The Mechanism of Kidney Disease Due to APOL1 Risk Variants

Etienne Pays

Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, Gosselies, Belgium

The apoL1 C-terminal variants G1 and G2 are linked to CKD.1 Expression of these variants induces effacement of podocyte foot processes, leading to the loss of these cells from glomeruli and impairment of the blood filtration activity of the kidney. A characteristic of G1/G2-linked disease is the strong association of glomerular pathology with the type I IFN inflammatory response, such as occurs with viral infection.1

In podocytes, either truncation of the C-terminal helix of apoL1 or deletion of apoL3 similarly induces reorganization of actomyosin activity, resulting in a reduction of their cellular size and adherence, and an increase of motility.2 This phenotype is linked to a reduction of Golgi phosphatidylinositol-4-phosphate [PI(4)P] levels, suggesting inactivation of the Golgi phosphatidylinositol 4-kinase PI4KB. In various podocyte lines derived from G1, G2, or G1/G2 patients, significant reduction of Golgi PI(4)P levels has also been observed.2 ApoL3 was found to stimulate Golgi PI(4)P synthesis by PI4KB, and C-terminal apoL1 variants exhibit interaction with apoL3 due to increased hydrophobicity.2 Thus, the apoL1 variants appear to inhibit the PI4KB-stimulating activity of apoL3 through apoL3 binding.2

The reduction of PI(4)P levels is known to affect actomyosin activity and cellular mobility.3 Moreover, type I IFN strongly stimulates the expression of apoL1 and apoL3, and these proteins are involved in the initiation of autophagy, mitochondrial fission, and apoptosis.2,4,5 Such activities may depend on the apoL3-mediated control of PI4KB, because autophagy and mitochondrial fission are dependent on the transfer of PI4KB-containing vesicles carrying the autophagy marker ATG9A from the Golgi to the endoplasmic reticulum (ER).4,6,7

Thus, podocyte dysfunctions could result from interference of apoL1 G1/G2 variants with PI4KB activity. This conclusion differs from that of many studies, which have concluded that G1 and G2 induce nonspecific cytotoxicity.1 Whereas some observed dysfunction traits could be explained by PI(4)P reduction (altered vesicular trafficking and mitochondrial activity, for instance), I argue that cytotoxicity could only result from an experimental increase of natural apoL1 levels.

- Either in natural G1 or G2 podocytes, or in genetically edited podocytes expressing C-terminal truncated apoL1 without change of gene expression level, no sign of cytotoxicity was detected.2 C-terminal apoL1 truncation even induced a strongly reduced potential for apoptosis, in striking contrast with the induction of apoptosis by ectopic G1 or G2 in various experimental settings.

- If overexpressed as occurs after ectopic expression, even wild-type apoL1 triggers nonspecific toxicity.8 This finding is in keeping with the toxicity exhibited by wild-type apoL1 when ingested in trypanosomes or transfected in yeast or *Escherichia coli*.2

In this respect, it is worth noting that the strong increase of apoL1 expression induced by the viral mimetic poly(I:C) is linked to apoL1 involvement in apoptosis.2,5 It is tempting to propose that, as occurs for apoptotic Bcl2 proteins,9 apoL oligomerization resulting from high protein expression triggers the formation of mitochondrial megapores.10 As occurs for Bcl2 homologous antagonist killer (Bak) or Bcl2–associated X (Bax), hydrophobic residues exposed on apoL helices may contribute to generate lipidic membrane pores by surface interaction with cardiolipin (CL).4,9

- In podocytes, intracellular apoL1 is processed differently from the secreted version of this protein (isoform 1).2 ApoL1 isoform 3, which is encoded by a transcript variant, is not targeted to the secretory pathway, but resides at the cytoplasmic face of the ER, together with apoL3.2,11 Interestingly, this isoform lacks the exon 4 sequence, which exhibits strong cytotoxicity when present in ectopically expressed apoL1.12

ApoL1-mediated cytotoxicity is reported to result from cationic pore formation at the plasma membrane.1,11,13

Because podocytes secrete apoL1

Published online ahead of print. Publication date available at www.jasn.org

Correspondence: Dr. Etienne Pays, Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, Gosselies, Belgium. Email: epays@ulb.ac.be

Copyright © 2020 by the American Society of Nephrology
isoform 1, it is not surprising to find this isoform in the secretory pathway and on the cell surface. However, given its dependence on acidic pH for membrane insertion, in order to generate surface pores apoL1 must travel through the endocytic pathway and be recycled back to the plasma membrane. Because independent studies did not detect apoL1 in the endocytic pathway, this hypothesis can be ruled out. Moreover, if apoL1 recycling cannot be envisaged to explain trypanosome lysis, this process is difficult to justify in podocytes, and does not explain why the G1 or G2 variants would trigger toxic pore activity. Conversely, reducing Golgi PI(4)P levels can affect cation transport at the cell surface.

