American Journal of Pathology, Vol. 140, No. 5, May 1992
Copyright © American Association of Pathologists

Mechanism of Complement Activation in the Hyperacute Rejection of Porcine Organs Transplanted into Primate Recipients

Agustin P. Dalmasso,*†‡ Gregory M. Vercellotti,‖ Richard J. Fischel,¶ R. Morton Bolman,¶ Fritz H. Bach,†‡¶ and Jeffrey L. Platt†#**

From the Veterans Affairs Medical Center, Minneapolis, and the Immunobiology Research Center,† and the Departments of Laboratory Medicine and Pathology,‡ Medicine,‖ Surgery,¶ Pediatrics,# and Cell Biology and Neuroanatomy,** University of Minnesota Medical School, Minneapolis, Minnesota*

The authors investigated the importance of natural antibody and complement in the pathogenesis of hyperacute xenograft rejection using* in vivo *and* in vitro *pig to primate models. Studies were carried out in rhesus monkeys transplanted with a pig heart or kidney in which hyperacute rejection was observed within a few hours. The rejected organs showed deposits of IgM, C3, C4, C5, and C9 neoantigen along small blood vessels, but few deposits of factors B and P. Removal of anti-endothelial cell "natural" antibodies by plasmapheresis, immunoabsorbtion, and immunosuppression techniques resulted in marked prolongation of the survival of a subsequently transplanted heart, even when complement levels were within the normal range. Thus, complement, in the absence of natural antibodies, did not initiate hyperacute rejection in this species combination. The requirements for complement activation in human serum to cause cytotoxicity of porcine endothelial cells were then evaluated. Cytotoxicity was abrogated by depleting human serum of IgM, C2, or C5, but not of factor B. Restoration of the effect of serum on endothelial cells was achieved by reconstitution of the respective depleted sera with purified IgM or with the corresponding complement proteins, indicating that IgM and the classical, but not the alternative, pathway of complement, were involved. Identical conclusions were drawn from experiments to ascertain the requirements for complement activation in human serum to mediate binding of iC3b to porcine endothelial cells. The authors conclude that in a pig to primate xenograft complement does not directly initiate injury to the graft but rather requires activation by bound xenoreactive natural antibodies; IgM antibodies directed against endothelial cells activate the classical complement pathway, which then contributes to endothelial cell activation and subsequent events characteristic of hyperacute rejection. *(Am J Pathol 1992, 140:1157–1166)*

Activation of the complement system is considered to be essential in the pathogenesis of hyperacute rejection of immediately vascularized xenogenic organs such as the heart or kidney (reviewed in [1,2]). The central role of complement in hyperacute rejection was demonstrated in decomplemented or congenitally complement deficient animals that showed significant prolongation in the survival of organ xenografts in comparison to normocomplementemic controls.[3–9] It has been believed that complement activation in hyperacute rejection is triggered by the deposition of "natural" antibody in a graft.[1,2,10] However, in some species combinations the alternative pathway may be activated by xenogeneic erythrocytes without involvement of antibodies.[11,12] Moreover, reports suggest that the alternative complement pathway may initiate rejection in guinea pig to rat[13] and in rabbit to newborn pig[14] xenografts. We have found that primate serum has naturally occurring IgM antibodies that bind to porcine endothelial cells, suggesting that complement activation might occur

Supported by the Department of Veterans Affairs Medical Research, an NIH grant (DK13083)), a Clinical Research grant (no. 6-572) from the March of Dimes Birth Defects Foundation, and the Minnesota Medical Foundation. J. L. Platt is an Established Investigator of the American Heart Association. F. H. Bach holds the Helen and Milton Kimmelman Chair in Immunobiology. This article is publication number 578 from the Immunobiology Research Center, Veterans Affairs, Minneapolis, Minnesota.

Accepted for publication December 26, 1991.

Address reprint requests to Dr. Agustin P. Dalmasso, Veterans Affairs Medical Center, One Veterans Drive, Minneapolis, MN 55417.

via the classical pathway.[2,15] We asked whether the alternative pathway might contribute to hyperacute rejection in a model that is likely more clinically relevant and also tested whether endothelial cells, the apparent target of hyperacute rejection,[2] would activate complement directly.

We studied the mechanism of complement activation as it contributes to the rejection of porcine organs transplanted into primates. We first investigated complement deposition as it occurs in porcine hearts and kidneys transplanted into rhesus monkeys and found that, during rejection, components of the classical pathway along with IgM are codeposited on vessels. We also found that xenografts could survive in recipients with normal serum complement levels but in which natural antibodies had been depleted, suggesting that complement was not directly activated by the graft. The role of complement was also evaluated in an *in vitro* model of xenotransplantation consisting of porcine endothelial cell monolayers as targets and human serum as source of natural antibody and complement. Our findings indicate that in pig to primate combinations, hyperacute rejection is initiated when natural antibodies bind to porcine endothelium and trigger the activation of the classical complement pathway. In this combination, no evidence was obtained of antibody-independent direct activation of either the classical or alternative pathway by the endothelium of the donor organ.

