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THE COMPLEX FUNCTIONING OF THE COMPLEMENT SYSTEM IN XENOTRANSPLANTATION

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Abstract

The role of complement in xenotransplantation is well-known, and is a topic that has been reviewed previously. However, our understanding of the immense complexity of its interaction with other constituents of the innate immune response and of the coagulation, adaptive immune, and inflammatory responses to a xenograft is steadily increasing. In addition, the complement system plays a function in metabolism and homeostasis. New reviews at intervals are therefore clearly warranted. The pathways of complement activation, the function of the complement system, and the interaction between complement and coagulation, inflammation, and the adaptive immune system in relation to xenotransplantation are reviewed. Through several different mechanisms, complement activation is a major factor in contributing to xenograft failure. In the organ-source pig, the detrimental influence of the complement system is seen during organ harvest and preservation, e.g., in ischemia-reperfusion injury. In the recipient, the effect of complement can be seen through its interaction with the immune, coagulation, and inflammatory responses. Genetic-engineering and other therapeutic methods by which the xenograft can be protected from the effects of complement activation are discussed. The review provides an updated source of reference to this increasingly complex subject.

Keywords

Adaptive immune response; coagulation; complement; inflammation; innate immune response; ischemia-reperfusion injury; thromboinflammation; xenotransplantation

Introduction

The role of complement in xenotransplantation is well-known, and is a topic that has been reviewed previously. In xenotransplantation, as part of the innate immune system, the

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Conflict of interest

The authors declare no conflicts of interest.

complement system plays an essential role in the hyperacute rejection seen when a wild-type (i.e., genetically-unmodified) pig organ is transplanted into a human or Old World nonhuman primate. Its rapid destruction of a pig organ graft through the classical pathway is probably its most important role in xenotransplantation. However, our understanding of the immense complexity of its interaction with other constituents of the innate immune response and of the coagulation, adaptive immune, and inflammatory responses to a xenograft is steadily increasing. Furthermore, complement plays a role in metabolism and homeostasis that may also impact function and survival of a pig xenograft, but is possibly of less importance. New reviews at intervals are therefore clearly warranted.

Pathways of complement activation

There are three known pathways of activation of the complement system, with different ‘triggers’ (Table 1), recognizers, receptors, and regulators⁴ (Figure 1).

The *classical pathway* (CP) is activated by binding of antibodies to antigens, and is the major mechanism by which an organ from a wild-type pig (i.e., a pig that expresses galactose- α 1,3-galactose [Gal] and the other known pig antigens against which humans have natural [preformed] antibodies) is rejected after transplantation into a primate (that produces anti-pig antibodies). Antibodies bind to xenoantigens (e.g., Gal), trigger C1q, activate C1r, C1s, then cleave C4 and C2 to form C4b2a (C3 convertase) (Figure 1). This pathway can also be activated occasionally independent of antigen-antibody binding.¹ The *alternative pathway* (AP) is activated in the absence of antibody-antigen binding.^{1,5} The *lectin pathway* (LP) was the last discovered and is the most complicated.^{6,7} Details of its mechanism remain incomplete. The C5 convertase of each of the three pathways cleaves C5 into C5a and C5b, the latter interacting with C6–C9 to form the membrane attack complex (MAC) (C5b-9), which in turn results in lysis, damage, or activation of target cells^{8,9} (Figure 1).

These three pathways sometimes play a cooperative role. LP activation may also be mediated through IgM antibodies.¹⁰ Components of the coagulation cascade (e.g., thrombin and plasmin) can directly activate C3 and/or C5.¹¹ Some complement-independent enzymes (e.g., neutrophil elastase and macrophage serine protease) may activate C5, providing an additional, context-specific ‘extrinsic’ pathway of complement activation.^{12,13}

Receptors and regulators of the complement system

Complement factors function through specific receptors¹⁴. To protect self-tissues from damage, complement regulators^{10,15–18} (soluble or membrane-binding cofactor proteins, listed in Table 2) monitor the activation of complement factors, and control the reaction to a certain degree and in certain locations.¹⁷ Complement receptor 1 (CR1), membrane cofactor protein (MCP, CD46), decay-accelerating factor (DAF, CD55), and membrane attack complex inhibitor protein (CD59) are membrane-bound proteins.¹⁹ The latter three have been the main regulators studied in xenotransplantation to protect donor pig cells.²⁰ CR1, CD46, and CD55 participate in the control of C4 and C3 activation. CD55 has been reported to be more efficacious than CD46 against the AP.²¹ CD59 competes with C9, and hinders the formation of the MAC, and thus has been named ‘protectin’.²²

There are several non-classic regulators of complement, including von Willebrand factor (vWF), thrombomodulin, and plasminogen. Some of these have also been the targets of gene modification in xenotransplantation to improve xenograft survival.²⁰ In time, more molecules may be found to be involved in the delicate regulation of complement (Table 2), which would be consistent with the complicated physiologic and pathologic functions of this system.

