Non-HLA Antibodies in Renal Transplantation, Where Do We Stand?

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Abstract

Objective: This review discusses current findings in the subject and addresses the clinical relevance of Non-HLA antibodies.

Methods: This traditional narrative review used PubMed and Medline searches for English language reports on Non HLA abs during last 20 years. The search included the key words: non-human leukocyte; antibodies; kidney; transplantation.

Results: 65 related articles and review were found.

Conclusion: Non-HLA immunity is associated with poor graft survival, rejection and chronic graft loss. Moreover, they could be used as biomarkers of ongoing immune response and as predictors of graft failure.

Keywords: Non-human leukocyte; Antibodies; Kidney; Transplantation

Abbreviations: Abs: Antibodies; AEPCA: Anti-Endothelial Precursor Cell Antibodies; AECA: Anti-Endothelial cell Antibodies; AMR: Antibody-Mediated Rejection; Anti-Col: Anti-Collagens; AT1R: Angiotensin II Type 1 Receptor; Anti-Ka1 tubulin: Anti-Tubulin ; AVA: Anti-Vimentin Antibody; C4D: Complement Component 4d; DSA: Donor-Specific Antibodies; EC: Endothelial Cell; ELISA: Enzyme-Linked Immunosorbent Assay; ETAR: Endothelin-1 Type A Receptor; FSGS: Focal Segmental Glomerulosclerosis; HLA: Human Leukocyte Antigen; MHC: Major Histocompatibility Complex; MICA: Major Histocompatibility Complex Class I Chain-Related Gene A; Non-HLA abs: Non-Human Leukocyte antibodies; XM: Crossmatch

Introduction

Non-HLA antigens include antigens expressed on endothelial, epithelial cells, parenchymal cells and circulating immune cells [1-3]. Non HLA abs can be directed against auto- or allo-antigens and be either present pre-transplant or de novo formed post transplantation [3]. Furthermore, The most reported Non-HLA abs include those directed against Angiotensin II Type 1 Receptor (AT1R-Ab), Endothelin Type A Receptor (ETAR), MHC Class I Chain-Related Antigen A (MICA-Ab), Vimentin (AVA), Tubulin (anti-Ka1 tubulin), Collagens (anti-Col) Anti Endothelial Cell Antibodies (AECA), anti-heat shock protein, and anti-phospholipid (Table 1) [4,5].

Moreover, the triggers of activation or transition of these Non-HLA abs toward pathogenicity are likely acute rejection, hyperperfusion, ischemia reperfusion, calcineurin toxicity, infection, and recurrent diseases [6].

Non-HLA abs have a stronger role in graft dysfunction and rejection; Antibody-Mediated Rejection (AMR) or C4d deposition in the absence of circulating donor specific Non-HLA abs than previously thought [1,5-7]. The aim of this review is to shed light on Non-HLA abs development, mechanism of action, clinical relevance, and treatment.

Mechanism of NON-HLA antibodies production

Injury of graft endothelium by Non HLA abs can lead to exposure of neo-antigens which consecutively stimulate the production of antibodies against non-HLA antigens [1,4,5,7-9]. Furthermore, Cytokine storm during brain death and inflammation associated with an ischemia–reperfusion injury, vascular injury, and/or rejection may cause increased expression of cryptic autoantigens, and may stimulate Non-HLA abs production. Additionally, immune activation, tapered immunosuppression in transplant recipients may stimulate Non HLA abs production [10].

However, several studies reported other ways of Non-HLA abs development other than sensitization [1,11-13]. For example, an A5.1 mutation in the donor, which is related to the MICA*008 allele, is associated with a strongly increased MICA expression on donor endothelial cells compared to wild type donors and therefore these mutated MICA molecules are important targets for antibody formation [14]. Additionally, mismatching on certain amino acid residues leads to increased MICA antibody formation and it can be that based on the 3D-structure of MICA, these structures are more accessible for antibodies [1,13].

HLA antibodies and Non-HLA antibodies correlation

Conversely, inflammatory response induced by Non-HLA abs could sequentially upregulate HLA expression, increase the risk for a patient to develop HLA-specific antibodies and thus make the allograft more susceptible to an allo-immune response involving both humoral and cellular [1,2,4,5,8,15].

