mTOR signaling cascade: novel clinical implications in HLA and non-HLA antibody-mediated vasculopathies?

Kevin Louis[1,2], Alexandre Loupy[1,3] and Carmen Lefaucheur[1,2]

Antibodies targeting endothelial antigens represent major threats to the integrity of endothelial vasculature, contributing to development of organ transplant rejection and vasculopathies. In this issue, Catar *et al.* present the mechanisms by which non-human leukocyte antigen angiotensin II type 1 receptor and endothelin-1 type A receptor antibodies mediate impairment of endothelial repair via β2-arrestin link to the mammalian target of rapamycin pathway. This commentary discusses the mechanisms of human leukocyte antigen and non-human leukocyte antigen antibody-endothelium interactions, and the clinical implications and challenges of the use of mammalian target of rapamycin signaling activation as biomarker and therapeutic target in vasculopathies.

Kidney International (2022) **101,** 451–454; https://doi.org/10.1016/j.kint.2021.12.008

see basic research on page 498

As potent mediators of the adaptive immune system, antibodies directed against antigens expressed by endothelial cells represent an ongoing obstacle to the integrity and function of the endothelial vasculature. The deleterious interaction between antibodies and the endothelium has been identified as a major pathomechanism responsible for the acute inflammatory process and chronic progression of vascular lesions in vasculopathic disorders in both autoimmunity and alloimmunity.[1] In transplantation, human leukocyte antigen (HLA) antibodies are well recognized to induce changes in the allograft vasculature resulting in acute antibody-mediated rejection and progression to transplant vasculopathy leading to allograft destruction.[2] In contrast, the clinical effects induced by non-HLA antibodies have been much less appreciated and the description of their mechanisms of action on allograft vasculature remains at a preliminary stage.

HLA antibodies exert effector functions that mediate endothelium damage by several mechanisms. The crystallizable fragment (Fc)-dependent effector functions of HLA antibodies are the most characterized and include effector leukocyte (natural killer cells, T cells, and monocytes) recruitment and activation as well as macrophage polarization. Glycosylation status of HLA antibodies can also significantly modulate and alter these outcomes. In addition, HLA antibodies can activate the classical pathway of the complement system by binding of their Fc region to the complement protein C1q, which initiates a cascade of proteolytic cleavage steps and results in formation of membrane attack complexes (consisting of the complement products C5b to C9) on endothelial cell surface leading to cell destruction.[3] Although most effector functions of HLA antibodies are elicited via their Fc region, recent evidence describes that binding of antigen-binding fragment regions to HLA molecules triggers intracellular signaling downstream in endothelial cells leading to their upregulation of adhesion molecules and their production of inflammatory cytokines and chemokines as well as anaphylatoxins, both of which favor further leukocyte recruitment and activation. Interestingly, evidence suggests that HLA class I and class II antibodies may exert differential and nonredundant Fc-independent effects of endothelial cells. HLA class I antibodies bind to class I molecules and integrins and transduce signals leading to the activation of endothelial cell survival and proliferation pathways including Src, focal adhesion kinase, and mammalian target of rapamycin (mTOR), as well as the downstream targets extracellular-signal regulated kinase (ERK), S6 kinase (S6K), and S6 ribosomal protein (S6RP). These events lead to increased intercellular adhesion molecule 1 expression and clustering and favor monocyte adhesion to the endothelium.[4] HLA class II antibodies bind to class II molecules and activate mTOR/Akt, S6K, and S6RP, leading to interleukin-6 and regulated on activation, T-cell expressed, and secreted production[5] (Figure 1).

Compared with the accumulating knowledge in the deleterious effects of HLA antibodies on endothelial vasculature, that of non-HLA antibodies is still emerging. Although it has been implied that non-HLA antibodies in the pathogenesis of antibody-mediated rejection is suspected and considered complement-independent, so far, little was known regarding the mechanisms by which non-HLA antibodies mediate endothelial damage.[1]

[1]*Paris Translational Research Center for Organ Transplantation, Institut National de la Santé et de la Recherche Médicale, Unite de Mixte Recherche S970, Université de Paris, Paris, France;* [2]*Kidney Transplant Department, Saint Louis Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France; and* [3]*Kidney Transplant Department, Necker Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France*

