

HUMAN SERUM-INDUCED EXPRESSION OF E-SELECTIN ON PORCINE AORTIC ENDOTHELIAL CELLS IN VITRO IS TOTALLY COMPLEMENT MEDIATED¹

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TRANSPLANTATION 2001;72:1967-1973

Background. Whereas complement is a key mediator of hyperacute xenograft rejection, its role in acute vascular rejection (AVR) is a matter of controversy. AVR is associated with de novo synthesis of endothelial cell-derived inflammatory mediators, including the leukocyte-recruiting adhesion molecule E-selectin. Here we investigate the role and mechanism of complement in human serum-induced porcine endothelial cell activation.

Methods. An in vitro xenotransplantation method was designed using porcine aortic endothelial cells stimulated with human serum in microculture wells. E-selectin expression was measured by cell-enzyme immunoassay. Complement inhibitors acting at different levels in the cascade were investigated for their effect on E-selectin expression.

Results. E-selectin was strongly induced by normal human serum but not by heat-inactivated serum. Compstatin, a synthetic C3 inhibitor, markedly reduced human serum-induced E-selectin expression. Purified C1-inhibitor suppressed E-selectin induction completely, indicating activation through the classical or lectin pathway. Furthermore, a monoclonal antibody (mAb) that inhibits cleavage of C5 or another mAb that blocks the function of C7, completely inhibited the expression of serum-induced E-selectin, consistent with the terminal C5b-9 complement complex being the mediator of the endothelial cell activation. Inhibition of the alternative pathway using a novel antifactor D mAb did not reduce E-selectin expression.

Conclusion. Human serum-induced expression of porcine E-selectin is totally complement dependent, induced by a C1-inhibitor regulated pathway and mediated through the terminal complement complex. The data may have implications for therapeutic strategies, particularly of C1-inhibitor and anti-C5 mAb, to protect against endothelial cell activation and subsequent AVR of porcine xenografts.

INTRODUCTION

Hyperacute rejection (HAR) of porcine-to-primate xenografts is initiated by the binding of xenoreactive natural antibodies to donor endothelial cells (EC) followed by the activation of complement, predominantly via the classical pathway. Complement activation products induce type I activation of the endothelium, a rapid process independent of protein synthesis and characterized by reversible cell retraction, translocation of P-selectin to the luminal surface, generation of vasoactive substances, and release of heparan sulphate. These events make blood vessels more susceptible to procoagulant and complement-mediated injury. Subsequent to the loss of critical EC functions, the characteristic features of HAR manifest within minutes to a few hours, including interstitial hemorrhage, diffuse thrombosis, and irreversible organ damage (1).

HAR can be prevented by manipulating either the xenoreactive antibodies or complement, for example, by introduction of human complement regulatory proteins in pigs (2,3). Thus, the problems concerning HAR have principally been solved. However, in the absence of HAR, the xenograft will be rejected after a few days by a process known as acute vascular rejection (AVR) or delayed xenograft rejection (DXR). AVR is characterized by infiltration of the graft by host inflammatory cells and by diffuse intravascular blood clotting. In contrast to HAR, AVR seems to depend on protein synthesis-dependent EC activation (type II EC activation) (4). Although xenoreactive antibodies are definitely of importance for AVR (5), this process has until recently been implicated to be largely complement independent. However, a role for complement in AVR should be reconsidered. In fact, AVR in a life-supporting, pig-to-primate kidney model correlated with both fluid-phase and tissue complement activation in pig organs expressing human decay accelerating factor (hDAF) (6). Inhibition of complement by soluble complement receptor type 1 (sCR1) combined with immunosuppression delayed the occurrence of AVR of porcine hearts transplanted to cynomolgus monkeys (7). Furthermore, hDAF-transgenic porcine kidneys transplanted to cynomolgus monkeys were to some extent protected against AVR (8). Sublytic activation of complement might fail to induce HAR but still contribute to EC activation and subsequent AVR. In support for the occurrence of such activation, addition of anti-EC antibodies and complement in sublytic doses induced expression of tissue factor (9). Furthermore, porcine EC exposed to human serum expressed plasminogen activator inhibitor (10) and increased levels of inflammatory chemokines (11), the latter being dependent on complement activation and formation of the terminal C5b-9 complex. Similarly, C5b-9 and other pore-forming structures were recently shown to induce mRNA synthesis of several adhesion molecules including E-selectin (12).

