The Emerging Importance of Non-HLA Autoantibodies in Kidney Transplant Complications

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ABSTRACT

Antibodies that are specific to organ donor HLA have been involved in the majority of cases of antibody-mediated rejection in solid organ transplant recipients. However, recent data show that production of non-HLA autoantibodies can occur before transplant in the form of natural autoantibodies. In contrast to HLAs, which are constitutively expressed on the cell surface of the allograft endothelium, autoantigens are usually cryptic. Tissue damage associated with ischemia-reperfusion, vascular injury, and/or rejection creates permissive conditions for the expression of cryptic autoantigens, allowing these autoantibodies to bind antigenic targets and further enhance vascular inflammation and renal dysfunction. Antiperlecan/LG3 antibodies and antiangiotensin II type 1 receptor antibodies have been found before transplant in patients with de novo transplants and portend negative long-term outcome in patients with renal transplants. Here, we review mounting evidence suggesting an important role for autoantibodies to cryptic antigens as novel accelerators of kidney dysfunction and acute or chronic allograft rejection.


Successful management of ESRD through kidney transplantation (KT) is one of the most important advances in medical care achieved in the second half of the 20th century. KT improves both life expectancy and quality of life compared with dialysis.1,2 With current immunosuppressive regimens, the incidence of acute rejection is approximately 15%–20% in kidney transplant recipients.3 Although this figure is inferior to the rates reported two decades ago, rejection still represents a challenge for transplant physicians. Treatment with increased immunosuppression leads to excellent recovery of graft function in many patients, but some patients experience treatment failure and subsequent progression to graft loss. Among the factors relevant to the prognosis of rejection, the presence of antibody-mediated damage to the microcirculation is associated with adverse long-term graft outcomes.4–6

Although circulating, anti–HLA, donor-specific antibodies (DSAs) have been involved in the majority of patients with antibody-mediated rejection (ABMR), this type of rejection can also occur in patients who are DSA negative.7,8 Although adsorption of DSA within the allograft has been proposed to explain the occurrence of ABMR in DSA-negative patients,9 mounting evidence has pointed to the role of non-HLA antibodies as important contributors to ABMR. Non-HLA autoantibodies have been associated with rejection in kidney, heart, and lung transplant recipients.7 In contrast to HLAs, which are constitutively expressed on the cell surface of the allograft endothelium, autoantigens are usually cryptic and become exposed after tissue damage prompted by ischemia-reperfusion or allograft rejection. Tissue damage seems to play an important role in both fueling the production of these autoantibodies and if persistent, allowing these autoantibodies to react with their antigenic target, therefore enhancing inflammation at sites of injury.10,11 Although some autoantibodies have been described in patients with classic autoimmune conditions, such as SLE, others have been reported in absence of autoimmune diseases or sensitizing events.12,13 In this work, we review the mechanistic role of autoantibodies in accentuating renal damage and dysfunction and discuss recent evidence pointing to vascular injury as an important contributor to both their production and effect.

ALLOIMMUNE GRAFT INJURY LEADS TO THE PRODUCTION OF AUTOANTIBODIES

The concept of renal damage–mediated autoantibody production leading to enhanced renal injury is not novel. In the 1960s, Milgrom and coworkers14 reported that rabbits that had rejected mismatched kidney transplants developed antibody-mediated
lesions in their native kidneys. This early work showed that alloimmune attack to a kidney graft can lead to the development of autoantibodies, possibly through the release or increased immunogenicity of kidney-specific autoantigens. Although these autoantibodies were able to induce disease in the rabbits’ native kidneys, whether this occurred through complement-dependent mechanisms was not studied at the time.

The involvement of acute and chronic rejection processes in the development of autoantibodies has also been examined in patients with transplants. For instance, polyreactive, natural IgG autoantibodies against apoptotic Jurkat cells were isolated from the sera of kidney transplant recipients with ABMR. Whether these autoantibodies participated in accelerated rejection was not addressed in this cross-sectional study, but the purified IgGs led to C4d deposition at the surface of targeted apoptotic cells in the presence of complement. The capacity of these autoantibodies to activate complement through the classical pathway suggested a potential role in enhancing allograft damage. In another study, kidney transplant recipients with transplant glomerulopathy, a key feature of chronic ABMR, were shown to have increased levels of autoantibodies to agrin, a component of the vascular basement membrane. The number of previous acute rejection episodes was higher in patients with antiagrin antibodies, again suggesting that alloimmune graft damage may have fueled the production of autoantibodies. Lastly, vimentin, an intracellular intermediate filament protein, can be expressed at the surface of apoptotic T cells and neutrophils as well as endothelial cells. Increased anti-vimentin antibodies have been reported in patients with chronic rejection and failed kidney allografts, whereas the levels observed in transplant-naïve patients with end stage CKD were similar to those observed in blood donors.

