Human Immunology 80 (2019) 614–620

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

Review

Sensitization to endothelial cell antigens: Unraveling the cause or effect paradox

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ARTICLE INFO

Keywords:
Anti-endothelial cell antibodies
Antibody mediated rejection
Exosomes
Non-HLA antibody
Transplantation

ABSTRACT

Anti-endothelial cell antibodies (AECAs) have been correlated with increased acute and chronic rejection across all organ types and early graft dysfunction in kidney and heart transplantation. Nevertheless, the lack of appropriate tools and clear criteria for defining injurious versus non-injurious AECAs prohibits their routine inclusion in clinical risk assessments and diagnostic algorithms for antibody mediated injury. Clinical characterization of AECAs is complicated due to the wide range of polymorphic and non-polymorphic antigens expressed across different vascular tissues and the diverse array of specificities observed between individuals. This complexity is also reflected in the broad spectrum of reported injury phenotypes. AECAs detected at time of allograft dysfunction may represent biomarkers of past vascular injury or active contributors to a current rejection process. New tools within the fields of proteomics, genomics, bioinformatics, and imaging are currently being validated and hold great promise for unraveling the AECA paradox.

1. Introduction

There is growing interest surrounding the role of anti-endothelial cell antibodies (AECAs) in allograft injury following kidney, heart, lung, and liver transplantation [1–4]. Given that the vascular endothelium serves as the primary barrier between the patient's immune system and the transplanted allograft, transplant immunologists have long been interested in how AECAs may influence activation and leukocyte infiltration. Current studies have revealed multiple pathways for AECAs to contribute to endothelial cell dysfunction to include apoptosis, hypercoagulability, permeability, pro-inflammatory cytokine production, and complement activation [3,5,6]. Detection of preformed or *de novo* AECAs have been correlated with an increased risk for allograft dysfunction, acute and chronic rejection, and failure. Humoral immunity is now thought to be the primary barrier to improving long-term allograft survival; therefore, greater insight into how AECAs develop and their injury potential is greatly needed.

Clinical investigators are particularly keen in developing platforms for detecting and defining injurious AECAs to guide intervention and mitigate antibody mediated injury. However, this is not an easy task given the heterogeneity of the non-HLA, endothelial cell (EC) targets and the broad spectrum of reported injury phenotypes. Additionally, it remains uncertain whether all AECAs are active contributors to allograft injury or biomarkers of past tissue injury arising from end-stage organ failure or underlying autoimmune diseases. New tools in the laboratory hold great promise for unraveling this paradox with improved detection sensitivity and specificity as well as the ability to define injury phenotypes with greater accuracy. Only then can we provide adequate AECA risk assessments and intervention strategies to mitigate their impact on transplantation outcomes.

Abbreviations: ABMR, antibody mediated rejection; AECA, anti-endothelial cell antibodies; AMS, allogenomics mismatch score; AT1R, angiotensin II type 1 receptor; BOS, bronchiolitis obliterans syndrome; AV, allograft vasculopathy; C3d, complement 3 deposition; C4d, complement 4 deposition; EC, endothelial cell; EDIL3, EGF like repeats and discoidin I-like domains 3; ETAR, endothelin receptors type A; FLT3, fms-like tyrosine kinase-3; HEV, high endothelial venules; HLA-DSA, donor-specific HLA antibody; HUVEC, human umbilical vein endothelial cell; ICAM4, intercellular adhesion molecule 4; LG3, perlecan LG3 fragment; MICA, major histocompatibility complex class I related chain A; nsSNP, non-synonymous single nucleotide polymorphisms; STORM, stochastic optical reconstruction microscopy; ZG16B, zymogen granule protein 16B

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https://doi.org/10.1016/j.humimm.2019.04.014
Received 21 February 2019; Received in revised form 2 April 2019; Accepted 23 April 2019
Available online 01 May 2019