To evaluate the contribution of complement in induction of porcine E-selectin, we tested the effect of different complement inhibitors in an *in vitro* system of porcine-to-human xenotransplantation. Both compstatin, a synthetic C3-binding peptide, which inhibits the C3 convertase activity, and C1-inhibitor, a physiological regulator of the classical and mannose binding lectin (MBL) complement pathways, inhibited HAR efficiently in *ex vivo* studies from our group (13,14). Here we show that compstatin, C1-inhibitor, and a novel inhibitory mAb to C5 abolished induction of E-selectin in porcine EC exposed to human serum. The effect was mediated by the terminal C5b-9 complement complex (TCC) because blocking of C7 abolished the expression of E-selectin. Blocking of the alternative pathway factor D had no effect on E-selectin expression. The data indicate an important role of complement in type II activation of xenograft vascular endothelium and points to C1-inhibitor and anti-C5 mAb as interesting candidates for future therapeutic strategies to prevent AVR.

MATERIALS AND METHODS

Reagents

Endothelial cell buffer (140 mM of NaCl, 4 mM of KCl, 1 mM of Na₂PO₄·2H₂O, 11 mM of glucose,

pH 7.4) was produced at the hospital pharmacy. Amphotericin B, gentamicin, endothelial serum-free medium (Endothelial-SFM) and Trypsin-EDTA were obtained from Life Technologies (Paisley, Scotland). Collagenase A was from Boehringer Mannheim (Mannheim, Germany) and 96-well cell culture clusters were purchased from Costar (Cambridge, MA). Gelatin from porcine skin and human recombinant tumor necrosis factor α (TNF- α) were obtained from Sigma Chemical Co. (St. Louis, MO) and 25-cm² culture flasks from Falcon (Becton Dickinson, Mountain View, CA). Human AB serum pooled from 10 individuals (Blood Bank, Ullevaal Hospital, Oslo, Norway) was immediately frozen and stored at -70°C. The following murine mAbs were used in cell-based ELISA (CELISA): antihuman C4d (Quidel, San Diego, CA), antihuman C3d (Quidel), antihuman TCC recognizing a neoepitope of C9 (15) to detect human complement deposition, and antihuman E-selectin (Endogen, Woburn, MA) cross-reacting with the porcine analogue (16) to detect porcine EC E-selectin expression. Rabbit-antimouse immunoglobulin (Ig) and horseradish peroxidase (HRP)-conjugated swine antirabbit Ig were purchased from DAKO (Glostrup, Denmark). Antibodies for the C3bBbP ELISA mAb to human properdin (clone #2) were purchased from Quidel, polyclonal rabbit antihuman C3c from Behringwerke AG (Marburg, Germany), and HRP-conjugated donkey antirabbit Ig from Amersham International (Little Chalfont, U.K.).

Complement Inhibitors

Compstatin and the corresponding control peptide have been described in detail previously (17). Purified C1-inhibitor from serum pools of blood donors was obtained from Immuno AG (Vienna, Austria). mAbs to human C5 (clone 137-76, IgG1) to factor D (clone 166-32, IgG1) and an isotype-matched control (IMC) mAb were all produced and purified under identical conditions in one of the author's laboratory (M.F.). Anti-C7 mAb was purchased from Quidel. According to the manufacture it inhibits complement lysis, which we confirmed in a standard complement hemolytic assay in our own laboratory. Finally, heat-inactivated human AB serum pool (identical to the test pool but heated to 56°C for 30 min) was included as a serum sample without complement activity.