Materials and Methods

Source of Complement and Antibodies Against Porcine Endothelial Cells

Serum from normal human subjects and from rhesus monkeys was used as a source of natural antibodies and heterologous complement. The sera were stored at −70°C and thawed only once. Serum from a patient with C2 deficiency was also used.[16] Human serum pools that were selectively depleted of C5 or factor B by affinity chromatography were purchased from Quidel (San Diego, CA). Restoration of hemolytic activity of C2-deficient serum to low normal CH50 was achieved by reconstitution with 2000 units of purified C2 (Diamedix, Miami, FL) per ml of serum. The reconstituted serum was dialyzed against RPMI medium 1640 containing 25 mmol/l HEPES (pH 7.4) (RPMI, United Life Technologies, Grand Island, NY) before use. Restoration of hemolytic activity in C5-depleted serum to normal CH50 was performed by addition of 75 ug of purified C5[17] per ml of serum. Reactivity of antibodies against porcine endothelial cells was measured by an enzyme-linked immunosorbant assay (ELISA), as previously described.[15] Classical pathway complement activity (CH50) was quantitated with sheep red cells sensitized with rabbit hemolysin.[18,19] Alternative pathway complement activity (AP50) was assessed with a rabbit red cell assay.[11]

IgM-depleted human serum was prepared by affinity chromatography of a serum pool obtained from 12 normal blood donors. Chromatography was carried out on agarose (Affi-gel 10, Biorad Laboratories, Richmond, CA) conjugated to rabbit IgG anti-human IgM, in the presence of 0.01 mol/l Na_2 ethylenediaminetetraacetic acid (Na_2 EDTA). IgM-depleted serum was concentrated, dialyzed against RPMI, and reconstituted with 0.5 mg of purified human C1q [20] per ml of serum. Removal of IgM was then determined by radial immunodiffusion on low level IgM plates (Kallestad Laboratories, Chaska, MN) and found to be greater than 95%. The CH50 of this reagent, after reconstitution with C1q, was 92% of that in the original serum. In some experiments, the IgM-depleted serum was reconstituted with IgM isolated from a human serum with a high titer of anti-endothelial cell antibodies.[15] Solid polyethylene glycol 6000 was added to the serum to a concentration of 4% (w/v), the precipitate was solubilized in phosphate-buffered saline (pH 7.4) and chromatographed on Sepharose 6B. The fractions containing IgM were pooled and concentrated to 3.2 mg of protein/ml.

Endothelial Cells

Endothelial cells were explanted from porcine aortae and cultured as described previously.[21] Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, United Life Technologies) containing 10% fetal bovine serum with L-glutamine and penicillin/streptomycin. The cells were identified as endothelial cells by their morphology and their ability to take up acetylated LDL.[22] Experiments were performed with cells in passages 5 to 16 which were grown to confluency in 24-well Costar plates (Cambridge, MA).

Endothelial Cell Cytotoxicity Assay

Endothelial cells were labeled with ^{51}Cr in RPMI as previously described.[23] Labeled cells were incubated with primate serum diluted in RPMI. Incubation was carried out at 37°C in a 5% CO_2 atmosphere, usually during 4 hours. After incubation supernatants were removed and the radioactivity was counted in the supernatants and cell monolayers. Percent specific lysis of endothelial cells was calculated as previously described.[23]

Assay of Endothelial Cell iC3b Binding

The binding of iC3b to porcine endothelial cells was evaluated by ELISA in triplicate wells as follows. Endothelial

cell monolayers were cultured in 96-well plates (Flow Laboratories, Inc., McLean, VA) and were 2 days postconfluency at the time of use. The cells were first incubated during 1 hour with 25% heat inactivated (30 minutes at 56°C) human serum as source of antiporcine endothelial cell antibodies. After washing with DMEM, they were incubated for 1 hour with 25% normal or complement-deficient human serum diluted in DMEM. Incubations were carried out at 37°C in a 5% CO_2 atmosphere. After incubation supernatants were removed, and the cells were washed three times with phosphate buffered saline (pH 7.4), fixed with 0.1% glutaraldehyde, and blocked with 1% bovine serum albumin. Deposition of iC3b on the cells was then detected with a murine monoclonal antibody against iC3b neoantigen (Quidel, San Diego, CA) and affinity-isolated, alkaline phosphatase-conjugated goat anti-mouse IgG (Zymed, South San Francisco, CA). Reaction product was generated using p-nitrophenylphosphate as substrate and was assayed at 405 nm using a Titertek Multiscan MCC plate reader (Flow Laboratories, McLean, VA).

Organ Transplantation in Rhesus Monkeys

Porcine hearts and kidneys were obtained from heparinized 10-kg to 20-kg outbred pigs. The organs were perfused with cold Ringer's lactate solution until all visible blood had been removed and then placed in the circulation of anesthetized male rhesus monkeys weighing 8 to 10 kg. Untreated rhesus monkeys received a porcine heart or kidney xenograft as described previously.[24,25] Natural anti-porcine endothelial cell antibodies were depleted from two rhesus monkeys by two exchanges of two plasma volumes each, using human serum albumin as replacement fluid, as described previously,[24] and by circulating the blood of a heparin-treated rhesus monkey sequentially through two porcine kidneys for 30 minutes each at room temperature, as described previously.[24,25] This treatment resulted in significant depletion of the levels of natural antibodies to porcine endothelial cells in both monkeys. Immunosuppressive agents were given intravenously and consisted of Minnesota anti-lymphoblast globulin (a gift of Dr. Richard Condie), 100 mg/kg/day; cyclosporine A (a gift of Sandoz Corporation), 2 mg/kg/day for 2 days followed by 1.5 mg/kg/day; azathioprine, 5 mg/kg/day for 2 days followed by 2.5 mg/kg/day; and prednisone, 2 mg/kg/day, tapered every other day by 0.25 mg/kg. All immunosuppressive agents were started on day −2, with day of transplant being day 0. The animals were transplanted with a porcine heart. One rhesus monkey died at day 1 due to hemodynamic problems and the other rhesus monkey had to be killed at day 8 due to wound infection. However, there were no signs of rejection detected up to the time of death.

Immunofluorescence

Tissue samples were snapfrozen in precooled isopentane and processed for immunohistologic techniques as previously described.[26] Goat antibodies to human immunoglobulins, complement components, albumin, and α2-macroglobulin were obtained from Organon-Teknika (West Chester, PA). Goat antisera to human factor B and factor P were purchased from Calbiochem (San Diego, CA). A mouse monoclonal antibody against a neoantigen of polymerized human C9 was used as described previously to represent the membrane attack complex of complement.[27] All polyclonal antisera to human proteins gave strong crossreactivity with rhesus monkey proteins when tested by double immunodiffusion techniques. Antibodies and antisera were absorbed with pig serum before use. Positive controls for all anticomplement reagents were performed with rhesus kidney tissues containing glomerular immune deposits.

