Introduction
The development of post-transplantation antibodies against non-human leukocyte antigens (non-HLA) is associated with rejection and decreased long-term graft survival.

The principal targets of the humoral immune-response to allograft are highly polymorphic HLA antigens, but several studies have also documented the role of non-HLA antibodies, either in the process of antibody mediated rejection (AMR) or in long-term graft dysfunction. The occurrence of AMR in recipients of renal transplants from HLA identical siblings (1) is the most convincing evidence of the role of non-HLA antibodies. Two other multicenter studies (2, 3) documented the role of non-HLA immunity to allografts in chronic rejection. In this review, we will provide a classification of these non-HLA antibodies, identify of non-HLA antibodies that are frequently involved, and discuss the mechanisms involved in antibody production, the interplay between alloimmunity and autoimmunity, and the mechanisms of non-HLA antibody mediated graft damage.

Classification of Non-HLA Antibodies Involved in Transplantation

Alloantibodies Directed Against Polymorphic Antigens
These antigens differ between the recipient and donor. The major histocompatibility complex class I chain related gene A (MICA) is a relevant example of these antibodies (4).

Keywords: non-HLA antibodies; MICA antibodies, preformed antibodies, anti-endothelial cells antibodies, antibodies against G protein-coupled receptors, natural antibodies.
Antibodies that Recognize Self-Antigens (Auto Antibodies)

As the vasculature is the interface between the recipient immune system and the transplanted organ, a relevant proportion of the non-HLA antibodies recognize auto-antigens expressed by endothelial cells (5). These anti-endothelial cells antibodies (AECA) include the vast majority of the non-HLA auto-antibodies. AECA have been frequently reported to be higher in renal recipients with failed transplants (6) and in pre-transplant sera from HLA-sensitized renal transplant candidates (7). Several studies document that recipients from deceased donors have higher levels of non-HLA antibodies than those from living donors (8).

Identification of Most Frequently Involved Non-HLA Antibodies

The most frequently involved non-HLA antibodies are represented in table 1.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Organ</th>
<th>Reference</th>
<th>N° of Patients</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>MICA</td>
<td>Kidney</td>
<td>4</td>
<td>1910</td>
<td>MICA antibodies are associated with increased graft loss</td>
</tr>
<tr>
<td>MICA</td>
<td>Heart</td>
<td>16</td>
<td>44</td>
<td>MICA antibodies precede acute rejection</td>
</tr>
<tr>
<td>Angiotensin II type 1 receptor</td>
<td>Kidney</td>
<td>20</td>
<td>33</td>
<td>AT₁R detected in 16 out of 33 patients with acute rejection</td>
</tr>
<tr>
<td>AT₁R</td>
<td>Heart</td>
<td>21</td>
<td>200</td>
<td>Co-presence of AT₁R and anti HLA antibodies increases the rate of AMR</td>
</tr>
<tr>
<td>Endothelin-1 type A (ET₁R)</td>
<td>Heart</td>
<td>30</td>
<td>30</td>
<td>Increased levels of ET₁R and AT₁R are associated with acute rejection</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Kidney</td>
<td>40</td>
<td>70</td>
<td>Vimentin IgG are elevated in patients with IF/TA</td>
</tr>
<tr>
<td>Myosin</td>
<td>Heart</td>
<td>41</td>
<td>72</td>
<td>Anti-myosin antibodies are associated with CAV</td>
</tr>
<tr>
<td>LG3 (perlecan)</td>
<td>Kidney</td>
<td>45</td>
<td>60</td>
<td>Antibodies anti LG3 are present in patients with AMR</td>
</tr>
<tr>
<td>Other non-HLA endothelial cell antigens</td>
<td>Kidney</td>
<td>51</td>
<td>150</td>
<td>Antibodies against endoglin, Fms-like tyrosine kinase-3 ligand, discoidin I-like domains 3 and ICAM 4 are associated with graft dysfunction</td>
</tr>
</tbody>
</table>

MICA: Major histocompatibility complex class I chain related gene A; AT₁R: Angiotensin II type 1 receptor; AMR: antibody-mediated rejection; ET₁R: Endothelin-1 type A receptor; IF: interstitial fibrosis; TA: tubular atrophy; AMR: antibody-mediated rejection; ET₁R: Endothelin-1 type A receptor; IF: interstitial fibrosis; TA: tubular atrophy; LG3: laminin globular 3; ICAM 4: intercellular adhesion molecule 4

