Colonization of prospective heart graft with recipient bone marrow dendritic cells prior to transplantation does not prolong graft survival

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Abstract

Dendritic cells (DC) have been implicated in the induction of tolerance. We studied the tolerogenic effect of recipient DCs on the heart allografts. The prospective grafts were populated prior to transplantation by recipient bone marrow DCs and subsequently transplanted to the recipient. Briefly, LEW rats were depopulated from own DCs by irradiation. Then, they received vascularized bone marrow graft from BN rats (in transplanted hind-limb). Subsequently, LEW hearts and skin with BN bone marrow derived DCs were transplanted to normal and tacrolimus-treated BN rats. Replacement of LEW donor heart and skin with either of mature or immature BN DCs did not prolong graft survival in BN recipient. The intragraft microchimerism did not mitigate the allogeneic rejection reaction. To the contrary, the BN donor DCs repopulating LEW heart or skin initiated the against-recipient reaction in the prospective graft already during the repopulation process. Immunosuppression with Tacrolimus prolonged the BN DC-repopulated LEW grafts survival as long as it was administered. Weaning from immunosuppression was followed by rejection of heart grafts after 7-14 days, whereas those of skin after 3 days. LEW hearts populated with BN DCs and transplanted back to normal LEW rats underwent chronic rejection and stopped contracting 2 to 12 weeks after grafting. Taken together, replacement of donor hearts with recipient DC did not result in prolongation of graft survival. It initiated the alloreaction in the graft already during repopulation. Recipient DC retained their immunogenic properties also when the graft was returned back to the donor.

Key words: transplantation, dendritic cells, tolerance, bone marrow, heart.

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Introduction

Allograft passenger dendritic cells (DC) and intravascular lymphocytes leave the transplanted organ after grafting and migrate to recipient lymphoid tissues. The graft DC belong to the group of professional antigen presenting cells (APC) evoking a "direct" allogeneic response by the recipient. They may play opposing roles in peripheral tolerance and allograft rejection [1]. The tolerogenic versus immunogenic effects of the micro-chimerism related to DC depends on the host's immunological maturity and the antigenic disparities [2]. It is not known whether DC exert their tolerogenic function in recipient lymphoid tissue, where they process the shed alloantigen or in the graft itself. In man rejection of solid organ grafts rather than tolerance is generally observed. Tolerance may be limited to the chimeric cells but not to graft antigens that are not expressed on the chimeric cells [2]. Creating chimeric organs with recipient-type DC would eliminate the effect of donor passenger cells on the rejection process (direct pathway) and expose the role of graft antigens via the indirect allogenic response. The question arises as to whether the chimeric organ deprived its own DC and repopulated by recipient DC elicits

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a weaker recipient reaction thereby resulting in prolonged graft survival.

The aim of the study was to follow the potential tolerogenic effect of recipient DCs on the allogeneic graft. Prospective donor transplants were populated with recipient DCs prior to transplantation by grafting the donor with vascularized bone (hind-limb transplantation) of recipient origin. Replacement of LEW donor heart with either of mature or immature BN DCs did not prolong graft survival in BN recipient. The intragraft microchimerism did not mitigate the allogeneic rejection reaction. To the contrary, the BN donor DCs repopulating LEW heart or skin initiated the against-recipient reaction in the prospective graft already during the repopulation process.

Material and methods

Animals

Three-month-old male Lewis (RT1^l) and female Brown Norway (RT1^n) rats bred and maintained in our facility were used throughout the study. The Lewis (LEW) rats served as donors and Brown Norway (BN) rats as recipients in the allogeneic combination. LEW rats served as recipients and donors in the syngeneic graft combination.

Vascularized bone marrow transplantation technique for repopulation of LEW heart and skin with BN DCs.

Total body irradiation

Recipient animals were exposed to 8.0 Gy of gamma-radiation from a cobalt-60 source (Theatron, Atomic Energy of Canada, Mississauga, Ontario, Canada) at a dose rate of 150 cGy/mir. This dose was selected because it caused death in 100 percent of irradiated animals without bone marrow transplantation.

