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IS SENSITIZATION TO PIG ANTIGENS DETRIMENTAL TO SUBSEQUENT ALLOTRANSPLANTATION?

Qi Li^{1,2}, Hidetaka Hara¹, Zhongqiang Zhang^{3,4}, Michael E. Breimer⁵, Yi Wang^{2,*}, and David K.C. Cooper^{1,*}

¹Xenotransplantation Program, Department of Surgery, University of Alabama at Birmingham, Birmingham AL, USA

²Second Affiliated Hospital, University of South China, Hengyang City, Hunan, China

³Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, USA

⁴Department of Organ Transplantation and General Surgery, Second Xiangya Hospital of Central South University, Changsha, Hunan, China

⁵Department of Surgery, Institute of Clinical Sciences, Sahlgrenska Academy at University of Gothenburg, Sweden

Abstract

An important question in xenotransplantation is whether an allotransplant can safely be carried out in a patient who has become sensitized to a pig xenograft. To answer this question, we have searched the literature. We primarily limited our review to the clinically-relevant pig-to nonhuman primate (NHP) model, and found five studies that explored this topic. No NHP that had received a pig graft developed antibodies to alloantigens, and in vitro studies indicated no increased humoral and/or cellular alloreactivity. We carried out a small in vitro study ourselves that confirmed this conclusion. There have been three experiments in which patients undergoing dialysis were exposed to wild-type pig kidneys, and three clinical studies related to bridging a patient in hepatic failure to liver allotransplantation. Despite the development of anti-pig antibodies, all subsequent organ (kidney or liver) allografts were successful (except possibly in one case). In addition, pig fetal islets were transplanted into patients with kidney allografts; there was no increase in panel-reactive allo-antibodies and the kidney grafts continued to function satisfactorily. In conclusion, the limited data suggest that, after sensitization to pig antigens, there is no evidence of antibody-mediated or accelerated cellular rejection of a subsequent allograft.

Address for correspondence: David K.C. Cooper MD, PhD, Xenotransplantation Program, University of Alabama at Birmingham (UAB), ZRB 701, 1720 2nd Avenue South, Birmingham, AL 35294-0007, USA, Tel: 205-996-7772; Fax: 205-934-8344, dlkcooper@uabmc.edu.

*Joint senior authors

MISS QI LI (Orcid ID : 0000-0002-7835-5886)

DR. ZHONGQIANG ZHANG (Orcid ID : 0000-0002-4410-4675)

PROF. MICHAEL E BREIMER (Orcid ID : 0000-0003-4279-0736)

DR. DAVID K.C. COOPER (Orcid ID : 0000-0002-8899-9431)

Conflict of interest

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Keywords

Antibodies, cross-reacting; Clinical trial; Immune response, human; Nonhuman primate; Pig; Sensitization; Xenotransplantation

Introduction

There is a continuing shortage of organs from deceased human donors for the purpose of transplantation into patients with end-stage organ failure (1). Xenotransplantation could provide an alternative source of organs. Recently, there has been substantial progress in overcoming the barriers to xenotransplantation, especially through the transplantation of organs from genetically-engineered pigs combined with effective immunosuppressive therapy (2).

Initial patients might receive a pig graft as a 'bridge' to maintain life until a suitable allograft becomes available. Others might require allotransplantation in the event that a xenograft fails. If a pig xenograft were to fail, and a suitable allograft became available, could the patient undergo allotransplantation without a detrimental effect from the previous xenotransplant? In other words, do elicited xenoreactive antibodies cross-react with alloantigens? This important question has not yet been definitively answered. We have searched the literature, and identified a small number of studies of relevance.

We primarily limited our search to studies directly relevant to pig organ or cell transplantation in humans, which largely related to studies in pig-to-nonhuman primate (NHP) (discordant) models. However, some other experimental studies, including those relating to xenotransplantation between closely-related NHP (concordant) models, will be briefly summarized. We also searched for experience in clinical xenotransplantation.

