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Xenotransplantation – State of the Art

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Xenotransplantation

Xenotransplantation is defined as the transplantation of tissues or cells between two zoologically different species. Due to the shortage of human organs for allotransplantation, i.e. transplantation between humans, essential efforts aimed at development of a donor for animal-human xenotransplantation have been performed over the last decade.

Rejections caused by xenotransplantation

Transplantation between two different species may give rise to two types of graft rejection. Between so-called *discordant* species a hyperacute rejection (HAR) occurs within minutes or hours. In so-called *concordant* species this type of rejection does not occur, but over days a *delayed xenograft rejection* (DXG) (6) will occur. In all transplanted organs, xeno- as well as allotransplanted, chronic graft rejection (CRG) will occur after years.

Man is concordant with old world primates, but due to several reasons, such as breeding difficulties, risk of retroviral epidemics, concerns for the use of endangered species, etc., the discordant pig is considered the donor of choice.

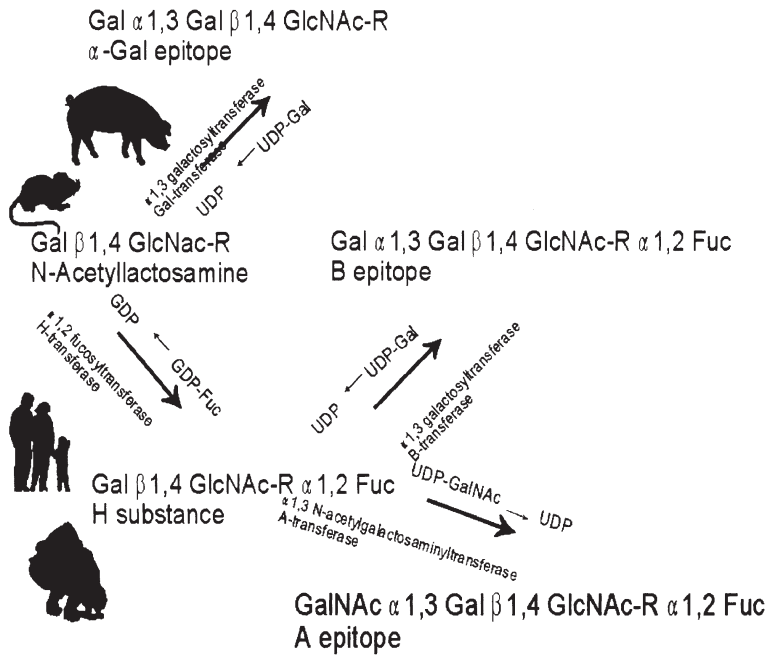
HAR is primarily caused by initiation of the complement cascade. The antigen known to be activating the classical pathway is the alpha-gal epitope (10) (Figure 1); the epitope alternative to the primate AB0 blood group system in most non-primate mammals. In species, in which the antigen is absent, circulating antibodies are present. In humans IgG against the alpha-gal epitope counts for up to a total of 1% of circulating IgG (11). These antibodies probably derive from a reaction to members of the intestinal flora, especially Enterobacteriaceae spp., but also other types of infectious agents possess the alpha-gal epitope as a structural element in their cell walls (10).

Strategies to prevent rejection

Acute rejections after allotransplantation are generally prevented by immunosuppressive treatment, but although HAR may be prevented by cobra snake venom (12) and certain complement regulatory immunocomponents (27), it seems unlikely that complement cascade activation can be prevented by the same means without seriously interfering with the health and well-being of the recipient. Therefore, the donor or source animal needs to be modified to prevent recognition of complement-activating antigens. Transgenic techniques are the tools for this.

Inserting genes

Insertion of genes may be done by the pronucleus microinjection method (14), which functions well in a range of animal species, including pigs. Three types of genes may be attractive



F i g u r e 1. Synthesis of the α-gal epitope.
N-acetyllactosamine is in most mammals converted to the alpha-gal epitope by the gal-transferase. In certain primate species, including humans, N-acetyllactosamine is converted to the H-subtance by the H-transferase and by the A- or B-transferases further on to the epitopes of the AB0 blood groups, i.e. either the A or B substances, respectively. Strategies for xenoacceptance of porcine organs in humans would be removal of the gal-transferase or introduction of the human H-transferase in the transplanted organ.