Numerous studies showed that patients with both HLA and non-HLA antibodies had lower graft survival rates compared to patients with either one of them [16]. It is assumed that HLA and non-HLA

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Non-HLA antibodies have a synergistic effect [4,15].

Non-HLA antibodies incidence and pathogenicity

Non-HLA abs may function as complement- and non-complement-fixing antibodies and they may induce a large variety of allograft injuries, reflecting the complexity of their acute and chronic actions [17].

Complement-dependent and complement-independent mechanisms are not mutually exclusive [8,18]. For example, Anti-Vimentin Antibodies (AVA) seem to fix complement [19]. Similarly, MICA Abs have been shown to be more efficient at complement activation and have been associated with C4d * AMR [2]. In contrast, 40%-50% of cases with severe vascular changes such as fibrinoid necrosis are C4d-negative, implicating involvement of either non-complement-fixing antibodies or other mediators, as noticed in cases of AMR in the presence of AT1R-Ab or AECA that occurred without evidence of complement activation [2, 20,21].

Besides, antibodies can induce lysis of target-cells with membrane bound antibodies through activation of natural-killer cells, a process called antibody-dependent cell mediated cytotoxicity [1,22]. Furthermore, Non-HLA abs may also contribute to short and long-term injury may depend on their specificity and affinity, density of the target antigens (78%). The compartment specificity of selected antibodies was confirmed by IHC [7].

Compartment specificity

Non-HLA immune responses, including anti-MICA antibodies, were detected against kidney compartment-specific antigens, with highest post-transplant recognition for renal pelvis and cortex specific antigens (78%). The compartment specificity of selected antibodies was confirmed by IHC [7].

Table 2: Non-HLA antibodies incidence and mechanism of action.

<table>
<thead>
<tr>
<th>Antibody Incidence and Mechanism of Action</th>
<th>Antibody</th>
<th>Incidence</th>
<th>Mechanism of Action</th>
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<tbody>
<tr>
<td>Major histocompatibility complex class I chain-related gene A (MICA)</td>
<td>*13.9% and 5.4% pre and post-transplant, respectively [25].</td>
<td>*Complement-activating antibodies (fix C1q) [2,23].</td>
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<tr>
<td>*Activate NK cell via MICA/NKG2D interactions with subsequent cytotoxic proteins and IFN-γ release [26].</td>
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<td>Angiotensin II type 1 receptor (AT1R)</td>
<td>*22% [27], 23% [28], 47%-59% [29] and 59% [30] using a cutoff ≥ 9 units/ml.</td>
<td>*Activate complement independent pathways. In addition, increased tissue factor expression and thrombotic occlusion [18,23].</td>
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<td>*Induce Erk1/2 signal transduction cascade that directly affect endothelial and vascular smooth muscle cells [23].</td>
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<td>*Increase DNA binding activity of NF-B transcription factor, and increase expression of NF-B proinflammatory target genes such as chemokines 1 and RANTES [23].</td>
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<td>Endothelin-1 type A receptor (ETAR)</td>
<td>*23 % [1,7].</td>
<td>*Damaging endothelial cells and increasing downstream effectors of GPCR signaling.</td>
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<td>*Activate endothelial cell and produce of inflammatory cytokines [2,3].</td>
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<td>*Increase endothelial cell injury, which may explain the severity of antibody-mediated injury in recipients when both AECA and HLA-DSA were detected [2].</td>
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<tr>
<td>*Lead to AMR by activating complement [34].</td>
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<tr>
<td>Antiendothelial cell antibodies (AECA)</td>
<td>*Increase DNA binding activity of NF-B transcription factor, and increase expression and thrombotic occlusion [18,23].</td>
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<td>*Activate complement independent pathways. In addition, increased tissue factor expression and thrombotic occlusion [18,23].</td>
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<tr>
<td>LG3 (Perlecan)</td>
<td>*Increase DNA binding activity of NF-B transcription factor, and increase expression and thrombotic occlusion [18,23].</td>
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<tr>
<td>*Activate endothelial cell via induction of downstream proinflammatory signaling pathways [36].</td>
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<tr>
<td>Intercellular adhesion molecule 4 (ICAM4)</td>
<td>*Increase DNA binding activity of NF-B transcription factor, and increase expression and thrombotic occlusion [18,23].</td>
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<tr>
<td>*Activate endothelial cell via induction of downstream proinflammatory signaling pathways [36].</td>
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<tr>
<td>Anti-GBM</td>
<td>*Targeting perlecan via proteolysis and degradation of perlecan induce profound changes in its biological activity [37].</td>
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</table>

Table 1: Targets for Non-HLA Antibodies.