Functions of complement

The main mechanisms of complement action against microbes include anaphylaxis, opsonization (to enable phagocytosis), and the initiation of inflammation. After binding to the membranes of target cells or pathogens, MAC leads to osmotic lysis of the target. Macrophages, including Kupffer cells, help to clear the opsonized pathogens.²³ Complement (C1q,²⁴ iC3b/C3R²⁵) facilitates clearance of apoptotic (damaged) cells. Complement also acts as a bridge between innate and adaptive immunity, and is involved in the disposal of immune complexes and the products of inflammatory injury (damaged cells or modified molecules). It cross-reacts with the coagulation system, and is involved in metabolism. Accumulative research has revealed the functional versatility of the complement system.²⁶

Complement factors and inflammation

Complement factors help induce inflammation against microbial invasion, and cross-react with toll-like receptors (TLRs), inflammasomes, and the acute-phase protein (or C-reactive protein, C-RP). C1q partially shares the same role as TNF- α .²⁷

Innate immune cells (neutrophils macrophages, dendritic cells [DCs], natural killer cells), and some adaptive immune cells (T and B lymphocytes) and non-immune cells (epithelial and endothelial cells, and fibroblasts) respond to pathogens through pattern-recognition receptors, e.g., toll-like receptors (TLRs), to initiate reactions for eradicating an infectious microorganism. In this respect, TLRs and the complement system work cooperatively.^{10,28} The complement system helps regulate the activity of TLRs. In contrast, TLR activation can regulate the function of complement factors.

The inflammasome is a multimeric protein complex assembled in the cytosol. Ultimately, the inflammasome activates pro-caspase-1, which activates the pro-inflammatory cytokines, IL-1 β and IL-18, resulting in inflammation. Complement cross-reacts with the inflammasome,²⁹ and both can be activated by the same triggers, namely infection, tissue damage, or metabolic dysfunction.³⁰

The defensive action of complement may be enhanced by C-RP, as is the clearance of damaged cells. Membrane-bound C-RP is a strong opsonin with partial complement-activating activity,³¹ but C-RP also helps restrict excessive complement activation, allowing for temporal AP activity against invading microbes, promoting phagocytosis by macrophages, and contributing to the non-inflammatory clearance of damaged cells mediated by the complement system.³²

Interaction of complement with coagulation factors and platelets - thromboinflammation

Complement cross-reacts with coagulation factors and platelets, and plays an important role in the development of consumptive coagulopathy, which develops as a consequence of thromboinflammation (Figure 2).³³ Thromboinflammation refers to the response that occurs when intravascular foreign substances, including microorganisms, foreign bodies, immune complexes, and debris from apoptotic or necrotic cells, activate the various cascade systems, e.g., the contact, coagulation, fibrinolytic, and complement systems, which in turn activate cells (monocytes, polymorphonuclear cells, endothelial cells) and platelets, and orchestrate the inflammatory and thrombotic responses.^{33,34}

The complement and coagulation systems share some trigger molecules. For example, C1q and Factor XII recognize negatively-charged molecules (DNA, lipopolysaccharides, and heparin).³⁵ These two systems also share some regulators, e.g., C1-INH, an inhibitor of the CP and LP,³⁶ also acts as a modulator of the coagulation contact system (factor XIIa, factor XIIIa, and kallikrein),³⁷ and the fibrinolytic system (tissue plasminogen activator and plasmin).³⁸ Thrombomodulin may inhibit thrombin, and also favor C3b inactivation and C3a and C5a degradation.^{11,39} von Willebrand factor also interacts with complement components.^{40,41} Complement may thus activate the coagulation system and, in turn, the coagulation system may regulate complement.

Platelets interact with both the complement and coagulation systems. Immune complexes may bind to Fc γ RIIA on the platelets, thus activating them, and also bind to C1q to activate the complement system.⁴² The activated platelets may bind ficolin-1, -2 and -3, and activate mannose-binding lectin-associated serine proteases (MASP-1 and 2).⁴² Activated platelets bind to plasma proteins, initiating contact activation (factors XIIa and XIa, and kallikrein),⁴³ and may induce C3 and factor XI phosphorylation, which may enhance their activities.⁴⁴

