Transpl Int (2002) 15: 212–219
DOI 10.1007/s00147-002-0405-1

ORIGINAL ARTICLE

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Adenovirus-mediated gene transfer of triple human complement regulating proteins (DAF, MCP and CD59) in the xenogeneic porcine-to-human transplantation model

Part II: xenogeneic perfusion of the porcine liver in vivo

Received: 4 September 2000
Revised: 7 June 2001
Accepted: 18 October 2001
Published online: 11 April 2002

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Abstract In this study, the adenovirus-mediated gene transfer of triple human complement regulating proteins was investigated in xenogeneic pig liver perfusion. The porcine liver was perfused in situ at 4 °C under a pump-driven veno-venous shunt of the portal vein and inferior vena cava, with 5 to 15×10^{11} plaque-forming units (pfu) of adenovirus vector (group 1: AxCALacZ; 2: AxCACD59; 3: AxCACD59 + AxCADAF; 4: AxCACD59 + AxCADAF + AxCAMCP) for 1 h (for each, $n=3$). The livers were harvested 24 h after gene transfer and then were reperfused ex-vivo with fresh human blood for 2 h. In immunohistochemical staining, each complement regulating protein (CRP) showed a distribution similar to that of the LacZ expression. The C3 levels in the perfusate were also maintained at higher levels in group 4 from 60 to 120 min after reperfusion (C3: 85% to 95% of the initial level) than in groups 1 to 3 (C3: 80% to 90% of the initial level) from 60 to 120 min after reperfusion. The complement deposition on the porcine liver [C3, membrane attack component (MAC)] decreased significantly more in group 4 than in groups 1 to 3. In conclusion, the adenovirus-mediated multiple gene transfer of human CRPs (hCRPs) was found to effectively suppress the complement activation in xenogeneic pig liver perfusion.

Keywords Hyperacute rejection · Adenovirus vector · MCP · DAF · CD59 · Xenoperfusion

Introduction

In xenogeneic transplantation or xenoperfusion, the rapid destruction of the xenoperfused organs is caused by hyperacute rejection (HAR), in which the complement activation on the endothelial cells plays a fundamental role [15, 18]. Because the complement activation is caused by the inability of complement regulating protein (CRP) to act beyond species [19], transgenic pigs which express human CRPs have been developed to cope with the HAR. As a result, the extra-hepatic organs, such as the heart, lung and kidney, from these transgenic pigs were successfully transplanted into non-human primates [9]. Since some of these xenogeneic organs survived from a few days to several weeks, HAR already seems to have been overcome in these organs. Recently, the first successful xenogeneic liver transplantation was reported by Ramirez et al., in which two livers from decay-accelerating factor (DAF) transgenic pigs were transplanted into baboons and survived 4 and 8 days without HAR [17]. In other papers, however, these transgenic livers could not sustain their functions for more than a few hours, even in the xenogeneic liver perfusion [10, 14, 16]. These facts thus indicated that such transgenic livers still need to be improved before xenogeneic perfusion and eventually xenogeneic liver transplantation can be successfully performed.

In this context, the transgene expression of the human CRPs in the transgenic liver might not effectively suppress HAR. Transgenic pig livers expressing human CRPs, such as DAF [14, 16], have been investigated in xenogeneic perfusion with human blood. In spite of the fact that no simultaneous or equal expression of multiple CRPs could be established in these models, human complement activation was reported to be effectively suppressed. Human CRPs, however, can be divided into four major proteins, namely membrane cofactor protein (MCP), DAF, CD59, and complement receptor type 1 (CR1) [1, 4, 5, 8, 13], which inhibit complement activation at different levels. DAF inhibits the converting enzymes of C3 and C5, while CR1 and MCP accelerate I-factor mediated inactivation of C3b, and CD59 inhibits the formation of membrane attack component (MAC). At least theoretically, the multiple and synchronous expression of these CRPs should thus be required to achieve effective human complement inactivation. Although the double expression of CRPs (DAF and CD59) has also been reported in transgenic pigs, no equal expression of more than two CRPs has yet been achieved in these pigs.

