1704

Mechanisms of Hamster Kidney Graft Rejection in the Rat

H. Miyazawa, N. Murase, A.J. Demetris, K. Nakamura, K. Matsumoto, Q. Ye, R. Manez, S. Todo, and T.E. Starzl

HAMSTER-to-rat transplantation has been widely used to study xenotransplantation between the relatively closely related species. Recently, we developed the surgical procedure for kidney transplantation in this species combination. This study describes mechanisms of hamster kidney graft rejection in the rat and the effect of different treatment protocols, including FK 506, cyclophosphamide, and hamster organ perfusion, on graft survival.

MATERIALS AND METHODS

Animals

Male Lewis rats (LEW, Harlan Sprague-Dawley, Indianapolis, Ind), weighing 200 to 300 g, and Golden Syrian hamsters (Charles River, Wilmington, Mass), weighing 100 to 150 g, were used as recipients and donors, respectively.

Orthotopic Kidney Transplantation

The left hamster kidney was isolated with the left renal artery on a segment of the aorta and the left renal vein with a cuff of inferior vena cava. The ureter was mobilized together with a small portion of the bladder. After donor heparinization (200 U), the graft was excised and perfused with 1 to 3 mL cold-lactated Ringer's solution via the aorta. Vascular reconstructions were by end-to-side anastomoses of graft aorta to recipient infrarenal aorta and graft left renal vein to recipient inferior vena cava. Urinary tract was reconstructed by suturing recipient and donor bladders. Bilateral native nephrectomy was performed at the time of transplantation. Graft rejection was defined by animal death.

Liver or Kidney Perfusion

In order to deplete preformed antihamster antibodies in the rat recipient, a hamster liver or kidney was temporarily connected to the recipient jugular vein and carotid artery by the cuff technique. Perfusion of either organ was initiated 1 hour before intra-abdominal kidney transplantation and continued for 1.5 to 2 hours.

Immunosuppressive Agents

FK 506 (Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan) was dissolved in normal saline and given intramuscularly at doses of 1 to 2 mg/kg per day. Cyclophosphamide (CP) was administered orally by gastric installation at a dose of 10 mg/kg per day. Experimental groups and treatment regimens are shown in Fig 1.

Lymphocytotoxic Antibody Assay

A complement-fixing lymphocytotoxic antibody assay was performed using hamster lymphocytes as targets by Terasaki's method.[1] The lymphocytotoxic antibody titer was defined as the most diluted serum sample with greater than 50% cell lysis.

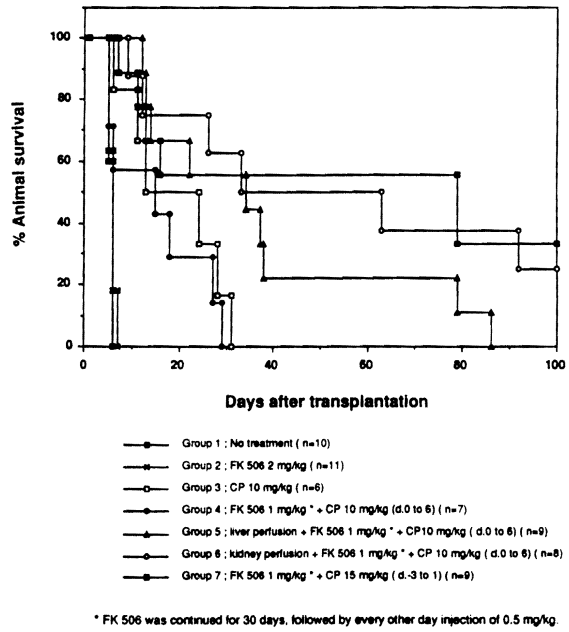

Fig 1. Animal survival after kidney transplantation from hamster to rat. (—□—) Group 1, no treatment (n=10); (—×—) group 2, FK 506 2 mg/kg (n=11); (—□—) group 3, CP 10 mg/kg (n=6); (—●—) group 4, FK 506 1 mg/kg* + CP 10 mg/kg (days 0 to 6) (n=7); (—▲—) group 5, liver perfusion + FK 506 1 mg/kg* + CP 10 mg/kg (days 0 to 6) (n=9); (—○—) group 6, kidney perfusion + FK 506 1 mg/kg* + CP 10 mg/kg (days 0 to 6) (n=8); (—■—) group 7, FK 506 1 mg/kg* + CP 15 mg/kg (days 3 to 1) (n=9). * FK 506 was continued for 30 days, followed by every other day injection of 0.5 mg/kg.

