USE OF ANTI-LYMPHOCYTE SERUM TO PROLONG DOG HOMOGRAFT SURVIVAL

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EARLY RESEARCH with anti-lymphocyte serum (1-6) involved the use of inbred rats, mice, or guinea pigs. The development of a safe horse-anti-dog lymphocyte serum to be described has gone through 3 stages.

In the first stage, anti-lymphocyte plasma (ALP) was prepared by subcutaneously injecting 4 to 20 million dog lymph node lymphocytes into 6 horses from 4 to 12 times at intervals of 1 to 8 weeks. The heparinized plasma was heated at 56° C. for 30 min. Leuko-agglutinin titers of the horse plasma against dog white cells rose from control to

1:16 to 1:128; lympho-agglutinin titers spot checked were essentially the same as with unseparated dog white cells. The resulting pooled ALP, which caused marked lymphopenia, was toxic. Of 36 dogs to which it was given IP (1 to 4 ml./kg./day), 11 died within 15 days. Anaphylactic reactions were common. Anemia was invariable; 26 dogs studied for 15 days had a hematocrit value fall from 40.1 ± 4.1 (SD)% to 30.8 ± 6.3 (SD)%. The 25 remaining dogs, subjected to bilateral nephrectomy and renal homotransplantation from nonrelated donors, subsequently lived 4 to 144 days (mean 32.3 ± 33.3 (SD) days); 10 lived longer than a month. Twelve of the 25 dogs which had received thymectomy in advance did not fare better than the other 13. The ALP was thus markedly immunosuppressive but excessively dangerous.

In the second stage, anti-lymphocyte serum (ALS) was prepared from the same horses. Heteroagglutinins were partially absorbed with 1 part dog red cell pack against 10 parts ALS. This product given IP was also lymphopenic, but it did not cause any deaths among 19 dogs treated daily for 16 to 22 days. However, in 19 dogs the fall in hematocrit value during 15 days was from 45.8 ± 4.4 (SD)% to 34 ± 5 (SD)%. Six dogs pretreated for 16 to 18 days were then provided with renal homografts. Survival was 13 to 49 days 27.2 ± 13 (SD) days), 2 of the 6 living more than a month. Nine more pretreated animals were given orthotopic liver transplants. The present mean survival is 22.9 ± 20.4 (SD) days; 2 dogs are still alive after 37 and 68 days. One of the latter dogs was only treated preoperatively. The other (the longest survivor) had therapy stopped 3 weeks after operation with no subsequent deterioration. Both now have marked lymphocytosis. The effect of ALS tends to be lasting and is not dependent on continued lymphopenia. Finally, 6 dogs had renal homotransplantation without pretreatment. Survival was 12.8 ± 12.5 (SD) days. Only 1 dog lived more than 12 days. Pretreatment is therefore an important factor.

The features of rejection in the homografted organs did not differ from those seen after prolongation of survival with agents such as azathioprine. Dogs who received either ALS or ALP with or without transplantation had depletion of the small lymphocytes in all lymphoid depots. In transplanted dogs, pyroninophilic cells were present in varying numbers and many were in mitosis. Nontransplanted dogs receiving ALS or ALP intraperitoneally did not have injury of their kidneys but all had received treatment for 14 days or less. In 10 other dogs given 2 to 6 ml. ALS/kg. IV for 18 days to 2 months in a toxicity study, subepithelial deposits were present on the renal glomerular capillary basement membrane in 6.

In the third stage, the immunizing doses of white cells were drastically increased by giving 5 to 95 billion spleen white cells to each

horse 1 to 3 times a week for 4 weeks. Leuko-agglutinin titers rose to as high as 1:32,000. In an additional horse, similar immunization with human spleen resulted in a comparable anti-human lymphocyte response. The ALS was repeatedly absorbed with 10% to 30% of dog red cells and in some cases also with 5% serum, 10% to 25% kidney, and 10% to 25% liver. Red cell and serum absorption caused little titer loss but 75% to 95% was lost with liver and kidney. The absorbed serum was precipitated first with 0.35 and then with 0.5 sat NH_4SO_4; most of the activity was in the 0.35 portion. The 0.35 portion was lyophilized, reconstituted, and tested, or alternatively passed through a diethylaminoethanol cellulose column. With column separation, 2 principal fractions were collected, neither completely pure. The first (principally γ globulin G) contained a high leuko-agglutinin titer. The second, which had a low titer, was principally γ globulin M. The active principle is probably an immunoglobulin G (7S).

Lymphopenia was caused by all the altered products providing a sufficient SC dose was used. Anemia did not occur; anaphylactic reaction has not been seen in any of 24 test dogs. The acute risk with the material absorbed only with red blood cells and serum was not greater than with that also absorbed with kidney and liver. Using small doses of 0.35 saturated precipitated material which did not cause lymphopenia, immunosuppression was evident under certain circumstances. Six dogs, which had therapy started the day before operation, survived only 4 to 13 days after renal homotransplantation. In contrast, 3 of 4 dogs which received orthotopic liver transplants with the same therapy are well after 16 to 21 days. Six more dogs received renal homografts with the same dose, started 12 days preoperatively, and continued postoperatively. Four died after 9 to 13 days but the other 2 are still alive. These findings confirm the value of pretreatment cited earlier, prove that peripheral lymphopenia is not a prerequisite to success, and suggest that it is fundamentally easier to prevent repudiation of the liver than of the kidney.

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