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Antibody binding to endothelial and epithelial antigens triggers pig-to-rabbit xenograft rejection and its absence results in atypical complement deposition

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Abstract. In pig-to-rabbit kidney xenograft (PRKX), endothelial antigen determinants (EAD) are immediately recognized by IgG and IgA, while IgM does not react with them. The purpose of this study was to investigate the different roles of IgG, IgA, IgM, and complement in the hyperacute rejection of a PRKX model. Nine isolated Landrace pig kidneys were each perfused with10 ml normal New Zealand rabbit serum. Perfusates (serum A) were collected after discarding the first 0.5 ml. Serum A and rabbit complement were then incubated for 30 min with frozen sections of normal pig kidney. After washing with buffer solution all the specimens were treated for immunohistochemistry. Three frozen sections of normal Landrace pig kidney and three samples of normal New Zealand rabbit serum were used as controls. Immunohistochemical analysis of the nine perfused kidneys demonstrated IgG, IgA and C3 deposition on the peritubular and glomerular vascular endothelium. No IgM reactivity was shown. In the frozen sections exposed to serum A, immunofluorescence showed minimal IgG, IgA and C3 reactivity while IgM deposition was clearly evident on the tubular epithelium. Immunofluorescence of frozen sections exposed to rabbit complement, done by fluorescein-labeled goat anti-rabbit C3 antibodies were positive only in the glomerular endothelium. The same rabbit complement was active in antibody dependent cytotoxicity on human T cells. Our results indicated that in the PRKX model, IgG and IgA acted as preformed antibodies recognizing endothelial EAD. IgM did not bind to any endothelial molecules, but recognized antigens located on the brush border of the tubular epithelium. Furthermore, in this model, absence of antigen-antibody complexes resulted in atypical complement deposition.

Key words: Xenotransplantation – Natural antibodies – Hyperacute rejection

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Transplantation is currently the treatment of choice for several end-stage organ diseases [10–13]. Considering that the increasing demand for human organs for transplantation far exceeds their availability, xenogeneic transplantation is probably the most realistic solution to the problem [1, 3]. Hyperacute rejection of discordant xenografts is triggered by natural antibodies binding to endothelial antigen determinants (EAD) of the graft, and by complement activation [1]. We have already reported [7–9] that IgG and IgA are responsible for triggering the hyperacute rejection of the pig kidney in a pig-to-rabbit kidney xenograft model (PRKX). In PRKX, EADs are immediately recognized by IgA and IgG, while IgM does not react with them [7–9]. The purpose of this study was to investigate the different roles played by IgG, IgA, IgM and complement in the hyperacute kidney rejection of PRKX. We were interested in identifying the antigen determinants recognized by the different Ig classes, and in ascertaining whether complement can be activated in the absence of antibody binding.

Materials and methods

Five female Landrace pigs weighing 2.0–2.5 kg were used as donors from which nine kidneys were harvested. The donor operation technique has already been reported in detail elsewhere [7]. The kidneys underwent an initial vascular isolation in situ. The infrarenal aorta and the left renal vein were cannulated in order to perfuse the left kidney, while the suprarenal aorta and the cava were cannulated in order to perfuse the right kidney. Fifteen New Zealand female rabbits weighing 4.1–5.0 kg were used as donors of a total of 180 ml normal rabbit serum. Of this normal rabbit serum 90 ml was used to perfuse the nine isolated Landrace pig kidneys (10 ml each). The perfusion of the kidneys was performed through the aorta; 10 ml rabbit serum was infused in each kidney (serum A), and then collected through the renal vein (left kidney) or through the cava (right kidney). The first 0.5 ml of each perfusate was discarded. Following the perfusion with serum A, kidney tissue samples were taken and embedded in the optimum cutting temperature medium (Miles Scientific, Naperville, Ill.), Snap frozen in liquid nitrogen, sectioned and prepared for immunohistochemical analysis. The sections were incubated with fluorescein isothiocyanate conjugated (FICT) goat

