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Fakulteten för veterinärmedicin och husdjursvetenskap

# **A survey of xenograft rejection mechanisms**

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## **A survey of xenograft rejection mechanisms**

Avstötningsmekanismer vid xenotransplantation- En översikt

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## **SUMMARY**

There are four types of xenograft rejection. They are referred to as hyperacute rejection, acute humoral xenograft rejection, acute cellular xenograft rejection and chronic rejection. Hyperacute rejection is mediated by natural xenoreactive antibodies (mainly directed against the antigen  $\alpha$ -Gal found on porcine cells) that activates the classical complement pathway. Acute cellular xenograft rejection is mediated by xenoreactive IgM and IgG, that seems to damage the xenograft by among other things activating complement and endothelial cells. Acute cellular xenograft rejection is mediated by T cells and NK cells that act cytotoxic on the endothelium. Chronic rejection results in four different types of vasculopathy, which lead to occlusion of arteries, and in vascular fibrosis. Although a lot of research has been conducted in recent years, for example transgenic swines that does not express  $\alpha$ -Gal have been generated, more remains to be done before xenotransplantation can be conducted clinically.

## **SAMMANFATTNING**

Det finns idag fyra mekanismer beskrivna för avstötning av xenotransplantat. Dessa är hyperakut avstötning, akut humoral xenotransplantat avstötning, akut cellulär xenotransplantat avstötning och kronisk avstötning. Hyperakut avstötning medieras av naturliga xenoreaktiva antikroppar, vilka främst är riktade mot antigenet  $\alpha$ -Gal på svinceller och aktiverar komplement systemet (den klassiska vägen). Akut humoral xenotransplantat avstötning medieras också av xenoreaktiva IgM och IgG, som verkar skada xenotransplantatet bland annat genom aktivering av komplementsystemet och endotelceller. Akut cellulär xenotransplantat avstötning medieras av T-celler och NK-celler, som verkar cytotoxiskt på endotelet. Kronisk avstötning ger upphov till fyra typer av vaskulopati, vilket leder till förträngning av artärer, och till fibros av kärl. Trots att framsteg har gjorts inom forskningen, till exempel har transgena grisar som inte uttrycker  $\alpha$ -Gal framställts, måste mer göras innan xenotransplantation kan komma till klinisk användning.

## INTRODUCTION

Experiments with organ transplantation have been carried out since the beginning of the 20<sup>th</sup> century (Starzl, 2000). Since the first successful human transplantation was conducted in 1954 between two identical twins organ transplantations have been used as treatment for end-stage diseases. Due to the lack of available organs, the possibility of xenotransplantation, i.e. the transplantation of organs between different species, has been studied (Gonzalez-Stawinski et al., 2002). Xenografts are rejected by the recipient's immune system in a sequence of rejection mechanisms (Gonzalez-Stawinski, 2002), that all are an obstacle to the clinical use of xenotransplantation.

The aim of this essay is to summarize the mechanisms for immunological xenograft rejection known today and which parts of the immune system that are involved in these.

My intent is to describe the immune reactions by which xenografts transplanted from animals (mainly swine) to humans are rejected. There are however more difficulties in using xenotransplantation clinically than rejection by the immune system. These include microbiological (transfer of microorganisms and infectious agents from the donor to the recipient) and physiological differences between humans and other species (Cooper, 2003). Because of lack of space these will only briefly be discussed here. Neither is it not within the scope of this essay to discuss immune suppression drugs and other methods used to prevent immune rejection. I only mention generation of transgenic pigs lacking the  $\alpha$ -Gal epitope because of its great importance.

### Concept explanations and abbreviations

<b>Xenotransplantation</b>	Transplantation of organs between different species.
<b>Xenograft</b>	A graft that is transplanted between different species.
<b>Heterotopic transplantation</b>	Transplantation of an organ to another site than its natural anatomical position.
<b>HAR</b>	Hyperacute rejection
<b>AHXR</b>	Acute humoral xenograft rejection
<b>ACXR</b>	Acute cellular xenograft rejection
<b>Delayed xenograft rejection</b>	Delayed xenograft rejection (DXR), also called acute vascular xenograft rejection (McCurry et al., 1997), is a generally acknowledged notion for acute rejection of xenografts, but there exists disagreement as to how the notion is used. Chen et al. (2004) mean that DXR is rejection mediated by both antibodies and cells. However Hisashi et al. (1998) divide acute rejection into two parts. These authors propose that DXR only is an antibody-mediated rejection (in their article DXR is also synonymous with acute humoral xenograft rejection), while the cellular rejection is referred to as acute cellular xenograft rejection. To avoid confusion in this essay the acute antibody-mediated rejection will be designated "acute humoral

xenograft rejection” and for the acute cellular rejection “acute cellular xenograft rejection” will be used, despite that DXR might have been used in the articles referred to.