2. Endothelial cells Diversity: are we using the right tools?

Early investigative studies used serological methods and immortal EC lines or primary EC cultures to detect AECAs in patient sera [7–9]. However, the clinical outcome correlations with AECA positivity must be viewed with caution given the possible presence of unrecognized HLA-antibody bound to HLA on the EC target cells. In 2009, Breimer et al. reported prospective, multicenter clinical trial data that utilized endothelial precursor cells isolated from donor blood to identify donor-specific AECAs in a flow cytometric crossmatch [10]. All centers utilized Luminex® based platforms for donor-specific HLA antibody detection and biopsies were used to confirm kidney rejection. This study found a significantly higher rejection rate within the first 3 months post-transplantation among patients testing positive in a donor-specific EC crossmatch (16 of 35, 46%) compared to patients that tested negative (13 of 112, 12%; $P < 0.00005$). The use of donor-derived endothelial precursor cells allows for detection of sensitization toward polymorphic proteins, which may differ between donors [11]. However, the question remains as to whether these immature endothelial precursor cells express all of the relevant non-HLA proteins expressed by mature ECs within different allograft tissues.

In the current era, determining the best ECs to use in AECA detection is under debate given their vast heterogeneity [12–14]. ECs contribute to multiple physiological processes to include: hemodynamics, coagulation, permeability and nutrient exchange, angiogenesis, leukocyte trafficking, and innate and adaptive immunity. Chi et al. revealed the extent of this diversity using gene expression data from 53 different ECs isolated from venous and arterial vessels across different tissues. This specialization of EC structure and physiology creates considerable diversity at the level of gene expression, which is tightly controlled by the microenvironment [15,16]. Heterotopic transplantation of ECs into different tissues results in significant morphological changes, demonstrating the importance of environmental cues in maintaining EC phenotypes. Perhaps more striking was the study by Lacorre et al. that demonstrated gene expression differences between human tonsil high endothelial venules (HEVs) measured directly ex vivo and following 2 days of in vitro culture [16].

EC diversity and its consequence for AECA assessment was recently highlighted by Delville et al., who characterized AECAs targeting microvascular glomerular ECs, but not macrovascular arterial ECs, in kidney transplant recipients with early and severe allograft injury in the absence of HLA-DSA [17]. While previous studies aiming at developing cell-based assays used various ECs (i.e. human umbilical vein endothelial cells, HUVECs) [9], primary cultures of macrovascular arterial ECs [8], and circulating EC progenitors [10], none utilized tissue-specific ECs representing the target cells involved the immune mediated injury. Using glomerulus derived ECs, Delville et al. demonstrated a specific AECA response to renal microvascular ECs and this specificity may explain why their pathogenicity was confined to the allograft [17].

3. Polymorphisms outside of HLA

To develop the optimal AECA detection strategies, the most clinically relevant EC antigens must be identified and correlated with organ specific injury phenotypes and clinical outcomes. Once identified, solid phase assays (i.e. ELISA or Luminex®) can be developed to eliminate the logistical problems of cell based assays. Early investigations into AECA antigens utilized cell extracts to immunoprecipitate immunoglobulin-antigen complexes followed by mass spectrometry to determine antigen specificity [7,18–20]. More recent work has tested sera across large protein arrays with the capacity to survey up to 9000 human antigenic targets simultaneously [17,21–25]. Cumulatively, these studies have revealed a broad array of non-HLA antigens expressed on the vascular endothelium that include both polymorphic and non-polymorphic proteins.

Major histocompatibility complex class I related chain A (MICA) was an early reported polymorphic antigen that has been correlated to allograft injury and loss [26,27]. Interestingly, MICA is not constitutively expressed, but is upregulated in response to stress from infection, malignancy, and rejection; thus, making it an interesting target in the setting of transplantation [28]. Cell surface antigens, beyond HLA, may serve as the stimuli as well as the targets for the antibody-mediated immune responses and influence long-term graft outcomes. Following exome sequencing of donor and recipient pairs, Mesnard et al. calculated an allogenomics mismatch score (AMS) and demonstrated that AMS had a significant effect on graft function that was independent of the HLA-A, B, DR matching, donor age, and time post-transplantation [29]. The iGeneTRAiN consortium performed genome-wide genotyping of 477 kidney recipient/donor pairs using a transplant-specific gene array that distinguished non-synonymous single nucleotide polymorphisms (nsSNPs) within transmembrane and secreted proteins [30]. They found a significant association between nsSNP mismatch and early functional graft loss that was independent of HLA incompatibility. Pineda et al. further explored polymorphic proteins, outside of HLA, by performing exome sequencing and computational gene expression analysis in kidney recipient/donor pairs with biopsy proven rejection and identified 94 exome variants within 72 unique genes that associated strongly with the risk of ABMR [31]. These variants were enriched within immune-related genes and genes encoding cell-surface proteins, suggesting possible targets for alloantibodies. Delville et al. also identified a large array of AECAs directed against constitutively expressed antigens of human glomerular ECs [17]. Using a combination of transcriptomic and proteomic approaches, new targets of non-HLA antibodies with little redundancy among individuals were identified.