Some factors may dysregulate the complement and coagulation systems, activate platelets, and result in consumptive coagulopathy, which is a main manifestation hindering xenograft survival.^{45,46} For example, (i) after adhering to activated platelets, pentameric C-RP complex dissociates into monomeric C-RP and recruits more platelets, and activates the AP; (ii) antibodies against platelet membrane glycoproteins may activate the CP through C1q; (iii) antibodies against CD55 and CD59 on platelets may interfere with complement regulation and cause exacerbated injury to platelets and immune thrombocytopenia;⁴⁷ (iv) after being stimulated by histamine, human umbilical vein endothelial cells may become enriched in local acute inflammatory molecules, including molecules involved in both the complement and coagulation cascades;⁴⁸ (v) dysregulation of the complement system⁴⁹ and drug-induced injury, e.g., by sirolimus, cyclosporine, tacrolimus,^{50,51} may decrease expression of vascular endothelial growth factor in a graft, resulting in endothelial injury and complement activation,⁵² leading to thrombotic microangiopathy and consumptive coagulopathy.

Interaction between complement and the adaptive immune system

Both innate and adaptive immunity are essential for protection against invading microorganisms. Complement can regulate adaptive immunity,⁵³ by indirectly modulating the development and function of antigen-presenting cells, or by direct ligation to complement receptors/regulators expressed on T and/or B cells, affecting T and B cell activation, differentiation, and function.⁵⁴

Antigen-presenting DCs are critical in adaptive immune defense, and their function is influenced by complement.⁴⁰ C1q, iC3b, C5a-C5aR, CR1 and factor H are reported to regulate the maturation and function of DCs.^{24,25,55–57}

CR2 is part of the co-receptor (along with CD19, CD81, and Leu13) of B lymphocytes.^{58,59} In the germinal centers, C3 degradation fragment-coated immune complexes are recognized and retained by follicular DCs, enhancing the differentiation of memory and effector B cells.^{60,61} Regulators of complement also limit over-reaction of the immune system, thus reducing inflammation, but nevertheless allow an antigen-specific response by B cells.^{62,63} CR2, C4 binding protein, and CD46 modulate immunoglobulin class-switching of B cells.^{64–66}

Immune cell-derived complement plays a fundamental role in T cell differentiation, activation, and expansion.^{67,68} Complement components (C1q, C3a, C4, C5a, CD46, CD55) are reported to affect T cell differentiation, proliferation, and activity, with or without the help of DCs.^{68,69}

C1q-polarized macrophage/DCs may inhibit allogeneic and autologous T helper cell type 17 (Th17) and 1 (Th1) proliferation, and induce differentiation of T regulatory cells.²⁴ C3aR expressed on CD4⁺T cells has been shown to be critical in the regulation of their development and function.^{68,69} C3a and C5a inhibit natural regulatory T cell induction, function, and stability.⁷⁰ When co-cultured with DCs, C4 was found to increase the regulatory T cell count.⁷¹ Expansion of T cell numbers and destabilization of regulatory T cells results in T cell-dependent transplant rejection.⁵⁷

As mentioned above, synergistic function between complement and TLRs may enhance adaptive immunity.⁷² However, complement may also inhibit the adaptive immune response. CR1g is a strong regulator of T cell proliferation and Th1 cytokines.⁷³ CR1g⁺DCs (i.e., DCs with CR1g binding) may facilitate immunosuppression and promote tolerance.⁷³ TLR2/4-CR3 has been shown to suppress IL-12 expression and inhibit the Th1 response.²⁸ CD46 acts as a stimulatory factor to generate IL-10-producing T regulatory cells and other immune cells.^{65,66,74} CD46 contains mutually exclusive cytoplasmic tails, namely CYT1 and CYT2.^{75,76} CD46-CYT1 induces Th1 cells and IFN- γ production, and then differentiation to T regulatory cells, secreting IL-10 (Tr1s). CD46-CYT2 terminates the Tr1s response and restores homeostasis.⁷⁷

Thus, the complement system is not confined to innate immunity, but is an integral component of adaptive immunity. Both innate and adaptive immunity participate in the immune response to a xenograft.

Complement and metabolism

Metabolism provides basic substances and the energy required for construction, survival, and functioning of cells. Complement receptor/regulators help regulate nutrient channels (e.g., influx of glucose and amino acids), levels of glycolysis, and oxidative phosphorylation, which affect cell function.^{78,79} A novel 'complement-metabolism-inflammasome axis' has been proposed to describe complement regulation of metabolism during inflammation.^{79,80} There is growing acknowledgement that complement may also play a role in cell homeostasis, wound repair, and in liver, bone, and retinal regeneration.^{81–83}

Intracellular complement

Complement may be activated intracellularly,^{77,84} the intracellular receptors may convey distinct signaling pathways from those expressed on the cell surface,^{53,69} and regulate the adaptive immune system. The hydrolytic product of C3 in plasma may be loaded intracellularly in a rapid, saturable manner. This uptake alters activated CD4⁺T cell cytokine secretion. Under stable conditions, most (80%) of the incorporated C3 is returned to the extracellular space.⁸⁵

