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B. Lukomska
M. Durlik
E. Cybulska
W. L. Olszewski

Comparative analysis of immunological reconstitution induced by vascularized bone marrow versus bone marrow cell transplantation

B. Lukomska (✉) · M. Durlik · E. Cybulska · W. L. Olszewski
Department for Surgical Research and Transplantology, Medical Research Center Institute, Polish Academy of Sciences, 5, Chalubinskiego Street, 02 004 Warsaw, Poland

Abstract We have reported previously that vascularized bone marrow transplantation (VBMT) in an orthotopic hind limb graft brings about complete repopulation of bone marrow cavities in lethally irradiated syngeneic recipients within 10 days. Intravenous infusion of an equivalent volume of bone marrow cell suspension was evidently less effective. The purpose of this study was to investigate the reconstitution of immunocompetent compartments of lethally irradiated syngeneic rats after VBMT. Lewis rat hind limbs were transplanted orthotopically into irradiated recipients. Ten days after irradiation and bone marrow transplantation, bone marrow, mesenteric lymph nodes, and sera from rats were harvested. Mesenteric lymph node lymphocytes were analyzed. The responsiveness fo mesenteric lymph node lymphocytes (MLNL) to mitogens and cell proliferation in the presence of sera and bone marrow cell (BMC) culture supernatants were measured. Our studies have shown that vascularized bone marrow transplantation brings about rapid replenishment of lymphoid organs of lethally irradiated syngeneic recipients. The repopulating subsets are fully responsive to mitogens. Sera from reconstituting rats had no effect on the proliferation of mature lymphocytes. Intravenous infusion of a number of BMC in suspension equivalent to that grafted in hind limb transplant was less efficient in reconstitution of lymphoid tissue.

Key words Lymphopoiesis · Bone marrow transplantation · Mitogenic response · Stromal cells

Introduction

Immunodeficiency after bone marrow transplantation (BMT) is one of the major problems encountered in bone marrow recipients. The chemoradiotherapy used to prepare marrow graft recipients ablates immune cells to permit engraftment of hematopoietic elements. Although the immunodeficiency is created under controlled circumstances, bone marrow transplanted patients can experience life-threatening infection with bacterial, viral, and fungal antigens, especially in terms of the first 3 months postgrafting. Deppresion in the generation of the immunological response against different pathogens has been shown to correlate with impaired myelopoiesis. Recent observations provide evidence that the immune system regulates hemopoiesis. Lymphocytes seem to be required for the optimal growth of bone marrow progenitor cells [7, 8, 14]. Therefore the study of the recovery process of the immune system in such bone marrow graft recipients is of great interest.

We have reported previously that vascularized bone marrow transplantation (VBMT) in an orthotopic hind limb graft brings about complete repopulation of bone marrow cavities in lethally irradiated syngeneic recipients within 10 days [5]. Intravenous infusion of an equivalent volume of bone marrow cells (BMC) was evidently less effective. The question arises whether