**$\alpha$ -Gal**

Short for galactose- $\alpha$ (1,3)-galactose. It is a carbohydrate epitope found on porcine cells (but not cells from human, apes and Old World monkeys). It is the main antigen that the antibodies, which cause hyperacute rejection, bind to (Chen et al., 1999) and is also involved in acute humoral xenograft rejection (McCurry et al., 1997).

**GalT-KO swine**

Short for  $\alpha$ 1,3-galactosyltransferase gene-knockout swine. They are transgenic pigs that lack the enzyme 1,3-galactosyltransferase, which generates  $\alpha$ -Gal.

**Xenoreactive antibodies**

Antibodies that is directed against epitopes on a xenograft.

**Natural antibodies**

Antibodies that an individual possesses without prior immunisation against the antigen. The individual however might have been immunised by crossreaction from bacterial epitopes.

**MATERIALS AND METHODS**

Articles were searched on the Internet using three different databases. These were PubMed, Science Direct and Web of Knowledge. On PubMed “mechanism of hyperacute xenograft rejection” gave 58 results. On Science Direct it gave 17 results. On Web of Knowledge it gave 219 results. “Mechanism of delayed xenograft rejection” gave 26 results on PubMed, on Science Direct 25 and 105 on Web of Knowledge. ”History of clinical transplantation” gave 3310 results on PubMed. Searching for ” $\alpha$ -Gal AND xenograft” on PubMed gave 140 results. Articles containing experiments with transplantations of organs from pigs to baboons were preferred.

**LITERATURE REVIEW**

**Immunological rejection of xenografts**

Xenografts are rejected by the recipient’s immune system by four different rejection mechanisms (Hisashi et al., 2008). These are referred to as hyperacute rejection, acute humoral xenograft rejection, acute cellular xenograft rejection and chronic rejection. They are mentioned below in the chronological order that they are responsible for the rejection of a xenograft. If the graft is rejected within 48 hours after transplantation the rejection is hyperacute. If the graft is rejected up to 7 days post-transplantation the rejection is denominated acute. Chronic rejection occurs months after transplantation. Xenograft rejection is often defined as the loss of function of the organ, for example the termination of beating of a heart (McCurry et al., 1997; Chen et al., 2000).

***Hyperacute rejection***

Chen et al. (1999) found that heterotopic xenotransplantation of hearts from pigs to baboons resulted in hyperacute rejection (HAR) 30-60 minutes post transplantation. If HAR occurs

after transplantation the species are termed discordant, whereas if HAR does not transpire the species are said to be concordant (McCurry et al., 1997).

HAR is seen histologically as diffuse interstitial oedema and focal haemorrhage with thrombi (consisting of fibrin and thrombocytes) in the smaller blood vessels (Dalmaso et al., 1992). Such lesions were observed in cardiac and renal xenografts from pigs transplanted to rhesus monkeys. Furthermore components of the complement system (C3, C4, C5 and C9 with the membrane attack complex, MAC) and IgM were found on the endothelial surface of the xenograft's blood vessels. The authors could not find depositions of factor B and only small amounts of factor P, also components of the complement system. The complement system consists of three distinct cascade reactions, three "pathways". Complement proteins cleave each other in the cascade reactions. The three pathways end in the same terminal pathway, to which C5, C3 and C9 belong. The terminal pathway ends with polymerisation of C9, that together with C5b, C6, C7 and C8 form MAC. MAC inserts in the cell membrane of a foreign cell, forms a pore and the cell dies by osmotic lysis. C4 is together with subunits of C1 the major constituent of the classical pathway, which is activated by IgM and IgG. Factor B and P participate in the alternative pathway that is activated by spontaneous breakdown of C3. According to Dalmaso et al (1992) their findings indicate that complement caused damage to xenografts during HAR is generated by the classical pathway. Further studies done in vitro by the same authors, investigating human serum toxicity on porcine endothelial cells, came to the same conclusion. Serum deficient in components of the classical pathway did not trigger an immune response, but when lacking components of the alternative pathway a response was observed (Dalmaso et al., 1992).

