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Role of complement and NK cells in antibody mediated rejection

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Abstract

Despite extensive research on T cells and potent immunosuppressive regimens that target cellular mediated rejection, few regimens have been proved to be effective on antibody-mediated rejection (AMR), particularly in the chronic setting. C4d deposition in the graft has been proved to be a useful marker for AMR; however, there is an imperfect association between C4d and AMR. While complement has been considered as the main player in acute AMR, the effector mechanisms in chronic AMR are still debated. Recent studies support the role of NK cells and direct effects of antibody on endothelium cells in a mechanism suggesting the presence of a complement-independent pathway. Here, we review the history, currently available systems and progress in experimental animal research. Although there are consistent findings from human and animal research, transposing the experimental results from rodent to human has been hampered by the differences in endothelial functions between species. We briefly describe the findings from patients and compare them with results from animals, to propose a combined perspective.

1. Introduction

T cells have been considered as central regulatory and effector cells in graft rejection, since animals that lack T cells do not reject allografts. Thus, most current therapies generally target T cells in order to prevent rejection. These therapies remarkably improve short-term outcome; however, long-term allografts often develop chronic rejection. The evidence of the correlation of graft pathology with the presence of circulating antibody indicates that antibody is commonly involved in these rejection episodes. Numerous studies have demonstrated involvement of complement in the rejection process which has supported the opinion that C4d is a reliable marker of antibody-mediated rejection. Nevertheless, recent studies have implicated the presence of complement-independent pathways in antibody-mediated rejection. In this review, we will focus on the roles of complement and NK cells in antibody mediated rejection in experimental systems.

Early work by Peter Gorer and others demonstrated that mouse tumor allografts induced alloantibodies that could agglutinate erythrocytes [1]. However, passive transfer of antibody at the time of engraftment failed to cause accelerated rejection of skin allografts in naïve mice that had grafts from the same donor [2], while the adoptive transfer of sensitized lymphocytes caused prompt rejection of grafts [3]. Therefore, antibody was not thought to

mediate rejection. In contrast, Winn and co-workers showed that if rat skin grafts were allowed to heal in over 2 weeks in immunosuppressed mice, grafts became highly susceptible to acute rejection by adoptive transfer of mouse anti-rat serum with immediate effects evident 10 min after serum administration and total graft loss in 1–2 days [4]. Gerlag and co-workers confirmed the sensitivity of healed in skin grafts to donor specific antiserum in analogous studies in mouse skin allografts [5].

In contrast to the animal studies, donor reactive antibody in presensitized patients was shown to predict and precipitate hyperacute kidney rejection in patients [6]. This was potentially avoidable by screening serum from the recipient with cells from the donor (crossmatch). In 1970, de novo alloantibodies, arising in crossmatch negative patients, were first implicated in chronic rejection by Russell and co-workers [7]. They reported that chronic allograft arteriopathy in renal allografts arose only in patients who developed de novo antibodies against donor HLA specificities. Terasaki et al. and other investigators discovered an association of circulating HLA antibodies with an increased rate of graft loss [8].

A close association of antibodies with specific forms of graft pathology was amplified by Halloran and co-workers [9,10]. They described distinct pathological features such as neutrophils in peritubular capillaries (PTC) during acute renal allograft rejection in patients with donor specific anti-class I HLA antibodies. However, despite the presence of anti-class I antibodies, little or no immunoglobulin deposition was detected in the biopsies showing microvascular injuries and they did not provide a direct causal link of the pathology with the antibodies. A breakthrough discovery was made by Helmut Feucht in 1991, who first demonstrated deposition of complement fragment C4d in peritubular capillaries (PTCs) of kidneys that were acutely rejected [11]. Collins and coworkers [12,13] later tied C4d deposition to circulating donor specific antibodies (DSA) and to the graft pathology described by the Edmonton group [9,10]. These findings led to the introduction of the diagnosis ‘acute antibody-mediated rejection’ in the Banff classification. In human renal allografts, acute antibody-mediated rejection is defined by three criteria: histological evidence of acute tissue injury, immunopathological evidence for the action of antibodies in the graft and serological evidence of HLA-specific antibodies or other donor-specific antibodies at the time of biopsy [14,15]. The NIH classification adds clinical graft dysfunction to distinguish clinical from subclinical antibody mediated rejection, the Banff criteria does not separate these conditions. Although there is a variation by institution, detection of C4d deposition in capillaries has been proved to be the most reliable marker of acute antibody-mediated rejection [16].

Mauiyyedi et al further discovered that chronic transplant glomerulopathy or arteriopathy was linked to C4d deposition in PTCs and donor specific antibody [17]. They proposed a new term ‘chronic humoral rejection’ (CHR) for this entity. Regele and others extended the features of CHR to include capillaritis with mononuclear leukocytes and basement membrane multilamination in PTCs and confirmed that about 50% of patients with transplant glomerulopathy or arteriopathy have C4d deposition in PTCs [18,19]. These features were used to define CHR, which was incorporated in subsequent Banff criteria [20].

2. Complement system

Since complement fixation is naturally suspected to be an important mediator in acute and hyperacute rejection, we will briefly review the importance of its C4 and C5–9 components in the cascade. Activation of C1 can be initiated by interaction of the globular domains of C1q with IgG or IgM bound to antigen epitopes on the graft endothelium. The C1q-binding potential of human IgG subclasses in order of decreasing capacity is IgG3, IgG1, IgG2 and

IgG4. C4 is cleaved by C1s into the small fragment C4a and the large fragment C4b. After inactivation of C4b to C4d by factor I, C4d remains covalently bound to the tissue and is thereby a durable marker of in situ complement activation. Eventually, C4d is cleared from the tissue, after the antibody response has ended: loss of C4d has been documented as early as 8 days after treatment [13]. Activation and regulation of the steps of the complement cascade after C4 are crucial, because the pathophysiological effects of complement require activation of the final common pathway. Cleavage of C5 then release the bioactive peptide C5a, and C5b. C5b initiates formation of the membrane attack complex (MAC), which causes cell lysis.

