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Innate networking: Thrombotic microangiopathy, the activation of coagulation and complement in the sensitized kidney transplant recipient

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Abstract

Thrombotic microangiopathy (TMA) is a histological feature of antibody-mediated rejection and has the potential to cause problematic graft dysfunction, particularly for highly sensitized cross-match positive kidney transplant recipients. Prompt recognition of pertinent histopathological and systemic features of TMA in kidney transplantation is necessary. Underlying mechanisms of this process involve the activation of both complement and coagulation systems as a response to HLA antibody. As serine proteases, coagulation and complement cascades exhibit similar characteristics with respect to homeostatic function. Increasing evidence now exists for the interaction between these innate defenses in both activation and regulation, lending scope for intervention.

Understanding the complexities of these interactions remains a challenge. This review provides an overview of the current understanding, particularly with respect to the activation of coagulation and complement by HLA antibody in the setting of highly sensitized kidney transplantation.

Keywords

complement; coagulation; highly sensitized; thrombotic microangiopathy; kidney transplantation; antibody incompatible transplantation; HLA-incompatible transplantation

Introduction

Thrombotic microangiopathies (TMAs) are characterized by a tendency to develop microvascular thrombi in conjunction with endothelial cell dysfunction, thrombocytopenia

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and hemolytic anemia. TMA may result in end-organ dysfunction as a result of thrombosis, has a broad range of associations, and is manifest in the kidney in a number of disease entities (1, 2). Following kidney transplantation, a wide range of associations with new presentations of TMA is recognized in the transplanted kidney (3), including immunosuppressive drugs (cyclosporine, calcineurin inhibitors, sirolimus (3–5), antibody mediated rejection (4), and recurrent and de novo hemolytic uremic syndrome (HUS) (6). In this regard, TMA can be seen as a terminal feature of a process that may have many triggers, as well as an underlying predisposition which facilitates this phenomenon.

In the early era of kidney transplantation, localized TMA in the kidney graft leading to significant thrombocytopenia, in conjunction with platelet and fibrin deposition, was recognized as a form of delayed hyperacute rejection, and attributed to HLA antibody-mediated vascular endothelial injury (7, 8). More recently, the importance of post-transplant TMA associated with antibody-mediated rejection (AMR), particularly in the highly sensitized recipient, has been highlighted particularly with the advent of clinical therapies targeting terminal complement complexes (9). Graft survival in patients with TMA but no AMR has been shown to be significantly better when compared to AMR-positive TMA (10). Improved understanding of TMA, particularly with respect to HUS and the recognition of the range of etiological triggers, has focused on the role of complement protein expression and regulation (11). However, given the importance of thrombosis in the pathology, TMA may be viewed as a thromboinflammatory disorder in which complement and coagulation cascades are intimately linked in a network of activation and inhibition.

TMA in the highly sensitized kidney transplant recipient is not a commonly occurring pathology. Nonetheless, it is a rapidly evolving condition with non-specific early markers, which may lead to a severe kidney injury and even rapid graft loss. Early recognition and treatment, as well as an understanding of the likely pathological mechanisms, is vital to reverse TMA in a timely manner. This review combines a clinical description of TMA associated with the highly sensitized kidney transplant recipient with underlying pathophysiology and potential therapeutic targets.

Definition of post-kidney transplant TMA

The diagnosis of TMA is broadly defined as the presence of thrombocytopenia in addition to microangiopathic hemolytic anemia (MAHA)—that is to say, the presence of red cell fragments in the form of schistocytes, which develop as a consequence of the sheer stress on red blood cells as they pass through vessels narrowed by microthrombi. Additional systemic markers of hemolysis, such as lactate dehydrogenase (LDH), haptoglobin and bilirubin, may also be raised, while acute severe TMA may be accompanied by a consumptive coagulopathy in which fibrinogen and platelet levels fall. Diagnosing the underlying cause of TMA is more difficult, and the recent improvement in understanding TMA has led to increased clarity with respect to disease-specific diagnosis and treatment (12).

Clinical guidelines relating to the diagnosis of TMA after bone marrow transplantation, provide diagnostic criteria and classification of TMA severity (13); however, there are no such tools following renal transplantation. TMA following kidney transplantation is fundamentally different from that following bone marrow transplantation in that, while

significant systemic evidence of anemia, thrombocytopenia and raised LDH levels may be observed, end-organ damage is limited to the kidney graft itself. Because of this, initiation of TMA after kidney transplantation may be considered to be a local phenomenon. The systemic effects of this, if left unchecked, have the potential to lead to systemically observed effects on platelet counts and hemoglobin; however, unlike other causes of TMA, multi-organ damage in the form of neurological and gastrointestinal injury in addition to renal dysfunction is rare.

Diagnosis of TMA in the sensitized kidney transplant recipient is therefore predominantly confirmed by histological findings; however, given the thrombocytopenia in severe cases of TMA, obtaining a biopsy at the time may be judged too risky, and systemic features and clinical suspicion may suffice to implement initial treatment.

Histopathological findings—As described, TMA is, in itself, a description of the histopathological finding of thrombosis within the capillaries and arterioles of the affected organ—namely, the transplanted kidney. In many cases, histopathological assessment of TMA severity within the transplanted kidney is subjective and based on the presence of microthrombi in the glomeruli and arterioles, the presence of fibrin in the glomeruli, the thickening and double contours of the glomerular basement membrane, fibrinoid necrosis of the glomeruli, and arteriolar endothelial swelling (14). Satoskar et al. describe evaluation of 10 different histological features of TMA, including tubular isometric vacuolation of proximal tubules and mucoid arteriolar wall thickening, as well as systemic features of anemia, thrombocytopenia and elevated LDH (15). They found that interstitial fibrosis, tubular atrophy, degree of glomerular sclerosis and transplant glomerulopathy, which represent changes consistent with chronic graft injury, were all associated with graft loss, but that acute tubular necrosis was seen in grafts that subsequently recovered function, suggesting that elements of TMA are reversible. Indeed, in donor organs with TMA present at the time of implantation, recovery from TMA and lack of effect on long-term outcomes suggest that the presence or absence of the initiating trigger may be the most important predictor of resolution (16, 17).

Meehan et al., in their histological evaluation of kidney biopsies with TMA, observed that C4d positivity with TMA is both more likely in biopsies taken in the first 3 months post-transplant and more likely to be associated with graft loss (18). In addition, like the findings of Satoskar et al., the TMA+C4d+ group had evidence of donor-specific alloreactivity in both pre- (positive cross-match) and post-transplant (positive DSA) specimens. When assessing kidney biopsies with TMA from both native and allograft kidneys with HUS, drug toxicity and rejection as known underlying initiators of the TMA response, Chua et al. found that C4d positivity was the common denominator amongst various underlying precipitators, suggesting that complement activation, whether by classical or lectin pathways may be considered to be one of the hallmarks of the pathology (14).