Results

Immunofluorescence Studies of Rejected Organs

Hyperacute rejection of porcine heart or kidney transplanted into rhesus monkey occurs 2 to 3 hours after vessel anastomosis.[1,2] The histology of tissues from xenografts undergoing rejection was characterized by diffuse interstitial edema and focal hemorrhage and prominent platelet and fibrin thrombi in small blood vessels. Immunopathologic analysis of tissues from xenografts in the process of rejection demonstrated deposits of C3, C4, C5 (not shown), and C9 along endothelial surfaces, in a distribution similar to that of IgM (Figure 1). No deposits of factors B and trace amounts of factor P were observed (Figure 1). The distribution of IgG deposits was the same as that of albumin and α2-macroglobulin (not shown), suggesting that the presence of IgG in rejected tissues may reflect exudation from blood vessels.[28] Similar results were obtained with kidney xenografts. Thus, rejection of porcine organs by rhesus monkeys is accompanied by endothelial cell binding of complement components of the classical pathway and membrane attack complex but minimal deposits of complement proteins that participate exclusively in the alternative pathway.

Survival of Xenografts in Animals with Normal Complement Levels

Rhesus monkeys that had their blood perfused over porcine kidneys and plasma exchange exhibited significant

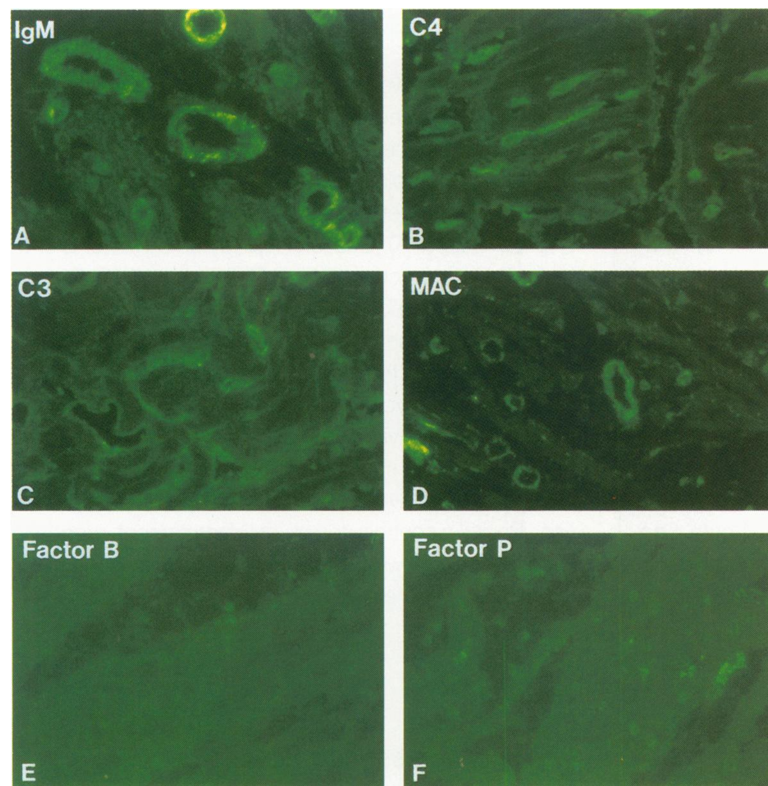

Figure 1. *Immunofluorescence studies of biopsy specimen from porcine heart undergoing rejection in an untreated normal rhesus monkey. Immunofluorescence for IgM, C4, C3, and C9 neoantigen (MAC, membrane attack complex of complement) is seen along capillary endothelium. Factor B and factor P are negative.*

depletion of their natural antibody levels[2] and prolonged survival of subsequently engrafted porcine hearts.[24] Biopsy tissue obtained a few hours after transplantation showed no deposits of antibody or complement in small blood vessels (Figure 2A, compare with Figure 1). In one case, a cardiac xenograft survived and continued to function for 8 days, and showed no evidence of rejection when the animal was sacrificed on that day. In this case, immunopathologic techniques revealed diffuse deposition of IgM along endothelial surfaces but only a few focal deposits of C3 and C9 neoantigen (Figure 2B).

Serum samples were obtained serially from the two rhesus monkeys in which prolonged cardiac xenograft survival has been achieved. In repeated experiments (Table 1) serum complement activity was normal. These findings suggested that in rhesus monkeys with normal complement levels, complement was not activated directly by a porcine xenograft and would not by itself trigger xenograft rejection.

In Vitro *Studies*

Initial experiments showed that fresh human serum was cytotoxic to porcine endothelial cells and that cytotoxicity was abrogated by heat inactivation of human serum at 56°C for 30 minutes, suggesting that cytotoxicity of human serum to porcine endothelial cells is a complement-dependent process. Figure 3 shows the kinetics of killing in the cytotoxicity assay. When porcine endothelial cells were incubated in 10% human serum or 25% human serum, significant killing (> 5% specific lysis) was observed after 1 hour of incubation at 37°C. The cytotoxicity endpoint with respect to time was achieved at 3 hours. All subsequent cytotoxicity experiments were carried out by incubation of endothelial cells with human serum for 4 hours. Figure 4 shows the dose–response relationship between endothelial cell cytotoxicity and increasing concentrations of fresh human serum.

The following experiments address which pathway is involved in activation of complement when porcine endothelial cells are incubated with human serum. The essential requirement for participation of the classical pathway is demonstrated by the inability of C2-deficient serum to produce endothelial cell cytotoxicity; cytotoxicity was restored by reconstitution with purified C2 (Figure 5). In contrast, activation of the alternative pathway does not appear to be primarily involved, as factor B-depleted serum caused cytotoxicity comparable to that induced by unmodified serum. Cytotoxicity is mediated by the membrane attack complex of complement since cytotoxicity was not observed with C5-depleted serum and could be restored by addition of purified C5 (Figure 5).

