BH3 domain-independent apolipoprotein L1 toxicity rescued by BCL2 prosurvival proteins

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The identification of a putative BH3-like domain within the APOL1 polypeptide was predicted to constitute a pore-forming domain, based on homology to E. coli colicin (44). Indeed, native APOL1 within the purified heteromeric serum complex, trypanolytic factor 1 (TLF1) (34), recombinant APOL1 pore-forming domain (44), and recombinant holo-APOL1 (52) each has been shown to confer increased ion permeability to liposomes and/or planar lipid bilayers. However, reported ion selectivities have differed, and secondary ion transport events may be triggered in intact cells (30, 34, 44, 52).

The central portion of the APOL1 polypeptide was predicted to constitute a pore-forming domain, based on homology to E. coli colicin (44). Indeed, native APOL1 within the purified heteromeric serum complex, trypanolytic factor 1 (TLF1) (34), recombinant APOL1 pore-forming domain (44), and recombinant holo-APOL1 (52) each has been shown to confer increased ion permeability to liposomes and/or planar lipid bilayers. However, reported ion selectivities have differed, and secondary ion transport events may be triggered in intact cells (30, 34, 44, 52).

The identification of a putative BH3-like domain within the APOL1 sequence (58) suggested APOL1 as a novel, atypical BH3-only protein, promoting autophagic cell death (60) by a mechanism potentially similar to that of Beclin-1 (47). However, increased autophagy might equally be attributed to functions of a pore-forming domain (9, 10, 17, 28).
to correlate the functions of transmembrane ion channels and transporters with cellular toxicity associated with transgene expression (2, 5, 8, 12, 13, 51, 55). We have used the Xenopus oocyte to study the importance of the putative BH3 domain of APOL1 in mediating the cell toxicity and ion transport activities associated with heterologous expression of APOL1.

METHODS

Materials. Na<sup>36</sup>Cl was from ICN (Irvine, CA). <sup>45</sup>CaCl<sub>2</sub> was from PerkinElmer (Waltham, MA). Restriction enzymes and T4 DNA ligase were from New England Biolabs (Beverly, MA). EXPES High-fidelity PCR System was from Roche (Indianapolis, IN). 4,4-Diisothiocyanostilbene-2,2-disulfonic acid (DIDS) was from Calbiochem (La Jolla, CA). 4,4-Dinitrostilbene-2,2-disulfonic acid (DNDs) was from Pfalz & Bauer (Waterbury, CT). ZVAD-FMK was from Tocris (R&D Systems, Minneapolis, MN). Trametinib and obatoclax were from LC Labs (Woburn, MA). MBQ was from Santa Cruz (Dallas, TX). Necrostatin-1 was from Enzo (Farmington, NY). Spautin-1 was from Junying Yuan (Harvard Med. School). Other reagent-grade reagents were from Sigma-Aldrich (St. Louis, MO) or Fluka (Milwaukee, WI).

Solutions. MBS consisted of (in mM) 16 NaCl, 2.4 KCl, 1.8 NaHCO<sub>3</sub>, 1 MgCl<sub>2</sub>, 0.8 MgSO<sub>4</sub>, 0.33 Ca(NO<sub>3</sub>)<sub>2</sub>, 0.41 CaCl<sub>2</sub>, and 10 HEPES (pH 7.40 and pH 8.50). ND-96 consisted of (in mM) 96 NaCl, 2 KCl, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, and 5 HEPES free acid (adjusted to pH 7.40 or pH 8.50 with HCl). For all experiments at pH 5, HEPES was replaced by equimolar MES, and pH was adjusted accordingly. NMDG-97 consisted of (in mM) 97.3 N-methyl-D-glucamine, 2 KCl, 1.2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, and 5 HEPES free acid (pH 7.40 and pH 8.50). Cl<sup>-</sup> substitution was achieved by mole-for-mole replacement with Na cyclamate. Cl<sup>-</sup> salts of K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> were substituted on an equimolar basis with the corresponding gluconate salts as needed.

Synthesis of cDNA and cRNA. APOL1 WT cDNA (BC141823 encoding AA418;2: Open Biosystems, Huntsville, AL) was modified to retain the native Kozak sequence and 35 nt of native 3'-UTR and subcloned into transcription vector pTX7 (11), hereafter described as APOL1 WT. The amino acid sequence encoded by BC141823 (and this APOL1 WT cDNA) is identical to NP_003652 (APOL1 isoform a, also known as APOL1 G0) with the exceptions of known coding polymorphisms E150K, M228I, and NP_003652=G0). Expression of the putative BH3 domain of APOL1 in mediating the cell toxicity and ion transport activities associated with heterologous expression of APOL1.

cRNA expression in Xenopus oocytes. Mature female Xenopus laevis (Dept. Systems Biology, Harvard Medical School) were subjected to partial ovariectomy under hypothermic tricaine anesthesia following protocols approved by the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center. Stage VI oocytes were prepared by overnight incubation of ovarian fragments in MBS with 2 mg/ml collagenase B (Alfa Aesar, Ward Hills, MA), followed by a 20 min rinse in Ca<sup>2+</sup>-free MBS, with subsequent manual selection and defolliculation as needed. Oocytes were injected with cRNA on the day of isolation and maintained at 17.5°C in MBS or HiK MBS supplemented with gentamicin (10 μg/ml) for the times indicated.