I propose that kidney disease results from cellular reorganization induced by apoL1-mediated inactivation of apoL3 that, via PI4KB, controls vesicular trafficking at the Golgi (Figure 1, hit 1) and membrane fusion activities at ER–mitochondrion contact sites (MERCs) (Figure 1, hit 2).

- In accordance with the phenotype expected after PI(4)P reduction, kidney disease induced by apoL1 variants in transgenic mice involves modifications of podocyte vesicular traffic and autophagy.
- Significantly reduced levels of PI(4)P were measured not only in various podocytes expressing C-terminal apoL1 variants, but also in glomeruli of kidney biopsies from human G1 or G2 patients.
- In line with observations regarding PI(4)P reduction in podocytes, kidney disease is not only genetically linked to apoL1 G1 and G2, but also to natural apoL3 knockout. The podocyte dysfunction phenotype resulting from apoL3 knockout in either podocyte lines or human individuals cannot be explained by the cytotoxicity of apoL1 variants.
- The proposed pathogenic effect of increased hydrophobicity in apoL1 variants easily explains the puzzling observation that the recruitment of these variants to lipid droplets attenuates the effects of G1/G2.
- The fact that kidney disease results from actomyosin reorganization is in line with the induction of this disease after mutations in actomyosin components such as those involved in mitochondrial fission. Given the particular cytoskeleton constraints required for the biology of podocytes, such as the architecture of foot processes, the high susceptibility of these cells to actomyosin changes can readily be explained.
- The increased mitochondrial fission observed after either apoL3 inactivation or deletion strikingly parallels the spectacular inhibition of mitochondrial fission observed in trypanosomes with ingested apoL1, proving that apoL1 or apoL3 can control mitochondrial fusion/fission in quite distinct cellular types. Accordingly, the G1 and G2 variants, but not wild-type apoL1, trigger mitochondrial fission. Given the importance of CL and phosphatidic acid in mitochondrial fission or fusion, the strong binding of apoL1 and apoL3 to both lipids is likely to be involved in the control of this activity.

**Figure 1.** ApoL1 G1- or G2-mediated inhibition of apoL3 can explain kidney disease. ApoL3 controls PI(4)P synthesis by PI4KB. Under normal conditions, inhibition of PI4KB at the trans-Golgi network triggers reorganization of actomyosin-driven vesicular trafficking (hit 1). Under starvation or inflammatory conditions like viral infection, induction of autophagy and mitochondrial fission occur through actomyosin-driven transfer of PI4KB-carrying ATG9A vesicles from the Golgi to the ER, coupled with the reciprocal transfer of the PI(4)P phosphatase Suppressor of actin (Sac1) from the ER to the Golgi. Thus, the site of PI(4)P synthesis moves from the Golgi to the ER to trigger expansion of autophagy membranes rather than expansion of vesicular membranes. Inhibition of PI4KB at the ER-mitochondrion contact sites (MERCs) interferes with initiation of autophagy and mitochondrial membrane fusion/fission activities, preventing autophagy-mediated rescue from infection damage (hit 2). NM2A, nonmuscle myosin 2A, strongly associated with apoL1 and apoL3.
Mitochondrial fission can be involved in autophagy.23,24 The binding of apol3 to mitochondrial phosphatidic acid and CL could promote membrane fusion required for phagophore extension.24 Apol3 also stimulates PI4KB, which triggers the initiation of autophagy.6 Presumably due to the low renewal rate of podocytes, interference with autophagy is expected to exacerbate the pathology of kidney disease.25 Indeed, the inhibition of autophagy due to the inactivation of apol3 is expected to result in a loss of podocyte ability to rescue from infection damage. This could explain the effect of viral infection on G1/G2-linked kidney disease.

Given their effects on Golgi PI(4)P levels, the G1 and G2 variants should not only affect podocytes, but also other cell types with intense intracellular membrane trafficking. This could be the case of neurons, where neurotransmission heavily involves secretion and where autophagy is important to compensate for low cellular renewal. This could account for the genetic association of apol3s with neurotransmission disorders.4 Thus, it seems worth investigating whether neurotransmission is affected in G1 or G2 individuals.

To solve the G1/G2-linked pathology, increased production of lipid droplets can probably rescue the pathology due to increased hydrophobicity of the apol1 variants.18 Considering that apol1 appears to be dispensable in the absence of African trypanosomases,28 strategies aiming to achieve apol1 elimination could probably also solve the problem without side effects. Whether apol1 is already under counterselection out of Africa is not known, but the late appearance of the disease during life could possibly limit the strength of such a process.

FUNDING

This work was financially supported by the European Research Council grant 669007-APOLa.

ACKNOWLEDGMENTS

The content of this article reflects the personal experience and views of the author(s) and should not be considered medical advice or recommendations. The content does not reflect the views or opinions of the American Society of Nephrology (ASN) or JASN. Responsibility for the information and views expressed herein lies entirely with the author(s).

REFERENCES


DISCLOSURES

The author has nothing to disclose.
27. Del Bel LM, Brill JA: Sac1, a lipid phosphatase at the interface of vesicular and non-vesicular transport. Traffic 19: 301–318, 2018