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Mechanisms of antibody-mediated acute and chronic rejection of kidney allografts

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Abstract

Purpose of the review—Antibody-mediated rejection is responsible for up to half of acute rejection episodes in kidney transplant patients and more than half of late graft failures. Antibodies cause acute graft pathologies that are distinct from T cell mediated rejection and at later times post-transplant a distinct pathology associated with capillary basement membrane multilayering and glomerulopathy. Despite the importance of donor-reactive antibodies as the leading cause of kidney graft failure, mechanisms underlying antibody-mediated acute and chronic kidney graft injury are poorly understood. Here we review recent insights provided from clinical studies as well as from animal models that may help to identify new targets for therapy.

Recent findings—Studies of biopsies from kidney grafts in patients with donor-specific antibody versus those without have utilized analysis of pathology and gene expression to identify the distinct characteristics of antibody-mediated rejection. These analyses have indicated the presence of NK cells and their activation during antibody-mediated rejection. The impact of studies of antibody-mediated allograft injury in animal models have lagged behind these clinical studies, but have been useful in testing the activation of innate immune components within allografts in the presence of donor-specific antibodies.

Summary—Most insights into processes of antibody-mediated rejection of kidney grafts have come from carefully designed clinical studies. However, several new mouse models of antibody-mediated kidney allograft rejection may replicate the pathologies observed in clinical kidney grafts and may be useful in directly testing mechanisms that underlie acute and chronic antibody-mediated graft injury.

Keywords

antibody-mediated rejection; platelets; natural killer cells

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Conflicts of interest

The authors have no conflicts to declare.

INTRODUCTION

The problem

The use of calcineurin inhibitor-based immunosuppression has resulted in marked decreases in T cell mediated graft rejection; however, there is increased observation of antibody-mediated graft injury and rejection (1–4). Antibodies are reported to be the cause of 30–50% of acute rejection episodes in kidney transplants and more than 60% of late graft failure and are the leading cause of kidney graft failure in the U.S. (3–7). The increased incidence and difficulty in treating antibody-mediating rejection (AMR) has underscored the need to identify the cellular and molecular components underlying antibody-mediated kidney graft rejection in order to develop more efficacious therapeutic strategies to prevent or attenuate antibody-mediated graft injury.

Acute T cell-mediated and antibody-mediated kidney graft rejection have distinct histopathologic features (8–10). T cell mediated rejection includes mononuclear interstitial infiltration and tubulitis with intimal endarteritis whereas features of AMR include thrombotic microangiopathy, neutrophil and macrophage margination in the microcirculation (capillaritis) and often within the glomeruli. Chronic AMR results in late transplant glomerulopathy with interstitial fibrosis and capillary basement membrane multilayering. In many cases donor-specific antibody (DSA) doesn't mediate acute graft rejection as a solitary event, but includes activation of other components of innate and adaptive immunity that contribute to graft injury. T cell infiltration and tubulitis is often observed in for-cause biopsies with diagnosis of antibody-mediated rejection and such mixed T cell-antibody mediated pathology may occur in 10–90% of graft biopsies (5, 9, 11, 12).

PATHOLOGY AND GENE EXPRESSION SIGNATURES DURING AMR OF CLINICAL KIDNEY TRANSPLANTS

The importance of AMR in undermining kidney graft outcome has spurred a concerted effort by several groups to identify genetic markers that distinguish T cell-mediated and antibody-mediated kidney graft rejection. These studies have yielded gene signatures that appear to be restricted to AMR and have provided new insights into potential mechanisms that could underlie AMR in these grafts.

Studies from the Edmonton group have used kidney graft biopsies to examine tissue pathology and identify gene signatures at early and later times following kidney transplantation (13¹⁴). Histological examination of a total of 703 biopsies taken up to 35 years after transplant identified 205 with pathology indicating T cell- and/or antibody-mediated rejection. Gene expression analysis by microarray was consistent with the histopathologic evaluation of the biopsies. T cell mediated rejection was observed in biopsies with rejection taken early after transplant and up to one year post-transplant, then decreased over time and was absent by 10 years post-transplant, whereas AMR was common in in biopsies from grafts with rejection taken after the first year. Importantly, the presence of AMR and its gene signature in the biopsy strongly associated with graft loss, whereas pathologic evidence of T cell mediated rejection or its gene signature did not.

Surprisingly, mixed T cell/antibody rejection was only observed in about 14% of the biopsies.

