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Donor DNA can be detected in recipient tissues during rejection of allograft

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Abstract The main source of donor DNA in recipients of allograft are "passenger" cells. It is claimed that they are responsible for the post-transplantation microchimerism and prolongation of allograft survival. We have observed that besides cellular microchimerism, donor DNA can be found in the recipient tissues at the time of rejection of the allograft. In this study, we provide evidence for the presence in the recipient of both DNA in "passenger cells" and free DNA in tissues at the terminal stage of rejection. Male BN (RT1 n) rat heart or skin was transplanted to female LEW (RT1 l) rats followed by a vascularized bone marrow in a hind-limb transplant. In another group, heart and skin were transplanted followed by immediate i. v. infusion of donor-type bone marrow cells. CsA was given in a dose of 17 mg/kg body weight for 30 days, then the rats were followed up until day 100 unless rejection occurred earlier. LEW blood, spleen, mesenteric node and bone marrow cells were stained with moAb OX27 specific for BN but not LEW. Genomic male DNA was isolated and amplified with SRY oligonucleotide. At day 30 and day 100 cellular microchimerism was detected in blood, spleen, nodes and bone marrow cells. Donor DNA was detected in recipient skin, liver and heart extracts, as well as lymphoid organs, at the time of rejection of allograft, but not when the rats were maintained on CsA. Taken together, donor DNA was detected in recipient tissues at the time of heart or skin rejection. It appeared to be released from cells of rejecting grafts and not from "passenger" cells, representing only a minor cellular mass compared with the graft.

Key words Tolerance · Allograft · DNA · Microchimerism

Introduction

Microchimerism developing after allogeneic organ transplantation may be responsible for the partial tolerance to MHC antigens [4]. 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 [6]. Also in humans, organ allograft survival time has been prolonged in recipients of donor BMC [1, 3, 4]. There are also reports on the lack of correlation between microchimerism and tolerance [2, 5]. It is expected that chimerism raised after allotransplantation will create a state of decreased responsiveness to donor antigens and slow down rejection of the transplanted tissues or organ. The question arises as to how the presence of microchimerism can be objectively documented and whether the live donor "passenger" cells or donor DNA are important in prolongation of allograft survival time. Donor "passenger" cells can be detected with the use of specific monoclonal antibodies. Documenting

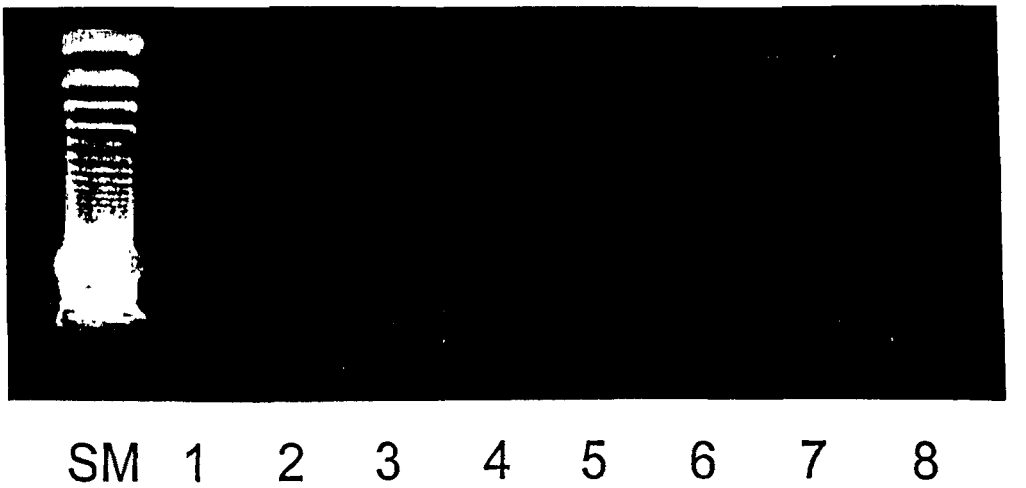

Fig. 1 Determination of donor DNA by semiquantitative PCR. The total DNA was obtained from skin (1), peripheral blood cells (2), spleen (3), liver (4), mesenteric lymph nodes (5), heart (6), bone marrow of transplanted BN tibia (7) and bone marrow of own tibia (8) of female LEW rat after hind limb and free skin flap transplantation from BN male rat, on day 30 of CsA administration. Note that traces of donor DNA were detected only in donor and recipient bone marrow

the presence of "passenger" cells by isolating donor DNA from recipient tissues may be burdened by admixture of donor DNA originating from the graft parenchymal cells.

The aim of this study was to develop microchimerism by transplantation of a BN rat limb with its BMC or by injection of a BMC suspension to a LEW recipient, followed by a free skin or heart graft from the same donor and to observe the rejection process of this graft. The presence of donor BN cells and BN DNA in recipient tissues was investigated.

Material and methods

Rats

Male BN ($RT1A^n$) served as donors and female LEW ($RT1A^l$) as recipients.

