1525

ong-Term Survival of Heart and Liver Xenografts With Splenectomy id FK 506

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WE HAVE reported that while hamster-to-rat liver xenograft survival is prolonged by FK 506, ham- hearts undergo humoral rejection under this immuno- pressant.[1] Because the spleen has been shown to be of the main sources of xenoantibodies,[2,3] and in vivo eriments have demonstrated that splenectomy (Spx) is to increase the efficacy of various immunosuppressive nts after xenotransplantation,[4–6] we combined Spx h FK 506 to test whether survival of the intractable diac xenografts is prolonged, and of liver xenografts roved.

TERIALS AND METHODS

is rats (250 to 280 g) and Golden Syrian hamsters (120 to 180 ere used as recipients and donors, respectively. Heterotopic rt transplantation (HTx) was performed according to the hod of Ono and Lindsey, while orthotopic liver transplanta- (OLT) was performed according to the cuff technique. enectomy (Spx) was performed at the end of the transplant ration. The animals were divided in 12 groups, as shown in the le 1.

complement-dependent cytotoxicity test (CDC) was used ally to detect, in the recipient's serum, the amount of antiham- antibodies following heart and liver transplantation. Grafts of erent groups were studied with light microscopy to create a tionship between the immunosuppressive therapy and the ence of rejection. At 100 days after transplantation, two animals in group 6 received intravenously 0.5 mL of serum taken from a group 1 rat at the time of rejection.

RESULTS

Table 1 shows xenograft survival. Among the animals that received heart transplantation, the best results were achieved with the combination of Spx and FK 506 2 mg/kg/d (group 6). In this group, all grafts survived beyond 100 days. The therapy with FK 506 1 mg/kg/d + Spx was effective in only two animals. Spx or FK 506 alone, even at high doses, did not significantly prolong the survival of the transplanted hearts. Among the animals that received liver transplantation, the best results were obtained with Spx + FK 506 1 mg/kg/d (group 11). Group 12, that received Spx + 2 mg/kg/d of FK 506, showed poorer results than group 11 and the groups that received FK 506 alone. In groups 8, 9, 11, and 12, all animals that died had severe jaundice and biliary dilatation at autopsy. In these cases, the histologi-

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Table 1. Survival of Hamster Cardiac and Liver Xenografts in Lewis Rat Recipients

ip	Treatment	N	Graft Survival Days	100-Day Survival	Percent
	Untreated	6	3, 3, 3, 3, 3, 3	0/6	0%
	FK 506 1 mg/kg/d	6	3, 3, 3, 3, 3, 4	0/6	0%
	FK 506 2 mg/kg/d	6	4, 4, 4, 4, 5, 5	0/6	0%
	Spx	6	5, 5, 5, 5, 5, 5	0/6	0%
	Spx + FK 1 mg/kg/d*	8	6, 7, 7, 7, 7, 8, >100, >100	2/8	25%
	Spx + FK 2 mg/kg/d†	10	>100 × 10	10/10	100%
	Untreated	8	6, 7, 7, 7, 7, 7, 7, 8	0/8	0%
	FK 506 1 mg/kg/d*	10	12, 20, 27, 29, 33, 48, 66, >100 × 3	3/10	30%
	FK 506 2 mg/kg/d‡	10	10, 13, 20, 23, 34, 34, 50, >100 × 3	3/10	30%
).	Spx	8	7, 7, 7, 7, 7, 7, 8, 8	0/8	0%
1.	Spx + FK 1 mg/kg/d*	10	9, 21, 26, 40, 48, >100 × 5	5/10	50%
2.	Spx + FK 2 mg/kg/d‡	8	7, 9, 9, 12, 17, 20, 30, 31	0/8	0%

K 506 was administered in a dose of 1 mg/kg/d during the first month, and then 0.5 mg/kg every other day, stopping treatment on day 100.
K 506 2 mg/kg/d × 15, then 1 mg/kg until day 30, then 0.5 every other day until day 100.
K 506 was given in a dose of 2 mg/kg/d for the first month, then 0.5 mg/kg every other day until day 100.

cal study showed important biliary proliferation without signs of rejection. Heterophilic antibody production after cardiac xenografting was completely suppressed in group 6 (combined treatment). The recipients of the other groups showed peaks of antibody titers around the third postoperative day (range 1:8 to 1:512). In contrast, all animals that underwent liver transplantation developed high titers of cytotoxic antibodies around the seventh postoperative day (range 1:512 to 1:8182). Lower titers were found in the group treated with Spx and 1 mg/kg/d of FK 506 (1:128). Cytotoxicity in the serum of long-term survival liver recipients became negative by 3 to 4 weeks, regardless of the treatment. Long-term surviving cardiac xenografts which received hyperimmune antihamster serum underwent hyperacute rejection 3 minutes after injection.

DISCUSSION

Splenectomy seems to have a different impact after liver or heart xenotransplantation. While after heart xenografting, Spx + FK 506 2 mg/kg/d resulted in long-term graft survival in all recipients, confirming Carobbi's experiments,[7] with the same therapy the percentage of long-term surviving liver xenografts was zero. The best protocol of immunosuppression for liver xenografts proved to be Spx + 1 mg/kg/d FK 506. However, even with this therapy, 100-day recipient survival was increased to only 50%. In both liver and heart xenotransplantation, there was a correlation between the presence of cytotoxicity in the serum of xenograft recipients and graft survival. Absence of cytotoxicity resulted in long-term survival. In contrast, high cytotoxic activity in the recipient sera meant rejection in the heart recipients—but only hepatic damage in the liver recipients. In fact, liver xenograft recipients that died while under treatment had severe jaundice with biliary dilatation. The histological findings of the liver grafts showed severe biliary proliferation, without signs of active rejection. According with these results, it seems possible to correlate the failure of the liver grafts to the damage induced by the first humoral attack of the natural and induced antibodies, mainly focalized in the biliary tract.[8] This is in agreement with the observation that, after liver xenotransplantation, when FK 506 is combined with a drug that effectively prevents antibody production (antiproliferative drugs), we have never observed a hepatic failure with the characteristics described above.[9]

Paradoxically, after liver xenotransplantation, the animals receiving Spx + FK 506 2 mg/kg/d had higher mortality than the other groups. One explanation could be that high levels of FK 506 present in these rats increase the production of IL-10,[10] a potent growth and differentiation factor for activated T-independent B lymphocytes.[11]

The hyperacute rejection of long-term surviving cardiac xenografts after injection with hyperimmune serum indicates that the vascular endothelium of the graft remains susceptible to humoral injury.

The liver seems to be more antigenic than the heart, as indicated by the huge splenomegaly,[4] the massive splenic lymphocyte proliferation,[2] and the very high cytotoxic titer observed after liver xenotransplantation. This would explain the slight improvement in survival of liver xenograft recipients. In conclusion, Spx + FK 506 can overcome the antibody barrier in the hamster-to-rat cardiac xenograft model with excellent graft survival.

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