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APOL1 nephrotoxicity: what does ion transport got to do with it?

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Summary

Apolipoprotein L1 (APOL1) protein is the human serum factor that protect humans against *Trypanosoma brucei brucei*, the cause of trypanosomiasis. Sub species of *T.b. brucei* that cause human sleeping sickness — *T.b. gambiense* and *T.b. rhodesiense* evolved molecular mechanisms that enabled them to evade killing by APOL1. Sequence changes (termed G1 and G2) in APOL1 gene that restored its ability to kill *T.b. rhodesiense* also increase risk of developing glomerular diseases and accelerate progression to end stage kidney disease. To lyse trypanosome parasites, APOL1 forms pores in the trypanosome endolysosomal and mitochondrial membrane resulting in rapid membrane depolarization. However, the molecular mechanism underlying APOL1 nephropathy is unknown. Recent experimental evidence shows that aberrant efflux of intracellular potassium is an early event in APOL1-induced death of human embryonic kidney (HEK) cells. Here we discuss the possibility that abnormal efflux of cellular potassium or other cations may be relevant to the pathogenesis of APOL1 nephropathy.

Keywords

Apolipoprotein L1; focal segmental glomerulosclerosis; African-American; end stage kidney disease

Introduction

African Americans develop end stage kidney disease (ESKD) at rates that are up to five times higher than Americans of European descent (1). This excess risk of ESKD is largely attributable to 2 coding mutations (G1 and G2) in the C terminus of the APOL1 gene. Compared to the most common variant of APOL1 (G0), G1 contains 2 amino acid substitutions (S342G and I384M), while G2 is a result of a 2 amino acid deletion

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(del388N389Y) (2, 3). The specific molecular mechanisms by which renal risk variants APOL1 (G1 and G2) cause and accelerate kidney disease in human has been elusive. Expression of either G1 or G2 in cultured human cells elicits heightened cytotoxicity compared to G0 expression, and thus have been the model for research efforts at elucidating the mechanisms of APOL1 nephropathy by (4–7). *In vitro* expression of G1 or G2 APOL1 in various cells of human origin has been reported to increase cell death by several mechanisms including necrosis, pyroptosis, apoptosis and autophagy (6–12). The exact mechanism by which G1 and G2 APOL1 cause mammalian cell death is the focus of ongoing research.

Recent experimental evidence suggests an interesting parallel between APOL1 trypanocidal activity and APOL1-induced mammalian cell death. Trypanocidal activity of APOL1 resides in its ability to form lethal ion pores in trypanosome lysosomal and mitochondrial membranes (13–16). The exact ions transported by APOL1 are the subject of ongoing debate. In planar lipid bilayers, APOL1 forms cation-selective pores that are permeable to sodium and potassium ions (13, 15, 17). Therefore, recent report that in human embryonic kidney (HEK) cells, the cytotoxicity of G1 and G2 APOL1 is mediated by aberrant efflux of cellular potassium (7) raises the possibility that the mechanism of APOL1 trypanocidal activity may be related to mechanism of APOL1 mammalian cytotoxicity. Here, we reviewed the literature on APOL1 as an ion channel, discussed cell-based models of APOL1 toxicity and how understanding of APOL1 channel function in human cell culture models could advance our understanding of APOL1 nephropathy.

PATHOMECHANISM OF APOL1 TOXICITY TO MAMMALIAN CELLS

The pore forming domain of APOL1: a story of pluses and minuses

In the most recent reviews, cytotoxic effects of APOL1 are postulated to stem from an aberrant pore formation in a cellular membrane, allowing a flow of ions, disrupting homeostasis, eventually leading to a cascade of death pathways (18, 19). However, the most fundamental aspect of the pore has been controversial, with some reports proposing cytotoxicity stems primarily from a flow of negatively charged ions (5, 6, 14, 16, 20) while others find a more primary role for the flux of positively charged ions (Table 1) (7, 13, 15, 21, 22). Although the opposite nature of cations and anions may seem to facilitate clear conclusions, the true effects in a cellular system may be difficult to parse, since the disruption of one type of charge in a cellular system will often elicit a compensatory mechanism involving flux of the opposite charge.