A similar set of experiments was then carried out to confirm the pathway of complement activation that is involved when porcine endothelial cells are incubated with human serum. An assay of complement activation that is probably more sensitive than cytotoxicity was selected,

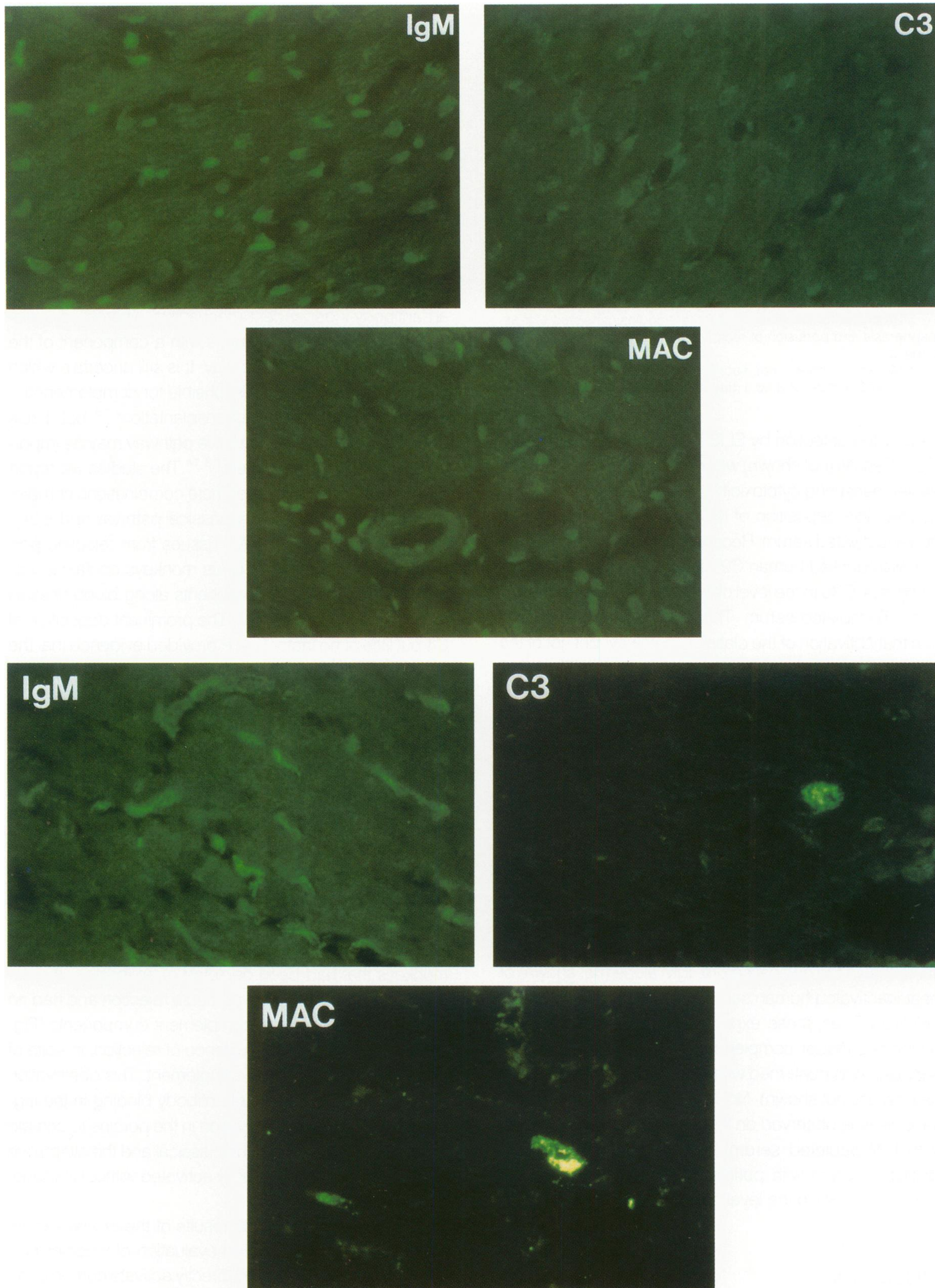

Figure 2. *Immunofluorescence studies of functioning porcine heart xenografts after transplantation into a rhesus monkey that had been pretreated by plasmapheresis and absorption of the monkey's blood over porcine kidneys, as described in Materials and Methods.* **A:** *Biopsy obtained 2 hours after transplantation shows no deposition of IgM, C3, or C9 neoantigen (MAC) in small blood vessels (nuclei are weakly reactive with anti-IgM reagent).* **B:** *Tissue sample from cardiac xenograft on the eighth day after transplantation shows IgM is deposited on small blood vessels, whereas C3 and C9 are present in only one focus.*

Table 1. *Serum Complement Levels in Rhesus Monkeys Depleted of Natural Antibodies Against Porcine Endothelial Cells* Bearing a Surviving Porcine Heart Transplant*

Rhesus number†	Time (days) in relation to procedure	Complement titers	
		CH50	AP50
1	Pretreatment	123	22
	2 days after transplantation	77	19
	4 days after transplantation	71	15
2	Pretreatment	88	11
	1 day after transplantation	72	10

* Treatment for removal of natural antibodies consisted of plasmapheresis and perfusion of recipient's blood through two porcine kidneys.

† Monkey number 1 was sacrificed 8 days after transplantation and monkey number 2 died 1 day after transplantation.

namely the detection by ELISA of endothelial cell-bound iC3b. Results (not shown) were similar to those described earlier measuring cytotoxicity. C2-deficient human serum caused little deposition of iC3b beyond that found with heat-inactivated serum. Reconstitution of C2-deficient serum with purified human C2 restored the endothelial cell binding of iC3b to the level obtained with normal serum or factor B-depleted serum. These experiments thus confirm that activation of the classical pathway, but not of the alternative pathway, takes place when porcine endothelial cells are incubated with human serum.