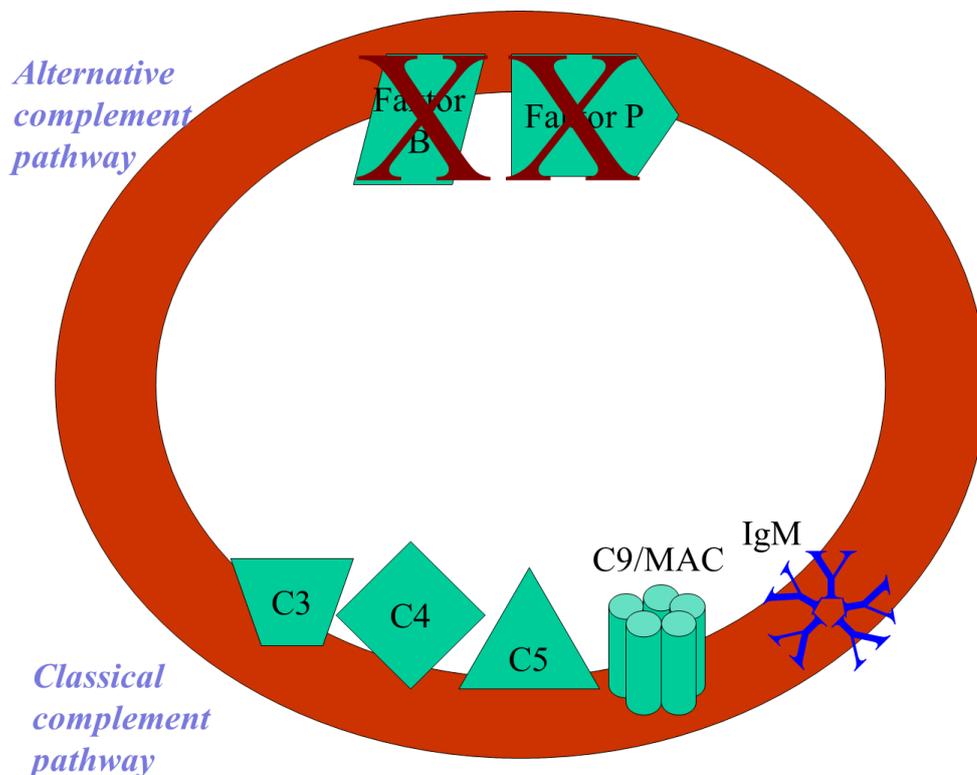


Figure 1. The depositions found on xenogeneic blood vessels rejected by HAR. Modified from Dalmaso et al. (1992).

In the study mentioned above (Dalmaso et al., 1992) removal of antibodies from the blood of the monkeys prevented the deposition of complement components and antibodies in the

xenografts's blood vessels a few hours post-transplantation. Furthermore human serum lacking IgM was not cytotoxic on porcine endothelial cells in vitro.

HAR of porcine xenografts transplanted to Old World monkeys is mainly caused by the binding of natural antibodies from the recipient to carbohydrate structures on the endothelial surface of blood vessels of the xenografts (Chen et al., 1999). The principal carbohydrate epitope is named galactose- $\alpha$ (1,3)-galactose ( $\alpha$ -Gal) (Chen et al., 1999). Most mammals (including swines) possess it, only excluding humans, apes and Old World monkeys. Chen et al. (1999) found that  $\alpha$ -Gal epitopes were expressed in a higher amount in small blood vessels than larger. In consistence with this, IgM and MAC were found in a higher quantity in small vessels. Lack of  $\alpha$ -Gal on porcine cells leads to reduced cell lysis by complement (Baumann et al., 2004).

Also subclasses of IgG can provoke HAR (Ding et al., 2008). In accordance with Chen et al. (1999) these are directed against  $\alpha$ -Gal. Hearts from rats, expressing  $\alpha$ -Gal, were transplanted into the abdomens of mice that did not express  $\alpha$ -Gal. Injection of these mice with anti-Gal IgG provoked HAR and the grafts were rejected after 5-7 days.