Porcine Aortic Endothelial Cell Culture

Fresh porcine aortae obtained from a local slaughterhouse were cut distal to the aortic arch and immediately placed into a sterile beaker containing endothelial cell buffer, 2.5 μ g/ml of amphotericin B, and 50 μ g/ml of gentamicin. The aortae were transported to the laboratory within 30 min and transferred to a second beaker containing fresh endothelial cell buffer with antibiotics at 4°C. The intercostal vessels were clamped with LigaClips (Ethicon, Cincinnati, OH) and the aorta was cannulated at both ends with wide-bore suction tips that were fixed with self-locking cable strips. The vessel was flushed with endothelial cell buffer to remove blood cells, subsequently filled with 0.1% collagenase, and incubated in a water bath at 37°C for 4–8 min. The collagenase solution was then flushed back and forth through the aorta 3–4 times to detach endothelial cells and transferred to a tube containing Endothelial-SFM with 5% heat inactivated fetal calf serum (FCS) and antibiotics. The cells were centrifuged (170 g, 10 min) and resuspended in fresh medium before plating in gelatin-coated (1%) culture flasks (25 cm²). The concentration of serum and amphotericin B was reduced to 1% and 0.5 μ g/ml after 1 and 7 days in culture, respectively. Subconfluent primary cultures were trypsinized and cultured to confluence in the first passage before being frozen for storage in aliquots.

Porcine-to-Human In Vitro Method

Porcine aortic endothelial cells (PAEC) were plated in 96-well microculture plates and grown to confluence in Endothelial-SFM containing 1% FCS, 0.5 μ g/ml of amphotericin B, and 50 μ g/ml of gentamicin. The cells were exposed to 100 μ l/well of pooled human AB serum (as a source of xenoreactive antibodies and complement) and different concentrations of complement inhibitors for 4 hr at 37°C. After pilot experiments, we decided to use 25% or 50% serum. The different activation markers were analyzed by means of a cell-based ELISA (CELISA) described in detail elsewhere

(18). In brief, the cells were fixed in 0.5% periodate-lysine-paraformaldehyde (10 min, 20°C) and incubated with 50 μ l of primary mAb (45 min, 20°C) followed by three washes with phosphate-buffered saline (PBS). To measure the induction of E-selectin, we used a mAb-to-human E-selectin that has been found to cross-react with the porcine homolog (16). The secondary rabbit antimouse Ig and the final HRP-conjugated swine antirabbit Ig were applied in the same manner. O-phenylenediamine in citrate buffer was used as peroxidase substrate solution, and the color reaction was read at 490 nm. The microculture plates were subsequently stained with 0.1% crystal violet, washed in tap water, and the nuclear stain eluted in acetic acid before reading the optical density (OD) at 550 nm. Thus, the OD ratios presented are corrected for the number of cells present in each well. Cells incubated with medium alone and stained with anti-E-selectin or cells incubated with human serum and stained with an isotype- and concentration-matched control Ab served as negative controls, whereas cells stimulated with TNF- α (100 U/ml) served as a positive control.

Assays for Complement Activation Products

The ELISAs for C5a and TCC determination have been described in detail previously (19,20). The reagents for the C5a assay were a kind gift from Prof. Kåre Bergh, Trondheim, Norway. The assay for the alternative convertase C3bBbP was performed as follows: capture Ab was mouse monoclonal antihuman properdin diluted 1:1000. Test samples were diluted 1:25. Detection was made by polyclonal rabbit antihuman C3c diluted 1:1000 and HRP-conjugated antirabbit Ig diluted 1:1000. The rest of the assay was performed as for the C5a and TCC assays. The standard for all three assays was zymosan-activated human serum pool (ZAS) made by incubating serum with zymosan (Sigma), 10 mg/ml, for 60 min at 37°C. After centrifugation, the supernatant was split and stored in aliquots at -70°C. The ZAS was defined to contain 1000 arbitrary units (AU)/ml. For the C5 mAb experiments, 10% human serum was activated by 1 mg/ml of zymosan.

