Molecular Medicine 4: 629-637, 1998

Molecular Medicine

Decreased Fibrinolytic Activity in Porcine-to-Primate Cardiac Xenotransplantation

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Accepted July 15, 1998

Abstract

Background: One major barrier to successful xenotransplantation is acute vascular rejection, a process pathologically characterized by microvascular thrombosis and diffuse fibrin deposition in transplant blood vessels. This pathologic picture may result from a disturbance in the coagulant or fibrinolytic pathways that regulate normal vascular patency. This study evaluated the regulation of fibrinolytic activity defined by tissue plasminogen activator and plasminogen activator inhibitor-1 as it may exist in the setting of acute vascular rejection.

Materials and Methods, Results: Serial biopsies from cardiac xenotransplants evaluated by immunofluorescence microscopy demonstrated progressive decreases in tissue plasminogen activator and increases in plasminogen activator inhibitor-1. In vitro studies measuring fibrinolytic activity of cell culture medium from porcine aortic endothelial cells stimulated with human serum or autologous porcine serum revealed that human serum triggered as much as 93% increase in antifibrinolytic activity.

Conclusions: These findings demonstrate that porcine vascular endothelial cells change toward an antifibrinolytic state following stimulation with human xenoreactive antibodies and complement. The shift is at least partly explained by an increased ratio of plasminogen activator inhibitor-1 to tissue plasminogen activator, and is at least in part mediated by the activation of complement. This increased antifibrinolytic activity may contribute to the thrombotic diathesis seen in acute vascular rejection in pig-to-primate xenografts.

Introduction

The clinical application of solid organ transplantation is limited by a severe shortage of donor organs. One possible solution to this problem is the use of organs from other species for transplantation into humans, i.e., xenotransplantation. The initial barrier to successful transplantation of organs from animals such as the pig into humans is hyperacute rejection, which leads to organ dysfunction within minutes to hours (1,2). Hyperacute rejection is characterized pathologically by interstitial edema, hemorrhage, and platelet thrombi resulting from loss of endothelial function and integrity (1,3,4). Hyperacute rejection can be prevented through the depletion of xenoreactive antibodies (5) or partial inhibition of complement activation (6,7). Recent work in our laboratory has demonstrated that depletion of host anti-graft antibodies or the inhibition of complement activation increases xenograft survival in porcine-to-baboon transplants (8–10). When hyperacute rejection is avoided, the graft often succumbs to "acute vascular rejection," which is characterized pathologically by endothelial injury, focal ischemia, and diffuse microvascular fibrin deposition and thrombosis (11).

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Fibrin is the physiological endproduct of the coagulation cascade that is formed in response to vascular injury. In the final step of the coagulation pathway, thrombin converts fibrinogen to fibrin, which forms the biochemical matrix that composes occlusive vascular thrombi. Vascular patency is restored via the proteolytic breakdown of fibrin via a series of complex interactions between serine proteases and their inhibitors. Thus, fibrin is degraded by the protease plasmin which is formed from its inactive zymogen, plasminogen. The main biological plasminogen activator, tissue plasminogen activator, is secreted from endothelial cells (12,13) and is involved in the dissolution of fibrin in both normal physiologic and disease states (14). Inhibitors of plasmin generation act by inhibiting plasminogen activators, of which plasminogen activator inhibitor-1 (PAI-1) is the major active protein (15,16). The intricate balance between plasminogen activators and their inhibitors, therefore, tightly regulates fibrinolytic activity.

Vascular endothelial cells play a pivotal role in the regulation of fibrinolysis (17). In addition to constituting a nonthrombogenic barrier between circulating blood and subendothelial components (18), vascular endothelial cells produce and secrete integral proteins of the fibrinolytic cascade, such as tissue plasminogen activator and PAI-1 (17). Disturbance in the production of these components has been implicated in or correlated with thrombotic and hemorrhagic disease states including deep vein thrombosis (19,20), coronary artery disease (21,22), allograft rejection (23), and inherited hemorrhagic disorders (24,25). In addition, the clinical utility of fibrinolytic regulation has been demonstrated by the widespread use of fibrinolytics such as tissue plasminogen activator for acute thrombotic events (26–28).