These studies were extended in microarray analyses of biopsy RNA to identify gene signatures that distinguished T cell-mediated rejection in early biopsies obtained from kidney grafts with rejection versus AMR occurring in biopsies obtained from kidney grafts with rejection at later times post-transplant (14). T cell mediated rejection was indicated by kidney graft expression of CD3D, TcR α , CXCR6, GPR171 and NELL2. Consistent with their previous studies, observation of this signature and pathology in biopsies decreases within the first year and later rejection episodes were indicative of antibody-mediated injury, which had a distinct gene signature. Ongoing AMR was indicated by expression of CX₃CR1, KLRF1, MYBL1, and Sh2D1B, genes associated with the presence and activation of NK cells. These T cell- and NK cell-associated gene signatures correlated with previous immunohistochemical staining studies indicating the presence of T cells in biopsies during early rejection episodes and the presence of NK cells in peritubular capillaries during AMR (15). In support of the potential role of NK cells during AMR, kidney patients with high serum anti-donor HLA antibodies had substantial increases in circulating NK (CD56^{high} and NKG2A⁺) cells (16).

MECHANISMS OF ACUTE AMR

While the use of gene signatures that correlate with pathology in kidney graft biopsies in patients has provided important insights into the processes of AMR, the ability to assign cause and effect of specific cells or molecular mediators to mechanisms and to identify new potential therapeutic targets can only be inferred. There has been considerable concern that AMR of kidney allografts in animal models does not accurately replicate the pathology observed during AMR in clinical kidney transplants. For the most part, studies of AMR in rodent models have relied either on transfer of polyclonal or monoclonal antibodies that are specific for donor MHC and other antigens or on priming recipients with donor cells or tissues (e.g. skin grafts) several weeks before challenge with the allograft. As with all animal models, each of these approaches has limitations and although there has been considerable concern that AMR of kidney allografts in these animal models does not accurately replicate the pathology observed during AMR in clinical kidney transplants, findings in animal models have been important for providing mechanistic insights into the effects of graft reactive antibodies on the graft endothelium and other tissues.

Role of complement

Although deposition of C4d in biopsies was the first reliable marker of AMR, C4d was always recognized as an imperfect surrogate for complement-mediated injury to transplants (17). Complement is a multi-dimensional system that progresses from a largely anti-inflammatory series of mediators to extremely pro-inflammatory mediators (18). The initiating components of the classical and lectin pathways of complement, C1q and MBL, are crucially important in clearing apoptotic and necrotic cells by macrophages without inciting inflammation (19, 20*). The final split products of C4 activation, C4a and C4d have no established biological activities (21). Recently, evidence that C3a decreases neutrophil recruitment, while enhancing monocyte/macrophage function has been reviewed (22).

Similarly, receptors for iC3b have been shown to modulate interstitial inflammation in kidneys (23²²). However, the progression of complement activation from C3 to C5 generates the soluble split product C5a, which is an extremely potent chemoattractant and activator for neutrophils and monocytes (22). Of relevance to AMR, C5a stimulates macrophages to down-regulate inhibitory FcγR and upregulate activating FcγR (24).

It is important to note that the potential harmful effects of the pro-inflammatory terminal complement components on endothelial cells are controlled by multiple regulators of complement, many of which act on the enzymatic components of C3 and C5 convertases. As a result, C5 and the subsequent complement components are only activated when the capacity for regulation is exceeded or the expression of regulators is decreased (25). Overall, the function of complement regulatory proteins on endothelium limits the direct contribution of complement to graft injury during AMR.

Macrophages and platelets

Antibodies with sufficient avidity to cross-link MHC class I antigens stimulate endothelial cells to exocytose von Willebrand factor (vWf) and P-selectin in vitro and in vivo (26–33). These mediators rapidly bind and activate platelets and macrophages (26, 32–35). Experiments investigating platelet activation by alloantibodies in skin allografts allowed direct visualization of alloantibody induced platelet tethering in capillaries by intravital imaging (30). The platelet response to DSA is extremely rapid and dynamic. Within 1 hour platelets were induced to roll and adhere to capillary endothelium. Even platelets that roll along capillaries and then return to the circulation deposit trails of RANTES on vascular endothelium (36). Although there is increasing recognition that platelets modify vascular diseases (37–40), only limited data are available about the potential contribution of platelets to AMR (26, 30–35).

