MECHANISM OF RENAL ALLOGRAFT REJECTION

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Although the precise mechanisms of renal allograft rejection are unknown, it is well established that allograft rejection is an immunological phenomenon due to recognition of immunogenetic differences between donor and recipient. Renal transplantation between inbred strains of rats provides an opportunity to study the mechanisms of allograft rejection under controlled immunogenetic conditions. The results of our studies on renal allograft rejection mechanisms in the inbred rat are the basis for the present discussion.

Experimental model

Renal transplantation between inbred strains of rats was accomplished by microsurgical techniques using Lewis rats as donors and Lewis and (Lewis × BN) F₁ hybrids as donors for isografts and allografts respectively. Lewis and Brown Norway (BN) strains of rats were selected for study as an example of strong histocompatibility difference. These strains of rats probably differ at 14-16 histocompatibility loci, and they have different blood group antigens (Billingham et al., 1962). The use of F₁ hybrid donors rather than BN donors allows study of a one way host vs. graft reaction and eliminates possible graft vs. reaction.

For further description of this system and for details of the materials and methods employed in the functional, immunologic, morphologic and immunohistochemical studies see Guttmann et al. (1967) and Lindquist et al. (1967).

Primary site of immunologic attack during allograft rejection

Evidence points to the peritubular capillary as the primary site of immunologic attack during allograft rejection. Two to three days after allotransplantation, immunofluorescent study reveals immunoglobulin G (IgG) deposition on peritubular capillaries as a thin homogeneous layer and as granular deposits with the former pattern predominating (Figs. 2 and 3). This IgG may represent antibody directed against transplantation antigen(s) residing on the capillary wall. β₁c globulin (C₃), presumably bound after antigen-antibody interaction, is also located on peritubular capillaries and supports the interpretation that an immunologic reaction occurs at this site. The immunologic specificity of these findings is emphasized by the absence of the non-immunoreactants fibrinogen and α₂-macroglobulin.

Once antibody interacts with antigens on the peritubular capillaries, change in vascular permeability probably follows. The early focal and later diffuse interstitial edema and accu-
**Fig. 1.** Allograft 2 days after transplantation. Peritubular capillary contains mononuclear cells with large vesicular nuclei and prominent nucleoli. Some cells (lower left and lower right) are adherent to the capillary walls. Toluidine blue O-borax; ×1000.

**Fig. 2.** IgG localization in 2 day renal allograft. IgG is deposited on peritubular capillaries as a thin homogeneous layer and as granular deposits. β_v globulin, but not fibrinogen or α_2-macroglobulin is
mulation of IgG, $\beta_{1c}$ globulin, fibrinogen and $\alpha_2$-macroglobulin (Fig. 8) is a reflection of this increased vascular permeability with the diffusion and nonspecific accumulation of these proteins in the renal interstitium. Peritubular capillaries also become more permeable to passage by circulating mononuclear cells. Not infrequently mononuclear cells are seen going through peritubular capillaries. Progressive dissolution of peritubular capillaries with interstitial haemorrhage has been seen in rejecting canine allografts (Kountz et al., 1963), but it is not a prominent feature in the present, unsensitized rat model. Vascular spasm is also known to follow antibody-antigen interaction on vessels and may occur in rat renal allografts. The functional significance of capillary spasm, however, is not well defined. Although significant decrease in effective renal plasma flow (ERPF) is seen in this model 5 days after allografting, at day 3 when IgG and $\beta_{1c}$ globulin are first detectable on peritubular capillaries, ERPF is normal; however, only total ERPF and not compartmental flow was measured. Vasospastic phenomena have been demonstrated in rejecting canine renal allografts. Rejecting canine allografts show marked diminution in cortical blood flow, at a time when total blood flow is still normal, which approaches normal compartmental size if vasodilators are infused into the kidney (Hollenberg, 1967).

**Cellular vs. humoral mechanisms**

Renal transplantation in the inbred rat has provided information relevant to the question of cellular vs. humoral mechanisms of allograft rejection. Concomitant with the deposition of IgG and $\beta_{1c}$ globulin on peritubular capillaries, frequent mononuclear cells with large vesicular nuclei and one or more prominent nucleoli are seen within these vessels and some cells are adherent to the endothelium (Fig. 1). These mononuclear cells contain abundant IgG (Fig. 3) but not $\beta_{1c}$ globulin, fibrinogen or $\alpha_2$-macroglobulin, and they may transfer their contents to peritubular capillaries when they come in contact with the endothelium.

Fig. 3. IgG localization in 2 day renal allograft. A thin layer of IgG is deposited on a peritubular capillary. Two mononuclear cells contain IgG. One is in contact with the capillary wall and may transfer its IgG to the capillary wall. × 500.