Antibodies to Mica

The MICA protein, which shares a similar structure with HLA class I but does not associate with β2 microglobulin, is highly polymorphic with approximately 100 alleles identified by July 2016. The recipient’s immune system may develop antibodies against donor specific MICA alleles (12). The contribution of MICA antibodies to AMR pathogenesis was demonstrated by Zou et al (4). This study was debated by other authors (13-15). Later, the relevance of MICA antibodies in heart and pancreas transplantation was also documented (16, 17).

Natural Antibodies (Nabs)

Natural antibodies (Nabs) are most often commonly defined as immunoglobulins present in the absence of exogenous antigen stimulation (9). In many cases, they provide immediate protection against infections. Recent studies (10, 11) have documented that natural polyreactive serum antibodies correlate with AMR of kidney grafts, the pre-transplant serum Nabs are associated with late kidney graft loss and that post-transplant Nabs are associated with kidney allograft injury and a reduced long term survival. These antibodies may be directed against foreign proteins as well as against self-molecules (auto antibodies). Due to their particular nature, they will be treated separately at the end of the next chapter.

Table 1. Non-human leukocyte antigen (HLA) antibodies in solid organ transplantation

Antibodies to Mica

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Relevance of Non-HLA Antibodies in Transplantation

such as tumor necrosis factor alpha (TNFα) (18). This up regulation is mediated by NFkB, and, subsequently, MICA can activate natural killer (NK) cells via the interaction with the immunoreceptor NKG2D , (19) which is a glycoprotein activating receptor expressed on NK cells.

**Auto Antibodies Against G Protein-Coupled Receptors (GPCRs)**

Angiotensin II type I receptor (AT₁R) and endothelin type A receptor (ETₐR) belong to the GPCR family, which has seven transmembrane domains. Antibodies to the GPCRs may be important due to their endothelial cell surface expression accessibility to antibodies.

After the first description of 33 patients with steroid-refractory rejection and with antibodies against AT₁R in the blood (20), several studies (21-32) have documented the association of serum AT₁R antibodies and AMR or abnormal renal or heart function (Table 2). In one study (30) concerning heart transplantation, high ETₐR antibody levels were associated with AMR.

**Table 2. AT₁R antibodies in human organ transplantation**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Time of Ab detection</th>
<th>Key findings</th>
<th>Sinergy with HLA-DSA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney recipients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33 patients with steroid-refractory rejection</td>
<td>Post-Tx</td>
<td>Biopsy samples not showing C4d. Treatment with plasmapheresis, IVIG and losartan improved graft survival.</td>
<td>NA</td>
<td>20</td>
</tr>
<tr>
<td>63 patients with no HLA-DSA or MICA-DSA</td>
<td>Pre-Tx and post-Tx</td>
<td>AT₁R Ab associated with AMR.</td>
<td>NA</td>
<td>21</td>
</tr>
<tr>
<td>-134 patients with abnormal biopsies-217 control patients</td>
<td>Pre-Tx and post-Tx</td>
<td>AT₁R Ab associated with abnormal biopsies samples.</td>
<td>Yes</td>
<td>22</td>
</tr>
<tr>
<td>-283 AT₁R Ab+ patients-316 AT₁R Ab- patients</td>
<td>Pre-Tx</td>
<td>Preformed AT₁R Ab associated with increased AR.</td>
<td>NA</td>
<td>23</td>
</tr>
<tr>
<td>-7 AT₁R Ab+ patients-72 AT₁R Ab- patients</td>
<td>Pre-Tx</td>
<td>AT₁R Ab associated with increased AMR</td>
<td>NA</td>
<td>24</td>
</tr>
<tr>
<td>11 patients with AR and no HLA-DSA</td>
<td>Pre-Tx and post-Tx</td>
<td>10 of 11 patients had no C4d deposition</td>
<td>NA</td>
<td>26</td>
</tr>
<tr>
<td>12 patients with AMR and no HLA-DSA</td>
<td>Pre-Tx and post-Tx</td>
<td>AT₁R Ab increased the risk of AMR</td>
<td>NA</td>
<td>27</td>
</tr>
<tr>
<td>-98 AT₁R Ab+ patients-64 AT₁R Ab- patients</td>
<td>Pre-Tx</td>
<td>Pre-Tx AT₁R Ab associated with increased AR</td>
<td>NA</td>
<td>28</td>
</tr>
<tr>
<td>Pediatric recipients:10auto-Ab+; 10 auto-Ab-</td>
<td>Pre-Tx and post-TX</td>
<td>50% of patients developed de novo AT₁R Abs; they did not correlate with graft function</td>
<td>NA</td>
<td>29</td>
</tr>
</tbody>
</table>