Moreover, in these transgenic livers, transgene expression was distributed exclusively on the intralobular vascular endothelium but not on parenchymal sinusoidal endothelial cells (SEC) [2]. In hepatic HAR, Satoh et al. defined a MAC formation on the SEC as a central mechanism in complement-mediated destruction of the xenoperfused pig liver [20]. In this context, limited expression of human CRPs on the interlobular vascular endothelium of the transgenic pig liver might thus not be sufficient to inhibit MAC deposition of the sinusoidal area. In previous reports from our laboratory, we reported the adenovirus-mediated gene transfer to porcine liver in vivo, using in-situ perfusion of the liver [21, 22]. In this procedure, pig liver was perfused in-situ with high-titer adenovirus vectors under cold preservation and systemic immunosuppression. The aim of this procedure was to avoid diluting the adenovirus vector, to prevent exclusion of the vectors by the host complement and/or humoral immunological attack, and to enable the long contact of high-titer adenovirus vector with the porcine liver. As a result, marker gene was recognized on the SEC as well as on the vascular endothelium of the portal tract.

In part I of the present study (in vitro), we confirmed the complement inhibitory functions of the triple human CRPs transfected with adenovirus vector to porcine endothelial cells. These triple CRPs were also found to work synergistically in suppressing a human complement activation in vitro. In the present part-II study, we used the above-described in-situ perfusion method to achieve the simultaneous gene transfer of three different human CRPs (DAF, CD59, and MCP) to the parenchymal SEC in porcine liver. The inhibitory effects of human complement activation were then assessed using xenoperfusion of the porcine liver with fresh human blood.

Materials and methods

Construction of adenovirus vectors

All adenovirus vectors were constructed with the "Adenovirus Expression Vector Kit" (TaKaRa Biomedical, Kyoto, Japan). Full-length cDNA of MCP was kindly provided from John P. Atkinson, M.D. (Department of Medicine, Washington University School of Medicine, St. Louis, Mo., USA), DAF from Edward M. Medof, M.D., Ph.D. (Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, USA), and CD59 from Alexandra Davies, B.A., Ph.D. (Molecular Immunopathology Unit, Medical Research Council Centre, Cambridge, UK). These cDNAs were adjusted by the restriction enzyme and were subcloned into the SwaI site of the cosmid vector (pAxCAwt) which had a CAG promoter (cytomegalovirus enhancer, chicken β-actin promoter, rabbit β-actin polyA signal; "Adenovirus Expression Vector Kit"). Similarly the cosmid vector coding *E.Coli* β-galactosidase (pAxCALacZ) was prepared [7]. These cosmid vectors and the restriction enzyme which digests DNA-TPC were co-transfected into 293-kidney embryonal cells [11]. The supernatant containing the adenovirus vector, was collected by four freeze-and-thaw cycles. These steps were repeated four times without significantly expanding the culture volume in order to obtain a high-titer vector solution. Viral titers were determined by a 50% tissue culture infectious dose ($TCID_{50}$), in which the virus titer in $TCID_{50}$ was approximately the same as that of plaque-forming units (pfu) [25]. These virus vectors of AxCALacZ (6×10^9 pfu/ml), AxCAMCP (3×10^9 pfu/ml), AxCADAF (3×10^9 pfu/ml), and AxCACD59 (1×10^9 pfu/ml), were stored at –80 °C until required.

In-situ perfusion of the porcine liver

The in-vivo gene transfer to the porcine liver was performed with the in-situ perfusion technique based on our previous reports [21, 22]. Adult female pigs weighing from 15 to 20 kg were immunosuppressed with 1 mg/kg/day of cyclophosphamide, 2 mg/kg/day of prednisolone and 3 mg/kg/day of cyclosporine from 2 days before gene transfer to 24 h after gene transfer, to prevent the host immunological removal of the virus vector or the infected cells with those vectors. All animals were laparotomized under general anesthesia and the liver was then surgically isolated from the surrounding tissue, except for the bile duct or vessels, such as the superior and inferior hepatic vena cava (SHVC; IHVC), portal vein (PV), and the hepatic artery (HA). The blood flow from the PV and IHVC was bypassed with a centrifugal pump (Biopump, Biomedicus, Minn., USA), to the internal jugular vein. The HA was also clamped and perfused through the excised stump of the gastroduodenal artery with cold lactate for precooling. The livers were then flushed with a cold lactate Ringer's solution at 4 °C through the PV, and then were kept at 4 °C. The adenovirus-mediated gene transfer to the porcine liver was performed under five different combinations of vectors (pAxCALacZ in group 1; AxCADAF in group 2; AxCADAF + AxCACD59 in group 3; AxCADAF + AxCACD59 + AxCAMCP in group 4; and xenoperfusion without vectors in group 5). In groups 1 to 4 ($n=3$), 5×10^{11} pfu of adenovirus vectors in 20 ml of culture medium were injected through the excised stump of the gastroduodenal artery into the common HA for 60 min under cold ischemia. The laparotomized wound was closed and the pig was allowed to live until the liver was harvested.

