of total body irradiation and intensive combination chemotherapy as adjuvant therapy for Ewing's sarcoma. When compared to other patients receiving similar treatment, leukocyte recovery was accelerated by 3-4 wk, and occurred at a rate comparable to that induced by infusion of autologous cryopreserved marrow. Recovery of granulocytes, monocytes, and platelets was not accelerated. The low number of CFU-C present in the preparation used (one-eighth the number of CFU-C we usually obtain from bone marrow autograft collections) may have led to the pattern of hematopoietic recovery we observed in this patient.

The ability of infusions of human bone marrow cells to effect hematopoietic recovery in appropriate clinical settings is well documented. Although an operative procedure under general or regional anesthesia is necessary in order to collect the large numbers of bone marrow cells required (2.0-3.0 x $10^9$/kg of recipient), in allogeneic and isogeneic settings utilizing normal donors this requirement has not been of great concern. In autologous settings, however, the requirement for bulk bone marrow collection may be a more significant limitation for efforts at hematopoietic reconstitution. Bulk bone marrow collections are obtained in large part from the bony pelvis; if the pelvis has been compromised by tumor or prior radiotherapy, autologous marrow collection may not be possible. Additionally, patients with primary pulmonary neoplasms, such as small cell carcinoma of the lung, or pulmonary metastases may be judged to be at increased risk for the anesthesia required for these collections.

Observations regarding the presence of circulating hematopoietic stem cells in the peripheral blood of rodents, dogs, and humans have raised the question of whether circulating hematopoietic stem cells could be collected in numbers adequate to permit autologous, hematopoietic, reconstitution. Using continuous flow centrifugation techniques, large numbers of peripheral blood mononuclear cells (upwards of $1-2 \times 10^9$) have been collected from dogs and shown to be capable of rescuing animals from the otherwise hematopoietically lethal insults induced by high-dose cyclophosphamide or total body irradiation.

Recent work in the setting of human chronic myelogenous leukemia (CML) has further supported this possibility. Normally, the number of hematopoietic stem cells in human peripheral blood is very low as compared to marrow. However, in untreated chronic phase CML, the numbers of hematopoietic stem cells present in both the marrow and the peripheral blood are greatly expanded above normal levels. Goldman et al., using continuous flow centrifugation techniques, have been able to demonstrate that in this setting, autologous hematopoietic stem cells can be collected and cryopreserved, and subsequently used to effect hematopoietic reconstitution when ablative levels of combined modality therapy are used in an effort to control the accelerated phase of CML.

Other workers have shown that collections of peripheral blood mononuclear cells obtained from normal human volunteers also contain hematopoietic stem cells and have speculated that such collections would be capable of effecting hematopoietic reconstitution. If so, the relative ease with which such peripheral blood mononuclear cell collections could be obtained might well broaden the applicability and desirability of using hematopoietic reconstitution in association with intensive antineoplastic treatment.

The case report study we describe was undertaken in an effort to test the ability of collections of peripheral blood mononuclear cells to accelerate hematopoietic recovery in a clinical setting utilizing intensive myelosuppressive therapy.  

CASE REPORT AND METHODS

A 16-yr-old white male weighing 45 kg was referred to the Pediatric Oncology Branch of the National Cancer Institute (NCI) for management of his histologically documented Ewing's sarcoma that involved the left posterior ilium and sacral wing of his pelvis. Current treatment of such patients at the NCI consists of 5000 rad regional radiotherapy to a port designed to encompass both the entire bone containing the primary tumor and any soft tissue...
component associated with it. Two courses of systemic chemotherapy (vincristine 2 mg/m², 2.0 mg maximum dose; Actinomycin-D 2 mg/m², and cyclophosphamide 40 mg/kg all given i.v. on day 1 of each cycle) are given, one at the start of radiotherapy and the second 4 wk later. Following this therapy, whenever possible, autologous bone marrow is harvested in anticipation of combined modality intensive therapy with fractionated total body irradiation (TBI) and systemic chemotherapy. In this patient, marrow could not be harvested because most of the pelvic sites from which bone marrow cells are usually aspirated for storage were encompassed within the radiotherapy ports required for treating his primary tumor.

This patient was fortunate, however, in having a healthy identical twin brother who was willing to serve as a donor of hematopoietic stem cells. Their relationship as identical twins was confirmed by physical appearance, report of a common placenta at birth, and serologic identity of HLA loci and red blood cell surface antigen phenotypes.

At our institution, patients with primary Ewing's sarcoma involving the torso receive the following combined modality intensive therapy after the completion of initial treatment. The first half of the combined modality intensive therapy consists of fractionated total body irradiation (TBI) given in 10 equal fractions over 5 wk to a total midplane dose of 150 rad (i.e., 15 rad/fraction, 2 fractions/week for 5 wk). The second half of the intensive therapy consists of intravenous chemotherapy. The chemotherapy is not begun until 7–10 days following the last TBI fraction and consists of cyclophosphamide 40 mg/kg on days 1 and 2, doxorubicin (Adria-myacin) 35 mg/m² on days 1 and 2, imidazole carboxamide (DTIC) 250 mg/m² on days 1, 2, and 3, and vincristine 2.0 mg/m² (maximum of 2.0 mg) on days 1 and 8.