| Heart recipients | | | | |
| -14 AT₁R Ab+ patients -16 AT₁R Ab- patients | Pre-Tx and post-Tx | Pre and post-Tx AT₁R Ab associated with increased AMR | NA | 30 |
| -76 AT₁R Ab+ patients -124 AT₁R Ab- patients | Pre-Tx and post-Tx | Pre and post-Tx AT₁R Ab did not correlate with AMR | Yes | 31 |
| Patients with AT₁R with LVAD | Pre-Tx | 3 of 5 patients with primary graft dysfunction had AT₁R Ab | NA | 32 |

Abbreviations: Ab, antibody; AT₁R: angiotensin II type 1 receptor; ACR: acute cellular rejection; AMR: antibody mediated rejection; CAV: coronary artery vasculopathy; C4d: complement degradation product; DSA: donor specific antibody; IVIG: intravenous immunoglobulin; LVAD: left ventricular assist device; NA: not assessed; Tx: transplant
Many of the cited studies documented the co-presence and the synergistic effect of AT, R and HLA-donor specific antibodies (HLA-DSA). This fact represents an intersection of alloimmune and autoimmune responses (33, 34). Tissue inflammation inflicted by anti-HLA antibodies may result in exposure and loss of tolerance to auto antigens (35).

Ischemia reperfusion injury (IRI) also may induce auto antibody production. Indeed, IRI may unmask neoepitopes that, once exposed, can serve as auto antibody targets (36, 37).

AT, R antibodies are principally represented by IgG1 and IgG3 isotypes and can activate complement, but, C4d deposition is not found in several patients. In such cases, antibodies against AT, R can trigger ERK kinase phosphorylation and activate the transcription factors AP-1 and NFkB on endothelial and smooth cells (20).

Anti-Vimentin Antibodies

Production of anti-vimentin antibodies is common after solid organ transplantation. Recent evidence demonstrates that cell surface expression of vimentin is not unusual. Vimentin is also secreted by macrophages, endothelial cells, activated platelets and neutrophils (38). Nath et al (39) reported higher anti-vimentin antibody levels in patients who develop cardiac allograft vasculopathy (CAV). A study by Basarani et al (40) suggested that IgG anti-vimentin antibodies have a role in determining interstitial fibrosis and tubular atrophy in renal transplant patients.

Anti-Myosin Antibodies

The presence of myosin antibodies has been associated with AMR and CAV in heart transplantation. In a study by Kalache et al (41), the detection of either cardiac myosin (CM) reactive T cells or anti-CM antibodies was highly indicative of CAV. Three hundred single nucleotide polymorphisms have been identified in the myosin motor domain of cardiac myosin. Additionally, several mutations have been recognized in the region head (S1) and in the myosin tail (S2) and light meromyosin (LMM). This fact causes that in general the myosin antibodies detected in the recipients are donor specific and not auto antibodies (42). The presence of donor HLA specific antibodies in these patients precedes the detection of myosin and vimentin antibodies. This fact again suggests the interplay of allo and autoimmune responses (39).

Anti-Perlecan Antibodies

Perlecan, a large heparin sulphate proteoglycan, is a major component of the vessel wall. The C-terminal domain of perlecan, endorepellin, has antiangiogenic properties and contains three laminin like globular domains (LG) separated by two sets of epidermal growth factor (EGF)-like repeats. The antiangiogenic properties are linked to the LG3 domains. Apoptotic endothelial cells liberate cathepsin L, which cleaves the LG3 domain from the terminal fragment of perlecan (43). An important question, is whether LG3 also behaves as a neoantigen that drives the production of LG3 antibodies. Studies have shown that vascular injury promptly releases apoptotic exosome-like vesicles, which trigger the production of antibodies against LG3 (44).