Although C4d was previously believed to be strongly associated with AMR, as reflected in the Banff 2007 criteria, recent evidence regarding C4d-negative AMR has led to the inclusion in Banff 2013 of C4d-negative AMR, in which evidence of microvascular injury, glomerulitis (g), peritubular capillaritis (ptc) and TMA in combination with donor-specific

antibody (DSA) but no C4d deposition may still be classified as AMR (19, 20). At present, it is not clear whether C4d-negative AMR represents a complement-independent mechanism of graft injury and inflammation, or whether TMA associated AMR should be considered as a particularly acute form of aggressive AMR in which thrombo-inflammatory cross talk between complement and coagulation presents a very clear threat of graft loss if left unchecked.

Systemic features—Detection of the presence of donor-specific antibody is one of the Banff criteria of AMR, and also a prerequisite of identifying the highly sensitized recipient. Other systemic markers of TMA, such as anemia, thrombocytopenia, raised LDH levels and disseminated intravascular coagulopathy, are reported in the xenotransplant literature of acute AMR (21–25), and they form the basis of early reports of TMA in the kidney allograft (8). In the modern era, clinicians undertaking positive crossmatch transplantation are familiar with a clinical presentation of aggressive, severe AMR presenting with rapid onset of anuria, thrombocytopenia and evidence of hemolysis, requiring radical therapies in order to prevent graft loss (26). It should, however, be noted that our understanding of the wide array of phenotypes of antibody mediated rejection are advancing (27).

Incidence in sensitized patients post-transplant

TMA is an infrequent phenomenon, hence the reason for multicenter registry groups such as the Harvard TMA Research Collaborative. Satoskar et al., in their histological evaluation of the features of TMA, found the incidence to be low overall. Of 960 patients, 59 (6.1%) patients showed de novo TMA post-transplant. Of those, over half ($n = 33$, 55%) had diffuse C4d staining, and of those, 84% were associated with a high panel reactive antibody (PRA) (15). The true incidence of TMA in cross-match positive kidney transplantation is not described.

Pathophysiology: Coagulation and complement description and cross-talk

Thrombotic microangiopathy is a product of an acute coagulopathic process in combination with complement activation. Complement and coagulation systems are serine protease systems with homeostatic function found in primitive ‘living fossil’ life forms such as the horseshoe crab (28). These interactions between the systems are not surprising; however, the complexity of this cross-talk to both activate and regulate is only now being understood through in vitro, animal and xenotransplantation models. Rose and Cooper, in their examination of allografts from clinical and experimental recipients, together with xenografts, observed that venous thrombosis was present early in the time course of AMR and posit that venular thrombosis is a key event in the development of AMR, however it may be more accurate to consider thrombosis (and TMA) and AMR to be overlapping entities (29).

The complement system refers to a primitive innate host defense system, consisting of a cascade or chain of more than 30 linked plasma proteins and cell surface receptors. Its name results from its being a “heat-labile aspect of plasma serum that ‘complemented’ antibodies to kill bacteria” (30, 31). It is activated by three initial pathways: the classical pathway (initiated by immune complexes), lectin pathway (in which mannose binding lectin (MBL)

or other lectins, bind on to a variety of membrane-bound glycoproteins or glycolipids), and alternative pathway (considered to predominantly describe the function of amplification of C3b cleaved through classical and lectin pathways, although it also describes the mechanism in which activating surfaces may directly activate C3) (Figure 1). Complement activation results in the generation of effectors to mediate the functions of complement, either by directly inserting into the cell membrane (C5b-9, membrane attack complex (MAC)), or by receptor-mediated cell activation. Complement control proteins act to regulate the formation of the converting enzyme complexes which are assembled by the respective activation pathways, and are therefore regulators of complement activation; alternatively, other regulators limit the insertion of the MAC into the activating cell surface. This broad defense mechanism is critical to the host response to pathogen-induced danger and integral to initiation of the inflammatory and adaptive immune response (32). The nomenclature of complement follows the numbering in which factors were discovered. Complement has many roles, but essentially serves three physiologic purposes.

First, it is an innate defense against infection. C3 and C4 are involved in opsonization, following which C3a, C5a and C4a induce chemotaxis and a leucocyte response. Additionally, complement results in direct damage to cells via the MAC, which causes cell membrane perforation (33). All routes of complement activation converge on the C3 to C5 positive feedback loop by a chain effect of enzyme cleavage in which the activation of the initial protein cleaves and activates the next zymogen in the complement pathway (34), leading to the production of the MAC. Second, activated complement products are involved in the development of a specific adaptive response. The opsonization of antigen with complement products has been shown to have an effect on both antigen presentation of both T and B cell responses in transplantation (35–37). Third, complement is involved in the clearance of waste: C1q binds directly to apoptotic cells and promotes clearance through C1q receptors on phagocytic cells (38), as well as binding directly to immune complexes. The complement cascade is therefore a system that both recognizes and eliminates the dangers of invading microbial pathogens, immune complexes, apoptotic cells and cellular debris by opsonization and induction of inflammation (39).

Like complement, the coagulation cascade is a system of serine proteases, but in this case, activation leads to thrombin-activated platelet aggregation in primary hemostasis. In secondary hemostasis, the activation of thrombin results in cleavage of soluble fibrinogen to fibrin monomers to create a stable plug, which is later degraded by plasmin in the fibrinolytic portion. Two activation pathways for this cascade are commonly described (Figure 2): the contact or ‘intrinsic’ pathway in which local damage to the endothelium results in contact activation, and the extrinsic pathway in which tissue factor (TF) released by injured cells complexes with Factor VIIa, resulting in a proteolytic chain reaction in which thrombin is generated from prothrombin.

Thrombin may be considered the central pivot within the coagulation cascade; it also exerts many non-hemostatic effects as both regulator and inhibitor through the activation of protease-activated receptors (PAR) (40).

The effect of thrombin has been studied in a xenotransplant model of pig-to-baboon liver transplant, in which significant thrombocytopenia and bleeding risk together with TMA limit graft survival in control animals. Exogenous administration of coagulation factors either in the form of human prothrombin concentrate complex (Octoplex) maintained platelet counts, but resulted in large vessel and graft thrombosis with concurrent TMA. In the same model, the continuous administration of human recombinant Factor VIIa resulted in preservation of platelet counts in the absence of TMA, confirming the integral role thrombin plays in TMA in activating platelets in addition to its role in secondary hemostasis (41).