Recent studies evaluating tissue plasminogen activator levels following cardiac allotransplantation have demonstrated that endothelial cell–derived tissue plasminogen activator is depleted after cardiac allotransplantation and this depletion correlates with graft failure (23). On the basis of these studies, we have evaluated whether acute vascular rejection, which is characterized by diffuse intravascular thrombosis, might be associated with an aberrant balance of tissue plasminogen activator and plasminogen activator inhibitor-1, and whether this imbalance may be brought about by activation of small amounts of complement within the porcine-to-primate xenograft.

Materials and Methods

Grade L human fibrinogen, human glu-plasminogen, goat anti-porcine tissue plasminogen activator antibody, and goat anti-human plasminogen activator inhibitor-1 antibody were purchased from American Diagnostica (Greenwich, CT). Plasminogen activator inhibitor-1 was purchased from Calbiochem-Novabiochem International (La Jolla, CA), and flourescein-conjugated rabbit F(Ab')2 to goat IgG, from Organa Teknika Corp. (Durham, NC). Dulbecco's modified Eagle medium (DMEM), and phosphate-buffered saline (PBS) were purchased from Gibco Biological Research Laboratories (Grand Island, NY); borate and acetone, from Mallinckrodt Inc., (Paris, KT); and agar was from DIFCO Laboratories (Detroit, MI). Human recombinant tissue plasminogen activator (Activase®) was supplied by Genentech, Inc. (San Francisco, CA), and human thrombin was purchased from Haematologic Technologies, Inc. (Essex Junction, VT). Aortic endothelial cells were harvested from outbred pigs; culture of these cells is described below. Aortic endothelial cells were harvested from transgenic pigs that expressed CD59 and decay accelerating factor (29). Serum was collected from normal pigs by routine venipuncture, and human serum was collected by venipuncture from normal human subjects. Following clotting of blood and centrifugation, all serum was stored at −80°C until ready for use. Human serum used in experiments was a pooled sample of equal proportion from four individuals. Fetal calf serum (FCS) was purchased from Hyclone (Salt Lake City, UT).

Tissue Specimens and Immunocytochemistry

Hearts from transgenic pigs expressing human complement proteins were transplanted into baboons as previously described (30). The hearts were biopsied as previously described (8) and the cardiac tissue was snap frozen at −80°C. Frozen tissue sections of 4 μm thickness were prepared in a Leica cryostat (Heidelberg, Germany). Sections were air dried, fixed with acetone, and washed with PBS as previously described (31). Sections were incubated for 45 min with anti-tissue plasminogen activator antibody (0.2 mg/ml) or anti-plasminogen activator inhibitor-1 antibody (0.2 mg/ml) in the dark at room temperature. The sections were washed with PBS and incubated with FITC-conjugated rabbit anti-goat IgG (0.8 μg/ml) for 1 hr in the dark at room temperature. The sections were then

washed with PBS and mounted. Tissue sections were examined using a Leitz DMRB epifluorescence microscope (Wetzlar, Germany). Immunofluorescence was graded on a scale of 0–4 (4 = maximum) by three independent reviewers. Background immunofluorescence was evaluated using tissue sections as described above but omitting the primary antibody. The specificity of anti-tissue plasminogen activator antibody was assessed in a blocking experiment in which tissue plasminogen activator (final concentration 2.9 μM) was incubated with anti-porcine tissue plasminogen activator antibody (final concentration 1.3 μM) for 2 hr at room temperature, then applied to the tissue section. Antibody binding was tested by immunofluorescence as described above. The specificity of anti-plasminogen activator inhibitor-1 antibody was assessed in a blocking experiment in which plasminogen activator inhibitor-1 (final concentration 4.8 μM) was incubated with anti-human plasminogen activator inhibitor-1 antibody (final concentration 1.3 μM) for 2 hr at room temperature, then applied to the tissue section. Antibody binding was evaluated and scored by immunofluorescence as described above.