Besides the classical pathway that has been explained above, there are two other pathways for complement activation: the alternative pathways and the lectin pathways. The alternative pathway is initiated by the spontaneous hydrolysis of C3, which is abundant in the plasma in the blood. Complement activation on the host cells are prevented by several kinds of regulatory proteins such as Complement Receptor 1 (CD35), Decay Accelerating Factor, Factor 1 (CD55), Complement Factor H etc. Since foreign pathogens do not possess such protective mechanism, the C3b protein directly binds the microbe and the complement system follows the same path of activation. The lectin pathway is similar in structure to the classical pathway. It proceeds through the action of C4 and C2 to produce activated complement proteins further down the cascade. However, the lectin pathway does not require binding of antibody to its target. Instead, the lectin pathway starts with mannose-binding lectin that is produced by the liver and can initiate the complement cascade by binding to pathogen surface. These pathways serve as innate component of the immune system's natural defense against infections. However, it should be remembered that lectin pathway involve cleavage of C4 which eventually produce C4d deposition. To date there is no clear evidence that either the alternative or the lectin pathways contribute substantially to the graft lesions.

3. Acute AMR: experimental studies

As mentioned in the previous section, Winn and co-workers conducted a series of experiments with mice receiving rat skin grafts [4]. Rejection was shown to be dependent on complement, as judged by the inability of non-complement fixing antibodies or $F(ab')_2$ fragments to mediate rejection, prevention by depletion of complement by cobra venom factor and resistance to rejection in C5 deficient recipients. They found a notable time dependence of susceptibility to antibody mediated rejection. Grafts were resistant to mouse anti-rat serum during the first 7–10 days after transplantation, but gradually became susceptible to immune serum and reached a peak of sensitivity at 14–16 days after grafting [21,22]. If the graft survived beyond that time, the graft once again became resistant to antiserum for as long as they survived [21]. Deposits of C3 and injected immunoglobulin were found in the graft capillary walls. In a following study, they discovered that antibody can initiate immunological assault only if it reacts with antigens of donor vessels [22]. Thus, the unresponsiveness to antiserum in early grafts was attributed to the lack of vascularization and the resistance of long terms grafts to replacement of graft endothelium cells by host cells [22]. These findings are not, of course, transposable to vascularized solid organ transplantation, since replacement of donor endothelium is quite limited, if it occurs at all, and rat heart grafts in mice retain their sensitivity to antiserum indefinitely [23]. However, these findings are particularly relevant to the topics we are discussing in this review, because they demonstrated the central importance of endothelium as the target of antibody or complement action in the immunological process of antibody-mediated rejection.

Chong and co-workers have conducted a series of experiments to investigate the role of NK cells and complement in acute rejection of murine cardiac xenografts [24] using monoclonal

antibodies to the xenoantigen, galactosyl- α (1–3)-galactose determinant (Gal). They discovered that IgG1, a poor activator of complement, possessed the ability to induce rejection primarily through activation through Fc γ R-mediated interaction and NK cells. On the other hand, anti-Gal IgG3 mAb induced hyperacute rejection only through complement activation. These observations suggest that there are two types of IgG-mediated rejection, confirming the finding of Winn and co-workers that interaction between complement and endothelium is important in antibody mediated rejection. It has also been reported that depletion of NK cells with anti- α 1-antitrypsin-GM1 prolongs the survival of mouse to rat cardiac xenografts [25]. However, the fact that this treatment also affects macrophages limits the conclusions. The NK-cell-dependent arm of the mechanism will be discussed in the later section.

There has been some debate about the relevance of experiments with rodents to study mechanisms in antibody-mediated rejection in patients. But the involvement of complement components has been illuminated by reagents to detect C4d and C3d deposition [26]. With these reagents, Baldwin and co-workers demonstrated that the extent, degree and clearance of C4d deposition correlated with alloantibody levels and rejection in rat cardiac transplants [26–28]. These findings showed that alloantibody to the endothelium of donor vessels led to the subsequent deposition of C4d in murine cardiac allografts. Furthermore, they revealed synergism between non-complement-fixing antibody (IgG1) and complement fixing antibody (IgG2b) in this system which used IgG-KO mice as recipients [29–31].

Wang et al reported clear evidence that complement fixation is necessary for acute antibody mediated rejection in mice. It is known that the terminal components (C5 through C9) possess potent proinflammatory and cell lytic properties. Because all three distinctive pathways in the complement cascade share the same terminal components (C5 through C9), they are attractive targets for potential therapies. They induced long-term cardiac allograft survival in pre-sensitized mice by triple therapy consisting of cyclosporine, cyclophosphamide and anti-C5 mAb. These heart grafts are resistant to antibody-mediated rejection despite systemic and intragraft anti-donor antibodies [32]. This study not only confirmed the effector role of complement in antibody-mediated rejection in mice, but also provided the basis for a clinically applicable strategy.

Although the usefulness of experiments with rodents is supported by these studies, they were performed with presensitized recipients to mimic highly presensitized patients in clinical settings, and it is impossible to exclude a concomitant effect of memory cells. One option to exclude contribution of memory cells is to use RAG-KO mice which do not have functional T cells or B cells. However, it is difficult to induce antibody-mediated-rejection by passive transfer of antibody in RAG-KO mice. Fairchild and co-workers concluded that the titer of transferred antibody is critical for successful induction of antibody-mediated rejection in RAG-KO mice [33]. They discovered that CCR5-KO recipients of murine cardiac transplants produce higher titers of alloantibody compared to that of WT recipients. While passive transfer of undiluted sera of CCR5-KO recipient successfully induced antibody-mediated rejection, antibody-mediated rejection did not occur when the sera was diluted to levels found in wild type recipients.

4. Chronic AMR: experimental studies

Many groups have tried to elucidate the pathogenesis and etiology of AMR by using experimental animals in which antibody, complement and potential effector cells can be directly manipulated. The most commonly used experimental system is heart allografts in mice. In early studies by our group, the arteriopathy of heart transplants in mice was more severe in a mouse strain combination known to produce detectable antibodies to donor

antigens compared to a strain combination that did not produce antibodies [34]. The adoptive transfer of alloantibodies resulted in chronic allograft arteriopathy in normal or immunodeficient mice, whereas B-cell-deficient mice did not develop fibrotic arterial lesions [35]. However, as discussed in the previous section, since the presence of alloantibodies indicates presensitization to donor antigens, and presumably primed T cells, it was difficult to exclude the contribution of other immunological components such as T cells and macrophages in animals with intact immunity. Nevertheless, it was confirmed that alloantibodies could instigate vascular changes in transplanted hearts by showing that infusion of class I MHC donor-specific antiserum significantly increased coronary lesions in a dose-dependent manner. In the following study, by using B cell deficient mice, we showed that fully developed fibrous, chronic graft arteriopathy was observed only in the presence of alloantibodies [34]. We have also shown that chronic allograft vasculopathy can be produced by adoptive transfer of class I MHC DSA to immunodeficient RAG1-KO mice [36]. Interestingly, severe arteriopathy was elicited in only 4 weeks even with transient DSA and C4d and even by non-complement fixing IgG1 alloantibody.

