Non-HLA antibodies against endothelial targets bridging allo- and autoimmunity

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Detrimental actions of donor-specific antibodies (DSAs) directed against both major histocompatibility antigens (human leukocyte antigen [HLA]) and specific non-HLA antigens expressed on the allograft endothelium are a flourishing research area in kidney transplantation. Newly developed solid-phase assays enabling detection of functional non-HLA antibodies targeting G protein–coupled receptors such as angiotensin type I receptor and endothelin type A receptor were instrumental in providing long-awaited confirmation of their broad clinical relevance. Numerous recent clinical studies implicate angiotensin type I receptor and endothelin type A receptor antibodies as prognostic biomarkers for earlier occurrence and severity of acute and chronic immunologic complications in solid organ transplantation, stem cell transplantation, and systemic autoimmune vascular disease. Angiotensin type 1 receptor and endothelin type A receptor antibodies exert their pathophysiologic effects alone and in synergy with HLA-DSA. Recently identified antiperlecan antibodies are also implicated in accelerated allograft vascular pathology. In parallel, protein array technology platforms enabled recognition of new endothelial surface antigens implicated in endothelial cell activation. Upon target antigen recognition, non-HLA antibodies act as powerful inducers of phenotypic perturbations in endothelial cells via activation of distinct intracellular cell-signaling cascades. Comprehensive diagnostic assessment strategies focusing on both HLA-DSA and non-HLA antibody responses could substantially improve immunologic risk stratification before transplantation, help to better define subphenotypes of antibody-mediated rejection, and lead to timely initiation of targeted therapies. Better understanding of similarities and dissimilarities in HLA-DSA and distinct non-HLA antibody–related mechanisms of endothelial damage should facilitate discovery of common downstream signaling targets and pave the way for the development of endothelium-centered therapeutic strategies to accompany intensified immunosuppression and/or mechanical removal of antibodies.

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Efforts to understand mechanisms of allorecognition and to modulate the effectors of cellular immunity dominated the field of transplantation medicine for a long period of time. Increasing importance of antibody (Ab)-mediated rejection (ABMR) shifted the attention of the transplant community toward allo- and autoantibody-mediated mechanisms of transplant damage during the past decade. ABMR is a major barrier to tolerance and a leading cause of kidney allograft loss.[1] Lack of adequate therapies to effectively modulate or reverse Ab-mediated injury illustrates the pressing need to identify new diagnostic and therapeutic concepts beyond traditional targeting of the immune system effectors. Vascular endothelium comprises a critical interphase between parenchymal cells of the transplanted organ and the immune system of the recipient. Homeostatic functions of the endothelium are regulation of organ perfusion, regulation of nutrient/waste/gas exchange between blood and tissue, regulation of hemostasis, and participation in immune surveillance.[2] Injuries to macrovascular and microvascular endothelium disturb endothelial homeostasis in the transplanted kidney at multiple levels and the cardiovascular system of the recipient.[3] Prominent upregulation of endothelial transcripts indicative of endothelial stress and microscopic microcirculation changes also underscore importance of the endothelium as a primary target for the effectors of cellular and humoral immunity after kidney transplantation.[4] Repetitive microvascular endothelial injuries and repair attempts lead to impairment in intrarenal oxygen delivery, activating various vasoactive systems and eventually resulting in fibrosis of the allograft.[5] Although critically important, the endothelium remains an unfortunately largely untapped therapeutic target in kidney and other forms of solid organ transplantation. Discovery of several endothelial antigenic targets on macrovascular and microvascular endothelium including G protein–coupled receptors (GPCRs); first, the angiotensin type I receptor (AT_1R) and later the endothelin type A receptor (ET_AR), as well as the bioactive C-terminal fragment of perlecan (referred to as LG3) with consecutive diagnostic developments, sparked the interest of the transplant community

in nonhuman leukocyte antigen (HLA) Ab responses and their actions on the endothelium. Protein array studies enabled an unbiased search for other targets. After providing a brief general overview of kidney endothelium with the pitfalls of older studies on antiendothelial cell Abs (AECAs), we focus on recent clinical and experimental studies investigating the impact of anti-GPCR and antiperlecan Abs, followed by discovery of proteomics-based developments. As ABMR is frequently C4d negative,[6] which underscores the potential relevance of complement-independent mechanisms, we further address similarities and dissimilarities of specific Ab-related intracellular signaling pathways elicited in endothelial cells. In order to address current critical gaps between HLA and non-HLA Ab responses, we attempted to identify potential leads and common targets for future therapeutic improvements.