Hind-limb transplantation

The hind limb of the donor was amputated at the mid-thigh level and anastomosed with the proximal stump of the previously amputated thigh of the recipient. End-to-end anastomoses of the femoral artery and vein were made with recipient vessels using 10-0 monofilament sutures. The stumps of the sciatic nerve were sutured. The femoral bone stumps were connected with use of an intramedullary metallic stent. The muscles and skin were sutured.

Identification of BN bone marrow cells in LEW tissues

For identification of BN cells in LEW tissues flow cytometry analysis of blood, bone marrow, spleen, and mesenteric lymph node cells was carried out on one-color (green fluorescence at 535 mm) FACStar (Becton Dickinson, San Jose, Calif.) using anti-OX27 antibody directed against major histocompatibility complex class I,

polymorphic, RT1^{c1a} specifically staining Brown Norway but not Lewis class I antigens.

There exist no specific markers for rat DC. However, according to our observations they should be OX6 (class II$^+$) and OX62$^+$. To check depletion of LEW heart and skin DCs, histochemical staining of the irradiated donor tissues destined for transplantation was carried out with OX6 (anti-class II monoclonal antibody). To prove repopulation of LEW heart and skin with BN DCs, the OX27 mAb specific for BN (but not LEW) and OX6 were applied.

Mature and immature DC

To obtain immature DCs, BN rats were given an 8-Gy radiation dose and 10^7 BN BM cells were administered intravenously. Irradiation depleted BN BM cells and subsequent infusion of normal BN BM cells stimulated hemopoiesis. BM cells harvested from these rats did not express CD86 and were OX6^{low}.

Polymerase chain reaction analysis of BN DNA in LEW tissues

To detect BN DNA, as a prove for repopulation of LEW tissues by BN DCs, genomic DNA was prepared from the LEW peripheral blood mononuclear cells, mesenteric lymph node lymphocytes, bone marrow cells, liver, splenocytes, heart and skin. Quantitation of DNA was performed spectrophotometrically. The DNA products were amplified with sex-determining region Y gene-specific oligonucleotide primers. The products were then analyzed on ethidium bromide stained 1% agarose gel electrophoregrams. The results were expressed in units of optical density.

Heart and skin transplantation technique

Heart graft was routinely transplanted to abdominal large vessels. Skin grafts were placed on the dorsum of the recipient. Rejection of heart graft was recognized upon cessation of pulsation using palpation method. Skin graft rejection was diagnosed as lack of capillary bleeding from the punctured graft.

Histochemistry of transplanted heart and skin

The transplanted rejected heart and skin were stained with monoclonal antibodies OX27 (class I specific for BN), W3/13 (CD3), W3/25 (CD4), OX8 (CD8), ED1 (CD68), 62 and OX6 (class II).

Mixed lymphocytes reaction

Mixed lymphocytes reaction MLR was used to examine the responsiveness of naive BN recipient lymphocytes stimulated with LEW cells (BN DC repopulated donor) as well as with BN splenocytes obtained 5 days after LEW heart transplantation (with BN DC) and naive LEW splenocytes.

Immunosuppression

Tacrolimus was given in a dose of 0.1 mg/kg b.w. for 30 days.

Experimental groups

In group 1 ($n = 7$), Lewis (LEW) rats irradiated with 8 Gy underwent 24 hours later transplantation with a BN vascularized hind-limb graft as a source of mature bone marrow (BM)-derived DCs. Fourteen days later hearts and skin from the chimeric LEW rats (with BN DCs) were transplanted into untreated BN hosts (Table 1, Fig. 1).

In group 2 ($n = 7$), LEW rats irradiated 8 Gy underwent transplantation with immature BM-derived DCs. Briefly, to obtain immature DCs, BN rats were given an 8-Gy radiation dose and 10^7 BN BM cells intravenously. Irradiation depleted BN BM cells and infusion of normal BN BM cells stimulated hemopoesis. Fourteen days later

