# Recent Advances in Pig-to-Primate and Related Xenotransplantation: A Brief Review of Presentations Relating to Xenotransplantation at the 18th International Congress of The Transplantation Society, Rome, August 2000

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## A Brief Review of Presentations Relating to Xenotransplantation at the 18th International Congress of The Transplantation Society, Rome, August 2000

*Ian F.C. McKenzie and Mauro S. Sandrin*

We here review the presentations made at the recent Congress of The Transplantation Society held in Rome, and attempt to summarize recent progress in xenotransplantation, particularly with regard to advances in porcine transplants into primates or humans. There is clearly a desperate need for more transplants to be done, and the technology is now available in regard to the engineering of genes, the manipulation of large animal embryos and oocytes, and the cloning of pigs. With the editor's instructions in mind, we attended the Congress with a number of preconceived ideas and with questions to be answered. Our preconceptions will be noted as background, and the perceived "advances" will be reported in the light of how they addressed our preconceived ideas.

### Questions to be Answered

1. Hyperacute rejection (HAR) is due to natural anti-Gal antibodies and complement—what progress is being made towards developing a pig with decreased or no Gal antigen expression?
2. What exactly is the role of transgenic expression of human complement regulatory proteins. It is clear that complement is a crucial component in HAR and, in experimental pig-to-primate models, the use of pigs expressing human complement regulatory proteins (CRPs—CD46, CD55, CD59) has provided evidence that the incidence of HAR can be decreased. The evidence presented (mostly from commercial companies) suggests that HAR can be overcome almost entirely by the presence of human CD55 (hDAF) in the transplanted pig organs. Firstly, how good is hDAF? Secondly, what are the details of the immunosuppressive regimens used, and how important are variations in these protocols?
3. Are several CRP's better than one?
4. What is the progress in tolerance induction?
5. Have there been any other advances of significance?

### The Congress

This international meeting had 6,000 participants, making it difficult to see all of the appropriate people and hear all of the relevant presentations. However, xenografting was well-represented with 11/2 plenary sessions, 2 symposia, and 9 specific oral sessions, with many posters. It was a pity that the posters did not receive greater prominence. There were two outstanding social events, both held in the evening, one at Hadrian's Villa and the other in the Piazza Navona. There was a little confusion about the former, which was held to popularize transplantation in Italy, but neither venue had been used for a major social event for 2,000 years, which may explain the confusion. There was remarkable event—the Pope visiting the Congress on the Tuesday morning and giving a clear and forthright statement of the Catholic Church's view on transplantation. His Holiness's speech referred to a number of aspects of transplantation, but

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in particular in reference to xenotransplantation the following statement was made:

"I would merely like to recall that already in 1956 Pope Pius XII raised the question of their legitimacy. He did so when commenting on the scientific possibility, then being presaged, of transplanting animal corneas to humans. His response is still enlightening for us today: In principle, he stated, for a xenotransplant to be licit, the transplanted organ must not impair the integrity of the psychological or genetic identity of the person receiving it; and there must also be a proven biological possibility that the transplant will be successful and will not expose the recipient to inordinate risk."

Raffaello Cortesini is to be congratulated on his organizational skills, and it is appropriate to note Cortesini's original work in antibody-mediated graft rejection conducted in the 1960s.

Enormous numbers of cells have to be given in mice to adsorb out the existing anti-Gal antibodies, and whether this can be done in humans (or even baboons) is not clear.

## Progress with the Problem of Gal Antigen/Anti-Gal Antibodies

The cloning of pigs from two centers was noted by several speakers. In the PPL studies (published in *Nature*), an in vitro knockout of the galactosyltransferase gene had been carried out, and so it may well be just a matter of time before such cells are transferred to a pig and a Gal-knockout pig produced. However, the frequency of success with nuclear transfer cloning is small, and we may well be waiting a long time for the Gal knockout pig. The introduction of a gene for a human CRP—more work has been done with hDAF than with others (see below)—apparently avoids anti-Gal-mediated HAR. However, delayed rejection occurs, which is almost certainly due to smaller amounts of residual anti-Gal antibody. The importance of removing all, or nearly all, expression of Gal is therefore clear. Indeed, Platt has estimated that removing 80% of the antigen can avoid HAR, but 98% of the antigen has to be removed to avoid the antibody-mediated rejection occurring 5 days or so later, which has been called acute vascular rejection (AVR).

