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Tolerance induction following allogeneic vascularized bone marrow transplantation – the possible role of microchimerism

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Abstract We have noticed that bone marrow transplanted in a vascularized limb graft, providing a continuous supply of donor bone marrow cells (BMC), may prolong the survival time of a skin graft from the same donor. The question arises whether the microchimerism raised plays a role in the prolonged survival of skin allografts. The aim of the study was to follow the development of microchimerism after allogeneic vascularized bone marrow transplantation (VBMTx) concomitantly with the rejection process of transplanted skin. Brown Norway (BN) rats served as donors and Lewis rats as recipients of VBMTx and free skin flap allografts. A hind limb was transplanted, followed by a full-thickness skin graft on the dorsum. Cellular microchimerism was investigated in recipients of VBMTx and skin grafts in blood, spleen, mesenteric lymph node, and bone marrow with the monoclonal antibody OX27 directed against MHC class I polymorphic RT1 on BN cells and quantitatively analyzed in a FACStar. In the VBMTx group, the free skin flap survived 70 days after weaning off cyclosporine A (CsA). An intravenous infusion of BMC in suspension equivalent to that grafted in the hind limb did not prolong skin graft survival after cessation of CsA therapy. Donor-derived cells could be detected in VBMTx recipients as long 70 days after weaning off CsA but not in recipients of i. v. suspension BMC grafting.

Key words Tolerance · Allograft · Bone marrow · Microchimerism

Introduction

It has been suggested that microchimerism developing after allogeneic organ transplantation may be responsible for partial tolerance to MHC antigens [3, 5, 6, 11, 13]. There is evidence that dendritic cells and lymphocytes from transplanted organs migrate to recipient lymphoid organs and survive for considerable periods of time. Further evidence that microchimerism may play a role is that allograft tolerance has been achieved in many species following administration of donor bone marrow cells (BMC) to organ recipients [10, 12, 14, 15]. Recently, in humans also, organ allograft survival time has been prolonged in recipients of donor BMC [1, 9, 12]. There are also reports on the lack of correlation between microchimerism and tolerance [4, 14]. Irrespective of the controversies, one condition should be met for raising and maintaining microchimerism: a continuous supply of donor BMC to the recipient. This can be achieved by transplantation of a live source of donor BMC in the vascularized bone marrow in a limb graft [2, 7, 8]. The advantages of the model are maintaining the proliferative capacity of donor BMC in their own environment (stromal cells, hemopoietic cytokines) and continuous seeding of donor BMC and homing in recipient tissues. It is expected that chimerism raised in this fashion will create a state of decreased responsiveness to donor antigens and slow down rejection of the transplanted skin.

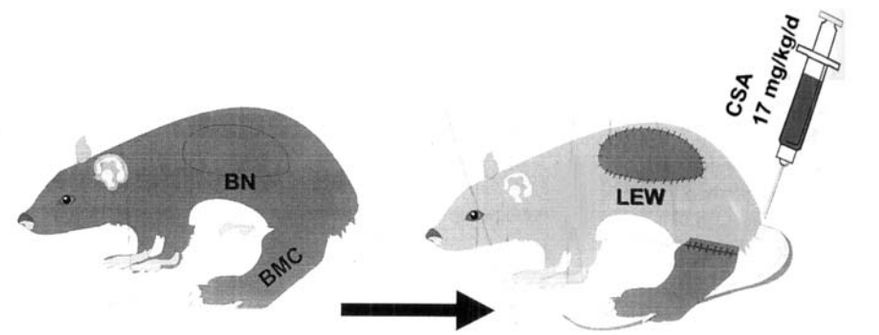

Fig. 1 The experimental model of vascularized bone marrow transplantation in a hind limb graft (*BN* Brown Norway rat, *LEW* Lewis rat, *BMC* bone marrow cells, *CSA* cyclosporine A)

The aim of the study was to develop microchimerism by transplantation of Brown Norway (BN) rat limb with its BMC to Lewis (LEW) rat recipient, followed by a free skin graft from the same donor and to observe the presence and distribution of donor BMC in the recipient lymphoid tissues and, simultaneously, the rejection process of the BN skin grafts.

Materials and methods

Rats

Bn ($RT1^n$) served as donors and LEW ($TR1^l$) as recipients.