Cardinal et al (45) and Soulez et al (46) reported that pre- or post-transplant anti-LG3 antibodies are related to AMR and graft dysfunction. In addition, several studies (45) reported that HLA-DSA and LG3 auto antibodies can synergize to elicit endothelial cell and graft damage. This damage may be caused in two ways. LG3 may cause vascular injury and neointimal formation by stimulating autoantibody production and/or by promoting the migration of vascular smooth muscle cells (vSMCs).

Anti bodies against Collagen V and Ko1tubulin.

Collagen V is present on airway epithelial cells and the clinical relevance of collagen auto antibodies has been documented in lung transplantation. Post-transplantation development of collagen V antibodies is associated with the development of bronchiolitis obliterans syndrome (BOS) (47). Similar to collagen V, ko1 tubulin was found to be an antigen capable of inducing humoral responses that facilitate BOS.

A recent study that evaluated the incidence of col V and anti-ko1 tubulin antibodies surprisingly demonstrated the common presence of both antibodies in lung transplant recipients (48).

In addition, antibodies against col V and ko1 tubulin may also be present in cardiac transplant recipients with AMR and/or CAV (49). Interestingly, in the above-mentioned study (48), almost all patients who had antibodies against HLA-DSA also had antibodies against col V and ko1 tubulin documenting once again the interrelationship between allo and auto immunity.
Antibodies against Col IV and Fibronectin

The development of auto antibodies against collagen IV and fibronectin has been reported in renal transplant patients with transplant glomerulopathy (TG) (50). These patients had an increased frequency of self Ag specific interferon gamma (IFNγ) and IL-17 cells with reduced IL-10, which demonstrates a tolerance break down to self Ag, which may have a role in the pathogenesis of TG.

Other Non-HLA Antibodies

As aforementioned, the endothelium lines the interface between the graft and the recipient. Antigens expressed by these cells are the first line targets of the recipient immune system. All of these antibodies belong to the AEC class (5). The most frequently involved have already been described. Recently, Jackson et al (51) described four new AECAs in 24% of pre-transplant sera that were associated with post-transplant donor specific HLA antibodies, AMR and transplant glomerulopathy. In vitro, AECAs stimulated endothelial cell cultures increased adhesion molecule expression and of inflammatory cytokines productions, which activated the vascular endothelium, amplified the alloimmune response and increased micro vascular damage.

Endoglin is a membrane glycoprotein primarily expressed on vascular endothelium. The importance of endoglin in IRI is sustained by the fact that the reduction of endoglin expression by gene disruption or by neutralizing antibodies reduces angiogenesis and revascularization (52).

Fms-like tyrosine kinase-3 (FLT3) is a receptor tyrosine kinase that regulates cell differentiation, survival and proliferation. Its stimulation can increase CCL5 production by renal endothelial cells and increased mononuclear cells recruitments (53).

Discoidin I-like domains 3 (EDIL3) is secreted by endothelial cells and is associated with extracellular matrix. EDIL3 deficiency or antibody blockade increases LFA-1 dependent leukocyte adhesion (54).

Intercellular adhesion molecule 4 (ICAM4) is a transmembrane protein that mediates binding of leukocytes via its interaction with integrins. Anti-ICAM4 antibodies are associated with AMR and reduced graft survival (51).

Natural Antibodies

As mentioned, natural antibodies (Nabs) are defined as immunoglobulins that are present in the absence of exogenous antigen stimulation. Nabs provide immediate protections from infections. Beyond this, Nabs have been shown to play different roles in the immune system, such as contributing to apoptosis debris clearance, suppression of allergic responses, regulation of B cell responses and development, protection from cancer and atherosclerosis (9). Nabs are often present at birth, and are often polyreactive antibodies by their ability to bind to multiple different ligands, including self-antigens (10). Polyreactive antibodies have been shown to react with a wide variety of antigens, including nucleic acids, carbohydrates, proteins and lipids (55).