The capacity of human serum to lyse certain trypanosomes was first reported over a century ago, and the identification of HDL particles as the vehicle for trypanosome lytic factor (TLF) has been known for over two decades (23). Alternatively, the identification of APOL1 as the key component of the TLF was reported a little over a decade ago (24) and the association of APOL1 variants with kidney disease was reported within the previous decade (2). It's uncertain that the biochemical mechanisms of APOL1 cytotoxicity in *Trypanosoma brucei* are similar or the same to the biochemical mechanisms of toxicity to podocytes by the APOL1 variants. Still much has been learned by studying the effects of TLF on trypanosomes thus providing the initial basis for the investigation APOL1 variants in FSGS.

Much of the early work on TLF examined how the HDL particle was taken up by trypanosomes and the morphological changes in the pathogens associated with death. However, in 1984 Rifkin proposed ionic flux as a connection between the endocytosis of TLF and the subsequent lysis of *Trypanosoma Brucei*. Using radiolabeled isotopes of monovalent and bivalent cations, Rifkin determined normal human serum (NHS) dramatically increased flux of both potassium and calcium, but concluded the primary event in the cytotoxic cascade was a loss of K^+ (22). Rifkin's work was not expanded upon for many years, although one other investigation found that the trypanolytic factor is inhibited by Zn^{2+} at a maximal concentration of 0.5 mM (25). While not specifically stated at the time, these data could be seen as consistent with a cationic pore block.

More than 20 years after Rifkin made the connection between ion gradients and cytotoxic effects of APOL1 two important papers came out in 2005, almost concurrently, addressing the subject of ionic conductance through the trypanosome lytic factor (TLF). The first paper from the lab of Etienne Pays put forth a model in which APOL1 transports negatively charged anions, while the second paper from the lab of Jayne Raper argued that the TLF was selective for positively charged cations. These papers established the controversy between anionic or cationic conductance through APOL1, even though both papers reported use of planar lipid bilayers to investigate the function of the trypanolytic factor appeared (13, 14). Planar lipid bilayers are aptly named: two monolayers of lipid molecules which form a small planar surface to separate two aqueous compartments. Into this simplified recapitulation of a cellular membrane, proteins may be incorporated and the flux of ions activity can be measured as electrical current.

The first of the two papers is arguably the most seminal paper on structure and function of APOL1 (14). In it Perez Morgia and colleagues predicted the function of APOL1 based on putative structural homology to other proteins with known function. The authors proposed that the N-terminal portion of APOL1 forms a pore based on its homology to the bacterial pore forming toxin, colicin A (26, 27). Although colicin A was reported to be highly specific transporter of K^+ (26) the authors of the study determined APOL1 to have evolved to transport Cl^- . They concluded that APOL1 is activated on the lysosomal membrane, allowing a flow of chloride into the lysosome. To compensate for the chloride depletion in the cytoplasm a 4,4'-Diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS) sensitive chloride transporter at the cell surface is upregulated. In this same paper Perez Morgia and colleagues were able to rescue trypanosomes from the toxic effects of APOL1 using either exogenous DIDS or chloride depleted extracellular medium.

Although the second paper presented evidence that cytotoxicity stems from cation flux (13), Molina-Portela and colleagues corroborated several of the findings by Perez Morgia *et al.* Both papers assumed APOL1 was initially activated by low pH at the lysosome but the cationic model posited that some component of the TLF translocated to the cell surface to allow the flux of cations. Also, both papers detected an increase in chloride flux through the plasma membrane, but Molina-Portela *et al.*, assumed that the increased chloride flux was a compensation for the increased cationic current, specifically a sodium current.

There were striking differences between the two experimental approaches in the opposing papers. Perez Morga and colleagues had used a truncated portion of APOL1 whereas Molina-Portela and colleagues had used a presumable full-length APOL1, but as a part of large complex of other proteins and lipids. Thomson and Finkelstein addressed both caveats a decade later when they incorporated full length recombinant APOL1 into planar lipid bilayers and investigated its functional properties (15). They found APOL1 induced conductance that is “ideally cation selective.” Moreover, they found the APOL1-induced conductance required an initial exposure to acidic environment (pH 5.3) but alkalization of environment would increase the magnitude of conductance by 300 orders of magnitude with a pKa of 7.1. These data taken together would validate the model put forth by Molina-Portela et al.