**AUTOANTIBODIES AGGRAVATE ACUTE OR CHRONIC REJECTION**

Although allograft injury can lead to the production of autoantibodies, both human and animal data have shown that autoantibodies can, in turn, accelerate and/or enhance renal allograft damage. In the seminal work published a decade ago, Dragun *et al.* showed that agonistic autoantibodies to angiotensin II type 1 receptors (AT1-R-Abs) were associated with a severe form of acute vascular rejection with refractory hypertension in patients with renal transplants who were DSA negative. Passive transfer of AT1-R-Abs in a rat model of KT accentuated allograft vascular injury and hypertension, therefore replicating the salient clinical features observed in patients with kidney transplants and showing the pathogenic effect of these autoantibodies.

Previous work from our group showed that endothelial apoptosis leads to the release of LG3, a fragment of perlecan, a large proteoglycan normally present within the vascular and to a lesser extent, the tubular basement membrane. Because endothelial apoptosis is a prominent feature of acute vascular rejection in KT, we measured LG3 in patients with acute vascular rejection and observed elevated circulating levels compared with those in patients with kidney transplants with normal function and tubulointerstitial rejection. We hypothesized that LG3, a C-terminal laminin G motif normally embedded within perlecan but released in the circulation in association with vascular apoptosis, could behave as a neoantigen and fuel the development of autoantibodies. In other work, we showed that patients with kidney transplants and acute vascular rejection have significantly higher anti-LG3 antibody titers at the time of rejection compared with controls. The anti-LG3 antibodies that we identified were of complement-activating IgG isotypes. To characterize the functional effect of anti-LG3 antibodies in acceleration of rejection, we turned to a murine model of vascular rejection on the basis of aorta transplantation between two fully mismatched donors and recipients. Passive transfer of anti-LG3 antibodies accelerated vascular inflammation, obliterator remodeling, and C4d deposition, showing their mechanistic effect in complement activation and aggravation of vascular injury (Figure 1). In patients with kidney transplants, the acute vascular rejection episodes associated with high anti-LG3 titers were early events, occurring at a median of 12 days after transplantation. Because this was unexpectedly early for a *de novo* antibody response, we evaluated whether anti-LG3 antibodies were present before transplantation. We observed that anti-LG3 titers were significantly higher before transplantation in patients with kidney transplants who went on to develop acute vascular rejection, therefore identifying pretransplant anti-LG3 titers as predictors of rejection.

The effect of pretransplant autoantibodies on post–transplant graft outcomes has also been documented by others. Elevated pretransplant levels of AT1-R-Abs have been associated with an increased risk of post–transplant hypertensive emergencies and severe rejection episodes targeting the vascular compartment. Similarly, pretransplant levels of polyspecific, natural autoantibodies reactive to apoptotic cells have been associated with increased risk of rejection and reduced allograft survival in kidney transplant recipients. Additionally, both *de novo* formation and pretransplant levels of anti-fibronectin and anticollagen type 4 autoantibodies were linked with transplant glomerulopathy in patients with kidney transplants. Autoantibodies have also been linked to acute and chronic allograft rejection in patients with heart and lung transplants. Despite the ample correlational evidence, the clinical factors that influence the levels of these autoantibodies before transplantation remain unclear. AT1-R-Abs have been associated with scleroderma, lupus, and preeclampsia. However, no correlations between allosensitizing events, classic autoimmune diseases before transplantation, and anti-LG3 or antiapoptotic cell autoantibodies have been observed.