Polyreactive antibodies, mostly IgM, are principally present in healthy individuals, but aberrant levels of IgGNAbs are detected in various autoimmune diseases (56) and in transplantation. Porcharay et al. (57) detected Nab IgG in the serum of kidney graft patients with AMR. A study from Gao et al. (58) observed higher levels of IgGNAbs in the sera of pre-transplant sera. An important characteristic of these natural antibodies found in the sera of uremic or pre-transplant patients is their capacity to react to apoptotic cells, as observed by others (59, 60). It is still unclear as to what antigens are recognized by these antibodies at the surface of the apoptotic cells. In the study by Gao et al (58) two polyreactive antibodies bound either to phosphatidylserine and lysophosphatidylcholine: both antigens are exposed at the surface of apoptotic cells.

The relevance of the Gao study is that the survival analysis revealed that patients with high pre-transplant IgG reactivity to apoptotic cells had a worse post-transplant outcome.

The origin of these polyreactive Nabs is not clear. They may be present in the sera independently or before transplantation. After transplantation, IRI may have a critical role. During IRI, ischemic endothelial cells expose self-antigens that may produce Nabs (61).

Oxidation related antigens are additional immunogenic targets of Nabs resulting from lipid peroxidation as a response to oxidative stress (62). Peroxidation generates highly reactive products as malondialdehyde (MDA) that binds to lipid proteins.
After transplantation, oxidative stress and local production of MDA have been shown to cause graft injury (63) and chronic rejection as well as graft dysfunction (64).

Recently, several studies reported the association between IgGNAbs, AMR and worse long-term graft outcomes after kidney transplantation (11, 57, 58). Nabs were also associated with primary graft dysfunction in heart transplant recipients, principally in patients who received mechanical support before transplantation (65).

**Mechanisms Involved In Antibody Formation**

Several mechanisms may be involved in autoantibody production after transplantation, including IRI, alloimmunity, the formation of extracellular vesicles and the activation of T,17 cells and tertiary lymphoid tissue (TLT). These mechanisms can cause intracellular proteins to be expressed on the surface of apoptotic cells. The auto antigens are then presented to auto reactive T and B cells, which produce auto antibodies.

**Ischemia-Reperfusion Injury**

IRI is a process that involves the generation of reactive oxygen species (ROS), complement activation, coagulation, endothelial activation and leukocyte recruitment. IRI induced damage to vascular endothelial and tubular epithelial cells triggers the release of donor extracellular vesicles and damage associated molecular patterns (DAMPs), including nucleic acids, histones and high mobility group protein B1 (HMGB1). In addition, extracellular vesicles can serve as carriers of DAMPs and auto antigens. DAMPs interact with pattern recognition receptors (66).

Ligation of Toll-like receptor (TLR) 2 and TLR4 on myeloid (67) and dendritic cells (68, 69) activates the MyD88 and NFκB pathways, resulting in the production of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF, which promote the activation of adaptive immune-responses that can exacerbate allograft damage and exposure to auto antigens (69). Auto antigens released from an injured allograft are processed and presented to auto reactive T cells by antigen presenting cells (APCs) that are recruited during IRI (70).

**Role of Alloimmunity**

The involvement of acute and chronic rejection in the development of auto antibodies has been documented in kidney transplant recipients (57). The capacity of these antibodies to activate complement through the classical pathway suggests a potential role in enhancing allograft damage. In another study, Joosten et al (71) documented that renal transplanted patients with transplant glomerulopathy had increased levels of anti-agrin auto antibodies. Agrin is a component of the vascular basement membrane. In another study, increased anti-vimentin antibodies have been reported in patients with chronic rejection and failed kidney allograft (72). In conclusion, alloimmune attack to the graft creates permissive conditions for the enhanced availability of cryptic antigens and increased interaction with antigens present on apoptotic cells. Preexisting circulating auto antibodies, such as LG3, AT1R abs or antibodies directed toward apoptotic cells bind to their targets and increase vascular inflammation leading to increased severity of rejection (20, 45, 57, 58).