Sodium Dodecyl Sulfate/Polyacrylamide Gel Electrophoresis and Western Blot of Human C5

Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot were performed as described (21), using 7.5% homogenous Excel SDS Gel (Amersham Pharmacia Biotech, Uppsala, Sweden). Five microliters of purified protein C5 (10 μ g/ml)/lane (Quidel) was used as antigen. The protein was reduced by dithiothreitol (Amersham Pharmacia Biotech) after boiling the protein with SDS. Rainbow Molecular Weight Protein Markers (Amersham Pharmacy Biotech) were included. The separated proteins were blotted onto 0.2- μ m pore nitrocellulose membrane (Amersham) and incubated overnight with goat antihuman C5 (Quidel), diluted 1/500, or mouse antihuman C5 (clone 137-76) at 8 μ /ml. HRP-conjugated mouse antigoat (Jackson ImmunoResearch, West Grove, PA) 1:25,000 and HRP-conjugated sheep antimouse (Amersham Pharmacia Biotech) 1:1000, incubated for 2 hr at room temperature, served as detection antibodies, respectively. All dilutions were performed in PBS containing 0.2% tween 20 (Sigma) at room temperature. Between each incubation, the blots were washed three times for 10 min in PBS with 0.1% tween 20. Finally, the blots were developed using 3,3'-diaminobenzidine (Sigma) and H₂O₂.

RESULTS

In preliminary experiments, we established that a pool of human AB serum induced the deposit of C3d and TCC, as well as the expression of E-selectin on PAEC. The level of E-selectin expression induced by 25% or 50% human serum peaked at 4 hr and was similar to that induced by 100 U/ml of TNF- α , whereas virtually no expression was seen using heat-inactivated serum (HIS) (Fig. 1). To establish a role for complement in this activation, we initially used the C3-binding peptide, compstatin, which blocks the activation of C3 and thus inhibits further activation from all initial pathways of complement. Compstatin (10–240 μ M) inhibited human serum-induced E-selectin expression in a dose-dependent manner and markedly reduced the deposition of C3d and TCC in the PAEC cultures.

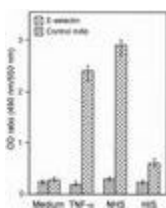


FIGURE 1. Effect of TNF- α , normal human serum, and heat-inactivated serum on porcine endothelial cell expression of E-selectin. Porcine endothelial cells were incubated for 4 hr with medium, 100 U/ml of TNF- α , a 50% dilution of a pool of normal human AB serum (NHS), or the same serum pool heat inactivated serum (HIS). Expression of E-selectin (crosshatched bars) was measured by a cell enzyme-linked immunosorbent assay and compared with the signal obtained using an isotype matched control (IMC) antibody (single-hatched bars). Median and 20–80 percentile of six parallel observations in 1 experiment out of more than 20 are shown. Values are given as OD ratios correcting for the amount of cells present in each well.

To further dissect the mechanism of activation and establish the mediator level for this complement-induced protein synthesis-dependent (type II) EC activation, we next used purified C1-inhibitor, a major control protein of the classical and lectin pathways. C1-inhibitor reduced the human serum-induced deposition of C4d (Fig. 2, left panel) and TCC (Fig. 2, right panel) on PAEC in a dose-dependent manner, confirming its complement inhibitory effect although not excluding its noncomplement associated effects. Human serum-induced E-selectin expression was reduced in the presence of C1-inhibitor, reaching a 90% inhibition level at 4 mg/ml and being completely abolished at higher concentrations (Fig. 3).

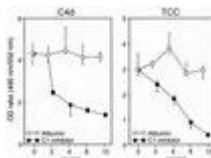


FIGURE 2. Effect of C1-inhibitor on complement activation. Effect of C1-inhibitor (closed circles) and albumin (open circles) on deposition of C4d (left panel) and TCC (right panel) in PAEC cultures exposed to 50% human serum. Median and 20–80 percentile of six parallel observations in one representative experiment out of three is shown. Values are given as OD ratios correcting for the amount of cells present in each well.

