233

Modification of Xenograft Response by Selective Plasmapheresis

By F. K. MERKEL, M. BIER, C. D. BEAVERS, W. G. MERRIMAN, C. WILSON AND T. E. STARZL

HYPERACUTE REJECTION of human renal allografts in ABO compatible cases was first reported by Terasaki.[1] Kissmeyer-Nielson,[2] Williams,[3] and Starzl[4] have further documented this problem, which has now been encountered in many active transplant centers. Hyperacute rejection is initiated by preformed antidonor antibodies and may result in the destruction of the transplant within minutes of vascularization. A characteristic of this reaction is thrombotic occlusion of the microvasculature.[4,5]

The rejection of xenografts in widely divergent species, such as the pig to dog combination, is similar to hyperacute rejection in humans. Naturally occurring heterospecific antibodies apparently cause rejection of renal xenografts within 6–15 minutes.[6] The following experiments were designed to determine whether the xenograft response could be modified by selective plasmapheresis (SPF), an electrophoretic process which can extract gamma globulin from the circulating blood.

MATERIALS AND METHODS

The SPF apparatus was developed by Bier in 1957.[7] Two plastic endplates, containing platinum electrodes, surround a series of spacers, making up a cell pack much like some in use for hemodialysis. An external buffer carries away the toxic products formed at the endplates and makes contact with the cell. Temperature, pH, hemolysis, and fluid shifts are controlled by an internal buffer.[8] The membranes between the spacers are heavy regenerated cellulose except between the blood and the globulin channel, where a Millipore filter is used. A gradient of 6–8 volts/cm. results in globulin extraction and prevents clogging of the Millipore filter by the red cells. The apparatus is attached to unanesthetized, heparinized mongrel dogs by silastic extracorporeal shunts placed in the neck vessels.

Three dogs underwent SPF for approximately four hours on two successive days and received porcine renal xenografts toward the end of the second SPF (Fig. 1). One dog received the graft

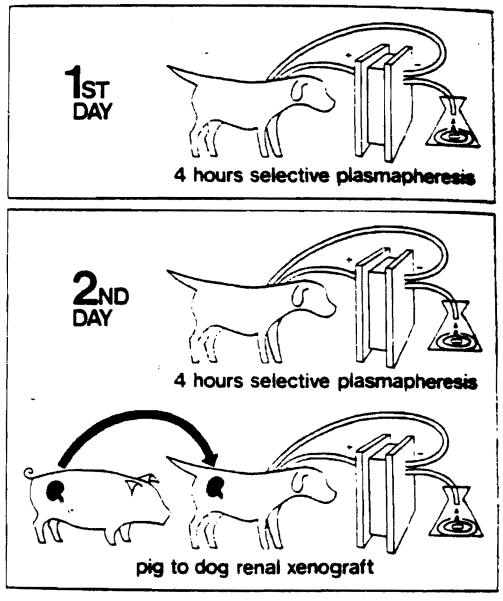

Fig. 1.—Experimental model. After two 4-hour periods of selective plasmapheresis on 2 successive days, pig-to-dog xenograft is carried out and selective plasmapheresis is continued.

From the Departments of Surgery, University of Colorado Medical Center, The Denver General Hospital, and the Denver Veterans Administration Hospital, Denver, Colo.; the Tucson Veterans Administration Hospital, Tucson, Ariz.; and the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

Supported in part by Public Health Service Contract PH 43-68-621, and Public Health Service Grants AI 04152, AI-AM 08898, AM 12148, AM 07772, RR 00051 and RR 00069, and by grants from the American Diabetes Association, the Colorado Heart Association, and the Eli Lilly Co.

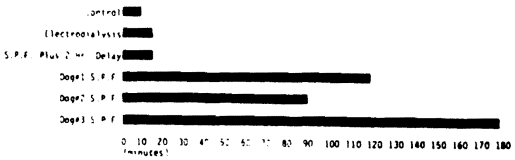

Fig. 2.—Survival of pig-to-dog renal xenografts. Untreated pig-to-dog xenografts rejected in 10 minutes. Electrodialysis (a process similar to selective plasmapheresis but without the Millipore filter essential for globulin extraction) and SPF with a 2-hour delay failed to prolong graft survival significantly. SPF dogs rejected their xenografts in 120, 90, and 180 minutes, extending survival by as much as eighteen times normal.

two hours after cessation of the second SPF. Electrodialysis was carried out on two dogs instead of SPF and followed by the transplant. In electrodialysis, the same apparatus is employed, without the Millipore filter compartment. The difference is that no globulin extraction occurs.