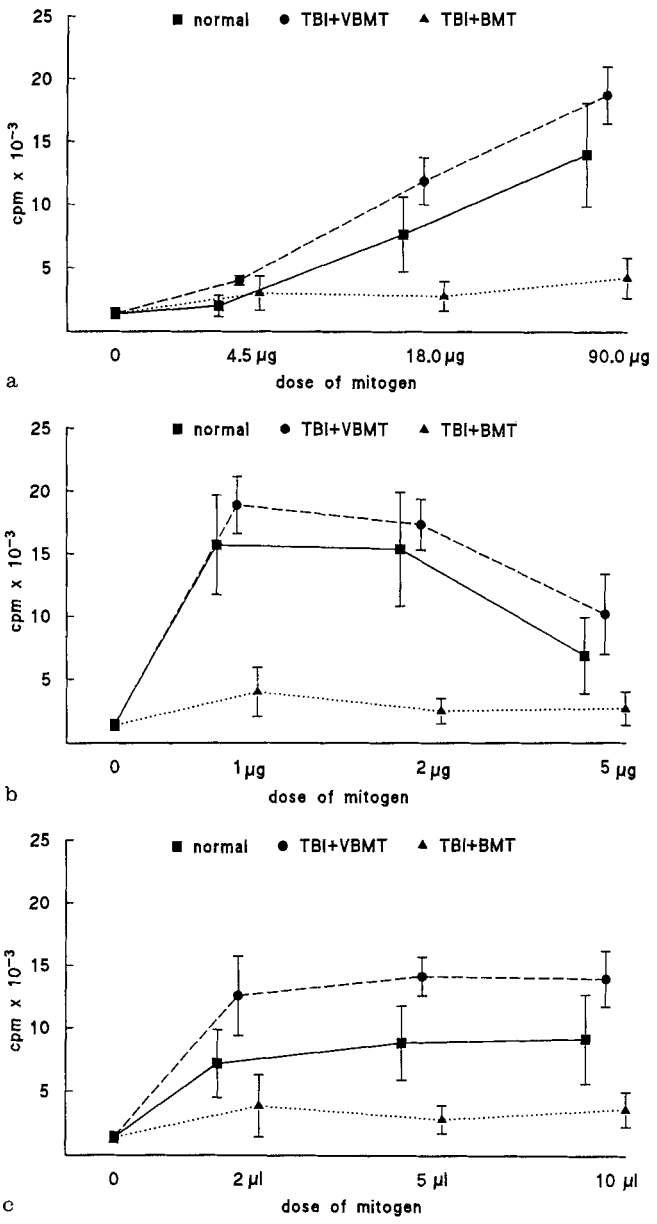

Fig. 1 Responsiveness of mesenteric lymph node lymphocytes (MLNL) to different doses of mitogens: **(a)** phytohemagglutinin (PHA); **(b)** concanavalin A; **(c)** pokeweed mitogen (mean values ± SD, $n = 6$), *TBI* total body irradiation, *VBMT* vascularized bone marrow transplantation, *BMT* bone marrow transplantation)

VBMT also promotes rapid replenishment of lymphoid organs.

The aim of the study was to investigate the reconstitution of immunocompetent compartments of lethally irradiated syngeneic rats after VBMT.

Materials and methods

Animals

Three-month-old male Lewis rats (RT1l) bred and maintained in our own animal facility, were used throughout this study. Recipients were exposed to 8 Gy of r irradiation from a ^{60}Co source (Theratron) at a dose rate of 150 cGy/min.

Hind limb transplantation

The donor hind limb was amputated at the groin level with a long vascular stump. The hind limb of the recipient was amputated at the mid-thigh level. End-to-end anastomoses of the graft and recipient vessels were performed with the use of 10-0 monofilament sutures. The stumps of sciatic nerves were stiched. Femurs were anastomosed with an intramedullary metallic stent.

Experimental design

Group I: Total body irradiation (TBI) was followed by transplantation of a syngeneic hind limb. Group II: TBI was followed by i.v. infusion of syngeneic BMC ($6 \times 10_7$). Group III: TBI without subsequent treatment. Normal non-treated rats served as a control.

Cell collection

Ten days after irradiation and transplantation, BMC, mesenteric lymph node lymphocytes (MLNL), and peripheral blood were harvested.

Condition supernatants

Bone marrow cells were grown for 24 h in D-MEM medium supplemented with 15 % fetal calf serum and antibiotics. Supernatants were passed through a 0.2-µm filter and stored at – 70 °C.

Proliferation assay

Responsiveness of MLNL isolated from different experimental groups to the mitogens phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) was measured in 72-h cultures. The effect of sera and BMC supernatants on normal MLNL responsiveness to PHA (90 µg/ml) was studied in a 72-h assay. Incorporation of methyl [^{3}H]-thymidine, added 18 h before cell harvesting, was measured in a Beckman liquid-scintillation radiation counter.

Results

Histological analysis

Group I. Ten days after TBI and hind limb transplantation, the normal cellular pattern was observed in mesenteric lymph nodes (MLN). The cortex contained lymphoid follicles with active germinal centers. Group II. After BMC infusion, MLN remained largely depleted of lymphocytes. Dilated sinuses filled with erythrocytes