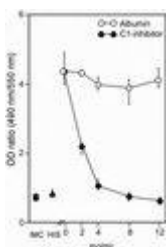


FIGURE 3. Effect of C1-inhibitor on E-selectin expression. Effect of C1-inhibitor (closed circles) or albumin (open circles) on E-selectin expression levels in PAEC cultures exposed to 50% human serum. Closed square represents the isotype matched control (IMC) of cells incubated with serum. Closed triangle represents E-selectin expression on cells incubated with heat inactivated serum (HIS). Median and 20–80 percentile of six parallel observations in one representative experiment out of six is shown. Values are given as OD ratios correcting for the amount of cells present in each well.

To ascertain a role for the terminal pathway in human serum-induced E-selectin expression, we studied the effect of inhibition of C5 activation using the C5-specific mAb 137-76. This antibody specifically binds the β -chain of human C5 in Western blot (Fig. 4) and detects a single band in human serum corresponding to the molecular weight of nonreduced C5. The antibody completely blocked C5 cleavage and the formation of C5a and TCC (Fig. 5, left and middle panels), whereas the isotype-matched negative-control mAb had no effect. As expected, the anti-C5 mAb had no effect when tested for alternative pathway convertase formation (C3bBbP) (Fig. 5, right panel). Notably, mAb 137-76 reduced E-selectin expression on PAEC in a dose-dependent manner to baseline levels at 10 μ g/ml (Fig. 6), indicating that the EC activation was not only complement dependent but totally mediated through the terminal pathway.

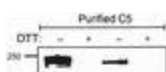


FIGURE 4. Reactivity of anti-C5 (clone 137-76) in Western blot. Western blot of

purified C5 in the presence or absence of dithiothreitol (DTT) reacted with a polyclonal antiserum to C5 (anti-C5 pAb) or with the monoclonal antibody 137-76 (anti-C5 mAb). First lane identifies native C5 and the second lane the alpha and beta chains (115 and 75 Kd, respectively). mAb 137-75 reacts with native C5 (third lane) and the epitope is located on the beta-chain (last lane).

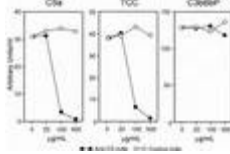


FIGURE 5. Effect of anti-C5 on terminal complement pathway activation. Serum (10%) was activated with zymosan (1 mg/ml) in the presence of anti-C5 (clone 137-76) (closed circles) or IMC mAb (open circles). Anti-C5 completely blocked formation of C5a (left panel) and TCC (middle panel), whereas no effect was seen on formation of the alternative C3BbP convertase (right panel). The control mAb had no effect in either of the assays. One representative experiment out of three is shown. Values are given in arbitrary units (AU) using a standard of 100% zymosan-activated serum defined to contain 1000 AU/ml.

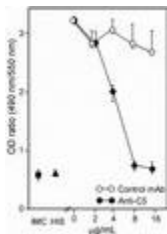


FIGURE 6. Effect of anti-C5 mAb on E-selectin expression. Effect of anti-C5 (clone 137-76) (closed circles) or isotype matched control (IMC) mAb (open circles) on E-selectin expression levels in PAEC cultures exposed to 25% human serum. Closed square represents the IMC of cells incubated with serum. Closed triangle represents E-selectin expression on cells incubated with heat inactivated serum (HIS). Median and 20–80 percentile of six parallel observations in one representative experiment out of four is shown. Values are given as OD ratios correcting for the amount of cells present in each well.

We next investigated the mechanism of the terminal pathway by which the anti-C5 mAb inhibited E-selectin expression. Anti-C7 mAb inhibited E-selectin expression virtually completely (Fig. 7, left panel), indicating that the terminal C5b-9 complement complex was responsible for the complement-mediated effect. Finally, we investigated a possible role for the alternative complement pathway in inducing the E-selectin expression. Addition of antifactor D mAb in doses up to 100 μ g/ml failed to reduce the expression of E-selectin (Fig. 7, right panel), although the antibody neutralized hemolytic factor D activity completely at a 1:2 molar ratio (22).