4. Sensitization to non-polymorphic proteins

AECA targets also include non-polymorphic (autologous) surface proteins such as: protein kinase Cζ, perlecan (LG3), EGF like repeats and discoidin I-like domains 3 (EDIL3), fms-like tyrosine kinase-3 (FLT3) ligand, intercellular adhesion molecule 4 (ICAM4), endoglin, and others [3,32]. Angiotensin II type 1 receptor (AT1R) and endothelin receptors type A (ETAR) are not known to harbor polymorphisms within their protein coding regions, but can vary in non-coding regions that impact protein expression levels and isoforms expressed in different tissues [33,34]. Pathogenic AECAs directed toward autoantigens have also been identified across multiple autoimmune diseases [35]. The prevalence of autoantibodies in transplant patients differs by study cohort and detection method. The identification of a wide breadth of autoantibody specificities with little overlap between patients suggests systemic B-cell deregulation and loss of tolerance checkpoints in transplant candidates [17,36–38]. The events or triggers that precipitate sensitization toward autoantigens in transplant patients are likely multifactorial and may include underlying autoimmune diseases (ie diabetes, lupus) or the chronic injury and inflammation associated with end-stage organ failure itself [4]. Once transplanted, recipients experience additional vascular injury stemming from ischemia and reperfusion, infection, and alloimmune rejection. Throughout all of these injurious processes, cryptic or nonnative epitopes on autoantigens may be exposed creating neoantigens [39–43]. Autoimmunity research has highlighted the role for inflammatory induced posttranslational modifications of autoantigens in promoting autoantibody development. Oxidative stress can induce citrullination, the conversion of arginine to citrulline, within proteins such as collagen II, fibrinogen, and vimentin promoting autoantibody development [39,41]. Plasma transglutaminase was shown to post-translationally modify AT1R, contributing to autoantibody production and disease development in a murine model of preeclampsia [42]. Zorn and colleagues have described polyreactive or natural antibodies in the sera of kidney recipients that bind apoptotic cells and/or oxidative induced epitopes induced by endothelial lipid peroxidation [38,40,43].

5. Exosomes and AECA development: do exosomes impact AECA pathogenicity?

Research into the biology of exosomes have shown patients with end stage organ failure or ischemic injury have elevated circulating endothelial derived exosomes in their plasma [44,45]. Exosomes are small extracellular vesicles approximately 50–150 nm in diameter, produced by most cell types [46]. They are formed by invagination and membrane budding from late endosomes, resulting in vesicles that contained cytosol and extracellular proteins. Exosomes may contain DNA, coding or noncoding RNA, proteins, or lipids and the content is dynamic, reflecting the external and internal microenvironment of the originating cell. Exosomes participate in intercellular communication to include the exchange of membrane proteins and cytosol between two cell types. Exosomes have been hypothesized to be protective under cases of cellular stress through the release of RNA and proteins from the cell. However, immune-stimulatory functions have also been attributed to exosomes released from ECs following activation, hypoxia, or rejection [44,47–51]. The altered content of exosomes released from activated EC include pro-inflammatory cytokines, chemokines and costimulatory molecules with the potential to potentiate HLA antibody and AECA development [52].

Elevated circulating endothelial derived exosomes occur in patients exhibiting arterial dysfunction and reduced NO production and were shown *in vitro* to contribute to EC dysfunction [44,47]. These studies concluded that exosomes may represent a new biomarker to track cardiovascular disease and endothelial dysfunction in patients with end stage renal failure. Dieudé et al. have shown that vascular ischemic injury induces caspase 3 activation and release of apoptotic exosome-like vesicles that differ in protein content from apoptotic bodies [52]. They demonstrated the presence of LG3, an immunogenic proteolytic byproduct of the autoantigen perlecan, within these exosome-like vesicles resulted in downstream B cell activation, germinal center formation, and LG3 specific autoantibody production.

