Understanding the Immune Protection Afforded by Endogenous Complement Regulatory Molecules

Carmen W. van den Berg and B. Paul Morgan

Graft 2001; 4; 63

Understanding the Immune Protection Afforded by Endogenous Complement Regulatory Molecules

Carmen W. van den Berg and B. Paul Morgan

The first hurdle to be cleared for successful pig-to-primate xenotransplantation is hyperacute rejection mediated by natural antibody and complement (C). Self-cells are protected from the harmful effects of uncontrolled C activation by the expression of membrane-bound C-regulatory molecules. In man and most other species studied the key players are decay accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46) and CD59.[1] The concept that these membrane C regulators in a given species are in some way specialized to regulate C from that same species ("homologous restriction") has become dogma, despite a lack of supporting evidence. This dogma has been central to the investment in creating transgenic pigs expressing human membrane C regulators, also detailed elsewhere in this volume. Pigs have been produced by transgenesis that express one or several of the human membrane C regulators and organs from these pigs escape hyperacute rejection following transplantation into primates.

Missing from these transgenic strategies is any consideration of the roles of the endogenously expressed pig membrane C regulators, ignored because of the assumption, inherent in the concept of "homologous restriction," that pig regulators inhibit only pig C. We began to question this assumption in the early 1990s, initially with respect to CD59. We found that human CD59 and CD59 analogs from different species were all able to inhibit C from many different sources.[2,3] Further analyses of pig CD59, involving comparisons of recombinant pig and human CD59 expressed in human cell lines lacking CD59, demonstrated that pig CD59 was rather better on a molecule for molecule basis at inhibiting human C than was human CD59.[4] Encouraged by these findings, we examined other membrane C regulators in the pig. MCP was isolated from pig erythrocytes and shown to have a structure very similar to that of human MCP.[5,6] Functional analysis of pig MCP demonstrated cofactor activity for human C3b (with human factor I) equivalent to that of human MCP in the same assay.[5] Very recently, we have identified and characterized the pig analog of DAF.[7] Pig DAF differed in several important structural features from human DAF, being transmembrane anchored on most cells and containing only three of the short consensus repeat domains that typify this family of regulators. Even more unexpected were the findings from functional analyses—pig DAF displayed little or no regulatory activity for pig C but did regulate human C.

These data demonstrate that the concept of "homologous restriction" as applied to membrane C regulators is fundamentally flawed. Indeed, there was no logical reason to suspect that membrane C regulators were restricted in this fashion as there can be no evolutionary pressure to "specialize" in controlling C from the same species. What we observe experimentally is that membrane regulators work across species barriers. In the case of the pig, each of the membrane C regulators characterized regulates human C, in some cases more efficiently than do the human regulators.[2,5,7]

Why then is the non-transgenic pig organ hyperacutely rejected in the pig-to-primate model? One possibility is that pig regulators are not expressed at the relevant sites—on endothelium. To test this we have generated monoclonal antibodies against each of the pig regulators and characterized their distribution. Both CD59 and MCP in the pig were

B. Paul Morgan
Department of Medical Biochemistry
Tenovus Building
University of Wales
College of Medicine
Heath Park, Cardiff CF14 4XN, United Kingdom
Tel.: 44.29.20.742809
Fax: 44.29.20.744305
email: MorganBP@cardiff.ac.uk

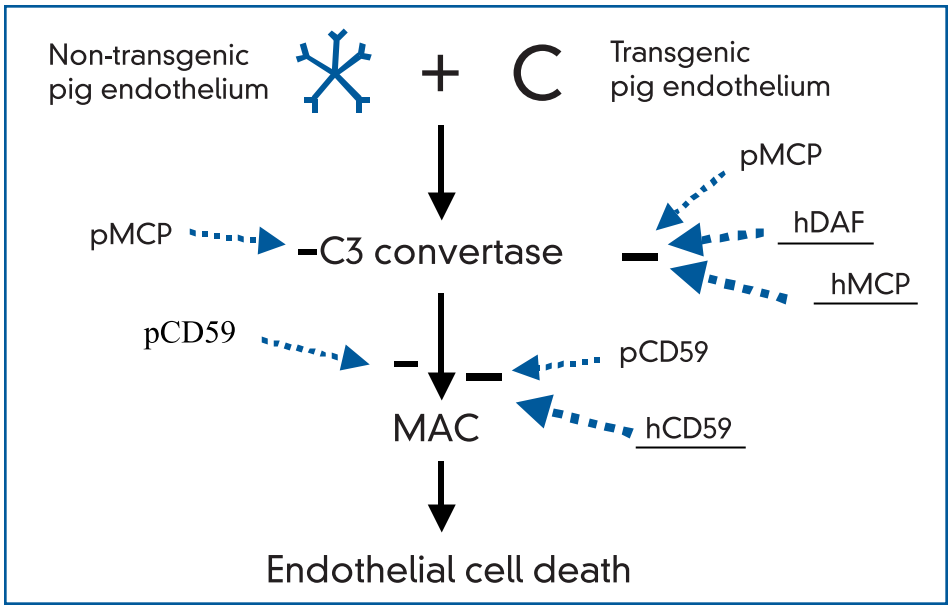

Figure 1. Natural antibody and human complement (C) target the endothelium in the xenografted pig organ. In non-transgenic endothelium, endogenous C regulators pMCP and pCD59 inhibit C activation but are overwhelmed by the intensity of activation and endothelial cell destruction occurs. In the transgenic pig endothelium, the endogenous regulators are complemented by the hyperexpressed human C regulators (hDAF, hMCP, hCD59), providing sufficient regulation to withstand the intense C activation and permitting endothelial survival.