Experimental secondary allotransplantation after xenosensitization in discordant models

'Discordant' relates to models in which transplantation is carried out between widely-disparate species where hyperacute rejection usually results, e.g., wild-type (WT, i.e., genetically-unmodified) pig-to-NHP (3). We identified only five relevant reports (Table 1). Within these reports were three in which in vitro studies were carried out after in vivo exposure to pig antigens, and three in which both in vitro studies and additional allotransplantation were carried out. In addition, however, we have carried out a new in vitro study which we report below

Ye et al (4) were the first (in 1995) to investigate this topic. In immunosuppressed baboons that were sensitized by WT pig heart transplants (n=2, with the grafts undergoing hyperacute rejection) or by pig erythrocytes (n=1), subsequent baboon liver allografts survived without evidence of antibody-mediated or accelerated cellular rejection. No baboon developed antibodies that cross-reacted with alloantigens.

This group also tested sera from baboons that had rejected a WT pig liver (n=2) or heart (n=1) graft against a panel of baboon lymphocytes (n=6). No cytotoxicity of the baboon lymphocytes was documented, supporting a conclusion that sensitization to pig antigens did not result in allo-sensitization.

In 2004, Baertschiger et al (5) carried out in vitro studies using baboon serum and peripheral blood mononuclear cells (PBMC) exposed to WT pig antigens. Serum and PBMCs from four groups of baboons were studied: (i) naïve baboons (n=4); (ii) baboons sensitized to galactose- α 1,3-galactose (Gal) antigens (n=2) by prior in vivo exposure to a pig heart or pig red blood cells; (iii) baboons sensitized to Gal+nonGal antigens (n=2) by prior in vivo exposure to a pig heart or pig PBMC; and (iv) baboons sensitized to alloantigens (n=2). In an antibody assay, baboon serum containing anti-pig xenoantibodies was cultured with baboon or pig PBMCs. There was no cross-reactivity between xenoantibodies and alloantigens. A complement-dependent cytotoxicity assay indicated no killing of baboon PBMC, and mixed lymphocyte reaction suggested no increased T cell proliferative response to alloantigens. Although the study was limited, it produced no evidence that a previous pig xenograft would be detrimental to a secondary allograft.

Key et al (6) studied 52 conventionally-immunosuppressed cynomolgus monkeys that had received pig kidneys from hCD55 (human decay-accelerating factor; hDAF) transgenic pigs to investigate whether anti-swine leukocyte antigen (SLA) antibodies cross-reacted with human leukocyte antigens (HLA). Graft survival was for a mean of 20 days (range 1–53 days). Pre-transplant and post-transplant serum from each monkey was incubated with “pooled, purified” HLA. There was no detectable increase in anti-HLA IgG antibodies after pig kidney transplantation.

In a WT pig-to-Chinese rhesus monkey decellularized corneal xenotransplantation model, porcine corneal lamellar (anterior partial thickness) xenografts were followed by full-thickness corneal allografts (n=5) (7). All recipients received immunosuppressive therapy (topical prednisolone acetate, subconjunctival dexamethasone, systemic methylprednisolone). Only one of five pig corneal grafts was rejected, with four grafts surviving for 7–13 months, at which time the monkey received an allograft. On in vitro assays, there was no evidence that any humoral or cellular immune response to the xenograft adversely affected the survival of the allograft. However, the fact that 4 of the 5 xenografts were not rejected suggests that no xenoreactive antibodies developed, thus reducing the likelihood of an immune response to the allograft. Furthermore, there are differences in the mechanism of rejection between an organ and a cornea.

In a pig-to-baboon skin xenotransplantation model, Albritton et al (8) studied four groups: (i) a primary baboon skin allograft (survival for 12–13 days) followed by a secondary skin graft from an α 1,3-galactosyltransferase gene-knockout (GTKO) pig (survival for 10–13 days); (ii) a primary GTKO pig skin xenograft (survival for 11–13 days) followed by a secondary baboon skin allograft (survival for 10–14 days); (iii) a primary GTKO pig xenograft (survival for 7–11 days) followed by a secondary GTKO pig xenograft (survival for 1 day); and (iv) a primary allograft (survival for 7–11 days) followed by a secondary allograft (survival for 4 days). These results suggested that primary allograft or xenograft

rejection did not accelerate the rejection of, respectively, a subsequent xenograft or allograft. However, initial sensitization to xenoantigens or alloantigens accelerated rejection of, respectively, a subsequent xenograft or allograft. A primary skin allograft was associated with the production of anti-allogeneic antibody, but not anti-xenogeneic antibody, and a primary xenograft did not induce antibodies directed to an allograft. In the highly-immunogenic skin transplant model, therefore, there was no cross-reactivity between xenoantibodies and alloantigens or between alloantibodies and xenoantigens.