HLA antibody activation of complement—The primary mechanism by which HLA antibody activates complement is the classical pathway, in which antigen-antibody complexes reveal the C1q binding site on the Fc portion of the bound immunoglobulin, thereby initiating the C1q-C1r-C1s complex, which in turn leads to the activation of C4 and C2 (42). This is confirmed by the finding that an anti-C1s antibody inhibits HLA-ab-dependent complement activation (43).

HLA antibody activation of the endothelium—Endothelial cell (EC) activation occurs by transcription dependent or independent mechanisms. It has been demonstrated that HLA antibody activates ECs in a number of ways. HLA antibody induced exocytosis of the endothelial cell (EC) stimulates enhanced expression of p-selectin, promoting leukocyte migration (44). In addition, in vitro, HLA class 1 antibody induces an up-regulation of intracellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs) (45), as does thrombin, thereby supporting monocyte adhesion (46).

HLA antibody EC activation has been demonstrated to activate both complement and coagulation pathways. The importance of local synthesis of complement in transplantation has been advocated for some time(37). In vitro stimulation experiments have demonstrated the role of HLA-antibody activated ECs to produce local C4 (47).

In vitro work also suggests that incubation with specific anti-HLA antibody against a line of human umbilical ECs induces up-regulation of TF expression and activity, defining a role in direct initiation of coagulation by HLA antibody binding (48).

Yamakuchi et al. have shown that, both in vitro and in vivo, anti-HLA antibody induces endothelial cell exocytosis, and, as a consequence, increased expression of von Willebrand Factor (vWF)(44). Von Willebrand Factor is an important multimeric glycoprotein that is key to both primary and secondary hemostasis. It also promotes platelet adhesion to thrombogenic surfaces as well as platelet-to-platelet cohesion during the initial phase of thrombus formation, and additionally has an indirect effect on Factor VIII, reducing its clearance (49). Because it is such an important player in hemostasis, secretion of vWF from Weibel-Palade bodies is kept under sophisticated levels of control in order to ensure timely and effective delivery of this multimeric protein (50). Since raised plasma levels of vWF are associated with an increased risk of arterial thrombosis and are largely genetically determined, there is increasing interest in genetic studies of vWF variation antigen and activity and associations with thrombotic disease (51). Interestingly, ADAMTS-13 (a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13) is an

enzymatic regulator of vWF multimer size, which is important for controlling thrombus formation. Clinically, ADAMTS-13 deficiency is increasingly recognized as a cause of arterial thrombosis, as well as thrombotic thrombocytopenic purpura (TTP), a potentially life-threatening form of systemic TMA (52). In a recent stringent registry study of all forms of TMA/TTP in the context of ADAMTS-13 deficiency, transplantation was associated with a less severe form of the deficiency, that is to say, higher levels of ADAMTS-13 activity. However, this broad categorization of transplantation includes both solid organ transplantation and hematopoietic stem cell transplantation, so understanding the role of ADAMTS-13 in positive cross-match transplantation is, as yet, unexplored (53).

The primary ignition mechanism—whether complement or coagulation activation—at the site of antibody binding is not clear; however, once activated, complement and coagulation then engage in a complex set of interactions(54). TMA may be considered to be a consequence of this thromboinflammatory fire, and its severity potentially related to the combination of donor and recipient characteristics to both propel or inhibit the ensuing effects.

Coagulation initiation of the complement cascade: Previously, it has been demonstrated that thrombin is able to directly activate complement at the level of C3. It has also been shown that thrombin can cleave C5 directly, independent of C3 (55). Both of these mechanisms would suggest that thrombin in and of itself, is able to activate complement independent of the classical pathway, thus providing additional amplification of the complement response. The importance of thrombin-mediated cleavage of C5 in vivo has been questioned, particularly in the light of the findings by Foley et al. (56). In their in vivo model of thrombosis, the efficiency and rate of complement cleavage by thrombin was found likely to be related to indirect effects through plasmin-mediated activity, rather than the generation of C5 by thrombin directly, since this thrombin produced too little C5a to be measured, while plasmin, the generation of which is amplified as a response to thrombin in combination with C5 convertase, was demonstrated to be a major mediator of the activation of C5. An alternative explanation, as Krisinger et al have demonstrated, for the efficacy of thrombin activation of complement, is that despite thrombin generating relatively small quantities of C5 effectors, the lytic complement products (MAC) generated by thrombin (C5-T and C5b-T) are more profoundly effective than conventional C5b (57). Thus it may be the case that thrombin induced complement end products are, in and of themselves, more damaging in the setting of TMA.

Additionally, evidence is accumulating about the role of platelet mediated complement activation, with the hypothesis that the interaction between platelets and complement induce inflammatory mediators and combine to produce acute and chronic inflammation, in addition to thrombosis(58).

Coagulation regulation of complement: In their examination of the effect of vWF expression on blood outgrowth ECs (ECs derived from peripheral blood), Noone et al. observed that contrary to expectation, vWF-deficient cells had greater C3 deposition than cells expressing vWF. In addition, they observed greater complement-dependent cytotoxicity of the vWF-deficient cells compared to controls. These results concur with earlier findings

that vWF is secreted in response to complement fixation and antibody induced EC activation (44, 59), but they also suggest that vWF has additional role in subsequently serving as a complement regulator on EC surfaces, protecting cells from the effects of complement (60). This finding adds additional mechanistic understanding to the association of increased vWF in endothelial transcripts of C4d-positive AMR (61), as well as introducing a complex role for vWF in HLA antibody-induced injury as both an initiator of coagulation and subsequently a regulator of complement (Figure 3).

Once activated, thrombin itself may act to regulate further complement activation by inducing increased expression of decay accelerating factor (CD55, an important complement regulator inhibiting the C3 and C5 convertases required for assembly of the MAC) by PAR signaling pathways (62, 63).

Complement initiation of the coagulation cascade: There are multiple ways in which the terminal complex of complement activation (MAC: C5b-9) initiates coagulation. In response to antibody-mediated complement activation, TF activity is increased, thus initiating coagulation (64). Ikeda et al have demonstrated that C5a is able to induce TF activity of human endothelial cells(65). Similarly, in vitro, Hattori et al. demonstrated that exposure of human endothelial cells to the MAC (C5b-9) induces both increased exocytosis of vWF (59), as well as exposing the catalytic binding site for assembling the prothrombinase complex (66, 67).