Xenoperfusion

Twenty-four hours after gene transfer, the pig was re-laparotomized and the liver was perfused with cold lactate Ringer's solution at 4 °C through the PV and HA. Next, the liver was harvested and transferred to the perfusion machine based on the previously described method with some modifications [14]. Fresh human blood from two healthy donors of identical blood type (A, B, or O) and Rh factor was diluted with lactate Ringer's solution to a hematocrit level of 25%. The donor blood perfusate was heparinized (10 IU/ml) during collection [23] and was oxygenated by a membrane oxygenator (MENOX AL-6000, Getz Bros., Tokyo, Japan). The priming volume was adjusted to approximately 800 ml. The livers were perfused for 2 h, and the flow volume was adjusted to 240 ml/min for the portal flow and to 60 ml/min for the hepatic arterial flow. The duration of xenoperfusion was adjusted to 2 h based on the findings from preliminary xenoperfusion studies, in which porcine liver was perfused for various lengths of time ranging from 60 min to 6 h. In those livers that were perfused for more than 3 h, overwhelming intrahepatic coagulation, occurring independently from complement activation, made comparative analysis of histology or others impossible. The blood temperature, pH, flow rates, perfusion pressures (PV and HA), partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2), pO_2 in the liver tissue, and liver appearance were all continuously monitored. The blood gases were frequently measured in the in- and outgoing vessels with a blood-gas analyzer.

Sample processing

Perfusate specimens were taken to determine the enzymes, electrolytes, complement levels, etc., at nine time points (before perfusion, and after 0, 5, 15, 30, 60, 90, and 120 min of perfusion). The complement levels of C3 and C4 were determined by a turbidimetric immunoassay, which is specific for human complement. The bile output was also measured continuously. The collected samples were centrifuged (KN70, Kubota, Tokyo, Japan) and stored at –80 °C until required for further analysis. Liver biopsies for histological analysis were performed at the end of perfusion, from both the left and right lobes, any superficial locations being avoided. Tissue specimens were prepared for routine histological analyses, the immunohistochemical detection of DAF, MCP, CD59, C3, C4, and MAC. For routine histological analyses and immunohistological detection of IgG, and IgM, samples were fixed in formalin and embedded in paraffin, and then 6-μm sections were routinely stained with hematoxylin and eosin. An immunohistological analysis of cryostat was also performed to confirm the expression of transferred human CRPs (DAF and MCP) and complement deposition (C3, C4, MAC).

X-gal staining

The marker LacZ gene expression was assessed by X-gal staining. Frozen sections 8 μm thick were produced on a cryostat, fixed with 1.25% glutaraldehyde at 4 °C for 10 min. After the glutaraldehyde had been removed, the preparations were immersed in X-gal solution and then counterstained with eosin. The cells expressing β-galactosidase turned blue in the presence of X-gal, while the non-expressing cells were either clear or faintly yellow. The stained sections were thereafter examined by light microscopy.

Immunohistology

Biopsies were snap-frozen in liquid nitrogen after being embedded in OCT (TissueTek; Miles, Elkhart, Ind., USA), and were kept at –80 °C. Frozen tissue samples were cut into 4-μm serial sections with a cryostat (CM502, Sakura, Tokyo, Japan), air dried for 15 min, and then fixed with 4% paraformaldehyde/PBS for 15 min. A standard indirect immunoperoxidase technique (biotin-streptavidin system) (ENVISION kit; Dako Japan, Kyoto, Japan) was used after each primary antibody had been applied. At least four sections from each of the major right and left lobes, any superficial parts of the liver being avoided, were stained with murine anti-human monoclonal antibodies (mAbs) specific for human MCP (1:5; Dako), DAF (1:10; Dako), C3 (1:5; Dako), MAC (1:25; Dako), and fibrinogen (1:100; Dako). Negative control sections were stained by the same procedure without any primary antibodies being used. Paraffin-embedded sections were also cut into 4-μm serial sections and were similarly stained by the same standard indirect immunoperoxidase technique used for frozen sections after the application of the primary antibodies. These paraffin sections were stained with murine anti-human mAbs specific for human CD59 (1:10; Dako), and IgM (1:25; Dako).

