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ORIGINAL ARTICLE

Hyperacute rejection in *ex vivo*-perfused porcine lungs transgenic for human complement regulatory proteins

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Summary

Inhibition of complement activation via human membrane-associated complement regulators is known to prevent hyperacute rejection in heart and kidney pig-to-primate transplantation. The protective effect of such strategies in pulmonary xenografts, however, seems to be insufficient. In an *ex vivo* perfusion, model lungs from donor pigs transgenic for human CD55 ($n = 6$) or human CD59 ($n = 5$) were perfused with fresh human blood and compared with nontransgenic organs ($n = 6$). In addition, a soluble complement component 1 esterase inhibitor (C1-Inh) was applied in h-CD55 transgenic lungs ($n = 3$). In the h-CD55 transgenic group, survival was prolonged ($P < 0.05$), quality and maximal time of oxygenation significantly improved and pulmonary vascular resistance reduced compared with the control group. There was a decreased sequestration of platelets, less parenchymal injury and reduced deposition of C_{5b-9} in the h-CD55 transgenic group. Additional soluble complement inhibition (C1-Inh) did not prolong survival of h-CD55 transgenic lungs. Survival and pulmonary function in lungs expressing h-CD59 was not significantly different from parameters observed in nontransgenic lungs. In this *ex vivo* model of pig-to-primate lung transplantation, membrane-based complement inhibition resulted in significantly improved pulmonary function. However, minor histopathological injuries observed in these transgenic xenografts suggested only partial protection from pulmonary dysfunction by complement inhibition alone.

Introduction

Pig-to-primate xenografts undergo hyperacute rejection (HAR) within minutes after reperfusion. Hyperacute rejection results predominantly from the binding of xenoreactive antibodies to the donor endothelium with subsequent uncontrolled activation of complement, leading to loss of endothelial cell integrity, tissue edema, hemorrhage, fibrin deposition and micro-vascular thrombosis [1–3]. Strategies to prevent HAR in xenotransplantation focused on the deletion of antiporcine antibodies or on the inhibition of activation of complement by various agents [4,5]. Over the past 10 years, several donor pigs transgenic for human complement regulatory proteins (CRP), including CD55 (h-DAF), MCP, CD59, or combinations, have been generated [5]. Both, MCP and CD55 inhibit complement activation at the C3/C5 step. CD59 prevents the complete assembly of the membrane attack complex of complement by blocking the binding of C9 to C_{5b-8} and inhibiting the polymerization of C9.

Hyperacute rejection of such transgenic donor kidneys and hearts in nonhuman primates was found to be prevented and graft survival of up to 3 month has been reported [2,5–7]. However, in pig-to-nonhuman primate

lung transplantation, donor organs from pigs transgenic for CD55/CD59 or CD46 failed to overcome HAR [8–11].

Hyperacute rejection of the lung occurs in a way that makes it unique compared with other solid organs such as the heart or the kidney. The term pulmonary xenograft dysfunction describes the failure of a pulmonary xenograft, which is associated with a marked rise in pulmonary vascular resistance (PVR).

Complement plays a central role in HAR and in inflammatory reactions of the lung [12]. Its contribution to pulmonary xenograft dysfunction, however, has not been fully elucidated. There is a speculation in as far complement inhibition of the lung by membrane-based CRPs is insufficient, or organ-specific factors independent of complement are predominantly responsible for the poor results achieved in pulmonary xenografts [13,14]. In *ex vivo* studies, contradictory results concerning the prevention of HAR in porcine lungs transgenic for human CRPs perfused with human plasma or blood have been reported. Some investigators supported the dominant role of complement in HAR of the lung [11,15,16]. However, in other models, h-CD55 transgenic donor pulmonary xenografts failed to survive [17,18].

In an *ex vivo* perfusion model, we investigated the potential of h-CD55 and h-CD59 transgenic lungs to prevent pulmonary dysfunction and HAR. In addition to membrane-based complement inhibition, we tested the effects of additional soluble complement inhibition by a complement C1 esterase inhibitor (C1-Inh), inhibiting the classical pathway of complement activation by binding to activated C1 (C1r, C1s) [19].