The absence of a functioning DAF molecule on pig endothelium is surprising and it remains possible that another, so far unidentified, C regulator substitutes for DAF.

broadly distributed and abundant on endothelium.[8,9] Pig DAF, in contrast, showed a restricted pattern of expression and was absent from endothelium.[7] CD59 and MCP are thus likely to perform important physiological roles in protecting pig endothelium from C. The absence of a functioning DAF molecule on pig endothelium is surprising and it remains possible that another, so far unidentified, C regulator substitutes for DAF. Given that pig endothelium abundantly expresses two membrane C regulators that efficiently inhibit human C, it is surprising that the endothelium is so rapidly destroyed by C activation in the pig-to-primate model. Our explanation is that, in the face of overwhelming attack by natural antibody and C, the endogenous regulators are exhausted and endothelial damage with resultant hyperacute rejection follows (Fig. 1). The fact that hyperacute rejection can be seen in the context of ABO-incompatible allotransplantation is evidence in support of this. Further support is provided by the finding that a proportion of non-transgenic pig hearts transplanted into primates as controls for the transgenic organs are not hyperacutely rejected.[10] Although not experimentally tested, we suggest that these represent organs that have "coped" with C attack by virtue of higher expression of the endogenously expressed regulators.

The strategy of transgenic expression of human regulators clearly does afford protection from C damage but we believe that this protection is a consequence of the amount of regulator expressed on the endothelium rather than the species source. Although the relative amounts of human C regulators expressed in the pig organs has not been well-addressed, most transgenic approaches aim to hyperexpress the protein of interest. Organs taken from, for example, DAF-transgenic pigs, will express several fold more human DAF than is expressed endogenously on the human organ. Expression of more regulator protects but the species of regulator is not relevant. Of note, hearts from mice transgenic for human or porcine CD59 are protected from human serum to a similar degree in an ex vivo perfusion model.

It is likely that if pig organs were induced to express on endothelium more of the endogenous regulators, then a similar protective effect would be observed. The challenge is to achieve this without resorting to transgenesis. Recent reports of up-regulation of CD59 on pig endothelium in vitro

following exposure to specific lectins and consequent protection of the endothelium from C damage suggest that non-transgenic approaches might work.[11] Our strategy is to analyze promoter regions in the genes encoding pig regulators in order to identify logical approaches to inducing up-regulation in vivo. The take-home message: don't forget, pigs have regulators too!

References

1. Morgan BP, Harris CL. Complement Regulatory Proteins. **London; Academic Press 1999:41-169.**
2. van den Berg C W, Morgan B P. Complement-inhibiting activities of human CD59 and analogues from rat, sheep, and pig are not homologously restricted. **J Immunol 1994; 152:4095-4101**.
3. van den Berg C W, Harrison RA, Morgan BP. A rapid method for the isolation of analogues of human CD59 by preparative SDS-PAGE: application to pig CD59. **J Immunol Methods. 1995; 179:223-231**.
4. Hinchliffe S J, Rushmere NK, Hanna SM, Morgan BP. Molecular cloning and functional characterization of the pig analogue of CD59: relevance to xenotransplantation. **J Immunol 1998; 160:3924-3932**.
5. van den Berg C W, Perez de la Lastra JM, Llanes D et al. Purification and characterization of the pig analogue of human membrane cofactor protein (CD46/MCP). **J Immunol 1997; 158:1703-1709**.
6. Toyomura K, Fujimura T, Murakami H et al. Molecular cloning of a pig homologue of membrane cofactor protein (CD46). **Int Immunol 1997; 9:869-876**.
7. Perez de la Lastra J.M, Harris CL, Hinchliffe SJ et al. Pigs express multiple forms of decay accelerating factor (CD55), all of which contain only three short consensus repeats (SCR). **J Immunol 2000; 165:2563-2573**.
8. Hanna S M, Williams GT, van den Berg CW et al. Characterization in vitro and in vivo of the pig analogue of human CD59 using new monoclonal antibodies. **Immunology 1998; 95:450-459**.
9. Perez de la Lastra J M, Hanna SM, Morgan BP. Distribution of membrane cofactor protein (MCP/CD46) on pig tissues. Relevance to xenotransplantation. **Immunology. 1999; 98:144-151**.
10. Carrington CA, Richards AC, van den Bogaerde J et al. Complement activation, its consequences and blockade by gene transfer. **World J Surg 1997; 21:907-912**.
11. Dalmasso A P, Benson BA, Johnson JS et al. Resistance against the membrane attack complex of complement induced in porcine endothelial cells with a Gal alpha(1-3)Gal binding lectin: up-regulation of CD59 expression. **J Immunol 2000; 164:3764-3773**.