

Role of complement in xenotransplantation

The xenotransplantation research is driven by the increasing gap between the number of patients with end-stage organ failure on waiting lists for transplantation and the supply of allografts. The lack of success in developing suitable artificial organs for permanent treatment of organ failure has further strengthened the need for xenotransplantation research. Pigs are now generally accepted to be the source animal of choice. Transplantation of pig organs to humans faces several barriers which have to be overcome before it comes to clinical application: (1) anatomical and physiological conditions; (2) immunological rejection mechanisms; (3) molecular compatibility between signal molecules of the two species; (4) risk of transmission of microorganisms, particularly pig endogenous retroviruses; and (5) legal and ethical aspects both with respect to the animal and the recipient. Here we will focus on the role of the complement system in the rejection of immediately vascularized pig-to-primate xenografts. The hyperacute rejection occurring within minutes after transplantation is mediated by binding of natural antibodies to the Gal α (1-3)Gal epitope on the endothelial cells with subsequent complement activation. Whereas inhibition of complement activation protects against hyperacute rejection, the role of complement in the later rejection phases is less clarified.

T. E. Mollnes, A. E. Fiane

Institute of Immunology and Department of
Cardiothoracic Surgery, The National Hospital,
University of Oslo, Norway

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Tom Eirik Mollnes
Institute of Immunology
The National Hospital
N-0027 Oslo
Norway

Rejection of immediately vascularized xenografts

The major barrier to the use of pigs as a source of organs for clinical transplantation is that the organs are subject to severe and irreversible rejection (1) and potentially to other biological barriers (2). Until recently these rejection reactions seemed to pose an insurmountable hurdle to clinical application of xenotransplantation. Studies during the past few years, however, have revealed much about the molecular basis of xenograft rejection, particularly humoral rejection, and have encouraged the view that these reactions and other biological problems might be addressed by novel and specific therapies (3–9). The reaction phases are similar to those we know from allotransplantation and are divided into hyperacute rejection, acute vascular rejection (AVR), cellular rejection, and possibly also a chronic rejection with gradual loss of organ function (Fig. 1).

Hyperacute rejection

The expression of a functional α (1-3)-galactosyltransferase gene in lower mammals leading to the synthesis of galactose α (1-3)galactose (Gal α (1-3)Gal) as the terminus of some oligosaccharide chains, as occurs in mammals (10), establishes a critical immune barrier (11, 12). This saccharide is recognized by naturally occurring antibodies, mainly IgM, made by all immunocompetent humans and higher primates which do not express that

sugar (10). This antigen-antibody system activates the classical complement pathway and leads to destruction of the graft within minutes to few hours (1). Activation of complement occurs partially as a result of binding of antibodies to the graft, and partially by the failure of complement regulation in the foreign organ, since several complement regulatory proteins are species specific. Endothelial cells are the primary target of host immunity during hyperacute rejection (13, 14).

Delayed vascular xenograft rejection

Different terminologies have been used to characterize this form of rejection. AVR (15) or delayed xenograft rejection (DXR) (16) have been used to describe the same phenomenon, namely the rejection occurring after a few hours to a few days after transplantation. The mechanism of this rejection is not fully elucidated, but antibodies are regarded to be essential, and platelets, macrophages and NK-cells have been postulated to be involved (15, 16). The role of complement in AVR has been doubted, but recent studies indicate that complement activation may in fact contribute to development of AVR (17–20).

Cellular and chronic xenograft rejection

The cellular rejection is mainly T-cell mediated as in the allograft counterpart, but seems to be more pronounced in xenografts than in allografts (6). The role of

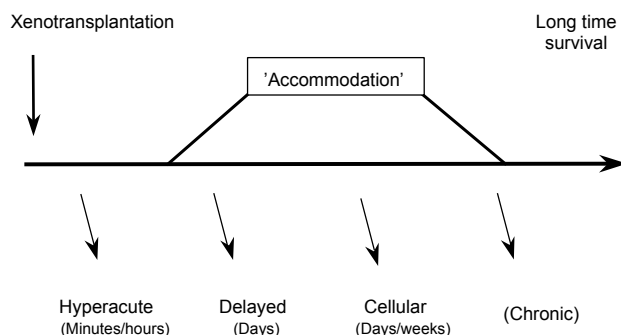


Figure 1. Rejection mechanisms in discordant xenograft transplantation, e.g. the pig-to-human combination. 'Accommodation' indicates a condition where rejection does not occur despite the presence of antibodies and complement.

complement in the late rejection phases may be limited, although it should be emphasized that an initial low-grade complement activation not leading to hyperacute rejection, may induce a certain degree of endothelial cell activation which may contribute to later rejection, including both AVR, cellular and chronic rejection. In this context it should be noted that complement has been found to contribute to general vascular destruction in the process of arteriosclerosis (21).