**ISCHEMIA-REPERFUSION CREATES A PERMISSIVE ENVIRONMENT FOR AUTOANTIBODIES TO ENHANCE TISSUE INJURY**

The concept of “innate autoimmunity,” as conceived by Carroll and coworkers, has been observed.
Figure 1. Autoantibodies aggregate rejection. Ischemia-reperfusion injury at or near the time of transplantation or alloimmune attack to the graft creates permissive conditions for the enhanced availability of cryptic antigens, such as LG3, and increased interactions with antigens present on apoptotic cells. Preexisting circulating autoantibodies (anti-LG3, AT1R-Abs, or antibodies directed toward apoptotic cells) bind to their target and increase vascular rarefaction and increased tubulointerstitial fibrosis (Figure 2).39 Ischemia increases the contractile activity of AT1R-Abs agonistic autoantibodies in isolated renal artery rings,41 which in turn, could potentially enhance renal vasoconstriction and distal ischemia. These observations suggest that, in nontransplanted patients with AKI, a potential aggravating role of autoantibodies deserves additional investigation.

MECHANISMS OF AUTOANTIBODY FORMATION IN HEALTH AND DISEASE: A PARADIGM SHIFT

In contrast to natural IgM autoantibodies, autoreactive IgGs have traditionally been linked to a break in self-tolerance and pathogenicity.42 In recent years, however, microarray techniques have revealed that both polyspecific and monospecific autoreactive IgG antibodies directed at multiple proteins expressed in human tissues are abundant and ubiquitous in normal human sera.43 Similar to their IgM counterparts, they are thought to be important players in the clearance of danger-associated antigens that was unmasked by tissue injury.10,34 found to bind to a cryptic self-antigen usually occurring IgM antibodies were enhanced tissue injury. This natural antibody was polyspecific, because it was shown to bind nonmuscle myosin heavy chain in ischemic intestinal tissue35 and glycogen phosphorylase in skeletal muscle36,37 exposed to ischemia-reperfusion injury. This natural antibody diminished tissue damage in a model of cardiac ischemia-reperfusion injury.38

We also found an association between ischemia-reperfusion and the capacity of anti-LG3 antibodies to aggravate vascular damage. Using a murine model of HLA–mismatched aortic transplantation, we noted that passive transfer of anti-LG3 accelerated vascular inflammation and complement deposition but only when the allograft was made ischemic before transplantation.24 More recently, we reported that, in patients with ESRD awaiting a kidney transplant, pretransplant anti–LG3 levels were associated with an increased risk of delayed graft function. Using a murine model of renal ischemia-reperfusion injury and passive transfer of anti-LG3 antibodies, we explored the mechanisms supporting the association between anti–LG3 and aggravation of renal dysfunction. We found that anti–LG3 antibodies aggravate AKI at least in part through increased complement activation within peritubular capillaries, leading to microvascular rarefaction and increased tubulointerstitial fibrosis (Figure 2).39

Along the same lines, lung transplant recipients with elevated pretransplant titers of autoantibodies against type 5 collagen and K-α1 tubulin were shown to be at increased risk of severe ischemia-reperfusion injury during lung transplantation.28 The bronchoalveolar lavage fluid from patients with primary graft dysfunction contained higher levels of C4d, suggesting that these autoantibodies activate complement through the classic complement pathway. In a murine model of syngeneic lung transplantation, passive transfer of autoantibodies to collagen type 5 and K-α1 tubulin induced graft inflammation and fibrosis in the transplanted lung but not in native uninjured lungs.11 Collectively, these results suggest that ischemia-reperfusion, independent of alloimmunity, can create permissive conditions needed for autoantibodies to enhance graft damage.

Whether autoantibodies, such as anti-LG3 antibodies or AT1R-Abs, can enhance the severity of AKI in patients with native kidney disease exposed to ischemia-reperfusion remains to be determined. LG3, the antigenic target of anti-LG3 antibodies, seems to be one of the earliest biomarkers rising in patients with AKI,40 which could create a favorable setting for anti-LG3 to bind to its target, activate complement, and enhance tissue damage. As mentioned above, passive transfer of anti-LG3 antibodies in association with ischemia-reperfusion of native kidneys in mice also accentuates renal dysfunction and aggravates microvascular rarefaction and fibrosis.39 Ischemia increases the contractile activity of AT1R-Abs agonistic autoantibodies in isolated renal artery rings,41 which in turn, could potentially enhance renal vasoconstriction and distal ischemia. These observations suggest that, in nontransplanted patients with AKI, a potential aggravating role of autoantibodies deserves additional investigation.