In an attempt to preclude HAR, consistent with the finding of Baumann et al. (2004), production of  $\alpha$ 1,3-galactosyltransferase-deficient swines has been performed (Phelps et al., 2003).  $\alpha$ -1,3-galactosyltransferase is the enzyme that generates  $\alpha$ -Gal. Phelps et al. (2003) accomplished production of  $\alpha$ 1,3-galactosyltransferase-deficient swine by somatic cell nuclear transfer (cloning). The production of pigs lacking  $\alpha$ -Gal was considered a possible way to overcome the obstacle of HAR (Itescu et al., 1998). This is proved by Hisashi et al (2008) in an experiment where hearts from  $\alpha$ 1,3-galactosyltransferase gene-knockout (GalT-KO) pigs were transplanted into baboons. No HAR was observed and one graft survived for nearly 6 months.

### ***Acute humoral xenograft rejection***

The  $\alpha$ -Gal epitope is not only the key factor causing HAR (Chen et al., 1999), it also has a vital part in eliciting acute humoral xenograft rejection (AHXR) (McCurry et al., 1997). In cardiac xenografts from swine heterotopically transplanted into baboons 70-95% of the xenoreactive IgM and 85-95% of IgG were directed against  $\alpha$ -Gal. The authors believe that antibodies directed against other antigens also contributed to the rejection. These antigens were integrins  $\alpha$ 1,  $\alpha$ v,  $\alpha$ 3/  $\alpha$ 5,  $\beta$ 1 and  $\beta$ 3 chains. Also Hisashi et al. (2008) demonstrated that other antigen than  $\alpha$ -Gal mediate AHXR. In their study AHXR occurred in baboon recipients, whose porcine cardiac xenografts did not express  $\alpha$ -Gal.

Xenoreactive antibodies align alongside the xenogeneic vascular endothelium (McCurry et al., 1997). These authors thus conclude that when a graft is in situ the antibodies are within the graft and for that reason the amount in serum is small. After extraction of a graft antibodies can be detected in sera. Chen et al. (2004) observed the same result independent on the findings of McCurry et al. (1997), where the amount of xenoreactive antibodies in serum decreased after transplantation.

Hisashi et al. (2008) transplanted hearts from GalT-KO swines into baboons. Their histological findings were numerous, including arterial fibrinoid necrosis. They also found microthrombi in the blood vessels (thrombotic microangiopathy) and neutrophils and foci of haemorrhage in the interstitium. Thrombosis occurred in capillaries (in the cases where rejection was slow) and also in arteries (in the cases where rejection was swift). In the blood

vessels IgM, IgG, C3, C4d, and C5b-C9 were deposited. This is consistent with the finding of Chen et al. (2004), who found the same depositions in hearts transplanted from pigs to rhesus monkeys. Hisashi et al. (2008) report that the amount of complement components and antibodies increased when the quantity of microthrombi increased. The results of Chen et al. (2004) and Hisashi et al. (2008) have previously been demonstrated by Chen et al. (2000), who found IgM and MAC deposited along the small blood vessels of porcine cardiac xenografts heterotopically transplanted to baboons.

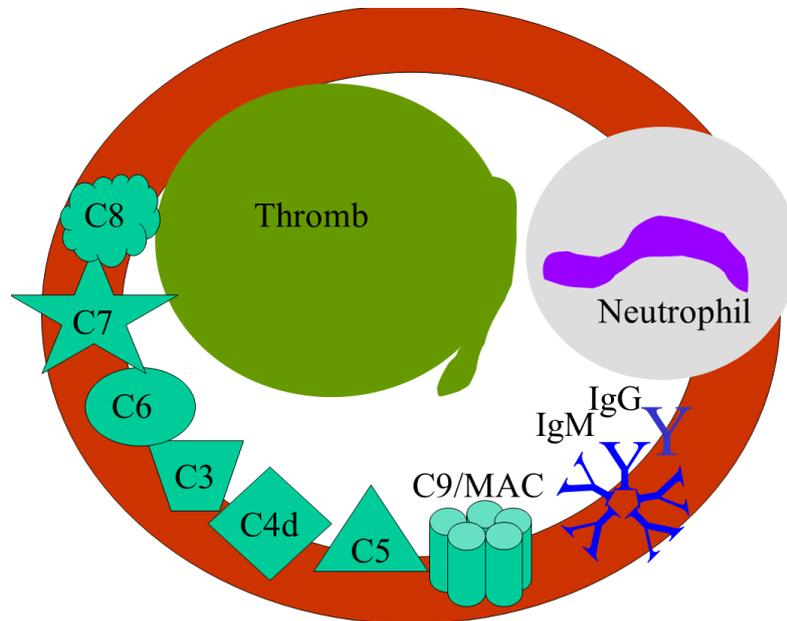


Figure 2. Some of the histological findings and depositions found in AHXR-rejected xenografts. Modified from Hisashi et al. (2008).