Confirming this finding, Rataj et al have shown that inhibition of the terminal complement pathway and formation of the MAC with eculizumab resulted in less antibody-mediated injury in a xenogeneic ex-vivo model of coagulation. Porcine ECs incubated with human plasma in the presence of a C5 inhibitor resulted in reduced EC activation, platelet activation and thrombus size, despite retaining the capacity to generate thrombin (68). The MAC (C5b-9) is known to be an initiator of the TF-dependent pathway of coagulation and has also been demonstrated to increase vWF expression; thus C5 inhibition is likely to down-regulate the coagulation cascade in addition to preventing direct complement-mediated cell damage (59).

In addition to being a key initiator of the lectin pathway, MBL-associated serine protease 1 (MASP-1) has been shown to directly activate prothrombin to produce thrombin (69), as well as activating FXIII and thrombin activatable fibrinolysis inhibitor (70). In addition, MASP-2 has been demonstrated to lead directly to clot formation, similar to that generated by thrombin (71). Kozarcanin et al. have demonstrated that proteases from the lectin pathway (MASP-1 and MASP-2) are produced in increased amounts when they themselves are induced by activated platelets and fibrin. This is supported by findings in polytrauma patients of low fibrin levels being associated with MASP/AT (MASP-antithrombin) complexes (72) and suggests that the lectin pathway both activates and amplifies the coagulation cascade.

Complement regulation of coagulation: The C1-esterase inhibitor is able to inhibit all three complement activation pathways, and in vitro, it is able to inhibit thrombin activity by forming enzyme inhibitor complexes (73) that are demonstrated to be more effective when

on the vascular endothelium (74). In addition, C1-inh also inactivates kallikrein and FXIIa (75, 76), suggesting a potential mechanism by which treatment with recombinant C1-inh may affect coagulation, although this has not been clinically confirmed in transplant studies.

Treatment of thrombotic microangiopathy – targeting complement & coagulation

Conventional treatment of TMA has focused on trying to remove the underlying cause. In the case of AMR-induced TMA, the mainstay of treatment is plasmapheresis (PP), with or without IVIg and additional immunosuppression. Treatment with IVIg is known to have the potential for prothrombotic side effects, the reasons for which are not fully elucidated, although it has been demonstrated that exogenous FXI is present in 90% of IVIg preparations tested (77). Overall, complications from PP are rare: in the UK, a rate of 1.7% is reported (78). Of the reported PP complications, perhaps most concerning for patients requiring a kidney biopsy for definitive diagnosis of TMA is that of iatrogenic coagulopathy, in addition to any systemic disseminated intravascular coagulopathy seen in response to the TMA. Interestingly, as reported by Montgomery et al., PP may itself promote complement activation by removing endogenous C1-inh (79).

The use of terminal complement inhibition (anti-C5 mAb) to prevent acute AMR in highly sensitized recipients was initially demonstrated in a murine model (80). In humans, it has been demonstrated that death-censored graft outcomes for patients with AMR-negative TMA are considerably better than AMR-positive TMA, despite a similar response to treatment (10). In keeping with this finding, the use of eculizumab (anti-C5 therapy) appeared to be extremely promising in reducing early AMR following crossmatch-positive (HLA-incompatible (HLAi)) transplantation. An important pair of studies by Stegall et al. highlight the use of terminal complement inhibition in highly sensitized patients in order to salvage grafts undergoing acute AMR that may be likely to be lost as a result of the aggressive end-organ damage(81); unfortunately, this early abrogation of AMR does not result in long term benefit(82). Taken together, these studies suggest that TMA comprises a significant clinico-pathological manifestation of AMR, capable of inducing graft loss. Full data from a multicenter trial of eculizumab prophylaxis in highly sensitized kidney transplants has yet to fully report; however, while successful as rescue therapy for grafts at risk of early failure due to AMR & TMA, the role of prophylactic complement inhibition, and the question of whether preventing or ameliorating the effect of TMA through complement inhibition prevents long-term damage induced by antibody is yet to be established.

The successful use of recombinant C1-inh has been reported for aggressive AMR in xenotransplant non-human primate models (83–85) and shows an organ-protective effect in a severe sepsis model (86). In highly sensitized (HLAi) transplantation, a small study using C1-inh (C1-INH, Berinert) as preventative therapy in adult patients desensitized with IVIg, rituximab and plasma exchange showed that, like the eculizumab studies, AMR was prevented during the treatment period, although this effect was not maintained (87). Interestingly, less delayed graft function was observed in the treatment group compared to the control group, suggesting a protective effect of C1-inhibition for against complement-mediated ischemia-reperfusion injury (87).

C1-inh therapy has also been used as a treatment for AMR post-transplant to prevent chronic inflammation leading to transplant glomerulopathy. In a study by Montgomery et al., patients with aggressive AMR presenting with a rapid onset of “severe oliguric AMR” were deliberately excluded—likely excluding patients with TMA associated with AMR. There was no difference in histological outcome when C1-inh was added to standard care, although it was noted that PP itself depleted endogenous C1-inh levels, thereby potentially increasing complement activation (79).

While complement-targeted therapies are currently of interest, there are fewer options for therapies targeting the coagulation cascade. Recombinant soluble human TBM—which has been used as a treatment for disseminated intravascular coagulation in Japan—has been successfully used as a means of treating TMA after liver transplantation (88) and shows promise as a therapeutic agent (89). Treatment of the graft itself with a locally active cell-localized (cytotoxic) anti-thrombin agent, Thrombalexin, has been used in a rat model of aggressive AMR, where it successfully prolonged graft survival compared to controls (90). Presently, we are investigating Thrombalexin in a non-human primate model of sensitized transplantation in which TMA is a predominant feature (91). Using Thrombalexin infused kidneys, histological evidence of TMA severity was reduced in treated animals compared to controls, while platelet and fibrin deposition were minimal. Additionally, although non-significant, there was a trend towards decreased deposition of C4d, suggesting an additional role of thrombin in earlier activation of the complement pathway which requires mechanistic exploration (92).

Using a similar model of organ-specific treatment, inhibition of the C3-convertase with Mirococept (APT070) is being clinically investigated for the treatment of ischemia-reperfusion injury (93, 94), but it may also have utility to protect the kidney graft for patients at risk of antibody-induced TMA, either by reducing sensitization against alloantigen or inhibiting the effector function of alloantibody on the endothelial surface.