Results

Expression of the marker LacZ genes in the X-gal staining

The tissue distribution of the marker LacZ gene was confirmed by X-gal staining. In the LacZ transfected group 1, positive staining was observed mainly around the portal tract. More specifically, the vascular endothelium in the portal tract, especially on the HA and sinusoidal endothelium in the hepatic parenchymal area, were both found to be positive for marker LacZ expression (Fig. 1). No such expression was observed in groups 2 to 4.

Expression of human complement regulating protein

The expression of each CRP was found to be positive only in those livers transfected with corresponding adenovirus vectors carrying each type of CRP. Although the strength of protein expression was not uniform between different CRPs with a relatively weak expression in CD59, such expression was exclusively detected around the portal tract, in which both the vascular endothelium and sinusoidal endothelium stained positively (Fig. 2).

Macroscopic appearance of the liver and circulation

During the 2-h of xenoperfusion, no apparent macroscopic findings, such as hemorrhagic or anemic ischemia were observed in any of the groups. Mild exudate from the liver surface was observed after 90 min of xenoperfusion in all the livers, thus resulting in a reduction (10%) of the total volume of perfusate and a mild hemoconcentration of the perfusate. Portal venous pressure was maintained at a lower level in groups 3 and 4 (85% to 90% of the initial flow) than in groups 1 and 2

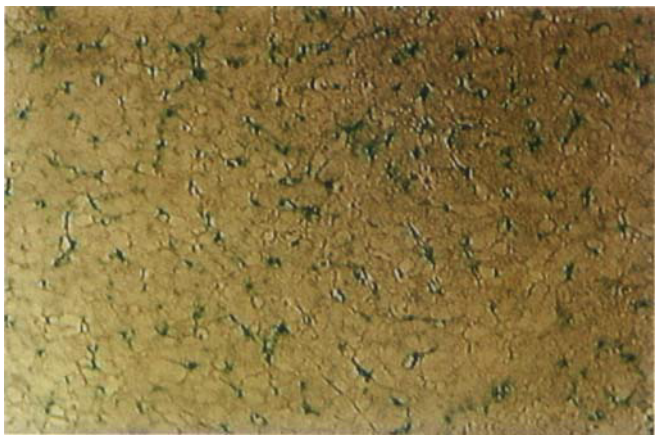

Fig. 1. To confirm the effectiveness of gene transfer in porcine liver, we enzymatically studied the protein expression from the transfected marker LacZ cDNA by detecting the β galactosidase activities with X-gal staining (group 1; $n=3$). Positive staining was recognized in the parenchymal area, in which the sinusoidal endothelial cells showed linear staining (×400)

(105% to 120% of the initial flow) from 40 to 120 min after reperfusion (Fig. 3).

Blood chemistry

In the perfusate, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations during xenoperfusion showed similar elevations in all groups, along with the development of xenoperfusion, without any significant difference between the groups (AST: 532 ± 468 IU/l in group 1 and 447 ± 383 IU/l in group 4). The prothrombin time (PT) was larger than the measurable limits (> 100 s) in all specimens, due to heparinization.

Complement levels in the perfusate

The assay, used to detect the complement (C) levels in the perfusate, could not detect normal C levels in the porcine blood. This assay was therefore human complement specific. The initial levels of the C activity were normalized to 100% to ensure comparability. The human C3 levels in the perfusate remained significantly higher in group 4 from 60 to 80 min after reperfusion (C3: 85% to 95% of the initial level) than in groups 1 to 3 (C3: 80% to 90% of the initial level) (Fig. 4a). The human C4 levels were also lower in group 4 during xenoperfusion, but it was not significantly lower than in the other groups (Fig. 4b).