McCurry et al. (1997) tested specificity differences of antibodies in serum taken before transplantation (preimmune sera) and after (immune sera). In most animals no difference in the binding of IgM was found. The authors conclude that the binding of natural IgM antibodies (which existed in both the preimmune sera and the immune sera) to foreign antigens on the transplant prevented antibodies formed during transplantation to bind. The specificity of IgG antibodies in the immune serum was however different from that of IgG in the preimmune sera.

Xenoreactive antibodies may mediate AXHR in four different ways (McCurry et al., 1997):

- ❖ Activation of the endothelium, which results in a change of function and structure of endothelial cells. For example it can lead to a rise in intracellular pH (which increases cell division) and a change in permeability of the cell membrane to molecules.
- ❖ Activation of the complement system, which acts on the endothelium and leads to an increase in neutrophil adhesion to the endothelium.
- ❖ Mediate the activity of Natural Killer cells (NK cells) and other cells with F<sub>c</sub>-receptors.
- ❖ Antibodies bind to essential endothelial molecules, such as integrins. In doing so the antibodies may disturb the molecules' function, which for integrins comprises the control of diapedesis.

Porcine hearts survive up to 12 days before rejection by AHXR (McCurry et al., 1997). 12-24 hours before rejection the graft gets bradycardia and lengthened QRS complexes on electrocardiography (Chen et al., 2000).

### ***Acute cellular xenograft rejection***

Acute cellular xenograft rejection (ACXR) is often masked by the more rapid AHXR (Hisashi et al., 2008). In absence of HAR, cardiac xenografts from infant piglets transplanted into baby baboons, survived for 83-96 hours (Itescu et al., 1998). Though some indications of HAR were detected the rejected grafts were mainly infiltrated by macrophages, NK cells and T cells. This finding corresponds with experiments where a porcine kidney ex vivo was perfused with human blood leukocytes (Khalfoun et al., 2000). Mainly T cells and NK cells were retained within the graft, because they bound to the endothelium of porcine renal capillaries. Their adherence led to endothelial damage in form of cell hypertrophy and changes in the basement membrane and podocytes.

T cells alone can provoke xenograft rejection (Lin et al., 1999). CD4<sup>+</sup> T cells (usually considered helper T cells, T<sub>H</sub>) or CD8<sup>+</sup> T cells were injected into nude rats (which lack T cells of their own) after they had received a hamster heart xenograft. CD4<sup>+</sup> T cells mediated production of IgG2a and IgG2b and a low quantity of IgG1 and IgG2c and IgM. Mononuclear cells infiltrated the graft. The CD4<sup>+</sup> T cell-mediated rejection was histologically characterized by oedema in the interstitium, haemorrhage, necrosis in the myocardium and thrombi in the blood vessels. CD8<sup>+</sup> T cells, considered cytotoxic T cells (CTLs), mediated rejection without production of xenoreactive antibodies. The grafts of these rats were infiltrated by mononuclear cells (some were CD8<sup>+</sup>) and the myocardium damaged. Lin et al. (1999) suggest that the rejection was mediated by CTLs that can “activate” themselves the absence of CD4<sup>+</sup> helper T cells. CTLs kill foreign cells by two ways: the perforin pathway and the CD95 pathway. In the perforin pathway the CTL secretes the proteins perforins and granzymes, which induces apoptosis of the foreign cell. In the other pathway binding of CD95L (CD178) to CD95 on the foreign cell also induces apoptosis.

Human NK cells damage porcine endothelial cells by direct cell lysis and indirect lysis by antibody-dependent cell-mediated cytotoxicity (ADCC) (Baumann et al., 2004). In ADCC cells such as NK cells, monocytes and neutrophils bind to foreign cells via antibodies and kill the cells. The mechanism is not completely understood. NK cells kill foreign cells by inducing apoptosis both via the intrinsic pathway (by perforins, granulysin and NK lysin) and the extrinsic pathway (mediated via binding of CD95L to CD95). As proved by Baumann et al. (2004) direct NK cell lysis is independent on binding to  $\alpha$ -Gal, whereas ADCC activity is reduced in the absence of  $\alpha$ -Gal. Besides binding to antigens NK cell activity is influenced by antibodies and cytokines. Xenoreactive IgG antibodies evoke a weak NK cell lysis on porcine endothelial cells, whereas cytokines (such as interleukin-2) provoke a stronger response (Itescu et al., 1998). However Itescu et al. (1998) suggest that the response provoked by xenoreactive IgG antibodies are more significant biologically.