Studies on the role of human natural antibodies in cytotoxicity of human serum against porcine endothelial cells are shown in Figure 6. Human serum immunochemically depleted of IgM, which retained normal level of complement, lost the ability to cause cytotoxicity; this property was restored in a dose-dependent manner by reconstitution of the IgM-depleted serum with purified human IgM (Figure 6). Full restoration of the cytotoxic activity of IgM-depleted serum was achieved with 1 mg of purified IgM per ml of serum, an IgM concentration similar to that in the original serum. IgM alone (not shown) or heat-inactivated human serum did not cause endothelial cell lysis. Thus, these experiments showed that natural antibodies trigger complement-mediated lysis. This observation was confirmed with the ELISA iC3b binding assay (results not shown). No iC3b binding to the endothelial cells was observed on incubation of endothelial cells with IgM-depleted serum. Reconstitution of the IgM-depleted serum with purified human IgM restored the binding of iC3b to the level obtained with normal serum.

Discussion

The participation of complement in hyperacute rejection of immediately vascularized xenografts has been documented in a number of models. Complement is consumed during xenograft rejection[13] and complement proteins are deposited in the rejected organ.[1,2,9] Decomplementation with cobra venom factor temporarily suppresses hyperacute rejection [3–7] and prolonged survival is also seen in animals congenitally deficient in C6.[8,9] Given the importance of complement in the pathogenesis of hyperacute rejection, a question of interest is the mechanism by which complement is activated. Complement might be activated by natural, antidonor antibodies in the recipient which bind to the endothelium of the grafted organ or complement activation might occur by an antibody-independent mechanism whereby a xenogenic surface directly interacts with a component of the classical or alternative pathway. It is still uncertain which of these mechanisms is responsible for complement activation in discordant xenotransplantation[2,13] but it has been claimed that the alternative pathway may be important in certain animal models.[13,14] The studies we report demonstrate that in pig to primate combinations complement activation involves the classical pathway and is triggered by deposition of IgM. Tissues from rejecting porcine heart xenografts in rhesus monkeys contained deposits of complement components along blood vessels in the same location as IgM. The prominent deposition of C4 but little or no factor B or P provided evidence that the classical rather than the alternative pathway had been activated.

Although we could not find any direct evidence of alternative pathway participation, it might have been secondarily recruited through classical pathway activation.[29] Our methods, which depend on persistence of complement components in tissues, might have been insufficiently sensitive to detect this involvement. Evidence against antibody-independent complement activation by the xenogenic endothelium was derived from two experiments in which xenografts continued to function in recipients with normal complement levels. In these rhesus monkeys that had been depleted of antibodies, the xenograft did not undergo hyperacute rejection and had no or minimal deposition of complement components (Figure 2) and no histologic evidence of rejection, in spite of adequate levels of serum complement. This observation highlights the importance of antibody binding in the triggering of complement activation in the porcine to primate model and suggests that the classical and the alternative pathway would not be directly activated without immunoglobulin binding.

Our interpretation of the results of the *in vivo* experiments was confirmed by an evaluation of whether porcine endothelial cells would directly activate human complement *in vitro* and whether alternative or classical pathway would be involved. Studies were performed with a model of xenogenic hyperacute rejection consisting of cultured porcine endothelial cells incubated with human

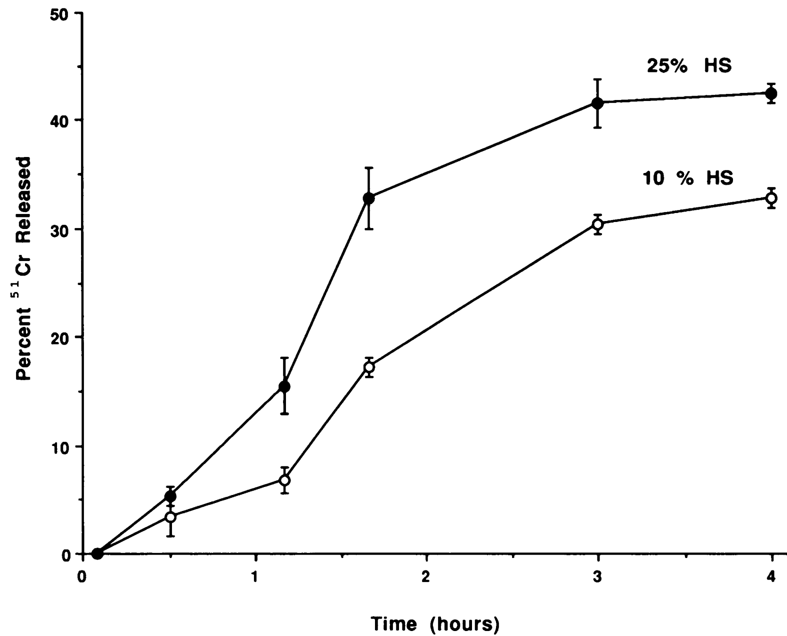

Figure 3. *Effect of time of incubation at 37°C on cytotoxicity of porcine endothelial cells by human serum. Endothelial cells were labeled with ⁵¹Cr, washed and incubated in RPMI containing 10% human serum or 25% human serum; after the indicated incubation times the radioactivity in the culture fluid and in the cells was measured and the percentage of cells killed was calculated as described.*[23]

serum as source of xenogenic natural antibodies and complement.[2,23] Previously we had shown by immunofluorescence microscopic examination that the addition of human serum to endothelial cells results in binding of iC3b to the endothelial cells.[30] The present experiments showed that complement activation occurs via the classical pathway through the presence in human serum of IgM natural antibody to porcine endothelial cells. Because cytotoxicity of human serum against porcine endothelial cells and binding of iC3b to endothelial cells was abrogated by removal of IgM, which could be restored by reconstitution with purified human IgM, we concluded that IgM natural antibodies against porcine endothelial cells accounts for most if not all the complement activation that occurs in this model. The *in vitro* experiments also showed that the anti-endothelial cell antibodies activate the classical pathway and not the alternative pathway.