The onset of rejection was determined by a mottled appearance of the kidney and was judged complete when urine production ceased. Blood samples were taken at the beginning and end of each SPF and at close intervals during and after the transplantation. These were analyzed for formed blood elements, plasma hemoglobin, serum proteins, fibrinogen, and complement. Biopsies for

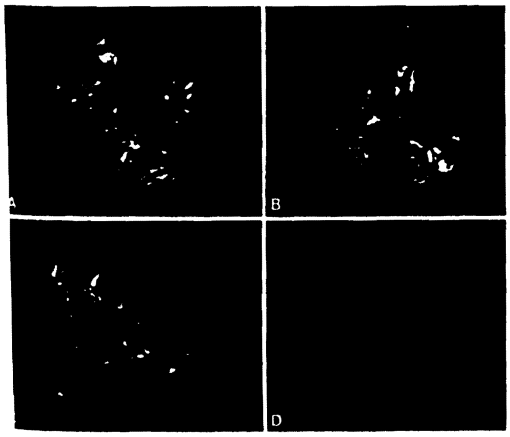

Fig. 3.—Fluorescent micrographs taken from PF dog 3. Stain is for fibrin. Samples A and B were taken from the pig xenograft at 30 minutes and sample C was at 60 minutes. Section D is dog's own kidney at 60 minutes. Fibrin deposits seen in xenograft are type seen in unmodified recipients. It is not possible to quantiate this reaction.

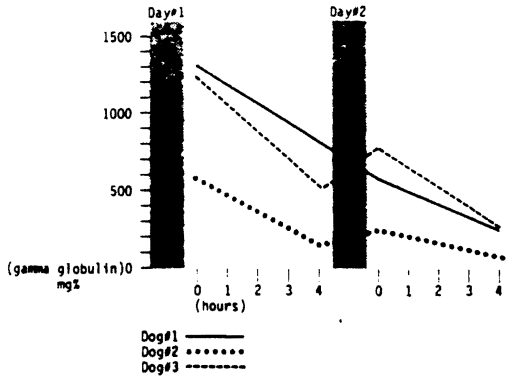

Fig. 4.—Effect of selective plasmapheresis on gamma globulin.

light and fluorescent microscopy were taken at the first sign of rejection and at selected periods until rejection was complete.

Results

Pig-to-dog renal xenografts without SPF rejected within 10 minutes. Xenografts in SPF recipients survived with urine output of 120, 90 and 180 minutes. Mottling occurred about 30–45 minutes after grafting and increased in amount until urine output ceased, when the kidneys were solid purple in color. In the dog that had a 2-hour delay between SPF and transplantation, rejection occurred within 15 minutes. If electrodialysis was substituted for SPF, the xenograft response was equally rapid (Fig. 2).

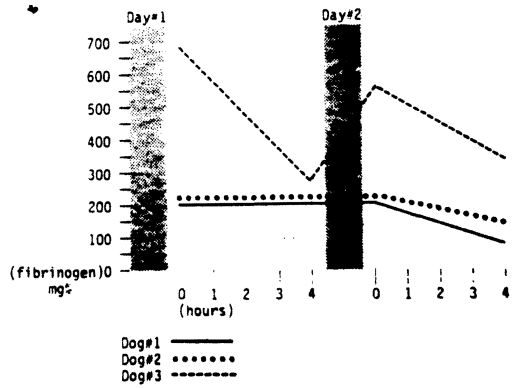

Fig. 5.—Effect of selective plasmapheresis on fibrinogen. Effect appears more variable than with gamma globulin.

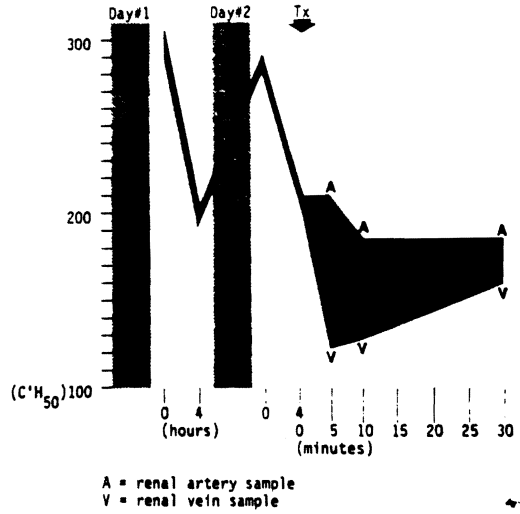

Fig. 6.—Effect of selective plasmapheresis in complement. Note return to normal within 12 hours of SPF. Within 5 minutes of xenografting, marked arterial-venous differences in complement could be detected across transplant, persisting for at least 30 minutes. This is in striking contrast to control grafts, where differences rarely persist for more than a few minutes.

