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Special Theme Issue:
NON-HLA ANTIBODIES IN THORACIC TRANSPLANTATION

REVIEW:

Introduction

The clinical impact of non-HLA specific antibodies either alone or in concert with donor HLA specific antibodies (DSA) has been an intense area of research in kidney transplantation and more recently in thoracic transplantation as well. These non-HLA antigens include receptors expressed by the vascular endothelium such as the G protein coupled receptors (GPCRs), Major Histocompatibility Complex Class I Chain-related Gene A (MICA) and antigens expressed on the surface of stressed endothelial cells such as myosin, vimentin, collagen V, and K α 1 tubulin. Of these antigens, commercially distributed reagents are only available for detection of the non-HLA specific antibodies to AT₁R, ET_AR, and MICA. Further, proficiency testing programs are also available for these assays making their implementation in testing for clinical transplantation more applicable. A growing body of evidence supports the role of alloimmune and autoimmune mechanisms involving antibodies directed against non-HLA antigens in transplant allograft damage. This review focuses on some of the most important studies of antibodies specific to non-HLA antigens and their impact on thoracic allograft outcome.

Antibodies to GPCRs: Angiotensin-II type 1 receptor (AT₁R) and endothelin type A receptor (ET_AR)

Several endothelial antigenic targets have been discovered including to the GPCRs, AT₁R and ET_AR. These 7-transmembrane spanning receptors appear particularly relevant due to their cell surface expression on the endothelium and their prominent extracellular regions accessible to antibodies. Newly developed ELISA solid-phase assays have allowed a reliable means of detecting antibodies to these GPCRs. The first case of kidney allograft antibody mediated rejection (AMR) with clinical features similar to preeclampsia and with presence of AT₁R antibodies in the absence of HLA mismatch was reported in 2005 (1). AMR in the absence of DSA and MICA was further associated with the presence of high-binding antibodies to AT₁R in kidney transplant recipients (2). Subsequent studies showed the synergistic effect of strongly binding AT₁R antibodies and HLA class II DSA in kidney transplant recipients with accelerated rejection and hypertensive encephalopathy (3, 4). Recipients having both antibodies to AT₁R and DSA had the worst graft survival (5). Pre-transplant anti-AT₁R antibodies are also a risk factor for long-term graft loss and early AMR in kidney transplant recipients (6).

In heart transplant recipients, cardiac allograft vasculopathy (CAV) is a major factor for morbidity and mortality in long-term graft outcome. Elevated levels of AT₁R and ET_AR have been associated with early onset of microvasculopathy as well as with AMR and cellular rejection (CMR) (7). In a study of 30 cardiac transplant recipients, those with pretransplant high levels of AT₁R and ET_AR antibodies presented more often with CMR (P=0.041), AMR (P=0.0002), and microvasculopathy (P=0.048) than patients without these antibodies at one year posttransplant. The presence of both DSA and non-HLA specific antibodies appeared to increase the risk of heart allograft rejection (8). In 200 heart recipients, freedom from AMR and/or CMR was significantly decreased at two years post-transplant when both de novo DSA and increased AT₁R antibodies levels were considered; the hazard ratio was 7.1 for patients with de novo DSA (P=<0.0002), 2.0 for patients with AT₁R antibody levels >12 (P=0.20), and 10.5 when both de novo DSA and AT₁R antibody levels >12 were considered (P=<0.0001). These two studies in heart transplantation indicate a negative impact of antibodies to the non-HLA antigens AT₁R and ET_AR on heart allograft outcome.

A recent report in lung transplant recipients also indicates worse allograft outcomes when antibodies to both HLA and either AT₁R or ET_AR are present (9). Pre and posttransplant sera from 162 lung recipients transplanted at 3 different centers showed a lower freedom from de novo DSA when the pretransplant antibody status of HLA specific antibodies (HR=1.69) together with either antibodies to AT₁R (HR2.26) or ET_AR (HR=2.38) in the strong binding range were considered. Lower freedom from CMR was observed for recipients with intermediate or strong levels of antibody to AT₁R and ET_AR.