Vallabhajosyula et al. showed a distinct change in microRNA and proteomic profiles of serum exosomes obtained at time of rejection in islet transplant recipients and this profile mirrored changes in clinical parameters such as serum glucose and c-reactive peptide levels [49]. The content of these exosomes allowed for the identification of the originating tissue; therefore, they proposed quantitative and qualitative monitoring of circulating exosomes may provide a noninvasive biomarker to assess the conditional status of transplanted allografts. Mohanakumar and colleagues have examined exosomes isolated from bronchoalveolar lavage fluid from lung transplant recipients and found expression of donor HLA molecules and autoantigens (annexin V, collagen V, and Kα1 tubulin) only at time of acute rejection or chronic bronchiolitis obliterans syndrome (BOS) diagnosis and not at time of stable function [50,51]. Global microRNA profiling of exosomes at time of rejection showed immunoregulatory signatures associated with inflammation, endothelial activation, antibody mediated rejection. They also revealed differential expression of costimulatory molecules and transcription factors in exosomes isolated from the sera of lung transplant recipients diagnosed with BOS compared to stable recipients. Concurrent with severe kidney rejection, Delville et al. identified AECAs to the Zymogen Granule Protein 16B (ZG16B) [17], a protein that has been previously identified in urinary exosomes [53], again suggesting that exosomes released at time of rejection may trigger autoantibody production.

6. AECAs: biomarkers of past injury or active contributors to rejection?

Eloquent studies have demonstrated strong correlations between preformed or post-transplant, *de novo* AECAs in the serum of transplant recipients at the time of acute and chronic rejection. Autoantibodies directed against G protein coupled receptors AT1R and ETAR, expressed on vascular endothelium, have been shown to correlate with an increased risk for donor-specific HLA antibody (HLA-DSA) development and acute and chronic rejection in kidney, heart, and lung transplant recipients [37,54–58]. Taniguchi et al. reported on 351 consecutive kidney transplants and found anti-AT1R in combination with HLA-DSAs experienced lower allograft survival compared to patients with only HLA-DSA alone, suggesting a synergistic pathogenic effect [59]. Philogene et al. demonstrated a direct correlation between AT1R antibody strength and allograft injury scores, which was enhanced in the presence of HLA-DSA providing further evidence for synergy between all EC bound antibodies [58].

Antibodies specific for MICA have been identified in kidney and heart transplant recipients with severe acute rejection and/or allograft loss [60,61]. Suarez-Allvarez et al. reported *de novo* detection of MICA antibodies in heart transplant patients preceded acute rejection and that higher levels of MICA expression within endomyocardial biopsies directly correlated with severity of histological scores for rejection [61]. Hébert and colleagues have demonstrated elevated levels of LG3 and subsequent anti-LG3 antibodies in the sera of kidney transplant recipients experiencing acute vascular rejection [48,52,62,63]. Research by Zorn and colleagues have shown that post-transplant development of polyreactive antibodies, within the first year post-transplant, was associated with increased allograft injury scores and a significant risk for graft loss [38,40,43]. Both polyreactive antibodies and AT1R antibodies have been detected in the sera of heart transplant recipients with primary graft dysfunction, especially those bridged to transplant with ventricular assist devices [64,65].

These clinical studies have been primarily retrospective in nature and many were cross-sectional analyses. The cumulative data have provided a substantial correlation between AECA detection and allograft injury, but most fall short of proving causation. A recent multicenter Clinical Trials in Organ Transplantation-05, correlating biomarkers and early post-transplant outcomes in 200 heart recipients, found no association between the detection of myosin or vimentin specific autoantibodies and acute rejection or transplant vasculopathy [66]. In a longitudinal study of kidney recipients, Gareau et al. found pre-transplant sensitization toward AT1R increased the incidence of *de novo* HLA-DSA, but had no impact on transplant outcomes independent of HLA-DSA [37]. These latter studies question our current ability to decipher when autoantibodies alone are active contributors to allograft injury or biomarkers of past tissue injury.