Platelet storage granules contain a wide array of mediators that could potentially promote acute and chronic AMR (26, 34–36). Of all these mediators, platelet factor 4 (PF4; CXCL4) is quantitatively most abundant (41, 42). In an experimental model of acute AMR, PF4 and serotonin were recovered from renal allografts in much greater quantities (10–30 ng/mg tissue) than other cytokines (5–150 pg/mg tissue for IL-1β, MCP-1/CCL2, MIP-1α/CCL3 and RANTES/CCL5) stored in platelet alpha granules (26²⁶).

The localization of large quantities of PF4 in the allograft has multiple consequences. Although the independent chemotactic effects of PF4 are modest, PF4 associates with the glycosaminoglycans of endothelial cells and modulates the effects of chemokines (43). By forming heterodimers with RANTES and IL-8, PF4 enhances the chemotactic properties of RANTES, but decreases those of IL-8 (44–46). In addition to chemotaxis, PF4 promotes monocyte survival and macrophage differentiation (47). In vitro studies have demonstrated that PF4 can stimulate monocytes to cause apoptosis of endothelial cells (48). These interactions are particularly relevant because macrophage infiltrates in human biopsies are a characteristic of AMR in renal and cardiac transplants (49, 50). In renal transplants the presence of glomerular monocytes predict poorer graft outcomes (51).

The expression of P-selectin by activated endothelial cells and platelets promotes interaction with neutrophils and monocytes through P-selectin glycoprotein ligand-1 (PSGL-1). The expression of P-selectin in human renal allografts is associated with the localization of neutrophils and monocytes in the first hours after transplantation (52). Similarly, passive transfer of antibodies to donor MHC class I antigens causes the upregulation of P-selectin and increased macrophage accumulation in experimental cardiac allografts, which can be blocked by administration of the fusion molecule recombinant soluble PSGL-Ig (32).

In conjunction with the release of chemokines, the expression of P-selectin on activated platelets promotes interactions with macrophages (53). P-selectin expressing platelets attached to macrophages are evident by immunohistology in capillaries and venules of mouse and human renal allografts (27). The formation of platelet-leukocyte conjugates is known to result in increased activation and localization of leukocytes in inflammatory sites (54–56). However, the effects of platelets on skewing macrophage function towards inflammatory or wound-healing functions in AMR remain unknown. PF4 has been demonstrated to elicit a distinct differentiation profile in macrophages in vitro that has been labeled M4 (57). Macrophages with an M4 phenotype have been demonstrated in atherosclerotic lesions (58). It is possible that M4 macrophages may be relevant to coronary allograft vasculopathy in chronically rejected cardiac transplants.

Release of serotonin from platelets also has potential consequences in renal allografts. Platelets express serotonin reuptake transporter (SERT) proteins and store serotonin in their dense granules in quantities that make platelets the major source of serotonin in the circulation. In models of acute inflammation, serotonin promotes recruitment of leukocytes by causing endothelial cells to exocytose vWf and P-selectin (59). Recently, increased vascular permeability caused by platelet-derived serotonin has been found to be a critical step in the inflammatory lesions of rheumatoid arthritis and lupus (37, 38).

In addition to regulating inflammatory responses by cross-linking MHC antigens on endothelial cells, antibodies can regulate neutrophil and monocyte function by binding to different types of Fc receptors (FcRs). Although the modulatory effects of activating (human FcγRI, FcγRIIA and FcγRIIIA) and suppressive (human FcγRIIB) receptors for IgG have been appreciated for a long time (60), new aspects of FcR interactions and functions have been discovered in the last few years (61). An increasing number of genetic polymorphisms have been identified that impose functional modifications on human FcRs (62[•]). Notably, some single nucleotide polymorphisms and splice variants result in increased expression and function of specific FcRs on neutrophils and monocytes. Using rolling velocity on human aortic endothelial cells coated with IgG1 or IgG2 antibodies, Valenzuela and colleagues demonstrated that monocytic cell lines that express high affinity variants of FcγRIIA had greater interactions with the endothelial cells (33^{••}). The antibodies were also demonstrated to cause the release of P-selectin and therefore the FcR functioned served as an additional adhesion element.

