1835

Pretreatment of Recipients (Nude Rat) With Donor Antigens Leads to Prolonged Survival of Hamster Heart Xenografts

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THE INITIAL barrier to successful organ xenotransplantation in discordant species is hyperacute rejection (HAR) mediated by either natural (preformed) or induced antibodies.[1,2] Hamster cardiac xenografts are normally rejected by this mechanism within 3 days when transplanted into naive LEW or athymic (nude) rats,[3] which in the latter is supposedly caused by a T-cell independent process. This is contrary to the prevailing tenet that dictates that T-cell help is required for the initiation of HAR following presensitization with xenoantigens. Accordingly, we hypothesized that prior exposure of T-cell-deficient nude rats to donor antigens would not precipitate HAR of a subsequently transplanted hamster heart. This study was therefore designed to test this hypothesis.

MATERIALS AND METHODS

Syrian hamsters and NIH athymic nude (*mu/mu*) rats were used as donors and recipients, respectively. Naive LEW rats were used as immunocompetent controls. LEW as well as nude rat recipients were pretreated with either heparinized hamster whole blood (donor-specific transfusion [DST]; 1 mL, IV) or a suspension of minced hamster heart (MH, IP) 7 days prior to heterotopic cardiac (hamster → rat) xenotransplantation. The production of xenoantibodies was determined in serum samples of the recipients by a two-step complement-dependent cytotoxicity assay (CDC). The quantity of both rat IgM and IgG was measured using a sandwich enzyme-linked immunosorbent assay (ELISA), the details of which are described elsewhere.[4] Additionally, donor-specific IgM and IgG were also quantified using an ELISA method described previously by Platt *et al*[5] with several modifications.[6]

RESULTS AND DISCUSSION

Although challenge hamster hearts underwent HAR (<10 minutes) when transplanted into presensitized LEW recipients, they nevertheless enjoyed prolonged survival (median of >100 days; group V, Table 1) when transplanted into presensitized nude rats. Furthermore, the loss of heart xenografts due to HAR in naive LEW (but not nude) rats presensitized with donor antigens was associated with marked elevation of donor-specific antibodies (IgM) in the recipient's serum when tested on the day of grafting (groups II and III). Repetition of CDC assays 7 days postcardiac transplantation revealed a minimal elevation of rat α hamster antibodies (groups V and VI), which was appreciably lower than that observed in a similarly treated immunocompetent rat. In light of these observations, it is tempting to speculate that prior infusion of donor-specific xenoantigens into nude rats with inherently deficient T-dependent immune responses may lead to prolonged survival of a subsequently transplanted xenograft. The precise explication of

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Table 1. Survival of Hamster Cardiac Xenografts in Sensitized Nude and LEW Rats and the Quantification of Rat Anti-Hamster IgM in the Serum of Variably Treated Nude Rat Recipients

Group	Recipient	Sensitization	Survival (d)	Median (d)	Rat Anti-Hamster IgM (ng)*	
					d 0	d 7
I	LEW	None	3, 3, 3, 3, 3	3	86 ± 5	ND
II	LEW	DST	<10 min × 5	<10 min	232 ± 5	ND
III	LEW	MH	<10 min × 5	<10 min	177 ± 25	ND
IV	Nude rat	None	3, 3, 3, 3, 3	3	41 ± 3	47 ± 3
V	Nude rat	DST	43, 63 > 100 × 3	>100	64 ± 2†	101 ± 11†
VI	Nude rat	MH	5, 16, 48, 59 > 100	48	88 ± 11	76 ± 3

Abbreviation: ND, not determined.

*The same volume of serum from each recipient in a given experimental group was mixed and tested. Two million hamster splenocytes were incubated with 500 μL of serum for 60 min followed by their lysis. The quantity of rat anti-hamster IgM and IgG in the lysate was measured using the sandwich ELISA assay. All tests were performed in triplicate.

†$P < .05$.

the role of T-cell-independent humoral responses in the development of HAR may allow us to contrive an approach to attenuate or abrogate this response for successful xenotransplantation.

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