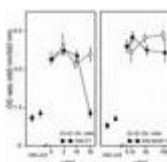


FIGURE 7. Effect of anti-C7 mAb and anti-factor D mAb on E-selectin expression. (Left panel) E-selectin expression levels in PAEC cultures exposed to 50% human serum in the presence of anti-C7 mAb (closed circles) or IMC mAb (open circles). (Right panel) E-selectin expression levels in PAEC cultures exposed to 50% human serum in the presence of antifactor D mAb (closed circles) or IMC mAb (open circles). (Both panels) Closed square represents E-selectin expression on cells incubated with HIS. Closed triangle represents E-selectin expression on cells incubated with 20 μ g/ml of anti-C5 mAb 137-76 (α C5). Median and 20–80 percentile of six parallel observations in one representative experiment out of four is shown. Values are given as OD ratios correcting for the amount of cells present in each well.

DISCUSSION

This study shows that human serum-induced expression of E-selectin in porcine EC cultures completely depends on complement activation. Induction of E-selectin was abolished by C1-

inhibitor, whereas antifactor D had no effect. Furthermore, we show unequivocally that the terminal complement pathway is responsible for the effect, because a mAb that inhibits cleavage of C5 abolished induction of E-selectin. Suppression of E-selectin expression by specific blocking of C7 revealed that the mediator is the terminal C5b-9 complement complex.

The major obstacle to clinical xenotransplantation to date is AVR. Although complement is clearly necessary for the pathogenesis of HAR, its role in the development of AVR is still unclear (23). A series of studies in small animal models observed AVR after a few days despite functional inactivation of the complement system, thus arguing that vascular rejection is complement-independent. However, inactivation of complement was in most of these studies achieved by means of cobra venom factor (CVF), which is an activator of complement rather than an inhibitor. Although usually well tolerated, CVF treatment elicits massive fluid-phase complement activation and adverse effects are regularly seen (24,25). Thus, vascular rejection in CVF-treated animals may instead be aggravated by complement activation. Furthermore, the CVF-induced complement activation is measured as total hemolytic activity, a read-out too insensitive to detect de novo synthesis of complement factors or exclude low levels of complement still present in the circulation. Moreover, the fact that sCR1 and CVF, more strongly than CVF alone, reduced macrophage activation and cytokine production in xenografts, indicates that CVF-mediated inactivation of complement is suboptimal (24). Complement has also been inactivated by blocking cleavage of C5, thereby preventing HAR but not AVR (26). However, some residual hemolytic activity was still present (26), which may have contributed to EC activation and AVR. Furthermore, AVR is also observed in C6-deficient rats that fail to mount HAR due to lack of TCC formation (27). However, the complement system of these animals is fully active down to C6, and C6 generated by the graft may have significant effects in the host (28). Thus, although the level of complement required to obtain substantial EC activation in vivo is currently unknown, it is tempting to speculate that small amounts of complement in the presence of antibodies may induce EC activation and AVR but fail to elicit HAR in these animal models.

A role for complement in AVR may be more obvious in porcine-to-primate xenograft models than in the small animal models, perhaps because complement inhibition was more directly targeted (7). Thus, in vivo studies with porcine transplants to cynomolgus monkeys indicated that complement inhibition partially protects against AVR (7,8) and that AVR in a similar model was closely associated with complement activation (6). Although it has been shown that immune sera from monkeys and different purified agonists binding to the galactosyl epitope on PAEC are able to induce type II activation in the absence of complement (29), our results indicate that such factors are not present in normal human serum, because the response we observed was fully complement dependent.

