The Role of Polymorphonuclear Leucocytes (PMNs) in Hyperacute Renal Homograft Rejection

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In this communication evidence will be presented that the polymorphonuclear leucocyte (PMN) plays an essential role in the mechanism of hyperacute rejection when a kidney is transplanted into a specifically sensitised host.

METHOD

The experimental model used was similar to the models used by other groups including MacDonald (MacDonald et al, 1970). Randomly selected pairs of adult mongrel dogs were used. Sensitisation of the recipients to a specific donor was achieved by repeated full thickness skin grafts, usually six, over a six week period. Seven days after the last skin graft a kidney was transplanted from the skin donor to the specifically sensitised recipient. The kidneys were revascularised by anastomosis to the iliac vessels, and the recipients own kidneys were both removed. Cutaneous ureterostomies were performed and the development of anuria was taken as the time of rejection. In 14 of 17 experiments in which the animal survived to the time of rejection, the mean rejection time was 18 hours, ranging from half an hour to 72 hours. All the kidneys were swollen, congested and had subcapsular haemorrhagic areas when removed at the time of rejection.

PMN counts were determined in the arterial blood supplying the homograft kidney and in the venous effluent blood draining from it, immediately before and at regular intervals after revascularisation of the homograft. The blood was collected into tubes containing EDTA, and total white blood cell counts were performed on the Coulter counter. Differential white blood cell counts were made on blood smears stained with a modification of the Giemsa-Wright stain to get the best possible definition of the PMN granules. The morphology of the PMN leucocytes was examined in detail.

RESULTS

The changes in the PMN counts in the arterial blood supplying the kidney

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homograft and in the blood draining from the effluent vein are shown in Figure 1. The values are expressed as a percentage of the PMN count immediately before revascularisation of the homograft. Two different types of response occurred. In 50% of the dogs (shown by the crosses in Figure 1) an immediate arteriovenous gradient occurred across the homograft, which was greatest at 10 minutes; by which time the PMN count in the venous effluent blood had fallen by 50%. Thus there was sequestration of PMN leucocytes in the graft. This was so extensive that the level of PMN leucocytes in the systemic arterial blood fell by 35% being greatest after 60 minutes. The arteriovenous gradient was eliminated by this time and the systemic arterial levels began to rise towards pre-existing levels. The changes in PMN leucocytes in the other 50% of the dogs shown by the solid circles in Figure 1 were unusual and rather surprising. The PMN leucocytes fell precipitously in the venous effluent blood, the fall being 70% within 1-2 minutes. The marked arteriovenous gradient which developed was not only eliminated within 20 minutes but the level of PMN leucocytes in the venous effluent blood leaving the homograft became greater than in the arterial systemic blood supplying it. The only possible explanation of this phenomenon is that PMN leucocytes initially sequestered in the homograft were later released in large numbers. Although this is most obvious in the group of dogs in which the
early sequestration was most marked, it probably occurred in all the animals.

That this is in fact the case is strongly supported by the appearance of large numbers of abnormal degranulated PMN leucocytes in the systemic and venous effluent blood shortly after revascularisation of the kidney homograft. One of these PMN leucocytes is shown in Figure 2. This PMN leucocyte has virtually no granules, and the cytoplasm is glassy and clear with a poorly defined margin. There is a suggestion of one or two vacuoles in the cytoplasm. The nucleus has lost its characteristic segmentation and is rather densely stained. It thus seems clear that the sequestration and release of PMN leucocytes by the homograft is a continuous dynamic process occurring soon after revascularisation, and that the PMN leucocytes have become degranulated whilst trapped in the graft.

DISCUSSION

Since the report by Kissmeyer-Nielsen (Kissmeyer-Nielsen et al, 1966) the role of circulating antibodies in hyperacute renal homograft rejection has been extensively investigated (Williams et al, 1968a, Terasaki et al, 1968, Starzl et al, 1968, Clarke et al, 1968, Klassen & Milgrom, 1969, Cochrum et al, 1969). There now seems little doubt that the rapid destruction of the homograft is mediated by humoral immunity mechanisms. Significance of
the various circulating antigraft antibodies described is controversial, especially in the case of lymphocytotoxins. It is interesting in this respect that only minimal absorption of lymphocytotoxins by the homograft was reported in a recent publication (Simpson et al., 1970). It now seems clear that the mixed agglutination test described by Milgrom (McDonald et al., 1965, Milgrom et al., 1966) is the most discriminating in detecting harmful antigraft antibodies directed against the histocompatibility antigens of the homograft.

Deposits of immunoglobulin and complement in the homograft have been demonstrated by several investigators (Busch et al., 1967, Williams et al., 1968b, Kincaid-Smith et al., 1969, Simpson et al., 1970). The controversial point at the moment is how the deposition of these immune complexes in the homograft lead to its destruction. An almost universal finding histologically is the presence of PMN infiltrates in the glomeruli of kidneys undergoing hyperacute rejection (Williams et al., 1968b, Starzl et al., 1968, Kincaid-Smith et al., 1969 and others). Indeed Kincaid-Smith (Kincaid-Smith et al., 1969), claims that heavy PMN infiltration of the graft in early post transplant biopsies is of grave prognostic significance, leading if not to rapid hyperacute rejection then to repeated rejection episodes with fibrin deposits in the vessels. Intravascular fibrin deposits in the graft microvasculature, thrombosis and cortical necrosis have been described by several investigators (Kissmeyer-Neilson et al., 1966, Starzl et al., 1968, Williams et al., 1968b, Busch et al., 1969). This finding has led to the incrimination of intravascular coagulation as the mechanism of graft destruction, a suggestion which is supported by MacDonald (MacDonald et al., 1970) who showed that hyperacute rejection of dog kidneys could be prevented by heparin. In spite of these findings, doubt has been thrown on the role of intravascular coagulation as a destructive mechanism because of the failure to find any localised or generalised consumption of clotting factors (Colman et al., 1969). More recently it has been shown that a local and systemic consumptive coagulopathy occurs in hyperacute renal homograft rejection in dogs (Simpson et al., 1970) and in humans (Starzl et al., 1970). It was also reported that fibrin split products were detected in the venous effluent blood draining from the homograft indicating fibrinolysis occurring within the graft.