Lin et al. (1999) further studied the influence of xenoreactive antibodies on ADCC mediated by unspecified cells. In their study naive nude rats were transplanted with hamster hearts and their complement system inhibited. They were then injected with serum containing xenoreactive IgG antibodies. Xenografts were rejected within 3.5 days (faster than for the control group). When the NK cells of the recipient were inhibited the grafts survived longer. The authors' conclusion was that IgG xenoantibodies mediate xenograft rejection by activating complement and by causing ADCC.

In addition Hisashi et al. (2008) found infiltration of mainly polymorphonuclear cells, some mononuclear cells and macrophages in lately rejected porcine cardiac xenografts (from GT-KO swine transplanted into baboons). The mononuclear cells however seemed not to have damaged the myocardium. In the more swiftly rejected grafts, in consistence with the studies mentioned above, mononuclear cells were identified. These were macrophages, B cells, CD4<sup>+</sup> T cells, CTLs and some NK cells. The authors suggest that both ACXR and AHXR might be mediated by T cells and thus be T cell-dependent.

The advances within xenotransplantation research that has found modes to overcome HAR opens up for ACXR to be the next obstacle to defeat (Hisashi et al., 2008).

### **Chronic rejection and vasculopathy**

Chronic xenograft rejection occurs 78-179 days post-transplantation (Hisashi et al., 2008). In the xenograft endothelialitis, a thicker arterial intima and arterial fibrinoid necrosis develop. The arteries occlude due to thickening of smooth muscle cells. Smaller arteries are worse afflicted. Hisashi et al. (2008) identified four types of chronic xenograft vasculopathy, presented in Table 1. They suggest that fully developed vasculopathy is the result of chronic humoral and chronic cellular rejection-associated vasculopathy, since fibrosis was usually preceded in the arteries by infiltration of cells and deposition of fibrins. The mechanisms by which chronic rejection and vasculopathy are mediated is at present not completely understood. However Hisashi et al. (2008) argue that both cellular and humoral responses are involved and that chronic humoral rejection is the dominant one.

*Table 1. The four types of vasculopathy identified by Hisashi et al. (2008).*

<b>Type of vasculopathy</b>	<b>Appearance</b>
Chronic humoral rejection-associated vasculopathy	<ul style="list-style-type: none"> <li>▪ Thickening of the intima of arteries</li> <li>▪ TUNEL<sup>+</sup> cells</li> <li>▪ Deposition of fibrin, IgM, IgG, C3,C4d and C5b-C9)</li> </ul>
Chronic cellular rejection-associated vasculopathy	<ul style="list-style-type: none"> <li>▪ Presence of T cells, CTLs, macrophages and B-cells in the intima</li> <li>▪ Endothelialitis</li> <li>▪ TUNEL<sup>+</sup> cells</li> </ul>
Combination of chronic humoral and cellular rejection-associated vasculopathy	<ul style="list-style-type: none"> <li>▪ Fibrinoid deposition</li> <li>▪ Infiltration of antibodies, complement, T cells, macrophages and polymorphnuclear leukocytes in the intima</li> </ul>
Fully developed vasculopathy	<ul style="list-style-type: none"> <li>▪ Fibrosis in the intima</li> <li>▪ No cellular infiltration or fibrinoid material present</li> </ul>

## **DISCUSSION**

The pathogenesis of HAR seems to be elucidated. Since depositions of C3, C4, C5 and C9 with MAC were found along the blood vessels of xenografts (Dalmaso et al., 1992) the simple conclusion is that natural xenoreactive antibodies activate the classical complement

pathway, resulting in MAC formation and osmotic lysis of xenogeneic cells. The finding that removal of antibodies in the blood abolished the rejection Dalmasso et al. (1992) further proves that antibodies initiate HAR. In HAR the main target of the antibodies or cells is the xenogeneic vascular endothelium (Dalmasso et al., 1992). This also seem to be the case for AXCR (Khalfoun et al., 2000), AHXR and chronic rejection (Hisashi et al., 2008).

