Non-MHC antigenic targets of the humoral immune response in transplantation
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There is a growing body of data supporting a role for non-HLA antibodies in acute and chronic rejection of solid organ transplants. While many of these non-HLA antigens remain poorly defined, the principal antigenic targets are expressed on cells of the allograft including endothelium and epithelium. These non-HLA antigens are classified as either alloantigens, such as the major histocompatibility complex class I chain-related gene A (MICA) or MICB, or tissue-specific autoantigens such as vimentin, cardiac myosin (CM), collagen V (Col V), agrin, and angiotensin II receptor type I (AT1). Herein we provide an overview of the non-MHC antigenic targets that have been implicated in graft rejection and discuss the interplay between alloimmunity and autoreactivity in graft rejection.

Non-MHC alloantigens

Anti-endothelial cell antibodies

Anti-endothelial cell antibodies (AECA) have been reported to mediate endothelial cell (EC) activation, apoptosis, and cell injury. AECA represent a heterogeneous group of antibodies comprising both IgM and IgG subclasses and are directed against a variety of antigenic determinants on ECs [7]. Pretransplant AECA are associated with increased frequency of acute renal rejection and decreased long-term graft survival [8]. To investigate how AECA are involved in acute renal allograft rejection, eluates from 25 renal allografts were tested for anti-EC antibodies. Eight of nine patients with irreversible vascular renal allograft rejection had IgM AECA eluted from the rejected kidney, but AECA were absent in the 13 kidneys lost to other types of rejection [9]. These AECA were able to activate EC resulting in upregulation of mRNAs encoding the adhesion molecules VACM-1 and ICAM-1. In a cohort of 57 renal transplant candidates, AECA were present in 47% of patients who were sensitized to HLA and in 16% of nonsensitized patients [10]. Although, these antibodies were mainly of the IgG isotype and did not mediate cytotoxicity, they were able to cause apoptosis of ECs in vitro. No significant correlation was found between the presence of AECA and graft outcome in this cohort.

In cardiac transplantation, 17/31 patients who developed posttransplant AECA experienced AMR compared with only 9 of 49 patients without AECA [11]. In addition, allograft survival at 2 years was significantly better in the AECA– group compared with the AECA+ group and AECA positivity was associated with cardiac allograft vasculopathy (CAV). Pretransplant cytotoxic IgM non-HLA antibodies were associated with a diagnosis of primary graft failure and worse survival of cardiac transplant recipients [12]. Collectively, these studies suggest that antibodies to non-MHC alloantigens may cause AMR and identify a high-risk group for CAV.

A major limitation of these studies is the lack of knowledge of the antigenic specificity of the AECA. Current lymphocyte crossmatching techniques fail to detect AECA. The XM-One assay is a novel endothelial cell
flow cytometry crossmatch technique that uses Tie-2 antibody coated magnetic beads to select precursor EC directly from donor blood [13**]. Results of a multicenter clinical trial evaluating the association of AECA with renal allograft rejection showed that pretransplant donor reactive AECA were present in significantly higher proportion of patients with rejection [13**]. Additional studies are needed to confirm if this crossmatch method is useful for identifying clinically relevant AECA.

MICA
MICA is encoded by genes located within the MHC region on chromosome 6 just centromeric to HLA-B. MICA is highly polymorphic with over 68 MICA alleles. MICA is considered as a plausible target of allograft response because of its polymorphic nature and the fact that endothelial cells can express MICA on their surface under stress due to ischemia reperfusion injury and rejection [14,15]. MICA antibodies associate with acute and chronic rejection of heart, renal, and pancreas transplants [14,16-19,20**]. In a large multicenter study, pretransplant MICA antibodies were found in 21% of the 1910 patients tested and were associated with renal graft rejection and lower 1-year survival [20**]. The long-term effect of anti-MICA antibodies was investigated in a prospective multicenter study of 1319 renal transplant recipients [19]. Patients developing posttransplant MICA antibodies had a significantly lower 4-year allograft survival (86%) compared with those without antibodies (98%). A limitation of these studies is that they failed to discriminate between donor specific vs. third party anti-MICA antibodies. Anti-MICA antibodies have been found to mediate complement dependent cytotoxicity in vitro [21] suggesting that they may contribute to the pathogenesis of AMR through complement mediated injury. A recent study addressed the question of C4d deposition in kidney biopsy and donor specific antibodies (DSA) to HLA, MICA, and GSTT1 [22]. They showed the majority of patients with C4d+ biopsies had DSA to HLA (47%), MICA (21%), or GSTT1.