In summary, TMA associated with HLAi transplantation is often an early and aggressive feature of AMR, occurring as a result of the complex interaction of complement and coagulation cascades. As Ekdahl and colleagues describe, the term thromboinflammation describes this cross-talk most effectively (95). TMA in the highly sensitized kidney transplant recipient is a complex interplay of coagulation and complement activation. The initial HLA antibody trigger may both activate the complement pathway, and initiate coagulation. In addition, as the presented evidence demonstrating the integral roles for C3, C5b-9, thrombin and plasmin indicate, the close interactions between complement and coagulation to both activate and inhibit are such that a pathological trigger of one system is likely to result in effects on the other, and the notion of ‘pure’ complement or coagulation activation is an over-simplification. Additionally, the changing roles of the key players under differing conditions suggest a sophistication that may prove difficult to parse with isolated mechanistic in vitro studies. Traditional views of HLA antibody have focused on the initiation of complement, and studies relating to the initiation of coagulation by HLA antibody activation are lacking. Therapies targeting complement and coagulation have the potential to reduce TMA, but despite promise, they do not so far appear to improve long-term AMR outcomes. It seems likely that, in such a complex system, a single intervention

may not be sufficient to target both systems adequately, and combination therapy may yield most benefit in preventing or treating TMA in the sensitized recipient. Future experimental directions in this field will advance mechanistic understanding of the interactions between coagulation and complement and define the relative importance of particular pathways under given clinical conditions. Additionally, further work will need to establish the optimal dosing and treatment periods for current anti-complement strategies, as well as alternative strategies to protect grafts from TMA, in order to enhance efficacy and limit potential side effects associated with complement inhibition.

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Abbreviations:

ABOi	ABO antibody incompatible
AIT	Antibody incompatible transplantation
AMR	Antibody-mediated rejection
DSA	Donor-specific antibody
EC	Endothelial cell
LDH	Lactate dehydrogenase
HLAi	Human leukocyte antigen–incompatible
HUS	Hemolytic uremic syndrome
ICAM	Intracellular adhesion molecule
MASP	MBL-associated serine protease
MAC	Membrane attack complex - C5b-9
MBL	Mannose binding lectin
PAR	Protease activated receptors
TF	Tissue factor
TMA	Thrombotic microangiopathy
vWF	von Willebrand factor
VCAM	Vascular cell adhesion molecule

References

1. Meri S Complement activation in diseases presenting with thrombotic microangiopathy. *Eur J Intern Med.* 2013;24(6):496–502. [PubMed: 23743117]
2. George JN, Nester CM. Syndromes of thrombotic microangiopathy. *The New England journal of medicine.* 2014;371(7):654–66. [PubMed: 25119611]
3. Chiurciu C, Ruggerenti P, Remuzzi G. Thrombotic microangiopathy in renal transplantation. *Ann Transplant.* 2002;7(1):28–33.
4. Tanabe K, Tokumoto T, Ishida H, Shimmura H, Omoto K, Makiyama K, et al. Prospective analysis of thrombotic microangiopathy after renal transplantation: comparison between cyclosporine and tacrolimus immunosuppression. *Transplant Proc.* 2002;34(5):1819–20. [PubMed: 12176590]
5. Ashman N, Chapagain A, Dobbie H, Raftery MJ, Sheaff MT, Yaqoob MM. Belatacept as maintenance immunosuppression for postrenal transplant de novo drug-induced thrombotic microangiopathy. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons.* 2009;9(2):424–7.
6. Caires RA, Marques ID, Repizo LP, Sato VA, Carmo LP, Machado DJ, et al. De novo thrombotic microangiopathy after kidney transplantation: clinical features, treatment, and long-term patient and graft survival. *Transplant Proc.* 2012;44(8):2388–90. [PubMed: 23026601]
7. Hutton MM, Prentice CR, Allison ME, Duguid WP, Kennedy AC, Struthers NW, et al. Renal homotransplant rejection associated with microangiopathic haemolytic anaemia. *Br Med J.* 1970;3(5714):87–8. [PubMed: 4913789]
8. Pillay VK, Kurtzman NA, Manaligod JR, Jonasson O. Selective thrombocytopenia due to localised microangiopathy of renal allografts. *Lancet.* 1973;302(7836):988–91. [PubMed: 4127277]
9. Ikeda T, Okumi M, Unagami K, Kanzawa T, Sawada A, Kawanishi K, et al. Two cases of kidney transplantation-associated thrombotic microangiopathy successfully treated with eculizumab. *Nephrology (Carlton, Vic).* 2016.
10. Wu K, Budde K, Schmidt D, Neumayer HH, Lehner L, Bamoulid J, et al. The inferior impact of antibody-mediated rejection on the clinical outcome of kidney allografts which develop de novo thrombotic microangiopathy. *Clinical transplantation.* 2015.
11. Kerr H, Richards A. Complement-mediated injury and protection of endothelium: lessons from atypical haemolytic uraemic syndrome. *Immunobiology.* 2012;217(2):195–203. [PubMed: 21855165]
12. Williams LA, Marques MB, Education Committee of the Academy of Clinical Laboratory P, Scientists. Pathology Consultation on the Diagnosis and Treatment of Thrombotic Microangiopathies (TMAs). *Am J Clin Pathol.* 2016;145(2):158–65. [PubMed: 27124904]
13. Ho VT, Cutler C, Carter S, Martin P, Adams R, Horowitz M, et al. Blood and marrow transplant clinical trials network toxicity committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2005;11(8):571–5.
14. Chua JS, Baelde HJ, Zandbergen M, Wilhelmus S, van Es LA, de Fijter JW, et al. Complement Factor C4d Is a Common Denominator in Thrombotic Microangiopathy. *Journal of the American Society of Nephrology : JASN.* 2015;26(9):2239–47. [PubMed: 25573909]
15. Satoskar AA, Pelletier R, Adams P, Nadasdy GM, Brodsky S, Pesavento T, et al. De novo thrombotic microangiopathy in renal allograft biopsies-role of antibody-mediated rejection. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons.* 2010;10(8):1804–11.
16. McCall SJ, Tuttle-Newhall JE, Howell DN, Fields TA. Prognostic significance of microvascular thrombosis in donor kidney allograft biopsies. *Transplantation.* 2003;75(11):1847–52. [PubMed: 12811244]
17. Soares KC, Arend LJ, Lonze BE, Desai NM, Alachkar N, Naqvi F, et al. Successful Renal Transplantation of Deceased Donor Kidneys With 100% Glomerular Fibrin Thrombi and Acute Renal Failure Due To Disseminated Intravascular Coagulation. *Transplantation.* 2016.