Fig. 4. Five day renal allograft. Hypercellular glomerulus with glomerular capillaries occluded by proliferation and swelling of mesangial and endothelial cells. An endothelial cell in mitosis is present between 4 and 5 o'clock. × 500.

Fig. 5. IgG localization in 5 day renal allograft. IgG is deposited in mesangial areas and on capillary walls. The granular character of the IgG deposition is seen at 6 o'clock. These deposits, it is postulated, represent immune complexes consisting of transplantation antigens and antibodies with bound complement that are deposited in glomeruli and initiate glomerular lesions. × 500.

Fig. 6. IgG localization in 7 day renal allograft. Five polymorphonuclear leukocytes contain IgG and IgG is no longer seen on glomerular walls. Careful inspection will reveal the typical trilobed nucleus of polymorphonuclear leukocytes. These cells probably phagocytize immune complexes deposited in glomeruli. PAS, × 500.

Fig. 7. Seven day renal allograft. Glomerular capillary loops are occluded by fibrillar, PAS positive material. Renal interstitium is infiltrated by mononuclear cells. × 250.

Fig. 8. Fibrinogen localization in 7 day renal allograft. Abundant fibrinogen is deposited in glomerulus. Fibrinogen is also present throughout renal interstitium. IgG, $\beta_{1c}$, and $\alpha_2$-macroglobulin are similarly deposited throughout the renal interstitium and reflect increased vascular permeability with diffusion and nonspecific accumulation of serum proteins in the renal interstitium. × 250.

Fig. 9. $\alpha_2$-Macroglobulin localization in 2 week renal allograft. Numerous cells contain $\alpha_2$-macroglobulin. Careful inspection will reveal a round area of nonfluorescence eccentrically placed in the cell and identify these cells as the mononuclear cells seen infiltrating the interstitium of rejecting renal allografts. These cells also contain IgG, $\beta_{1c}$ and fibrinogen at this time. When rejection is occurring during the first week after transplantation, these cells do not contain antibody, as indicated by absence of IgG and IgM. × 500.
Finding these IgG containing mononuclear cells within peritubular capillaries suggests participation of cell bound antibody; however, possible antibody manufacture at distant site reaching peritubular capillaries through circulation without either carriage or local manufacture by these mononuclear cells is not completely ruled out. The relatively small number of mononuclear cells containing IgG in comparison to the extensive localization of IgG along peritubular capillaries suggests that an additional mechanism of antibody deposition may be involved.

The infiltrating mononuclear cells seen in rejecting renal allografts have been considered as an example of cell mediated rejection. Abundant cytoplasmic RNA as indicated by pronounced cytoplasmic pyroninophilia and numerous free ribosomes, both suggesting protein synthesis, have been used as evidence for antibody production by the infiltrating cells and they have been called immunocytes. Our study of acute allograft rejection in the unsensitized host does not support this conclusion. These cells are not engaged in antibody synthesis, at a time when rejection is occurring as indicated by the absence of immunohistochemically detectable IgG and IgM. Low level synthesis of antibody below the sensitivity of immunohistochemical methods, though possible, seems unlikely. Since these infiltrating mononuclear cells are actively proliferating, the protein synthetic apparatus may be synthesizing cellular proteins necessary for growth and replication rather than antibody. Only after kidneys are rejected completely, do these mononuclear cells contain IgG. However, even then only a small percentage of them contain IgG, and at this time they also contain βlC globulin, fibrinogen and α2-macroglobulin (Fig. 9) suggesting the phagocytic accumulation of these proteins.

Search for specific circulating transplantation antibodies has not been carried out in this experimental model. Circulating serum hemagglutinating and cytotoxic antibodies, however, have been measured in rats with rejecting renal allografts even though participation of these antibodies in allograft rejection has not been established. These antibodies are not detectable in serum until 7 days after allotransplantation, a time when the kidney is completely rejected. Although this suggests that they have no primary role in the mechanism of allograft damage and rejection, the possibility of their early presence with fixation to the allograft cannot be excluded. Their detection at day 7 may represent antibody in excess of the available binding sites.

IgG is demonstrated immunohistochemically in the renal interstitium during rejection, first as focal collections and later diffusely throughout the renal cortex. It is not known at present if this IgG represents antibody directed against the graft; however, since βlC globulin, fibrinogen and α2-macroglobulin parallel the distribution of IgG (Fig. 8), we consider the interstitial accumulation of IgG a reflection of increased vascular permeability (vide supra) with the nonimmunologic accumulation of serum proteins.