- Major histocompatibility complex class I chain-related gene: A (MICA), B (MICB)
- Antibodies against G Protein-Coupled Receptors (GPCRs): AT1R (Angiotensin II type 1 receptor) and Endothelin-1 type A receptor (ETAR)
- Antiendothelial cell antibodies (AECA)
- Anti endothelial precursor cell antibodies (AECPCA)
- LG3 (Perlecan)
- Intercellular adhesion molecule 4 (ICAM4)
- Anti-GBM Glomerular basement membrane proteins and IFN-γ release [26].
- Anti-GBM Glomerular basement membrane proteins and IFN-γ release [26].
Clinical Relevance of Non-HLA antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clinical Relevance</th>
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<tr>
<td>Antivimentin (AVA)</td>
<td>*Expression increases during rejection [2].</td>
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<td>*Post-transplant development of IgG AVA was a risk factor associated with chronic injury such as interstitial fibrosis and tubular atrophy [27, 40-41].</td>
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<tr>
<td>Major histocompatibility complex class I chain-related gene: A (MICA)</td>
<td>*Correlated with rejection (acute and chronic) and poor allograft survival (only significant in low immunological risk transplantations: well matched for the HLA) [1,7,42,44].</td>
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<td>* Contrary to expectations, patients with positive pretransplant MICA antibodies had superior death-censored renal allograft survival when compared with MICA-negative patients [1].</td>
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<tr>
<td>Anti-endothelial precursor cell antibodies (AEPCA)</td>
<td>*Strongly associated with acute rejections and increased serum creatinine levels at 3 and 6 months post-Tx [45].</td>
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<td>Angiotensin II type 1 receptor (AT1R)</td>
<td>*Associated with a higher incidence of graft loss [1,28,49]. severe rejection (chronic and acute rejection (AMR and cellular mediated) and malignant hypertension [7,27-29,46].</td>
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<td>*Patients with both AT1R-Ab and HLA-DSA had greater incidence of allograft damage and graft loss [29,46-47].</td>
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<td>*Patients with anti-AT1R-Ab level &gt;9 U/ml run a higher risk of graft failure independently of classical immunological risk factors [28].</td>
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<td>*Patients with both anti-AT1R and DSA had lower graft survival than those with DSA alone [48].</td>
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<td>Endothelin-1 type A receptor (ETAR)</td>
<td>*Associated with a higher incidence of graft loss and rejection during the first post-transplant year [1,5,49].</td>
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<td>*Vasculopathy or arteritis were observed in patients with anti-ETAR ≥ 2.5 U/ml (p=0.0275) [9].</td>
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<tr>
<td>Duffy antibody (a chemokine receptor)</td>
<td>*Associated with chronic renal allograft histological injury [7].</td>
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<tr>
<td>Agrin antibody</td>
<td>*Associated with transplant glomerulopathy [7].</td>
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<tr>
<td>fibronectin and collagen IV antibodies</td>
<td>*A significant risk factor for development of transplant glomerulopathy, a chronic lesion characterized by duplication of glomerular basement [27,50].</td>
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<tr>
<td>Antiendothelial cell antibodies (AECA)</td>
<td>*AECAs are a risk marker for acute rejection [51]</td>
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<td></td>
<td>*associate with both severe rejection (cellular mediated rejection and (AMR)) in kidney transplant recipients [2,52].</td>
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<td></td>
<td>*high prevalence of C4d negative microcirculation injury [53].</td>
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</table>

Table 3: Clinical relevance of non-HLA antibodies.