The pathogenesis of AHXR is probably similar to that of HAR, because the depositions of antibodies and complement factors found in blood vessels of AHXR rejected xenografts (Chen et al., 2004; Hisashi et al., 2008) were similar to those found in HAR rejected xenografts (Hisashi et al., 2008). It is very probable that the vascular damage is mediated by the depositions since Hisashi et al. (2008) found that the amount of microthrombi was most extensive at the site of most dense deposition. The occurrence of thrombi in the xenograft's blood vessels as well as haemorrhage in the interstitium are other similarities between HAR and AHXR (Hisashi et al., 2008), which also indicate that their pathogenesises are alike.

The reason that McCurry et al. (1997) failed to find a broadened specificity for IgM antibodies in immune sera compared to the preimmune sera can be due to low sensitivity of the method. The authors suggest that the natural IgM antibodies blocked the antigen binding sites for any IgM with a new specificity, for example by steric hindrance. The antigen binding sites for any IgM with a new specificity (in the immune sera) would then have to be close to the binding sites for natural IgM. Then the natural IgM (which are large molecules) could then physically block the epitopes recognized by an IgM with a new specificity.

ACXR is mainly mediated by NK cells (Itescu et al., 1998; Khalfoun et al., 2000), CD4<sup>+</sup> and CD8<sup>+</sup> T cells (Lin et al., 1999). The histological image of CD4<sup>+</sup> T cell mediated ACXR (Lin et al., 1999) is similar to that of HAR (Dalmasso et al., 1992) and AHXR (Hisashi et al., 2008) (interstitial haemorrhage and vascular thrombosis) with myocardial necrosis as the new feature (Lin et al., 1999). However immune cells mediate the pathogenesis. The CD4<sup>+</sup> T cells are presumably at least partly T<sub>H-2</sub> cells, since production of subclasses of IgG and a little IgM was observed (Lin et al., 1999). These subclasses are likely produced by host B cells situated in the graft (also found in tissue by Hisashi et al. (2008)) after co-stimulation from T<sub>H-2</sub> cells. Lin et al. (1999) showed that CTLs could activate themselves in the absence of T<sub>H-1</sub> co-stimulation and mediate rejection. However in the mammalian body T<sub>H-1</sub> are commonly present. For that reason in the living body CTLs may not have the need to activate themselves when there are T<sub>H-1</sub> cells present that can activate them. For that reason some of the CD4<sup>+</sup> T cells can also be T<sub>H-1</sub> cells. Removal of NK cells from the recipient's blood resulted in longer graft survival (Lin et al., 1999), indicating that NK cells participate in ADCC-caused xenograft damage.

There is no consensus regarding the main celltypes that are infiltrating the xenograft. Hisashi et al. (2008) found polymorphonuclear cells (the mononuclear cells they found did not seem to damage the myocytes), while Khalfoun et al. (2000) and Itescu et al. (1998) found mainly T cells and NK cells (both mononuclear cells). The scientists used different techniques for their experiments. Itescu et al. (1998) used infant baboons and newborn piglets. Khalfoun et al. (2000) used human cells (not baboons' as the other two) on porcine kidneys, while Hisashi et al. (2008) used cardiac xenografts from GalT-KO swines transplanted to baboons. Newborn baboons have a different immune system (where all parts are not developed yet) than adult baboons. Their reaction to a xenograft for that reason probably differs. Human cells are possibly not identical to those of baboons. Perhaps  $\alpha$ -Gal has a role in influencing the cell

type, since the results of Hisashi et al. (2008), that used organs from GalT-KO, differed from the other two.

A key factor in all the reactions, except chronic rejection, is  $\alpha$ -Gal. It is the major antigen involved in HAR (Chen et al., 1999), but it is also involved in AHXR (McCurry et al., 1997). However unlike HAR (which is prevented in the absence of  $\alpha$ -Gal (Itescu et al., 1998)), AHXR still occurs (Hisashi et al., 2008). I have not found any article describing the histological image of  $\alpha$ -Gal-mediated AHXR, so I cannot compare if  $\alpha$ -Gal-dependent and  $\alpha$ -Gal-independent AHXR differ. Also ACXR is influenced by  $\alpha$ -Gal, since the absence of  $\alpha$ -Gal reduces ADCC (Baumann et al., 2004).