Challenge of kidney endothelium heterogeneity for AECA diagnostics

Endothelial heterogeneity evolved as a core feature of the endothelium, reflecting its role to meet the diverse needs in specific body tissues.[7] In the kidney, endothelial cells display functional heterogeneity in specific vasculature subsets.[8,9] An endothelial layer of renal arteries and veins is nonfenestrated and continuous.[8] Microvascular fenestrated endothelium of glomerular capillaries evolved at the site of increased filtration and fenestrated endothelium of peritubular capillaries to cope with increased transendothelial transport. Data on the expression of HLA class II antigens vary from reporting constitutively high levels on quiescent glomerular and peritubular microvascular endothelial cells[10] to opposing findings based on the traditional view that only HLA class I antigens but not HLA class II antigens are expressed on quiescent endothelium of different vascular beds.[11,12] Quiescent glomerular endothelial cells express very low levels of AT_1R.[13] The endothelium of preglomerular vessels expresses ET_AR.[14] Despite the mentioned specific features of the kidney endothelium, endothelial cell lines derived from the kidney micro- or macrovascular endothelium are only very rarely used in research, and the majority of the studies are generated in other cell systems such as human umbilical vein endothelial cells (HUVECs) or aortic macrovascular endothelium. Maintenance of functional specificity of macro- and microvascular endothelium is essential for the health of the transplanted kidney. Kidney allograft endothelium is, however, never quiescent and is prone to various injurious stimuli that disturb the homeostatic function of the endothelium. Endothelial activation leading to broad upregulation of both class I and II HLA antigens as well as non-HLA antigens such as AT_1R and ET_AR occurs already during brain death[15] and organ retrieval process. Processes associated with endothelial dysfunction continue during ischemia and reperfusion injury and can be augmented with calcineurin inhibitors, the mainstay immunosuppressants.[16]

Logically, AECAs represent a heterogeneous group of Abs directed against a variety of antigenic determinants on endothelial cells.[17] The most significant barrier to the general acceptance of AECAs as causative of allograft injury is the lack of standardized assays to determine their presence for a long time period. During the past years, 2 different detection platforms emerged. One is an indirect immunofluorescence test on slides covered with HUVECs.[4,18] Surface antigen expression in quiescent HUVECs may, however, differ from that in activated kidney allograft endothelium. Not surprisingly, *de novo* but not preexisting AECAs were associated with steroid refractory acute rejection.[18] The other modality is a flow cytometry–based endothelial crossmatch assay (ECXM) that relies on isolation of endothelial cell precursors positive for the angiopoietin receptor Tie-2 from the peripheral blood of organ donors.[19] An isolation procedure for ECXM largely relies on monocyte-rich hematopoietic progenitors with surface antigen expression that largely differs from patterns detected in activated endothelial cells. Accordingly, results of clinical studies showed a better correlation of positive ECXM results with cellular than with humoral rejection.[20] Donor-reactive AECAs detected on ECXM belonged to complement nonfixing IgG2 and IgG4 subclasses.[20] AECAs detected by ECXM may differ from AECAs detected by means of immunofluorescence on HUVEC coverslips. Neither of the mentioned test modalities are in broad clinical use. Development of experimental primary cultures of endothelial cells prospectively isolated from deceased transplant donors are probably biologically closest to a real-life transplant situation, yet technically tedious to become feasible for clinical routine use.[21] It is likely that the targeted detection of Igs against relevant endothelial antigens involved in allograft pathology (Figure 1) will offer a more practical diagnostic approach compared with measurements of broad reactivity to the endothelium. So far, most of the clinically relevant discovered endothelial Abs are directed against variety of proteins that display prominent extracellular regions (Figure 1). Perlecan shows a spatial difference as it is contained within the endothelial basement membrane and may act as a neoantigen.

G protein–coupled receptors acting as non-HLA endothelial antigens

G protein–coupled receptors (GPCRs) are the largest family of cell surface receptors and the most important drug targets because >40% of currently used drugs are GPCR modulators.[22] Structural organization of 7-transmembrane span GPCRs is complex, showing an extracellular region with an N terminus and 3 extracellular loops (ECL1–ECL3); the transmembrane (TM) regions (TM1–7), consisting of 7 α-helices, and the intracellular region, consisting of 3 intracellular loops, an intracellular amphipathic helix, and the C-terminus.[22] AT_1R belongs to class A of GPCRs and mediates most of the physiologic and pathophysiologic effects of angiotensin II (Ang II) by promoting vasoconstriction, inflammation, proliferation, and fibrosis.[23] Similar actions of endothelin-1 are exerted via ET_AR.[24] A low level of expression of both AT_1R and ET_AR has been detected on quiescent glomerular kidney