Enhancement of Hemopoietic Recovery After Bone Marrow Transplantation With the Addition of Nucleated Blood Cells in Mice

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Recovery of bone marrow cellularity, CFU-C, and CFU-S were studied sequentially over 90 days after syngeneic bone marrow transplantation in mice. A minimal cell dose of $2 \times 10^8$ bone marrow cells was given. At day 28 after transplantation, CFU-C reached more than 50% of the normal range whereas the CFU-S concentration was less than 15%. Normalization of CFU-S occurred at day 90. The effect of the addition of peripheral blood nucleated cells on bone marrow hemopoietic recovery was studied at day 28. The augmentation of CFU-C and CFU-S recovery were dose dependent. Optimal enhancement was seen with bone marrow to blood ratios of 1:1 and 1:2.5. This enhancement effect was lost when nucleated blood cells in a ratio of 1:10 were administered.

The addition of peripheral blood mononuclear cells to bone marrow cells lead to an enhancement of bone marrow engraftment in DLA matched canine litter mates and unrelated dogs. The phenomenon could be reproduced with the addition of thoracic duct thymocytes. The same principle has been applied to patients with aplastic anemia who have been previously transfused and thus run a great risk of rejecting their transplanted bone marrow. The combination has led to 70% survival in this patient population; previously achieved survival was 40%

The purpose of this study was to investigate the effect of the addition of various cell concentrations of blood cells on recovery of femoral and splenic CFU-C and CFU-S. We established a transplantation model for this study using a minimal cell dose of bone marrow cells and followed hemopoietic recovery over a 90-day period.

MATERIALS AND METHODS

Male BDF, mice, 8–10 wk old, were used for all experiments (Simonson Laboratory, Calif.)

Preparation of Bone Marrow Cells

Donor mice were sacrificed by cervical dislocation, and both femora dissected free. The distal femur tip was cut with scissors, and 2 ml of Hanks' balanced salt solution (HBSS) was forcefully injected through the needle into the proximal end of the femur, and the expelled marrow was collected and passed through a stainless steel filter.

Preparation of Peripheral Blood

Approximately 1 ml of peripheral blood was collected from retroorbital space of each mouse and anticoagulated with preservative-free heparin (Fisher, Pittsburgh, Penn.). Whole blood was diluted with HBSS to obtain the required concentration of white cells.

Preparation of Spleen Cells

Spleens were taken from three donor mice in a group and repeatedly aspirated through 22 and 25-gauge needles to achieve single cell suspensions. Cells were prepared in HBSS and passed through a fine mesh filter to remove tissue debris.
Colony Forming Units in Culture (CFU-C) Assay

Colony forming units were cultured according to modification of the method described by Metcalf.14 Marrow was harvested and diluted in HBSS. Double strength α-MEM (alpha modification of minimum essential medium, K. C. Biological, Lanexa, Kans.) was added to an equal volume of 0.6% bactoagar (Difco Laboratories, Detroit, Mich.). One hundred thousand cells of bone marrow and three hundred thousand spleen cells were plated per dish. Colony formation was stimulated by fibroblast conditioned medium.15

Colony Forming Units in Spleen (CFU-S) Assay

Recipient mice were irradiated with 1050 rads from a Cobalt 60 source. The focus to target distance was 130 cm and the dose rate 24 rads per minute. The following cell doses were injected into irradiated mice: 2.5 and 5 x 10⁶ of bone marrow cells, and 3 and 6 x 10⁶ of spleen cells.16

Bone Marrow Transplantation

Bone marrow cells were injected into the tail vein of supralethally irradiated animals. The mice were sacrificed at various time points from 1 wk to 90 days posttransplantation, and their femora and spleens were examined for the content of nucleated cells, CFU-S, and CFU-C.

Statistical Analysis

The different experimental groups are compared by the Student-Newman-Keuls procedure and the Chi-Square test.17

RESULTS

Figure 1 shows the effect of different doses of transplanted bone marrow cells on hemopoietic recovery measured 7 days after rescue from supralethal irradiation (1050 rads). Doses of 2 x 10⁵, 5 x 10⁵, and 2 x 10⁶ marrow cells were tested. The minimal dose of bone marrow to rescue 100% of supralethal irradiated mice under these experimental conditions was determined to be 2 x 10⁵. A tenfold increase in the dose injected does not lead to normalization of bone marrow cellularity, CFU-C, and CFU-S 1 wk postirradiation. To further characterize the hemopoietic recovery of nucleated cells, CFU-S, and CFU-C per femur, the mice were followed for up to 90 days posttransplantation (Fig. 2). Groups of four animals were sacrificed at day 14, 21, 28, 60, and 90. At day 21 and 28, near normal CFU-C levels were reached, and the bone marrow cellularity was 50% of normal on one mouse. The CFU-S concentration, at this time point, was less than 15% of the normal controls.