refers to natural autoantibodies that synergize with ischemia-reperfusion injury to enhance complement–mediated organ damage. It was initially reported in murine models of intestinal and skeletal ischemia-reperfusion injury, where naturally occurring IgM antibodies were found to bind to a cryptic self-antigen that was unmasked by tissue injury.10,34–36 This led to complement activation through the classic pathway and enhanced tissue injury. This natural antibody was polyspecific, because it was shown to bind nonmuscle myosin heavy chain in ischemic intestinal tissue35 and glycogen phosphorylase in skeletal muscle36,37 exposed to ischemia-reperfusion injury. This natural antibody diminished tissue damage in a model of cardiac ischemia-reperfusion injury.38

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molecular patterns and restraining the inflammatory response prompted by dying cells and tissue injury.\textsuperscript{44,45} Although the types and levels of natural IgG autoantibodies are somewhat influenced by age, sex, and disease states, the natural autoantibody profile of a given individual is considered to be relatively stable over time,\textsuperscript{43} and the factors that can lead to changes in autoantibody profiles remain to be fully characterized. Whether some of the autoantibodies associated with acceleration of rejection represent natural autoantibodies is not entirely clear, although many clues point in that direction. Autoantibodies reactive to apoptotic cells before transplantation in patients with de novo transplants are likely to represent natural antibodies implicated in the clearance of dying cells.\textsuperscript{13,15} Indeed, the LG3 autoantigen is released by apoptotic vascular and tubular cells,\textsuperscript{20,21,46,47} suggesting that anti-LG3 antibodies represent natural antibodies produced in response to tissue injury.

In further support of this hypothesis, we recently described a novel mechanism implicated in the production of anti-LG3 antibodies. We showed that, in endothelial and renal epithelial cells, activation of caspase-3, a cysteine protease central to the effector phase of apoptosis, leads to the release of small membrane-bound vesicles, called exosome–like apoptotic vesicles. These extracellular vesicles carry LG3 and an active 20S proteasome core complex.\textsuperscript{47} Immunization with exosome–like apoptotic vesicles triggers the production of anti-LG3 antibodies in naïve and transplanted mice.\textsuperscript{42} Exosome–like apoptotic vesicles induce aggravation of vascular rejection with complement deposition, increased T and B cell infiltration, and increased anti–LG3 antibodies without enhanced production of anti-HLA antibodies. Inhibition of proteasome activity specifically within exosome–like apoptotic vesicles reduces their capacity to trigger anti-LG3 production and aggravate vascular rejection, showing the key role for proteasome activity within these vesicles for controlling their immunogenicity and anti-LG3 production.\textsuperscript{47} In further support for an important role of vascular injury in triggering the production of exosome–like apoptotic vesicles that lead to anti-LG3 production, we showed that acute vascular injury in mice, in the form of hind limb ischemia or renal artery clamping, increases circulating levels of exosome–like vesicles followed by a surge of anti-LG3 production (Figure 3).\textsuperscript{47} Endothelial cells, vascular smooth muscle cells, and tubular epithelial cells all release proteasome-active vesicles when injured in vitro. These cell types are targets of injury during ischemia-reperfusion and could all be contributing to the release of immunogenic, apoptotic, exosome–like vesicles in vivo.\textsuperscript{47} However, additional studies are needed to assess whether episodes of vascular injury in patients awaiting transplantation also lead to the production of immunogenic, exosome–like apoptotic vesicles that can break tolerance to self and induce the production of autoantibodies. Various lines of evidence suggest that ischemia-reperfusion injury occurring either at the time of or before transplantation represents a clinical setting.