The H type α1,2fucosyltransferase concept to avoid Gal expression apparently does not work well in the pig (compared to the mouse, where almost total removal of Gal occurs in H transgenic mice). The reason for this is not clear—some suggested it was due to the pig expressing much more Gal antigen than the mouse, but there are other possibilities. In mouse systems, combining the introduction of the gene for galactosidase with that for the H antigen provides almost total removal of Gal. It remains to be seen whether this approach will work as well in the pig; at this time, it would seem unlikely, given that the presence of H does not seem to be of much benefit in the pig.

A number of studies again demonstrated the value of removing anti-Gal antibodies by extracorporeal immunoadsorption (although one study indicated a detrimental effect from immunoadsorption). Removal of antibodies by immunopheresis and selective immunosuppression can prevent antibody rebound and the induced antibody response, although natural antibody is still present and AVR still occurs. It would seem to us that removal of antibody by columns is satisfactory as a short-term procedure, but would be logistically difficult to do on repeated occasions if many transplants were being undertaken. It is perhaps more an experimental proof of principle, demonstrating that removal of antibody can avoid HAR. Thus, we await the Gal-knockout pig, probably produced by nuclear transfer and subsequent cloning, but nothing was reported to indicate how long we shall have to wait.

Other approaches are being examined, such as by introducing more than one gene or the intracellular expression of antibodies to transferases (all of which work spectacularly in mouse models). It could be that these will also work in the pig, and it is perhaps wise for these approaches to be taken to larger animals. The field needs to move along faster, and this can only be done when HAR is totally avoided (currently achieved) but delayed HAR or AVR is also avoided. Only then will it be possible to examine the severity of the cellular responses and the importance of other incompatibilities.

While it could be considered that the total removal of Gal may avoid antibody-mediated rejection, this is unlikely to be case. All that will happen is that pre-existing antibodies will not be a problem and secondary anti-Gal antibodies may not develop. But surely antibody-mediated rejection will occur in the pig-to-primate model (just as it can occur in human allografts) with an antibody response to other pig antigens.

## How Good Is hDAF?

There have been a number of papers over the last year or so suggesting that hDAF may be suitable agent to bind sufficient complement to avoid

HAR. The use of Imutran's hDAF pigs was reported from at least 3 centers independently, and there is no doubt that the presence of hDAF as a transgene gives rise to graft prolongation. The explanation for this is that a large amount of complement has to be fixed on the cell before any cell damage can occur. The presence of hDAF apparently maintains a low enough threshold of complement activation such that HAR does not occur.

Thus, several interesting points emerged.

1. There was some interest in the level of hDAF expressed by the Imutran pig. Other hDAF transgenic pigs were reported. Particularly high expression was noted in the transgenic pigs in Italy, which in vitro and ex vivo give interesting results. It will be of interest to see what happens when pig-to-baboon transplants are performed and, indeed, whether more hDAF is better, i.e., whether there are better pigs around than the Imutran pig.
2. In many of the studies, high levels of immunosuppression have been used. At the Congress, usually because of time constraints, it was difficult to obtain information on any individual animal. Although HAR is antibody/Gal antigen/complement-dependent, which would be largely unaffected by pharmacologic immunosuppression, immunosuppression could interfere with the involvement of platelets and polymorphs. With this in mind, d'Apice and his group have not used immunosuppression, and find that, in the presence of hDAF, the grafts are prolonged 2 or 3 days versus several hours, i.e., the presence of hDAF alone causes prolongation without the need for any other modification or immunosuppression (as previously reported by the Imutran group).
3. One study reported the occurrence of anti-hDAF antibodies in immunosuppressed cynomolgus monkeys, and this correlated with earlier and greater intensity of AVR. This finding lacks real explanation. Why should an immunosuppressed monkey make antibodies to hDAF? It is not known how hDAF and nonhuman primate DAF are related, but surely human and nonhuman primate DAF are more closely related than, say, pig DAF. Nonetheless, the observation has been made and must be kept in mind.
4. While the presence of hDAF seems to ameliorate HAR, it seems to be unable to prevent AVR. Presumably, additional factors come into play, other than complement, antibodies, platelets and polymorphs. At this later phase, endothelial cell activation and endothelial cell damage by other mechanisms may be of great importance, so that modifying the complement response may be insufficient.
5. An important finding was the prolongation of survival of an hDAF porcine liver in a nonhuman primate by Ramirez et al. Pig livers survived in baboons for 4-8 days. What was surprising was that the levels of coagulation proteins in the baboons were maintained for a week after the liver transplant.