Growth and Treatment of Porcine Aortic Endothelial Cells

Porcine aortic endothelial cells were isolated from freshly harvested aortae and grown in T-25 cell culture flasks in DMEM containing 20% autologous serum, L-glutamine (2.0 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were passed into 24-well plates (2 cm^2) and grown to confluence in 5–8 days. Autologous porcine serum was removed, the cells were washed three times with PBS, and then were incubated with 20% human serum in DMEM for up to 24 hr. Control porcine aortic endothelial cells were incubated with autologous serum for the same times. After incubation, control or experimental medium was removed from the cells, serum-free medium was added, and the cells were incubated for 24 hr. The conditioned medium was then collected, concentrated 10-fold, and assayed for fibrinolytic activity. The harvested pool of cultured medium for each sample was 6 ml prior to concentration and assay.

Aortic endothelial cells were isolated from aortae of transgenic pigs expressing human CD59 and decay accelerating factor. The cells were grown in T-25 cell culture flasks in DMEM containing 10% FCS, L-glutamine (2.0 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were passed into 24-well plates (2 cm^2) and grown to confluence in 5–8 days. Control growth medium was removed, cells were washed with PBS, and incubated with 20% human serum for the same times as given above. Culture medium was collected and assayed as described below.

In Vitro Fibrinolytic Activity Assay

Fibrin plates were prepared according to the methods of Soeda et al. (32) with the following modifications. Wells of 10 μl volume were carved in the matrix using a 1-ml glass pipette tip. A 10-μl sample of concentrated conditioned medium was placed in each well and the plate was incubated at 37°C. After 18 hr of incubation, the area of lysis was calculated using the mean of 2 perpendicular diameters. Fibrinolytic activity was determined using a standard curve generated from human recombinant tissue plasminogen activator. Plasminogen activator inhibitor-1 activity was determined using a standard curve of inhibition of tissue plasminogen activator by human recombinant plasminogen activator inhibitor-1.

Viability of Cultured Endothelial Cells

The viability of cultured endothelial cells exposed to human serum was evaluated by the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI). Endothelial cells were incubated with various concentrations of human serum for up to 24 hr and assayed for incorporation and bioreduction of MTS© to formazan by measuring absorbance of formazan at 550 nm.

Results

Fibrinolysis in Cardiac Tissue

Hearts from transgenic pigs were transplanted into baboons. The hearts underwent functional and pathological changes consistent with acute vascular rejection over a period ranging from 5 days to 12 days. The changes included deposition of IgM, IgG, and small amounts of complement on donor endothelium as previously described (8, 9, 11, 29) (not shown). Specimens from pretransplant porcine hearts reacted with anti-tissue plasminogen activator antibodies and not with anti-plasminogen activator inhibitor-1 antibodies (Fig. 1). Tissue plas-