Although antibodies against AT₁R and ET_AR have been shown to be of the IgG1 and IgG3 subclasses, biopsies of AT₁R antibody positive patients with graft dysfunction are frequently of the C4d negative rejection phenotype. Thus, the mode of graft destruction does not appear to be limited to complement-mediated injury but likely includes mechanisms of endothelial damage distinct to non-HLA antibodies which appear to synergize with HLA specific antibodies.

Antibodies to major histocompatibility complex class I chain-related antigens A (MICA)

The polymorphic MICA antigens are encoded within the MHC complex; however, they do not bind beta 2 microglobulin and cannot present peptides. Alloantibodies to MICA have been associated with acute and chronic vascular rejection. A retrospective study with MICA genotyping in donor and recipient and antibodies detection was done on 72 cardiac allograft recipients. A significantly higher proportion of patients developed DSA to MICA antigens amongst those with AMR: 5/19 developed DSA to MICA in patients with AMR versus 1/53 in those without AMR (p<0.01) (10). Non DSA MICA antibodies however were not associated with AMR. In patients with AMR, 11 developed DSA to HLA, 2 developed DSA to HLA and MICA, 3 had donor specific antibodies to MICA alone and 2 developed non-DSA antibodies. CAV-free survival at 2 years was significantly lower in recipients who had DSA to HLA and MICA compared to those without any DSA (p<0.02). The authors concluded that in the absence of HLA specific antibodies, recipients' sera should be tested for the presence of DSA to MICA.

Anti-vimentin antibodies and anti-myosin antibodies

Vimentin is expressed by endothelial cells, vascular smooth muscle cells, activated platelets and apoptotic T cells and neutrophils (11, 12). Myosin is an actin-binding protein part of the cytoskeleton. Development of antibodies targeting these two proteins has been associated with CAV and AMR in heart transplant recipients. Nath DS et al (12) published their findings in 65 heart transplant recipients transplanted <12 months ago and 83 patients transplanted >12 months ago. Ten of the recent transplant patients developed AMR; their anti-myosin and anti-vimentin antibodies titers were nearly 2 and 3 times higher than in patients without AMR. Detection of these antibodies preceded AMR diagnosis by 3.5 and 2 months respectively, and DSA detection preceded non-HLA antibodies by 1.7 and 3 months. In patients >12 months post-transplantation, 14 developed CAV. Anti-myosin and anti-vimentin antibodies titers were approximately 2 and 5 times higher respectively in

patients with CAV versus those without CAV. There was no difference in the antibody titers of patients without CAV regardless of DSA status. However, in patients with CAV, anti-myosin and anti-vimentin titers were significantly higher in the presence of DSA. CD4+ T cells specific to vimentin and myosin secreted predominantly IL-5 in patients with AMR and IL-17 in patients with CAV. Concomitant loss of IL-10 secreting CD4+ T cells was observed in both cases, indicating a breakdown in peripheral tolerance to self-antigens. Pre-transplant anti-vimentin antibody was also tested by another group and no correlation with 1-year rejection in cardiac transplant recipient was found. These results, however do not inform us of the potential pathogenicity of posttransplant anti-vimentin antibody(13).

Anti-collagen V and anti-K α 1 tubulin antibodies

Collagen V (Col V) is an extracellular matrix protein and K α 1 tubulin (K α 1T) is a gap junction protein. Antibodies targeting these two antigens are associated with poor graft outcome after lung transplant in multiple studies. The group from Washington University reported the detection of anti-Col-V and anti-K α 1T antibodies post-lung transplantation in 72/108 recipients (14). Fifty-four of them were treated for presence of DSA with Rituximab \pm IVIg; those who cleared their DSA but not their anti-Col-V and anti- K α 1T antibodies were significantly more likely to develop bronchiolitis obliterans syndrome (BOS) than those who cleared both their DSA and their anti-Col-V and anti- K α 1T antibodies. The same group published their observations in 2013 on pre-transplant antibodies to collagen-I (Col-I), Col-V and K α 1T in 317 lung transplant recipients (15). Antibodies against Col-I, Col-V and K α 1T were detected in 18 to 34% of patients pre-transplantation depending on their baseline lung disease, compared to only 3% of normal volunteers. The presence of these antibodies pre-transplantation significantly increased the odds of primary graft dysfunction by 7 and of BOS by 20. Trend for increased odds of DSA development in patients with these auto antibodies was observed, but it reached statistical significance only in cystic fibrosis patients (OR 3.3, 95% CI 1.0-11.2, p=0.046).

