



Modulation of Fluid-Phase Complement Activation Inhibits Hyperacute Rejection in a Porcine-to-Human Xenograft Model

A.E. Fiane, V. Videm, J.D. Lambris, O.R. Geiran, J.L. Svennevig, and T.E. Mollnes

HYPERACUTE rejection (HAR) of porcine organs is due to classical complement activation by naturally occurring human antibodies to the endothelium. Pigs that are transgenic for human regulators of complement (RCA) seem promising with respect to avoiding HAR, and their use in various studies has brought xenotransplantation closer to clinical reality. However, RCAs are usually expressed on the endothelial cells as clusters and the activation of complement may take place at some distance from RCA localisation. Thus, it should be emphasised that, although RCA pig xenografts may be protected against HAR, a certain degree of complement activation may still contribute to an inflammatory reaction with subsequent impaired graft function. Therefore, efforts should be taken to control the fluid-phase activation of complement, which was the main topic of our studies.

We have established an *ex vivo* perfusion model¹ to investigate the effect of complement manipulation by intravenous immunoglobulin (IVIG), a C3-binding peptide (Compstatin) and C1-inhibitor (C1-INH) on HAR.

MATERIAL AND METHODS

Pairs of pig kidneys (six in each study) were simultaneously perfused with fresh human blood containing either the active agent or a control. For details, see Fiane et al.¹

RESULTS

IVIG prolonged graft survival substantially and enhanced fluid-phase complement activation, consistent with C1q binding to IVIG and deviating complement activation from the endothelium.² The survival of the Compstatin-perfused organs (median, 380 minutes) was significantly ($P = .0036$) longer than that of the controls (median, 90 minutes). The classical complement pathway was significantly and equally

activated in both groups during the first 60 minutes, consistent with Compstatin acting at the C3 level. Thus, C3 activation products increased five-fold and the terminal complement complex (TCC) eight-fold in the control group, whereas no increase occurred in the Compstatin group during this period. Kidney depositions of C3, TCC, and fibrin were less in the Compstatin group. A significant leukocyte activation was observed in both groups, without any difference between the groups, as was also the case for platelet activation.³

C1-INH also significantly increased the survival (median, 315 minutes) compared with the control (median, 75 minutes). C1-INH markedly inhibited complement activation. This effect was at the level of C1 because the whole classical (C1rs-C1-INH and C4bc) as well as the terminal pathway was inhibited. C1-INH also reduced activation of leukocytes and platelets ($P < .02$). There were markedly less immunoglobulin (Ig) and complement deposits on the endothelium in the C1-INH group compared with the controls.⁴

CONCLUSION

Addition of IVIG showed a prolonged graft survival, despite an increased complement activation in the fluid-phase. The effect was presumably due to deviating the complement activation from the endothelium to the Ig in the fluid-phase. Addition of a new complement inhibitor, the C3 binding peptide Compstatin, to human blood, produced a substantial increase in the survival times of the pig kidneys. The effect was apparently mediated by inhibition of C3 and the terminal C5-9 pathway. No significant effects on white blood cells or platelets were shown.

Addition of C1-INH in supraphysiological doses to human blood significantly prolonged pig kidney survival. C1-INH efficiently inhibited activation of the classical and terminal complement pathways and similarly attenuated leukocyte and platelet activation. These latter effects are most likely secondary to the inhibition of complement because activation of the contact system was not shown. We conclude that IVIG Compstatin, and C1-INH prolonged graft survival and modulated complement activation, but through different mechanisms. They are all potential candidates for future clinical use in xenotransplantation.

From The National Hospital, University of Oslo (A.E.F., O.R.G., J.L.S.), Oslo, University of Trondheim, Trondheim, (V.V.), University of Tromsø, Tromsø, Norway, and The University of Pennsylvania, (J.D.L.), Philadelphia, Pennsylvania, United States.

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Address reprint requests to Arnt E. Fiane, MD, PhD, Department of Cardiothoracic Surgery, The National Hospital, Pilestredet 32, N-0027 Oslo, Norway.

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