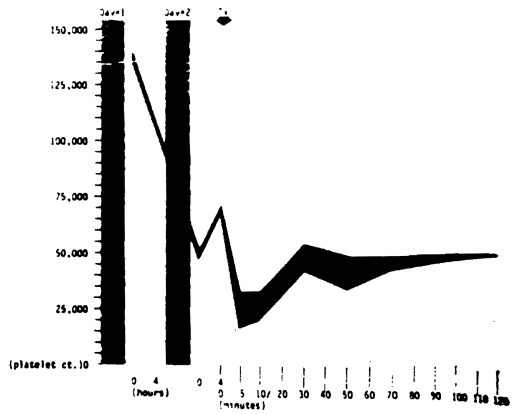

Fig. 7.—Effect of selective plasmapheresis on platelets. Depression of platelet count by SPF apparatus is substantial, but it is clear that across xenograft significant arterial-venous differences in platelets can be measured that also persist much longer than in untreated controls.

Light and fluorescent microscopy of the grafts in the SPF treated dogs showed fibrin deposits in the glomerular capillaries identical to that in the rapidly rejecting nontreated controls (Fig. 3). The hematoxylin and eosin light microscopy revealed an increasing polymorphonuclear leucocytic infiltrate with time. The abnormalities in the xenografts in treated and untreated recipients were the same, the only difference being the times for these changes to develop.

After 4 hours the SPF caused 60 per cent reductions of gamma globulin concentrations in the recipients with recovery to 74 per cent of pretreatment values overnight. At the end of a second SPF, globulin levels were about 25% (Fig. 4). Fibrinogen (Fig. 5) and complement (Fig. 6) were similarly effected. Platelets were reduced 40 per cent by SPF (Fig. 7). Red cell destruction was considerable, with the result of a rising plasma hemoglobin. White cells were not depleted.

During rejection by the conditioned or treated recipients, arterial-venous differences across the grafts of platelets and complement were detectable for 30–70 minutes. In the treated animals, the degree of prior gamma globulin reduction by SPF did not correlate well with the duration of graft survival.

Discussion

SPF provides an electrical means for modifying the xenograft response. It is clear that the present apparatus is a prototype and must be modified to obtain better and more consistent results. Nevertheless, significant prolongation of pig-to-dog renal xenografts was obtained, although eventually the rejection went to completion. This points up the possible use of SPF for more detailed study of the xenograft response since a reaction occurring within 5–15 minutes is stretched out to several hours.

The graft prolongation by SPF was most likely by globulin depletion. In the control studies using electrodialysis, but omitting the Millipore filter compartment which is

essential for globulin extraction, nonspecific changes occurred in fibrinogen and complement, but no graft protection resulted. In combination with other modes of immunotherapy, SPF may eventually aid in the prevention of hyperacute rejection.

Conclusions

SPF prolongs the response to procine renal xenografts in dogs. The effect of SPF is probably a result of globulin depletion and/or reduction of complement. The usefulness of this process in the study and prevention of hyperacute rejection has been suggested.

ACKNOWLEDGMENT

We wish to thank Drs. Charles Zudoski and Scott Clark, Miss Joyce Trembath, Mr. Paul Taylor, Mr. Yosh Arai, Mrs. Isabelle Snow, and Mrs. Ruth Gibbs for their assistance.

REFERENCES

1. Terasaki, P. I., Marchioro, T. L., and Starzl, T. E.: *In* Histocompatibility Testing. Washington D.C., National Acad. Sci.–National Res. Council, 1965, pp. 83-96.

2. Kissmeyer-Nielson, F., Peterson, V. P., Olson, S., and Fieldborg, O.: Lancet 11:6-62, 1966.

3. Williams, G. M., Lee, H. M., Weymouth, R. F., Harlan, W. R., Holden, K. R., Stanley, G. M., Millington, G. S., and Hume, D. M.: Surgery 62. 204, 1967.

4. Starzl, T. E., Lerner, R. A., Dixon, R. J., Groth, C. G., Brettschneider, L., and Terasaki, P. I.: New Eng. J. Med. 278:642, 1968.

5. Simpson, K., Bunch, D. L., Amemiya, H., Boehmig, H. J., Wilson, C. B., Dixon, F. J., Coburg, A. J., Hathaway, W. E., Giles, G. R., and Starzl, T. E.: Surgery 68:77, 1970.

6. Linn, B. S., Jenson, J. A., Porteil, P., and Snyder, G. B.: J. Surg. Res. 8:211, 1968.

7. Bier, M.: Science 125: 1684, 1957.

8. Bier, M., Beavers, C. E., Merriman, W. G., Merkel, F. K., Eiseman, B., and Starzl, T. E.: Trans. Amer. Soc. Artif. Int. Organs 16:325, 1970.