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## Effect of Other Complement Regulators

There were several papers on other complement regulators, such as CD46, which seems to be at least as beneficial as hDAF, although it was not clear whether it was more beneficial. d'Apice's studies indicate two regulators (CD55 and CD59) are better than one. In the absence of immunosuppression, these grafts function for 5 or more days (compared to 3 days).

The soluble complement inhibitor sCR1 (TP10) was used by a number of groups, and is clearly effective in delaying HAR. sCR1 has two effects—(a) it decreases the ischemic damage due to storage (pig kidneys were deliberately subjected to periods of 6-11 hours cold ischemia and sCR1 certainly improved their immediate function); and (b) it has an anti-complementary effect on HAR, and this was additive with hDAF.

## Intragraft and Intravascular Clotting

That antibody-mediated rejection can lead to intravascular coagulation was noted in the 1960s by Starzl (in a classical paper describing the Shwartzmann reaction) and also in classical experimental studies in the mouse where, depending on the amount of antibody used, Arthus or Shwartzmann reactions could be induced. It therefore comes as no surprise to see this in pig-to-primate studies. d'Apice reported that, in the presence of several transgenes and in the absence of immunosuppression, intragraft disseminated intravascular coagulation (DIC) was seen and was only slightly delayed with heparin. This has also been found in other studies,

particularly from Cooper and colleagues, where the removal of antibody combined with extensive immunosuppression, was associated with DIC. However, the beauty of the transgenic approach is that the problem can be examined using different genes expressed by transgenesis. While in vivo studies in the pig-to-primate model have not yet been reported, plans were presented to modify pig organs to express either tissue factor, plasminogen inhibitor, thrombomodulin, or other anticoagulant agents, such as hirudin. Other genes to inhibit thrombin activation could be useful.

### Tolerance Induction

There was little new presented in large animals. The anti-Gal HAR/AVR problem is totally obscuring the picture at present. There was, however, some interesting work in progress in the Sachs' group where thymus/kidney allografts are being performed. Autologous donor thymic tissue is transplanted under the kidney capsule, and this thymo-kidney subsequently transplanted into the recipient. A similar approach in the mouse gives rise to allograft and xenograft tolerance. In some of these experiments, tolerance extends to T and B cells, so that the anti-Gal response (in Gal-knockout mice) is totally abrogated. One approach that could could work in larger animals is the gene transfer of the Gal antigen to the Gal-negative bone marrow cells of the potential recipient. This could induce B and T cell tolerance. It remains to be seen whether this will totally abrogate the anti-Gal response in larger animals. Enormous numbers of cells have to be given in mice to adsorb out the existing anti-Gal antibodies, and whether this can be done in humans (or even baboons) is not clear. Perhaps prior lowering of the titer of anti-Gal antibodies with immunopheresis may allow this approach to be successful.

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### Other Studies

The use of a number of other genes as transgenes was discussed, e.g., anti-oxidant and anti-apototic genes. Some progress iss taking place but, again, the problems of HAR/AVR are obscuring the possible advances. The dramatic studies using anti-CD40 ligand monoclonal antibodies in allografts have not been reported with any great success in pig-to-primate xenografts, although Buhler et al have demonstrated that treatment with this monoclonal antibody can totally prevent the induced antibody response (both to Gal and non-Gal pig antigens) in pig-to-primate studies.

### Comment

This field is on the move. The presence of hDAF as a single transgene has certainly given reproducible graft prolongation; with immunosuppression, grafts survive for many weeks. In addition, it is likely that the introduction of more than one CRP is better than one. In Rome, however, there were no major breakthroughs, but perhaps we should not expect these. Rather, there was consolidation of findings from previous International Xenotransplantation Association and Transplantation Society Congresses, and maintained interest and enthusiasm in the prospect of ultimately transplanting pig organs into humans.

One small point is that in these studies, particularly in large animals, it is important to investigate all of the parameters—organ function, immune responses, degree of immunosuppression, etc,—and it is difficult to report on all of these within the confines of a large meeting. A workshop with a small number of participants actively involved in this area is required. These studies are expensive, difficult, and time-consuming. It is essential that all investigators make all of their data widely available.