Complement activity during organ transplantation

The complement system plays a significant role in allotransplantation, particularly when antibody-mediated rejection occurs,⁸⁶ but an even greater role in xenotransplantation. There is considerable overlap between its roles in allo- and xeno-transplantation. For example, the complement system is activated during ischemia-reperfusion injury, and is involved in antibody- and cell-mediated rejection and the development of thrombotic microangiopathy and transplant vasculopathy.⁸⁷

After ischemia, tissue damage is caused when, during reperfusion, there is reduced aerobic metabolism. Oxygen radicals are generated, accompanied by inflammation, resulting in IRI (ischemia-reperfusion injury). Complement is recognized as one of the markers of ischemia;⁸⁸ IRI may trigger damage-associated molecular patterns through all three complement pathways, inducing complement activation.⁸⁹

IRI is an inevitable process during donor organ procurement, organ transport, and transplantation into the recipient, usually manifest as delayed graft function. In kidney transplantation, it is associated with increased C3a production and degradation products locally and in the circulation, mainly from the graft.⁹⁰ C5a was shown to have a more significant impact on IRI than C3a, through regulation of pro-inflammatory cytokines (IL-1 β , IL-6 and IL-8).⁹¹ Both C5aR1 and C5aR2 contribute to IRI and neutrophil activation.⁹² Upregulation of complement activation during mouse kidney IRI is associated with impaired kidney function even one year later.⁹³

Complement activation in xenotransplantation

Complement activation is clearly detrimental to xenograft survival. The complement cascade is involved in many of the barriers that impair xenograft survival, e.g., IRI,⁹⁴ coagulation

dysfunction (thrombotic microangiopathy, consumptive coagulopathy),^{95,96} graft vasculopathy,⁹⁷ antibody-mediated (hyperacute, delayed) or cellular rejection,^{97,98} and systemic inflammation.⁹⁹

Following pig organ transplantation into a human¹⁰⁰ or nonhuman primate,¹⁰¹ hyperacute rejection frequently occurs within minutes. Binding of primate natural (preformed) anti-pig IgM antibodies to the vascular endothelial cells of the graft activates the CP of complement,¹⁰² resulting in injury to the endothelial cells, thrombosis, interstitial hemorrhage, and edema, that disrupts graft function.^{89,103} Hyperacute rejection results from the binding of primate antibodies (e.g., anti-Gal) to the graft, activating complement.⁶¹ Complement deposition has been found in corneal¹⁰⁴, islet¹⁰⁵, renal¹⁰⁶, cardiac¹⁰⁶, and hepatic¹⁰⁷ xenografts.

One of the first approaches to prevent hyperacute rejection was to administer an agent that depleted or inhibited complement, e.g., cobra venom factor or soluble complement receptor-1 (sCR-1), which extended graft survival significantly, but had only a temporary effect.^{108–110}

The presence of complement-regulatory proteins on the surface of human vascular endothelial cells, e.g., CD55, CD46, or CD59, to some extent protects the cells from complement-mediated injury. Pig cells have equivalent complement-regulatory proteins, but these are less able to provide protection from the effects of human complement.^{111–113}

When genetic modification of the organ-source pig became possible, the introduction into the pig of a transgene for a human complement-regulatory protein to prevent hyperacute rejection was suggested,^{113,114–116} and several groups investigated this approach.^{114,115,117–121} The expression of hCD55 and/or hCD46 and/or CD59 provided considerable protection to pig organs and islets from hyperacute rejection.^{122–125}

The incidence of hyperacute rejection was significantly reduced by the transplantation of organs from α 1,3-galactosyltransferase gene-knockout (GTKO) pigs,^{126,127} but as demonstrated in many in vitro and in vivo models,^{96,128–135} the complement system still played a role in graft rejection. Furthermore, IRI could still activate complement through all three pathways. Therefore, even in the GTKO era, the complement system is still activated.¹³⁶

Inhibition of complement activation by the introduction of one or more human complement regulators^{137,138} into the organ-source GTKO pig has therefore become a common, even standard, method of preventing or reducing the injury associated with the humoral response (Table 3). Because of their different specificities, expression of more than one human complement-regulatory protein has been recommended (Figure 3).^{20,151} Expression of CD46 and/or CD55 in GTKO pigs reduces complement deposition in GTKO pig kidney and heart grafts,¹⁵² and protects against antibody-mediated rejection.^{20,133,137,152} thus prolonging graft survival in non-human primates.^{151,154} (Table 3)