FcγRs are also the major receptors for C-reactive protein (CRP) and serum amyloid P (SAP) component (63). CRP and SAP facilitate clearance of phospholipids and nuclear components from late apoptotic and necrotic cells. Cardiac allografts to FcγRIII deficient

recipients undergo accelerated rejection accompanied by increased levels of circulating alloantibodies, perivascular margination of monocytes and neutrophils, and intense periarterial infiltrates with extensive accumulation of apoptotic bodies (64). These findings suggest that Fc γ RIII is essential for effective clearance of SAP-opsonized apoptotic bodies from transplants through a non-inflammatory pathway. Testing the effects of the FcR variants in vivo is poised for rapid advances by the availability of a variety of mice with transgenic expression of human FcRs (62).

NK cells

As discussed above, recent gene profiling in kidney biopsies from patients experiencing AMR indicate the presence of transcripts associated with NK cells and their activation within the allograft. To date the role of NK cells in mediating acute antibody-mediated allograft injury has mostly been examined using the heterotopic heart allograft model. Infusion of allograft reactive mAb or serum into RAG-1^{-/-} recipients of heart allografts caused chronic allograft vasculopathy within 3–4 weeks (65). Development of cardiac allograft vasculopathy (CAV) was attenuated when the recipients were treated with anti-NK1.1 mAb to deplete NK cells and required the Fc portion of the DSA as infusion of F(ab')₂ fragments prepared from DSA resulted in the absence of CAV. These studies suggest that DSA interaction with MHC molecules on the graft endothelium recruits and induces NK cell activation through the Fc receptor binding to the DSA. What those NK cell activities are that induce CAV are unknown but may include either IFN- γ production and/or antibody-mediated cytotoxicity.

As macrophages are quickly recruited to the allograft endothelium during the beginning of the DSA response and express multiple high affinity Fc receptors, they are certainly capable of mediating ADCC whereas NK cells express only CD16, low affinity Fc receptors. Recent clinical studies by Clatworthy and colleagues (66^{***}) have indicated that non-functional vs. functional polymorphisms in the inhibitory Fc γ RIIB, which is expressed on neutrophils and macrophages but not on NK cells, do not affect the incidence or intensity of renal allograft injury or 10-year graft survival. Furthermore, peripheral blood mononuclear cells pulsed with reactive anti-HLA mAb stimulate NK cells to produce IFN- γ and express CD107a, an indicator of perforin/granzyme B cytolytic activity (67). In conjunction with the studies of clinical kidney graft biopsies, these results support a role for NK cells in contributing to the graft tissue injury during acute and chronic antibody-mediated kidney graft injury.

New mouse models of acute AMR

A useful animal model to investigate mechanisms of antibody-mediated graft injury would be one in which the progression of AMR is dependent on de novo DSA produced in response to the allograft with sustained titers that recapitulates the histopathology observed during AMR in clinical transplants. In most donor-to-recipient strain combinations of mice, wild type recipients do not reject the majority of kidney allografts. Although there is moderate CD3 and macrophage infiltration during the first 10 days this quickly resolves and > 70% of the allografts will survive long-term with good function and histology (68, 69). A major mechanism mediating kidney allograft survival in these wild type recipient models is the rapid infiltration and action of FoxP3⁺ Tregs in the kidney allografts and depletion of

these Tregs does result in acute graft rejection (70). In contrast, it has recently been reported that CCR5-deficient mice produce de novo antibody with increasing high titers in response to heart and kidney allografts (71). The increases in serum DSA titers are associated with increased kidney graft histopathology and increasing levels of serum creatinine and ultimately result in failure of complete MHC mismatched kidney grafts between days 18 and 25 post-transplant. Strengths of this model include that rejection of the complete MHC disparate kidney grafts by CCR5^{-/-} recipients is dependent on the production of DSA and the pathology of injury is similar to that observed during AMR in clinical kidney allografts including margination of neutrophils and macrophages in the PTC, interstitial hemorrhage, edema and glomerular fibrin deposition. Kidney allograft rejection is also accompanied by large increases in allograft expression of IFN- γ , perforin, granzyme B, FasL and VCAM-1, which peak in the allografts about day 7 after transplant and are attenuated by recipient depletion of NK cells but not CD8 T cells (unpublished data). These data are in line with those suggesting a role for NK cells during AMR of kidney allografts and in preliminary experiments CCR5-deficient recipient depletion of NK cells results in long-term survival of kidney allografts. The limitations of the model are that the DSA response is dysregulated by an apparent defect in the function of CD4⁺ Tregs. Thus, this model may be of value for investigating mechanisms of DSA-mediated kidney allograft injury but is inappropriate for investigation into mechanisms underlying the production of DSA.

