

Prolonged Survival of Kidney Xenografts in Leucopenic Rabbits

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Summary. Rabbits rendered leucopenic by means of nitrogen mustard reject kidney xenografts more slowly than normal controls. It is therefore probable that polymorphonuclear leucocytes play an important role in the acute rejection of xenografts. The previously reported complement requirement for xenograft rejection was also confirmed in this work, by depleting the graft recipients of C3 with cobra venom factor.

INTRODUCTION

Some insight into mechanisms involved in the hyperacute rejection of kidney xenografts has been achieved recently as a result of experiments on the modification of complement levels in the recipient of the graft. The graft survival time is increased significantly when the third complement component is depleted with cobra venom factor (Nelson, 1966; Gewurz, Clark, Finstad, Kelly, Varco, Good and Gabrielsen, 1966). Prolonged renal xenograft survival is also obtained after depletion of the fourth complement component by means of shark C4 inactivator (Jensen, 1969). On the other hand, C6, C7, C8 and C9 are not required since a normal rejection pattern has been observed in C6 deficient rabbits (Mejía-Laguna, García-Cornejo, López-Soriano and Biro, 1970). In addition to the role of the first five complement components, natural agglutinins against recipient red blood cells that show cross reaction with recipient tissue antigens seem to take part in this process (Linn, Jensen, Portal and Snyder, 1968). These agglutinins seem to act by decreasing both complement levels in serum and the number of peripheral polymorphonuclear leucocytes (PMNs).

These observations, together with the finding of a significant number of PMNs in glomerular or peritubular capillaries (Gewurz *et al.*, 1966; Rowlands, Kirkpatrick, Vatter and Wilson, 1967; Mejía-Laguna *et al.*, 1970), suggest that the presence of PMNs may be related to the hyperacute rejection of kidney xenografts.

In the present study the effect of a reduction in the number of PMNs in the recipient, by means of nitrogen mustard, was investigated and the results were compared with those obtained when C₃ was depleted with cobra venom factor.

MATERIALS AND METHODS

Adult and young animals were used for the present experiment. Rabbits weighed be-

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tween 1.0 and 4.1 kg. Dogs weighed between 3.2 and 10 kg. Dogs were used as kidney donors, and rabbits as recipients. Three groups of transplants were performed:

- I. From a normal dog to a normal rabbit.
- II. From a normal dog to a rabbit injected with cobra venom factor.
- III. From a normal dog to a rabbit rendered leucopenic by the administration of nitrogen mustard.

The following data were obtained from recipient rabbits 1 hour before transplantation: (a) number of leucocytes in peripheral blood; (b) differential count of leucocytes and (c) estimation of the number of platelets.

Recipient rabbits in group II received an intravenous injection of 1.6 mg protein of cobra venom factor 12–24 hours before transplantation.

TABLE I
NUMBER OF LEUCOCYTES AND TIME OF REJECTION OF KIDNEY XENOGRAFTS IN NORMAL RABBITS, IN RABBITS TREATED WITH COBRA VENOM FACTOR AND IN RABBITS TREATED WITH NITROGEN MUSTARD

Donor (dog no.)	Recipient (rabbit no.)	No. of leucocytes/mm ³	No. of PMNs/mm ³	Rejection time (minutes)	Treatment
10	2200	Not done	Not done	16	None
12	2213	Not done	Not done	23	
19	2235	8250	3447	15	
20	2249	10600	4664	25	
23	2265	10050	4924	10	
26	2284	13300	7714	75	Cobra venom factor
27	2287	19250	10010	50*	
29	2289	13450	4707	52*	
30	2292	30500	18605	71	
31	2293	13100	7205	126*	
11	2201	2400	96	93	Nitrogen mustard
14	2219	2300	184	23	
15	2221	2250	22	105	
32	2295	1250	75	110	
34	2357	1450	43	50*	

* Minimal survival time of the xenograft, since the recipients died from venous anastomotic bleeding.

The surgical procedure for transplantation was similar to that described in detail in a previous publication (Mejía-Laguna *et al.*, 1970). Biopsies of the transplanted kidney were obtained 10, 20, and 30 minutes after releasing the arterial clamp, and after rejection occurred. Kidney biopsies 2 minutes after transplantation were performed in the last six cases.

The material was processed for light and for electron microscopy following the usual procedures. A detailed account of morphological data will be the subject of a separate report.

Renal transplant rejection was determined by cessation of urinary flow from, and by the appearance of oedema, cyanosis and venous congestion in the transplanted kidney.

RESULTS

The time of rejection of kidney xenotransplants was as follows: in normal recipients, 10–25 minutes; in recipients treated with cobra venom factor, 50–126 minutes; and in recipients treated with nitrogen mustard, 23–110 minutes. As expected, the time of rejection

was prolonged in most animals in group II. Rabbits in group III also exhibited delayed rejection, approximately 3.5 times that in normal recipients.

The treatment with cobra venom factor or with nitrogen mustard had no obvious effect on the general appearances of the recipient animals. Body weight fell by 100–200 g.