However, human complement regulators are used by many pathogens as co-receptors to enter host cells¹³⁶ and evade host immunity^{155,156} (Table 4), or as a receptor of toxins

produced by the pathogens.¹⁶⁶ There is, therefore, the potential that a pig expressing one or more human complement regulators might be at a higher risk for infection, particularly of the graft itself. Research aimed to create transgenic animals with modified CRPs that evade CRP-related pathogens would be a potential solution.^{167–169}

New intracellular mechanisms that mediate protection from complement injury include increased expression of claudin-5, and endothelial induction of adenIL-4; IL-4 is a potent inducer of protection.^{170–172} Importantly, this protection is associated with maintenance of mitochondrial morphology and membrane potential, and is not related to upregulation of complement-regulatory proteins.

Minimizing the effects of complement activation in organ transplantation

Many interventions have been introduced to prevent complement injury during xenotransplantation (Table 5)^{136,173,174}, and xenograft survival has been increased. Eculizumab, a recombinant antibody against complement C5, may reduce antibody-mediated rejection in recipients with high levels of donor-specific antibodies.¹⁷⁵ It may abrogate kidney allograft rejection,¹⁷⁶ and alleviate refractory thrombotic microangiopathy,¹⁷⁷ but a prospective randomized trial showed no convincing reduction in the incidence of antibody-mediated rejection after allotransplantation.¹⁷⁸ Neither does it appear to reduce the incidence of chronic rejection (transplant vasculopathy).¹⁷⁹ This implies that upstream complement components may be involved in antibody-mediated rejection and endothelial injury.

The C3 inhibitor, Cp40, inhibits activation of pig aortic endothelial cells and human leukocyte adhesion to the endothelium,¹⁷³ prevents complement activation, reducing cell damage and preserving heart graft function.¹⁸⁰ Furthermore, C4a is structurally similar to C3a and C5a and has been shown to have similar functions,¹⁸¹ which need to be considered when targeting C3.

C1q and related complex-targeting interventions are under investigation. Serum-derived C1-INH has been shown to be protective against antibody-mediated rejection in a baboon allotransplant model.¹⁸² In vitro studies show that C1q deletion or an anti-C1s antibody may significantly reduce monocyte adhesion to human aortic endothelial cells.¹⁸³ A C1s antibody effectively prevented late antibody-mediated rejection in renal allograft recipients.¹⁸⁴ Furthermore, C1-INH may inhibit the kinin B1 receptor, reducing the release of chemotactic microvesicles from injured donor tissue.¹⁸⁵ These results imply that C1q (C1r)-targeting might be beneficial in xenotransplantation.

Local synthesis of C3 from a renal graft contributes to 16% of systemic circulating C3 in mice with renal transplants.¹⁸⁶ Local expression of complement in renal grafts is associated negatively with early and late graft function.^{187,188} By avoiding the disruption of host defense mechanisms, local complement inhibition may be preferable to systemic inhibition, especially in immunocompromised patients. There are two ways to reduce complement-mediated injury to a graft, namely targeting complement molecules (i) during donor organ preservation, or (ii) subsequently when any one of the three pathways is activated. For

example, (a) Mirococept (APT070), a derivative of human CR1, is now under clinical trial for preventing IRI in kidney allografts during preservation.¹⁸⁹ Annexin IV is expressed in grafts,¹⁹⁰ and an anti-annexin-IV antibody tagged to a complement inhibitor (Crry) protected mouse cardiac allografts from IRI.¹⁹¹ (b) Many efforts have been made to directly target complement at sites of activation.¹⁹² For example, CR2–complement inhibitor fusion proteins (CR2-FH, CR2-Crry, and CR2-CD59)^{193,194} make local targeting feasible. Ergidina, a neutralizing recombinant anti-C5 antibody, protected rat kidneys from IRI by binding to injured endothelium.¹⁹⁵ The glycocalyx has been proven to be critical for protecting endothelial cells from activation during xenotransplantation.^{196,197} The application of heparin onto mesenchymal stem cells,¹⁹⁸ or endothelial cells,¹⁷⁴ which would help to avoiding or mitigate the shedding of the glycocalyx on the cell surface, may inhibit complement activation and improve graft cell viability.

Rapamycin may inhibit the expression of complement-regulatory proteins on endothelial cells,¹⁹⁹ but there is also evidence that rapamycin (but not cyclosporine, mycophenolate mofetil, or azathioprine) may induce CD55 expression, thus protecting endothelial cells from complement-mediated injury.^{200,201} These conflicting results may be associated with study design. For xenotransplantation, intensive immunosuppressive therapy may probably be required but, as complement is involved in calcineurin inhibitor-related renal injury,^{50–52} reducing this kind of xenograft injury warrants more research.