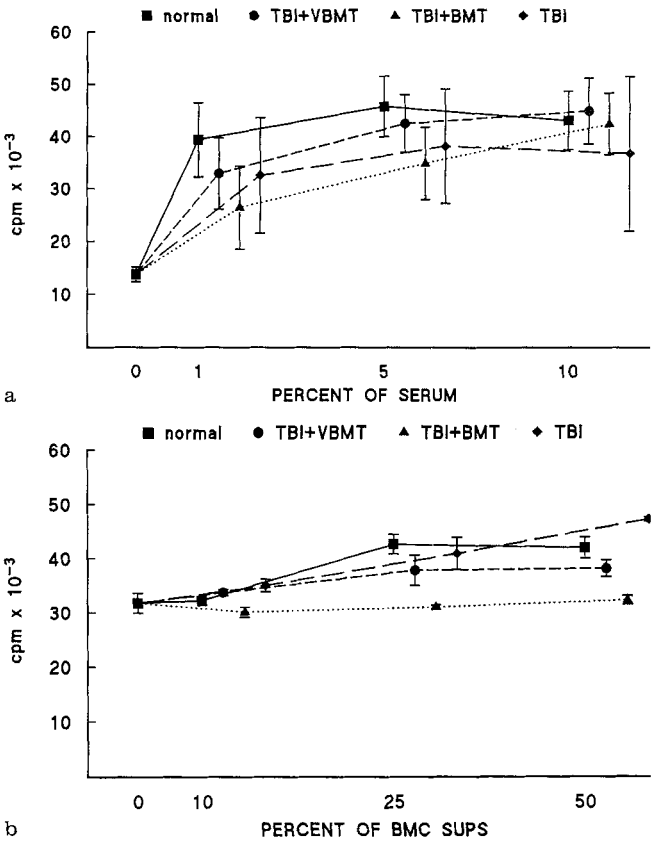

Fig. 2 Responsiveness of normal MLNL to PHA (90 μg/ml), **(a)** in the presence of sera from graft recipients; **(b)** in the presence of bone marrow cell *(BMC)* culture supernatants from graft recipients (mean values ± SD, $n = 6$)

and macrophages were observed. Group III: TBI rats left without any treatment presented a picture of total lymphoid depletion in MLN. No signs of regeneration were observed 10 days after TBI.

Cell yield

The MLNL yield of TBI rats with VBMT was $37.0 \pm 3.6 \times 10^7$/g of tissue on day 10 compared with $76.5 \pm 16.1 \times 10^7$/g in normal non-irradiated rats. In group II, the MLNL yield was found to be 80 % lower ($12.8 \pm 2.6 \times 10^7$/g of tissue). Total body irradiation in non-transplanted rats brought about a decrease of lymphocyte number to $1.4 \pm 1.2 \times 10^7$/g of the MLN.

Responsiveness to mitogens

Ten days after VBMT, the responsiveness of MLNL to PHA (4.5; 18.0; 90.0 μg) was comparable with control values (2026 ± 321 vs 2016 ± 840; 11871 ± 1867 vs 7665 ± 2958; 18767 ± 2280 vs 14011 ± 4134 cpm, respectively). Infusion of BMC revealed low PHA stimulation of MLNL (3020 ± 1367; 2806 ± 1168; 4256 ± 1580 cpm, at the different doses of mitogen) (Fig. 2a). Concanavalin A and PWM stimulation showed similar patterns of MLNL responsiveness to that of PHA. Proliferation of MLNL in the presence of Con A (1.0; 2.0; 5.0 μg) 10 days after VBMT reached normal rat MLNL levels (18895 ± 2280 vs 15708 ± 3973; 17322 ± 2028 vs 15350 ± 4549; 10150 ± 6878 vs 6878 ± 3030 cpm, respectively). Transplanted BMC suspensions gave lower values of MLNL responsiveness to 1.0, 2.0, and 5.0 μg of Con A (3995 ± 1929; 2503 ± 983; 2708 ± 13030 cpm, respectively). The responsiveness of MLNL from VBMT recipients to different doses of PWM (2.0; 5.0; 10.0 μl) was above that of control rats (12594 ± 3174 vs 7204 ± 2666; 14172 ± 1554 vs 8850 ± 2942; 13991 ± 2246 vs 9186 ± 3531 cpm, respectively). An infusion of BMC did not restore proliferation levels of MLNL to normal rat values (3878 ± 2431; 2836 ± 1095; 3665 ± 1367 cpm in the presence of 2.0, 5.0, and 10.0 μl of PWM, respectively) (Fig. 1).