Mechanistic studies linking AECAs with *in vitro* experiments or animal models of allograft injury have provided more causal evidence for the pathogenicity of these antibodies. Dragun and colleagues have investigated the capacity of autoantibodies specific for AT1R and ETAR to mimic natural ligands and elicit receptor activation and downstream effector functions [67]. Furthermore, adoptive transfer of AT1R autoantibodies into mice resulted in phenotypic changes resembling vascular disease in a preeclampsia model system [42]. Similarly, passive transfer of LG3 specific antibodies or LG3+ apoptotic exosomes into mice following aortic transplantation has been shown to induce allograft vasculopathy with neointima proliferation and complement split-product 4 deposition (C4d) [52,63,68]. Gao et al. immortalized B cell clones isolated from a rejected kidney allograft and induced the production of clonal polyreactive antibodies with reactivity toward apoptotic but not viable cells [40]. Functional studies showed that these clonal polyreactive antibodies were proficient in activating complement, resulting in the deposition of complement split-product 3 (C3d) and C4d at the surface of target cells.

It is well established that activated vascular endothelium plays an active, not passive, role in propagating immune responses [69]. Activated ECs not only increase leukocyte adhesion and diapedesis, but they can also influence the differentiation of the transmigrating leukocytes and promote their activation [70,71]. Previous studies have shown that AECA positive sera obtained at time of allograft rejection can stimulate EC activation *in vitro* [72]. Jackson et al. used EC adsorption-elution

strategies to isolate AECAs from sera at time of rejection and show that the AECAs fraction holds the capacity to upregulate adhesion molecules and inflammatory cytokine production of cultured ECs [25]. Interestingly, AECA stimulation also led to increased HLA surface expression on ECs, providing a possible mechanism for the synergistic action observed for AECAs and HLA-DSA in rejection severity.

7. AECA-induced injury: the clinical challenges

If AECAs are active contributors to allograft injury, this should translate into clear clinico-immunological phenotypes. However, in the absence of clinically actionable AECA testing, the identification of AECA-induced graft injury remains challenging. Similarly, transplant physicians are particularly keen in defining clinicopathological phenotypes associated with AECA to guide intervention and apply treatment targeting injurious antibodies.

The demonstration of microvascular inflammation (MVI), in the absence of HLA-DSA, is usually considered as an appropriate surrogate of an antibody-induced injury. However, MVI by mononuclear cells may also be due to antibody-independent allorecognition. Studies in mice models demonstrate that innate myeloid cells also engage in allorecognition [73,74], thus questioning a possible and still unrecognized allorecognition by innate myeloid cells in humans. In this scenario, an antibody-independent injury to the graft endothelium could release/expose endothelial antigens that could subsequently induce AECAs, the latter being considered as a consequence of the injured process. Philogene et al. showed that the degree of MVI directly correlated with increased AT1R antibody concentrations [58]. Similarly, Delville et al. correlated intense MVI with the presence of preformed microvascular specific AECAs, in the absence of HLA-DSA, very early after transplantation in kidney recipients suffering acute graft dysfunction [17]. This study also demonstrated that AECA-induced allograft injury often associated with severely injured microvascular wall and consequently interstitial hemorrhage and histological features of thrombotic microangiopathy. However, a recent report from Naesens and colleagues found no inferior long-term kidney allograft survival associated with HLA-DSA negative ABMR [75]. This study did not investigate the presence of AECAs at time of ABMR, but the results highlight the need for further vetting of ABMR and AECA characteristics that define pathogenicity with impact on long-term transplant outcomes.

C4d deposition in peritubular capillaries is also considered by transplant physicians as a surrogate of antibody-induced injury. However, increasing evidence suggest the relevance of complement-independent mechanisms in the context of HLA-DSA associated antibody-mediated allograft injury [76]. In suspected non-HLA, AECA-induced allograft injury, C4d deposition in peritubular capillaries is also often absent, thus supporting the hypothesis of a complement-independent injury [33,77]. Antigen density on the vascular endothelium may impact the ability of AECAs to activate complement. While HLA is highly expressed on ECs, the density of individual non-HLA targets may be insufficient to promote complement activation [78]. The upregulation of non-HLA antigen expression under inflammatory conditions or the contribution of multiple non-HLA targets in cases of broad AECA specificity may be what is required to initiate the complement cascade.