Experimental groups

Group 1 (n = 6)

BN hindlimbs were transplanted simultaneously with free skin flaps to LEW. Cyclosporine A (CsA) was given in a dose of 17 mg/kg b.w. for 30 days and tissue specimens and BMC were harvested.

Group 2 (n = 6)

The transplantation and immunosuppression protocol was the same as in group 1, however, the follow-up lasted for another 70 days after weaning off CsA.

Group 3 (n = 6)

From BN, 6×10^7 BMC in suspension were given i.v. to LEW and a free skin graft was performed. CsA was given for 30 days.

Group 4 (n = 6)

The transplantation and CsA administration protocol were the same as in group 3, however, the tissue specimens were taken after weaning off CsA at the first signs of skin graft rejection.

Limb transplantation

BN hindlimb was transplanted (HLTx) orthotopically to LEW. Blood vessels were anastomosed with 10-O Dermalon sutures (Davis and Geck) and the femur fragments were fixed with a metallic splint (Fig. 1).

Free flap skin transplantation

Free 2 × 2-cm skin flaps were taken from male BN and transplanted to the dorsum of LEW.

Identification of BN BMC in LEW lymphoid tissues

Cells were isolated from recipient blood (B), spleen (SPL), mesenteric lymph nodes (MLN), bone marrow (BM), and donor limb BM. They were stained with the monoclonal antibody OX27 directed against MHC class I, polymorphic, $RT1^{c+l-a-}$ on BN cells and analyzed in a FACStar (Beckton Dickinson). Mouse isotype IgG2 was used as the control.

Results

Group 1

Skin grafts survived the entire observation period after HLTx and 30 days of CsA therapy. The cytometric analysis of cellular microchimerism revealed the presence of BN cells in LEW B in $5.7 \pm 4.1\%$, SPL in $1.7 \pm 0.3\%$, MLN in $0.65 \pm 0.07\%$, BM in $0.3 \pm 0.4\%$ and in BN HLTx BM in $7.2 \pm 2.7\%$ (Fig. 2).

Group 2

Skin grafts found after HLTx, 30 days of CsA, and 70 days of weaning off CsA were contracted and partly fibrotic but with intact hair in the middle of the graft. The donor BN cells were found in LEW B in $3.3 \pm 5.2\%$, SPL in $2.0 \pm 2.7\%$, MLN in $0.7 \pm 1.4\%$, BM in $0.4 \pm 0.7\%$, and in BN HLTx BM in $1.5 \pm 2.5\%$.