Methods

In an *ex vivo* perfusion model, left porcine lungs were perfused with fresh whole human blood. Lungs from nontransgenic pigs (group I, $n = 6$), lungs from h-CD55 transgenic pigs (group II, $n = 6$), and lungs from h-CD59 transgenic donor pigs (group III, $n = 5$) were evaluated. One of the six lungs in group III had to be excluded because of an early technical failure of the perfusion circuit. In group IV ($n = 3$), 800 units of soluble C1 inhibitor (C1-Inh; Berinert HS, Aventis, Behring, Germany) were added for a concentration of 1 U/ml prior to the onset of perfusion of a h-CD55 transgenic lung.

In a previous study in this model porcine lungs perfused with autologous porcine blood, all survived the observation period of 240 min with a PVR below 0.16 ± 0.4 mmHg/ml/min and preserved oxygenation (Δ $AVpO_2$ 295 ± 77 mmHg at 240 min) and only minimal macroscopic and histopathological injury.

All animals received human care in accordance with the 'Principles of Laboratory Animal Care' formulated by the National Society for Medical Research and the 'Guide for the Care and Use of Laboratory Animals' published by the National Institute of Health (NIH publication No. 86–23, revised 1985), as well as the current version of the German law on the protection of animals. Donor pigs (12–20 kg) did not undergo blood typing tests. Animals transgenic (heterozygous) for the human CRP decay accelerating factor (h-CD55, h-DAF) were generated by Imutran Ltd, Cambridge, United Kingdom, and supplied by Harlan, Correzzana, Italy [20]. The heterozygous h-CD59 transgenic pigs were generated by the Department of Biotechnology, Institute für Tierzucht und Tierverhalten (FAL), Mariensee, Germany [21]. Porcine donor lungs were explanted using a low potassium dextran glucose solution (Perfadex; Virtolife, Upsalla, Sweden, 50 ml/kg) and stored inflated at 4 °C [22]. Donor animals received 300 IE/kg heparin. Cold ischemic time was 1 h, followed by 20 min of warm ischemia for dissection and connection of the left lung to the circuit.

Pooled fresh whole human blood (800 ml) was collected from 2–3 healthy volunteers (blood group A, rhesus positive) 1 h prior to the experiment in blood bags containing 4 IE/ml of heparin. Autologous porcine blood was collected from donor animals prior to aortic clamping. Human blood was not diluted. The activated clotting time (ACT) was adjusted to 400–500 s.

Left lungs were perfused with deoxygenated blood using a roller pump (Ismatec, Bioblock, Strassburg, France) for 240 min at 37 °C. Blood flow was adjusted to the donor animals' weight and kept constant throughout the experiments at 8 ml/kg/min. Pulmonary artery pressure (cm H_2O) was monitored. The pulmonary venous outflow was drained into a reservoir. For de-oxygenation, a mixture of carbon dioxide and nitrogen was applied via a pediatric membrane oxygenator unit (Polystan Safe Micro, Copenhagen, Denmark). The de-oxygenated blood was adjusted for a pO_2 of 40 mmHg and a pCO_2 of 60 mmHg. Lungs were mechanically ventilated with 50% oxygen and 50% nitrogen, a peak airway pressure of 35 cm H_2O, 20 breaths/min and a positive end expiratory pressure (PEEP) of 6 mmHg. Endpoint for perfusion was overwhelming edema ascending into the respirator tubing, preventing mechanical ventilation, in all experiments. Because pulmonary artery pressure was not limited, no case of failure of forward blood flow was observed. An arterio-venous difference in oxygen saturation of <100 was considered to be the endpoint for oxygenation.

Blood samples were collected from the pulmonary artery and the pulmonary venous outflow for standard blood gas analysis. White blood cells, differential blood cell count, red blood cells, and platelets were measured by

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standard hematology techniques. The activated coagulation time (ACT) was measured with the Hemochron device (International Technidyne Corporation, Edison, NJ, USA).

Soluble terminal complement complex (C_{5b-9}) in the plasma was analyzed by an enzyme-immunoassay (SC5b-9 Complex ELISA KIT, A009; Innogenetics, Heiden, Germany). The plasma concentration of C1-inhibitor was measured by using a commercially available kit (Berichrom C1-Inhibitor Kit; Dade-Behring, Marburg, Germany).