It should be noted that John Edwards also presented data on full length recombinant APOL1 incorporated into planar lipid bilayers. He also found that an acidic environment (pH 5) was necessary for incorporation of APOL1 into the membrane. However, the bulk of his data were in stark contrast to Thomson and Finkelstein’s. He found APOL1 to be Cl⁻ selective across a range of pHs and alkalization of the milieu did increase the magnitude of conductance.) (ASN poster presentation 2015).

Thus, there are two models of APOL1 ion conductance: a pH sensitive cationic model and a pH insensitive anionic model. A clearer understanding of how the G1 and G2 variants lead to nephropathy may come from analysis of the data from cell lines discussed later in this review.

Is the subcellular localization of APOL1 a relevant to its cytotoxicity?

Membrane localization of APOL1 (to endolysosome and mitochondrial) is essential to its trypanocidal activity (16). *T.b.rhodesiense* and *T.b. gambiense* were able to evade the trypanocidal effect of wild type APOL1 by evolving proteins (SRA and TgsGP, respectively) which prevent membrane insertion of APOL1. Sequence changes in G1 and G2 APOL1 restore their ability to lyse *T.b.rhodesiense* by reinstating their ability to once again insert into the endolysosomal membranes (28). This phenomenon raises the possibility that APOL1 toxicity in mammalian cell may also be a function of its subcellular localization. While the cytosolic localization of APOL1 is well known (see Fig. 1) and reported (29, 30), knowledge about the dynamic regulation of APOL1 subcellular localization in mammalian cell is lacking. Comprehensive study APOL1 subcellular localization both at baseline and in pathologic states could provide useful insights.

Target cells of APOL1 toxicity in the kidney?—Risk variants APOL1 are associated with increased risk of a wide spectrum of glomerular diseases including HIV associated nephropathy (HIVAN), FSGS, and lupus nephritis (2, 3, 31). APOL1 is expressed in many tissues including the kidney (32). In the glomeruli, APOL1 expression is seen in podocytes and endothelial cells but not in mesangial cells (29, 30). Because current understanding of pathomechanism of HIVAN and FSGS emphasizes the role of podocyte injury and loss, many investigations of APOL1 nephropathy presume that podocytes could be the targets of APOL1 cytotoxicity and that the subsequent podocyte loss could be the cause of the

glomerular sclerosis. This view is supported by the observation that *in vitro* expression of risk variants APOL1 in human podocytes increased cell death (5). However, a recent report on transgenic mice in which podocyte APOL1 expression is driven by nephrin promoter presents a challenge to the podocentric view of APOL1 nephropathy (33). Despite robust expression of G2 APOL1 in these mice podocytes, there was no visible podocyte injury or podocyte death nor did the mice develop evidence of kidney disease (33). One possible interpretation of these findings is that expression of risk variants APOL1 solely in the podocyte may not be sufficient to initiate or propagate APOL1- nephropathy in mice. Concurrent expression of APOL1 in glomerular endothelium may also be necessary. Study of transgenic mice with ubiquitous APOL1 expression under its own promoter in all relevant kidney compartments would be illuminating. In addition, a second event, concurrent or sequential to APOL1 expression may be necessary for the appearance of APOL1 nephropathy in this animal model.

A second finding from above mentioned transgenic APOL1 mice raises an alternate interpretation. By 200 days of age, transgenic mice that express G2 APOL1 (compared to those that express G0 APOL1) in their podocytes were found with significantly lower podocytes density—despite lack of visible evidence of podocyte injury or podocyte death (33). This observation raises the possibility that G2 APOL1 indeed cause podocyte loss perhaps through mechanism that was not detected or quantifiable by the chosen experimental method. Because a minimum threshold of podocyte loss is required for the emergence of glomerular sclerosis (34), the argument can be made that reason why the G2 APOL1 transgenic mice did not develop kidney disease was because the requisite podocyte loss was not achieved—either because more time was required or because the mouse podocytes have a cytoprotective mechanism(s) against APOL1 toxicity that human podocytes lack.