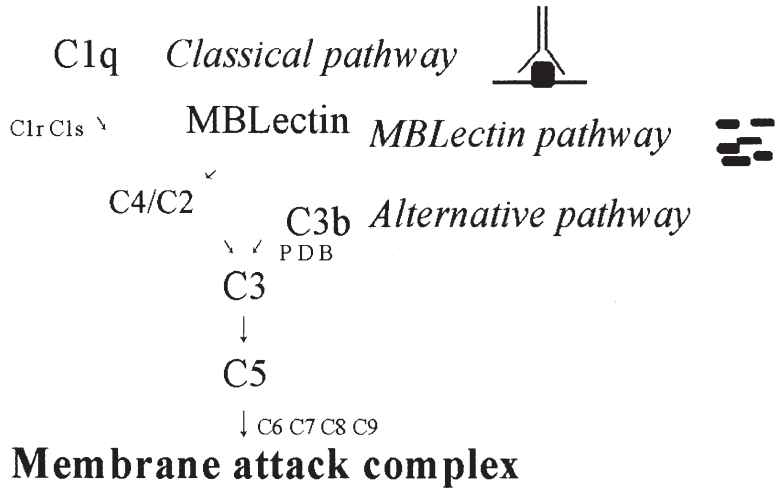
to insert in a xenograft donor. The H-transferase, which fucosylates N-acetyl-lactosamine in competition with the gal-transferase (Figure 1), has been inserted in both mice (5, 28) and pigs (20, 28). Cells of H-transferase transgenic animals show higher resistance towards human sera (20, 28), but in the mice produced by Chen and coworkers (5), it was found that although the alpha-gal epitope was only eliminated in those cells, in which it was also low in non-transgenic mice. Furthermore, it has been impossible to produce homozygotic H-transferase animals (21), i.e. too high an expression of the H-transferase in gal transferase producing animals seems to be toxic. The mechanisms behind this are not fully known, but lectin binding studies show that the structure of the cell walls of H-transferase transgenic animals is changed far beyond the sole deficit of the alpha-gal epitope and presence of the H antigen. Some crypt antigen, Tn and Forssman, may even become exposed on the cellular wall, which may increase the risk of DXG of organs from a H-transferase transgenic donor (23). This reduces the likelihood of such a transgenic animal becoming a future xenograft donor. Another approach attempted in mice is to insert genes coding for the enzyme, alpha-1,3-galatosidase, which resynthesises the alpha-gal epitope into a compound not attacked by natural antibodies

produced by humans. These mice tended to secrete more proteins in the urine than the wild type. Furthermore, low body weights, partial damage to hair growth and early death occurred more frequently (19). Therefore, neither does this approach seem to be applicable for the production of a xenograft source animal.

A third and more successful approach may be to insert genes, which identify the tissue as homologue to the complement system. Therefore, it binds parts of the proteins involved in the complement cascade, thereby disabling both the classical and the alternative pathway, so-called *complement-regulatory factors* (CRF) (Figure 2). The most important ones in xenotransplantation research are *human decay accelerating factor* (H-DAF), *membrane cofactor protein* (MCP) and CD 59. Both mice and pigs transgenic for CRF's have been produced, and the transgenic animals have been combined with one another and with animals changed on alpha-gal related genes.

Knock-outing genes

To knock-out loci for the upstart of a transgenic source animal colony demands the use of the embryonic stem cell method for transfection (15); a method, which so far is only available in mice. However, pig cloning and nuclear transfer techniques (9) represent a reasonable alternative. Two groups reported the successful production of alpha-gal knock out mice in the mid-nineties (30), and around New Year 2002/2003 two other groups reported the successful production of alpha-gal knock out pigs by cloning and nuclear transfer (8, 21). Although those pigs appearing in these two publications were hemizygotic, one of these groups has, by



F i g u r e 2. Possible pathways for complement activation; the key factor for hyperacute rejection (HAR). The classical pathway is activated by the presence of a specific antigen. In xenografts in humans this is the alpha-gal epitope against which humans have natural antibodies. The alternative pathway is normally activated by some non-specific cell surface structures, but activation of this pathway against xenografts may be prevented if the xenograft contains either membrane cofactor (MCP or CD 46) or decay accellerating factor (H-DAF or CD 55), which both prevent conversion of C3 to C5 or CD 59, which prevents the C6 C7 C8 C9 cascade.

cloning of cells from these pigs, successfully produced homozygotic alpha-gal knock out pigs, which seem to be fully viable (2).