IgG and IgM α-Gal antibodies were analyzed by a modified ELISA technique [23]. In brief, Maxisorb microtiter plates (Nunc Roskilde, Denmark) were coated with α-Gal conjugate Galα1–3′ LacNAc (Lectinity, Moscow, Russia) followed by an incubation with serum samples. The binding of IgG and IgM was analyzed by addition of affinity-purified alkaline phosphatase (AP)-conjugated goat antiprimate antibodies, which reacted with a chromogenic substrate containing OPD (Sigma-Aldrich, Deisenhofen, Germany), citric acid, and sodium phosphate.

Biopsies were taken from identical locations of the left upper lobe at 0, 15, 30, 60, 120, 180, and 240 min of perfusion. Lung specimens embedded in Tissue Tek (OCT compound; Miles Inc, Elkhart, IN, USA) were snap-frozen in liquid nitrogen. Paraffin sections of 5 μm were stained with hematotoxyline and eosine (HE), antihuman IgG (1/400, DAKO, Norden, Denmark), antihuman IgM (1/400; DAKO), and anti-C_3 (1/200; DAKO) as primary antirabbit antibodies. Cryosections were stained with anti-C_{5b-9} (1/50; DAKO) using a two-step method (EnVision®, DAKO, Hamburg, Germany). All donor lungs were evaluated for immunhistological expression of h-CD55 and h-CD59. Primary antibodies were a murine antihuman CD59 monoclonal antibody (clone p282; Becton Dickinson, Heidelberg, Germany) and a murine antihuman CD55 monoclonal antibody (Bric 216; Cymbus Biosciences Ltd, Southampton, UK). A standard two-step-indirect staining technique with a peroxidase-labeled goat antimouse secondary antibody was used. On HE-stained sections, interstitial edema, intraparenchymal, and intra-alveolar hemorrhage were evaluated via a semi-quantitative scale (0 = not apparent; 1 = minimal; 2 = moderate, focal; 3 = diffuse, generalized) by a blinded pathologist. Immunohistochemistry sections (C3, C_{5b-9}, human IGM and IgG, h-CD55 and h-CD59) were scored on an identical semi-quantitative scale.

Pulmonary vascular resistance was calculated from the recorded pulmonary artery blood pressure and the flow rate set by the roller pump as follows: PVR (mmHg/ml/min) = PAP (mmHg)/blood flow (ml/min). The arterio-venous oxygen difference (Δ AVO_2) was calculated from the formula: Δ AVO_2 (mlO_2/100 ml blood) = $(1.34 \times Hb \times S_{art}) - (1.34 \times Hb \times S_{ven})$, with S representing the arterial (S_{art}) or venous (S_{ven}) oxygen saturation. Data are presented as mean ± SD. Differences between the measurements and baseline were assessed by the Friedmans ANOVA test followed by the Wilcoxon–Wilcox test. For the statistical comparison of the groups, including the semi-quantitatively evaluated immunohistochemistry, the nonparametric Mann–Withney *U*-test was applied. Graft survival and duration of oxygenation was calculated via Kaplan–Meier analysis. A $P < 0.05$ was considered statistically significant. Statistical analysis was performed with the SPSS (version 12.0) software package (SPSS Inc., Chicago, IL, USA).

Results

Nontransgenic porcine lungs (group I, $n = 6$) were able to oxygenate the deoxygenated human blood for a mean of 55 (30–120) min, compared with h-CD55 transgenic lungs (group II, $n = 6$) with 238 (225–240) min ($P = 0.0004$, Fig. 1). The arterio-venous difference in oxygen partial pressure (Δ $AVpO_2$) was significantly higher in the h-CD55 transgenic group compared with the nontransgenic groups ($P < 0.05$, beginning at 60 min of perfusion, Fig. 2). Correspondingly, the arterio-venous difference in oxygen saturation (Δ AVO_2) was significantly better in h-CD55 transgenic lungs ($P < 0.05$, beginning at 30 min