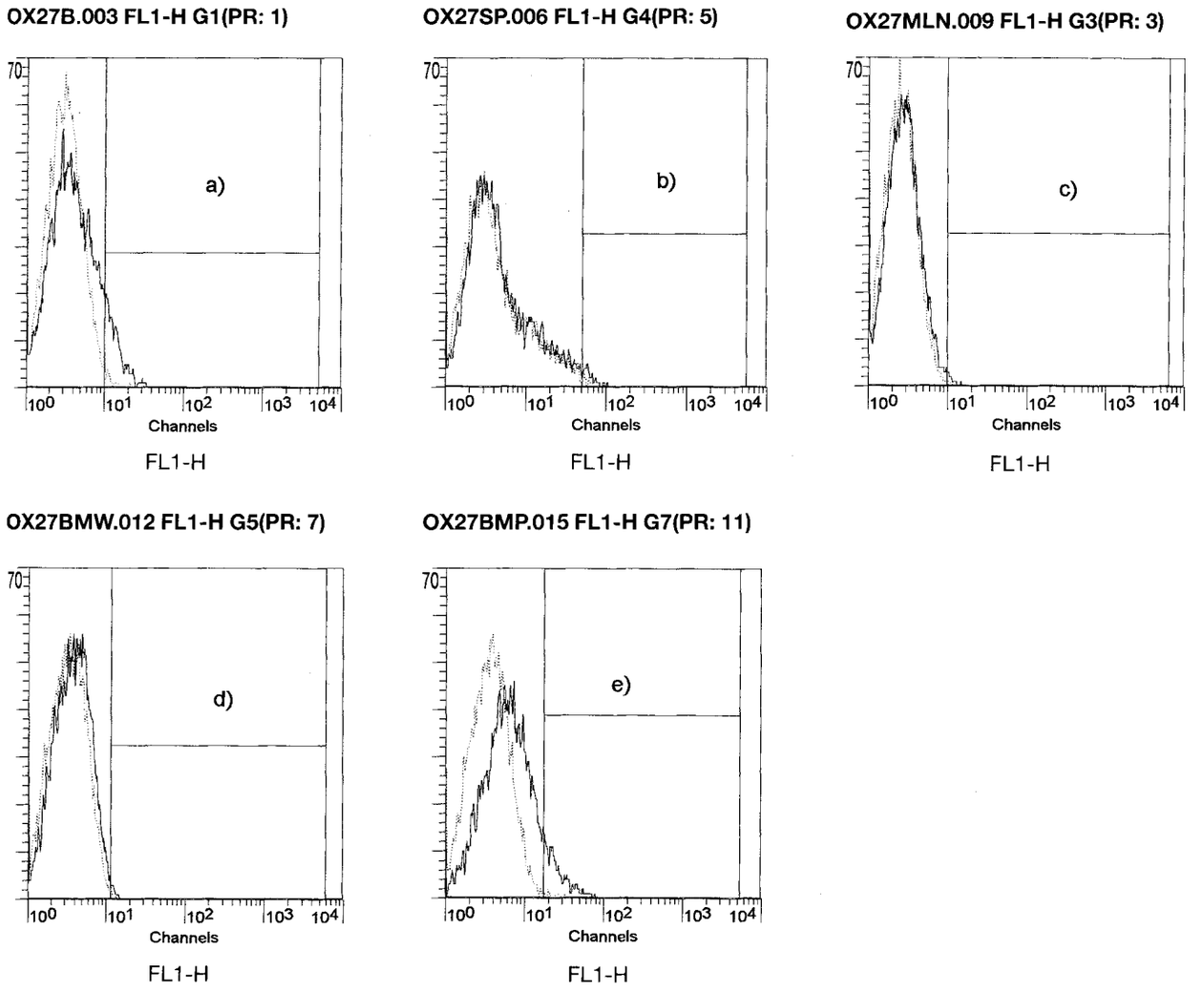

Fig. 2 One-color flow cytometry analysis of OX27 expression on peripheral blood *(a)*, spleen *(b)*, mesenteric lymph node *(c)*, bone marrow of own tibia *(d)*, and bone marrow of transplanted tibia *(e)* cells isolated from LEW rats 100 days after BN hind limb transplantation

Groups 3

Skin grafts retained a normal appearence after BMC infusion and 30 days of CsA administration.

Group 4

Skin grafts were rejected within 5–8 days after cessation of CsA administration. No BN cells were identified in recipient lymphoid organs.

Discussion

This study provided information that BMC seeded from the BM cavities of transplanted limbs migrate to recipient lymphoid tissues and can be detected there at day 30 of CsA therapy and as long as 70 days after weaning off CsA. During this observation period, skin grafts from the same donor show a relatively normal gross appearence. In contrast, skin grafts in recipients of i.v. transplants of BMC in suspension are rejected within 6–8 days after termination of 30-day CsA administration. In the group with vascularized BM, the initial 30-day immunosuppressive therapy prevented rejection of transplanted BMC as well as the skin graft. A relatively high number of donor BMC could be found in blood and recipient lymphoid tissues. The phenotypes of these cells were not defined. However, since the OX27 antibody labels BN class I antigens, all BM lin-

eages could be represented. A high number of donor BMC in recipient blood would point to their continuous release from the BM of the grafted limb. The BMC were transplanted together with stromal cells in their spatial relationship. The stromal cells produce hemopoietic cytokines. This natural environment could secure proliferation of transplanted allogeneic BMC and their release to the circulation and homing to lymphoid organs. In the model of i.v. suspension BMC transplantation, the grafted cells were deprived of their stromal cells, thus limiting their maturation and proliferation. In practice, their number detected in the recipient at the termination of CsA therapy was very low and nil 6–8 days later. Interestingly, skin grafts were rejected soon after discontinuation of CsA. Taken together, we have found that the presence of donor vascularized BM tissue has a beneficial effect on the survival of skin allografts after the termination of CsA administration. A low level of cellular microchimerism could be detected as long as 70 days after cessation of CsA therapy. Intravenous BMC grafts did not prolong skin graft survival time after termination of CsA. It seems that a continuous supply to the recipient of donor BMC may play a crucial role in the prolongation of allograft survival. Live donor lymphoid cells are necessary to obtain this beneficial effect.

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