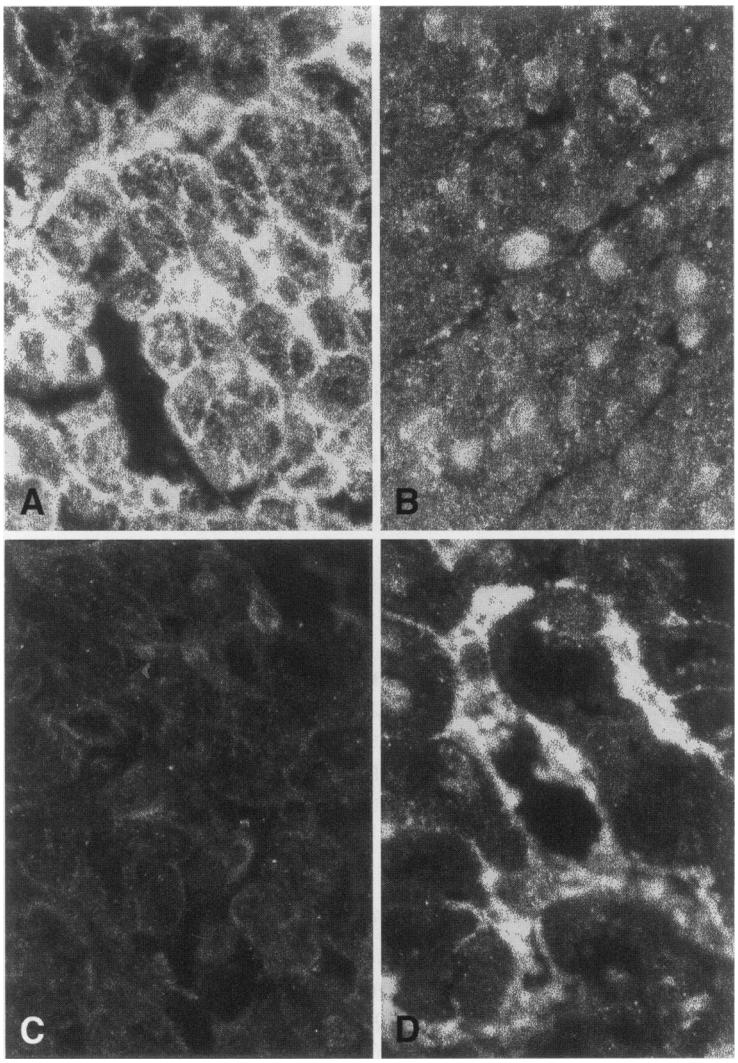

Fig. 1. Expression of tissue plasminogen activator and plasminogen activator inhibitor-1 expression in porcine-to-baboon cardiac xenografts. Tissue samples obtained from porcine hearts and porcine cardiac xenografts were studied by immunofluorescence for expression of tissue plasminogen activator and plasminogen activator inhibitor-1. **(A)** Normal porcine cardiac tissue stained for tissue plasminogen activator demonstrates staining throughout the cardiac microvasculature. **(B)** Normal porcine cardiac tissue stained for plasminogen activator inhibitor-1 demonstrates no detectable fluorescence above background. Hearts of transgenic pigs expressing human CD59 and decay accelerating factor were transplanted into baboons, and tissue biopsies were taken post-transplant. Cardiac xenografts with acute vascular rejection revealed a dramatic decrease in tissue plasminogen activator expression **(C)** and a significant increase in expression of plasminogen activator inhibitor-1 **(D)**. ×20. The increase in plasminogen activator inhibitor-1 is distributed along the vascular endothelium. The changes occurred prior to histologic evidence of acute vascular rejection. This suggests that the depletion of tissue plasminogen activator and upregulation of plasminogen activator inhibitor-1 contribute early to the pathogenesis of thrombosis seen in acute vascular rejection, and are not merely the result of tissue injury after acute vascular rejection has commenced.

minogen activator was located on vascular endothelium. Serial biopsies of porcine hearts transplanted into baboons exhibited an initial increase in tissue plasminogen activator at 1 hr post-transplant, followed by a progressive decrease to background levels at 120 hr. Plasminogen activator inhibitor-1 was positive by immunofluorescence 1 hr post-transplant and increased progressively up to 216 hr post-transplant (Figs. 1, 2). Plasminogen activator inhibitor-1 expression was also located along vascular endothelium in the transplant biopsy.

In Vitro Fibrinolytic Activity

Conditioned medium from porcine aortic endothelial cells grown in autologous serum had no detectable fibrinolytic activity. This finding is consistent with other studies of fibrinolytic proteins in endothelial cell cultures (33). Conditioned medium from porcine aortic endothelial cells grown in autologous serum had antifibrinolytic activity as defined by the ability of the sample to inhibit 7.35 nM tissue plasminogen activator in fibrin plate assays. When porcine aortic endothelial cells were stimulated with 20% human serum, the antifibrinolytic activity in the medium increased by 76% after 8 hr compared with that of medium from porcine aortic endothelial cells incubated with 20% autologous serum. A maximum increase in antifibrinolytic activity of 93% occurred in medium from cells that were stimulated for 18 hr (Fig. 3).