In order to add to the experience on this topic, we have carried out a further in vitro experiment. Flow cytometry of serum IgM and IgG binding to CD3⁺T cells (gated from PBMC) from either a GTKO/CD46 pig or a baboon was carried out. Serum was taken from (i) naïve baboons (n=8), (ii) baboons exposed to pig antigens (in the form of an organs or artery patch graft) that had not become sensitized (n=4), and (iii) baboons exposed to pig antigens that had become sensitized (n=4). Although there was minimal antibody binding of naïve and nonsensitized sera to GTKO/CD46 pig PBMCs, there was significant binding of sensitized serum (both IgM and IgG) to these cells ($p<0.05$) (Figure 1). In contrast, there was no significant binding of any baboon serum to baboon PBMC. This small study strengthens the conclusion that prior sensitization to a pig xenograft would not be detrimental to a subsequent allograft.

Additional study of relevance

Recently (2016), Kim et al (9) transplanted pig islets into mice that had previously been sensitized to either pig or mouse antigens. Survival of islet *allografts* in naïve mice (15.5+/-2.38 days) was no different from that of islet allografts in mice previously sensitized to pig antigens (14.4+/-1.41 days). Furthermore, there was no difference in survival of pig islets in naïve mice (5.8+/-2.04 days) or in allo-sensitized mice (6.4+/-2.26 days). In vitro assays indicated that (i) xeno-sensitized mice did not induce anti-allogeneic antibody, and there was (ii) no cross-reactivity between xenoantibody and alloantigens, and (iii) no accelerated cellular response to a subsequent allotransplant.

Experimental secondary allotransplantation after xenosensitization in concordant models

Although today clinical concordant xenotransplantation, i.e., between closely-related species where hyperacute rejection would not be anticipated, e.g., NHP-to-human (3), is not being considered, there have been a few experimental studies of secondary allotransplantation in a NHP after an initial concordant xenograft that are of interest. Given the closer evolutionary relationship between the initial donor and recipient species, with the likelihood of more conserved antigen structure, it might be anticipated that there would be greater cross-reactivity of antibodies that develop after rejection of a concordant xenograft.

However, three groups provided evidence to suggest that primary concordant xenografts in immunosuppressed NHPs did *not* induce a humoral or an accelerated cell-mediated immune response that jeopardized the survival of a secondary allograft (Table 2) (4,10,11).

Additional studies of relevance

There have also been studies in other animal models that provide further evidence. The groups of Gannedahl et al (12), Chice et al (13) and Di Stefano et al (14), respectively using initial mouse-to-Lewis rat, hamster-to Lewis-rat, and hamster-to Lewis-rat models followed by allotransplantation, independently concluded that an initial xenograft was not detrimental to a subsequent allograft.

In contrast, two groups provided data to suggest that secondary allografts are at risk after transplantation into a recipient previously sensitized to a xenograft, though these studies were not in the clinically more relevant pig-to-NHP model.

Hammer et al (15) carried out allogeneic and xenogeneic heterotopic heart transplantation. Six dog recipients accepted primary allogeneic hearts (with immunosuppressive therapy in the form of cyclosporine, azathioprine, and corticosteroids) with a mean allograft survival of 18 days. Heart xenotransplants between donor foxes and recipient dogs (under identical immunosuppressive therapy) were rejected in a mean of 10 days, and subsequent dog allograft hearts (under the same immunosuppressive regimen) were rejected in a mean of 5 days. Therefore, rejection of the concordant xenograft reduced survival of a subsequent allograft (from a mean of 18 days to 5 days).

