SCANNING ELECTRON MICROSCOPIC STUDY OF HYPERACUTE REJECTION IN THE INBRED RAT

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Summary

Twenty skin-presensitised Lewis rats received kidney transplants from (Lewis x BN)f1 rats. Two grafts each were withdrawn at intervals from 1–120 min and examined using a scanning electron microscope (SEM). A series of Lewis to Lewis isografts served as control. In hyperacute rejection at just 1 min spider-like fibrin fibres and platelets could be observed in small arteries, where the endothelium was severely altered. In these regions at 2 and particularly 5 min a fibrin network often contained platelet aggregates, mechanically altered erythrocytes as well as different kinds of leucocytes. This coagulation process progressed with time and resulted in a complete vascular occlusion at 30–60 min.

Introduction

The morphological alterations, which occur in hyperacute rejection of kidney transplants, have been extensively examined using light and transmission electron microscopy [1–3]. On the other hand a scanning electron microscopic (SEM) study has — as far as we know — not yet been reported. Since this method provides good opportunities for investigating surface changes, we thought that it would be interesting to demonstrate particularly the thrombotic lesions of blood vessels in hyperacute transplant rejection. For this method we chose a controlled sequential study in inbred rats.

Material and Methods

After presensitisation by 3 successive skin grafts, serum samples were obtained from 20 Lewis rats for testing the presence of lymphocytotoxic antibodies. These Lewis rats then received kidneys from (Lewis x BN)f1 rats using microsurgical techniques [4]. Two rats each were sacrificed at 1,2,3,5,7,10,15,30, 60 and 120 min by a perfusion fixation with cacodylate-buffered 2.5% glutar-
aldehyde (pH 7.4). A series of Lewis to Lewis isografts served for control. The kidneys were removed, washed in cacodylate buffer and cut in small tissue samples, which were dehydrated, broken, critical point dried, coated with gold-palladium and examined with a JEOL 100C.

Results

The lymphocytotoxic titre of the sensitised rats was in the range of 1/256–1/1024. In hyperacute rejection just 1 min after declamping the blood supply large areas

Figures 1–4. Scanning electron micrographs; original magnifications: x 8,000, x 6,000, x 3,000, x 6,000 (reproduced about half original size). For explanation please read results
of the endothelium in arteries had lost their normal smooth surface and looked rough and granular (see Figure 1). At this time the first spider-like fibrin fibres could be observed. Often they occurred in connection with platelets or — as seen in Figure 2 — with possibly mechanically altered erythrocytes. At 2 min the endothelial cells were severely damaged, in part disconnected and frequently in a stage of desquamation. Networks of fibrin were often attached to the vascular wall and contained erythrocytes and platelets (see Figure 3). In other localisations the parietal deposition of these cells was pronounced, but almost without fibrin (see Figure 4). At 5 min the fibrin networks frequently crossed
the lumen of the arteries, catching erythrocytes, platelet aggregates and different kinds of leucocytes (see Figure 5). These thrombotic alterations advanced with time and resulted in complete vascular occlusions (see Figure 6). The time of complete thrombosis varied from vessel to vessel, but occurred between the 30th and 60th min. Up to 15 min the podocytes of the glomeruli were looking quite normal. Later however hemispheric protrusions could be identified on the visceral layer of Bowman's capsule (see Figure 7). The glomerular capillaries were involved by thrombotic processes quite similar to the alterations in arterioles. In the arteries of the isografts only minor endothelial lesions, presumably a sequel of ischaemia, could be observed. In large areas the endothelium of glomerular capillaries — typically fenestrated — looked quite normal (see Figure 8).

Discussion

The hemispheric protrusions of the visceral layer of Bowman's capsule, which were found after 15 min, are interpreted by us as manifestations of severe alterations to the minor podocyte branches. When we consider the arterial lesions it seems to be obvious that immunological attack in hyperacute rejection causes a sudden and heavy damage to the endothelium, which is the barrier between transplant and host. The sequel is a sequence of events which occur with intravascular coagulation. Contrary to these findings, Forbes et al described in hyperacute cardiac allograft rejection an extensive platelet aggregation preceding the endothelial changes [5]. It may be that the different organs chosen for study are responsible for this discrepancy. However, we suggest that SEM — Forbes et al used transmission electron microscopy at this time — provides improved opportunities for examination of surface alterations as in endothelial lesions. Thus Bowyer and Reidy reported recently on aortic allografts in the rabbit [6]. We think that the importance of SEM for clinical and experimental transplantation studies will increase in future.

Acknowledgment

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References

4 Lee, S (1967) Surgery, 61, 771
6 Bowyer, DE and Reidy, MA (1977) J. Pathol., 123, 237
Open Discussion

MACPHERSON (Glasgow) I would like to hear your evidence that what you are describing is a feature of hyperacute rejection. It is a very difficult process to engender in this particular rat model, and I wonder if you followed some of these rats through, without taking the kidneys out for biopsy, or if you looked at the LBN f1 to Lewis in straightforward allograft rejection.

LEITHNER We made controls of these hyperacutely rejected grafts by normal light microscope and saw the type of alterations which occurred in hyperacute rejection.

MACPHERSON Did you look at straightforward allograft rejection with scanning electron microscopy?

LEITHNER No, we have no experience with acute or chronic allograft rejection, only with hyperacute rejection.

ROWINSKI (Warsaw) We have done similar studies in dogs having the model of hyperacute rejection and our electron microscopical pictures look almost the same, but I have one question. Do you have any proof that in your model you have had intravascular coagulation, besides the pictures showing aggregates, which are not specific at all and which might be just due to destroyed endothelium? Do you have any coagulation studies?

LEITHNER No, we had no coagulation studies. However, there are several reports about coagulation studies. It is a question whether the coagulation is a primary or secondary event.

KEMP (Odense) May I ask if you have any experience on xenografting because in renal xenografting, for instance as we have done it between rabbit kidneys transplanted to cats, you can see exactly the same sequence even more dramatic and even faster.

LEITHNER We have almost no experience with xenografting. I agree with you completely that the xenograft model is perhaps better than mine.