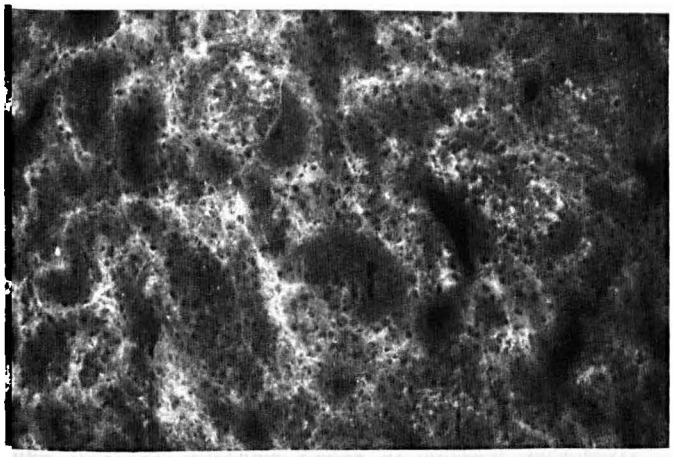

Fig. 1. Immunohistochemical analysis of a Landrace pig kidney perfused with normal New Zealand rabbit serum. The fluorescence staining was performed with goat anti-rabbit IgG. IgG deposits were present in the peritubular capillary walls and in the glomerular capillary loops (× 20). Deposits of IgA were present with similar distribution. Ig deposits were absent from the tubular epithelium

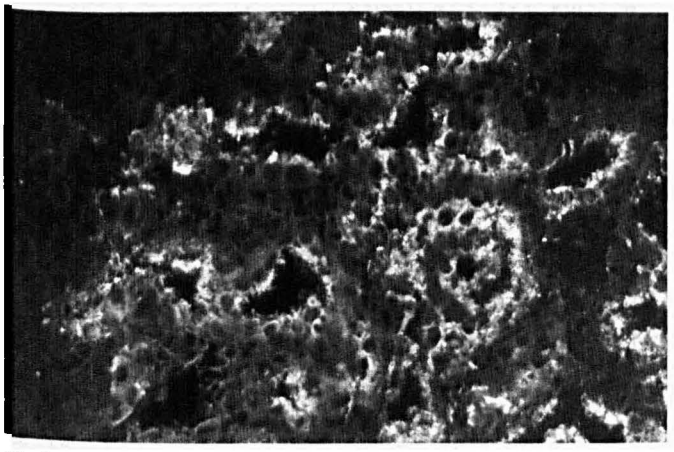

Fig. 2. Immunohistochemical analysis of a frozen section of tissue samples of Landrace pig kidney exposed to serum A (New Zealand rabbit serum infused through the renal artery of an isolated Landrace pig kidney and then collected from the renal vein). The fluorescence staining was performed with goat anti-rabbit IgM. IgM deposits are evident on the tubular epithelium (× 40)

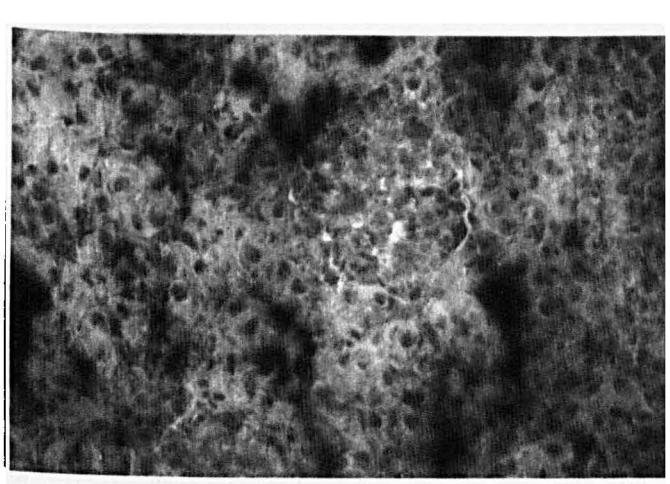

Fig. 3. Immunohistochemical analysis of a frozen section of Landrace pig kidney exposed to rabbit complement. The fluorescence staining was performed with fluorescein-labeled anti-rabbit C3 antibodies. Complement deposition is evident on the glomerular endothelium, while the other tissues did not take the stain (× 40)

anti-rabbit IgM, IgA, IgG, and C3 (Cappel, Organon Teknika, Veedijk, Belgium) for 60 min at room temperature, in a moist chamber.