To determine if the increase in antifibrinolytic activity in medium from cultured endothelial cells was due to an increase in plasminogen

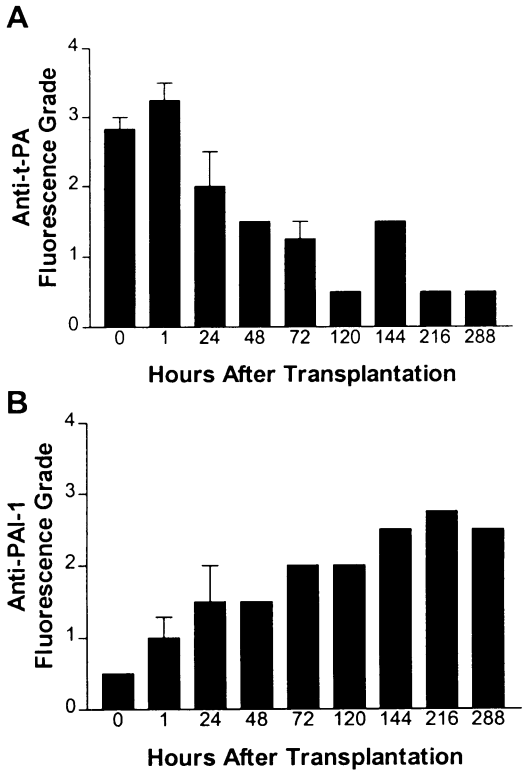

Fig. 2. Tissue plasminogen activator and plasminogen activator inhibitor-1 in pig-to-baboon cardiac xenografts. Tissue samples obtained normal porcine hearts and porcine cardiac xenografts were studied by immunofluorescence for expression of tissue plasminogen activator and plasminogen activator inhibitor-1. Fluorescence was graded on an assigned scale of 0–4 (4 = maximum). Average grades for the tissues at various times after transplantation are shown graphically. **(A)** Tissue plasminogen activator (t-PA). Tissue samples exhibited an initial increase in tissue plasminogen activator 24 hr after transplant, followed by a progressive decrease over time. **(B)** Plasminogen activator inhibitor-1 (PAI-1). The same biopsy tissues shown in A exhibited increased plasminogen activator inhibitor-1 beginning 1 hr after transplantation (B). These changes in tissue plasminogen activator and plasminogen activator inhibitor expression were seen before the development of histological evidence of acute vascular rejection, suggesting that a defect in fibrinolysis contributes to the pathogenesis of acute vascular rejection.

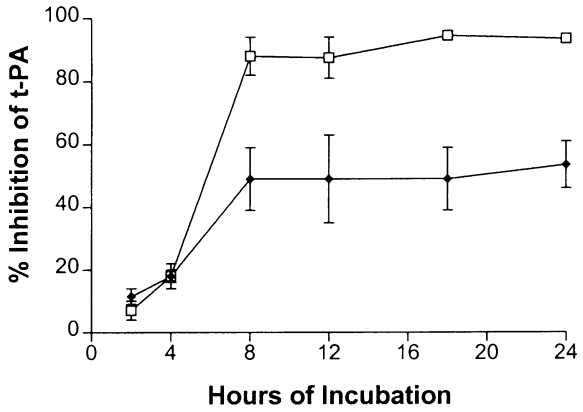

Fig. 3. Antifibrinolytic activity of conditioned cell culture medium from porcine aortic endothelial cells. Porcine endothelial cells were incubated with 20% autologous serum (diamonds) or 20% human serum (squares) for up to 24 hr. Serum was removed and the cells were incubated with serum-free medium for 24 hr. The cell culture medium was then harvested and assayed for antifibrinolytic activity by inhibition of 7.35 nM tissue plasminogen activator. The results are shown as the percentage of 7.35 nM tissue plasminogen activator that was inhibited by the conditioned medium. These findings suggest that stimulation with human antibody and complement alters the balance of expression and secretion of fibrinolytic and antifibrinolytic proteins.

