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Modification of established rejection of canine kidney and liver homografts with antilymphocyte gamma-G globulin

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The ability of heterologous antilymphocyte serum (ALS) or its globulin derivative (ALG) to mitigate or even prevent homograft rejection is well known. In early investigations with skin homotransplantation in inbred rodents, it was shown that ALS given even several days after arrival of the homograft resulted in definite prolongation of transplant survival.[4, 7] This communication concerns an extension of this kind of study in mongrel dogs that were subjected to renal or liver transplantation and were not treated until the onset of rejection.

METHODS

Renal homotransplantation and simultaneous bilateral nephrectomy were performed in 10 test and 10 control mongrel dogs which weighed 10 to 20 kilograms. Postoperatively, blood urea nitrogens (BUN's) were performed daily. In addition, one animal with orthotopic liver transplantation was included and followed with standard liver function tests. Autopsy was carried out in all but two dogs which are still alive after 45 and 100 days.

The sole antirejection therapy in the treated group was with horse antidog-lymphocyte globulin (ALG). The ALG consisted of a nearly pure gamma-G globulin that had been removed from the horse serum with a diethylaminoethyl cellulose batch technique.[5] The material had a protein content of 2.8 Gm. percent and leukoagglutinin, hemagglutin, hemolysin, and thromboagglutinin titers of 1:4,000, 1:1,000, 1:4, and 1:12, respectively. The individual intramuscular doses were based on the leukoagglutinin titers and were calculated to provide 1,000 units per kilogram[11] or about 0.7 mg. per kilogram per day. The same ALG when administered daily from the time of operation had previously been shown by Kashiwagi and co-workers[5] to permit a mean postoperative survival after renal homotransplantation of 23 days.

In the experiments with kidney transplantation, ALG therapy was not started until the BUN had begun to rise secondarily after several days of initial normal renal function. It was then given daily until death or for at least 2 months from the time the BUN reached or exceeded 40 mg. percent. One week after transplantation the recipient of the liver homograft developed rejection and was given injections of ALG for 3 consecutive days but not thereafter.

RESULTS

Control animals. For unexplained reasons, survival was unusually prolonged in the untreated recipients of renal homografts (Table

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Table I. *Data on untreated control dogs subjected to renal homotransplantation and test animals in which ALG was started after the onset of uremia*

Experiment No.	Day BUN > 40 mg. %		Total survival (Days)		Survival with BUN > 40 mg. % (Days)	
	Control	Treated	Control	Treated	Control	Treated
1	11	13	30	25	19	12
2	30	5	33	13	3	8
3	9	14	13	17	4	3
4	4	7	10	13	6	6
5	14	6	20	9	6	3
6	4	8	9	32	5	24
7	6	10	7	20	1	10
8	10	11	16	29	6	18
9	6	9	11	50*	5	41*
10	8	7	12	13	4	6
Mean	10.2	9.0	16.1	22.1	5.9	13.1

*Although survival credit was limited to 50 days, this animal is still alive with normal renal function after 100 days.

I), averaging 16.1 (range 7 to 33) days. Three of the animals lived for 20, 30, and 33 days.

BUN elevations to more than 40 mg. percent occurred between the fourth and thirtieth days (10.2 days average). Thereafter, the average survival was 5.9 days. The longest survival after this level of uremia became established was 19 days. The rise in BUN in the longest-surviving dog was at 30 days with death following 3 days later. There were no examples of spontaneous reversal of the azotemia. The histologic features of all the homografts were those of typical rejection.

Treated animals. One of the recipients of a renal homograft is still alive with a normal BUN after 100 days. Limiting credit for this animal to 50 days, the mean survival for the entire series was 22.1 days (Table I). Lymphopenia was commonly but not invariably observed after starting ALG treatment, but its presence or absence was not well correlated with subsequent survival.