Two reports assessed the effect of MICA DSA on cardiac allograft outcome. In a study of 44 heart recipients, 60% of patients with acute rejection produced MICA DSA compared with 14% without rejection [18]. In the second study, pretransplant and posttransplant sera from 491 heart transplant recipients were studied for DSA to MICA. They found no effect of sensitization to MICA on episodes of rejection or CAV. The lack of concordance between these studies may be due to small sample size and/or differences in the timing of sample collection. Interestingly, both groups reported an absence of MICA expression on cardiac endothelial cells suggesting that MICA is not constitutively expressed in the transplanted heart. However, MICA genes contain a heat shock response element promoter and their expression can be induced in response to cellular stress. Ischemia reperfusion injury and cytokines such as IL-2, IL-4, and IL-15 that are produced during inflammation and rejection can also upregulate the expression of MICA in the graft [23,24]. Further studies are needed to determine the expression pattern of MIC in solid organ transplants during quiescence and rejection.

Since membrane bound MICA proteins can be upregulated during inflammation and rejection, and soluble MICA is increased in the circulation of transplant recipients [25] we posit that MICA alloreactive T cells responding via the indirect pathway are primed to donor derived soluble MICA antigens in the context of self MHC class II and induce anti-MICA antibody responses. Consistent with this possibility, several studies have shown that antibodies to MICA are produced after transplantation and their frequency is higher in regraft patients [26]. Furthermore, the immune response to mismatched HLA lead to the development of antibodies to MICA antigens expressed on the airway epithelial cells of lung transplants [27].

**Tissue-specific antigens**

**Vimentin**
Vimentin is a non-polymorphic intermediate filament expressed in cytosol of endothelial, vascular smooth muscle cells, activated platelets and macrophages, renal tubular cells, mesangial cells, and renal stromal cells. Vimentin is strongly expressed in the intima and media of coronary arteries where vascular smooth muscle cells and fibroblasts locate. Autoimmune responses to vimentin are associated with both acute and chronic rejection of heart and renal allografts. Cardiac transplant recipients developing CAV show significantly higher titers of anti-vimentin antibodies in the first and second year post-transplant than patients who remained disease free [28]. Production of humoral immune responses to vimentin was also accompanied by the generation of vimentin-specific autoreactive CD8 positive T cells in cardiac transplant recipients [29]. Furthermore, sera containing anti-vimentin antibodies induced leukocytes, to release platelet activating factor that in turn caused the formation of platelet–leukocyte conjugates [30]. Studies in non-human primates confirmed the findings in human heart recipients and showed that development of cellular and humoral autoimmune responses to vimentin was a prominent feature of allograft rejection and CAV [31]. Immunization of mice with vimentin resulted in development of anti-vimentin antibodies and vimentin-specific T cells and accelerated rejection of cardiac allografts, but not isografts [32*]. Furthermore, anti-vimentin antibodies were necessary to cause rejection as shown by the ability of adoptively transferred serum to restore accelerated rejection in B cell deficient mice. Thus, it appears that anti-vimentin antibodies alone are insufficient to cause graft rejection and rather act in concert with the alloimmune response. An important question that emerges from
these findings is how vimentin antibodies are pathogenic to the process of rejection. One theory suggests that alloreactive immune response mediates graft injury, apoptosis of endothelial cells and subsequent exposure of neo-antigens such as vimentin causing an autoimmune response. Anti-vimentin antibodies bind to vimentin positive platelets, leukocytes, and endothelial cells causing complement deposition and leukocyte-platelet aggregation in the microcirculation of the graft [32]. In addition, cross-reactivity between anti-streptococcal antibodies and vimentin/cardiac myosin has been described, suggesting a possible mechanism contributing to myocyte injury [33].

Cardiac myosin
Cardiac myosin (CM) is a heart specific antigen implicated in allograft rejection [34]. Pretransplant myosin autoantibodies correlated with acute cardiac transplant rejection [35]. The expansion of alloreactive T cells was followed by an increase of cardiac myosin reactive T cells and development of anti-myosin IgG1 autoantibodies in a mouse heart transplant model mismatched for minor histocompatibility alloantigens [36]. This supports the idea that CM released during allograft immune injury of the allograft is recognized by CD4+ T helper autoreactive cells through indirect recognition pathway and triggers the generation of autoreactive CM antibodies. Notably, mature CM is not expressed in the thymus during development that may result in incomplete negative selection [37].