Proliferation assay in the presence of sera and BMC culture supernatants

Sera and supernatants from cultured BMC of VBMT or BMT recipients had no effect on third-party MLNL cultured with PHA. The responsiveness of normal MLNL to PHA (90 μg/ml) in the presence of sera (10 v/v) isolated from VBMT, BMT, and TBI recipients did not differ from that of normal rat serum (44920 ± 14786; 42437 ± 5931; 36789 ± 14786 vs 43053 ± 5640 cpm, respectively). The presence of BMC culture supernatants (50 v/v) from VBMT, BMT, and TBI did not change the responsiveness of third-party MLNL to PHA (90 μg) in comparison to the effect of normal BMC culture supernatants (38106 ± 1541; 32249 ± 790; 47185 ± 385 vs 41930 ± 1946 cpm, respectively) (Fig. 2).

Discussion

The survival of patients undergoing BMT is critically dependent on the nature and rate of reconstitution of the immune system. Immunological recovery requires both a quantitative and a qualitative repopulation of lymphocytes.

Multipotential stem cells differentiate into hemopoietic or lymphoid progenitors [2, 4, 19]. Specialized microenvironments and specific cytokines direct the fate of these multipotent progenitor cells [3, 13]. In contrast to other hematopoietic cells, lymphocyte development and differentiation occurs nor only within bone marrow cavities but also within peripheral lymphoid compart-

ments such as lymph nodes. The significance of extrathymic T-cell differentiation is underscored by the fact that BMT results in recovery of T-cell function in the absence of thymus [16]. Terminal differentiation for T-lymphocytes is linked to L-selectin expression allowing migration to lymph node compartments [20]. The finding that stem cells express L-selectin raises the possibility that, following BMT, early T-cells may directly migrate to lymph nodes [18]. The results of our previous studies revealed that a large proportion of BMC transplanted in suspension or, in bone, as a vascularized bone marrow graft into lethally irradiated rats accumulates in the lymph nodes, spleen, and gut [12].

Our experiments described here showed that the ability of lymphocytes to localize into lymph nodes was significantly different in VBMT and BMT recipients. The fast repopulation of lymphoid compartments from hind limb graft remains in sharp contrast to the results obtained in rats infused with BMC suspension. It seems that transplantation of stromal cells present in the bone promoted lymphopoiesis. There is evidence that stromal cells support expansion of lymphoid cells by cytokine production [1, 11, 15]. Growth factors released into the circulation can exert an effect on lymphocyte replenishment and maturation. Clinically, BMC are frequently transplanted into recipients with functional insufficiency of stromal cells. The preparatory regimens for BMT such as radio- and chemotherapy damage the host stroma and bring about loss of synthesis of different regulatory molecules [9, 10]. Bone marrow cell inoculum transplanted intravenously contains only hematopoietic and not stromal cells. This could be reflected in scanty replenishment of lymphoid organs in irradiated recipients repopulated with BMC suspension. Bone marrow transplantation has been shown to be associated not only with a decline in lymphoid cell number but also with a deficit in various T-cell mediating functions [6, 17, 21]. The results of our studies revealed more rapid reconstitution of function of the immune system after VBMT than after BMT. The repopulating subsets of MLNL isolated from hind limb recipients were fully responsive to mitogen stimulation whereas a low MLNL proliferation rate was seen in rats receiving BMC suspension.

Cell proliferation assays are useful for estimating cytokine production. For that purpose, the effect of sera or BMC culture supernatants from transplanted rats on PHA-induced proliferation of MLNL isolated from normal rats was measured. Our studies have shown that sera and supernatants of reconstituted bone marrow cells had no effect on the proliferation of normal third-party lymphocytes. However, they could contain factors required for the mobilization of lymphoid progenitors and their accelerated differentiation.

The results of our studies clearly indicate that bone marrow cell transplantation in hind limb graft is highly effective in the replenishment of lymphoid compartments of lethally irradiated rats. Two mechanisms may be responsible for the fast repopulation of lymph nodes. One would be immediate seeding of lymphoid precursors from the transplanted bone marrow in the hind limb graft, the other, the release of cytokines from transplanted stromal cells, facilitating local proliferation of lymphocytes in the secondary lymphoid compartments.

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