In order to explore the mechanisms triggered by alloantibodies further studies were performed by our group using three approaches: 1) DSA treatment with NK depletion in either B6.RAG1^{-/-}C3^{-/-} (H-2^b) recipients or B6.RAG1^{-/-} (H-2^b) recipients; 2) F(ab')₂ fragments of DSA (HB13, IgG2a mAb to H-2K^k in B6.RAG1^{-/-} (H-2^b) recipients; and 3) DSA treatment in B6.RAG1^{-/-} recipients (H-2^b) with B10.BRx B6RAG1^{-/-} (H-2^{kxb})F1 hearts, that is, F1 to parental combination.

In the first approach, NK cells were depleted using anti-NK1.1 mAb (PK136, anti-NK1.1) in B6.RAG1^{-/-}C3^{-/-} recipients because NK cells existed in the coronary lesions induced by DSA (36-7-5, IgG2a mAb to H-2K^k) in B6.RAG1^{-/-}C3^{-/-} recipients [37]. Complement fixing DSA (36-7-5, IgG2a mAb to H-2K^k) treatment alone induced coronary lesions in B6.RAG1^{-/-}C3^{-/-} recipients with B10.BR hearts (H-2^k). However NK cell depletion attenuated the frequency and severity of coronary lesions in this combination.

Furthermore, we used B6.RAG1^{-/-} as recipients to test whether or not NK cell depletion can attenuate coronary lesions in complement competent B6.RAG1^{-/-} recipients. NK cell depletion eliminated the development of coronary lesions in complement competent B6.RAG1^{-/-} recipients regardless of either complement fixing DSA (36-7-5, IgG2a mAb to H-2K^k) or non-complement fixing DSA (AF 3-12.1.3, IgG1 mAb to H-2K^k). These results indicate that antibody mediated rejection due to DSA requires NK cells even in the presence of complement fixation.

In the second approach to test whether Fc receptors on NK cells were important, we used F(ab')₂ fragments of complement fixing DSA (HB13, IgG2a mAb to H-2K^k), which is a different clone from the first set and was used to rule out a peculiarity of the 36-7-5 complement fixing DSA. The intact HB 13 complement fixing DSA treatment induced coronary lesions in B6.RAG1^{-/-} recipients as with the 36-7-5 complement fixing DSA, and NK cell depletion by anti-NK1.1 mAb (PK136) attenuated the development of coronary lesions. More importantly, F(ab')₂ fragments of complement fixing DSA (HB13, IgG2a mAb to H-2k^k), even with twice the dose of F(ab')₂ fragments, failed to induce coronary lesions. These results indicate that chronic antibody mediated rejection is induced through Fc receptors. Further experiments were performed to clarify the associations of NK cells, DSA, and endothelial activation by DSA. By using tissues of B10.BR hearts transplanted into B6.RAG1^{-/-} recipients treated with intact HB13 DSA (IgG2a mAb to H-2k^k), F(ab')₂ fragment of HB13 DSA (IgG2a mAb to H-2k^k), or intact HB13 DSA (IgG2a mAb to H-2k^k) plus NK cell depleting antibody (PK136), pERK expression, an endothelial cell activation marker, in myocardial microvascular endothelial cells was examined.

The lack of Fc portion in F(ab')₂ fragment of HB13 DSA(IgG2a mAb to H-2K^k) or the absence of NK cells using NK depleting antibody (PK136) significantly decreased pERK expression in myocardial microvascular endothelial cells compared with those induced by intact HB13 DSA (IgG2a mAb to H-2K^k). These results imply that endothelial cell activation in myocardial microvascular endothelial cells is induced by NK cells and intact DSA, not F(ab')₂ fragment of HB13 DSA (IgG2a mAb to H-2K^k).

The third approach was done to exclude the direct effects of NK cells to endothelial cells of B10.BR hearts (H-2^k). In the previous approaches, NK cells from B6RAG1^{-/-} recipients (H-2^b) can attack endothelial cells of B10.BR hearts (H-2^k) directly because NK cells from B6RAG1^{-/-} recipients can recognize absence of self antigen(H-2^b).

In the third experiment, (B10.BR × B6.RAG^{-/-}) F1 (H-2^{kxb}) hearts were transplanted into parental strain recipients (B6.RAG1^{-/-}, H-2^b). Since these F1 grafts contain all self-antigens (H-2^{kxb}), NK cells from the recipients of parental strain (H-2^b) do not react to graft antigens. As expected, a transfer of DSA IgG2a (HB13) induced CAV in these grafts while controls in same combination without DSA had no lesions. These results indicate that antibody mediated rejection was induced even without direct reactivity of NK cells to (B10.BR × B6.RAG^{-/-}) F1 (H-2^{kxb}) hearts.

Thus in mice, there is a complement independent pathway for CAV, which possibly explains the imperfect association of C4d deposition and chronic allograft vasculopathy in patients. Taken together, these results suggest that antibody mediated rejection is induced by NK cells, and through an Fc dependent manner [38].

NK cells are important players in the innate immunity system. NK cells constitute 5–20% of the mononuclear cells in the blood and spleen and are rare in other lymphoid organs. They do not express somatically rearranged, clonally distributed antigen receptors like immunoglobulin or T cell receptors. NK cells can affect various target cells through natural cytotoxicity or antibody dependent cellular toxicity, without a need for additional activation. In addition to killing infected cells directly, NK cells are a major source of IFN γ . NK cell activation is regulated by a balance between stimulatory and inhibitory receptors that recognize self-antigens expressed by the target cells. This is a theoretical mechanism in the “hybrid resistance” of F1 recipients to parental bone marrow transplants and chronic allograft vasculopathy that develops in parents to F1 heart allografts.