Collagen V
Both cellular and humoral responses to collagen V (Col V) act as a major risk factor in the development of bronchiolitis obliterans syndrome (BOS) after human lung transplantation [38]. Col V is usually interstitial and not normally exposed in healthy tissue. However, Col V is unveiled during ischemia reperfusion injury or in interstitial remodeling of lung transplants and can be detected in bronchoalveolar lavage fluid. Transfer of anti-Col V antibodies to rat lung isografts induced pathology consistent with primary graft dysfunction and mediated epithelial cell cytotoxicity [39]. Col V-specific T cells appear in human and rat lung transplant recipients before the clinical onset of BOS and adoptive transfer of Col V reactive T cells induced rejection [40]. Cellular injury to Col V was mediated by IL-17A that recruits monocytes and neutrophils and acts in synergy with other local inflammatory cytokines [38,41]. Th17 cells have been implicated in a number of autoimmune or inflammatory conditions and in models of allograft rejection [42]. In the absence of Th1-mediated alloimmune responses, CD4 Th17 cells mediate an aggressive proinflammatory response leading to cardiac allograft rejection and CAV [43]. Furthermore, Th17-mediated acute lung transplant rejection could be prevented by adoptive transfer of CD4+ Col V-specific T regulatory cells [40].

K-α1 tubulin
K-α1 tubulin is a glycoprotein expressed in airway epithelial cells and is constitutively associated to a guanosine triphosphate (GTP). It forms microtubules in cells and plays an important role in maintaining cellular structure, microtubule-based intracellular movement. K-α1 tubulin is not normally expressed on cell surface, however epithelial cell damage can result in the exposure of K-α1 tubulin that may promote autoimmune responses. Goers et al. [44] showed that 12/36 lung transplant recipients developed anti-K-α1 tubulin antibodies posttransplant in the absence of HLA sensitization and was strongly associated with BOS. The binding of K-α1 tubulin antibodies to airway epithelial cells activated a PKC-driven calcium maintenance pathway and stimulated expression of transcription factors and fibrogenic growth factors culminating in cell cycle signaling and fibroproliferation. To determine if alloimmunity induces pathogenic autoimmune responses, anti-MHC antibodies were administered intrabronchially into the native lungs of mice. Lungs of mice receiving anti-MHC class I antibodies showed increased expression of IL-17 and they developed antibodies to self-antigens K-α1 tubulin, and collagen V [45]. IL-17 neutralization resulted in reduction of autoantibody and lesions induced by anti-MHC class I antibodies. These results indicate that antibodies to donor MHC can induce pathogenic autoimmune response that may play a pivotal role in chronic rejection.

Angiotensin II receptor type I
Angiotensin type 1 receptor (AT1R) is the main receptor for angiotensin II in the glomerulus and mediates arterial blood pressure and salt balance. It is also expressed in the brush border and basolateral membranes of the proximal tubules, in the vasculature, and in other components of the kidney. Anti-AT1R-antibodies were found in 16/20 recipients with renal refractory vascular rejection who had no HLA antibodies [46]. Removal of AT1R-antibodies by plasmapheresis in combination with intravenous immunoglobulin (IVIG) and pharmacologic AT1R blockade improved renal function and graft survival in 7/16 patients as compared with the remaining 9 patients with conventional treatment. In addition, passive transfer of human AT1R-antibodies into rats induced endarteritis and intravascular infiltrates within 1 week. The AT1R-antibodies were complement-fixing IgG1 and IgG3 isotypes, however, C4d deposition was only detected in 5 out these 16 patients, suggesting the pathogenesis of AT1R-antibodies may be complement independent. AT1R-antibodies were shown to promote inflammatory responses and contribute to allograft rejection through the phosphorylation of ERK kinase and activation of AP-1 and nuclear factor κB (NF-κB) resulting in the production of tissue factor and reactive oxygen species. Blockade of NF-κB with decoy oligodeoxynucleotides reduced tubulointerstitial infiltration in rat renal allografts.
Natural antibodies and ischemia reperfusion injury
Recent studies have implicated IgM natural antibodies as self-targets in the pathogenesis of ischemia reperfusion injury (IRI). Studies of different animal IRI models showed that reperfusion of ischemic tissues elicits an acute inflammatory response involving the complement system that is activated by autoreactive natural IgM [47]. Mice deficient in complement are protected against IRI. These studies suggest that hypoxia triggers the expression of neo-antigens and upon binding of the natural antibodies, initiates cellular recruitment and complement activation. Recent studies confirmed that human natural IgM could induce IRI injury in a murine intestinal model suggesting that innate autoimmunity may operate under pathogenic conditions in human [48]. However, whether similar mechanisms operate in humans is unknown. Candidate natural antibodies that have been shown to bind to ischemic endothelial cells include the nonmuscle myosin heavy chain type II A and C IgM antibodies.