Correspondence: *Carmen Lefaucheur or Alexandre Loupy, Paris Translational Research Center for Organ Transplantation, Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche S970, Paris, France. E–mail: carmen.lefaucheur@wanadoo.fr or alexandreloupy@gmail.com*

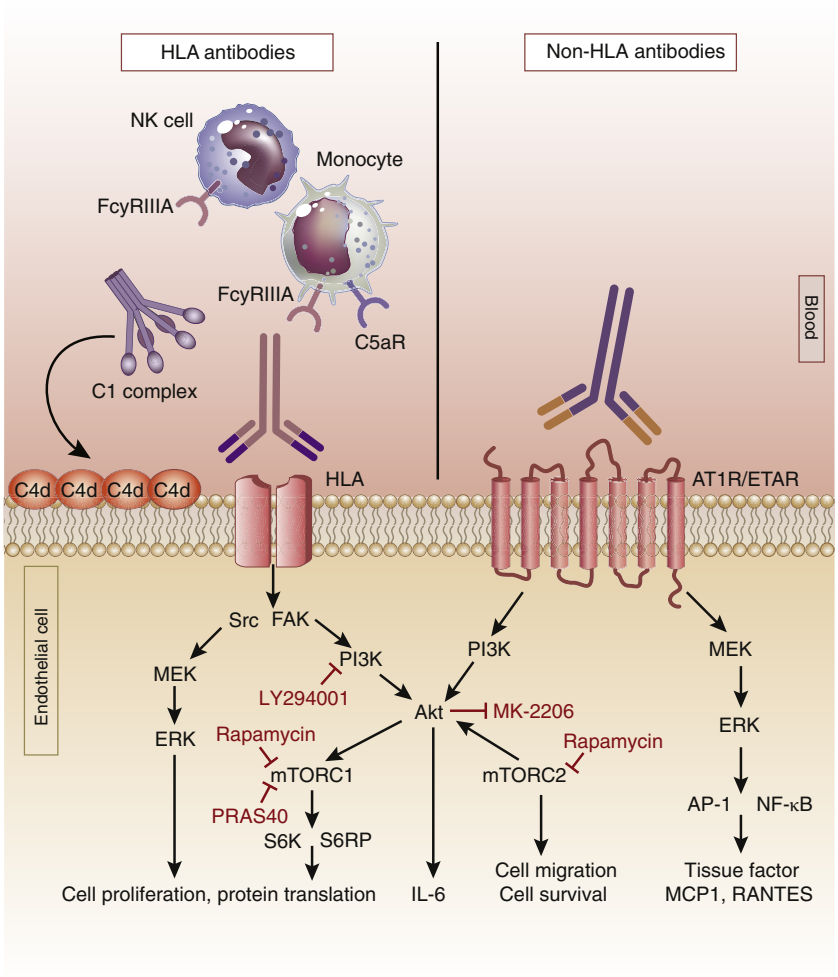

Figure 1 | Mechanisms of human leukocyte antigen (HLA) and non-HLA antibody-induced endothelial cell changes. AP-1, Activator protein-1; AT1R, angiotensin II type 1 receptor; C5aR, complement component 5a receptor; ETAR, endothelin-1 type A receptor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; IL, interleukin; MCP1, monocyte chemoattractant protein-1; MEK, Mitogen-activated protein kinase kinase; mTORC, mammalian target of rapamycin complex; NF-κB, nuclear factor-κB; NK, natural killer; PI3K, phosphatidyl-inositole-3 kinase; PRAS40. proline-rich Akt substrate-40; RANTES, regulated on activation, T-cell expressed, and secreted; S6K, S6 kinase; S6RP, S6 ribosomal protein.

In this issue, Catar *et al.*[6] sought to identify the mechanisms by which the 2 major non-HLA antibodies, angiotensin II type 1 receptor (AT1R) and endothelin-1 type A receptor (ETAR), may stimulate their endothelial receptor and impair endothelial cell activation and function. Based on their previous findings that suggest the involvement of activated signaling pathways in endothelial vasculature of patients who have received transplants undergoing AT1R and ETAR antibody-mediated rejection, Catar *et al.*[6] here hypothesized that AT1R and ETAR antibody binding to endothelial cells may transduce ERK and mTOR pathways, which may impair endothelial cell repair.