NK cells can also be activated by their Fc receptors (Fig. 1). Interaction of Fc γ RIII on NK cells with immunoglobulin bound to antigen on target cells will lead to activation of NK cells [39,40]. In vitro activation of NK cells via Fc γ RIII leads to production of IFN γ and TNF α among other effects [41,42] (Fig. 1). IFN γ is thought to play a central role in CAV, based on its ability to trigger CAV [43], the inhibition of CAV by neutralizing anti-IFN γ antibody in murine cardiac allografts [44], and the relative lack of CAV in IFN γ null mice [45,46]. Incubation of human NK cells with DSA and HLA bearing target cells leads to the production of IFN γ [47]. TNF α is another candidate mediator, since it induces vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells [48] as well as cytotoxicity. It is well established that most toll-like receptor (TLR)-mediated signaling is mediated by the signal adaptor, MyD88. It has been shown that IL-18 and IL-12, both cytokines secreted by DCs and macrophages, synergize for IFN γ production by NK cells. Sawaki and co-workers have also shown that TLR2 and TLR7 agonists can replace IL-18 for induction of IFN- γ by NK cells in the presence of IL-12. The increased expression of IFN γ by IL-12 was abrogated when using tlr2^{-/-} or tlr7^{-/-} NK cells, suggesting TLR signaling is necessary [49].

Identification of NK cells in tissues is difficult, since no one marker is uniquely specific and expressed on all NK cells. Furthermore NK cells change their phenotype with activation,

losing expression of certain surface markers such as CD56, and gaining others, such as NKG2D. Activation via the FC γ RIII leads to increased expression of the transcription factor, T-bet, and the production of multiple cytokines (Fig. 1). Therefore, the study of NK cells in grafts requires a multiparameter approach, for example combining antibodies for the activating receptor NKG2D expressed on NK and T cells with CD3.

Antibody can also induce phenotypic and metabolic changes in endothelium independent of complement and Fc receptors. For example, antibodies that are specific for MHC class I molecules increase tyrosine phosphorylation and NF- κ B levels in both human umbilical vein and heart microvascular endothelial cells, and they promote proliferation of these cells in vitro [50]. HLA-class-I-specific antibodies stimulate endothelial cells to express FGF receptors, to phosphorylate SRC and to proliferate [51]. Non-complement-fixing, MHC-class-I-specific antibodies activate cultured mouse endothelial cells to produce CCL2 and CXCL1 (also known as KC), an effect that is increased in the presence of tumor-necrosis factor (TNF) [30]. This transcriptional activation might be relevant to the arterial intimal proliferation that is characteristic of chronic antibody-mediated rejection. Other cell types such as bronchial epithelial cells can be triggered to proliferate by MHC-class-I-specific antibodies [52]. A comprehensive review on interaction between anti MHC class I/II antibodies and graft vascular cells is available [53].

Aortic transplants have also been used to investigate chronic rejection, either with mouse allografts or human to mouse xenografts. Observations of mouse aortic allografts are not readily transposable to patients because of some known differences in immune functions of human and murine vascular cells such as expression of MHC molecules and co-stimulators [54]. Pober and others have used human artery transplants in mice and performed extensive studies on T cell mediated immune reactions in this setting [55,56]. Antibody mediated effects in this system were first explored by Galvani and co-workers[57,58]. They highlighted a important role for MMP2 and neutral sphingomyelinase-2 in vasculopathy induced by humoral immunity [58]. To visualize events in vivo, we have used aortic transplants evaluated by two photon microscopy to visualize vascular dynamics in vivo [59]. Although the data are still preliminary, two photon microscopy captured cellular-level activities and endogenous fluorescent signature of the aortic transplant over the time course of disease progression in tissues up to a depth of ~150 μ m. The application of deep tissue two photon imaging in the study of aortic transplants may prove to be a new platform for investigating the dynamics of alloimmune reactions with vessels in vivo. These studies may show different results due to biological differences in vessel endothelium and structure [60]. There are number of difference in immune function between endothelial cells and smooth muscle cells such as MHC molecules expression, co-stimulators expression (OX-40L, ICOS-L, PDL-1, PDL-2 etc.), IL-6 production, IDO activity etc. Overall, smooth muscle cells are considered to produce “medial immunoprivilege” while endothelial cells are recognized as pro-immunogenic. Since arteries that are used in transplant model in mice possess smooth muscle layers, such difference may impact the experimental results and should be remembered in the interpretation of the experimental work.

Chronic AMR has been characterized in non-human primates as well. Nickleleit and co-workers have studied the pathologic sequence of chronic rejection under various immunosuppression regimens and showed that these were quite comparable to those in the human; however, these observations were made with non-life-supporting kidney transplants [61]. Through a series of studies in non-human primates using the mixed chimerism protocol, we have proposed that chronic antibody-mediated rejection progresses through 4 stages [62]. In these studies, we used monkeys that were not treated with long-term immunosuppressive drugs. The serologic and pathologic evidence indicates that the first event is alloantibody production (stage I), followed by antibody interaction with alloantigens

resulting in the deposition of C4d in PTC and possibly glomeruli (stage II), and followed by pathologic changes (stage III) and graft dysfunction (stage IV). A minority of animals develop transplant glomerulopathy without detectable C4d deposition (Smith et al., unpublished observations).

5. Comparison with human pathology

Chronic AMR is a major cause of late human renal allograft graft dysfunction. The majority of patients with late graft dysfunction (57–63%) had evidence of chronic AMR (C4d and/or DSA) in two large series [63,64]. Chronic AMR was described in 2001 by our group [17], has been widely confirmed [18,19] and is incorporated into the Banff classification [20]. The pathological features are neo-intimal arterial fibrosis and inflammation [65], endothelial activation [66], duplication of the microvascular basement membranes, mononuclear inflammation (capillaritis) and often, but not invariably, C4d deposition [67,68]. These pathologic features are well described and a comprehensive review of pathologic features is available [67]. Recent studies by three groups have underscored the major role of chronic antibody mediated rejection in graft failure [63,69,70]. Two prospectively studied kidney transplants that progressed to failure and discovered that most common cause for rejection losses is noncompliance with immunosuppressive medications resulting in antibody-mediated rejection [69,70]. Despite extensive knowledge of the pathology, the mechanisms of chronic AMR are incompletely understood. Identification of the pathogenesis should lead to new therapeutic strategies.