RESULTS

Animal Survival

Untreated recipients died between 5 and 6 days after transplantation (Fig 1, group 1). FK 506 alone at a daily dose of 2.0 mg/kg had no beneficial effect and animals died within 7 days (group 2). When used as a daily oral treatment, CP 10 mg/kg was more effective in prolonging graft survival, compared with FK 506, and median animal survival was 18.5 days (group 3). Combination treatment with FK 506 1.0 mg/kg per day and CP 10 mg/kg slightly

From the Pittsburgh Transplant Institute, University of Pittsburgh, Pittsburgh, Pennsylvania.

Address reprint requests to T.E. Starzl, 5C Falk Clinic, 3606 Fifth Avenue, Pittsburgh, PA 15213.

0041-1345/94/$3.00/+0

improved animal survival to 15 days (group 4). When performed antibodies were depleted before transplantation, better survival was obtained. Hamster liver perfusion with FK 506 and CP treatment significantly prolonged the kidney xenograft survival (group 5, median survival 34 days). Better animal survival was also obtained after hamster kidney perfusion with FK 506 and CP treatment (group 6). Median animal survival was 48 days, and two of eight animals survived for more than 100 days. The best result was obtained when CP 15.0 mg/kg per day was started 3 days before grafting. Three of 9 animals survived over 100 days (group 7).

Antihamster Lymphocytotoxic Antibodies

Antibody titer in untreated animals increased with time after transplantation and reached 2^{11} to 2^{13} when the animal died of rejection. This increase was slightly inhibited by FK 506 treatment and completely abolished with CP 10 mg/kg administration. FK 506 and CP combination reduced the increase of the antihamster antibodies in all groups of animals in this study.

Histopathology and Direct Immunofluorescence

Untreated kidney grafts failed because of pure humoral rejection with numerous platelet-fibrin thrombi in the vasculature and widespread vascular and glomerular necrosis. No mononuclear infiltrates were seen. Intense IgM>>IgG deposits in major arteries were seen as early as 1 hour after revascularization. The deposits increased with time and glomerular capillary loops and interstitium became IgM positive by 24 hours to 3 days. Liver or kidney perfusion, or cyclophosphamide pretreatment significantly decreased the IgM deposits in large arteries when examined 1 hour after grafting.

DISCUSSION

This study demonstrates that hamster kidney graft rejection is entirely mediated by humoral mechanisms in the rat. Deposition of IgM>>IgG antibodies to the endothelium of main vessels was observed as early as 1 hour after revascularization and extended to glomerular capillary loops and interstitium with time. No mononuclear cell infiltrate was present.

We have previously shown that long-term (>100 days) hamster heart and liver xenograft survival in the rat was routinely obtained using FK 506 and a short induction course of antiproliferative drugs.[1] When the same treatment strategy was applied to the kidney graft in this study, the results were not satisfactory and all animals died within 30 days. Because of early antibody deposition and subsequent vascular damage seen in untreated animals, the effect of preformed antibody depletion on animal survival was also examined. Donor organ perfusion, which has been shown to mitigate hyperacute rejection in many studies,[2] significantly improved hamster kidney graft survival in this study. Three-day pretreatment with CP was also effective to obtain better survival, suggesting the excellent effect of CP to suppress antibody production in this species combination.

The critical role of preformed xenospecific natural antibodies has been well demonstrated when grafts from widely separated species were hyperacutely rejected. In the hamster-to-rat combination, which is considered closely related xenotransplantation, the low levels of preformed antibodies did not have a significant role for heart and liver grafting.[3] This study shows that kidney grafts were more susceptible to xenospecific antibody damage than heart and liver grafts and that treatment for preformed antibodies was necessary to obtain long-term survival.

REFERENCES

1. Murase N, Starzl TE, Demetris AJ, et al: Transplantation 55:701, 1993

2. Giles GR, Boehmig HJ, Lilly J, et al: Transplant Proc 2:522, 1970

3. Langer A, Valdivia LA, Murase N, et al: Am J Pathol 143:85, 1993