Previous studies of discordant xenotransplantation in rodents in the guinea pig heart to rat combination have not demonstrated participation of natural antibodies and suggested that hyperacute rejection in this model may be caused by primary activation of complement via the alternative pathway.[13] It has been proposed that hyper-

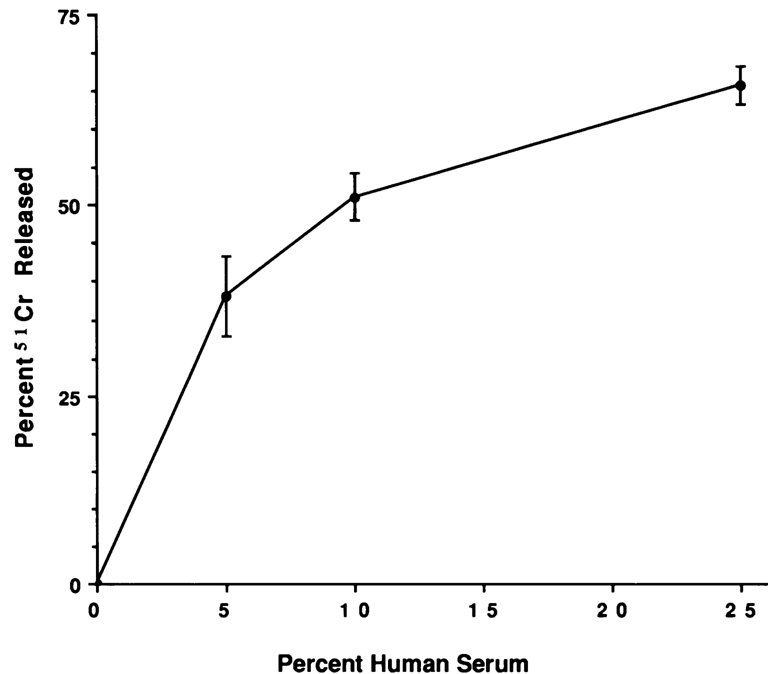

Figure 4. *Effect of concentration of human serum on cytotoxicity of porcine endothelial cells. ⁵¹Cr-labeled endothelial cells were incubated at 37°C for 4 hours with different concentrations of human serum in RPMI. Percentage of cells killed was calculated as described.*[23]

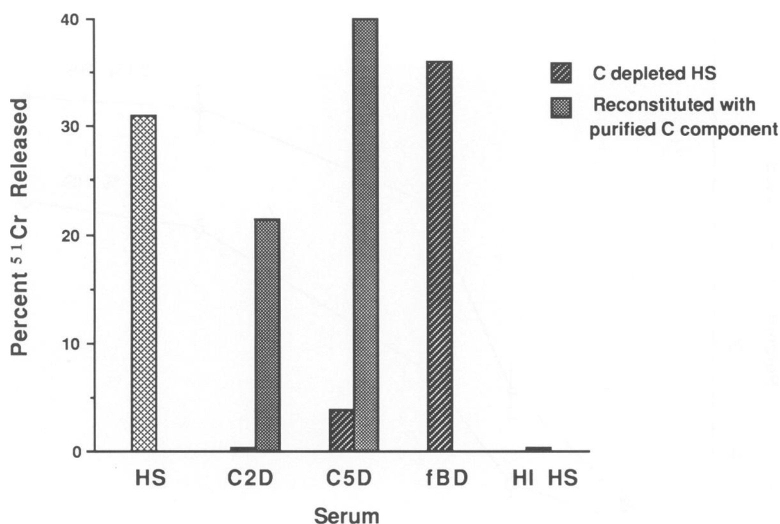

Figure 5. *Role of the classical complement pathway in lysis of porcine endothelial cells by normal human serum. ^{51}Cr-labeled endothelial cells were incubated at 37°C for 4 hours with 10% human serum (HS), human serum deficient in complement components C2 or C5 (C2D and C5D, respectively), factor B-depleted human serum (fBD), or human serum that was heat-inactivated at 56°C for 30 minutes (HI HS). Percentage of cells killed was calculated as described.*[23]

acute rejection in this model may be due to rat IgM natural antibodies against guinea pig endothelial cells,[31] suggesting that the classical pathway may also be involved.

Identification of the multiple steps of the complement reaction that participate in hyperacute rejection is needed to define the mechanisms ultimately responsible for the rapid and massive rejection of a discordant xenograft. Complement fragments may participate in production of tissue pathology in hyperacute rejection.[2] C3a and C5a cause vasoconstriction and polymorphonuclear leukocyte adherence to the vascular endothelium, which may be associated with hyperacute rejection. We recently found that C5a in conjunction with natural antibodies causes loss of heparan sulfate from the endothelium,[32] possibly contributing to loss of blood vessel integrity and intravascular coagulation, which are components of hyperacute rejection. Binding of iC3b to endothelium markedly enhances leukocyte adherence.[30,33] The membrane attack complex, in sublytic amounts, can produce loss of membrane fragments and vesiculation, which in turn promotes coagulation.[34] These processes may initiate thrombosis and necrosis of the grafted organ before complement-mediated endothelial cell killing takes place.[2]