Catar *et al.*[6] established an *in vitro* cellular model and microscopic studies based on purified AT1R and ETAR IgG antibodies, from sera of recipients of kidney transplants undergoing obliterative transplant vasculopathy, incubated with cotransfected human embryonic kidney 293 cells or human microvascular endothelial cells-1. The intracellular downstream signaling, dependency on the cellular partner (β-arrestin, a G-protein coupled receptor adapter protein regulating receptor signaling), and functional capacity to repair after injury of human microvascular endothelial cells-1 were analyzed. The cell cultures were also done in the presence of the natural ligands angiotensin II and endothelin-1, pharmacologic antagonists of AT1R and ETAR, and inhibitors of mTOR complexes (mTORC1 and mTORC2) and ERK pathways. Catar *et al.*[6] found that AT1R/ETAR antibodies from patients with transplant vasculopathy impaired endothelial repair in an AT1/ETAR-dependent manner involving phosphatidylinositol-3′-kinase (PI3K)-dependent mTORC2 hyperactivation and sustained activation was supported by β2-arrestin recruitment. In their study, Catar *et al.*[6] provided the following lines of evidence for this mechanism: (i) AT1R/ETAR antibodies activate both mTORC1 and mTORC2 via AT1R and ETAR; (ii) upstream regulation of mTORC1 and mTORC2 activation is

PI3K-dependent but ERK-independent; (iii) the mTOR inhibitor rapamycin abolishes activation of mTORC1 and mTORC2 induced by AT1R/ETAR antibodies; (iv) β2-arrestin, but not β1-arrestin, is recruited to ETAR in response to AT1R/ETAR antibodies and β2-arrestin silencing significantly reduces ERK and mTORC2 activation; (v) AT1R/ETAR antibodies impair endothelial repair by AT1R- and ETAR-induced mTORC2. However, rapamycin (which inhibits both mTORC1 and mTORC2) worsen wound healing. Catar *et al.*[6] concluded that AT1R/ETAR antibodies induced mTORC2 activation involving β2-arrestin in vascular endothelium and that mTORC2 inhibitors may represent a therapeutic option for patients who have received transplants with transplant vasculopathy-associated with AT1R/ETAR antibodies. In this study, Catar *et al.* [6] establish a novel mechanism of action of non-HLA AT1R/ETAR antibodies on the vascular endothelium and provide a translational rational for the potential use of mTORC2 inhibitors, in combination with AT1R/ETAR blockers for therapeutic blockade.

Despite the detailed mechanistic analyses and exploration of several signaling pathways using sera from transplant patients, there are several limitations in Catar *et al.*[6] study that should be discussed. First, the findings rely solely on *in vitro* data, without of evidence of similar effects of AT1R/ETAR antibodies or of systemic drug administration *in vivo* in transplant animal models or patients who have received transplants. Second, the limited number of patients included significantly restricts the exportability of the findings in patient populations and cannot address the heterogeneity in individual patients. Third, the limited amount of patient material available has also significantly curbed the capacity to perform quantifications, biological replicates, or dynamic microscopic studies. However, this seminal study opens several avenues of investigation for future efforts of the transplant community for improving of the understanding of pathomechanisms of actions of non-HLA antibodies.

Importantly, the study by Catar *et al.*[6] highlights 2 potential clinical implications: (i) the use of mTOR complexes' components as biomarkers of endothelial cell activation; and (ii) targeting mTOR pathway or their specific mTOR complexes to preserve endothelial cell function during antibody-mediated vasculopathies. In the study by Catar *et al.*,[6] S6K phosphorylation, reflecting mTORC1 activation was induced after ligation of AT1R/ETAR antibodies to their receptors. Interestingly, S6K phosphorylation has also been previously shown to be induced after ligation of HLA antibodies to HLA class I or class II molecules, leading to endothelial cell proliferation.[3] S6K phosphorylation also manifested as a biomarker with good sensitivity and specificity in heart allograft biopsies, showing antibody-mediated rejection from patients with donor-specific HLA class I and class II antibodies, regardless of the presence of C4d deposition. S6RP phosphorylation was also found induced in heart antibody-mediated rejection and during antiphospholipid syndrome, which is another severe vasculopathic disorder.[7] In the study by Catar *et al.*,[6] phosphorylated Akt, reflecting mTORC2 activation, was induced after ligation of AT1R/ETAR antibodies to their receptors, which is also consistent with its induction after HLA class II or antiphospholipid antibody stimulation of endothelial cells. However, before phosphorylated components of mTORC1 or mTORC2 pathways can be implemented as biomarkers in vasculopathies, larger independent studies are needed for clinical validation.