Experimental groups

In group 1 ($n = 6$), male BN hind limbs were transplanted simultaneously with heart or free skin flaps to female LEW, CsA was given in a dose of 17 mg/kg body weight for 30 days, and tissue specimens and BM were harvested. In group 2 ($n = 6$), the transplantation and immunosuppression protocol was the same as in group 1; however, follow-up lasted after cessation of CsA until rejection occurred. In group 3 ($n = 6$), male BN 6×10^7 BMC in suspension were given intravenously to female LEW, and a heart or free skin graft was performed. CsA was given for 30 days. In group 4 ($n = 6$), the transplantation and CsA administration protocol was the same as in group 3; however, the tissue specimens were taken after cessation of CsA at the first signs of skin graft rejection.

Limb transplantation

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

Heart and free flap skin transplantation

Hearts were transplanted to abdominal vessels and free 2 × 2 cm skin flaps were taken from male BN and transplanted to the dorsum of female LEW.

Identification of BN BMC in LEW lymphoid tissues

Cells were isolated from recipient blood (B), spleen (SPL), mesenteric lymph nodes (MLN), bone marrow (BMC) and donor limb BM. They were stained with the monoclonal antibody OX27 directed against MHC class I, polymorphic, RTl^{c+1-a-} on BN cells and analyaed in FACStar (Beckton&Dickinson). Mouse isotype IgG_2 was used as the control.

PCR analysis of BN DNA

Genomic DNA was prepared from peripheral blood mononuclear cells, lymph nodes, spleen and bone marrow isolated from own and transplanted tibias, and quantification of DNA was performed spectrophotometrically. DNA was isolated using DNA extraction buffer (Perkin Elmer). DNA products were amplified with SRY-specific oligonucleotide primers (KH-1,5-GAGAGAGGGCA-AGTTGGC-3 and KH-2,5-GCCTCCGGAAAAAGGGCC-3) in a thermal cycler by 35 cycles of denaturation (95 °C, 45 s) annealing (72 °C, 60 s), and extension (72 °C, 7 min) steps. The products were analysed by electrophoresis in 1 % agarose gel followed by ethidium bromide staining.

Results

In group 1, skin grafts survived the entire observation period after HLTx and 30 days of CsA. The cytometric analysis of microchimerism revealed the presence of BN cells in LEW B in 5.7 ± 4.1 % of cases, SPL in

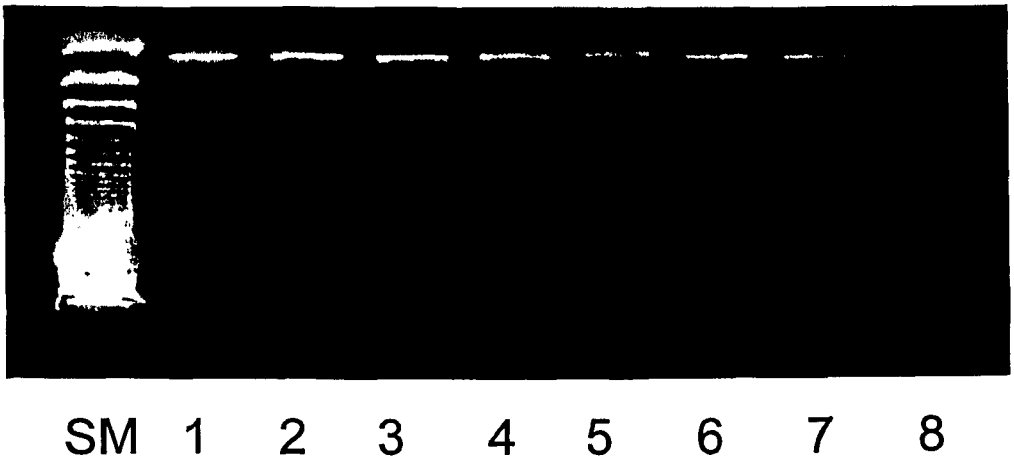

Fig. 2 Determination of donor DNA as in the previous figure. The total DNA was obtained from skin (1), blood (2), spleen (3), liver (4), mesenteric lymph node (5), heart (6), bone marrow from own tibia (7) and the free transplanted BN skin flap (8) in a female LEW rat after hind limb transplantation from a BN male rat on day 100 (30 days of CsA administration and 70 days after cessation of CsA). Note that donor DNA was present in all recipient tissues and least in the transplanted rejecting skin flap

$1.7 \pm 0.3\%$, MLN in $0.65 \pm 0.07\%$ and BM in $0.3 \pm 0.4\%$, and in BN HLTx BM, in $7.2 \pm 2.7\%$ of cases. The PCR analysis revealed the presence of male donor DNA in transplanted donor BM (Fig. 1).

In group 2, skin grafts were examined after HLTx and 30 days of CsA followed by 70 CsA-free days. They were found to be contracted, partly fibrotic but with intact hair in the middle of the graft. Donor BN cells were found in LEW B in $3.3 \pm 5.2\%$ of cases, SPL in $2.0 \pm 2.7\%$, MLN in $0.7 \pm 1.4\%$ and BM in $0.4 \pm 0.7\%$, and in BN HLTx BM in $1.5 \pm 2.5\%$ of cases. Donor DNA was detected in all donor tissues, but only trace levels were detected in the skin graft (Fig. 2).