The barrier to transplantation is usually visualized in

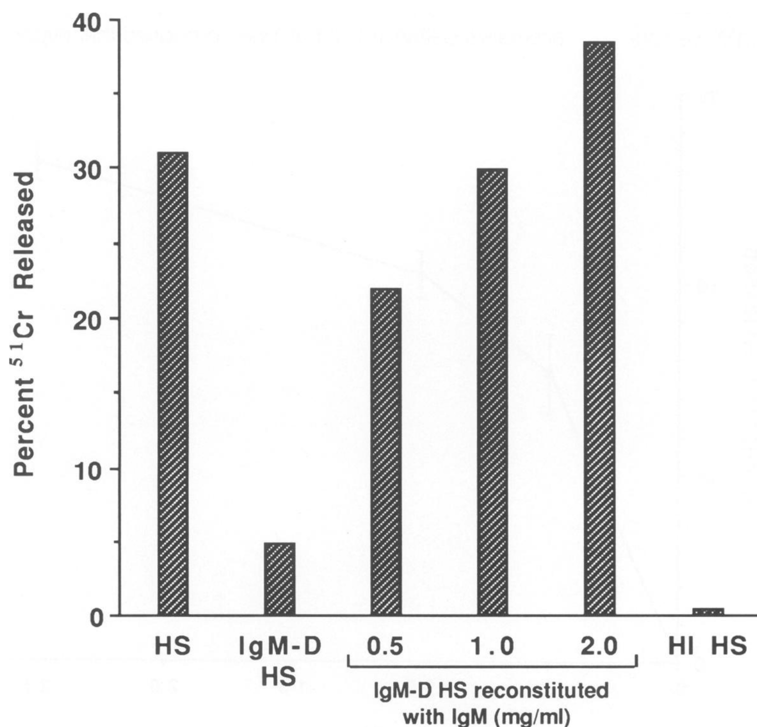

Figure 6. *Role of IgM in lysis of porcine endothelial cells by normal human serum. ^{51}Cr-labeled endothelial cells were incubated at 37°C for 4 hours with 10% human serum (HS), IgM-depleted human serum (IgM-D), IgM-D reconstituted with purified human IgM, or human serum that was heat-inactivated at 56°C for 30 minutes. Percentage of cells killed was calculated as described.*[23]

terms of cellular or humoral immunity.[1,2,10] It seems likely that complement itself may contribute to such a barrier.[2,35] In some donor–host combinations, alternative pathway activation without participation of antibodies may be operative.[13,14] Another element of the barrier may be related to the species specificity of membrane complement inhibitory proteins. Such inhibitors as decay-accelerating factor and homologous restriction factor may not inhibit complement of a different species while effectively inhibiting cell injury mediated by homologous complement.[36–38] If polyreactive autoantibodies constitute a part of the xenoreactive antibody repertoire as recent findings suggests,[39] then the species restriction of the complement inhibitory proteins might contribute to the definition of discordance versus concordance of species.

The mechanism of complement activation has important implications for the development of strategies to avert hyperacute rejection. To the extent that complement activation depends on antibody binding, the depletion of antibodies should protect the graft from injury. Our limited experience[2,24,25] and the results of others[40,41] suggest that antibody depletion significantly prolongs graft survival. We have suggested an alternative approach based on inhibition of the complement reaction at the level of the grafted organ.[2,42] We have proposed that incorporation of complement inhibitors of the host species into the endothelium of the donor organ may result in complement inhibition and prevention of hyperacute rejection. We have tested this hypothesis *in vitro* with human decay-accelerating factor added to porcine endothelial cells and found significant inhibition of cytotoxicity by human serum as source of natural antibodies and complement.[42] Thus, we suggested that inhibition of complement by incorporation of membrane complement inhibitors of host origin into the endothelial cells of the donor organ might represent a powerful approach to abrogate hyperacute rejection, particularly if used with other interventions.

References

1. Auchincloss H Jr: Xenogeneic transplantation. Transplantation 1988, 46:1–20
2. Platt JL, Vercellotti GM, Dalmasso AP, Matas AJ, Bolman RM, Najarian JS, Bach FH: Transplantation of discordant xenografts: a review of progress. Immunol Today 1990, 11:450–456
3. Snyder GB, Ballesteros E, Zarco RM, Linn BS: Prolongation of renal xenografts by complement suppression. Surg Forum 1966, 17:478–480
4. Gewurz H, Clark DS, Cooper MD, Varco RL, Good RA: Effect of cobra venom-induced inhibition of complement activity on allograft and xenograft rejection reactions. Transplantation 1967, 5:1296–1303
5. Mejia-Laguna JE, Martinez-Palomo A, Biro CE, Chavez B, Lopez-Soriano F, Garcia-Cornejo M: Morphologic study of the participation of the complement system in hyperacute rejection of renal xenotransplants. Am J Pathol 1972, 69:71–78
6. Kemp E, Steinbrüchel D, Starklint H, Larsen S, Henriksen I, Dieperink H: Renal xenograft rejection: prolonging effect of captopril, ACE-inhibitors, prostacyclin, and cobra venom factor. Transplant Proc 1987, 19:4471–4474
7. Adachi H, Rosengard BR, Hutchins GM, Hall TS, Baumgartner WA, Borkon AM, Reitz BA: Effects of cyclosporine, aspirin, and cobra venom factor on discordant cardiac xenograft survival in rats. Transplant Proc 1987, 19:1145–1148
8. Chartrand C, O'Regan S, Robitaille P, Pinto-Blonde M: Delayed rejection of cardiac xenografts in C6-deficient rabbits. Immunology 1979, 38:245–248
9. Zhow XJ, Niesen N, Pawlowski I, Biesecker G, Andres G, Brentjens J, Milgrom F: Prolongation of survival of discordant kidney xenografts by C6 deficiency. Transplantation 1990, 50:896–898
10. Hammer C: Isohemagglutinins and preformed natural antibodies in xenogeneic organ transplantation. Transplant Proc 1987, 19:4443–4447
11. Platts-Mills TAE, Ishizaka K: Activation of the alternate pathway of human complement by rabbit cells. J Immunol 1974, 113:348–358
12. Edwards J: Complement activation by xenogeneic red blood cells. Transplantation 1981, 31:226–227
13. Miyagawa S, Hirose H, Shirakura R, Naka Y, Nakata S, Kawashima Y, Seya T, Matsumoto M, Uenaka A, Kitamura H: The mechanism of discordant xenograft rejection. Transplantation 1988, 46:825–830
14. Johnston PS, Lim SML, Wang MW, Wright L, White DJG: Hyperacute rejection of xenografts in the complete absence of antibody. Transplant Proc 1991, 23:877–879
15. Platt JL, Turman MA, Noreen HJ, Fischel RJ, Bolman RM, Bach FH: An ELISA assay for xenoreactive natural antibodies. Transplantation 1990, 49:1000–1001
16. Mahowald ML, Dalmasso AP, Petzel RA, Yunis EJ: Linkage relationship of C2 deficiency, HLA and glyoxalase I loci. Vox Sangu 1979, 37:321–328
17. Tack BF, Morris SC, Prahl JW: Fifth component of human complement: Purification from plasma and polypeptide chain structure. Biochemistry 1979, 18:1490–1497
18. Mayer MM: Complement and complement fixation. Experimental Immunochemistry. Springfield, IL, Charles C Thomas 1961, 133–240
19. Dalmasso AP: Complement in the pathophysiology and diagnosis of human diseases. CRC Crit Rev Clin Lab Sci 1986, 24:123–183
20. Yonemasu K, Stroud RM: C1q: rapid purification method for preparation of monospecific antisera and biochemical studies. J Immunol 1971, 106:304–313
21. Ryan US, Maxwell G: Isolation, culture and subculture of endothelial cells: mechanical methods. J Tissue Cult Methods 1986, 10:3–5