What Manner of Death?—In order to understand the underlying mechanism of APOL1 nephropathy, some investigators approached the problem from a reductionist angle by expressing risk variants APOL1 gene in cultured human cells and study their effect on cell function and cell survival. Understanding how risk variants APOL1 perturb human cells in culture could provide insights into how they may dysregulate human kidney cells and cause kidney disease. One common finding that studies using this approach have reproducibly shown is that expression of G1 or G2 APOL1 results in significantly more cell death compared to wild type APOL1 (G0) in various human cells in culture (4–7, 12, 35). However, the reported mechanism of cell death varies. Necrosis, pyroptosis, apoptosis and autophagy have all been reported as causes of APOL1 induced cell death (6–12). It is possible that mechanisms of the cell death may be cell-type dependent. However, it is yet to be determined if these different types of APOL1 induced cell death share a common upstream step.

Depletion of cellular potassium is an early event in APOL1 mediated cytotoxicity of HEK 293 cell

Investigators, including our group have reported that expression of G1 or G2 APOL1 in HEK 293 cells results in necrotic cell death that is several folds higher than that caused by

G0 APOL1. We found that the earliest measurable cellular event that preceded APOL1-induced cell death was an abnormal efflux of cellular potassium which is measurable by 6 hours of G1 or G2 APOL1 induction but dramatic after 9 hours, (Fig.2) (7). The significance of this finding is underscored by the similarity between APOL1-induced efflux of cellular potassium and induced loss of cellular potassium by bacteria colicins and diphtheria toxins with which the pore forming domain of APOL1 share modest homology (36, 37). Notably, APOL1-induced depletion of cellular potassium, through yet unknown mechanism, triggered the activation of MAP kinases including stress-activated protein kinases, SAPK (p38, JNK) which are known mediators of podocyte injury, proteinuria and glomerulosclerosis (7). These findings also parallel the downstream signaling effect of bacteria pore-forming toxins which leak cation from mammalian cells (36, 37). Finally, elevation of extracellular potassium in culture media inhibited APOL1-induced potassium efflux which in turn abrogated the APOL1-induced activation of SAPK. These findings are summarized in Fig. 3.

It will be important to discover if this G1 or G2 APOL1-induced potassium depletion and activation of SAPKs are conserved mechanisms in human podocytes and glomerular endothelial cells. If potassium-SAPK axis is found to mediate APOL1 nephropathy, it could unveil potential druggable targets that could be part of therapeutic intervention of APOL1 nephropathy.

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Figure 1.

Confocal immunofluorescence of human embryonic kidney (HEK) 293 cells. Nine hours following stable induction of flag-tagged human APOL1 (G2) in HEK 293 cells, the cells were stained for APOL1 (green) B, lysotracker (red) C, and DAPI (blue) A. APOL1 appeared as punctate staining in the cytosolic space, and it does not appear to colocalize with marker of lysosome. Lysotracker is highly selective for acidic organelles such as lysosome.

Figure 2. Measurement of intracellular potassium in HEK 293 cells using XRpro X-ray fluorescence. Compared to cells expressing wild type APOL1 (G0), cells expressing G1, or G2 APOL1 had dramatic and incremental depletion of intracellular potassium that is most pronounced after 9 hours of APOL1 induction. EV represents empty vector. *, differences are statistically significant. Modified from (7)

Figure 3. Proposed model of G1 or G2 APOL1-induced cytotoxicity mediated by augmented K⁺ efflux and activation of stress activated protein kinase (p38 and JNK kinase) signaling. Modified from (7)

Table 1

Summary of reports on APOL1 ion selectivity from experiments using planar lipid bilayers.

Construct	Selectivity	pH sensitivity	Reference
TLF1- complex of lipids and proteins	Cation pK/pCl = 4.8	Activated by pH 5 Functions at pH 7	Molina-Portela et al. (2005)
Putative pore forming domain of APOL1 (M60–W235)	Anion pCl/pK = 3.2	Activated by pH 5	Perez-Morga et al. (2005)
Purified recombinant full length APOL1	Cation pK/pCl = high	Activated by pH 5 Increased by pH 7	Thomson and Finkelstein (2015)
Purified recombinant APOL1	Anion pCl/pK = high	Activated by pH 5 <i>Not</i> increased by pH 7	Edwards (2015)