Recent studies suggest that the NK-DSA pathway may also be operative in humans [71]. Chronic AMR in human renal allografts shows increased NK cell specific transcripts, accumulation of CD56⁺ mononuclear cells, and increased endothelial gene expression, often with no evidence of complement activation [72,73]. Blood NK cells are activated in patients with chronic rejection and the number of CD16⁺ NK cells increased in transplanted lung during the progression of chronic rejection [74]. The presence of C4d in tissue correlates with the ability of DSA to fix complement (C1q) in vitro (Farkash et al, unpublished observations).

While the primary targets of antibody mediated rejection are the conventional MHC class I and II antigens, other MHC related antigens (MICA), autoantigens, parenchymal cell antigens are also proposed as potential targets and are the presumptive explanation for the rare but occasionally observed case of antibody mediated rejection in HLA identical sibling grafts [75,76]. Major histocompatibility complex class I chain-related gene A (MICA) is encoded by genes located within the MHC region in chromosome 6 just centromeric to HLA-B. Because of its polymorphic nature and its expression on surface of endothelial cells during rejection and ischemic reperfusion injury, MICA is considered as possible target of alloimmune response. Indeed, anti-MICA antibodies have been found to mediate complement dependent cytotoxicity in vitro suggesting that they may contribute to the pathogenesis of antibody-mediated rejection through complement mediated injury. In clinical transplant, MICA antibodies are reported to associate with acute and chronic rejection of heart, renal and pancreas transplants. Tissue specific autoantigens such as vimentin and myosin are also proposed as potential targets. It is not yet fully deciphered how these antigens cause an immune response to transplanted organs. One theory suggests that exposure of tissue specific antigens due to alloimmune injury or ischemic reperfusion injury that are presented through indirect pathway to recipient's CD4⁺/CD8⁺ T cells cause autoimmune response and subsequently leads to humoral response. How alloimmunity leads to loss of tolerance to self-antigens and lead to alloimmune response against transplanted in turn is not well understood. Further studies are needed to determine interaction among

antibodies directed MHC and others in solid organ transplants during quiescence and rejection as well [77].

One especially interesting phenomenon in patients is the process of accommodation to ABO antibodies. West and co-workers have performed extensive research and have contributed a comprehensive review [78]. ABO antibodies can cause hyperacute or acute rejection, but patients in whom the antibody has been transiently reduced by various techniques have undergone successful transplantation [79]. Curiously, even though the ABO antibodies return after one month, and complement (C4d) is fixed in the graft vasculature, the transplants remain functional without appreciable pathology for extended periods. Accommodation to ABO-incompatible grafts is not due to a loss of the target, because C4d is deposited in the graft. The basis of this “accommodation” is not known. It has even been postulated that accommodation to ABO may protect against the CAV due to HLA DSA [80]. It is possible that C4d itself participates in the accommodation or that the IgM ABO antibodies are ineffective due to lack of Fc receptors on the relevant cells. Accommodation is particularly of interest since the mechanism behind accommodation may provide a clue to overcoming chronic AMR.

Although experimental animal studies have been useful in pursuit of the mechanism involved, the pathogenesis of human disease should be studied in an experimental system as “human” as possible for optimal mechanistic insights. For example, the major DSA involved are to HLA antigens, predominantly but not exclusively type II antigens [19,81]. In interpreting results from experiments in mice, it must be appreciated that humans express class II antigens constitutively on microvascular endothelium and mice do not [54,82,83].

Knowledge of the mechanisms should lead to more informed and possibly novel therapies. For example, anti-C5 (eculizumab) shows promise for prevention of acute AMR [84], but has not been evaluated in chronic AMR. If chronic AMR involves complement independent pathways this approach will be imperfect or even futile. In contrast, cytokines may be identified that have a significant role in chronic AMR and for which FDA approved antagonists are available (e.g., TNF α), the results could be the basis for clinical trials.

6. General comments and conclusions

While the existence of chronic antibody mediated rejection has become widely known and can be diagnosed reliably by pathologists, knowledge of the mechanism behind this phenomenon is still incomplete. Experimental evidence has identified three pathways by which antibodies can affect allografts: complement fixation, interaction with Fc γ receptors particularly on NK, and by direct effects of antibody on the endothelium [85]. Complement has received particular attention in both hyperacute and acute rejection; however, the pathophysiology of chronic AMR cannot be fully explained by complement. One possibility is a direct effect of antibody on endothelium. Another possible mechanism is an NK cell mediated pathway, as has been identified in allografts in mice and for which emerging evidence has been acquired in man. Delineation of this pathway may lead to new therapeutic approaches to slow or reverse the devastating consequences of chronic antibody mediated rejection.