Even though β10 globulin (C3) participates in allograft rejection as indicated by the localization of β10 globulin along with IgG on peritubular capillaries, no consistent or significant difference in serum β1C globulin levels occurs between rat renal allografts or isografts during the first week after transplantation. β1C globulin becomes elevated in allografts during the second and third weeks with a return towards normal at the end of the third week. Since serum complement rises in response to a variety of stimuli in the rat, the role of complement in allograft rejection may be masked by a nonspecific complement response to rejection. Furthermore, a considerable amount of complement can be fixed and not be detected by study of serum concentrations.

Phenomena secondary to immunologic rejection

With the development of immunosuppressive therapy, acute rejection episodes can be prevented or reversed. Nonetheless, human renal allografts, though prolonged in survival,
usually fail to survive. Vascular lesions in the form of obliterative arteritis and/or glomerular lesions are frequently the underlying cause for allograft failure. Although information regarding the development of arterial lesions has not been determined from study of renal transplantation in the inbred rat, at present, this model has provided information pertinent to the development of glomerular lesions in renal allografts.

Three to five days after transplantation glomerular lesions predominately characterized by swelling and proliferation of endothelial and mesangial cells develop in renal allografts (Fig. 4). Concomitant with the appearance of this proliferative glomerulonephritis, IgG is deposited in mesangial areas and along glomerular capillary walls in a granular pattern (Fig. 5). $\beta_{1C}$ globulin, but not fibrinogen or $\alpha_2$-macroglobulin is also detected in a similar pattern. The host's own kidney also develops similar, though less severe morphologic and immunohistochemical alterations if it remains in place. Increased proteinuria occurs in association with these glomerular lesions, reflecting increased glomerular permeability to protein. It is postulated that circulating immune complexes consisting of soluble organ specific and genetically specific transplantation antigens and antibodies with bound complement are deposited in glomeruli and initiate the glomerular lesions. The pathogenic role of circulating antibody-antigen complexes in the development of glomerular alterations is well established. The similar glomerular abnormalities and immune deposits that are seen in the host's own kidney emphasize the nonimmunogenetically directed deposition of immune complexes in the glomerulus, such as occurs in serum sickness nephritis. The glomerular immune complexes are short-lived in the rat allograft and concurrently with their disappearance, polymorphonuclear leukocytes appear within the glomeruli. Since the polymorphonuclear leukocytes contain IgG and $\beta_{1C}$ globulin (Fig. 6), phagocytosis of immune complexes by leukocytes is suggested.

A similar deposition of immune complexes consisting of soluble organ specific and genetically specific transplantation antigens and antibodies may be one mechanism producing glomerular alterations in long term human allografts. Suggestion of such a mechanism was recently seen in a patient with a renal allograft at the Peter Bent Brigham Hospital.

Not all of the glomerular abnormalities seen in clinical transplantation can be explained on the above mechanism and study of the rat model provides evidence of an additional mechanism. Seven days after renal allotransplantation glomerular capillary loops are occluded by fibrillar periodic acid Schiff positive material (Fig. 7) and renal function ceases. This fibrillar material fluoresces brilliantly when stained with fluorescent labeled antifibrinogen or antifibrin (Fig. 8). Glomerular fibrin deposition with little IgG localization is an immunofluorescent feature of some membranous glomerulopathies seen in long surviving renal allografts and fibrin deposition may represent an additional mechanism producing glomerular lesions in renal allografts. Whether fibrin is deposited secondary to antigen-antibody interaction or to platelet clumping or for hemodynamic reasons is not known at present.

Summary and conclusions

Study of renal allograft rejection in the inbred rat has provided information useful in partially elucidating the mechanisms of renal allograft rejection.

Peritubular capillaries are the primary sites of immunologic attack which is mediated, at least in part and not necessarily to the exclusion of humoral mechanisms, by cell bound antibody. Peritubular capillaries, altered by the interaction of antibody with transplantation antigens on the endothelium and the binding of complement, probably play an important role in the development of interstitial edema and renal ischemia; events that lead to destruction of the graft.
There is no evidence in the rat that the mononuclear cells infiltrating the interstitium make antibody, since IgG or IgM cannot be detected in these cells at a time when rejection is occurring. Only after rejection has occurred do these cells contain IgG and then β10 fibrinogen and αp-macroglubulin are also present.

Glomerular lesions develop in rat renal allografts secondary to probable deposition of circulating immune complexes consisting of organic specific and genetically specific transplantation antigens and antibody with bound complement. A similar mechanism may initiate glomerular alterations in some human renal allografts. Fibrin deposition may be an additional pathogenic mechanism leading to glomerular abnormalities.

REFERENCES