In addition, Etheredge et al (16) reported accelerated skin allograft rejection following xenogeneic sensitization in dog- (a distantly-related species) and guinea pig- (a closely related species) to-rabbit models. Five rabbit recipients accepted a primary allogeneic skin graft with a mean survival of 10 days. Fifteen rabbits received an allograft after being sensitized to a guinea pig skin xenograft, with rejection occurring in a mean of 7 days. Eighteen rabbits received an allograft following a dog skin xenograft, with rejection in a mean of 7 days. It would therefore appear that, in these models, an initial skin xenograft (whether discordant or concordant) was detrimental to survival of a subsequent skin allograft.

Clinical allotransplantation after (or before) exposure to pig antigens

Six clinical studies are of relevance. Three patients undergoing dialysis were exposed to pig kidneys that were inserted into the dialysis circuit, and there were three studies related to bridging a patient in hepatic failure to liver allotransplantation. In addition, there was one study in which patients with renal allografts were subsequently sensitized to pig antigens.

Kidney allotransplantation after exposure to pig antigens (Table 3A)

After a course of plasmapheresis to remove anti-pig antibodies, Welsh and his colleagues exposed a patient undergoing dialysis to WT pig kidneys on two occasions approximately one month apart (17,18). The patient received conventional immunosuppressive therapy to cover the “experimental period”. The two pig kidneys were perfused for 6 and 1.5 hours, respectively. There was only a weak anti-pig immune response. The patient successfully underwent renal allotransplantation.

In 1996, Breimer, Rydberg, and colleagues (19,20,21) reported extracorporeal blood perfusion of WT pig kidneys (after plasmapheresis but in the absence of any immunosuppressive therapy) in two patients undergoing dialysis. Patient 1 (HLA-sensitized with panel-reactive antibodies of 85%) was exposed to the pig kidney for 65 minutes (before hyperacute rejection occurred). The patient developed a high level of anti-pig antibodies (20,21), but there was no change in anti-HLA antibodies (panel-reactive antibodies remaining at 85%) (20). Three years later, the patient received a cadaveric renal allograft (with a negative cytotoxic crossmatch). Graft function was excellent for two years when, unexpectedly, function rapidly declined (for unclear reasons). Microscopy showed thrombotic microangiopathy, but chronic antibody-mediated rejection could not be excluded.

Patient 2 (HLA-non-sensitized) was exposed to a WT pig kidney for 15 minutes, when he developed anaphylaxis, necessitating discontinuation of the perfusion. He recovered quickly, and developed a weak anti-pig antibody response (20,21). No anti-HLA antibodies developed. Four years later, he received a cadaveric renal allograft, which had to be removed 18 days later for persistent bleeding associated with thrombocytopenia which had been problematic for years, even before exposure to the pig kidney (19). It therefore did not appear to be a complication of sensitization to the pig kidney. Microscopy showed features of cellular and vascular rejection.

Based on this, it can be concluded that the perfusion experiments were not detrimental to the patients in obtaining subsequent renal allografts, though there may be some doubt in the second patient. It should be borne in mind that a large number of passenger leukocytes are transferred from the organ to the recipient during *ex vivo* organ perfusion (22), increasing the risk of immunization to pig antigens.

Liver allotransplantation after exposure to pig antigens (Table 3A)

In 1994, Chari et al (23) reported successful liver allotransplantation in a patient who had undergone *ex vivo* perfusion of five pig livers during the previous few days, though no details were given on the anti-pig or anti-HLA antibody responses.

Baquerizo et al (24) provided data in 1999 from eight patients bridged by a bioartificial liver (BAL), that incorporated pig hepatocytes, who subsequently underwent successful liver allotransplantation. When BAL treatment was performed only once, there was no increase in anti-pig antibody. After two or more BAL treatments, however, there was a significant increase in anti-Gal IgG antibody, though no antibodies developed to nonGal pig specificities. Sensitization to pig antigens appeared to have no detrimental effect on the outcome of the subsequent liver allografts.