8. New tools to unravel the AEAC injury paradox

Together, the clinical and mechanistic data investigating the pathogenicity of AECAs in transplantation suggest an iterative process whereby native organ or allograft injury can initiate AECA development and, once formed, AECAs may elicit new or contribute to ongoing EC injury or dysfunction (Fig. 1). AECA induced injury of ECs may potentiate the release of pro-inflammatory exosomes leading to broader immune activation, while EC activation may trigger proliferative pathways leading to chronic rejection phenotypes. This positive feedback loop may amplify the humoral response and generate AECAs of greater strength, affinity, and pathogenicity. It must also be appreciated that AECAs may precede or follow HLA-DSA development and together they may escalate this feedback loop of injury and immune activation.

Defining pathogenic AECAs will rely on our ability to control for confounding factors when diagnosing antibody mediated injury. The development of commercial Luminex® bead panels coated with non-HLA targets, to include relevant polymorphic and non-polymorphic EC targets, will allow for a standard platform to test for AECAs and allow the determination of AECA prevalence, breadth, and specificities across transplant cohorts. Additionally, the efficiency of this platform will allow testing of large populations to control for confounding factors such as underlying disease, age, donor factors, and time post-transplant, all of which may impact AECA development and pathogenicity. Li et al. performed one of the first non-biased analyses of *de novo* non-HLA antibody development [22]. Using a large human protein array and tissue-specific gene expression data, they mapped the most immunogenic non-HLA antigens to the renal pelvis and cortex. Similar strategies were used to track the development of *de novo* non-HLA antibody over time in chronically rejected kidney allografts [23,24]. Collectively, these types of datasets are refining our understanding of the intersection and contribution of AECAs to allograft injury.

Recognizing different phenotypes of AECA mediated EC injury is also complicated given the functional heterogeneity of ECs and non-immune factors that contribute to EC dysfunction. EC injury or dysfunction may manifest clinically as impaired endothelial permeability, thrombosis, hypertension, or transplant vasculopathy. Current data from genomic profiling is revealing how the vascular endothelium responds to environmental and immune insults and may broaden our recognition of EC injury [14,22–24,79,80]. To refine our understanding of chronic injury progression following kidney transplantation, Naesens et al. performed whole-genome microarray profiling of 120 protocol or indication biopsies [79]. Patients with HLA-DSA or recurrent kidney disease were excluded and all biopsies were obtained within 2 years post-transplant. Surprisingly, a high prevalence of infiltrating immune cells and immune activation signatures was observed, in the absence of Banff grade rejection. These findings reveal the complexity of immunological processes that occur in clinically quiescent kidney allografts and the role of the activated infiltrating cells as triggers for the development and progression of chronic injury. Sensitive and reproducible diagnostic signatures for T cell mediated rejection and ABMR using gene expression profiling of transplant biopsies are being developed across organ types (heart, kidney, lung, and liver) [81–85]. These studies also reveal the tissue injury sustained from surgical donation/implantation and recurrence of primary disease as well as highlighting the overlap between rejection and wound-healing transcripts [86]. Whether or not molecular signatures of ABMR defined in the context of HLA-DSA will mirror non-HLA, AECA-induced allograft injury remains to be demonstrated. However, in the recent transcriptomic analysis of the BIOMARGIN consortium, while a significant subset of ABMR were HLA-DSA negative, a molecular signature suggestive of NK cell infiltration robustly associated with diagnosis and prognosis of ABMR, thus suggesting that common molecular pathways associate with MVI, independently of the underlying mechanism [87].