**Extracellular Vesicles**

Cell- to-cell communications through extracellular vesicles is now recognized as an important mechanism that elicits alloimmune responses (73). Based on their biogenesis, extracellular vesicles can be categorized into three classes –exosomes, micro particles and apoptotic bodies all of which contain numerous auto antigens. A study by Dieudé et al (44) provided an example of how vascular injury may trigger the release of exosome-like apoptotic vesicles that prompt anti-LG3 production. In this study, acute vascular injury led to increased circulating levels of exosome-like apoptotic vesicles that contained an active 20S proteasome complex. Proteasome activity in exosome-like apoptotic vesicles prompts the production of anti-LG3 antibodies.

Principally, auto antigen presentation to T cells by extracellular vesicles may happen in three ways:

- Donor derived extracellular vesicles containing major histocompatibility complexes bound to auto antigens released from the graft can directly activate recipient and auto reactive CD4+ and CD8+ Tcells. The antigen presentation by free extracellular vesicles is much less efficient in activating T cells than presentation by antigen presenting cells (APCs).
- Donor derived extracellular vesicles can be internalized by recipient APCs; the antigen is processed by APCs and presented to auto reactive cells.
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- Antigen presentation may happen via a cross dressing of recipient APCs. The cross dressing occurs when the donor derived extracellular vesicles are internalized and recycled to the surface of recipient APC or fused to the APC surface.

Interplay between Alloimmunity and Autoimmunity

We have already documented that the development of auto antibodies after transplantation takes place at the same time as the alloimmune response to the graft with the presence of AMR, CR and anti-HLA antibodies.

Figure 1 document how auto antibodies may aggravate rejection. In the case of alloimmune attack to the graft permissive conditions realize for the enhanced availability of cryptic antigens, such as LG3, and increased interactions with antigens present on apoptotic cells.

Relevance of Non-HLA Antibodies in Transplantation

Once initiated, the alloimmune response to the transplanted organ can spread to additional determinants in a process termed intra molecular epitope spreading (74, 75). This spreading is associated with the expansion of alloreactive T cells to donor derived MHC (major histocompatibility complex) antigens.

Similarly, the same pathway can also promote the development of autoimmune T and B cells that contribute to the rejection process (76).

This autoimmunity is probably triggered by antigen mimicry between auto antigen peptides and donor MHC peptides (77).

Several studies have shown that auto reactive pro-inflammatory T cells specific for collagen V and cardiac myosin are detected after lung and heart transplantation. This clonal expansion of T cells only occurs after an alloresponse and can induce rejection of the transplanted organ “per se” (49, 78, 79). Alternatively, the tissue damage caused by the alloresponse to donor HLA antigens results in the presentation of cryptic self-determinants and thereby triggers an autoimmune process at the graft site. Endothelial apoptosis is a prominent feature of acute vascular rejection in kidney transplantation (80). In a study by Cardinal et al (45), patients with kidney transplants and acute vascular rejection had significantly higher anti-LG3 antibody titers at the time of rejection. Two studies in heart transplant recipients showed results similar to those obtained in kidney transplantation studies implicating high pre- and post-transplantation levels of AT1R-Abs alone or along with HLA-DSA, which were associated with AMR and chronic vascular remodeling (30, 31).
Non-HLA Antibodies Induced Damage

Synergistic Effect with HLA Antibodies

We have already described that HLA and non-HLA antibodies have a synergistic effect. While HLA-antibodies can evoke endothelial damage and exposure to auto antigens, an inflammatory response induced by non-HLA antibodies can up regulate HLA expression and make an allograft more prone to an alloimmune response (81).

Complement Cascade Activation

Antibodies can fix C1q and activate the complement cascade. IgG1 and IgG3 subclasses are the most efficient and able to generate membrane attack complexes which cause hyper acute rejection. Sublytic amounts of complement may activate the endothelium and induce the release of heparin sulfates and induce pro-inflammatory conditions (82, 83).

Endothelial Cell Activation

Antibody binding may induce endothelial cell activation and a subsequent immune response that is independent of complement through activation of intracellular signaling cascades (84).

NK Cells Activations

Antibody binding can induce lysis of target cells with membrane bound antibodies through NK cell activation via a process called antibody-dependent cell mediated cytotoxicity (85).

Specific Mechanisms Related to Different Antibodies

MICA cannot fix complement, as C4d positivity is rare in anti-MICA antibody positive patients. MICA may lead to NK cell activation via MICA/NKG2D interactions with subsequent cytotoxic proteins and IFN-γ release (19).

