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Accelerated Graft Arteriosclerosis Is Enhanced by Sensitization of the Recipient to Donor Lymphocytes

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THE DEVELOPMENT of arteriosclerosis is the most serious and common complication in long-term survivors of cardiac transplantation.[1] We recently have demonstrated that heart grafts exchanged between inbred rat strains with selected histocompatibility differences develop accelerated graft arteriosclerosis.[2] Heterotopic cardiac allografts were exchanged between strains that differed for MHC class I (RT1.A or RT1.E) antigens or groups of minor, non-MHC antigens in MHC-compatible congenic combinations. In combinations in which the allograft reaction is mild and prolonged, the donor hearts exhibit pathological changes that include a diffuse, interstitial myocardial fibrosis, perivascular fibrosis, and intimal proliferation in arteries of the graft myocardium. The results suggested that the comparable pathological changes seen in long-term human cardiac survivors may reflect low-level, persistent allograft reactions rather than factors associated with graft anoxia or effects of immunotherapy to prevent graft rejection.

The observation that accelerated graft arteriosclerosis could be induced as the result of low-level, persistent allograft reactions suggests that manipulations of this allograft reaction should be associated with alterations in the severity or prevalence of the lesions in the heart graft. In the studies reported here, we have extended these early observations to examine the influence of immunization of recipient animals with donor tissues on the pathological lesions in the donor heart.

MATERIALS AND METHODS

Adult rats (10-16 weeks of age) bred in our colony at the University of Pittsburgh School of Medicine or LEW rats purchased from Harlan-Sprague-Dawley (Indianapolis, IN) were used in this study. The donor and recipient strains represented two combinations of MHC-matched congenic pairs (BN.1A → DA and DA.1N → BN) that differ for BN and DA strain minor histocompatibility antigens, respectively. A third combination, LEW → F344, is two strains that are matched for the major class I and II of the rat MHC. The rats were housed under conventional conditions and fed rat chow (Wayne Lab-Blox F-6, Chicago, IL), and tap water ad libitum. Intraabdominal heterotopic cardiac grafting was performed using a modification of the techniques described by Ono and Lindsey.[3] The donor animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and maintained on methoxyflurane via inhalation. The vena cavae and pulmonary veins were ligated with 5-0 silk, and the pulmonary artery and aorta were transected 2-3 mm above their origins in the heart. After perfusion of the ventricles and atria with lactated Ringer's solution (containing 200 U/ml of heparin), the heart was placed in a saline bath at 4°C. Recipient animals were anesthetized with pentobarbital, a midline incision made, and the great abdominal vessels were dissected free from the left renal vein to the bifurcation. The graft was implanted in the abdominal cavity, with both anastomoses done in a running end-to-side fashion with 10-0 Novafil on a TE-10 needle. Operative times ranged from 30 to 45 minutes, with a success rate of approximately 90%. The function of the heterotopic grafts was monitored by abdominal palpation, and the grafts were removed when they ceased to function or had reached the appropriate time posttransplantation. All grafts were fixed in 10% buffered formalin, biventricular sections of the myocardium were submitted for paraffin embedding and processing, and the sections were stained with H&E.

Table 1. Effect of Histocompatibility Differences on Accelerated Arteriosclerosis

Incompatibility	*n*	Histopathology			
		VIP*	VAS	MF	MI
BN.1A → DA					
3 months PT	6	++	+	+++	++
6 months PT	5	+++	++	++	++
DA.1N → BN					
3 months PT	10	++	++	+++	+++
LEW → F344					
49-90 days PT	8	++	+++	++++	++++

*VIP, vascular intimal proliferation; VAS, vasculitis; MF, myocardial fibrosis; MI, myocardial inflammation; PT, posttransplant.

RESULTS AND DISCUSSION

As we have reported previously, the BN.1A/DA and DA.1N/BN strain combinations differ for weak histocompatibility differences that allow for prolonged graft survival and the development of accelerated graft arteriosclerosis.[2] When the grafts are allowed to remain for longer periods of time, the severity and incidence of the vascular lesions increase. In the BN.1A → DA combination, we expect to see approximately 50% of the grafts with mild to moderate lesions of arteriosclerosis in several vessels (Table 1). In the grafts that were harvested at 6 months posttransplantation, 4/5 grafts exhibited myointimal proliferative changes that were classified as moderate to severe. The LEW → F344 combination has been reported by others[4] to induce accelerated arteriosclerosis, and this combination was included in these studies for comparison to our congenic models (Table 1). In general, the

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Table 2. Changes in Accelerated Arteriosclerosis Following Immunization

Incompatibility	*n*	VIP*	Histopathology VAS	MF	MI	NEC
BN.1A → DA						
3 months PT	6	++	+	+++	++	—
Skin graft	3	—	—	—	—	++++
DA.1N → BN						
3 months PT	10	++	++	+++	+++	—
Skin grafts	4	+	+	+++	+++	—
Splenic lymphs	7	+++	+++	++++	++++	—

*VIP, vascular intimal proliferation; VAS, vasulitis; MF, myocardial fibrosis; MI, myocardial inflammation; PT, posttransplant; NEC, acute hemorrhagic necrosis.

prevalence and severity of the lesions in this combination were similar to those seen with the two congenic combinations, although the inflammatory component of the intimal lesion was more pronounced, and a more severe rejection reaction resulted in the failure of 5/8 grafts at days 49-50 posttransplantation.

The effect of sensitization of the recipient to donor tissues was examined by placing a single skin graft from the BN.1A or DA.1N donor strains on DA or BN recipients 1 month before cardiac transplantation (Table 2). In the BN.1A → DA combination, this level of sensitization was sufficient to precipitate an acute rejection of the heart grafts. The grafts were rejected within 6 to 12 days and were characterized histologically by hemorrhagic necrosis of the grafted tissue. Sensitization via skin grafting in the DA.1N → BN combination resulted in a more severe cell-mediated rejection reaction, including a more severe vasculitis of the graft vessels and myocardial inflammatory disease. The severity and prevalence of the intimal lesions, however, were not accelerated and were less extensive than those seen in the 3-month control animals. These results suggest that the skin graft may not express the antigen(s) that serve as the target for the allograft reaction in this combination.

The BN recipient animals also were sensitized to the DA.1N donors by a single i.p. injection of 1×10^6 splenic lymphocytes 3 weeks before transplantation. This type of sensitization produced a more severe myocardial inflammatory reaction, vasculitis, and the appearance of a more pronounced myointimal proliferation in the graft vessels (Table 2).

We believe that the results of these experiments demonstrate that accelerated arteriosclerosis can be induced experimentally in cardiac allografts by grafting across a variety of weak histocompatibility barriers. This type of tissue incompatibility may allow for prolonged graft survival and the stimulation of myointimal proliferative changes secondary to a low-level rejection reaction directed, at least in part, at the vascular endothelium.

The arteriosclerosis can be stimulated by both MHC and non-MHC antigens and increases in severity in proportion to the length of the posttransplant period and the level of sensitization of the recipient to selected donor tissues. We anticipate that more careful selection of human donor–recipient pairs for cardiac transplantation and better control of allograft immunosuppression should prevent the development of this important complication of cardiac transplantation.

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