Complement deposition

In control group 1, C3 deposition, which reflects the activation of both the classical and alternative pathways, was also positively detected mainly in the hepatic arterial vascular endothelium (Fig. 5a). In the triple CRPs transfected group 4, C3 deposition on the hepatic arterial endothelium was hardly observed (Fig. 5b). No factor P (properdin) deposition could be observed in any of the groups regardless of the CRP transfection, thus indicating a negative activation of the alternative

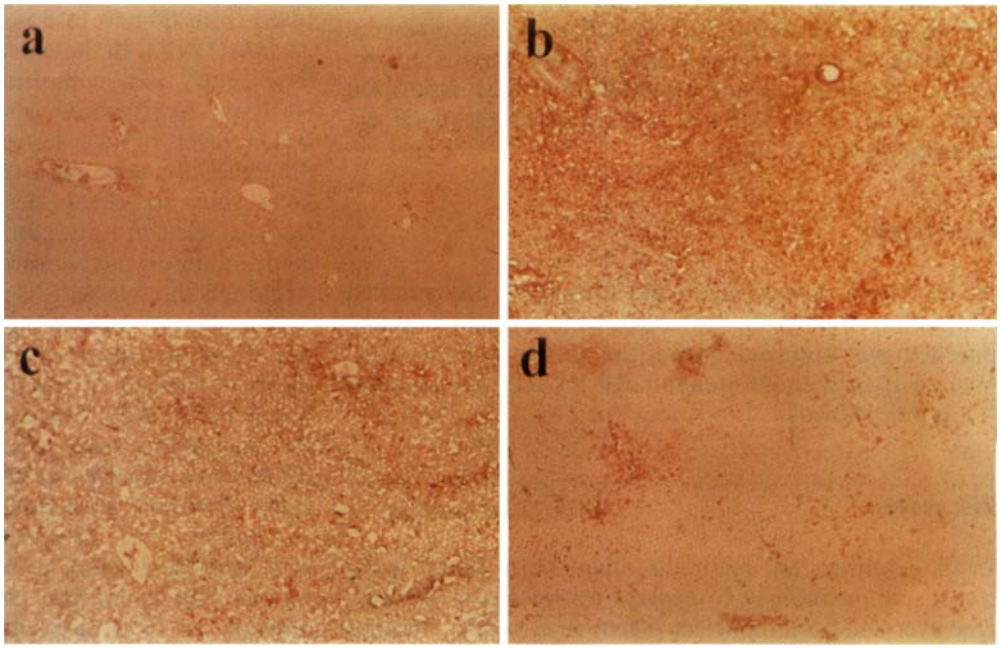

Fig. 2a–d. The protein expressions from the transferred hCRPs were assessed by immunohistochemical staining in groups 1 and 4. **a** There was no DAF staining in the marker LacZ transfected group 1. **b** DAF, **c** MCP, and **d** CD59 staining was mainly distributed in the periportal area in the triple-CRP transfected group 4. Strong expressions of **b** DAF and **c** MCP were observed, compared with the relatively weak expression of CD59 (**d**). (×100)

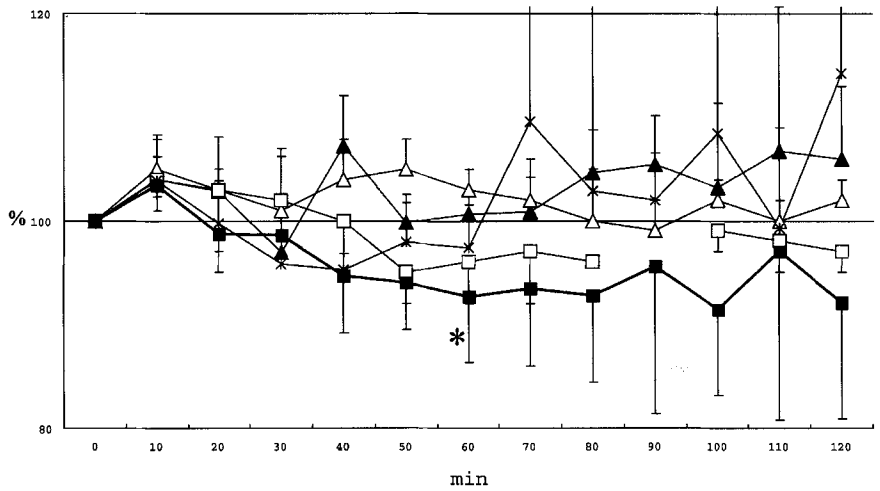

Fig. 3. To assess the hemodynamic effects of complement inhibition made by a gene transfer of hCRPs, we continuously monitored the portal venous pressure during xenoperfusion in groups 1 to 5 (for each, $n=3$). The initial levels of the portal venous pressure were normalized to 100%. The portal venous pressure remained lower in group 4, from 40 to 120 min after reperfusion, than in groups 1 to 3. *Solid triangle* group 1 (LacZ), *open triangle* group 2 (DAF), *open square* group 3 (CD59 + DAF), *solid square* group 4 (CD59 + DAF + MCP), * group 5 (untreated), * significantly lower than group 1 ($P<0.05$)