All AECAs may lead to AMR by activating complement. Several studies document the absence of C4d staining in biopsy samples from recipients with AECAs and AMR (86, 87). This fact may be due to the endothelial active antibodies enriched for the non-complement fixing subclasses, IgG2 and IgG4.

AT1R antibodies are involved in renal rejection, but the pathogenic mechanism of these antibodies often involves complement independent pathways. Indeed, Dragan et al (20) showed that binding of antibodies to AT1R mimicked the action of angiotensin II and triggered phosphorylation of ERK kinase and activation of the transcription factors AP-1 and NFkB, in endothelial and smooth muscle cells. In addition, increased tissue factor expression and thrombotic occlusion is also observed.

Perlecan mediated graft injury may happen in two different ways. First, Perlecan may cause vascular injury and neointimal formation, which promotes migration of donor vascular smooth muscle or recipient derived mesenchymal cells. Second, it may elicit humoral immune responses that accelerate immune-mediated vascular injury. Recent studies by Soulez et al (46) and by Pilon et al (88) suggest that LG3 may cause both of these reactions. Anti-LG3 auto antibodies enhance renal micro vascular injury post-ischemia-reperfusion in transplanted kidneys. Renal ischemia-reperfusion leads to initial micro vascular damage, which enhances the expression/availability of cryptic antigens, such as LG3. Circulating anti-LG3 reaches these antigenic targets and promotes further micro vascular damage, at least in part through complement-dependent mechanisms. Micro vascular damage leads to peritubular capillary dropout and enhanced renal fibrosis (figure 2).

Administration of antibodies to collagen V can induce BOS in animal studies. This happens via the involvement of specific Th17 (89). In particular, IL-17A and IL-17F secreted by Th17 cells are critical mediators of autoimmunity (90).

The pathogenic potential of polyreactive antibodies may develop in different ways. Polyreactive antibodies may activate the complement cascade leading to membrane attack complex formation. The finding of C4d deposition on target cells confirms this pathway (57). Polyreactive antibodies may also act via activation of allograft endothelial cells. Endothelial activation leads to pro-inflammation with immune cell recruitment and tissue damage (91). Finally, polyreactive antibodies can amplify micro vascular damage induced in grafts undergoing rejection by synergistic effects with DSA (92). Indeed, four separate monoclonal polyreactive antibodies have been isolated from kidney graft recipients and were found to cross-react to several HLA molecules, supporting a potential synergistic effect with DSA (92).
Current and Possible Future Treatment and Prevention Strategies

Interventions promoting endothelial health and resistance to non-HLA antibody-mediated injury would provide optimal strategy to face a non-HLA antibody attack on the endothelium. Current treatment strategies for AMR caused by auto antibodies are similar to those used for AMR caused by HLA auto antibodies, including antibody depletion (93), B cell depletion (94), IVIG (95), proteasome inhibitors (96) and complement inhibitors (97). In the case of auto antibodies against AT_1R the combination of sartanes and plasmapheresis was successful (98). The recent observation that bortezomib can block the production of anti-LG3 auto antibodies (44) represents a therapeutic option to prevent autoantibody production before transplantation.

In some patients the suppression of early events of endothelial activation, could be useful. Rapamycine documented to be effective on endothelial activation (99) and preliminary data over the efficacy of sirolimus and everolimus exist for AT_1R Abs and ET_αR Abs.

Conclusions

For a long time kidney transplant rejection has been considered the principal expression of donor-recipient MHC discrepancy. To date the production of auto antibodies in association with renal damage, at the time of rejection or in association with ischemia-reperfusion syndrome is emerging as a new way to produce renal dysfunction. In many circumstances the association of alloimmunity, autoimmunity and tissue injury may cause allograft dysfunction and reduced graft survival.

However the incomplete knowledge of the auto antigens that elicit immune response hampers diagnosis and treatment of AMR. Efforts to define the autoantibody network are necessary to understand the mechanisms and pathogenesis of auto antibodies and to develop strategies to improve long-term outcomes.

References


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Relevance of Non-HLA Antibodies in Transplantation


[80] Ishii Y, Sawada T, Kubota K, Fuchinoue S, Teraoka S, Shimizu A. Injury and progressive loss of