18. Meehan SM, Kremer J, Ali FN, Curley J, Marino S, Chang A, et al. Thrombotic microangiopathy and peritubular capillary C4d expression in renal allograft biopsies. *Clin J Am Soc Nephrol*. 2011;6(2):395–403. [PubMed: 20966124]
19. Haas M, Sis B, Racusen LC, Solez K, Glotz D, Colvin RB, et al. Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2014;14(2):272–83.
20. Haas M The Revised (2013) Banff Classification for Antibody-Mediated Rejection of Renal Allografts: Update, Difficulties, and Future Considerations. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2016;16(5):1352–7.
21. Ezzelarab M, Cortese-Hassett A, Cooper DK, Yazer MH. Extended coagulation profiles of healthy baboons and of baboons rejecting GT-KO pig heart grafts. *Xenotransplantation*. 2006;13(6):522–8. [PubMed: 17059579]
22. Lin CC, Cooper DK, Dorling A. Coagulation dysregulation as a barrier to xenotransplantation in the primate. *Transpl Immunol*. 2009;21(2):75–80. [PubMed: 19000927]
23. Ezzelarab M, Ekser B, Gridelli B, Iwase H, Ayares D, Cooper DK. Thrombocytopenia after pig-to-baboon liver xenotransplantation: where do platelets go? *Xenotransplantation*. 2011;18(6):320–7. [PubMed: 22168139]
24. Ekser B, Lin CC, Long C, Echeverri GJ, Hara H, Ezzelarab M, et al. Potential factors influencing the development of thrombocytopenia and consumptive coagulopathy after genetically modified pig liver xenotransplantation. *Transplant international : official journal of the European Society for Organ Transplantation*. 2012;25(8):882–96. [PubMed: 22642260]
25. Cooper DK, Ezzelarab MB, Hara H, Iwase H, Lee W, Wijkstrom M, et al. The pathobiology of pig-to-primate xenotransplantation: a historical review. *Xenotransplantation*. 2016.
26. Orandi BJ, Lonze BE, Jackson A, Terezakis S, Kraus ES, Alachkar N, et al. Splenic Irradiation for the Treatment of Severe Antibody-Mediated Rejection. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2016.
27. Garg N, Samaniego MD, Clark D, Djamali A. Defining the phenotype of antibody-mediated rejection in kidney transplantation: Advances in diagnosis of antibody injury. *Transplantation Reviews*. 2017;31(4):257–67. [PubMed: 28882367]
28. Zhu Y, Thangamani S, Ho B, Ding JL. The ancient origin of the complement system. *The EMBO journal*. 2005;24(2):382–94. [PubMed: 15616573]
29. Rose AG, Cooper DK. Venular thrombosis is the key event in the pathogenesis of antibody-mediated cardiac rejection. *Xenotransplantation*. 2000;7(1):31–41. [PubMed: 10809055]
30. Walport MJ. Complement. First of two parts. *The New England journal of medicine*. 2001;344(14):1058–66. [PubMed: 11287977]
31. Walport MJ. Complement. Second of two parts. *The New England journal of medicine*. 2001;344(15):1140–4. [PubMed: 11297706]
32. Ricklin D, Reis ES, Lambris JD. Complement in disease: a defence system turning offensive. *Nature reviews Nephrology*. 2016;12(7):383–401. [PubMed: 27211870]
33. Chowdhury P, Zhou W, Sacks SH. Complement in renal transplantation. *Nephron Clinical practice*. 2003;95(1):c3–8. [PubMed: 14520015]
34. Janeway CA TP Jr, Walport M, et al. The complement system and innate immunity. New York: Garland Science; 2001.
35. Kemper C, Chan AC, Green JM, Brett KA, Murphy KM, Atkinson JP. Activation of human CD4+ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. *Nature*. 2003;421(6921):388–92. [PubMed: 12540904]
36. Marsh JE, Farmer CK, Jurcevic S, Wang Y, Carroll MC, Sacks SH. The allogeneic T and B cell response is strongly dependent on complement components C3 and C4. *Transplantation*. 2001;72(7):1310–8. [PubMed: 11602861]
37. Pratt JR, Basheer SA, Sacks SH. Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nature medicine*. 2002;8(6):582–7.

38. Galvan MD, Greenlee-Wacker MC, Bohlson SS. C1q and phagocytosis: the perfect complement to a good meal. *J Leukoc Biol.* 2012;92(3):489–97. [PubMed: 22715140]
39. Nonaka M Evolution of the complement system. *Subcell Biochem.* 2014;80:31–43. [PubMed: 24798006]
40. Posma JJ, Posthuma JJ, Spronk HM. Coagulation and non-coagulation effects of thrombin. *Journal of thrombosis and haemostasis : JTH.* 2016.
41. Navarro-Alvarez N, Shah JA, Zhu A, Ligoeka J, Yeh H, Elias N, et al. The Effects of Exogenous Administration of Human Coagulation Factors Following Pig-to-Baboon Liver Xenotransplantation. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons.* 2016;16(6):1715–25.
42. Lintner KE, Wu YL, Yang Y, Spencer CH, Hauptmann G, Hebert LA, et al. Early Components of the Complement Classical Activation Pathway in Human Systemic Autoimmune Diseases. *Frontiers in immunology.* 2016;7:36. [PubMed: 26913032]
43. Thomas KA, Valenzuela NM, Gjertson D, Mulder A, Fishbein MC, Parry GC, et al. An Anti-C1s Monoclonal, TNT003, Inhibits Complement Activation Induced by Antibodies Against HLA. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons.* 2015;15(8):2037–49.
44. Yamakuchi M, Kirkiles-Smith NC, Ferlito M, Cameron SJ, Bao C, Fox-Talbot K, et al. Antibody to human leukocyte antigen triggers endothelial exocytosis. *Proceedings of the National Academy of Sciences of the United States of America.* 2007;104(4):1301–6. [PubMed: 17229850]
45. Naemi FM, Carter V, Kirby JA, Ali S. Anti-donor HLA class I antibodies: pathways to endothelial cell activation and cell-mediated allograft rejection. *Transplantation.* 2013;96(3):258–66. [PubMed: 23823649]
46. Kaplanski G, Marin V, Fabrigoule M, Boulay V, Benoliel A- M, Bongrand P, et al. Thrombin-Activated Human Endothelial Cells Support Monocyte Adhesion In Vitro Following Expression of Intercellular Adhesion Molecule-1 (ICAM-1; CD54) and Vascular Cell Adhesion Molecule-1 (VCAM-1; CD106). *Blood.* 1998;92(4):1259–67. [PubMed: 9694714]
47. Hamer R, Molostvov G, Lowe D, Satchell S, Mathieson P, Ilyas R, et al. Human leukocyte antigen-specific antibodies and gamma-interferon stimulate human microvascular and glomerular endothelial cells to produce complement factor C4. *Transplantation.* 2012;93(9):867–73. [PubMed: 22361472]
48. Naji A, Deschaseaux F, Racadot E, Ferrand C, Justrabo E, Guignier F, et al. Induction of tissue factor expression on human umbilical vein endothelial cells by cell-specific HLA class I antibody: preliminary data. *Transplantation proceedings.* 2005;37(6):2892–3. [PubMed: 16182846]
49. Ruggeri ZM. The role of von Willebrand factor in thrombus formation. *Thromb Res.* 2007;120 Suppl 1:S5–9. [PubMed: 17493665]
50. Nightingale T, Cutler D. The secretion of von Willebrand factor from endothelial cells; an increasingly complicated story. *Journal of thrombosis and haemostasis : JTH.* 2013;11 Suppl 1:192–201.
51. van Schie MC, van Loon JE, de Maat MP, Leebeek FW. Genetic determinants of von Willebrand factor levels and activity in relation to the risk of cardiovascular disease: a review. *Journal of thrombosis and haemostasis : JTH.* 2011;9(5):899–908. [PubMed: 21342431]
52. Sonneveld MA, de Maat MP, Leebeek FW. Von Willebrand factor and ADAMTS13 in arterial thrombosis: a systematic review and meta-analysis. *Blood reviews.* 2014;28(4):167–78. [PubMed: 24825749]
53. Bendapudi PK, Li A, Hamdan A, Uhl L, Kaufman R, Stowell C, et al. Impact of severe ADAMTS13 deficiency on clinical presentation and outcomes in patients with thrombotic microangiopathies: the experience of the Harvard TMA Research Collaborative. *Br J Haematol.* 2015;171(5):836–44. [PubMed: 26314936]
54. Huber-Lang M, Ekdahl KN, Wiegner R, Fromell K, Nilsson B. Auxiliary activation of the complement system and its importance for the pathophysiology of clinical conditions. *Seminars in immunopathology.* 2017.
55. Huber-Lang M, Sarma JV, Zetoune FS, Rittirsch D, Neff TA, McGuire SR, et al. Generation of C5a in the absence of C3: a new complement activation pathway. *Nature medicine.* 2006;12(6):682–7.