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References

1. Gorer P. The detection of antigenic differences in mouse erythrocytes by the employment of immune sera. *Br J Exp Pathol.* 1936; 17:42.
2. Billingham RE, Brent L. Further attempts to transfer transplantation immunity by means of serum. *Br J Exp Pathol.* 1956; 37:566. [PubMed: 13396140]
3. Makela O, Mitchison NA. The role of cell number and source in adoptive immunity. *Immunology.* 1965; 8(6):539. [PubMed: 5842810]
4. Winn HJ, Baldamus CA, Jooste SV, Russell PS. Acute destruction by humoral antibody of rat skin grafted to mice. *J Exp Med.* 1973; 137(4):893. [PubMed: 4120896]
5. Gerlag PG, Koene RA, Hagemann JF, Wijdeveld PG. Hyperacute rejection of skin allografts in the mouse. Sensitivity of ingrowing skin grafts to the action of alloantibody and rabbit complement. *Transplant.* 1975; 20(4):308.
6. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O. Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. *Lancet.* 1966; 2(7465):662. [PubMed: 4162350]
7. Jeannet M, Pinn VW, Flax MH, Winn HJ, Russell PS. Humoral antibodies in renal allotransplantation in man. *N Engl J Med.* 1970; 282(3):111. [PubMed: 4902226]
8. Terasaki PI, Ozawa M, Castro R. Four-year follow-up of a prospective trial of HLA and MICA antibodies on kidney graft survival. *Am J Transplant.* 2007; 7(2):408. [PubMed: 17229080]
9. Halloran PF, Wadgymar A, Ritchie S, Falk J, Solez K, Srinivasa NS. The significance of the anti-class I antibody response. I. Clinical and pathologic features of anti-class I-mediated rejection. *Transplant.* 1990; 49(1):85.
10. Trpkov K, Campbell P, Pazderka F, Cockfield S, Solez K, Halloran PF. Pathologic features of acute renal allograft rejection associated with donor-specific antibody, Analysis using the Banff grading schema. *Transplant.* 1996; 61(11):1586.
11. Feucht HE, Felber E, Gokel MJ, Hillebrand G, Nattermann U, Brockmeyer C, et al. Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. *Clin Exp Immunol.* 1991; 86(3):464. [PubMed: 1747954]
12. Mauiyyedi S, Crespo M, Collins AB, Schneeberger EE, Pascual MA, Saidman SL, et al. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J Am Soc Nephrol.* 2002; 13(3):779. [PubMed: 11856785]
13. Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Williams WW, Tolkoff-Rubin N, et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. *J Am Soc Nephrol.* 1999; 10(10):2208. [PubMed: 10505698]
14. Takemoto SK, Zeevi A, Feng S, Colvin RB, Jordan S, Kobashigawa J, et al. National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J Transplant.* 2004; 4(7):1033. [PubMed: 15196059]
15. Racusen LC, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, et al. Antibody-mediated rejection criteria - an addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant.* 2003; 3(6):708. [PubMed: 12780562]
16. Cohen D, Colvin RB, Daha MR, Drachenberg CB, Haas M, Nickleleit V, et al. Pros and cons for C4d as a biomarker. *Kidney Int.* 2012; 81(7):628. [PubMed: 22297669]
17. Mauiyyedi S, Pelle PD, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, et al. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol.* 2001; 12(3):574. [PubMed: 11181806]
18. Regele H, Bohmig GA, Habicht A, Gollowitzer D, Schillinger M, Rockenschaub S, et al. Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol.* 2002; 13(9):2371. [PubMed: 12191982]
19. Sis B, Campbell PM, Mueller T, Hunter C, Cockfield SM, Cruz J, et al. Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. *Am J Transplant.* 2007; 7(7):1743. [PubMed: 17564636]

20. Solez K, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, et al. Banff '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant.* 2007; 7(3):518. [PubMed: 17352710]
21. Jooste SV, Colvin RB, Soper WD, Winn HJ. The vascular bed as the primary target in the destruction of skin grafts by antiserum. I. Resistance of freshly placed xenografts of skin to antiserum. *J Exp Med.* 1981; 154(5):1319. [PubMed: 7028911]
22. Jooste SV, Colvin RB, Winn HJ. The vascular bed as the primary target in the destruction of skin grafts by antiserum. II. Loss of sensitivity to antiserum in long-term xenografts of skin. *J Exp Med.* 1981; 154(5):1332. [PubMed: 6795300]
23. Burdick JF, Russell PS, Winn HJ. Sensitivity of long-standing xenografts of rat hearts to humoral antibodies. *J Immunol.* 1979; 123(4):1732. [PubMed: 383839]
24. Yin D, Zeng H, Ma L, Shen J, Xu H, Byrne GW, et al. Cutting Edge: NK cells mediate IgG1-dependent hyperacute rejection of xenografts. *J Immunol.* 2004; 172(12):7235. [PubMed: 15187097]
25. Chen D, Weber M, Lechler R, Dorling A. NK-cell-dependent acute xenograft rejection in the mouse heart-to-rat model. *XenoTransplant.* 2006; 13(5):408.
26. Minami K, Murata K, Lee CY, Fox-Talbot K, Wasowska BA, Pescovitz MD, et al. C4d deposition and clearance in cardiac transplants correlates with alloantibody levels and rejection in rats. *Am J Transplant.* 2006; 6(5 Pt 1):923. [PubMed: 16611328]
27. Qian Z, Hu W, Liu J, Sanfilippo F, Hruban RH, Baldwin WM 3rd. Accelerated graft arteriosclerosis in cardiac transplants: complement activation promotes progression of lesions from medium to large arteries. *Transplant.* 2001; 72(5):900.
28. Qian Z, Lee CY, Murata K, Liu J, Fox-Talbot K, Wasowska BA, et al. Antibody and complement mediated injury in transplants following sensitization by allogeneic blood transfusion. *Transplant.* 2006; 82(7):857.
29. Murata K, Fox-Talbot K, Qian Z, Takahashi K, Stahl GL, Baldwin WM 3rd. Wasowska BA: Synergistic deposition of C4d by complement-activating and non-activating antibodies in cardiac transplants. *Am J Transplant.* 2007; 7(11):2605. [PubMed: 17868071]
30. Rahimi S, Qian Z, Layton J, Fox-Talbot K, Baldwin WM 3rd. Wasowska BA: Non-complement- and complement-activating antibodies synergize to cause rejection of cardiac allografts. *Am J Transplant.* 2004; 4(3):326. [PubMed: 14961984]
31. Wasowska BA, Qian Z, Cangello DL, Behrens E, Van Tran K, Layton J, et al. Passive transfer of alloantibodies restores acute cardiac rejection in IgKO mice. *Transplant.* 2001; 71(6):727.
32. 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. *J Immunol.* 2007; 179(7):4451. [PubMed: 17878341]
33. Nozaki T, Amano H, Bickerstaff A, Orosz CG, Novick AC, Tanabe K, et al. Antibody-mediated rejection of cardiac allografts in CCR5-deficient recipients. *J Immunol.* 2007; 179(8):5238. [PubMed: 17911609]
34. Russell PS, Chase CM, Winn HJ, Colvin RB. Coronary atherosclerosis in transplanted mouse hearts. II. Importance of humoral immunity. *J Immunol.* 1994; 152(10):5135. [PubMed: 8176230]
35. Russell PS, Chase CM, Colvin RB. Alloantibody- and T cell-mediated immunity in the pathogenesis of transplant arteriosclerosis: lack of progression to sclerotic lesions in B cell-deficient mice. *Transplant.* 1997; 64(11):1531.
36. Uehara S, Chase CM, Cornell LD, Madsen JC, Russell PS, Colvin RB. Chronic cardiac transplant arteriopathy in mice. relationship of alloantibody, C4d deposition and neointimal fibrosis. *Am J Transplant.* 2007; 7(1):57. [PubMed: 17227558]
37. Hirohashi T, Uehara S, Chase CM, DellaPelle P, Madsen JC, Russell PS, et al. Complement independent antibody-mediated endarteritis and transplant arteriopathy in mice. *Am J Transplant.* 2010; 10(3):510. [PubMed: 20055805]
38. Hirohashi T, Chase CM, Della Pelle P, Sebastian D, Alessandrini A, Madsen JC, Russell PS, Colvin RB: A novel pathway of chronic allograft rejection mediated by NK cells and alloantibody. *Am J Transplant.* 2012; 12(2):313. [PubMed: 22070565]