pathway of the complement activation. MAC deposition was found exclusively in the hepatic parenchymal area in all groups (data not shown).

IgM deposition

Regarding immunohistochemical staining, IgM deposition was mainly recognized on the hepatic vascular endothelium in the portal tract as well as on the sinusoidal endothelium in the broad area of the hepatic parenchyma. The intensity and distribution of IgM deposition were similar in all groups, regardless of the CRP expression (data not shown).

Fibrinogen

An extensive deposition of the fibrinogen and marked congestion were both simultaneously observed in the parenchymal sinusoidal area, in all the xenoperfused livers with or without human CRP (hCRP) transduction (Data not shown).

Discussion

Until recently, no methods other than those using transgenic technology to express transgenes in the porcine liver had been reported. Adenovirus vector, which is known to possess a wide range of target cells with a highly efficient transduction rate, has also been considered to be a possible candidate. With the same adenovirus vector used in the present study, we recently reported the successful marker-gene transfer to porcine endothelial cells in vitro [12], as well as to the whole liver in vivo [21, 22]. In our procedure of in-vivo gene transfer to porcine liver, the in-situ perfusion of the liver with adenovirus vector was performed under systemic immunosuppression, in order to increase the chance of infection, and to reduce the immunological elimination of the infected cells or vector itself [22]. In the present study, we used the same in-vivo procedure to transduce the porcine liver in vivo with three different hCRP genes, thus resulting in a high protein expression of the hCRPs on the SEC as well as on the vascular endothelium, mainly at the periportal area. In our preliminary experiments, more than five porcine livers were xenoperfused from 60 min to 6 h, with and without adenovirus vectors (LacZ). In these livers the histological findings of liver injury and complement deposition, complement levels in the perfusate, and liver function tests showed similar levels of liver destruction, without any detectable differences by adenovirus-mediated gene transfer. These facts thus indicated that adenovirus-mediated gene transfer itself did not contribute to any of the complement inhibitory activities observed in the present study. Although the strength of protein expression was somewhat different between the CRPs, their distributions were basically similar and were not significantly affected by the protocols of the single, double, and triple gene transfer of hCRPs. This can be explained by the fact that the adenovirus vectors carrying these three different CRPs have exactly the same structures, including the same promoter sequence. Other factors, which might also influence the protein expression, include the duration from gene transfection and the titers of adenovirus vector. The transduced liver was harvested for xenoperfusion at 24 h after gene transduction, when it is