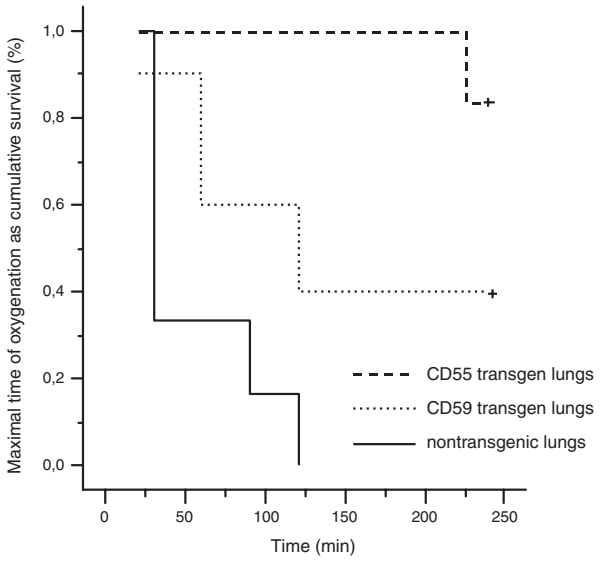

Figure 1 Time of oxygenation was significantly prolonged in CD55 transgenic lungs with 238 min (225–240), compared with controls with 55 min (30–120). Endpoint of oxygenation was defined as an arterio-venous difference in oxygen saturation (Δ AVO_2) of <100 ml/dl in this Kaplan–Meier analysis.

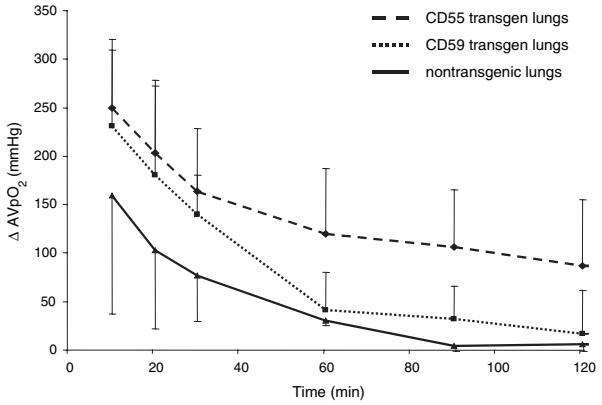

Figure 2 Arterio-venous difference in oxygen partial pressure (Δ $AVpO_2$) of CD55 and CD59 transgenic and nontransgenic lungs perfused with human blood. Data are expressed as means with SD. Arterio-venous difference in pO_2 was significantly higher in the CD55 transgenic group compared with the nontransgenic group from 60–240 min (Mann–Whitney *U*-test).

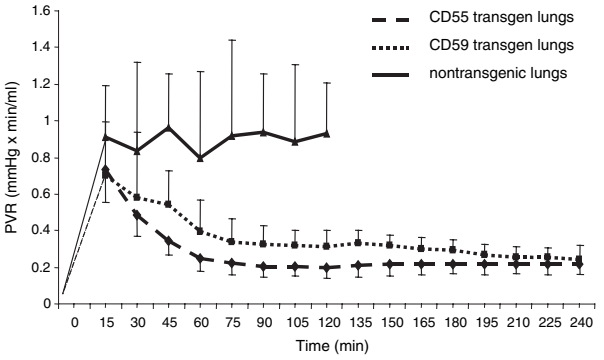

Figure 3 Pulmonary vascular resistance (PVR) was statistically significant ($P < 0.05$) between the h-CD 55 and the nontransgenic group from 30 to 240 min. Data are expressed as means with SD (Mann–Whitney *U*-test). At 120 min, six of six lungs in the h-CD55 group, two of five lungs in the h-CD59 group, and four of six lungs in the nontransgenic group were perfused. Five of six lungs in the CD55 group and two of five lungs in the h-CD59 group reached the end of the observation period at 240 min.

of perfusion). However, with time of perfusion porcine lungs from all groups showed a decline in their effectiveness to oxygenate. In h-CD55 transgenic lungs, the mean Δ $AVpO_2$ declined further from 87 ± 59 (24–157) at 120 min to 47 ± 39 (0–92) mmHg at the end of the observation period. After 60 min of perfusion, the relative change in hematocrite, an indirect marker for pulmonary edema, was 2.2 ± 5.9 % (−5–10) in the CD55 transgenic group in contrast to 16.1 ± 11.6% (6.9–38.9) in the nontransgenic group ($P < 0.005$).