Non-HLA IgM antibodies

Pre-formed IgM reacting antibodies detected against a panel of leukocytes but without any HLA specificity can be detected as a positive real-time complement dependent cytotoxicity (CDC) crossmatch. The CDC crossmatch is reduced to negative after serum treatment with dithiothreitol (DTT) to break up the IgM disulfide bonds. Most centers will not consider these IgM antibodies as significant and their presence will not preclude utilization of the organ. Smith et al. (16) challenged this concept as they found an increased risk of early cardiac graft failure in patients with pre-transplant non-HLA IgM. Fifty-nine of 616 recipients had pre-transplant non-HLA IgM, defined as: positive reaction by CDC tests against a panel of healthy volunteers peripheral blood mononuclear cells, no reactivity against mononuclear cells from patients with chronic B-cell lymphocytic leukemia and absence of HLA antibodies as determined by Luminex technology. Allograft survival was significantly lower in patients with non-HLA IgM than for those without any antibodies (respectively 1 year survival 55.9% versus 75.8 % and 10 year survival 43.3% versus 52.8%, p = 0.0085). In multivariate analysis, presence of non-HLA IgM persisted as a significant predictor of graft failure with a hazard ratio of 2.36 (p=0.0002). None of the patients with non-HLA IgM fulfilled AMR criteria but C4d+ biopsies were positive in three of those who died from primary graft dysfunction.

Antinuclear antibodies

Win et al. (17) used a chronic rejection mouse model of cardiac heterotopic transplantation to demonstrate the association of antinuclear antibodies and CAV. Donor mouse grafts differing from the recipient by only 3 amino acid residues in the MHC class II 1A antigen were slowly rejected with patchy inflammatory infiltrates and pathognomonic changes of humoral rejection including C4d and IgG deposition in majority of transplanted hearts. The explanted hearts also showed obliterative vasculopathy resembling closely to human CAV. No alloantibody was detected by flow cytometry in any recipient but they all showed strong antinuclear antibody responses. When using T cells deficient donors, no antinuclear antibody was detected. These grafts presented

the same time to rejection with similar amount of lymphocyte infiltrates as when using donor with preserved T cell function. However, they presented less severe vasculopathy and no finding of humoral rejection, indicating the critical role of passenger donor CD4+ T cells in the production of non-MHC antibody by the recipient. In a second experiment, recipients lacking MHC class II expression on B cells were transplanted with the same donor mice model with preserved T cells function and again no antinuclear antibody was detected. Passive transfer of autoantibody to these recipient mice did not induced vasculopathy or humoral rejection, showing the importance of recipient B cells in autoantibody production and graft induced damage. The authors concluded that donor CD4+ T cell passengers in the cardiac graft provide help for autoantibodies production via allorecognition of MHC class II on the recipient B cells and these autoantibodies contribute to humoral rejection and graft vasculopathy. No further typing of the antinuclear autoantibodies was provided but the authors reported a wide variation in the observed pattern of antinuclear antibodies between each animal, suggesting an antibody response targeting multiple antigens.

Conclusion

Are non-HLA antibodies really effectors in post-transplant graft damage or only nonpathogenic marker of immunologic activation? Growing number of studies now demonstrate an active participation of some non-HLA antibodies in premature graft deterioration. This area is still a relatively unknown field in solid-organ transplantation immunology but it is reasonable to expect that further investigations will allow the prevention of posttransplant immune complication. The studies presented here show the importance of determining the patient's immunologic risk by assessing the present of DSA and non-HLA specific antibodies. The mechanisms of endothelial cell damage by DSA to HLA and non-HLA antibodies needs to be investigated to distinguish similar and dissimilar modes of action and corresponding signaling pathways to allow for identification of therapeutic intervention aimed at protecting the endothelium before irreversible antibody mediated injury occurs.

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