**Figure 2.** Anti-LG3 autoantibodies enhance renal microvascular injury postischemia-reperfusion in native and transplanted kidneys. Renal ischemia-reperfusion leads to initial microvascular damage, which enhances the expression/availability of cryptic antigens, such as LG3. Circulating anti-LG3 reaches these antigenic targets and promotes further microvascular injury, at least in part through complement-dependent mechanisms. Microvascular damage leads to peritubular capillary dropout and enhanced renal fibrosis.\textsuperscript{39}

**Figure 3.** Vascular injury triggers the release of exosome–like apoptotic vesicles that prompt anti-LG3 production. Acute vascular injury in mice (hind limb ischemia or renal artery clamping) leads to increased circulating levels of exosome–like apoptotic vesicles containing an active 20S proteasome complex. Proteasome activity in exosome–like apoptotic vesicles prompts the production of anti-LG3 antibodies and antinuclear antibodies (ANAs).\textsuperscript{47} The mechanism underlying anti-LG3 production before transplantation in humans is unclear at this time. The role of acute vascular injuries (vascular access creation/manipulation and acute coronary and peripheral vascular events) in promoting anti-LG3 formation is currently being investigated.
prone to autoantibody production. Indeed, production of autoreactive IgGs directed against kidney-specific antigens has been associated with renal ischemia-reperfusion injury occurring at the time of transplantation.\textsuperscript{48} The most abundant autoreactive IgGs were directed against antigens present in the renal pelvis, a zone that is particularly sensitive to ischemia-reperfusion injury. Collectively, these results also raise the intriguing possibility that at least some of these autoantibodies could potentially contribute to renal dysfunction and progressive renal loss in patients without transplants as well. Anti-LG3, AT\textsubscript{1}R-Abs, antivimentin, and antibodies reactive to apoptotic cells have all been described in patients awaiting transplantation. Considering the role of AKI as a powerful predictor of progressive renal failure in patients with native kidney disease,\textsuperscript{49–51} it remains to be determined whether episodes of ischemia-reperfusion injury in native kidneys can prompt the production of autoantibodies that, in turn, can accelerate renal failure.

**CURRENT AND POSSIBLE FUTURE TREATMENT AND PREVENTION STRATEGIES**

Although clinical studies have yet to be performed, the various autoantibodies discussed above could serve as biomarkers to improve risk stratification for rejection or delayed graft function or as potential therapeutic targets. Pretransplant autoantibody levels could be added to the current clinical and laboratory variables used to assess the risk of rejection or delayed graft function, which in turn, could help transplant physicians select the most appropriate induction therapy. Current experimental data support the concept of a synergy between ischemia and autoantibodies in enhancing graft damage.\textsuperscript{24,39,41} If this is confirmed in larger cohort studies, pretransplant autoantibody titers could have implications in terms of organ allocation. For instance, an organ with expected long cold ischemic time or coming from a donor after circulatory arrest may not be best suited for patients with elevated pretransplant autoantibody titers. After transplantation, autoantibody titers could eventually serve as noninvasive biomarkers for the activity of antibody-mediated and/or vascular rejection.

Because in some patients, autoantibodies actively participate in accelerating graft damage, they could also serve as potential therapeutic targets. Plasma- pheresis could be used pre- and/or post-transplant to decrease circulating levels, and intravenous IgGs may also be beneficial to reduce their deleterious effect and their production. Angiotensin II receptor blockers represent an interesting therapeutic avenue in patients with AT\textsubscript{1}R-Ab–mediated rejection in combination with plasmapheresis.\textsuperscript{12,52} Finally, the recent observation that bortezomib can block the production of anti-LG3 autoantibodies triggered by exosome-like vesicles\textsuperscript{47} may prove useful to help define therapeutic options for preventing autoantibody production before transplantation.

**CONCLUSION**

For more than five decades, kidney transplant rejection has been considered the sole expression of donor-recipient MHC discrepancy that initiates allospecific immune responses. However, the notion needs revisiting in light of mounting evidence clearly delineating the contribution of autoimmune responses to the severity of allograft inflammation and damage. The production of autoantibodies in association with renal damage that may occur post-transplantation, at the time of rejection, at the time of transplantation, or before transplantation and in association with ischemia-reperfusion injury is emerging as an important contributor to the risk of renal dysfunction. A triad of alloimmunity, autoimmunity, and tissue injury can likely trigger the perfect storm for allograft dysfunction and reduced survival. Additional work will allow for better characterization of the molecular pathways that control contributing autoimmune responses and the role of these autoantibodies in promoting the severity of AKI in native kidneys as well as the identification of novel pharmacologic targets of intervention.

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**DISCLOSURES**

None.

**REFERENCES**