Nontransgenic porcine lungs perfused with human blood all failed after a mean time of perfusion of 110 min (30–150). Lungs transgenic for h-CD55 ($n = 6$) were perfused significantly longer for 238 min (225–240) with only one out of six lungs failing to reach the end of the observation period at 240 min ($P = 0.006$). In all failing lungs, the endpoint requiring termination was a fulminant edema preventing ventilation. In nontransgenic lungs perfused with human blood, PVR rapidly increased to 0.91 ± 0.4 mmHg/ml/min within the first 15 min and remained at a four- to fivefold elevation (Fig. 3), when compared with autologous pig blood perfusion. In contrast, PVR in h-CD55 transgenic lungs peaked at 0.73 ± 0.3 mmHg/ml/min and subsequently declined to 0.2 ± 0.05 mmHg/ml/min at 120 min, ($P < 0.05$, 30–240 min).

α-Gal antibodies were rapidly absorbed by perfused nontransgenic porcine lungs from human blood. IgG α-Gal antibodies decreased to 52.7 ± 26% (12.4–87.8) at 10 min and 16.3 ± 8.2% (7.4–25.3) at 120 min. The reduction of IgM was comparable. There was no statistical difference between transgenic and nontransgenic lungs in the absorbance of α-Gal antibodies during xenogeneic perfusion. Perfusion of porcine lungs was associated with a strong increase in activation of complement in the human blood at 10 min with a further increase at 60 min (Fig. 4). There was no statistical difference in formation of soluble C_{5b-9} between transgenic and nontransgenic groups. Human leukocytes were absorbed by the porcine lung. In the circulating blood, they were reduced to 69 ± 32% at 2 min and 29 ± 8% at 30 min of perfusion. There was no statistical difference between the groups. Platelets, however, decreased more rapidly and extensively in the nontransgenic group (Fig. 5).

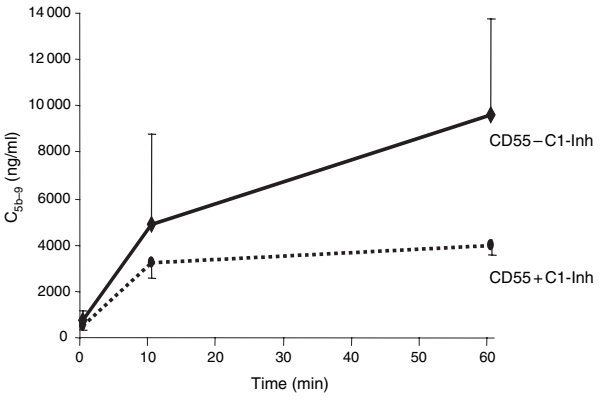

Figure 4 Significantly less soluble C_{5b-9} ($P < 0.05$) was found in the venous perfusion blood when h-CD55 transgenic lungs were perfused with C1-Inh ($n = 3$) compared with perfusion without C1-Inh ($n = 6$) at 10 and 60 min of perfusion (Mann–Whitney *U*-test). Data are expressed as means with SD.

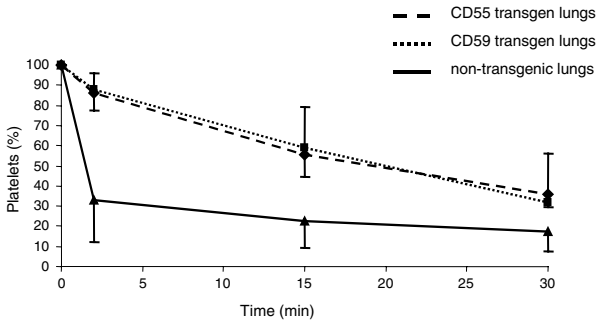

Figure 5 Platelets in the circulation were reduced significantly more in the nontransgenic group, compared with the h-CD55 and h-CD59 transgenic groups at 2, 25, and 30 min ($P < 0.005$). The loss of platelets was not prevented, but delayed in perfusion of transgenic lungs.