56. Foley JH, Walton BL, Aleman MM, O'Byrne AM, Lei V, Harrasser M, et al. Complement Activation in Arterial and Venous Thrombosis is Mediated by Plasmin. *EBioMedicine*. 2016;5:175–82. [PubMed: 27077125]
57. Krisinger MJ, Goebeler V, Lu Z, Meixner SC, Myles T, Pryzdial EL, et al. Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. *Blood*. 2012;120(8):1717–25. [PubMed: 22802338]
58. Peerschke EIB, Yin W, Ghebrehiwet B. Platelet Mediated Complement Activation. *Advances in experimental medicine and biology*. 2008;632:81–91. [PubMed: 19025116]
59. Hattori R, Hamilton KK, McEver RP, Sims PJ. Complement proteins C5b-9 induce secretion of high molecular weight multimers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. *The Journal of biological chemistry*. 1989;264(15):9053–60. [PubMed: 2470750]
60. Noone DG, Riedl M, Pluthero FG, Bowman ML, Liszewski MK, Lu L, et al. Von Willebrand factor regulates complement on endothelial cells. *Kidney international*. 2016;90(1):123–34. [PubMed: 27236750]
61. Sis B, Jhangri GS, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial Gene Expression in Kidney Transplants with Alloantibody Indicates Antibody-Mediated Damage Despite Lack of C4d Staining. *American Journal of Transplantation*. 2009;9(10):2312–23. [PubMed: 19681822]
62. Lidington EA, Haskard DO, Mason JC. Induction of decay-accelerating factor by thrombin through a protease-activated receptor 1 and protein kinase C-dependent pathway protects vascular endothelial cells from complement-mediated injury. *Blood*. 2000;96(8):2784–92. [PubMed: 11023512]
63. Lidington EA, Steinberg R, Kinderlerer AR, Landis RC, Ohba M, Samarel A, et al. A role for proteinase-activated receptor 2 and PKC-epsilon in thrombin-mediated induction of decay-accelerating factor on human endothelial cells. *American journal of physiology Cell physiology*. 2005;289(6):C1437–47. [PubMed: 16079188]
64. Carson SD, Johnson DR. Consecutive enzyme cascades: complement activation at the cell surface triggers increased tissue factor activity. *Blood*. 1990;76(2):361–7. [PubMed: 2369639]
65. Ikeda K, Nagasawa K, Horiuchi T, Tsuru T, Nishizaka H, Niho Y. C5a induces tissue factor activity on endothelial cells. *Thrombosis and haemostasis*. 1997;77(2):394–8. [PubMed: 9157602]
66. 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. *The Journal of biological chemistry*. 1990;265(7):3809–14. [PubMed: 2105954]
67. Sims PJ, Faioni EM, Wiedmer T, Shattil SJ. Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. *The Journal of biological chemistry*. 1988;263(34):18205–12. [PubMed: 2848029]
68. Rataj D, Werwitzke S, Haarmeijer B, Winkler M, Ramackers W, Petersen B, et al. Inhibition of complement component C5 prevents clotting in an ex vivo model of xenogeneic activation of coagulation. *Xenotransplantation*. 2016;23(2):117–27. [PubMed: 26773447]
69. Krarup A, Wallis R, Presanis JS, Gal P, Sim RB. Simultaneous activation of complement and coagulation by MBL-associated serine protease 2. *PloS one*. 2007;2(7):e623. [PubMed: 17637839]
70. Hess K, Ajjan R, Phoenix F, Dobo J, Gal P, Schroeder V. Effects of MASP-1 of the complement system on activation of coagulation factors and plasma clot formation. *PloS one*. 2012;7(4):e35690.
71. Gulla KC, Gupta K, Krarup A, Gal P, Schwaebler WJ, Sim RB, et al. Activation of mannan-binding lectin-associated serine proteases leads to generation of a fibrin clot. *Immunology*. 2010;129(4):482–95. [PubMed: 20002787]
72. Kozarcanin H, Lood C, Munthe-Fog L, Sandholm K, Hamad OA, Bengtsson AA, et al. The lectin complement pathway serine proteases (MASPs) represent a possible crossroad between the coagulation and complement systems in thromboinflammation. *Journal of thrombosis and haemostasis : JTH*. 2016;14(3):531–45. [PubMed: 26614707]