During the 5 wk required to administer the TBI of the intensive combined modality therapy to our patient, his normal twin underwent leukapheresis on three occasions, utilizing an Aminco continuous flow centrifuge apparatus. Collections were obtained at a bowlspeed of 1000 rpm. Using the white cell pump of the centrifuge, mononuclear cells were collected at a rate of about 4.0–5.0 x 10⁸/min. The location of the leukocyte–platelet interface between the plasma and red cell layers was positioned so that the hemoglobin content of the collected mononuclear cell suspension was kept at about 3.0–3.5 g/dl. Blood flow between the donor and the centrifuge was maintained at 40 ml/min, and the mononuclear cells were collected into a 600-mI Fenwal transfer pack to which had been added 2500 U of preservative-free heparin and 10 ml of acid citrate dextrose-NIH solution A. These three collections of mononuclear cells were cryopreserved using previously described techniques.17,18

Beginning 24 hr after our test patient received the last dose of DTIC in the intensive regimen, 5 additional collections of peripheral blood mononuclear cells were obtained (one daily) from his normal twin using the same technique as outlined above. These 8 collections (5 fresh, 3 cryopreserved) were infused on days 4–11 following the start of the intensive chemotherapy, as shown by the vertical arrows in Fig. 1. Data concerning the volume and cellular composition of these collections are summarized in Table 1.

CFU-C (granulocyte-macrophage precursors) were measured on 7 of these 8 collections in an effort to assess their hematopoietic stem cell content. On the cryopreserved collections, post-freeze CFU-C measurements were obtained using previously described techniques19 for thawing and diluting.

CFU-C were plated utilizing human placental conditioned medium in association with single layer agar suspensions without under layers. This methodology was adapted directly from the work of Burgess et al. Plating tubes were prepared using the following reagents which, after mixing, were maintained at 38°–39°C in a Sybron Thermolyne Dri-Bath: 1.000 ml of 0.75% agar (Bacto medium in association with single layer agar suspensions without under layers. This methodology was adapted directly from the work of Burgess et al. Plating tubes were prepared using the following reagents which, after mixing, were maintained at 38°–39°C in a Sybron Thermolyne Dri-Bath: 1.000 ml of 0.75% agar (Bacto

| Table 1. Mononuclear Cell Collections |
|-----------------------------|-------------|---------------------|----------------|----------------|
| Collection     | Volume (ml) | Hb (g/dl) | WBC/cu mm | Plates/ cu mm | Total Platelets |
| 1             | 500         | 3.4       | 37,200    | 1,900,000     | 9.5 x 10¹¹     |
| 2             | 365         | 3.5       | 29,000    | 1,500,000     | 5.5 x 10¹¹     |
| 3             | 400         | 2.5       | 31,400    | 1,050,000     | 4.2 x 10¹¹     |
| 4             | 440         | 2.6       | 33,000    | 1,380,000     | 6.1 x 10¹¹     |
| 5             | 495         | 2.7       | 24,400    | 830,000       | 4.1 x 10¹¹     |
| 6             | 476         | 3.9       | 29,500    | Not done      | 3.7 x 10¹¹     |
| 7             | 480         | 4.5       | 28,000    | 776,000       | 2.8 x 10¹¹     |
| 8             | 475         | 3.9       | 22,700    | 576,000       | 2.8 x 10¹¹     |

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<th>Percent Lymphocytes and Monocytes (Mononuclear Cells)</th>
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<td>Collection</td>
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Total mononuclear cells collected = 9.8 x 10¹⁰
RESULTS

Infusion of the isologous peripheral blood mononuclear cells was begun 24 hr after the last dose of DTIC (day 4, Figs. 1 and 2). CBC obtained immediately before and after each infusion revealed increments in hemoglobin, white count and platelets. However, of these, only the platelet increments were sustained from day to day during the period of mononuclear cell infusions (Fig. 1B). Thereafter, platelet counts declined, and platelet support was required through day 53 (Fig. 1B).

In order to evaluate the impact of the peripheral blood mononuclear cell infusions on hematopoietic recovery in our patient, the kinetics of his leukocyte, granulocyte, and platelet recovery were compared to those of 7 other patients with Ewing's sarcoma—all of whom underwent the same combined modality intensive therapy. Five of these patients were managed with infusions of autologous cryopreserved marrow given on day 4 or days 4 and 5 following the start of the chemotherapy segment of the intensive therapy (day 4, Fig. 2). The other 2 patients were managed without efforts aimed at accelerating hematopoietic recovery.

Beginning on day 11 and with a transient decline that was temporally related to the last dose of vincris-
RECONSTITUTION WITH PERIPHERAL CELLS

tine, our test patient’s total leukocyte recovery was comparable to that seen in patients given autologous marrow infusions (Fig. 2A) and was 3–4 wk faster than that seen in the 2 patients in whom hematopoietic reconstitution was not attempted (Fig. 2B). In contrast, as shown in Fig. 1A, 2C, and 2D, the granulocyte recovery of our test patient was not accelerated and his early leukocyte recovery consisted entirely of lymphocytic cells. Moreover, although patients managed with marrow demonstrated continued recovery of leukocyte, granulocyte, lymphocyte, and platelet counts towards normal levels, our test patient did not.