Because there are numerous immune and nonimmune effects of mTOR complexes on cell biology, targeting the mTOR pathway has long been an ambitious goal in organ transplantation. mTOR inhibitors represent an alternative to calcineurin inhibitors for preventing long-term nephrotoxicity as well as for limiting cardiovascular or carcinogenic events associated with use of calcineurin inhibitors.[8] Yet, although their favorable effects on organ function can be measured in the clinic, the mechanisms of action of mTOR inhibitors on vessels is a growing field and the fine balance needed between mTORC1 and mTORC2 activities in the endothelium remains to be investigated. In the study by Catar *et al.*,[6] mTORC2 inhibition via the Akt inhibitor MK-2206 and the PI3K inhibitor LY294001 significantly reversed the endothelial repair alteration induced by AT1R/ETAR antibodies, improving wound healing. However, treatment with rapamycin, which inhibits both mTORC1 and mTORC2, worsened wound healing, suggesting a paradoxical effect of nonspecific mTOR inhibition in comparison to specific mTORC2 inhibition. In *in vitro* models using HLA antibodies, mTORC2 inhibition by Akt inhibitor significantly diminished HLA class II antibody-induced endothelial cell activation and production of interleukin-6, and mTORC1 inhibition by proline-rich Akt substrate-40 also significantly reversed endothelial cell activation.[5–9] These findings suggest that HLA class II antibodies may signal along both mTORC1 and mTORC2 in endothelial cells. Antiphospholipid antibodies may also signal along both mTORC1 and mTORC2 *in vitro* and in vessels from patients with antiphospholipid syndrome. Consistently, patients who received kidney transplant and were receiving sirolimus had no recurrence of vascular lesions after transplant and had decreased vascular proliferation on allograft biopsy as compared to patients with antiphospholipid antibodies who did not receive sirolimus.[7] However, there is a lack of evidence in patients with HLA donor-specific antibodies receiving mTOR inhibitor on whether these patients develop less vascular lesions than those who do not receive mTOR inhibitor.

In conclusion, emerging data suggest a role for the activation of mTOR signaling cascade triggered by HLA and non-HLA antibodies in mediating endothelial dysfunction and in promoting human vasculopathies in transplant medicine and beyond. However, more studies investigating the clinical impact and mechanisms of action of non-HLA antibodies are warranted to identify the specificity and synergy of their effects with the HLA system. Interfering with mTOR complexes should be considered with

caution and should account for the fine balance that exists between mTORC1 and mTORC2 activities before it can be used for optimal protection against in HLA and non-HLA antibody-mediated injuries and preservation of homeostasis of the endothelial vasculature.

DISCLOSURE

All the authors declared no competing interests.

REFERENCES

1. Lefaucheur C, Louis K, Philippe A, et al. The emerging field of non-human leukocyte antigen antibodies in transplant medicine and beyond. *Kidney Int*. 2021;100:787–798.
2. Loupy A, Lefaucheur C. Antibody-mediated rejection of solid-organ allografts. *N Engl J Med*. 2018;379:1150–1160.
3. Valenzuela NM, Reed EF. Antibody-mediated rejection across solid organ transplants: manifestations, mechanisms, and therapies. *J Clin Invest*. 2017;127:2492–2504.
4. Salehi S, Sosa RA, Jin Y-P, et al. Outside-in HLA class I signaling regulates ICAM-1 clustering and endothelial cell-monocyte interactions via mTOR in transplant antibody-mediated rejection. *Am J Transplant*. 2018;18:1096–1109.
5. Cross AR, Lion J, Poussin K, et al. HLA-DQ alloantibodies directly activate the endothelium and compromise differentiation of FoxP3high regulatory T lymphocytes. *Kidney Int*. 2019;96:689–698.
6. Catar RA, Wischnewski O, Chen L, et al. Non-HLA antibodies targeting angiotensin II Type 1 receptor and endothelin-1 Type A receptors induce endothelial injury via β2-arrestin link to mTOR pathway. *Kidney Int*. 2022;101:498–509.
7. Canaud G, Bienaimé F, Tabarin F, et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. *N Engl J Med*. 2014;371:303–312.
8. Budde K, Becker T, Arns W, et al. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. *Lancet*. 2011;377:837–847.
9. Lion J, Taflin C, Cross AR, et al. HLA class II antibody activation of endothelial cells promotes Th17 and disrupts regulatory T lymphocyte expansion. *Am J Transplant*. 2016;16:1408–1420.