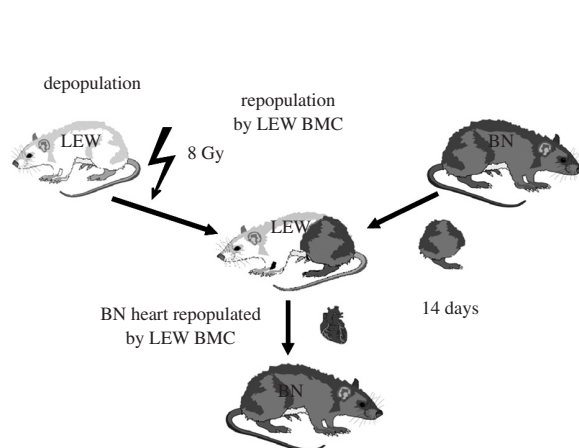hearts and skin from the chimeric LEW rats (with BN DCs) were transplanted into untreated BN hosts (Fig. 2).

In group 3 ($n = 7$), hearts and skin from irradiated and untreated LEW rats were grafted into normal BN rats. In group 4 ($n = 7$), hearts from chimeric LEW rats (with BN DCs) were transplanted to untreated LEW. In group 5 ($n = 7$), Lewis (LEW) rats irradiated with 8 Gy underwent 24 hours later transplantation of a BN vascularized hind-limb graft as a source of mature bone marrow (BM)-derived DCs. Fourteen days later hearts and skin from the chimeric LEW rats (with BN DCs) were transplanted into BN treated with Tacrolimus. In group 6 ($n = 3$), normal LEW hearts were transplanted to normal BN rats.

Results

Group 1. LEW hearts and other tissues became totally depopulated of their own DCs 3 days after irradiation and

Table 1. Experimental groups

|	Donor			Recipient		
	LEW	LEW	LEW	BN	BN	LEW
Group	Naive	Irradiated	Repopulated with BN BMC	No treatment	Tacrolimus	No treatment
1		+	+ mature DC	+		
2		+	+ immature DC	+		
3		+		+		
4		+	+ mature DC			+
5		+	+ mature DC		+	
6	+			+		

Fig. 1. Repopulation of prospective LEW heart with mature BN bone-marrow-derived dendritic cells (DCs). LEW irradiated 8 Gy receives hind-limb from BN. During 14 days LEW tissues become colonized by BN cells. LEW hearts containing BN DCs are transplanted to normal BNs
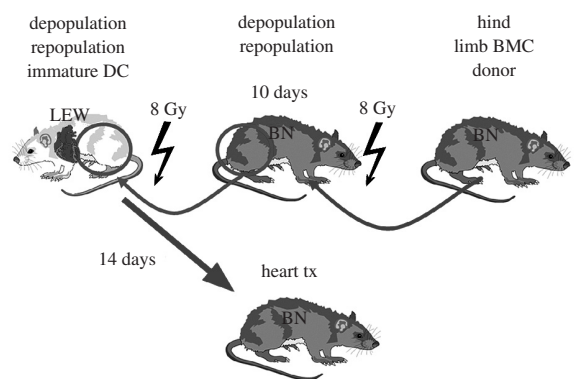
Fig. 2. Repopulation of prospective LEW heart with immature BN bone-marrow-derived dendritic cells (DCs). Irradiated BN rats (mid part of figure) receives BM cell suspension from a normal BN (right part of figure). BM cells of so treated BN contain immature (CD86^{low}) population. The irradiated and BM cell infused BN donate hind-limb to a LEW. Fourteen days later hearts from these rats are grafted to nontreated BNs