A major limitation of our serum based-study is the lack of xenoreactive factors present in whole blood. Thus, we limit our conclusion to the role of serum in type II EC activation and are cautious to interpret them with respect to complement as crucial for AVR. Despite these limitations, our data clearly point to complement as an important mediator of type II EC activation in the porcine-to-human combination. This is in accordance with the data from Selvan et al. (11), who demonstrated increased chemokine expression (monocyte chemoattractant protein-1, interleukin (IL)-8, and RANTES) when porcine EC were incubated with human serum. The mechanism was shown to be complement dependent, because it was inhibited with sCR1. Furthermore, C8-deficient serum failed to induce chemokine expression, indicating that C5b-9 was responsible for the effect. In further support of a role for complement, the same group demonstrated complement-dependent IL-1 production, leading to expression of mRNA for numerous adhesion molecules, including E-selectin, associated with type II EC activation by stimulating porcine EC with the C5b-9 complex and other pore-forming substances (12).

We studied the expression of E-selectin as a read-out for the type II EC, because this is an important adhesion molecule in leukocyte recruitment to inflammatory lesions and may be of importance not

only for AVR but even for cellular-mediated rejection of xenografts (30). Preliminary studies using compstatin or heat-inactivated serum indicated that the serum-induced activation was largely complement dependent. Porcine-to-primate HAR depends mainly on classical pathway activation, although an additional role for the alternative pathway has been suggested (31,32). The complete inhibition of E-selectin expression in the presence of C1-inhibitor may be explained by activation through the classical pathway, but it should be noted that C1-inhibitor also controls the MBL pathway (33), and as a serine protease inhibitor, it also has effects on other cascades like the kinin-kallikrin and coagulation systems (34). The recent observation that AVR can be attenuated by peptides that block binding of immunoglobulins to C1q points to a role for the classical pathway of complement in this rejection mechanism (35). The fact that C1-inhibitor and the anti-C5, mAb-inhibited E-selectin expression to the same extent indicates that the C1-inhibitor effect was complement mediated and not depending on noncomplement plasma cascade systems. Cleavage of C5 produces two potential candidates for cell activation, the inflammatory anaphylatoxin C5a and the terminal C5b-9 complex. In sublytic doses the latter induce inflammation as well. In our experimental system the expression of E-selectin was found to depend on C5b-9 formation, because the effect was abolished by blocking C7.

Inhibition of human complement-induced porcine EC activation type I and cell cytotoxicity, characteristics of HAR, has been shown previously. Thus, C1-inhibitor protected porcine EC against cytotoxicity (36) and against loss of heparan sulphate (37). Similarly, blocking of C5 and C8 also attenuated porcine EC endothelial cell cytotoxicity and prothrombinase activity (38). To our knowledge, this study is the first report that C1-inhibitor and blocking of C5, using a novel anti-C5 mAb, also very efficiently inhibits porcine EC type II, characteristic of AVR. C1-inhibitor acts at the very beginning of the activation and leaves the alternative and terminal pathways open for protection against infection. Furthermore, C1-inhibitor reduces formation of activation products from C4, C2, and C3. On the other hand, anti-C5 mAb also completely inhibited EC activation and would keep all three initial pathways open for host defense, which may be beneficial for long-term treatment.

In conclusion, we have shown that human serum-induced expression of porcine E-selectin is triggered through a C1-inhibitor-controlled complement pathway, activation of C5, and formation of the terminal C5b-9 complement complex. Although we cannot exclude amplification by the alternative pathway, its inhibition failed to abolish EC activation. Thus, C1-inhibitor and in particular the novel anti-C5 mAb 137-76 are promising candidates to inhibit porcine E-selectin induced by human serum. These findings may have significant implications for therapeutic strategies to protect against EC activation and prevent graft rejection in porcine-to-human xenotransplantation.

Acknowledgments.

The authors thank Grethe Bergseth for excellent technical assistance with the SDS-PAGE and Western blot experiments and Lynn Spruce for the peptide synthesis.

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¹ This study was financially supported by Novartis Norway, Tanox Inc., Houston, TX, The Research Council of Norway, The Norwegian Foundation for Health and Rehabilitation, and by NIH grant GM62134.

Received 29 March 2001.

Accepted 6 June 2001.

Transplantation 2001 December 27;72(12):1967-1973