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Prolonged Survival of Hearts Obtained From Chimeric Donors in a Mouse to Rat Xenotransplant Model

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UNTIL recently, it has been proposed that "indirect" recognition of xenogeneic antigens that were processed and presented by self-antigen presenting cells (APC) played a major role in the initiation of $CD4^+$ T-cell-dependent xenograft rejection.[1] However, the observation that the tempo of xenograft rejection remains unaltered in MHC class II "knock out" recipients provides evidence for direct antigen presentation by donor APCs.[2] The relative ease with which organs obtained from chimeric donors reconstituted with bone marrow (BM) cells of recipient origin are accepted across allogeneic barriers provides further credence to the latter observation.[3-6] We have therefore attempted to study the survival of heterotopically transplanted hearts obtained from mice reconstituted with recipient-type (rat) BM in a mouse to rat concordant xenotransplant model.

MATERIALS AND METHODS

Animals. B10.BR mice ($H\text{-}2^k$) were used as BM recipients and heart donors; LEW rats ($RT1^l$) were used as BM donors and heart transplant recipients.

Experimental Design. B10.BR mice were lethally irradiated (9.5 Gy) and reconstituted with 10^8 unfractionated LEW BM cells. At various times (48 and 130 days) after BM reconstitution, hearts from the chimeric mouse donors were heterotopically transplanted into LEW recipients, some of which also received tacrolimus (FK 506; 1 mg/kg/d IM) until rejection (Table 1).

Detection of Chimerism. The presence of cells of donor (LEW) origin in the lymphoid organs of mice reconstituted with rat BM was determined by flow cytometry using biotinylated mouse anti-LEW antibodies. Similar detection of rat and mouse cells in organs (heart and liver) obtained from chimeric mice was also attempted on cryosections using appropriate anti-MHC class II-specific antibodies.

Rat Antimouse Immune Responses. One-way MLR assays were performed using irradiated lymph node cells obtained from chimeric donors (B10.BR mice) as stimulators and naive LEW lymph node cells as responders. Antibody responses in the cardiac xenograft recipients were determined by a complement-dependent cytotoxicity assay.

RESULTS AND DISCUSSION

Of the mice reconstituted with xenogeneic BM, 20% died due to failure of engraftment, whereas an additional 15% suffered moderate to severe GVHD. Although these animals responded to FK 506 intervention, they were nevertheless not used as donors for subsequent heart transplantation. Hearts obtained from either naive or B10.BR donors reconstituted with syngeneic BM, when transplanted into untreated or FK 506-treated rats were rejected in <3 days (Table 1, groups I, II, and III). Furthermore, the tempo of graft rejection was similar when hearts obtained from mice 48 days postrat BM reconstitution, were transplanted into naive rat recipients (Table 1, group IV). A slight prolongation in heart survival was witnessed when organs transplanted into naive rat recipients were obtained from B10.BR donors 130 days postxenogeneic (rat to mouse) BM transplantation (Table 1, group V). However, with the addition of FK 506, hearts obtained 130 days postreconstitution from chimeric mice enjoyed a significantly prolonged

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Table 1. Survival of Cardiac Xenografts Transplanted From Chimeric B10.BR Mice Into LEW Recipients

Groups	Organ Donor (B10.BR)	n	Recipient (LEW) Treatment	Survival Days (X ± SD)	P
I	Normal	5	None	2.4 ± 0.4	
II	Normal	5	FK 506*	2.6 ± 0.4	
III	130 days postsyngeneic BMTx	10	None or FK 506*	3.0	
IV	48 days postxenogeneic BMTx†	5	None	2.6 ± 0.4	
V	130 days postxenogeneic BMTx	5	None	9.4 ± 5.7	.05 vs GIV
VI	130 days postxenogeneic BMTx	5	FK 506*	20.7 ± 8.5	.002 vs GV

*1 mg/kg/d IM.
†Rat to mouse BMTx.

survival when transplanted into LEW recipients ($P = .002$; Table 1, group VI). It is of interest to note that the appearance of antimouse cytotoxic antibodies in the serum of FK 506-treated (group VI) animals was significantly delayed. Additionally, in vitro MLR assays performed using irradiated lymphocytes from chimeric B10.BR mice revealed their inability to stimulate naive LEW lymphocytes.

When tested for chimerism at 48 and 130 days postreconstitution, 50% to 90% of cells in the lymphoid organs of mice were that of donor (rat) origin. A similar level of chimerism was also witnessed in immunostained cryosections of nonlymphoid (heart and liver) organs obtained from chimeric mice at 130 days postreconstitution. In conclusion, these experiments suggest that resident "passenger" leukocytes may play an important role in xenoantigen recognition, tempting us to speculate that the generation of a hematopoietic chimeric donor may be added to the therapeutic armamentarium currently employed to overcome concordant xenograft rejection.

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