repopulated by BN DCs from hind-limb transplants by day 14. More than 80% of blood mononuclear cells, BM, spleen, and mesenteric lymph node cells were of BN origin, staining with OX27 mAb (Fig. 3). Seventy percent of these cells in lymphoid organs were T lymphocytes. In the fraction of circulating blood cells there was 52% of W3/13, 14% of W3/25, 14% of OX8, 6% of ED1, 6% of OX62 and 18% of OX6 – classII cells. These cell repopulated LEW organs.
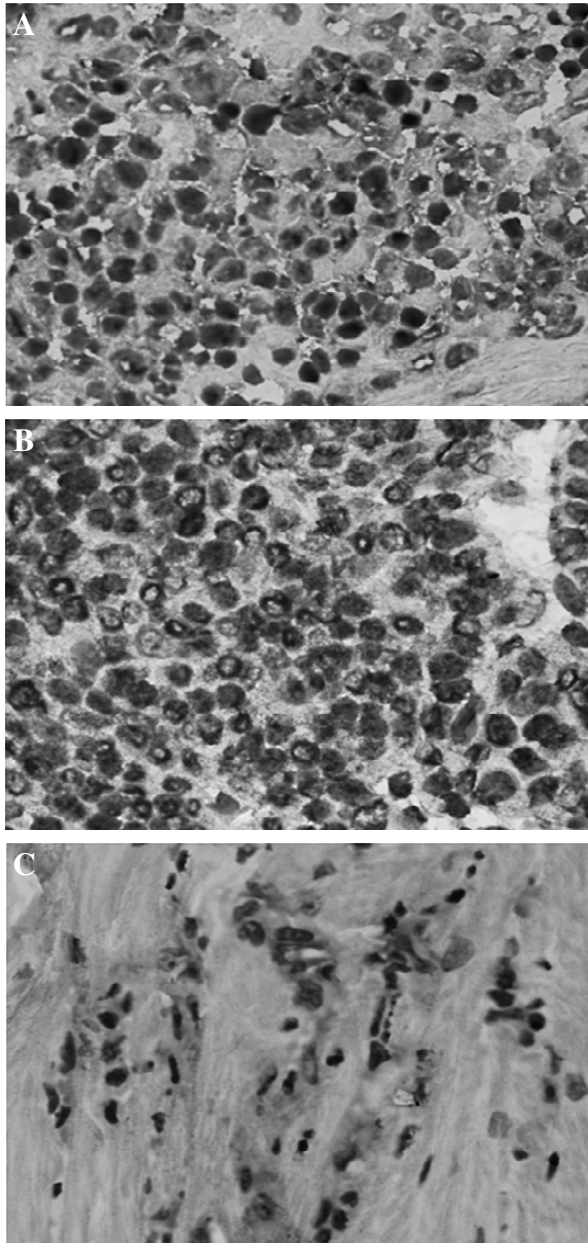

Fig. 3. Histological picture of irradiated LEW tissues replaced by BN BM-derived DCs. Spleen (A), mesenteric lymph node (B) and heart (C) containing OX27 BN cells (600×). Hearts were transplanted to BN rats

The BN DCs in repopulated hearts and skin and endothelial cells of their tissues strongly expressed class II antigens. Moreover, accumulation of BN-type mononuclear cells was seen in larger parenchymal vessels. The optical density of BN DNA Y-Sry fragment was in skin and heart around 6 and 4, respectively. This was only slightly less than in the spleen usually accumulating most donor DNA.

The BN DC-repopulated LEW hearts and skin were transplanted and rejected by naive BN hosts within 12 ±4 days. On histology on day 7-12 dense infiltrates of CD3, 4, 8, 68 and single CD62 cells were seen. All infiltrating cells and graft endothelial cells were OX6+. The infiltrating cells were of BN origin OX27+ (Fig. 4).