activator inhibitor-1, the ability of anti-plasminogen activator inhibitor-1 antibody to block antifibrinolytic activity in conditioned medium was tested. Culture medium taken from porcine aortic endothelial cells stimulated with human serum was incubated with 15 nM anti-plasminogen activator inhibitor-1 antibody for 15 min at room temperature and then fibrinolytic activity was assessed. This medium, following the block of plasminogen activator inhibitor-1, contained fibrinolytic activity (Fig. 4), suggesting that plasminogen activator inhibitor-1 in the medium is responsible for the inhibition of fibrinolytic activity. A fixed concentration of antibody was incubated with medium from cells stimulated with human serum for 2 hr and for 24 hr. There was 44% more antifibrinolytic activity in the media recovered from cells stimulated for 24 hr than from media from cells that were stimulated for only 2 hr. These results demonstrate that both plasminogen activator inhibitor-1 and tissue plasminogen activator are secreted from porcine aortic endothelial cells in culture. The increase in antifibrinolytic activity in response to stimulation with human antibody and complement is at least partly attributed to an increase in plasminogen activator inhibitor-1 production and secretion. In addition, the amount of plasminogen activator inhibitor-1 secretion increases with the length of stimulation up to 24 hr as tested in our experiments.

To test the role of complement activation in regulation of antifibrinolytic activity, fibrinolytic

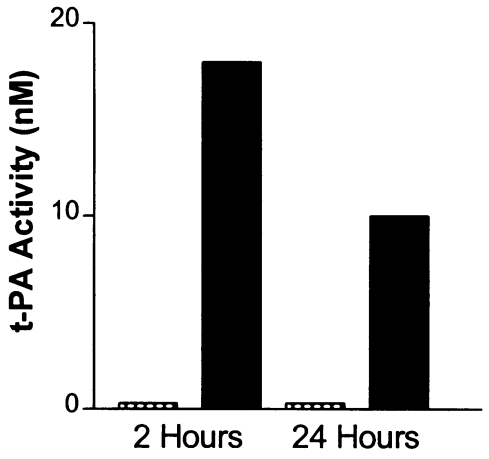

Fig. 4. Blocking of antifibrinolytic activity by anti-plasminogen activator-1 antibody. Wild-type porcine endothelial cells were stimulated with 20% human serum for 2 hr and 24 hr, then incubated with serum-free medium for 24 hr. The conditioned medium was then harvested. Medium samples were either assayed without antibody for fibrinolytic activity (stippled bars), or incubated with 15 nM anti-plasminogen activator-1 antibody (filled bars) for 15 min at room temperature before being assayed. Medium blocked with plasminogen activator-1 antibody demonstrated fibrinolytic activity, suggesting the presence of active plasminogen activator inhibitor-1 in the medium. Medium exposed to human serum for 24 hr before blocking had 44% less fibrinolytic activity than the medium exposed for 2 hr. Increases in antifibrinolytic activity in response to stimulation with human antibody and complement were at least partly attributable to an increase in plasminogen activator inhibitor-1 production and secretion. The amount of plasminogen activator inhibitor-1 secretion increased with a longer exposure to human serum.