Transplantation experiments from pigs to primates

The baboon, and to a lesser extent the rhesus monkey, are the animals of choice for modelling pig to human xenotransplantation. Transplantation of non-transgenic pig organs to these primates results in HAR within hours (24). Transplantation from CRF transgenic pigs to baboons has been attempted, primarily with the heart (1) and kidney (7) and it is evident that CRF's prevent HAR, while DXG occurs within one or two weeks (1, 4). Grafts become infiltrated by macrophages, T cells and B cells and the recipients develop thrombocytopenia and abnormalities in coagulation (7). Supplementary supportive treatments of recipients with antithymocyte serum (31), C1 inhibitor or cyclophosphamide may prolong graft survival up to 40 days (13). Extracorporal perfusion using CRF transgenic pig liver cells seem to offer an improvement compared to perfusion using non-transgenic pig cells (26). Results of transplantation of organs from alpha-gal knock out pigs to primates have not yet been published.

Xenozoonosis

The term xenozoonosis covers infections that may spread from the transplanted organ to the recipient. One might fear that ordinary zoonotic infections known to be present in pigs, such as encephalymyocarditis virus (3) and rotaviruses (17) may pose a risk for the recipients, but these infections are easy to handle during the animal production phase and would in any case mostly be a risk to the individual; a risk, that probably would be outweighed by the benefits from xenotransplantation.

Much more concern has been related to the risk of activating porcine endogenous retroviruses (PERV) in the recipients, which hereafter may spread the infection to relatives and, thereby, cause a world-wide retroviral epidemic. The discussion was initiated by the experience that retrovirus produced by the PK-15 kidney cell line (PERV-PK) infected human kidney 293 cell lines and co-cultivation of X-irradiated PK-15 cells with human cells resulted in a broader range of human cell infection, including human diploid fibroblasts and B- and T-cell lines (25). Host range analyses have shown that type A and B PERV Env's have wider host ranges including several human cell lines, compared with type C PERV, which infect only one human cell line (29). However, in a retrospective study 15 immunosuppressed baboons were tested for a specific immune response against PERV after transplantation of porcine endothelial cells, mononuclear blood cells, and lungs. Anti-PERV antibody expression was analyzed with peptide-based, enzyme-linked immunosorbent assays and highly sensitive Western Blot assay showing no evidence of PERV specific humoral immune response (22). Retrospective studies of patients exposed to living pig tissues have neither been able to show such cross species transmission (18).

Functional problems related to xenotransplanted organs

Physiological and anatomical differences between man and pig may be speculated to be obstructive for successful xenotransplantation in various ways. E.g. man is the only animal living in an upright position, and therefore gravity may have a different impact on the lung, heart, liver, and kidney. Differences on the humoral and enzymatic basis may be even more pronounced. Hormones and enzymes are regulated by complicated and often species-specific mechanisms, e.g. growth hormones may stimulate the xenografts to unrestricted growth. If enzymes are not removed by the liver they may

reach a level not compatible with life. Products like albumin are carriers for other molecules and need to be compatible for binding sites. Essential porcine and human proteins differ significantly, e.g. albumin have an amino acid identity of less than 65%, erythropoetin (EPO) of less than 82%, and complement of less than 70% (16). Therefore, especially the liver at present seems to be an unlikely candidate for xenotransplantation.

Future perspectives of xenotransplantation

The production of the alpha-gal knock out pig has been a major step-forward in xenotransplantation research. Although the road towards xenotransplantation still seems long, especially for complicated organs such as the liver, the fact that research in the future may be more directed against DXG, which may be handled by other means than transgenesis, e.g. immunosuppression, gives rise to optimism. Also, xenotransplantation with transgenic organs may in the future offer a higher quality of transplantation if the organs could be modified in such a way, that all types of rejection could be omitted, thus avoiding the life-long treatment with immunosuppressives,

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