The index of responsiveness of naive BN recipient lymphocytes stimulated with LEW cells ranged between 1.1
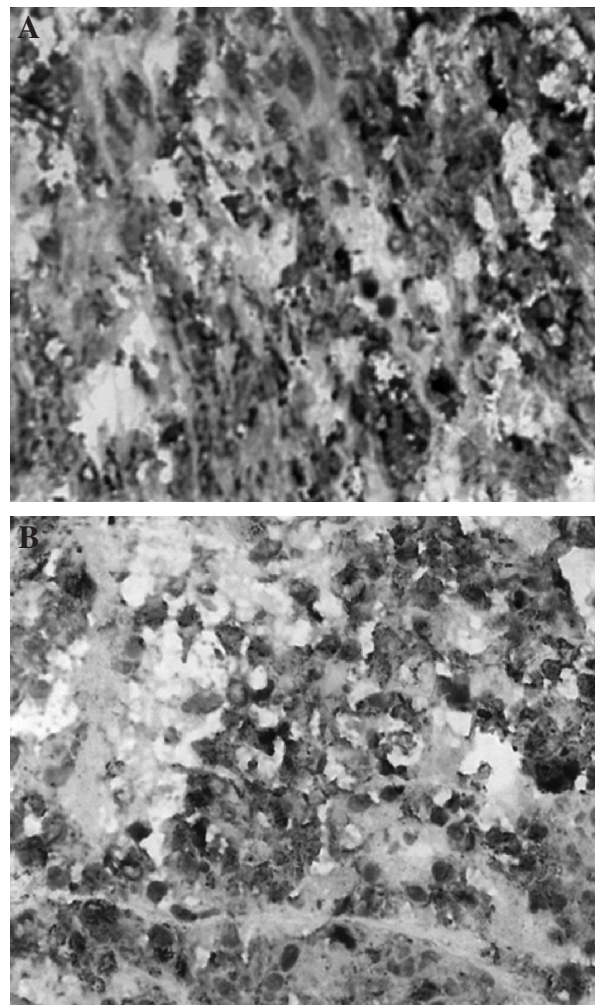
Fig. 4. Histological picture of LEW hearts (A, B) populated with BN DCs 7 days after transplantation to BN. Rejection by dense infiltrates of BN OX27 cells. Destruction of myocytes (600×)

and 1.4 and that of BN splenocytes obtained 5 days after LEW heart (with BN DC) transplantation and naive LEW splenocytes was 1.3 to 1.6.

Group 2. LEW DC-depopulated hearts and skin repopulated with BN immature DC – were rejected by BN naive hosts within 13 ±2 days. The histological picture was similar to that of group 1.

Group 3. In controls, the DC-depopulated LEW hearts and skin transplanted to naive BN rats underwent rejection within 13 ±2 days.

Group 4. The BN DC-repopulated LEW hearts stopped functioning in naive LEW in 40% of instances within 14 to 30 days after transplantation. There was strong expression of class II antigens on the endothelial cells of the graft with perivascular infiltrates on days 7 and 30 after transplantation. Interestingly, the heart grafts surviving 90 days showed subsidence of the perivascular infiltrates.

Group 5. LEW hearts and skin repopulated with BN DCs were transplanted to BN were treated with tacrolimus for 30 days. After weaning from immunosuppression hearts were rejected in 7 ±2 and skin in 4 ±2 days.

In group 6, normal LEW hearts transplanted to normal BN rats survived 7 ±2 days.

Discussion

This study provided the following observations: (a) lack of prolongation of survival time of heart and skin allografts deprived of its own dendritic cells and repopulated with recipient-type dendritic cells, (b) stimulation of allogeneic reactions in irradiated donor hearts, prepared for transplantation, by chimeric recipient-type bone marrow-derived dendritic cells during the repopulation process, (c) low stimulatory activity of splenocytes from the irradiated donor repopulated with recipient-type bone marrow cells to naive recipient splenocytes.

The first question to be answered was whether the prospective heart and skin grafts were populated by recipient DCs derived from transplanted hind-limb BM [3, 4]. This was proved on histological section showing BN phenotype cells in the niches usually occupied by DC. To detect the presence of even a minor concentration of donor cells, the genetic marker was used [5]. The recipient's blood cells, bone marrow cells, mesenteric lymph node lymphocytes, spleen, liver, skin and heart tissue were tested for the presence of donor sex-determining region Y gene nucleotide on days 7 and 14. The level of donor DNA in the recipients tissues was low but detectable in all tissues. The detected donor DNA originated from donor cells homing to the recipient organs, although the contribution of donor DNA from damaged transplant parenchymal cells could not be excluded. This test evidently showed prospective grafts to be populated by recipient cells.

The key observation of this study was lack of prolongation of survival time of heart allografts deprived of their own DCs and repopulated either with mature or immature BM-derived recipient – type DCs. DCs resident in a graft are potent antigen presenting cells that initiate the "direct" allogeneic recognition pathway. They are responsible for vigorous rejection of the allograft [6]. In our donor-recipient strain combination, depletion of donor DC antigen presenting cells, both mature and immature, did not mitigate the rejection process. Other authors have reported that deprivation of allograft passenger cells by irradiation prior to transplantation does not prolong graft survival, and even aborts the graft [7]. Krasinskas *et al.* [8] repopulated donor hearts with recipient cells using a bolus of BM cell infusion without affect on survival. Our model of repopulation was different because recipient BM cells were seeded into the prospective heart donor for 14 days, which could presumably "condition" the graft.