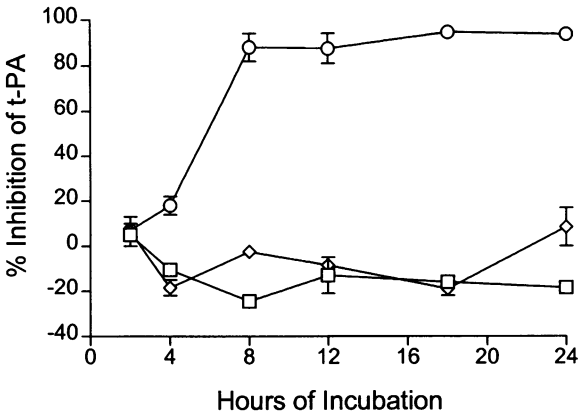

Fig. 5. Involvement of complement in the induction of antifibrinolytic activity in vascular endothelial cells. Porcine aortic endothelial cells were grown to confluence in autologous porcine serum. Transgenic porcine aortic endothelial cells that expressed human CD59 and DAF were grown to confluence in 10% FCS as a control medium. The cells were then stimulated with 20% human serum or control medium for times up to 24 hr. The cells were then stimulated with 20% human serum or control medium for times up to 24 hr. The serum was removed and the endothelial cells were incubated with serum-free medium for 24 hr. This conditioned medium was then harvested and assayed for fibrinolytic and antifibrinolytic activity. Wild-type porcine endothelial cells exhibited an increase in antifibrinolytic activity when stimulated with 20% human serum. Transgenic porcine endothelial cells did not have increased antifibrinolytic activity when stimulated with 20% human serum verses the control medium. These findings suggest that complement activation is the impetus that induces antifibrinolytic changes. Circles, wild-type porcine endothelial cells stimulated with human serum; diamonds, transgenic porcine endothelial cells stimulated with human serum; squares, transgenic porcine endothelial cells incubated with control FCS.

activity generated in porcine aortic endothelial cells expressing human CD59 and decay accelerating factor was compared with fibrinolytic activity generated in wild-type endothelial cells. The increase in antifibrinolytic activity that was observed following stimulation of wild-type porcine endothelial cells (Fig. 3) was prevented when similar endothelial cells that expressed human complement regulatory proteins were stimulated with human serum. The recovered medium from transgenic endothelial cells exposed to 20% human serum had no increased antifibrinolytic activity compared with wild-type porcine endothelial cells incubated under the same conditions (Fig. 5). The level of antifibrinolytic activity in the medium taken from transgenic cells was similar, regardless of whether the cells were incubated with human serum or the control growth medium containing 10% FCS (Fig. 5). Thus, when complement activation was impeded, the increase in antifibrinolytic activity was also impeded. These results suggest that the increase in antifibrinolytic activity observed was mediated at least in part by activation of complement by human antibodies during the 24-hr in vitro test period.

Discussion

The pathophysiology underlying the extensive vascular thrombosis observed during acute vascular rejection in xenotransplantation has not been thoroughly defined. Previous work has sug-

gested that inflammatory events activate vascular endothelium leading to procoagulant changes that account for the dramatic extent of fibrin deposition and thrombosis in acute vascular rejection (34). Experimental work from our laboratory and others has suggested that procoagulant activity seen in acute vascular might be caused by increased expression of tissue factor on vascular endothelium (35) and in inflammatory macrophages (36), loss of endothelial-associated heparan sulfate (37), and decreased availability and function of thrombomodulin (38,39). While these factors may contribute to increased generation of fibrin, the results of our study clearly demonstrate that there is also a fundamental disturbance in fibrinolysis owing to imbalance between tissue plasminogen activator and plasminogen activator inhibitor-1, resulting in a shift toward decreased fibrinolytic activity in porcine-to-primate xenotransplantation. Thus, to the extent that thrombosis in a xenotransplant is caused by aberration in the fibrinolytic cascade, therapeutic strategies aimed at acute vascular rejection may have to address this defect as well.

Current research supports the understanding that acute vascular rejection is associated with endothelial cell activation (1,4). In this report, we have attempted to establish what role alterations in the fibrinolytic system, as regulated by vascular endothelial cells and potentially arising in the context of endothelial cell activation, play in the process of acute vascular rejection. Our findings support the conclusion that changes in fibrinolysis might be involved in the pathogenesis of acute vascular rejection, rather than resulting as a consequence of tissue damage secondary to rejection because the increased expression of plasminogen activator inhibitor-1 and decreased expression of tissue plasminogen activator seen in xenograft biopsies preceded histologic evidence of tissue damage caused by acute vascular rejection.