Non-HLA antibodies monitoring and graft failure prediction

Many of the late graft failures attributable to non-HLA effects might be preventable [39]. The possibility of identifying recipients at increased risk of late graft loss before transplantation could be used to fashion specific immunosuppressive strategies for these patients [39-54]. For instance, the detection of anti-AT1R Abs seems to be a complementary risk factor for the identification of patients with higher immunological risk. Moreover, Banasik et al. proved that the occurrence of pre-transplant anti-AT1R Abs > 9 U/ml is an independent risk factor for graft failure [28,29]. Therefore, monitoring for Non-HLA abs should mirror that performed for HLA-DSA to identify those high risk patients [2].

Other possible uses of Non-HLA antibodies

Pre-transplant auto-antibody titers could have implications in terms of organ allocation. For instance, avoid use of organs with expected long cold ischemic time or coming from a donor after cardiocirculatory arrest for patients with elevated pre-transplant autoantibody titers [10].

Furthermore, Pre-transplant 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 [10]. For example, Pre-transplantation screening of recipients for AT1R-Abs may help to improve individual risk assessment and offer patients with AT1R-Abs preemptive specific treatment. Unfortunately, early AMR due to non-HLA antibodies is rare and seems difficult to predict by currently available assays including the AT1R-Ab-ELISA [53].

Who should be tested for Non-HLA antibodies?

Philogene et al. suggested performing pre-transplant Non HLA abs testing and post-transplant monitoring for high risk group of patients [2]. The risk factors include re-transplanted, male gender, young age, and those with FSGS at time of transplantation were positive for AT1R-Abs and AECA prior to transplantation [2]. Furthermore, testing for non-HLA antibodies is often performed when histological evidence suggests an antibody mediated process in the absence of HLA-DSA [2].

Non-HLA abs and Pediatric age group

Chaudhuri et al. reported that 24% of children with renal transplant have de novo antibodies, mostly directed against HLA. 6% of de novo antibodies were DSA Ab and 6% anti MHC class 1 related chain A (MICA), and were equally found either on steroid-free or steroid-based regimens. The presence of anti HLA and anti-MICA Ab was significantly associated with acute and chronic rejection with faster graft loss [54].

Interestingly, Matthew et al. reported a case of hyperacute rejection in 17 month old boy due to non-HLA antibodies. Pre-transplant Single antigen testing confirmed the absence of Donor Specific HLA Abs (DSA). Moreover, initial flow cross matches and 2 days post-Txp HLA-DSA were negative. Pre-Txp (pre-14 days) and post-Txp (post-24 days) samples were sent out for AT1R Abs screening and donor specific endothelial cell crossmatch (XM-One). The XM-One assay using endothelial precursors isolated from the donor as targets was strongly positive using a pre-Txp serum but negative using post-Txp serum. Approximately two month's post-Txp, the patient developed HLA Abs, on top of the AT1R antibodies [55].

Detection of Non-HLA Antibody

Considering the technical difficulties of current Non-HLA abs assays and the large variation in reported incidences of antibodies even with the same assays, continuous efforts to develop reliable and sensitive diagnostic tests are essential. Besides, measuring a panel of antibodies instead of one antibody at a time will provide valuable information regarding the role of Non-HLA abs in rejection and could eventually help identifying different risk profiles for rejection and impaired graft survival [1].

Currently, Non-HLA abs can be reliably detected by solid-phase assays (antibodies targeting G protein-coupled receptors (angiotensin type 1 receptor), MICA, collagen-V, vimentin), immunofluorescence
used as biomarkers of ongoing immune response and as predictors of survival, rejection and chronic graft loss. Moreover, they could be recognized. Non-HLA immunity is associated with poor graft outcomes. Angiotensin receptor blockers such as losartan have also been used to block the activity of angiotensin receptor in patients with AT1R-Ab-mediated rejection [54,57]. However, a more recent study shows that chronic use of losartan can upregulate AT1R expression resulting in worse outcomes [58].

**Conclusions**

The role of Non-HLA abs in renal transplantation is progressively being recognized. Non-HLA immunity is associated with poor graft survival, rejection and chronic graft loss. Moreover, they could be used as biomarkers of ongoing immune response and as predictors of graft failure. Therefore, they may herald the need for more suitable immunosuppression. Strong efforts to investigate Non HLA abs and their effect on graft outcome are still ongoing.

**Disclosure**

The author declared no competing interests.

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