Eight weeks after the infusion of peripheral blood mononuclear cells was begun, the experiment was terminated by the infusion of 1.33 × 10^10 nucleated bone marrow cells obtained from the normal twin. During the ensuing 4 wk, our test patient recovered all counts to normal levels. A bone marrow aspirate (from the sternum) and a bone marrow biopsy (from the pelvis) were obtained from our patient 2 wk prior to the bone marrow infusion. They were consistent with each other and revealed extreme hypocellularity and markedly diminished numbers of erythroid, granulocytic, and megakaryocytic cells. A sample of the sternal aspirate was found on CFU-C assay to produce no observable colonies.

As shown by the data in Table 2, the number of CFU-C present in the peripheral blood collections obtained from this donor ranged from 0.2 to 9.8/10^6 mononuclear cells. These relatively low values are within the range of CFU-C found in the peripheral blood of normals by other workers. On the 3 cryopreserved collections, the CFU-C appeared to survive the process of freezing and thawing (Table 2), and the median number of CFU-C present in these 8 collections was 3.0/10^6 mononuclear cells using either the prefreeze or the postfreeze CFU-C results from the 3 cryopreserved collections. Due to the slight differences observed between prefreeze and postfreeze CFU-C measurements, the mean number of CFU-C could be calculated as either 3.7 or 4.1/10^6 mononuclear cells. Since the total number of mononuclear cells collected was 9.8 × 10^9, we calculated the total number of CFU-C in these collections to be between 3.6 and 4.0 × 10^5.

**DISCUSSION**

In this report we have documented in man that infusion of a large dose (9.8 × 10^9) of isologous peripheral blood mononuclear cells was capable of accelerating total leukocyte recovery by 3–4 wk following the administration of combined modality therapy. Recovery of peripheral blood granulocyte and platelet counts, however, was not accelerated by the infusion of these peripheral blood mononuclear cells, and isologous bone marrow infusion was ultimately utilized to restore peripheral blood counts to normal levels in this patient. The median number of CFU-C present in these collections was 3/10^6 peripheral blood mononuclear cells. Since our usual range of CFU-C values from marrow is 200–500/10^6 nucleated marrow cells, it is likely that our failure to observe accelerated myeloid and thrombocyte recovery after the peripheral blood cell infusions was directly related to the relative paucity of hematopoietic stem cells (as reflected by CFU-C measurement) contained in these infusions as compared to bone marrow.

It is not likely that there was any intrinsic defect in the hematopoietic stem cells of the donor. He had normal circulating blood counts and recovered his counts promptly following leukapheresis. Moreover, his marrow cells (in contrast to his peripheral blood cells) were quite effective in prompting complete hematopoietic recovery in his twin brother.

The significance of the circulating CFU-C is largely unknown, and the suggestion has been made that there may be significant qualitative differences between bone marrow CFU-C and peripheral blood CFU-C. The minimum number of bone marrow CFU-C required to effect reconstitution is also unknown, but the number usually transfused with marrow as observed in our laboratory is about 6–7 × 10^6/kg—approximately eightfold more than we were able to collect from the peripheral blood of this donor utilizing intensive leukapheresis. Autologous marrow is usually administered in 1 or 2 closely-spaced transfusions. The extent to which administration of total stem cell doses in small aliquots over periods of time longer than 24–36 hr might adversely impact on hematopoietic recovery is uncertain. Whether CFU-C numbers are an accurate reflection of pluripotential hematopoietic stem cell numbers in the peripheral blood is also unclear, although such a correlation has been suggested for CFU-C measurement on human bone marrow preparations.
The acceleration of lymphocyte recovery that occurred following peripheral blood reconstitution in the absence of a measurable effect on granulocyte and platelet recovery suggests that lymphocyte precursors may be distinct from other hematopoietic precursors. This possibility is consistent both with clinical observations in aplastic anemia as well as with in vitro observations on hematopoietic stem cells. We observed an increase in peripheral blood CFU-C numbers in our donor during the week that he was leukapheresed 5 times (Table 2). This result has been previously observed by other workers, and its significance is presently unclear.

These findings extend the report by Hershko et al. in which isologous peripheral blood mononuclear cells were infused into a patient with paroxysmal nocturnal hemoglobinuria and aplastic anemia. In their experiment, no impact on peripheral blood leukocyte numbers was observed following the infusion of isologous peripheral blood mononuclear cells. This difference is most probably related to the fact that immunosuppressive therapy was not employed in their report and their patient-recipient had substantial numbers of circulating endogenous lymphocytes. In contrast, our patient had his lymphocyte count, as well as granulocyte and platelet counts, suppressed by intensive myelosuppressive therapy. Both our experience and that of Hershko et al. suggest that the clinical use of peripheral blood mononuclear cell transfusions with the intent of hematopoietic reconstitution should be approached with caution.

REFERENCES

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