The in vitro system that was employed in our studies involved stimulation of porcine aortic endothelial cells by human serum as a model for the reaction that occurs when a xenograft undergoes acute vascular rejection (1,37). In this system, the increase in antifibrinolytic activity was due at least in part to increases in plasminogen activator inhibitor-1 as antibodies against plasminogen activator-1 blocked the increased inhibitory activity. The fact that transgenic porcine cells expressing human complement regulatory proteins did not exhibit increased antifibrinolytic activity following stimulation with dilute human serum in vitro supports the conclusion that the activation of human complement on porcine endothelial cells may be one of the key steps in this process. In this setting, it is likely that complement activation directly stimulates endothelial cells, resulting in increased production and secretion of plasminogen activator inhibitor-1 and decreased production of tissue plasminogen activator, and thus altering the profibrinolytic state of the endothelial cells. This change in the fibrinolytic balance is likely one of the phenotypic events that leads to the thrombotic diathesis seen in acute vascular rejection. In considering this relationship between in vitro fibrinolysis and in vivo events, it is important to bear in mind that PAI-1 exists in an active form and as an inactive protein. The functional analysis of endothelial cell responses tests only the active form of the protein whereas immunohistochemistry as performed in this study might detect inactive protein as well as active protein.

One should note that we observed a significant difference in the protective effect of the transgenic expression of CD59 and decay accelerating factor between our in vitro and in vivo data. The results observed from tissue biopsies from transgenic animals demonstrate a progressive shift from a fibrinolytic to an antifibrinolytic state over the first 72 hr of the transplant. This is in contrast to the observation in vitro where the transgenic cells provided complete protection from antifibrinolytic activity following treatment with human serum. Although there are many potential interpretations of these data, one likely explanation for this difference is that the complement regulatory proteins prevent complement activation in the in vitro system in which a low concentration of complement (20% human serum) was used. On the other hand, it is likely that the protective effect of these proteins is overwhelmed when the endothelial cells are exposed to the normal levels of complement present in vivo (9,10). Consistent with this concept is the observation of Cozzi and co-workers that acute vascular rejection can sometimes be avoided if the xenogeneic organ graft expresses high levels of DAF (unpublished). Furthermore, the in vivo transplant may also be exposed to other inflammatory stimuli in addition to complement activation. These other stimuli, such as cytokines secreted by inflammatory cells are not affected by complement regulatory proteins.

The changes in tissue plasminogen activator and plasminogen activator inhibitor-1 expression may be explained by the following para-

digm. Upon exposure to human xenoreactive natural antibodies and complement, vascular endothelial cells in the graft are activated, resulting in an initial increase in both tissue plasminogen activator and plasminogen activator inhibitor-1. As the major source of tissue plasminogen activator, endothelial cells contribute to the total pool of tissue plasminogen activator through two mechanisms. At first there occurs a release of tissue plasminogen activator from cellular stores (40), secreted in response to stimuli such as shear stress (41) or thrombin (33,42). Initial stores are rapidly depleted and tissue plasminogen activator levels are reduced until an induced de novo synthesis occurs in 4–6 hr (40). Because endothelial cells do not contain stores of plasminogen activator inhibitor-1, the production of this molecule and secretion are delayed relative to the response of tissue plasminogen activator. Therefore, the increased ratio of plasminogen activator inhibitor-1 to tissue plasminogen activator, which impedes fibrinolysis, is delayed for a period of hours after endothelial cell activation. In addition, it has been shown that interleukin-1α is up-regulated in response to endothelial cell activation by complement. In turn, interleukin 1α suppresses the fibrinolytic system of endothelial cells by increasing activity of plasminogen activator inhibitor-1 (43,44) and decreasing activity of tissue plasminogen activator (43). This hypothesis is supported by the results of this study (Figs. 1, 2), which shows the course of the ratio of plasminogen activator inhibitor-1 to tissue plasminogen activator expression.

In summary, the findings of this study support the conclusion that the fibrinolytic process is altered during the process of acute vascular xenograft rejection. This suggests that further scientific and clinical experimentation that alters the fibrinolytic state of the xenograft may benefit graft survival in the setting of porcine-to-primate xenotransplantation.

Acknowledgments

This work was supported by grants from the National Institutes of Health (HL50985, HL52297, and HL46810). M. F. K. is a Howard Hughes Medical Institute Medical Student Research Training Fellow. J. H. L. is a Clinician Scientist Awardee of the American Heart Association. The authors thank Larkin Daniels and Michael Morowitz for their helpful suggestions as well as Tammy Moser for her technical expertise.

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