In all porcine lungs perfused with human blood typical signs of pulmonary injury because of HAR were observed, including progressive interstitial edema, interstitial, and intra-alveaolar hemorrhage, congestion of capillaries, and scattered thrombi in small pulmonary vessels (Table 1). On semi-quantitative evaluation, h-CD55 transgenic lungs had significantly less edema and hemorrhage after 120 min of perfusion compared with nontransgenic lungs. However, at 120 and 240 min, h-CD55 transgenic lungs were found to present with considerable histopathological injury (Table 1). In semi-quantitative analysis, epithelial and endothelial deposition of the human terminal complement complex C_{5b-9} in h-CD55 transgenic porcine lungs was observed to be significantly reduced compared with nontransgenic controls ($P < 0.05$, Table 1).

Mean survival of lungs transgenic for h-CD59 (group III, $n = 5$) was 144 (60–240) min, with three of five lungs failing. Similar to h-CD55 lungs, PVR in h-CD59 lungs showed an identical peak with a subsequent decrease (Fig. 3). Time of oxygenation was 136 min (20–240) in this group (Fig. 1). The rise in hematocrite at 60 min was 15.9 ± 12.7% (4.9–31.9). Compared with nontransgenic controls survival, PVR, time of oxygenation, Δ $AVpO_2$ (Fig. 2), Δ AVO_2, and hematocrite were not statistically different from the nontransgenic control group. In contrast to h-CD55 lungs, evaluation of histopathology revealed significantly more injury and increased deposition of the complement complex C_{5b-9} (Table 1).

The addition of low dose C1-Inh (group IV, $n = 3$) prior to perfusion of h-CD55 transgenic lungs resulted in a twofold increase of the C1-Inh concentration in the circulating blood and a reduction of complement activation during the perfusion experiments, as measured at the level of soluble C_{5b-9} formation ($P < 0.05$ at 60 min, Fig. 4). However, the addition of C1-Inh did not improve graft survival (180 vs. 238 min), time of oxygenation (170 vs. 238 min), the level of PVR (0.63 vs. 0.73 mmHg/ml/min at 15 min), or the increase of hematocrite (3.9 ± 1.5% vs. 2.2 ± 5.9% at 60 min) of h-CD55 transgenic lungs. The quality of oxygenation (Δ $AVpO_2$, Δ AVO_2) was even inferior with C1-Inh. The addition of C1-Inh to the heparinized blood resulted in a profound anticoagulative effect (ACT >1500 s) and was associated with increased parenchymal hemorrhage and edema. In contrast to the lower plasma levels of soluble C_{5b-9}, the deposition of C_{5b-9} in the h-CD55 transgenic lungs was not further reduced by application of C1-Inh.

Table 1. Parenchymal injury and complement activation in transgenic and nontransgenic lungs*.

	Hemorrhage			Edema			Deposition of C_{5b-9}		
	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
CD55 #1	0	1	2	0	0	0	1	2	2
CD55 #2	1	1	1	0	0	0	1	1	1
CD55 #3	2	2	2	0	1	1	1	1	2
CD55 #4	1	2	2	0	0	0	1	2	2
CD55 #5	1	1	1	1	1	1	1	1	1
CD55 #6	0	0	0	0	0	0	1	1	1
CD59 #1	3			2			3		
CD59 #2	1	2	2	0	1	2	1	2	2
CD59 #3	3	3		1	1		1	3	
CD59 #4	2	2	3	1	1	2	2	3	3
CD59 #5	2	2	3	1	1	2	2	2	3
Control #1	3	3	3	0	1	3	2	3	3
Control #2	3	3		2	3		3	3	
Control #3	3	3	3	1	2	2	2	2	3
Control #4	2	2	3	1	2	2	2	2	3
Control #5	2	3	3	1	2	3	2	2	3
Control #6	2			2			3		

*Semi-quantitative (0 = not apparent; 1 = minimal; 2 = moderate focal; 3 = diffuse, generalized) evaluation of hematotoxyline and eosine stains of biopsies from porcine lungs after 30, 60 and 120 min of perfusion with human blood. H-CD55 transgenic lungs had significantly less parenchymal edema and hemorrhage after 120 min of perfusion compared with nontransgenic lungs. Congestion of capillaries and scattered thrombi in small pulmonary vessels were found in all xenogenic perfused lungs. On semi-quantitative analysis, endothelial deposition of C_{5b-9} in CD55 transgenic porcine lungs was reduced, compared with nontransgenic controls ($P < 0.05$, Mann–Whitney *U*-test).