In group 3, skin graft retained a normal appearance after BMC infusion and 30 days of CsA administration. Donor DNA was found only in the graft (Fig. 3). In group 4, skin grafts were rejected within 5 to 8 days after cessation of CsA administration. Donor DNA was detected in recipient skin, blood, spleen, liver and BM cells, with traces in two out of five rejecting skin grafts (Fig. 4).

Discussion

In this study we showed that BMC seeded from the bone marrow cavities of transplanted limbs migrated to recipient lymphoid tissues and were detected there at day 30 of CsA therapy and as long as 70 days after cessation of CsA. During this observation period, skin grafts from the same donor showed a relatively normal gross

Fig. 3 Determination of donor DNA as in Fig. 1. The total DNA was obtained from transplanted skin flap (1), blood cells (2), spleen (3), liver (4), mesenteric lymph node (5), heart (6) and bone marrow of own tibia (7,8) of a female LEW rat 30 days after intravenous BMC infusion and CsA administration and BN skin grafting. Donor DNA was detected only in the nonrejected BN skin graft

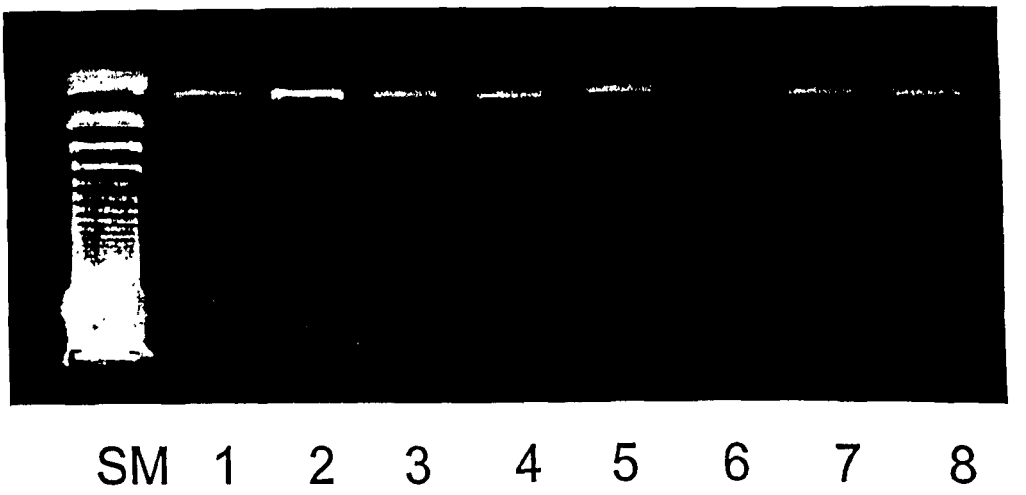

Fig. 4 Determination of donor DNA from tissues as in Fig. 3. LEW female rat received CsA for 30 days followed by 8 days without CsA until rejection of skin graft occurred. Note that donor DNA was present in all tissues

appearance. Interestingly, donor DNA was detected in recipient tissues during CsA therapy only at trace levels. At the time of overt graft rejection, donor DNA appeared in all recipient tissues investigated. It was still present in the skin graft and BM of the transplanted limb despite chronic rejection. In the group with vascularized BM, the initial 30-day immunosuppressive therapy prevented rejection of the transplanted BMC as well as the skin graft. Some few donor BMC were found in blood and other recipient tissues. The phenotypes of these cells were not defined. However, since the OX27 antibody labels BN class I antigens, all BM lineages were represented. The relatively stable number of donor BMC identified in recipient blood would point to their continuous release from the BM of the grafted limb. However, this number was still too low to produce strong DNA bands on the electrophoretic pictures. In contrast to the vascularized bone marrow graft group, skin grafts in recipients of intravenous transplants of BMC in suspension were rejected within 6–8 days after termination of the 30-day CsA administration. Donor DNA was not detected in recipient tissues during the period of CsA treatment, even in the recipient BM cell population. The latter was probably due to an extremely low number of surviving donor BMC after one intravenous injection. Similar to the case of the vascularized bone marrow graft, donor DNA appeared in blood and lymphoid tissue of the recipient as soon as the rejection of the skin graft occurred.

Taken together, we found that a low level of cellular microchimerism was detected in rats with BM transplanted in hind limb as long as 70 days after cessation of CsA therapy. Interestingly, donor DNA was not detected in recipient tissue extracts, except in the recipient BM, as long as CsA was given. DNA became detectable, however, when chronic rejection developed after discontinuation of CsA. Also, in the case of intravenous BM cell grafting, donor DNA was not detectable until rejection of the graft was evident. Detection of donor DNA in recipient tissue extracts preferentially reflects the process of the release of DNA from damaged graft cells and not from the level of existing cellular microchimerism.

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