Conclusions

Complement plays an important role in organ xenotransplantation, including its activation after anti-pig antibody binding to the pig vascular endothelial cells and during IRI, but also its subtle, more complex role in the coagulation, inflammatory, and adaptive immune responses. As complement can be activated through any of its three pathways, it would seem important to protect the transplanted organ by expression of one or more human complement-regulatory proteins, rather than rely on antigen-deletion alone. Furthermore, as it is also involved in the coagulation, inflammatory, and cellular responses to a xenograft, it is clearly key to the survival of the graft. Continued investigation is necessary to determine if further methods of inhibiting its effects can be achieved, either by genetic-engineering of the organ-source pig or by the administration of agents that prevent or interrupt the systemic complement cascade.

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Abbreviations

AP	alternative pathway of complement
C1-INH	C1 inhibitor
CP	classical pathway of complement

CR1	complement receptor 1
C-RP	C-reactive protein (acute-phase protein)
DC	dendritic cell
Gal	galactose- α 1,3-galactose
IRI	ischemia-reperfusion injury
LP	lectin pathway of complement
MAC	membrane attack complex
MASP	mannose-binding lectin-associated serine proteases
TLR	Toll-like receptor

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Figure 1: Schema of complement system.

Classical pathway (CP) (left): Activated by binding of antibodies to antigens, which triggers C1q, activates C1r, C1s, then cleaves C4 and C2 to form C4b2a (C3 convertase).

Lectin pathway (LP): One of MBL, ficolin -1, -2 and -3, and collectin 10/11 and collectin-P, recognizes lipopolysaccharides, etc., and binds to one of the MASP-1, MASP-2, and MASP-3, forming C3 convertase (C4b2a) (middle). C4b2a from the classical or lectin pathway cleaves C3 into C3a and C3b. C3b binds to C4b2a to form one of the C5 convertases (C4b2a3b).

Alternative pathway (AP): C3 undergoes spontaneous hydrolysis to form C3(H₂O), which binds to factor B, forming an unstable C3 convertase C3(H₂O)Bb, generating more C3b. Activation of C3 in the presence of factor B and factor D results in the formation of C3bBb (C3 convertase) (right). Properdin stabilizes C3 and C5 convertase, and enhances the amplification loop of C3 activation, then generating C5 convertases (C3bBb3b).

‘Extrinsic’ pathway: Some complement-independent enzymes (e.g., neutrophil elastase and macrophage serine protease) may activate C5, providing an additional, context-specific of complement activation.

Activation of MAC (bottom): The C5 convertase cleaves C5 into C5a and C5b, the latter interacting with C6–C9 to form the MAC (C5b-9), which in turn results in lysis, damage, or activation of target cells (lower part).

The complement system is tightly regulated by soluble and membrane inhibitors (red), including C1-INH, factor H (FH), factor I (FI), C4BP, vitronectin (VN, S-protein, Vn, and Clusterin (CL, apolipoprotein J, SP-40) . membrane inhibitors include CD46, CR1, CD55, thus controlling C4 and C3 activation. CD59 protects against assembly of the C5b-9 complex.

C1-INH = C1 inhibitor; C4BP = C4-binding protein; CR1 = CD35, Complement receptor 1; DAF = CD55, decay accelerating factor; MASP = Mannose-binding lectin-associated serine proteases; MBL = Mannose-binding lectin; MCP = CD46, membrane cofactor protein;

Figure 2: Thromboinflammation

Intravascular foreign substances (including foreign bodies, immune complexes, and debris from cells) activate the coagulation, fibrinolytic, and complement systems, which in turn activate cells (monocytes, polymorphonuclear cells, endothelial cells) and platelets, and orchestrate the inflammatory and thrombotic responses.

Complement may activate the coagulation system and, in turn, the coagulation system may regulate complement: C1-INH, an inhibitor of the CP and LP, also acts as a modulator of the coagulation contact system (factor X_{ia}, factor XII_a, and kallikrein), and the fibrinolytic system (tissue plasminogen activator and plasmin). Thrombomodulin (CD141) may inhibit thrombin, and also favor C3b inactivation and C3a and C5a degradation. von Willebrand factor also interacts with complement components.

Platelets interact with both the complement and coagulation systems. Immune complexes may activate platelets and also bind to C1q to activate the complement system. Activated platelets may induce C3 and factor XI phosphorylation, which may enhance their activities. (i) antibodies against platelet membrane glycoproteins may activate the CP through C1q; (ii) after adhering to activated platelets, pentameric C-RP complex dissociates into monomeric C-RP and recruits more platelets, and activates the AP; (iii) The activated platelets may bind ficolin-1, -2 and -3, and activate mannose-binding lectin-associated serine proteases (MASP-1 and 2), activate the LP.