Three female Landrace pigs weighing 2.0–2.5 kg were then used as donors from which three kidneys were procured. These three kidneys were used to prepare tissue samples of normal pig kidney. They were embedded in the optimum cutting temperature medium (Miles Scientific, Naperville, Ill.), frozen in liquid nitrogen and maintained at −80°C. Fifteen sections, 5 μm thick each, were then prepared from the frozen tissue. They were thaw-mounted on slides coated with poly-L-Lysine hydrobromide (Polysciences, Warrington, Pa.), and allowed to air dry for 3 h. Finally, they were fixed for 10 min in 100% acetone. Of these 15 sections, 10 were incubated for 30 min with serum A. The remaining five sections were incubated at 37°C with rabbit complement (Low-toxic-M, Cederlane, Ontario). The sections were then washed with buffer solution and incubated with FICT goat anti-rabbit IgM, IgA, IgG, and C3 (Cappel, Organon Teknika, Veedijk, Belgium) for 60 min at room temperature, in a moist chamber. Three frozen sections of normal pig kidneys that were incubated with three different samples of normal New Zealand rabbit serum were used as controls.

Results

Immunohistochemical analysis of nine Landrace pig kidneys perfused with serum A confirmed the results obtained in our previous in vivo and in vitro study [7–9]. Specifically, IgG, IgA and C3 deposition were evident with uniform distribution on the endothelium of the peritubular capillaries, and on the glomerular endothelium (Fig. 1). No IgM deposition was shown on the vascular endothelium. Immunohistochemical analysis of the ten frozen sections of tissue samples of normal pig kidneys exposed to serum A showed minimal IgG, IgA, and C3 reactivity was compared with the deposition obtained when perfusing normal pig kidneys with normal rabbit serum. Instead, IgM deposition was clearly evident on the tubular epithelium (Fig. 2). Immunofluorescence of the frozen sections exposed to rabbit complement, done by fluorescein-labeled anti-rabbit C3 antibodies demonstrated complement granular deposition only on the glomerular endothelium, while the other tissues did not take the stain (Fig. 3). The same rabbit complement was active in antibody-dependent cytotoxicity reactions on human T cells.

Discussion

Hyperacute rejection mechanisms in discordant xenogeneic transplantation are still unclear [3]. It is recognized that preformed antibodies mediate the hyperacute rejection of solid organs in discordant species combinations [2, 6, 10, 11]. However, it seems that the classes of Ig involved in the process are different in all the discordant xenogeneic transplantation models. IgM plays a primary role in mediating the rejection in many species combinations [4, 5]. In our previous reports [7–9] we have demonstrated that in a PRKX, IgA and IgG act as preformed antibodies and trigger the hyperacute rejection process by recognizing antigen determinants on the vascular endothelium. In this model, rabbit IgM does not react with the pig kidney vascular endothelium, but it recognizes antigen determinants located on the brush border of the tubular epithelium. This

happens at least 120 min following xenograft reperfusion [9]. The present study established that, at least in our model, preformed antibodies binding to endothelial targets (IgA and IgG) were different from those binding to epithelial targets (IgM). In fact, the fluorescence studies on the Landrace pig kidney, perfused with normal New Zealand rabbit serum, reproduced the fluorescence pictures obtained previously by our in vivo study [7]. The incubation of sections of normal Landrace pig kidneys with serum A demonstrated clearly two facts. First, that the passage of the New Zealand rabbit serum through the vascular bed of the Landrace pig kidney was characterized by IgG and IgA deposition on the capillary endothelium. Consequently, the effluent collected was IgG and IgA deprived. IgM was normally present in serum A because it did not bind to any endothelial antigen determinants. On the other hand IgM deposition was evident on the tubular epithelium when the sections of normal Landrace pig kidneys were incubated with serum A. Furthermore, in this model, in the absence of antigen-antibody complexes, complement deposits were detectable only on the glomerular endothelium. The endothelium of the peritubular capillaries did not show any complement deposition in the absence of antibody. In vivo [7–9], in PRKX the hyperacute rejection cascade starts as early as 15 min after kidney reperfusion in the wall of the peritubular capillaries. The glomeruli withstand antibody and complement activity longer than do the peritubular capillaries and this results in damage only 60–120 min after the organ reperfusion. The significance of the early complement deposition that we obtained in vitro on the glomerular endothelium remains uncertain and further studies should be conducted to clarify this point.

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