Interplay between alloimmunity and autoreactivity in graft rejection
It is increasingly recognized that alloimmune responses and tissue-specific autoimmune responses act in concert to promote graft rejection (Figure 1). Alloimmune responses occur through direct and indirect recognition. The direct pathway involves presentation of allogeneic MHC class I and II antigens on donor APCs to recipient T cells and is believed to be the primary mechanism of acute rejection mediated by alloreactive cytotoxic T lymphocytes and alloantibodies resulting in graft injury. Graft injury causes the release of alloantigens and self-antigens that can in turn be presented via the indirect recognition pathway to generate pathogenic allo and autoreactive cellular and humoral immune responses [45**]. The indirect pathway involves processing the donor alloantigens and/or self-antigens by recipient APCs and presentation to recipient T cells and is believed to be the major pathway for chronic rejection. Once initiated, the indirect alloimmune response can spread to additional determinants within the primary target antigen called intramolecular epitope spreading, or to epitopes on other allogeneic or self-antigens called intermolecular epitope spreading [49].

How alloimmunity leads to loss of tolerance to self-antigens in the transplant setting is not well understood but recent studies implicate alloreactive T cells in this process [36,45**]. Murine skin allograft studies have
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Zhang Q, Reed EF

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Current Opinion in Immunology 2010, 22:1–7

shown that activation of indirectly alloantigen primed T cells can result in determinant spreading and the generation of pathogenic autoreactive T cells [50**]. These findings suggest that the development of humoral responses to autoantigens could result as a consequence of alloimmune-mediated graft damage where repeated exposure of recipient CD4+ T cells to self-antigens surpasses the threshold of self-tolerance and leads to autoimmunity. Although the majority of autoreactive B cells in the periphery are functionally attenuated [51], they can pose a danger in the development of rejection if T cell tolerance is breached permitting T cell helper activation of these autoreactive B cells. Experimental studies suggest that chronic stimulation with autoantigens can break T cell self-tolerance. Tsumiyama et al. [52*] showed that repeated stimulation of CD4+ T cells with self-antigens led to the development of autoantibody-inducing CD4+ T cells. Thus autoimmunity resulted from over-stimulating the host’s immune response by repeated immunization with antigen.

Conclusions

Autoimmunity may be a consequence of alloreactivity induced after solid organ transplantation. Continued efforts to define the non-HLA alloantigens and tissue-specific autoantigens involved in transplant rejection are crucial to understanding the mechanisms and pathogenesis of non-HLA antibodies and development of treatment options.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Acknowledgements

Funding sources: This work was supported by the National Institute of Allergy and Infectious Diseases Grant RO1 AI 42819 and NIH U01AI077821 and the National Heart Lung and Blood Institute Grant RO1 HL 61518 to E.F.R.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest


Using a novel donor specific precursor endothelial cell crossmatch, this multicenter study demonstrated that sensitization to endothelial cell antigens was associated with acute renal allograft rejection.


A large multicenter study shows that presensitization to MICA is associated with renal allograft rejection and poorer allograft survival.
6 Immunogenetics and transplantation


39. Strong (v)-specific responses were associated with substantially increased incidence and severity of BOS. Co(v)-specific responses were dependent on both CD4+ T cells and monocytes and required both IL-17 and the monokines TNF-alpha and IL-1beta.


47. Native lungs treated with anti-MHC class I antibodies showed increased expression of chemokines and chemokine receptors, growth factors and induced IL-17. Antibodies to donor MHC antigens can induce autoimmune mediated by IL-17 that plays a role in chronic lung rejection.


This study shows that self-restricted, autoreactive T cells can be primed via the indirect recognition pathway. While the initial T cell priming is directed at alloantigenic determinants, chronic rejection involves autoreactive T cells.


52. Tsuniyama K, Miyazaki Y, Shiozawa S: Self-organized criticality theory of autoimmunity. PLoS One 2009, 4:e8382. This study demonstrates that naive CD4+ T cells can be activated through repeated antigenic exposure and acquire autoreactivity.