73. Cugno M, Bos I, Lubbers Y, Hack CE, Agostoni A. In vitro interaction of C1-inhibitor with thrombin. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. 2001;12(4):253–60. [PubMed: 11460008]
74. Caccia S, Castelli R, Maiocchi D, Bergamaschini L, Cugno M. Interaction of C1 inhibitor with thrombin on the endothelial surface. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. 2011;22(7):571–5. [PubMed: 21959589]
75. Davis AE 3rd., Biological effects of C1 inhibitor. *Drug news & perspectives*. 2004;17(7):439–46. [PubMed: 15514703]
76. van der Graaf F, Koedam JA, Bouma BN. Inactivation of kallikrein in human plasma. *J Clin Invest*. 1983;71(1):149–58. [PubMed: 6184384]
77. Paran D, Herishanu Y, Elkayam O, Shopin L, Ben-Ami R. Venous and arterial thrombosis following administration of intravenous immunoglobulins. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. 2005;16(5):313–8. [PubMed: 15970713]
78. Pusey CD, Levy JB. Plasmapheresis in immunologic renal disease. *Blood Purif*. 2012;33(1–3):190–8. [PubMed: 22269818]
79. Montgomery RA, Orandi BJ, Racusen L, Jackson AM, Garonzik-Wang JM, Shah T, et al. Plasma-Derived C1 Esterase Inhibitor for Acute Antibody-Mediated Rejection Following Kidney Transplantation: Results of a Randomized Double-Blind Placebo-Controlled Pilot Study. *Am J Transplant*. 2016.
80. Wang H, Arp J, Liu W, Faas SJ, Jiang J, Gies DR, et al. Inhibition of terminal complement components in presensitized transplant recipients prevents antibody-mediated rejection leading to long-term graft survival and accommodation. *Journal of immunology (Baltimore, Md : 1950)*. 2007;179(7):4451–63.
81. Stegall MD, Diwan T, Raghavaiah S, Cornell LD, Burns J, Dean PG, et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *American Journal of Transplantation*. 2011;11(11):2405–13. [PubMed: 21942930]
82. Cornell LD, Schinstock CA, Gandhi MJ, Kremers WK, Stegall MD. Positive crossmatch kidney transplant recipients treated with eculizumab: outcomes beyond 1 year. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2015;15(5):1293–302.
83. Tillou X, Poirier N, Le Bas-Bernardet S, Hervouet J, Minault D, Renaudin K, et al. Recombinant human C1-inhibitor prevents acute antibody-mediated rejection in alloimmunized baboons. *Kidney Int*. 2010;78(2):152–9. [PubMed: 20336054]
84. Vangerow B, Hecker JM, Lorenz R, Loss M, Przemeck M, Appiah R, et al. C1-Inhibitor for treatment of acute vascular xenograft rejection in cynomolgus recipients of h-DAF transgenic porcine kidneys. *Xenotransplantation*. 2001;8(4):266–72. [PubMed: 11737852]
85. Fiane AE, Videm V, Johansen HT, Mellbye OJ, Nielsen EW, Mollnes TE. C1-inhibitor attenuates hyperacute rejection and inhibits complement, leukocyte and platelet activation in an ex vivo pig-to-human perfusion model. *Immunopharmacology*. 1999;42(1–3):231–43. [PubMed: 10408384]
86. Caliezi C, Zeerleder S, Redondo M, Regli B, Rothen HU, Zurcher-Zenklusen R, et al. C1-inhibitor in patients with severe sepsis and septic shock: beneficial effect on renal dysfunction. *Critical care medicine*. 2002;30(8):1722–8. [PubMed: 12163783]
87. Vo AA, Zeevi A, Choi J, Cisneros K, Toyoda M, Kahwaji J, et al. A phase I/II placebo-controlled trial of C1-inhibitor for prevention of antibody-mediated rejection in HLA sensitized patients. *Transplantation*. 2015;99(2):299–308. [PubMed: 25606785]
88. Ito D, Akamatsu N, Ichida A, Kaneko J, Arita J, Hasegawa K, et al. Possible efficacy of recombinant human soluble thrombomodulin for the treatment of thrombotic microangiopathy after liver transplantation. *Liver Transpl*. 2016;22(5):689–92. [PubMed: 26850911]
89. Ito T, Kakihana Y, Maruyama I. Thrombomodulin as an intravascular safeguard against inflammatory and thrombotic diseases. *Expert opinion on therapeutic targets*. 2016;20(2):151–8. [PubMed: 26558419]
90. Kargli J, Melchionna T, Farrar CA, Greenlaw R, Smolarek D, Horsfield C, et al. Thrombalexins: Cell-localized inhibition of thrombin and its effects in a model of high-risk renal transplantation. *American Journal of Transplantation*. 2016:n/a-n/a.

91. Burghuber CK, Kwun J, Page EJ, Manook M, Gibby AC, Leopardi FV, et al. Antibody-Mediated Rejection in Sensitized Nonhuman Primates: Modeling Human Biology. *Am J Transplant*. 2016;16(6):1726–38. [PubMed: 26705099]
92. Manook M, Kwun J, Burghuber C, Samy K, Mulvihill M, Yoon J, et al. Thrombalexin: Use of a Cytotoxic Anticoagulant to Reduce Thrombotic Microangiopathy in a Highly Sensitized Model of Kidney Transplantation. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*. 2017.
93. EMPIRIKAL – A Double Blind Randomized Controlled Investigation into the efficacy of Mirococept (APT070) for preventing ischaemia-reperfusion injury in the kidney allograft (EMPIRIKAL) <http://public.ukcrn.org.uk/search/StudyDetail.aspx?StudyID=16181> [
94. Kassimatis T, Qasem A, Douiri A, Ryan EG, Rebollo-Mesa I, Nichols LL, et al. A double-blind randomised controlled investigation into the efficacy of Mirococept (APT070) for preventing ischaemia reperfusion injury in the kidney allograft (EMPIRIKAL): study protocol for a randomised controlled trial. *Trials*. 2017;18(1):255. [PubMed: 28587616]
95. Ekdahl KN, Teramura Y, Asif S, Jonsson N, Magnusson PU, Nilsson B. Thromboinflammation in Therapeutic Medicine. *Advances in experimental medicine and biology*. 2015;865:3–17. [PubMed: 26306440]

Figure 1:
Complement Cascade.

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Figure 2:
Coagulation Cascade.

Figure 3:
Complement and Coagulation Crosstalk in Thrombotic Microangiopathy in the Highly Sensitized Transplant Recipient.