In 2000, Levy et al (25) reported bridging of two patients to successful liver allotransplantation by *ex vivo* extracorporeal blood perfusion through livers from pigs transgenic for the human complement-regulatory proteins CD55 and CD59. The periods of perfusion were only 6.5 and 10 hours, respectively, because deceased human donor livers became available. Nevertheless, despite treatment of the recipient with tacrolimus-based immunosuppressive therapy, the level of anti-Gal antibody initially markedly increased (IgM

10-fold, IgG 25-fold). At 60 days after the transplant, IgG remained 20% higher than the pre-transplantation level. However, no anti-HLA antibodies developed.

Exposure to pig antigens, or at the time of, kidney allotransplantation (Table 3B)

There is one other clinical study of relevance. Groth and coworkers (26) reported on ten patients with Type 1 diabetes with long-standing renal allografts who received WT fetal pig islet-like cell clusters intraportally or under the kidney capsule (Table 3B). All patients developed xenoreactive antibodies against Gal antigens, which remained high for up to 6–8 years (27), but there was no increase in panel-reactive antibodies, and the kidney grafts continued to function well (28).

Conclusions

The data from discordant experimental pig-to-NHP models of xenotransplantation indicated that a primary xenograft did not induce a humoral or accelerated cell-mediated immune response that jeopardized the survival of a secondary allograft. The clinical experience, though also very limited, supports this conclusion.

Patients with a high level of anti-HLA antibodies (calculated panel-reactive antibodies) often wait many years before an organ from a deceased human donor becomes available. If there are no antibodies that cross-react between HLA and SLA, pig xenotransplantation would alleviate this problem. Several groups have investigated whether highly-allosensitized human serum cross-reacts with SLA (reviewed in 29) (Table 4). Some groups have concluded that human anti-HLA antibodies can cross-react with SLA and thus jeopardize the survival of a pig graft (30,31,32). Several other groups, however, have found no evidence of cross-reactivity between antibodies directed to HLA and SLA (Table 4).

If, indeed, HLA-specific antibodies do recognize SLA, then the question needs to be asked as to why no groups have reported a detrimental effect of initial exposure to SLA on the outcome of subsequent allotransplantation, as reviewed in the present report. HLA and SLA genes encode proteins on the cell surface (antigens), but the HLA genes are at least 100 kbp longer than SLA, and are greater than SLA in number (33). As the HLA system is more complex than the SLA system, the greater number and complexity of anti-HLA antibodies might result in their recognition of SLA. In contrast, anti-SLA antibodies may be insufficient in variety and number to recognize HLA. This hypothesis requires investigation.

The results of our review must be interpreted cautiously as not only are the numbers of reports very few, and in some cases almost anecdotal, but exposure to pig antigens was at times relatively brief. There were also varying periods between developing sensitization to pig antigens and subsequent exposure to alloantigens. With initial clinical trials of pig organ transplantation drawing closer, more data from the important pig-to-NHP model are required to allow a deeper understanding of the topic.

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Abbreviations

BAL	bioartificial liver
Gal	galactose- α 1,3-galactose
GTKO	α 1,3-galactosyltransferase gene-knockout
HLA	human leukocyte antigens
NHP	nonhuman primate
SLA	swine leukocyte antigens
WT	wild-type, i.e., genetically-unmodified

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Figure 1.

Flow cytometry of serum IgM (top) and IgG (bottom) binding to CD3⁺T cells (gated from PBMC) from (A) a GTKO/CD46 pig, and (B) a panel of cells from 4 baboons, using naïve baboon serum (n=8), nonsensitized baboon serum (n=4), and sensitized baboon serum (n=4). Although the sensitized baboon sera showed high IgM and IgG antibody binding to GTKO/CD46 pig PBMC, there was no increased binding to any of the panel of 4 baboon PBMC. (NS=not significant; *P<0.05).