Discussion

In an *ex vivo* working lung model, we demonstrated not only prolonged survival, but as well extended and improved oxygenation of porcine lungs transgenic for h-CD55 when compared with nontransgenic organs. This result was associated with a reduction in PVR, pulmonary edema, sequestration of human platelets, histopathological injury, and deposition of complement.

Oxygenation is considered to be the major parameter and the most sensitive marker of pulmonary function. Previous studies failed to show a difference in oxygenation. Some reports used human plasma instead of whole blood [15,16], and some strictly limited pulmonary artery pressure [3,4,11,17,18]. Even when a high initial blood flow was applied, within a few minutes, it decreased dramatically because of the rapid rise of PVR. Endpoint was an early failure of forward flow at a mean of 15–35 min [11,17,18]. In contrast, the perfusion system in this study maintained a steady, continuous, standardized flow that was below the level of the estimated physiological pulsatil flow of a left lung. Endpoint here was overwhelming pulmonary edema. Pulmonary artery pressures monitored did not exceed 65 mmHg in failing lungs.

In the h-CD55 transgenic group, we observed a significant reduction of PVR, which was associated with less pulmonary edema and prolonged survival. However, the initial peak at 15 min, not seen in autologous perfusion with porcine blood, was not blunted in lungs transgenic for h-CRPs. Kulick *et al.* found a reduction of PVR in h-CD59 transgenic lungs, in contrast to other authors who did not detect a difference in PVR [11,17,18].

Effective inhibition of complement has been found to be associated with the reduced sequestration and activation of neutrophils and platelets in a kidney perfusion model [24]. Lung perfusion models failed to demonstrate an effect of complement inhibition on platelets [17,18,23]. We hereby presented an evidence that inhibition of complement in lung xenografts resulted in reduced sequestration of platelets. Activated complement components such as 1Cq and C_{5b-9} can directly activate platelets. In addition, complement can activate platelets indirectly by causing injury to endothelial cells. Injured endothelial cells expose von Willebrand factor (vWF), which causes platelet adhesion [25]. Activated platelets have an integral role in HAR of the lung. They amplify the activation of the complement cascade and release mediators like thromboxane and participate in the disseminated intravascular coagulopathy (DIC) [9,26,27]. Thromboxane mediates pulmonary hypertension and lung inflammation during hyperacute lung rejection [26].

The lung seems much more sensitive to HAR injury and the role of complement in pig-to-human lung transplantation may be more complex. Different from other organs, the lung is not protected by soluble agents inhibiting complement [5,24,28,29]. In nonhuman primates, cobra verum factor (CVF) proofed to be ineffective [8]. In *ex vivo* models complement inactivation by heat, CVF, or inhibition by soluble inhibitor-like complement 1 receptor (sCR1, TP10) or low-dose C1-Inh were unable to prolong survival of a pulmonary xenograft [4,18,23].

In contrast to the deposition of C_{5b-9} in the lung xenograft, soluble phase complement activation seemed not to be effectively regulated by h-CD55 expression in our *ex vivo* perfusion experiments. The addition of C1-Inh was able to reduce activation of complement in the plasma, but no improvement of function and survival of h-CD55 grafts was observed. In addition, we found that the application of C1-Inh was limited by its profound anticoagulative properties (increased parenchymal hemorrhages) in the presence of heparin. In line with our findings, low concentrations of C1-Inh (1 and 5 U/ml) have been reported to be associated with adverse effects [23]. A very high, clinically not applicable dosage (10 U/ml) of C1-Inh was required to show an effect on survival of *ex vivo* perfused h-CD55 transgenic lungs [23]. However, even this – more than 10-fold higher than normal – concentration did not prevent platelet activation or neutrophil and platelet sequestration, nor did it prevent lung injury [23]. Improved survival, reduced PVR, and reduced deposition of C_{5b-9} have been reported by Azimzadeh *et al.* [18] in h-CD55 lungs when sCR1 was added. This synergistic effect of soluble and membrane-based complement inhibition may be explained by the insufficient effects of the expressed human CD55 transgene in the experimental setting applied. Lungs transgenic for h-CD55 had only a marginal improvement of survival and PVR was not reduced.