There is evidence that PMN leucocytes can initiate intravascular coagulation in the localised and generalised Shwartzman phenomenon, and it has been shown that these reactions do not occur in PMN depleted animals (McKay & Shapiro, 1958, Lee & Stetson, 1965, Horn & Collins, 1968). The data presented showing the sequestration of PMN leucocytes by the graft followed later by their release in a degranulated form is strong presumptive evidence that they play an essential role in hyperacute rejection. This association could only be proved by demonstrating the prevention of hyperacute rejection.
in PMN depleted animals. Foker (Foker et al, 1969) in an experimental model designed to investigate the role of complement and humoral antibodies in this phenomenon in dogs provides evidence that this might be the case. Kidneys transplanted into specifically sensitised but PMN depleted hosts showed no significant histological changes within 4 hours. When these kidneys were returned to the donors they rapidly became infiltrated with PMN leucocytes and hyperacute rejection followed in the animals to which they had originally belonged. When the donors were depleted of complement prior to return of the kidney infiltration of PMN leucocytes and hyperacute rejection did not occur. PMN infiltration is well recognised in several immunologically mediated vascular lesions such as the Arthus reaction (Cochrane, 1965), the localised Shwartzman phenomenon (Lee & Stetson, 1965), nephrotoxic nephritis (Cochrane et al, 1965), antigen-antibody-complex nephritis (Unanue & Dixon, 1967) and serum sickness vasculitis (Kniker & Cochrane, 1965). It has been shown that the PMN leucocytes are attracted by chemotactic factors released when complement is fixed by immune complexes deposited in relation to vascular endothelium (Ward & Cochrane, 1965). PMN depletion prevents the development of the classical lesions. Even so the PMN leucocytes seem to act by different mechanisms: in some they precipitate intravascular coagulation (as in the localised Shwartzman phenomenon) and in others damage the vascular endothelium and basement membrane (as in the Arthus reaction). In both instances the release of biologically active substances from the PMN granules has been suggested as a mediator of the changes which occur (Lee & Stetson, 1965, Cochrane, 1965).

Hyperacute rejection has been likened to the generalised Shwartzman phenomenon (Starzl et al, 1968, Myburgh et al, 1969), Arthus reaction (Williams et al, 1967, Cochrum et al, 1969, Myburgh et al, 1969) and inverse anaphylaxis (Williams et al, 1968b). It is probably preferable to regard hyperacute rejection as a unique phenomenon and it may be unwise to compare it with previously described pathological processes. Although the pathways leading to destruction of homograft kidneys, when transplanted into specifically sensitised hosts, are not known for certain, the following mechanism can be suggested from the available evidence. There is now overwhelming evidence that hyperacute rejection is immunologically mediated and that immune complexes are deposited in relation to vascular structures. Complement is fixed by these immune complexes and release of chemotactic factors attracts PMN leucocytes.

These may well play an essential role in several alternative pathways leading to homograft destruction. It is possible that release of the PMN granules may be a mediator in these processes. Damage to the vascular endothelium and basement membrane may occur leading to platelet deposition, formation of mural deposits of fibrin and later to thrombosis of the micro-
vasculature. It has been suggested that platelets may play a central role in acute rejection (Mowbray, 1966) and in heterograft rejection (Rosenberg et al., 1969). It has been shown (Simpson et al., 1970) that platelets are sequestered in kidneys undergoing hyperacute rejection shortly after revascularisation. Whether they play a central role in the initiation of the intravascular coagulation or merely become involved in the process once established has not been ascertained. Alternatively if the damage to the vascular endothelium and basement membrane caused by the PMN leucocytes is more severe increased permeability or complete loss of integrity of the vessel may occur. The ensuing tissue damage may result in the release of tissue thromboplastin precipitating intravascular coagulation. Possibly the PMN leucocytes may be capable of triggering intravascular coagulation directly leading to plugging of the microvasculature by fibrin. Although it must be admitted that direct evidence for the suggested roles of PMN leucocytes in hyperacute rejection is lacking they are almost certainly intimately involved with the destructive processes and the massive intravascular coagulation which is an almost universal finding.

The continuous turnover of PMN leucocytes in the homograft and the morphological changes described here have not been previously reported. These findings do however provide strong presumptive evidence that PMN leucocytes play an active if not essential role in hyperacute rejection. With such dramatic changes occurring in the transplanted kidney within minutes of revascularisation it remains unexplained why some of these kidneys are rejected rapidly and others continue to function for many hours. It could be that fibrinolysis plays a part in determining the ultimate outcome of the fibrin deposition and eventual vascular occlusion. Alternatively it could be because the pathway of destruction is variable and may be related to the intensity, composition and site of the immune complexes which are deposited.

SUMMARY

A canine model for the study of hyperacute rejection of renal homografts is described. Data showing that PMN leucocytes are continuously sequestered and released by the homograft is presented. Morphological changes in the PMN leucocytes presumed to have occurred in the homograft are described. The significance of these observations in the pathogenesis of hyperacute rejection is discussed in detail.

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