22. Nagelkerke JF, Barto KP, van Berkel TJC: *In vivo* and *in vitro* uptake and degradation of acetylated low density lipoprotein by rat liver endothelial, Kupffer, and parenchymal cells. J Biol Chem 1983, 258:12221–12227
23. Platt JL, Vercellotti GM, Lindman BJ, Oegema TR Jr, Bach FH, Dalmasso AP: Release of heparan sulfate from endothelial cells. Implications for pathogenesis of hyperacute rejection. J Exp Med 1990, 171:1363–1368
24. Fischel RJ, Platt JL, Matas AJ, Perry E, Dalmasso AP, Manivel C, Najarian JS, Bach FH, Bolman RM: Prolonged survival of a discordant cardiac xenograft in a rhesus monkey. Transplant Proc 1991, 23:589–590
25. Fischel RJ, Bolman RM, Platt JL, Najarian JS, Bach FH, Matas AJ: Removal of IgM anti-endothelial antibodies results in prolonged cardiac xenograft survival. Transplant Proc 1990, 22:1077–1078
26. Platt JL, LeBien TW, Michael AF: Interstitial mononuclear cell populations in renal graft rejection: identification by monoclonal antibodies in tissue sections. J Exp Med 1982, 155:17–30
27. Falk RJ, Dalmasso AP, Kim Y, Tsai CH, Scheinman JI, Gewurz H, Michael AF: Neoantigen of the polymerized ninth component of complement. Characterization of a monoclonal antibody and immunohistochemical localization in renal disease. J Clin Invest 1983, 72:560–573
28. Platt JL, Fischel RJ, Matas AJ, Reif SA, Bolman RM, Bach FH: Immunopathology of hyperacute xenograft rejection in a swine-to-primate model. Transplantation 1991, 52:214–220
29. Pangburn MK, Müller-Eberhard HJ: The alternative pathway of complement. Springer Semin Immunopathol 1984, 7:163–192
30. Vercellotti GM, Platt JL, Bach FH, Dalmasso AP: Neutrophil adhesion to xenogeneic endothelium via iC3b. J Immunol 1991, 146:730–734
31. Gambiez L, Weill BJ, Chereau C, Calmus Y, Houssin D: The hyperacute rejection of guinea pig to rat heart xenografts is mediated by preformed IgM. Transplant Proc 1990, 22:1058
32. Platt JL, Lindman BJ, Ihrcke N, Bach FH, Dalmasso AP: Activation of endothelial cells by C5a and antibody. Complement Inflamm 1991, 8:211 (abstract)
33. Marks RM, Todd RF III, Ward PA: Rapid induction of neutrophil-endothelial adhesion by endothelial complement fixation. Nature (Lond) 1989, 339:314–317
34. Hamilton KK, Hattori R, Esmon CT, Sims PJ: Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. J Biol Chem 1990, 265:3809–3814
35. Atkinson JP, Farries T: Separation of self from non-self in the complement system. Immunol Today 1987, 8:212–215
36. Nicholson-Weller A, March JP, Rosenfeld SI, Austen KF: Affected erythrocytes of patients with paroxysmal nocturnal hemoglobinuria are deficient in the complement regulatory protein, decay accelerating factor. Proc Natl Acad Sci USA 1983, 80:5066–5070
37. Hourcade D, Holers VM, Atkinson JP: The regulators of complement activation (RCA) gene cluster. Adv Immunnol 1989, 45:381–416
38. Müller-Eberhard HJ, Zalman LS, Chiu FJ, Jung G, Martin DE: Molecular mechanisms of cytotoxicity: comparison of complement and killer lymphocytes. J Rheumatol 1987, 14(s13)28–34
39. Turman MA, Casali P, Notkins AL, Bach FH, Platt JL: Polyreactivity and antigen specificity of human xenoreactive monoclonal and serum natural antibodies. Transplantation 1991, 52:710–717
40. Cooper DKC, Human PA, Lexer G, Rose AG, Rees J, Keraan M, Du Toit E: Effects of cyclosporine and antibody adsorption on pig cardiac xenograft survival in the baboon. J Heart Transplant 1988, 7:238–246
41. Henry ML, Han LK, Orosz CG, Sedmak D, Ferguson RM: Modification of xenograft hyperacute rejection via xenoantibody depletion. Transplant Proc 1990, 22:1081–1082
42. Dalmasso AP, Vercellotti GM, Platt JL, Bach FH: Inhibition of complement-mediated endothelial cell cytotoxicity by decay accelerating factor. Potential for prevention of xenograft hyperacute rejection. Transplantation 1991, 52:530–533