After being stimulated by histamine, endothelial cells may become enriched in local acute inflammatory molecules, including molecules involved in both the complement and coagulation cascades; rug-induced injury, e.g., by calcineurin inhibitors (CNI), including sirolimus, cyclosporine, tacrolimus, may decrease expression of vascular endothelial growth factor in a graft, resulting in endothelial injury and complement activation.

So, complement cross-reacts with coagulation factors and platelets, develops thromboinflammation.

CP = Classic pathway; LP = Lectin pathway; AP = Alternative pathway; PenC-RP = pentameric C-RP; MonC-RP = monomeric C-RP; EC = Endothelial cells; MASP = mannose-binding lectin-associated serine proteases; CNI = calcineurin inhibitors; C1-INH = C1 inhibitor; TAFI = Thrombin-activatable fibrinolysis inhibitor.

Figure 3: Human serum cytotoxicity (at 12.5% concentration) to WT, GTKO, GTKO/hCD46, and GTKO/hCD46/hCD55 pig corneal endothelial cells (pCECs) before and after activation with pig IFN- γ (40ng/ml for 48h)

In contrast to WT pCECs, there was no lysis of quiescent GTKO, GTKO/CD46, and GTKO/CD46/CD55 pCECs. After activation, although there was increased lysis of pCECs from all types of pig, there was significantly less lysis of GTKO/CD46 pCECs than of pCECs from WT pigs. Additional expression of CD55 on pig cells showed further resistance to lysis (n=3). (** $p < 0.01$). (Hara H, unpublished data).

Table 1:

Activators of the three pathways of complement

Pathway	Triggers	References
Classical pathway (CP)	Antibodies (immunoglobulins, Fc portion of IgG, especially IgG1 and IgG3, or IgM) binding to antigens, negatively-charged molecules (DNA, lipopolysaccharides, and heparin) Pentraxins (C-reactive protein [C-RP] and pentraxin-3) that recognize pathogen-associated molecular patterns (PAMPs, e.g., viral proteins) Certain phospholipids (amyloid)	1
Lectin pathway (LP)	Surface-linked carbohydrates (lipopolysaccharides, phosphatidylserine, and immunoglobulin) Acetyl groups on pathogens or damaged self-tissue	2
Alternative pathway (AP)	Hydrolysis (of the thioester bond in C3), bacteria or damaged cells	3

Regulators of the complement system^{10, 15–18}

Table 2:

Regulator	Target	Function	Pathway	Location
C1-INH	C1qrs, MASP5	Inactivates C1r and C1s, MASP-1 and MASP-2	CP/LP	Fluid
Small mannose-binding lectin-ficolin-associated protein (sMAP, MAP19)	MBL	Competes with MASP1–3	LP	Fluid
Mannose-binding lectin-ficolin-associated protein-1 (MAP-1)	MBL/ficolins	Competes with MASP	LP	Fluid
C4BP	C4b	Inhibits C3 convertase	CP/LP	Fluid
Factor H	C3b, Factor B, Bb	Blocks the formation of C3 convertases	CP/LP/AP	Membrane and fluid
Factor H-related proteins 1–5	C3b, Factor H	Competes with factor H	CP/AP	Membrane and fluid
Factor I	C3b, C4b	Degrades C3b or C4b with cofactor	CP/LP/AP	Fluid
CR1	C3b, C4b	Inhibits C3/C5 convertase	CP/LP/AP	Membrane
CD46 (MCP)	C3b, C4b	Inhibits C3/C5 convertase. Cofactor for Factor I.	CP/LP/AP	Membrane
CD55 (DAF)	C3b, C4b, C3convertase	C3/C5 convertase decay	CP/LP/AP	Membrane
Signal Regulatory Protein α (SIRPα and family)	MAC-1 (CR3)	Inhibits phagocytosis	CP/LP/AP	Membrane
Cry	C3/C5 convertase	Inhibits C3/C5 convertase	CP/LP/AP	Membrane
vWF	C3b	Helps with C3b cleavage	CP/LP/AP	Fluid
Thrombomodulin (CD141)	C3a, C3b, C4b, C5a	C3/C5 convertase decay. Anaphylatoxin inhibitor	CP/LP/AP	Membrane and fluid
Plasmin(ogen)	C3, C3b, C3d, and C5	C3/C5 convertase decay	CP/LP/AP	Membrane and fluid
Carboxypeptidase N, R, B (thrombin-activatable fibrinolysis inhibitor)	C3a, C5a	Anaphylatoxin inhibitor	CP/LP/AP	Fluid
Cartilage oligomeric matrix protein (thrombospondin 5)	C1q, MBL	Inhibits C3 convertase	CP/LP	Fluid
CUB (structural motif consists of C1r/C1s, Uegf, Bmp1) and sushi multiple domains 1 (CSMD1)	C3, Properdin	Activates AP	AP	Fluid
Sushi domain-containing protein 4 (SUSD4)	C3b, C4b	Cofactor of Factor I?	CP/LP?	Membrane
Clusterin (apolipoprotein 1, SP-40)	C1q, MBL, C3b?	Inhibits C3 convertase	CP/LP/AP	Membrane and fluid (?)
Vitronectin (VN, S-protein, S40)	C5b-8	Inhibits MAC formation	TP	Fluid
CD59 (HRF20, protein)	C5b-9	Inhibits MAC formation	TP	Fluid
	C8, C9	Inhibits MAC formation	TP	Membrane