Table 1 shows the numbers of leucocytes, differential counts and the time of rejection of their xenografts. The number of leucocytes and PMNs in the venom-treated rabbits was higher than in the control group, whereas a pronounced leucopenia and neutropenia was observed in rabbits treated with nitrogen mustard. Changes in platelet counts were never found.

DISCUSSION

The present study demonstrates that the rejection of renal xenografts is significantly delayed in leucopenic recipients. Also, as previously reported, a similar increase in the time of kidney xenograft survival was observed in animals depleted of the third complement component by administration of cobra venom factor. The results suggest, therefore, that PMNs play an important role in hyperacute rejection of kidney xenografts.

PMN infiltration in glomeruli of allotransplanted kidneys has been described by Weymouth, Seibel, Lee, Hume and Williams (1970), and by Kinkaid-Smith (1967), and in renal xenografts by Rowlands *et al.* (1967), Gewurz *et al.* (1966) and Mejía-Laguna *et al.* (1970). In this respect, it is interesting to recall that the presence of early PMN infiltration in human allotransplants is generally associated with a poor evolution of the transplanted kidney (Kinkaid-Smith, 1967; Weymouth *et al.*, 1970).

The infiltration of PMNs is probably a consequence of the fixation of natural antibodies pre-existing in the recipient to a structural element of the transplanted kidney. The subsequent series of events apparently includes the activation of the complement components C1, C4, C2, C3, C5 and the liberation from C3 and/or C5 of the chemotactic factors demonstrated by Bokisch, Müller-Eberhard and Cochrane (1969), and by Ward and Hill (1970). Evidence in support of this derives from the indirect demonstration of cross reaction of natural agglutinins against the red blood cells of the donor kidney (Rowlands *et al.*, 1967; Linn *et al.*, 1968) and from data on the participation of C3 (Nelson, 1966) and C4 (Jensen, 1969) in xenograft rejection. In addition, C6, C7, C8 and C9 are known not to be required for this form of hyperacute rejection. PMNs may act on renal structures through their lysosomal enzymes as suggested by the increment in survival time of renal xenotransplants in recipients treated with a stabilizing agent of PMNs lysosomal membranes (Nelson, 1966).

The prolonged survival of kidney xenografts in leucopenic rabbits observed in the present study is in disagreement with the result obtained by Gewurz *et al.* (1966). These investigators reported that hyperacute rejection is not prolonged in rabbits made leucopenic by irradiation. Further studies are required to elucidate the role of PMNs in the hyperacute rejection of kidney xenografts.

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REFERENCES

- BOKISCH, V. A., MÜLLER-EBERHARD, H. J. and COCHRANE, C. G. (1969). 'Isolation of a fragment (C3a) of the third component of human complement containing anaphylatoxin and chemotactic activity and description of an anaphylatoxin inactivator of human serum.' *J. exp. Med.*, **129**, 1109.
- GEWURZ, H., CLARK, D. S., FINSTAD, J., KELLY, W. D., VARCO, R. L., GOOD R. A. and GABRIELSEN, A. E. (1966). 'Role of the complement system in graft rejections in experimental animals and man.' *Ann. N.Y. Acad. Sci.*, **129**, 673.
- JENSEN, A. J. (1969). 'A specific inactivator of mammalian C4 isolated from nurse shark (*Ginglystoma cirratum*) serum.' *J. exp. Med.*, **130**, 217.
- KINKAID-SMITH, P. (1967). 'Histological diagnosis of rejection of renal homografts in man.' *Lancet*, **ii**, 849.
- LINN, B. S., JENSEN, J. A. PORTAL P. and SNYDER, G. B. (1968). 'Renal xenograft prolongation by suppression of natural antibody.' *J. surg. Res.*, **8**, 211.
- MEJÍA-LAGUNA, J. E., GARCÍA-CORNEJO, M., LÓPEZ-SORIANO, F. and BIRO, C. E. (1970). 'The role of the sixth component of complement in the rejection of kidney xenografts.' *Immunology*, **19**, 767.
- NELSON, R. A. JR (1966). 'A new concept of immunosuppression in hypersensitivity reactions and in transplantation immunity.' *Surv. Ophthalm.*, **11**, 498.
- ROWLANDS, D. T., KIRKPATRICK, C. H., VATTER, A. E. and WILSON, W. E. C. (1967). 'Immunologic studies in human organ transplantation IV. Serologic and pathologic studies following heterotransplantation of the kidney.' *Amer. J. Path.*, **50**, 605.
- WARD, P. A. and HILL, J. H. (1970). 'C5 chemotactic fragments produced by an enzyme in lysosomal granules of neutrophils.' *J. Immunol.*, **104**, 535.
- WEYMOUTH, R. J., SEIBEL, H. R., LEE, H. M., HUME, D. M. and WILLIAMS, G. M. (1970). 'The glomerulus in man one hour after transplantation. An electron microscopic study.' *Amer. J. Path.*, **58**, 85.