On immunohistological evaluation, the h-CD55 transgene was found to be expressed in porcine lungs in quantities comparable with levels in biopsies from human lungs. In contrast, we found a low level of expression with a patchy pattern of the human CD59 transgene in the porcine lungs examined. In contrast, high expression of h-CD59 was found in the pancreas, moderate expression on skin, muscle, heart, and kidney, but low expression in the lung, liver, and on endothelial cells [21]. Kidneys from these h-CD59 donor animals that underwent perfusion with human blood demonstrated improved survival and function as well as reduced histopathological injury [21]. We observed effects in some porcine lungs expressing h-CD59, including reduced PVR and less deposition of C_{5b-9}. However, these alterations as well as survival, oxygenation and histopathological injury were not statistically different compared with the nontransgenic control group. We and others observed considerable variability in performance of all xenogenic perfused groups [17,18]. This may be influenced by varying antibody concentrations in the human blood and varying amounts of reactive antigens in porcine lungs (no blood typing of donor pigs). Both the application of a CMV promoter and the technique of pronuclear microinjection may account for the variable levels of expression encountered in the h-CD59 donor pigs tested. Further

progress in xenotransplantation will be closely related to the development of more refined genetically modified pigs. Gene targeting and somatic cell cloning are promising techniques that will allow for modifying the pig genome precisely [2,30].

Our results underline the central role of membrane-based CRPs for the inhibition of complement activation in pig-to-primate xenotransplantation of the lung. We supported previous work and added important information. Although complement plays a critical role in HAR, efficient regulation of complement alone is inadequate to permit clinically useful function of a transplanted porcine lung. In contrast to the significant effects of membrane-based complement inhibition observed in *ex vivo* models, attempts to transplant porcine lungs transgenic for h-CRPs into nonhuman primates were not successful. Donor lungs from pigs transgenic for h-CD55/h-CD59 or h-CD46 transplanted into baboons were found to delay, but not to prevent HAR [8,9]. The blood flow of grafts was increased 12-fold in comparison with nontransgenic organs, but grafts failed in between 0 and 9 h of reperfusion. In association with the failing pulmonary xenograft, recipients developed DIC.

From the data currently available, it has to be suspected that lungs are much more susceptible for injury in pig-to-primate xenotransplantation than other organs. Complement deposition on h-CD55 transgenic pig endothelial cells has been found to be markedly reduced compared with unmodified cells, but not abolished [31]. Incomplete inhibition by CRPs may prevent destruction of endothelial cells by HAR, but does not prevent activation of endothelial cells and inflammatory responses known to be particularly injurious to the lung [18]. We found some evidence that protection was incomplete despite high level of expression of h-CD55. The typical histopathological features of HAR, including mild interstitial edema and hemorrhage, capillary congestion, micro-thrombi, and some deposition of C_{5b-9}, were still detectable in *ex vivo* perfused h-CD55 transgenic lungs. Compared with nontransgenic lungs, these appeared delayed and with much less intensity. Furthermore, the initial peak in PVR remained.

The additional removal of antiporcine antibodies improved survival of pulmonary xenografts transplanted and perfused *ex vivo*, but graft loss was not prevented [17,32]. Factors independent from xeno-reactive antibodies and complement activation are considered to be also important in pulmonary xenograft dysfunction. Activated platelets and activated endothelial cells, coagulation disorders including uncontrolled thrombin generation, as well as mediators like histamine, and thromboxane were found to participate in pulmonary xenograft dysfunction [2,13,14,18,26].

Further efforts to understand the pathomechanisms of immediate lung injury and pulmonary xenograft dysfunction seem to be required to finally allow for successful pig-to-primate lung transplantation.

Disclosures of authors

None.

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