

1840

# Tacrolimus (FK 506)-Dependent Tolerance After Liver and Heart Xenotransplantation: Inhibition of Humoral Response and Acceptance of Donor Organs

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**W**E HAVE previously reported that stable long-term survival of hamster heart and liver xenografts is successfully achieved in rat recipients by combining continuous tacrolimus treatment and a short induction course of antimetabolic drugs.<sup>1</sup> In this study we further examined the immunological status of long-surviving rat recipients of hamster heart and liver grafts.

## MATERIALS AND METHODS

Inbred male Lewis rats weighing 200 to 250 g (Harlan Sprague-Dawley Inc., Indianapolis, IN) and outbred male Syrian hamsters weighing 100 to 150 g (Charles River Lab., Wilmington, MA) were used for recipients and donors, respectively. Heart grafts were heterotopically transplanted into the abdomen or the neck of recipients using the methods previously described.<sup>2,3</sup> Liver grafts were orthotopically transplanted by the modified method of Kamada and Calne.<sup>4</sup> All recipients were treated with intramuscular tacrolimus administration at daily doses of 1 to 2 mg/kg from day 0 to day 30 with cyclophosphamide (CP) administration at daily oral doses of 7.5 to 15 mg/kg from day -1 to day 9 or 13. Tacrolimus was given from 30 days onward in every other day dose of 0.5 mg/kg until the time of challenge transplantation and continued indefinitely thereafter in experiments 2 and 3 (see below).

### Experiment 1

Tacrolimus therapy was stopped 30, 100, and >150 days after heart or liver transplantation, and animals were followed without immunosuppression for another 100 days or until death.

### Experiment 2

Long-surviving recipients were challenged to accept another hamster graft under continuing reduced-dose tacrolimus. Fifty to 100 days after the primary hamster heart or liver transplantation, recipients were challenged with second hamster grafts. For the treatment control, normal Lewis rats were treated with the same immunosuppressive protocol without the primary transplantation, and they received the hamster grafts 50 days after the initiation of treatment.

### Experiment 3

Hamster heart grafts surviving more than 50 days under continuous immunosuppression were harvested and retransplanted into naive Lewis rat recipients.

## RESULTS

### Experiment 1

All hamster grafts were eventually rejected after the cessation of maintenance tacrolimus treatment. When immunosuppression was stopped at 30, 100, or more than 150 days

after transplantation, median drug-free heart xenograft survivals were 43.5, 33, and 32 days, respectively. After discontinuation of tacrolimus at 30, 100, or >150 days after transplantation, liver grafts survived significantly longer than heart grafts for 97 ( $P = .01$ ), 121.5 ( $P < .05$ ), and 47.5 days, respectively.

### Experiment 2

Untreated (Group 1, Table 1) and treated control recipients not given primary heart grafts (Group 2) rejected challenge hamster heart grafts in 3 days. In contrast, challenge heart grafts were protected from rejection and survived for more than 90 days when these had been preceded by either a priming heart graft under protocol immunosuppression (Group 3) or by a priming liver (Group 4).

Hamster liver grafts were rejected by untreated recipients within 8 days (Group 5). This survival was prolonged to 33 days by immunosuppression (control Group 6). However, liver recipients primed with hearts had median survival of 85 days (Group 7). Two animals whose survival was 39 and 97 days had cause of death other than rejection.

### Experiment 3

Long-surviving hamster heart grafts were normally rejected in 4 days when retransplanted into untreated ( $n = 4$ ) or intramuscular tacrolimus (2 mg/kg/d) treated ( $n = 5$ ) naive rats.

## DISCUSSION

As previously reported,<sup>1</sup> hamster heart and liver transplants were able to consistently survive >100 days with the immunosuppression used in the experiments. However, grafts were slowly rejected with the discontinuation of maintenance tacrolimus, even after 100 days. This study also showed the same generic phenomena of organ tolerogenicity.

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Table 1. Survival of Secondary Challenged Hamster Heart and Liver Grafts Transplanted Into Long-Surviving Xenorecipients

Group	First Graft	Treatment* (tacrolimus + CP)	Second graft <sup>†</sup>	n	Survival (Second graft) (days)	Median Survival (days)	P value
1	—	—	Heart	6	3, 3, 3, 3, 3, 3	3	—
2	—	+	Heart	4	3, 3, 3, 4	3	—
3	Heart	+	Heart	7	50, >100 × 6	>100	<.01 <sup>‡</sup>
4	Liver	+	Heart	5	39 <sup>‡</sup> , 67 <sup>‡</sup> , 97 <sup>‡</sup> , >100 × 2	97	<.02 <sup>‡</sup>
5	—	—	Liver	8	6, 7, 7, 7, 7, 7, 8	7	—
6	—	+	Liver	2	33, 33	33	—
7	Heart	+	Liver	4	41 <sup>‡</sup> , 70 <sup>‡</sup> , >100 × 2	85	<.1 <sup>§</sup>

\*Tacrolimus (1–2 mg/kg day) was intramuscularly injected for 30 days after first grafting, followed by 0.5 mg/kg injection on every other day thereafter. Cyclophosphamide (CP) was given only after first grafting at oral doses of 7.5–15 mg/kg/day (day –1 to 13).

<sup>†</sup>Second grafts were transplanted between 50 and 100 days after first transplantation, when tacrolimus was injected at a dose of 0.5 mg/kg/every other day.

<sup>‡</sup>vs Group 2.

<sup>§</sup>vs Group 6.

<sup>‡</sup>Animal died with functioning graft without signs of rejection.

ity that we recently documented in allografts.<sup>5</sup> Although the liver is known to be more self-tolerogenic than the heart,<sup>4,5</sup> as was evident in the control experiments (compare control groups 2 vs 6, Table 1), the best results were actually obtained using the heart as the priming organ in the experiments described herein. This misleading result was caused by late death not related to rejection when the more difficult liver replacement was used for either the priming (Group 4) or challenge xenotransplantation (Group 7).

The results strengthen the contention that the same principles underlying allograft acceptance apply to xenotransplantation, but with a more resistant immunologic barrier.

The tolerogenicity of organ allograft has been associated with the chimerism they produce, which in turn simulates the effect of leukocyte cell suspensions. We previously documented that long-surviving rat recipients of hamster

organs have such chimerism.<sup>6</sup> The retransplantation experiments showed that alteration of the transplanted organ by residence in a xenogenic host is insufficient by itself to profoundly influence the xenograft reaction.

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