Another mouse model has been recently reported that utilizes the transfer of donor antigen-primed CD4 T cells to naïve recipients that are then challenged with an allograft. This strategy was initially used to study the induction of pathogenic DSA and the impact on complete MHC mismatched heterotopically transplant heart allografts (72, 73) and has been extended to investigate AMR of kidney allografts (A. Valujskikh, submitted for publication).

New models of chronic AMR

The major problem in clinical kidney transplantation is that while short-term success rates are high due to modern immunosuppression regimens, long-term outcomes have not improved. Grafts are being lost to chronic injury and this is probably due to DSA. This has been much harder to model in animal models and to date there has been very little investigation into this problem other than the CAV models induced following infusion of DSA.

The CCR5-deficient mice were crossed with transgenic mice expressing human CD20 on B cells to generate huCD20/CCR5^{-/-} mice (74^{***}). Complete MHC mismatched kidney allografts are rejected by these recipients with the same pathology as by the CCR5^{-/-} recipients. huCD20/CCR5^{-/-} recipient treatment with anti-human CD20 mAb at the time of the transplant and then weekly for 7 weeks resulted in the long-term survival of about 70% of the allografts with good graft function and normal glomerular and kidney histology when examined 100 days after transplant. However, when recipients are treated with anti-human CD20 mAb beginning at the time DSA is first detectable in recipient serum on day 5 post-transplant followed by two more doses on days 8 and 12, kidney allograft survival is extended from day 18–25 observed in control Ig treated recipients to day 60–80. Rather than

the pathology of acute AMR observed in kidney allografts in control treated recipients, the failed grafts in huCD20/CCR5^{-/-} recipients treated with the delayed course of anti-CD20 mAb have severe interstitial fibrosis and glomerulopathy similar that seen during late graft failure in clinical kidney transplants. These late failing grafts expressed 2–4-fold increases in many genes associated with fibrogenesis including E- and N-cadherin and connective tissue growth factor (CTGF) and high (>100-fold) increases in VCAM-1 and P-selectin, but did not express the high levels of many of the genes observed during acute AMR, including IFN- γ , perforin, and granzyme B. It is worth noting, however, that similar increases in the expression of E- and N-cadherin, CTGF, VCAM-1 and P-selectin were observed in kidneys undergoing acute AMR, suggesting that acute AMR may also initiate fibrogenesis in the kidney allograft. These initial studies suggest a novel animal model of the indolent antibody-mediated chronic injury that is proposed to account for most clinical kidney graft failures.

CONCLUSION

Antibody-mediated graft failure is one of the most important problems in kidney transplantation as well as in other organ transplants. Carefully designed clinical studies have established the unique pathologies of antibody-mediated acute and chronic injury of kidney transplants. These studies have also provided unique gene signatures in biopsies from kidney grafts experiencing these injuries that implicate specific gene expression and cellular targets as mechanisms linking DSA binding to target antigens on the graft endothelium to the injury observed during histopathologic analysis of the graft. The potential role of NK cells in acute and/or chronic AMR is intriguing, but at this point the mechanisms that NK cells would utilize to promote the pathology observed is entirely unclear. There remain other leukocyte populations infiltrating kidney grafts that are common to the antibody-mediated acute and/or the chronic injury but again mechanistic evidence linking such cells and their activation within the graft is at this point only correlative.

Animal models have been previously used to test the consequence of allograft-specific antibody on the phenotype and intensity of cells infiltrating the graft and on gene expression of inflammatory mediators within the graft. However, passive transfer of such antibodies or the use of donor-sensitized recipients has not reproduced the pathology observed during AMR of kidney grafts in the clinic. Several new models have been reported that appear to, at least in part, solve this problem with discrepancy in graft pathology in animal models versus the clinic. The development of these and other models may be more useful in robustly determining the requirements for specific molecules and cells during antibody-mediated acute and chronic injury of kidney allografts that leads to graft failure.

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- Antibody-mediated rejection is the major cause of early and late kidney graft failure
- Clinical studies have indicated unique gene signatures in kidney graft biopsies during acute rejection mediated by T cells vs. donor-specific antibody
- Activation of NK cells is observed in kidney allografts during AMR in clinical transplants and in mouse models
- Platelets circulate in high concentration adjacent to vessel walls and quickly amplify leukocyte responses to activated endothelium
- Kidney allografts in $CCR5^{-/-}$ recipients are subjected to acute and chronic antibody-mediated graft injury that shares many of the histopathologic features observed during AMR in clinical kidney transplants