Two points should be clarified, the in vivo identification of mature and immature DCs and which cell subsets populate the prospective heart graft in addition to DCs. There are no specific markers for rat parenchymatous tissue resident DCs. In rat hearts, DCs are located between myocardial fibers, are class II-positive, ED1+ and weakly OX62+ (antigen specific for migrating DCs). Mature DCs strongly express surface antigens in contrast to the immature subsets. This last is seen on smears of BM regenerating after irradiation [9]. The migrating BM-derived DCs populating peripheral tissues are immature [10]. Seeding of the prospective graft with recipient DCs could lower the antigenity of the graft, because DCs as passenger cells might become tolerogenic after 14 days of contact with graft antigens and dampen recipient reaction after "adoptive" transfer with the graft. Moreover "conditioning" could be achieved by replacing of the prospective graft endothelial cells by BM-derived cells of recipient phenotype. A period of 14 days may be long enough for this process [11]. In our model, LEW blood repopulated by BN bone marrow contained more than 90% BN cells. These cells, both mature and immature, remained in contact with LEW heart cells for a period of 14 days.

Interestingly, hearts populated with allogeneic DC and returned to the donor revealed major inflammatory changes with major histocompatibility complex DR-positive cell infiltrates. Some heart grafts stopped beating. The infiltrates consisted of allogeneic DC repopulating the heart as well as the host's own bystander lymphocytes. These findings are a clear example of immunogenic but not tolerogenic properties of DC in our model.

Taken together, "conditioning" of LEW heart with BN DCs, either mature or immature, was ineffective. The intragraft microchimerism did not prolong graft survival. Repopulation of prospective graft with recipient DC prior to transplantation initiated allogeneic reaction in the graft.

Acknowledgments

References

1. Fairchild PJ, Waldmann H (2000): Dendritic cells and prospects for transplantation tolerance. Curr Opin Immunol 12: 528-535.
2. Anderson CC, Matzinger P (2001): Immunity or tolerance: opposite outcomes of microchimerism from skin grafts. Nature Med 7: 80-87.
3. Maksymowicz M, Olszewski WL, Cybulska E (2003): Repopulation of donor heart by recipient bone marrow-derived dendritic cells prior to transplantation brings about acute rejection by both the allogeneic and syngeneic recipient. Transplantation Proc 35: 2374-2375.
4. Olszewski WL, Interewicz B, Maksymowicz M, Durlik M (2003): Biological aspects of limb transplantation: I. migration of transplanted bone marrow cells into recipient. Plast Reconstr Surg 112: 1628-1635.
5. Olszewski WL, Interewicz B, Maksymowicz M, Stanislawska J (2005): Transplantation of organs is transplantation of donor DNA: fate of DNA disseminated in recipient. Transpl Int 18: 412-418.
6. Lindahl KF, Wilson DB (1977): Histocompatibility antigen-activated cytotoxic T lymphocytes. II. Estimates of the frequency and specificity of precursors. J Exp Med 145: 508-522.
7. Sun J, McCaughan GW, Gallagher ND, et al. (1995): Deletion of spontaneous rat liver allograft acceptance by donor irradiation. Transplantation 60: 233-236.
8. Krasinskas AM, Eiref SD, MacLean AD, et al. (2000): Replacement of graft-resident donor-type antigen presenting cells alters the tempo and pathogenesis of murine cardiac allograft rejection. Transplantation 70: 514-521.
9. Lukomska B, Durlik M, Morzycka-Michalik M, et al. (1991): Transplantation of vascularized bone marrow. Transplantation Proc 23: 887-888.
10. Flores-Romo L (2001): In vivo maturation and migration of dendritic cells. Immunology 102: 255-262.
11. Durlik M, Lukomska B, Olszewski WL, et al. (1998): Tolerance induction following allogeneic vascularized bone marrow transplantation- the possible role of microchimerism. Transpl Internat 1: S299-302.