The elevations in BUN to more than 40 mg. percent were observed 5 to 14 days after operation. Subsequent survival, with the ceiling of 50 days for the longest-living dog, was 13.1 days. Four of the animals had some definite evidence of reversal of the azotemia although in only two did the BUN revert to normal (Fig. 1); in the two latter experiments, the peak BUN was recorded at 141 and 53 mg. percent, respectively. Ultimately, all the animals but the one still alive developed fatal uremia. All of the nine kidneys examined after death had histologic evidence of rejection although his was less marked than in the control experiments.

The recipient of the liver homograft developed rejection 7 days after transplantation, was given three injections of ALG, and is still alive 45 days postoperatively despite slowly progressive deterioration of hepatic function.

DISCUSSION

In these experiments, there was evidence that ALG therapy slowed the tempo and vigor of rejection even when treatment was started after this process was well advanced. The crude survival was increased by about a week. Of even greater significance was the fact that death after the onset of uremia was delayed from 5.9 days in the control animals to 13.1 days in the test series. Finally, there was apparent reversal of rejection, at least for brief periods, in four of the ten kidney recipients and in the liver recipient.

These findings were not surprising in view of the previous reports that ALS or ALG can prevent or blunt the expression of pre-existing immunization states[1-3, 8, 12-14] including that of a second-set reaction.[6, 7, 9, 10]

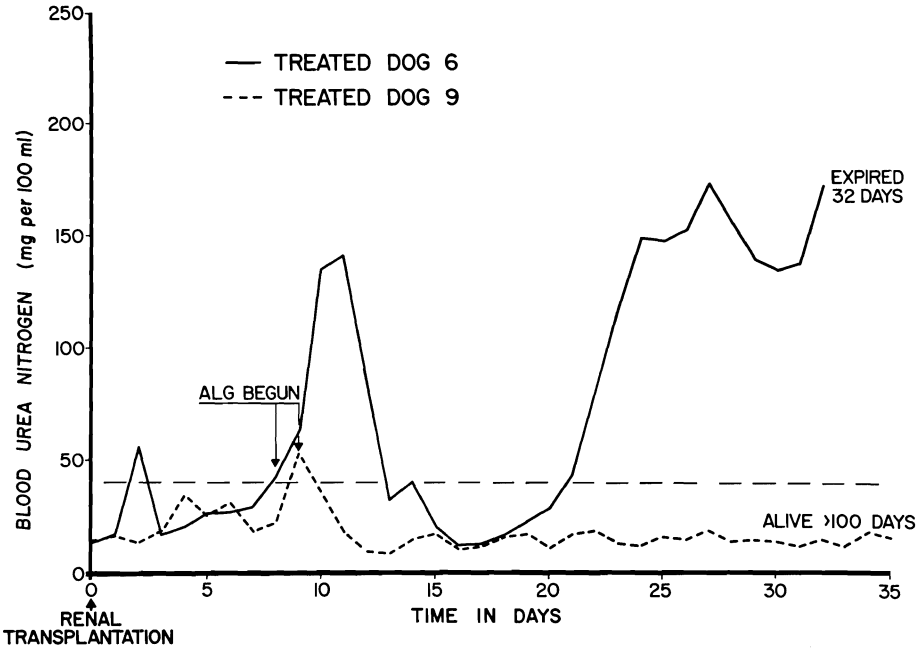

Fig. 1. Blood urea nitrogen levels in two dogs which received delayed treatment with antilymphocyte gamma-G globulin (ALG). Therapy was initiated *(arrows)* when the BUN secondarily rose above 40 mg. percent, 8 and 9 days, respectively, after renal transplantation, and continued thereafter in doses of 1,000 units per kilogram per day. Dog 6 died of uremia 32 days postoperatively, 24 days after the onset of rejection. Dog 9 is alive with normal renal function more than 100 days after transplantation.

However, the magnitude of the effect was limited and resulted in only one long-term survivor in the kidney series. Presumably, a greater therapeutic benefit might have been obtained if the doses had been larger or if treatment had been started at an earlier time.

SUMMARY

Heterologous ALG was administered to ten canine recipients of renal homografts and one recipient of an orthotopic liver. Treatment was started after rejection was well established. Death from homograft failure was thereby significantly delayed and in several experiments the rejection was at least partially reversed.

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