Complement regulation and therapeutic intervention

Under physiological conditions the activation of complement on autologous cells is controlled by cell-associated glycoproteins such as decay accelerating factor (DAF, CD55) and membrane cofactor protein (MCP, CD46), which inhibit complement activation at the C3 convertase step, and CD59, which prevents formation of the terminal C5b-9 complement complex (TCC).

Since antibodies and complement are the main contributors to hyperacute rejection, these have also been the goal for manipulation to avoid this immediate type of rejection. The major breakthrough has not been obtained by manipulation of antigen or antibodies, but rather of complement. There are several approaches to modulation of complement: (1) depletion of complement using cobra venom factor; (2) inhibition of complement using unspecific proteases; (3) binding of complement using intravenous immunoglobulins; (4) production of animals transgenic for human complement regulators; and (5) specific inhibition of complement using soluble complement inhibitors or antibodies to complement components. The first three approaches are hampered either by the generation of large amounts of activation products or by various unspecific effects. The last two approaches are briefly discussed below.

Pigs have been made transgenic for human complement regulators, particularly the naturally occurring membrane proteins DAF, MCP and CD59. Successful transplantation with organs from pigs transgenic for

one or more of these regulators to baboons has been obtained, in the sense that hyperacute rejection has been avoided (19, 22–27). Recently, 12 week survival after life-supporting hDAF transgenic pig heart transplantation to primates was demonstrated (28). The results with transgenic pigs have been so promising that the hyperacute rejection problems principally have been regarded as resolved. It is of importance, however, to add that recent data on pig complement regulators (DAF and CD59) have shown that they may be as efficient as human regulators in protecting pig endothelial cells against human complement (29, 30). Thus, increased expression of pig complement regulators on pig endothelial cells may be as efficient as making human transgenic pigs. In addition to avoiding hyperacute rejection the transgenic pigs also have shown attenuation of AVR, indicating that complement may contribute to this phase of rejection (19). This suggestion is supported by data indicating that complement activation correlates with graft rejection in AVR (17).

The use of transgenic pigs seems promising and has brought xenotransplantation nearer clinical reality. However, the membrane regulators are usually expressed on the endothelial cells as clusters and the activation of complement may take place at the membrane in between these clusters (31). It should thus be emphasized that although organs from these pigs may be protected against hyperacute rejection, a certain degree of complement activation may still contribute to an inflammatory reaction with subsequent impaired graft function. Efforts should therefore be taken to control the fluid-phase activation of complement as well. A few methods are today considered clinically applicable to inhibit complement activation. Recombinant soluble complement regulators like the soluble form of complement receptor 1 (sCR1) antibodies to complement components, particularly C5, have shown promising results and are candidates for future clinical trials (32, 33). A number of small peptides binding to receptors or blocking the activation of complement components are under development and should be useful for future therapeutic modulation of complement.

We have established an *ex vivo* perfusion model for the study of hyperacute rejection in a pig-to-human combination (pig kidneys perfused with fresh human whole blood) (34) and investigated the effect on inflammatory reaction and graft survival by modulating complement activation by the C3 inhibiting peptide Compstatin (35) and by C1-inhibitor (36). Both reagents completely inhibited hyperacute rejection and markedly reduced the inflammatory reaction. In a model of AVR we are currently investigating the effect of various complement inhibitors on endothelial cell activation.

Conclusion

Complement activation plays an essential role in hyperacute xenograft rejection and may be involved in later rejection mechanisms as well. Genetic manipulation of complement regulatory molecules in the source animal and inhibition of the recipient's complement system may both be required in order to obtain long-time survival of xenografts.

Note added in proof

A technical breakthrough was recently reported, where cloned pigs knocked out for the galactosyl transferase gene were successfully born (Lai L., et al. *Science Express*, 3 January 2002). Other groups report similar data still not published in peer-reviewed journals at the time of this article going to press.

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