Figure 3 shows the results of the experiment where nucleated blood cells were added to bone marrow cells at a ratio of 1:0, 1:1, 1:2.5, and 1:10. The CFU-S per 10⁵ injected cells were 13 ± 2.1, 12.8 ± 1.79, 21.2 ± 1.2, and 8.6 ± 2.07. Group 3 was significantly different from Group 1 and 2 (p < 0.001). The CFU-S calculated per femur revealed 623.3 ± 100, 816 ± 115, 1208 ± 73, and 648 ± 153. Group 2 and 3 were significantly different from Group 1 and 4. There was not a statistical difference between Group 1 and 4. The CFU-C per femur were determined simultaneously and 3935 ± 303, 7585 ± 217, 9575 ± 674, and 4522 ± 222. Group 2 and 3 were significantly different from Group 1 and 4.

Figure 4 shows the effect of the addition of nucleated blood cells on recovery of CFU-C and CFU-
S by day 28 in the spleen from the same experimental animals. The CFU-S value per spleen were 546 ± 80, 1298 ± 149, 482 ± 229, and 462 ± 228. Group 2 was statistically different from Group 1, 3, and 4 (p < 0.001). Simultaneous CFU-C determination revealed 3948 ± 235, 11,687 ± 525, 5908 ± 856, and 3425 ± 170. Group 2 and 3 are significantly different from Group 1 and 4 (p < 0.001, p < 0.005).

DISCUSSION

The addition of blood nucleated cells to bone marrow lead to consistent improvement of hemopoietic recovery after supralethal irradiation. This helper effect of blood cells appears to be dose dependent with an optimal ratio of 1:1 for recovery of CFU-S and CFU-C per spleen and 1:2.5 in recovery of CFU-S and CFU-C per femur. Beneficial effect of the addition of lymph node cells to bone marrow in supralethally irradiated mice given syngeneic bone marrow in supralethally irradiated mice given syngeneic bone marrow has been reported by Loutit. These early investigations showed improved survival and weight gain when lymph node cells were given. The addition of lymph node cells to syngeneic fetal liver cells led to faster recovery of cellularity and cellular composition of the lymphoid tissues and to a decrease in protracted infections seen with animals reconstituted by stem cell preparations alone.

In establishing the pattern of hemopoietic recovery after a minimal cell dose of bone marrow, we found, at various time points, a tendency towards differentiation. This favored the production of mature nucleated bone marrow cells and granulocyte macrophage precursors over reconstitution of the CFU-S compartment. Normalization of the CFU-S level was reached not earlier than by day 90.

The addition of thymocytes to syngeneic bone marrow led to an increase in number and size of splenic colonies in irradiated mice. The addition of blood nucleated cells in our experiments did not lead to an increase in colonies in the CFU-S assay by day 10. It should be noted that the absolute number injected (3 × 10⁴ to 3 × 10⁵) was 1 to 2 log lower than the experiments of Basford and Goodman. However, even at these low cell numbers, we could observe significant increase of CFU-S and CFU-C at day 28 after transplantation. The ratios of 1:1 and 1:10 were chosen because they lie within the range that can be obtained in clinical bone marrow transplantation. The implication of these findings for human bone transplantations are of considerable importance if one considers the very variable methods of bone marrow procurement in man. It has been shown by Gale and Fauci that the contamination of collected bone marrow cells with
peripheral blood cells increases significantly with larger aspiration volumes.\textsuperscript{18,19} Contamination with blood cells has been recognized as a potential problem in allogeneic transplantation since peripheral blood cells have a significantly higher capacity to induce graft versus host disease than bone marrow cells.\textsuperscript{20,21} It could be of importance for autologous transplantation to standardize the bone marrow aspiration procedure to be able to evaluate if different degrees of contamination with blood cells influence hematopoietic recovery and if potential benefits can be achieved with addition of a leukocyte to autologous transplantation in man.

The observed increased effectiveness of peripheral blood nucleated cells over thymocytes might be due to potential synergism between various blood populations. Synergism between T lymphocytes and macrophages has been documented in the production of colony-stimulating factor by Verma et al. who demonstrated that this CSA release was optimal at macrophage to T cell ratios of 1:3.\textsuperscript{13} Increased addition of T cells lead to a decrease in CSA production. It is possible that this phenomenon is due to a suppressor cell activity non-effective in lower concentrations.

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