Table 1

Secondary allotransplantation after xeno-sensitization in discordant NHP models

Author	Year	Recipient (n)	Primary xenograft donor	Primary organ/tissue /cell	Graft survival	Secondary allograft donor (n)	Secondary organ/tissue /cell	Graft survival	REF.
Ye et al	1995	Baboon (n=3)	Pig	Heart, Blood	20 min	Baboon (n=3)	Liver	6>62 days	4
Ye et al	1995	Baboon (n=3)	Pig	Liver, Heart	1h–6 days	-	-	-	4
Baertschiger et al	2004	Baboon (n=4)	Pig	Heart, RBCs, PBMCs	-	-	-	-	5
Key et al	2004	Cynomolgus monkey (n=52)	Pig	Kidney	1–53 days	-	-	-	6
Choi et al	2014	Rhesus monkey (n=5)	Pig	Cornea	49 days	Rhesus monkey (n=5)	Cornea	35>324 days	7
Albritton et al	2014	Baboon (n= 4)	Pig	Skin	11–13 days	Baboon (n=4)	Skin	10–14 days	8

Table 2

Secondary allotransplantation after xeno-sensitization in concordant NHP models

Author	Year	Recipient (n)	Primary xenograft donor	Primary organ/tissue /cell	Graft survival	Secondary allograft donor (n)	Secondary organ/tissue /cell	Graft survival	REF.
Alonso de Begona et al ^(a)	1992	Baboon (n=5)	African green monkey	Heart	5–65 days	Baboon (n=5)	Heart	10 198 days	10
Ye et al ^(b)	1995	Baboon (n=6)	African green monkey	Liver	10–120 days	Baboon (n=1)	Heart	>30 days	4
Michler et al ^(c)	1996	Baboon (n=4)	Cynomolgus monkey	Heart	>14 days	Baboon (n=4)	Heart	>56 days	11

^(a)Two baboons with allografts survived for 164 and 198 days (until they were euthanized) without evidence of rejection.

^(b)The baboon in which the xenograft survived 120 days received a secondary baboon heart allotransplant, which was followed for >30 days without features of rejection (until elective euthanasia). At the time of allotransplantation, a lymphocytotoxicity assay carried out with serum from the recipient and cells from the baboon heart donor was negative.

^(c)Despite the fact that, after xenotransplantation, 50% of the baboons developed cytotoxic antibodies against the MHC class II-like antigens expressed by lymphocytes of more than half of a panel of 12 baboons, neither the presence of these antibodies nor the severity of the prior xenograft rejection impacted the histology of allograft rejection. When T cell lines were developed from T cells isolated from xenograft biopsies, none demonstrated cell-mediated proliferative or cytotoxic activity against cells from the secondary allograft donor. These data suggested that a prior concordant xenograft was not detrimental to a subsequent allograft.

Table 3

Clinical allotransplantation after (or before) exposure to pig antigens

A: Clinical allotransplantation after exposure to pig antigens							
Author	Year	Patients (n)	Primary perfusion	Primary tissue	Secondary allograft donor	Secondary organ/tissue /cell	REF.
Welsh et al	1991	1	Pig	Kidney	Human	Kidney	17,18
Breimer et al	1996	2	Pig	Kidney	Human	Kidney	19,20,21
Chari et al	1994	1	Pig	Liver	Human	Liver	23
Baquerizo et al	1999	8	Pig	Liver	Human	Liver	24
Levy et al	2000	2	Pig	Liver	Human	Liver	25

B: Exposure to pig antigens after clinical allotransplantation							
Author	Year	Patients (n)	Primary allograft donor	Primary organ	Secondary xenograft donor	Secondary organ/tissue /cell	REF.
Groth et al	1994	10	Human	Kidney	Pig	Islet	26

Table 4

Studies relating to cross-reactivity between the anti-HLA immune response and pig antigens to investigate whether HLA sensitization is detrimental to pig xenotransplantation

<u>HLA sensitization is detrimental</u>	
Author/Year	REF.
Naziruddin et al, 1998	30
Taylor et al, 1998	31
Barreau et al, 2000	35
Popma et al, 2000	36
Mulder et al, 2000	37
Oostingh et al, 2002	38
Varela et al, 2003	39
Mulder et al, 2010	42
Martens et al, 2017	32
<u>HLA sensitization is not detrimental</u>	
Author/Year	REF.
Bartholomew et al, 1997	34
Wong et al, 2006	40
Hara et al, 2006	41
Zhang et al	29