I have found only little information about chronic xenograft rejection, probably because the mechanism is not completely understood (Hisashi et al., 2008). In chronological order it is the final rejection mechanism, perhaps making it a low priority to research (the other rejections, except HAR, (Itescu et al., 1998) are still obstacles that needs to be overcome). Chronic xenograft rejection perhaps is similar to chronic allograft rejection. Allotransplantation is transplantation of an organ between individuals of the same species. Allograft vasculopathy is characterized by occlusion of the arteries due to smooth muscle cell proliferation, which leads to ischemic damage of the graft. The result of the ischemic damage is graft fibrosis (influenced by interleukin-13, transforming growth factor- $\beta$  and fibroblast growth factor), a reaction called chronic allograft rejection. Also chronic xenograft rejection shows arterial occlusion with thickening of smooth muscle cells (Hisashi et al., 2008), but no graft fibrosis. Yet allograft and xenograft chronic rejection might be mediated by similar immune reactions. The lack of xenograft fibrosis might be due to extraction of the graft before long lasting damage (ie. to which fibrosis is a normal consequence) occurs.

Although I have divided xenograft rejection into different parts according to Hisashi et al. (2008) it is important to realize that in reality the mechanisms may co-operate. For example both HAR (Chen et al., 1999) and AHXR (McCurry et al., 1997) are caused by xenoreactive antibodies. The difference between these reactions according to definition is that HAR is only caused by natural antibodies (Chen et al., 1999), whereas AHXR also can be mediated by antibodies formed post-transplantation (McCurry et al., 1997). In reality the borderline between the two reactions are thin. How are we to know if only natural antibodies or both natural antibodies and antibodies formed post-transplantation contribute to xenograft rejection?

Ethical considerations are another obstacle to clinical xenotransplantation. Since they are not immunological aspects they will only briefly be discussed here. Xenotransplantation is an advantage for the human recipient, who is saved from dying of end-stage diseases (Cooper et al., 2000). Religious opposition can be due to considering that humans should protect all animals (Buddhism). However Judaism and Islam may accept xenotransplantation as a way of saving lives (Cooper et al., 2000). Some people deem xenotransplantation unacceptable, meaning that it disobeys the laws of nature. Another anxiety is the well-being of the pigs used. They are likely going to be bred separately from other pigs (though they probably will live in groups) in isolated areas (preventing them from being infected by infectious agents) (Cooper et al., 2000).

I do not intend to judge the ethical aspects, only clarifying them. In the end it will be up to every candidate for xenotransplantation to decide if he or she wishes to live with a porcine organ. If xenotransplantation becomes a reality it is possible that religious leaders around the

world will decide their religion's standpoint. For that reason it is now hard to know how different religions will react to xenotransplantation. The well-being of the pigs will probably be regulated by laws, but these may be different in different countries. This is how it is now for the laboratory animals. Precisely as for the situation of laboratory animals I do not think it is an issue that can be solved easily. Another ethical point I can see regarding the initial stages of clinical transplantations is the uncertainty of how long the graft will function. Even if researchers demonstrate long survival in baboons, we do not know for sure that a graft will function similarly in humans. The conclusion is that even if we can start transplanting we will not know the extent of success and the extent of survival until perhaps twenty years into the future (depending on the age of the recipients). That raises another ethical issue, if one graft fails after ten years, is it then okay to transplant another? How many pigs can be used per human?

Another obstacle to xenotransplantation is the risk of transfer of microbiological agents, especially porcine endogenous retroviruses and exogenous viruses such as cytomegalovirus, from pigs to humans (Cooper, 2003). One step to prevent transfer of cytomegalovirus is to isolate and wean newborn piglets from their infected parents at an early age. Although not studied this method may also be used to prevent infections from other viruses. One risk I see is the risk for undetected viruses. I hardly believe that mankind has as of now detected all porcine viruses (especially those viruses that do not cause disease in the pigs themselves). For that reason we cannot prevent the donor pigs from being infected if we do not know of the existence of the viruses. A further threat is the ability of viruses to mutate. Even if the porcine virus itself does not cause disease in humans it may combine with a human virus. This mutated virus may be virulent and pose a threat to the health of not only the recipient but to other humans that the recipient is in contact with.

In conclusion, although major research about xenotransplantation has been conducted in recent years and progress has been made, a lot of research remains to be conducted before the dream of clinical xenotransplantation as treatment for end-stage diseases can be realised.

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