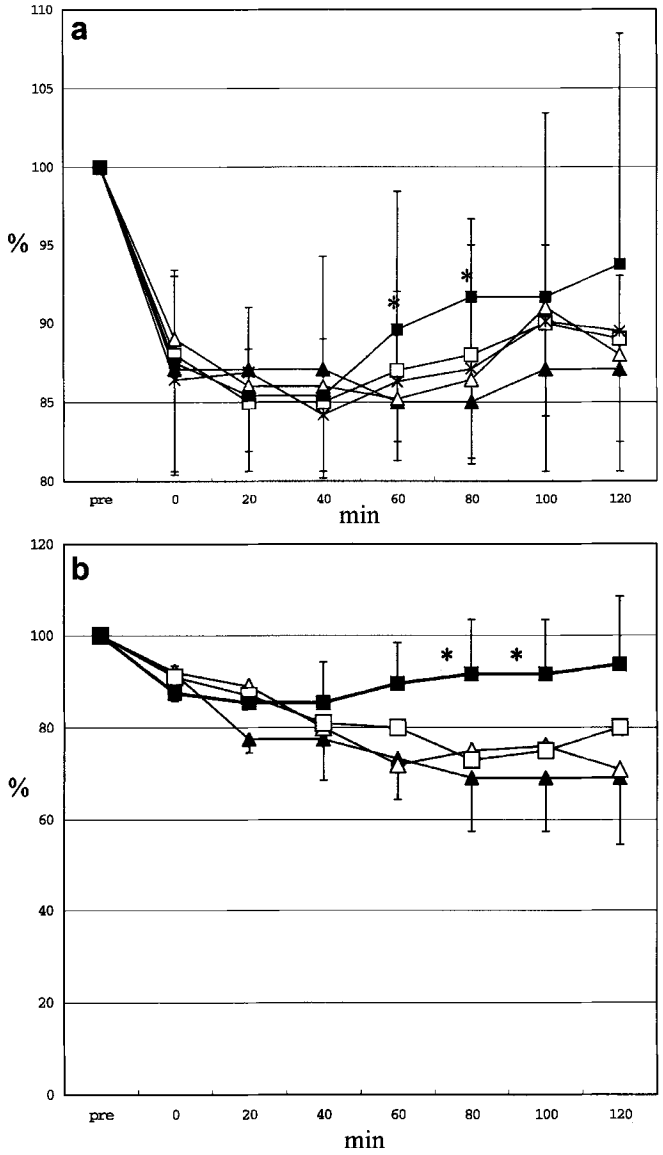

Fig. 4a, b. To assess the effects of complement inhibition made by the gene transfer of hCRPs, we monitored the dynamics of **a** C3 and **b** C4 levels in the perfusate, during xenoperfusion in groups 1 to 5 (for each, $n=3$). The initial levels of the complement were normalized to 100% to ensure comparability. **a** The human C3 levels in the perfusate remained significantly higher in group 4 (CD59 + DAF; DAF + MCP) from 60 to 80 min after reperfusion than in groups 1 to 3. **b** The human C4 levels were also lower in group 4 during xenoperfusion, but were not significantly lower than in the other groups. *Solid triangle* group 1 (LacZ), *Open triangle* group 2 (DAF), *open square* group 3 (CD59 + DAF), *solid square* group 4 (CD59 + DAF + MCP), * group 5 (untreated), * significantly higher than group 1 ($P<0.05$)

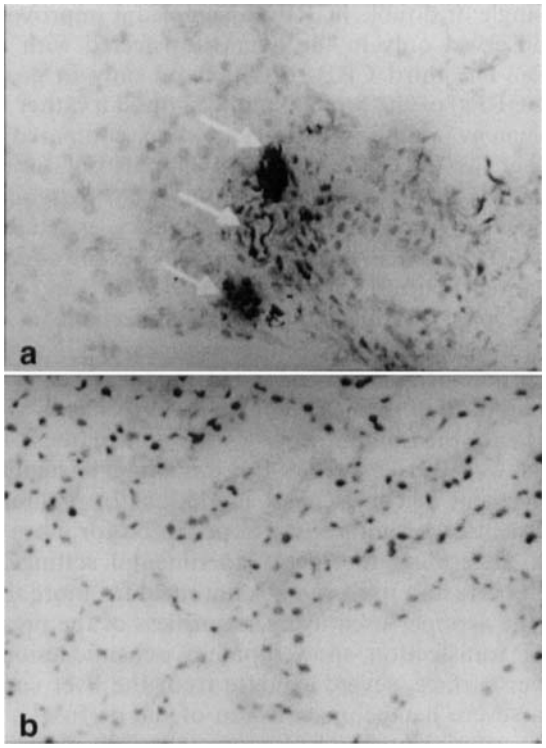

Fig. 5a, b. C3 deposition, which reflects the activation of both the classical and alternative pathways of complement, was immunohistologically assessed in a frozen section of xenoperfused porcine liver, using monoclonal antibodies specific for human C3, in groups 1 (transfected with LacZ (**a**), $n=3$) and 4 (transfected with triple human complement regulating proteins (**b**), $n=3$) (× 100). **a** In the control LacZ transfected group 1, C3 deposition was positively detected mainly in the hepatic arterial vascular endothelium. **b** In the triple CRPs transfected group 4, C3 deposition on the hepatic arterial endothelium was hardly observed

known to show a maximum transgene expression [22]. The titer used for the present in-vivo study was determined from our previous in-vitro data, in which the adenovirus vector-mediated gene transfer was possible in 100% of the porcine aortic endothelial cells, with 10^3 multiplicities of infection (MOI) over the target cells and 30 to 60 min of incubation time [12]. Since porcine liver contains 10^7 sinusoidal endothelial cells [3], at least 10^{10} pfu of adenovirus vector might be required to maintain a level of more than 10^3 MOI over the SEC. In another line of our experiments, porcine aortic endothelium cells (PAECs) were transfected with triple hCRP genes in vitro, the same adenovirus vector being used as in the present study.