Advances in diagnostic imaging are also increasing our ability to detect and study the pathophysiology of EC activation, injury, and dysfunction. The use of fluorescence proteins in single live-cell imaging have revolutionized EC research. Fluorescence resonance energy transfer based biosensors allow subcellular localization and interactions of molecules in the cytosol or plasma membrane and has been used to study EC migration and the dynamics of EC junction integrity [80,88]. Magnetization transfer imaging was able to noninvasively detect and monitor the progression of renal fibrosis in mice following unilateral renal artery stent placement. Near-infrared fluorescence optical imaging has shown the ability to measure post-stimulatory increases in

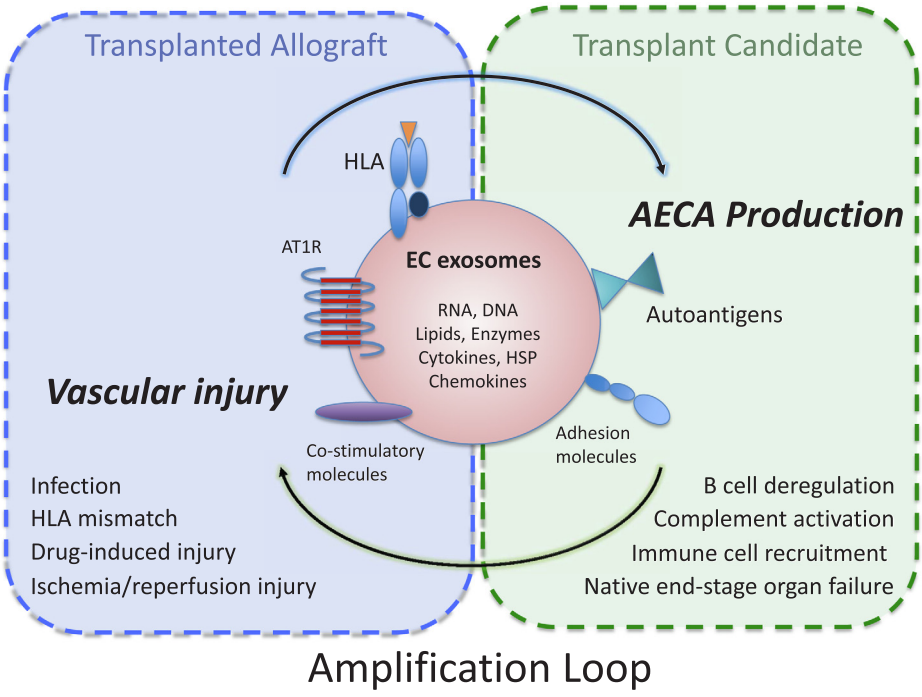

Fig. 1. Vascular injury can initiate AECA development or, alternatively, AECAs may elicit new or contribute to ongoing vascular injury or dysfunction potentiating the release of pro-inflammatory exosomes leading to broader immune activation.

adhesion molecules on brain microvascular EC in vivo [89]. Sub-diffraction resolution stochastic optical reconstruction microscopy (STORM) analysis of kidney tissue is revealing the protein organization within the kidney glomerular basement membrane and its interactions with podocytes and endothelial cells [90]. Refining these phenotypes of EC dysfunction may provide critical information for differentiating immune insults from recurrent kidney disease.

Allograft vasculopathy (AV) is a major barrier to long-term allograft survival and is propagated by immune and non-immune factors that promote vascular inflammation and fibroproliferative disease [91]. Evidence linking AECAs and/or HLA-DSA with AV development in humans has been limited, in part, by the inability to detect AV in its early stages [92]. However, non-invasive techniques such as cardiac magnetic resonance and positron emission tomography are now being tested in heart transplant recipients to provide detailed imaging of macro and microvasculature and quantitative assessments of microvascular function [93,94]. This improvement in imaging holds the potential for early detection of transplant microvasculopathy and opportunities for investigating the triggers of inflammation and disease progression.

9. Conclusion

The role of AECAs in allograft injury is complex, dynamic, and may manifest differently across transplant recipients and across organ types. Some transplant candidates may harbor more pathogenic AECAs at time of transplant, others may generate them *de novo* post-transplant, or not at all. Advances in transplantation proteomics and genomics will refine our understanding of AECA characteristics and EC targets that predict pathogenicity. Furthermore, earlier detection and a greater understanding of EC dysfunction and injury will generate clinicopathological phenotypes to guide clinical intervention. It will require this type of cross-disciplinary approach to decipher whether an AECA detected at time of allograft dysfunction is an active, injury contributor or a biomarker of past vascular insults.

Conflict of interest

AMJ is on the speaker bureau for One Lambda, ThermoFisher.

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