LPCing through the nephron accelerates diabetic kidney disease

Jeffrey D. Pressly[1], Mengyuan Ge[1] and Alessia Fornoni[1]

Lipid dysmetabolism is emerging as an important contributor to diabetic kidney disease, suggesting that intrarenal lipid accumulation is detrimental to kidney function. This commentary discusses the finding by Yoshioka *et al.*, connecting tubular lipotoxicity induced by an increase in locally produced lysophosphatidylcholine in patients with a fast progression of diabetic kidney disease, known as "fast decliner." Insight into the lipid species in the kidney may prove beneficial for the diagnosis and stratification of patients with diabetic kidney disease.

Kidney International (2022) **101,** 454–456; https://doi.org/10.1016/j.kint.2021.12.013

see basic research on page 510

[1]*Katz Family Division of Nephrology and Hypertension/Peggy and Harold Katz Drug Discovery Center, Department of Medicine, University of Miami Miller School of Medicine, Miami, Florida, USA*

Correspondence: *Alessia Fornoni, Katz Family Division of Nephrology and Hypertension/Peggy and Harold Katz Family Drug Discovery Center, University of Miami, 1580 NW 10th Avenue, Miami, Florida 33136, USA. E-mail: afornoni@med.miami.edu*

Lipids are essential constituents of cell membranes and important components in energy production. Dysregulation of lipid metabolism contributes to several forms of chronic kidney diseases, including diabetic kidney disease (DKD).[1] Although circulating lipids have been reported to play a role in kidney disease development and progression, the contribution of renal accumulation of lipids and impaired renal lipid metabolism to the progression of DKD has recently gained attention. Excess lipids are stored in lipid droplets (LDs), the lipid-rich intracellular organelles that are composed of triglyceride and cholesterol ester core and are shielded by a phospholipid monolayer and an outer layer of protein. LDs can be degraded via lipolysis and lipophagy (autophagic degradation of LD), which tightly regulate the turnover of intracellular lipids. LD contents, including cholesterol and phospholipid, can also be effluxed from the cell by adenosine triphosphate–binding cassette transporters subclass A (ABCA1), and reduced ABCA1 expression has been reported in glomeruli of patients with DKD, in normal human podocytes exposed to serum from patients with DKD, and in an experimental mouse model of DKD, where ABCA1 deficiency contributes to mitochondrial dysfunction and disease progression.[2]

Among several "toxic" lipids, including oxysterols, diacylglycerol, fatty acids, and cholesterol, lysophosphatidylcholine (LPC) is a phospholipid derived from the cleavage of phosphatidylcholine by phospholipase A2 and/or the transfer of fatty acids from phosphatidylcholine to cholesterol via lecithin-cholesterol acyltransferase. On the contrary, LPC can be converted back to phosphatidylcholine by acyl-CoA:LPC acyltransferase. The increase of LPC levels is associated with diseases, such as diabetes, cardiovascular diseases, cancers, and kidney failure. LPC is enriched in oxidized low-density lipoprotein, and the accumulation of LPC in low-density lipoprotein particles and tissue causes lipotoxicity and is positively correlated with disease development. The evidence that lecithin-cholesterol acyltransferase deficiency causes nephrotic range proteinuria, leading to end-stage kidney failure in affected patients, strongly supports the possibility that LPC may be a key mediator of this phenotype. Furthermore, increased LPC production may occur at the early stage of DKD, as demonstrated in more comprehensive lipidome profiling of rats with DKD.[3]