In the present study, a weak C3 deposition was recognized mainly in the hepatic arterial endothelial cells in LacZ transfected control livers, whereas it was reduced by the expression of triple hCRPs which coincided with relatively high C3 levels in the perfusate of these livers. As described in part I of the in-vitro study, the simultaneous expression of triple hCRPs on PAECs thus resulted in a significantly effective inhibition of human complement activation in the xenoperfused porcine liver in vivo, compared with those with either single CRP or none at all. Although these improvements in complement inhibition were also recognized in the livers transfected

with single or double hCRPs, a significant improvement was observed only in the livers transfected with three hCRPs. The third CRP (MCP), used only in group 4 (triple CRPs) of the present study, showed a rather weak complement inhibitory activity in vitro, compared with the other two CRPs (DAF, CD59), in part I of our serial studies. MCP alone might not thus yield a significant improvement in complement inhibitory activities of group 4 (triple CRPs). As described in the Introduction, these three major hCRPs of DAF, MCP, and CD59 cover all major complement inhibitory mechanisms, such as inhibition of the converting enzymes of C3 and C5, acceleration of I factor-mediated inactivation of C3b, and inhibition of formation of MAC, respectively. From these facts, the simultaneous expression of these three hCRPs was thus essential for obtaining a significant improvement in complement inhibition. In preliminary experiments, we continued xenoperfusion for more than 3 h to determine the ideal experimental settings. Although perfusion itself can be continued for more than 6 h, all the xenoperfused livers, regardless of the presence of CRP transfection, showed patchy ischemic lesions on the liver surface, severe exudate from the liver surface, with a severe hemoconcentration of the perfusate, high hepatic arterial pressure of more than 200 mmHg, increased hemolysis, and low tissue blood flow after 3 to 4 h. Based on a histological study of xenoperfused porcine liver and hepatic enzyme release into the perfusate, hepatic destruction or disturbance in microcirculation was thus thought to be caused by a progressive coagulation process, which can be initiated independently from the complement activation.

Such hepatic destruction itself has also been reported in the xenoperfusion of DAF transgenic livers, and was believed to be caused by such immunological influences as antibody deposition [14]. In these xenoperfused livers, however, the hypercoagulability status, such as a marked fibrin deposition on the porcine endothelium, can be induced independently either from the natural antibody deposition or from complement deposition [6]. These findings indicated that neither complement inhibition nor natural antibody depletion alone could sufficiently eliminate HAR of the hepatic xenograft, without controlling the hypercoagulability. Even in xenoperfusion of the transgenic porcine livers expressing hCRP, such progressive destruction could not be completely eliminated [16]. These facts thus indicated that additional therapies such as anti-coagulation therapy might be required in addition to complement inhibition, to achieve successful xenoperfusion or xenotransplantation of porcine liver. Since this hypercoagulability derives at least partially from the incompatibility beyond species of physiological anti-hemostatic molecules such as thrombomodulin (TM) [24], additional gene transfer of the anti-hemostatic molecules, besides CRPs, is thus considered to be necessary to achieve complete inhibition of hepatic HAR. In these contexts, the adenovirus-mediated gene transfer and in-situ perfusion of the liver might also be helpful for additional gene delivery to porcine liver including the transgenic pig liver.

In conclusion, the adenovirus-mediated gene transfer of triple CRP genes to porcine liver was found to effectively inhibit both complement activation and early endothelial activation, especially in the sinusoidal area. Successful complement inhibition, however, could not prevent progressive hepatic destruction, due to overwhelming hypercoagulability in the xenoperfused liver. Combined with the additional gene transfer of anti-hemostatic molecules and elimination of natural antibodies, adenovirus-mediated gene transfer of multiple CRP genes might therefore be useful as a clinically relevant model of either xenotransplantation or xenoperfusion.

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