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Mechanisms of Protection From Humoral Rejection by a Xenografted Liver

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WE PREVIOUSLY reported the immunological protection from humoral rejection afforded to a hamster heart that was heterotopically placed into a rat 2 months after hamster liver transplantation. Furthermore, this protection was so robust that injection of specific hyperimmune serum was unable to reverse this process. We attributed these observations to a switch of the recipient's complement (C) to that of the donor type, shielding the subsequently transplanted heart from the deleterious effects of antibody- and complement-mediated injury.[1,2] In the present study, we assessed whether this protective effect would also manifest itself in the early postliver transplant period, when the titer of xenospecific antibodies is extremely high in the recipient and the transformation of the C from the recipient to that of the donor type is underway.

MATERIALS AND METHODS

Animals

Syrian Golden hamsters (100 to 120 g) and Lewis rats (250 to 270 g) were used as donors and recipients, respectively.

Transplants

Liver was placed orthotopically according to the cuff technique. Heart was transplanted heterotopically into the abdominal cavity on day 1 after liver grafting. Immunosuppression was with FK 506 (1 mg/kg), which was given daily intramuscularly (IM) for 30 days. The experimental design is described in Table 1.

Complement-Dependent Cytotoxicity Assay

Cytotoxic antibody titers in the recipients were determined using hamster lymphocytes as targets and baby rabbit serum as the source of C.

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Table 1. Survival of Hamster Hearts Transplanted Into Lewis Rats on Day 1 After Receiving a Rat or a Hamster Liver Xenograft

OLTX (Treatment)	OLTX (Survival Days)	MST**	HTX (Survival Days)	MST**
1. None	—	—	3 × 5	3.0
2. Lew→Lew (none)	>100 × 5	>100	3, 3, 3, 3, 4	3.2 ± 0.4
3. Lew→Lew (FK 506*)	>100 × 5	>100	3 × 5	3.0
4. Ham→Lew (none)	7 × 5	7	6 × 5	6.0
5. Ham→Lew (FK 506*)	16, 34, 51, 51	38.2 ± 16.6	15, 33, 50, 50	37.2 ± 16.6

*FK 506 (1 mg/kg/d) was given daily IM for 30 days.
**Mean survival time (days).
OLT = orthotopic liver transplantation; HTX = heart transplantation.

Radial Immunodiffusion Assay

Goat antirat C3 was used for the quantitation of C3 in the serum of liver recipients. This polyclonal antibody crossreacts with hamster C3.

Electrophoresis and Immunofixation

These techniques were used to follow temporally the transformation of the recipient C to that of the donor type. Plasma samples from liver xenograft recipients on postoperative days 1, 2, and 3 were tested by a method described elsewhere.[4]

RESULTS

As depicted in Table 1, there was no difference in the survival of hamster hearts transplanted into Lewis rats in groups 1, 2, and 3, indicating that a syngeneic liver transplantation with or without FK 506 treatment does not influence the rejection of subsequently transplanted hamster hearts. On the contrary, the survival of hamster hearts was significantly prolonged in groups 4 and 5, in which the rats had previously received a hamster liver; all recipients, however, died with a beating heart graft. Furthermore, it is interesting to note that the recipients in group 4 died due to severe hepatic rejection with minimal histopathological changes in the secondary cardiac xenotransplants.

The gel electrophoresis of plasma obtained after liver xenotransplantation showed the coexistence of recipient and donor C3 on days 1 and 2, with almost a complete switchover to donor-type C3 by day 3 after transplantation (Fig 1).

Figure 2 shows the levels of C3 in the serum of liver recipients. On the first postoperative day, the level of circulating C3 in the serum was 50% of that found in normal rats; however, by day 5 after transplantation, it had reverted back to the baseline.

The determination of antibody titers in the rat recipients of either hamster liver alone or combined liver and heart transplants showed no statistically significant difference between the two groups, with the peak titer of the antibodies being observed at the end of the first postoperative week (data not shown).

DISCUSSION

We had previously demonstrated that the almost complete replacement of recipient C with that of the donor type after liver transplantation plays an important role in the prevention of humoral rejection of secondarily transplanted hearts from the same donor species but not from others.[2] Furthermore, in the present experiments we have shown that after liver transplantation, 72 hours were required for a complete conversion of C to donor type. The eventual loss of circulating rat C might be attributed to the orthotopic replacement of Lewis liver with that of hamster, thus eliminating the main source of C synthesis, and to the absorbion of activated C by liver grafts, leading to precipitous depletion of recipient type C. Although on postoperative day 3 the levels of circulating C3 were markedly reduced in recipients of hamster liver, we were able to detect their presence on the vasculature of the liver and heart xenografts. Taken together, these observations suggest that the apparent inability of a Lewis rat recipient of a

Fig 1. Gel electrophoresis test of sera obtained from rats on days 1, 2, and 3 after hamster liver transplantation. This indicates that by day 3, C had switched to that of the donor type. HAM: hamster.

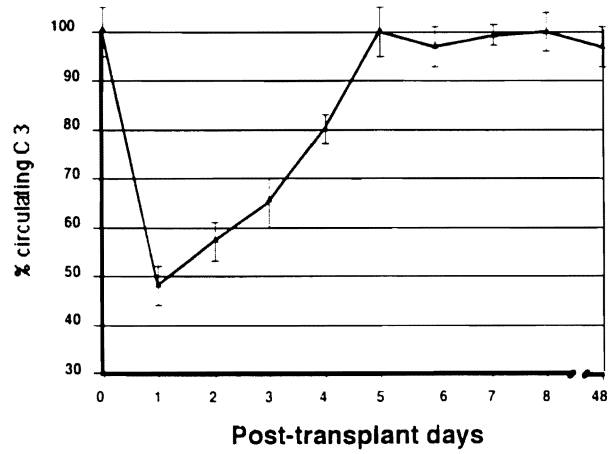

Fig 2. Amount of circulating C3 (radial immunodiffusion) in liver recipients after transplantation. The value obtained from normal rat sera was considered 100%.

hamster liver to mount a humoral rejection of a subsequently transplanted hamster heart hinges on the presence of C of the xenodonor type. This underscores their inherent deficiency to form an effective membrane attack complex due to the presence of species-specific regulators of C activation on the endothelium of the xenotransplanted hamster tissue.[5] Furthermore, this study has unequivocally established that a xenografted hamster liver protects a subsequently transplanted hamster heart during the first week after liver placement, when the antibody titer in the recipient is extremely high.

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