39. Arase N, Arase H, Hirano S, Yokosuka T, Sakurai D, Saito T. IgE-mediated activation of NK cells through Fc gamma RIII. *J Immunol.* 2003; 170(6):3054. [PubMed: 12626560]
40. Arase N, Arase H, Park SY, Ohno H, Ra C, Saito T. Association with FcRgamma is essential for activation signal through NKR-P1 (CD161) in natural killer (NK) cells and NK1. 1+ T cells. *J Exp Med.* 1997; 186(12):1957. [PubMed: 9396764]
41. Trotta R, Col JD, Yu J, Ciarlariello D, Thomas B, Zhang X, et al. Wei M, Mao H, Byrd JC, Perrotti D, Caligiuri MA: TGF-beta utilizes SMAD3 to inhibit CD16-mediated IFN-gamma production and antibody-dependent cellular cytotoxicity in human NK cells. *J Immunol.* 2008; 181(6):3784. [PubMed: 18768831]
42. Roda JM, Parihar R, Lehman A, Mani A, Tridandapani S, Carson WE 3rd. Interleukin-21 enhances NK cell activation in response to antibody-coated targets. *J Immunol.* 2006; 177(1):120. [PubMed: 16785506]
43. Tellides G, Tereb DA, Kirkiles-Smith NC, Kim RW, Wilson JH, Schechner JS, et al. Interferon-gamma elicits arteriosclerosis in the absence of leukocytes. *Nature.* 2000; 403(6766):207. [PubMed: 10646607]
44. Russell PS, Chase CM, Winn HJ, Colvin RB. Coronary atherosclerosis in transplanted mouse hearts. III. Effects of recipient treatment with a monoclonal antibody to interferon-gamma. *Transplant.* 1994; 57(9):1367.
45. Nagano H, Mitchell RN, Taylor MK, Hasegawa S, Tilney NL, Libby P. Interferon-gamma deficiency prevents coronary arteriosclerosis but not myocardial rejection in transplanted mouse hearts. *J Clin Invest.* 1997; 100(3):550. [PubMed: 9239401]
46. Nagano H, Libby P, Taylor MK, Hasegawa S, Stinn JL, Becker G, et al. Coronary arteriosclerosis after T-cell-mediated injury in transplanted mouse hearts: role of interferon-gamma. *Am J Pathol.* 1998; 152(5):1187. [PubMed: 9588888]
47. Toyoda M, Ge S, Suviolahti E, Pichurin P, Shin B, Pao A, et al. IFNgamma production by NK cells from HLA-sensitized patients after in vitro exposure to allo-antigens. *Transpl Immunol.* 2012; 26(2-3):107. [PubMed: 22101306]
48. Bergese S, Pelletier R, Vallera D, Widmer M, Orosz C. Regulation of endothelial VCAM-1 expression in murine cardiac grafts. Roles for TNF and IL4. *Am J Pathol.* 1995; 146(4):989. [PubMed: 7536398]
49. Sawaki J, Tsutsui H, Hayashi N, Yasuda K, Akira S, Tanizawa T, et al. Type 1 cytokine/chemokine production by mouse NK cells following activation of their TLR/MyD88-mediated pathways. *Int Immunol.* 2007; 19(3):311. [PubMed: 17289654]
50. Smith JD, Lawson C, Yacoub MH, Rose ML. Activation of NF-kappa B in human endothelial cells induced by monoclonal and allospecific HLA antibodies. *Int Immunol.* 2000; 12(4):563. [PubMed: 10744658]
51. Jin YP, Singh RP, Du ZY, Rajasekaran AK, Rozengurt E, Reed EF. Ligation of HLA class I molecules on endothelial cells induces phosphorylation of Src, paxillin, and focal adhesion kinase in an actin-dependent manner. *J Immunol.* 2002; 168(11):5415. [PubMed: 12023334]
52. Jaramillo A, Smith CR, Maruyama T, Zhang L, Patterson GA, Mohanakumar T. Anti-HLA class I antibody binding to airway epithelial cells induces production of fibrogenic growth factors and apoptotic cell death: a possible mechanism for bronchiolitis obliterans syndrome. *Hum Immunol.* 2003; 64(5):521. [PubMed: 12691702]
53. Valenzuela NM, Reed EF. The link between major histocompatibility complex antibodies and cell proliferation. *Transplant Rev (Orlando).* 2011; 25(4):154. [PubMed: 21803559]
54. Houser SL, Benjamin LC, Wain JC, Madsen JC, Allan JS. Constitutive expression of major histocompatibility complex class II antigens in pulmonary epithelium and endothelium varies among different species. *Transplant.* 2004; 77(4):605.
55. Lorber MI, Wilson JH, Robert ME, Schechner JS, Kirkiles N, Qian HY, et al. Human allogeneic vascular rejection after arterial transplantation and peripheral lymphoid reconstitution in severe combined immunodeficient mice. *Transplant.* 1999; 67(6):897.
56. Wang Y, Burns WR, Tang PC, Yi T, Schechner JS, Zerwes HG, et al. Interferon-gamma plays a nonredundant role in mediating T cell-dependent outward vascular remodeling of allogeneic human coronary arteries. *FASEB J.* 2004; 18(3):606. [PubMed: 14734640]