Abbreviations: AP = Alternative pathway; C1-INH = C1 inhibitor; C4BP = C4-binding protein; CR1 = Complement receptor 1 (CD35); CP = Classical pathway; Cry = Complement receptor-related protein, expressed in mouse and rat; DAF = CD55, decay-accelerating factor; LP = Lectin pathway; MASP = Mannose-binding lectin-associated serine proteases; MBL = Mannose-binding lectin; MCP = CD46, membrane cofactor protein; TP = Terminal pathway; vWF = von Willebrand factor.

Transgenic expression of one or more human complement-regulatory proteins protects pig-to-nonhuman primate heart grafts

Table 3:

Genetic background (or model)	Survival without immunosuppressive therapy (Ref)	Survival with immunosuppressive therapy (Ref)
Wild-type	Mean 20–80 minutes ¹³⁹	15–75 minutes (n=4), 5 days (n=1) ¹³⁹
Anti-Gal antibody depletion	Mean 15 days ¹⁴¹	<15 days ¹⁴²
GTKO		Mean 78 days (59–179d) ¹⁴²
hCD46 expression	Mean 5.25 days (60min–16d) ¹³⁹	Mean 96 days (15–137d, n=7) ¹⁴³
hCD55	Mean 5.1 days ¹⁴⁴	Mean 27 days (4–139d) ¹⁴⁵
hCD59		Mean 2.5–3.0 hours ¹⁴⁶
CD55+CD59		85–130 hours ¹⁴⁷
GTKO+hCD46		Up to 236 days ¹⁴⁸
GTKO+ hCD55		Mean 28 days ¹³⁴
GTKO+ hCD55+CD46		Mean 21 days (15–33d) ¹⁴⁹
GTKO+hCD46+hTBM		<945days ¹⁵⁰

Abbreviations: TBM=thrombomodulin.

Table 4:

Examples of complement regulators that act as co-receptors of pathogens

Co-receptor	Pathogen	References
CD46	Measles virus, herpes virus-6, adenovirus, human immunodeficiency virus (HIV) Streptococcus pyogenes, pathogenic Neisseria species Opsonized Escherichia coli, Helicobacter pylori	157–159
CD55	Enteroviruses (Coxsackievirus, Echovirus 11, Echovirus 7) Hantaviruses: (Hantaan virus, Puumala virus, Sin Nombre virus); HIV Helicobacter pylori, Escherichia coli, Neisseria gonorrhoeae, Staphylococcus aureus	158–164
CD59	HIV Human cytomegalovirus and vaccinia Streptococcus intermedius, Streptococcus suis	158, 165

Table 5:

Timeline of Interventions against complement injury in xenotransplantation models (in vitro and in vivo)^{136,173,174}

Time	Intervention
1967	CVF (cobra venom factor)
1991	hDAF protein
1992	Serine protease inhibitor
1993	Dextran sulfate
1994	CD59 transgene in pig
1995	C5 mAb
1996	sCD46
1997	IVIg (Intravenous immunoglobulin)
1998	PI-anchored-C4BP
1999	Compstatin (C3 antibody)
2001	Soluble Thrombomodulin (TBM)
2002	Melagatran
2004	PI-anchored-factor I
2009	hTBM + hDAF + hCD59 transgenes in pigs
2013	C5 Ab
2015	Cp40 (Compstatin analog)
2017	CHC (Corline Heparin Conjugate)

Abbreviations: sCR1 = soluble complement receptor 1; (s = soluble); FUT-175 = Nafamstat mesilate (synthesized low-molecular-weight protease inhibitor); K76-COOH = Oxidation of the natural product of *Stachybotrys* complementi, nov. sp. K-76, yielded K-76 COOH, which has complement inhibitory activity; PI-anchored-C4BP = consisting of a short consensus repeat 1–8 of the alpha-chain of C4bp and a glycosyl phosphatidylinositol (GPI) of decay-accelerating factor (CD55).