57. Galvani S, Auge N, Calise D, Thiers JC, Canivet C, Kamar N, et al. HLA class I antibodies provoke graft arteriosclerosis in human arteries transplanted into SCID/beige mice. *Am J Transplant.* 2009; 9(11):2607. [PubMed: 19843036]
58. Galvani S, Trayssac M, Auge N, Thiers JC, Calise D, Krell HW, et al. A Key Role for Matrix Metalloproteinases and Neutral Sphingomyelinase-2 in Transplant Vasculopathy Triggered by Anti-HLA Antibody. *Circulation.* 2011; 124(24):2725. [PubMed: 22082680]
59. Akiyoshi, TCC.; Alessandrini, A.; Sebastian, DP.; Della Pelle, P.; Lee, WM.; Connolly, SE.; Farkash, E.; Yun, SH.; Russell, PS.; Colvin, RB. Combination of aortic transplant and in vivo microscopy: A new tool for dynamic investigation of Allo-immune reaction. *Annual Scientific Exchange*; San Antonio, TX. 2011.
60. Aird WC. Endothelial cell heterogeneity. *Cold Spring Harb Perspect Med.* 2012; 2(1):a006429. [PubMed: 22315715]
61. Wieczorek G, Bigaud M, Menninger K, Riesen S, Quesniaux V, Schuurman HJ, et al. Acute and chronic vascular rejection in nonhuman primate kidney transplantation. *Am J Transplant.* 2006; 6(6):1285. [PubMed: 16686753]
62. Smith RN, Kawai T, Boskovic S, Nadazdin O, Sachs DH, Cosimi AB, et al. Four stages and lack of stable accommodation in chronic alloantibody-mediated renal allograft rejection in Cynomolgus monkeys. *Am J Transplant.* 2008; 8(8):1662. [PubMed: 18557724]
63. Gaston RS, Cecka JM, Kasiske BL, Fieberg AM, Leduc R, Cosio FC, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. *Transplant.* 2010; 90(1):68.
64. Einecke G, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant.* 2009; 9(11):2520. [PubMed: 19843030]
65. Hill GS, Nochy D, Bruneval P, Duong van Huyen JP, Glotz D, Suberbielle C, Zuber J, Anglicheau D, Empana JP, Legendre C, Loupy A: Donor-specific antibodies accelerate arteriosclerosis after kidney transplantation. *J Am Soc Nephrol.* 2011; 22(5):975. [PubMed: 21493773]
66. Lepin EJ, Zhang Q, Zhang X, Jindra PT, Hong LS, Ayele P, et al. Phosphorylated S6 ribosomal protein: a novel biomarker of antibody-mediated rejection in heart allografts. *Am J Transplant.* 2006; 6(7):1560. [PubMed: 16827856]
67. Colvin RB. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J Am Soc Nephrol.* 2007; 18(4):1046. [PubMed: 17360947]
68. 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. *Am J Transplant.* 2009; 9(10):2312. [PubMed: 19681822]
69. Sellares J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant.* 2012; 12(2):388. [PubMed: 22081892]
70. Wiebe C, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *Am J Transplant.* 2012; 12(5):1157. [PubMed: 22429309]
71. Hidalgo LG, Sis B, Sellares J, Campbell PM, Mengel M, Einecke G, et al. NK cell transcripts and NK cells in kidney biopsies from patients with donor-specific antibodies: evidence for NK cell involvement in antibody-mediated rejection. *Am J Transplant.* 2010; 10(8):1812. [PubMed: 20659089]
72. Sis B, Halloran PF. Endothelial transcripts uncover a previously unknown phenotype: C4d-negative antibody-mediated rejection. *Curr Opin Organ Transplant.* 2010; 15(1):42. [PubMed: 20009933]
73. Loupy A, Hill GS, Suberbielle C, Charron D, Anglicheau D, Zuber J, et al. Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). *Am J Transplant.* 2011; 11(1):56. [PubMed: 21199348]
74. Fildes JE, Yonan N, Tunstall K, Walker AH, Griffiths-Davies L, Bishop P, et al. Natural killer cells in peripheral blood and lung tissue are associated with chronic rejection after lung transplantation. *J Heart Lung Transplant.* 2008; 27(2):203. [PubMed: 18267228]

75. Collins AB, Chicano SL, Cornell LD, Tolkoff-Rubin N, Goes NB, Saidman SL, et al. Putative antibody-mediated rejection with C4d deposition in HLA-identical, ABO-compatible renal allografts. *Transplant Proc.* 2006; 38(10):3427. [PubMed: 17175293]
76. Grafft CA, Cornell LD, Gloor JM, Cosio FG, Gandhi MJ, Dean PG, et al. Antibody-mediated rejection following transplantation from an HLA-identical sibling. *Nephrol Dial Transplant.* 2010; 25(1):307. [PubMed: 19846396]
77. Zhang Q, Reed EF. Non-MHC antigenic targets of the humoral immune response in transplantation. *Curr Opin Immunol.* 2010; 22(5):682. [PubMed: 20833523]
78. West LJ. ABO-incompatible hearts for infant transplantation. *Curr Opin Organ Transplant.* 2011; 16(5):548. [PubMed: 21836514]
79. Takahashi K, Saito K, Nakagawa Y, Tasaki M, Hara N, Imai N. Mechanism of acute antibody-mediated rejection in ABO-incompatible kidney transplantation: which anti-A/anti-B antibodies are responsible, natural or de novo? *Transplant.* 2010; 89(5):635.
80. Haas M, Segev DL, Racusen LC, Bagnasco SM, Locke JE, Warren DS, et al. C4d deposition without rejection correlates with reduced early scarring in ABO-incompatible renal allografts. *J Am Soc Nephrol.* 2009; 20(1):197. [PubMed: 18776120]
81. Issa N, Cosio FG, Gloor JM, Sethi S, Dean PG, Moore SB, et al. Transplant glomerulopathy: risk and prognosis related to anti-human leukocyte antigen class II antibody levels. *Transplant.* 2008; 86(5):681.
82. Benson EM, Colvin RB, Russell PS. Induction of IA antigens in murine renal transplants. *J Immunol.* 1985; 134(1):7. [PubMed: 3917285]
83. Muczynski KA, Ekle DM, Coder DM, Anderson SK. Normal human kidney HLA-DR-expressing renal microvascular endothelial cells: characterization, isolation, and regulation of MHC class II expression. *J Am Soc Nephrol.* 2003; 14(5):1336. [PubMed: 12707403]
84. 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. *Am J Transplant.* 2011; 11(11):2405. [PubMed: 21942930]
85. Farkash EA, Colvin RB. Diagnostic challenges in chronic antibody-mediated rejection. *Nat Rev Nephrol.* 2012; 8(5):255. [PubMed: 22450441]

Fig. 1.

Activation of NK cells through the CD16/FcγRIII (CD16) triggers increased maturation of NK cells, loss of CD56, expression of the transcription factor T-bet and production of cytokine mediators. One or more of these mechanisms is hypothesized to be involved in the induction of CAV by NK cells and antibody.