Oocyte morphology assessment. Preliminary experiments monitored changes in morphological degradation in groups of oocytes in 60 mm polystyrene dishes containing 10 ml of MBS, using a Nikon SMZ-1 microscope equipped with an Olympus D-510 2.5 MP digital camera (data not shown). Subsequent experiments (all those reported with statistical analysis) monitored the progression of morphological deterioration by individual oocytes in single wells of 96-well round bottom polystyrene plates (Thermo Scientific Nunc). Individual oocytes were imaged (×18 magnification) at 24, 48, and 72 h post-cRNA injection with a CPW1308C 8bit CCD digital camera (Scion) through an Olympus S7X7 microscope. Images saved as tif files were scored as “unaffected” (green), “discolored” (yellow), or blebbing/oozing (red) and similarly color-coded in Figs. 1–3 and 7. In each experiment, comparisons were restricted to groups of cRNA-injected and uninjected oocytes harvested from the same frog(s) and prepared at the same time, to minimize lot-to-lot variation of oocyte responses to heterologous expression of APOL1 and to BCL2 family members.

Isotopic influx experiments. Unidirectional <sup>45</sup>Ca<sup>2+</sup> influx studies were carried out for periods of 30 min in 148 μL ND-96 and 2 μL <sup>45</sup>CaCl<sub>2</sub> (−2 μCi; final bath [Ca<sup>2+</sup>] = 1.8 mM, where brackets denote concentration). Unidirectional <sup>36</sup>Cl<sup>-</sup> influx studies were carried out for periods of 30 min in 148 μL ND-96 and 2 μL Na<sup>36</sup>Cl<sub>2</sub> (−0.2 μCi; final bath [Cl<sup>-</sup>] = 107 mM). Bath solutions for all <sup>36</sup>Cl<sup>-</sup> influx experiments were supplemented with 10 μM bumetanide to block Cl<sup>-</sup> influx by native oocyte Na<sup>-</sup>-Cl<sup>-</sup> cotransporter-1. Influx experiments were terminated with three washes in ice-cold isotonic Na cyclamate solution. Washed oocytes were individually lysed in 150 μL 2% sodium dodecyl sulfate (SDS). Triplicate 10 μl aliquots of influx bath solution were used to calculate specific activity of radiolabeled substrate ions. Oocyte ion uptake experiments were calculated from cpm values of cold-washed oocytes and from bath specific activity.

Two-electrode voltage clamp measurements. Microelectrodes from borosilicate glass made with a Sutter P-87 puller (Sutter Instruments, Novato, CA) were filled with 3 M KCl and had resistances of 2–3 MΩ. Oocytes previously injected with cRNA were placed in a 1 ml chamber (model RC-11, Warner Instruments, Hamden, CT) on the stage of a dissecting microscope and impaled with microelectrodes under direct view. Steady-state currents achieved within 2–5 min following bath change were measured with a Geneclamp 500 amplifier (Molecular Devices, Burlingame, CA) interfaced to a Dell computer with a Digidata 1322A digitizer (Molecular Devices).

Data acquisition and analysis utilized pCLAMP 8.0 software (Molecular Devices). The voltage pulse protocol generated with the Clampex software comprised of 20 mV steps between −100 mV and +40 mV, with durations of 738 ms, and separated by 30 ms at the holding potential of −30 mV. Bath resistance was minimized by 3 M KCl agar bridges. A virtual ground circuit clamped bath potential to zero.

Raising anti-APOL1 antibody. Synthetic peptide NH<sub>2</sub>-[CPSPGT-DTGDQPSKPLGDW-CONH<sub>2</sub> encoding aa 39–55 of the 398 aa isoform of human APOL1 with an N-terminal Cys-tag was HPLC purified (>85% purity), sequence validated by mass spectroscopy, and verified by formamide gel electrophoresis.

Nanodrop spectrometer (ThermoFisher, Waltham, MA). RNA integrity was ensured by formaldehyde gel electrophoresis.

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C333
APOL1 mutant oligonucleotides for PCR experiments.

Starting from APOL1 “WT”:

- **APOL1.D163A**

Starting from APOL1.D163A:

- **APOL1.L158A/D163A/V165A**

Starting from APOL1.D163A:

- **APOL1.R159A**

Starting from APOL1.R159A:

- **APOL1.L158A**

Mutagenic oligonucleotides

**BCL2 family member oligonucleotides for RP-PCR.**

**Human BCL2 Family Member**

- **BCL2**
  - NM_000633
- **BCLXL**
  - NM_138578
- **BCLW**
  - NM_004050
- **BCL2A1**
  - NM_004049
- **MCL-1**
  - NM_021960

**APOL1 mutant oligonucleotides for PRG977 mouse expression vector.**

Starting from APOL1 “WT”:

- **APOL1.D163A**

Starting from APOL1.D163A:

- **APOL1.L158A/R159A/D163A**

and haptenized by Cys-coupling with m-maleimidobenzoyl-N-hydroxysuccinimide ester. Haptenized peptide was used to raise polyclonal antiserum in rabbits, and antisera were affinity-purified (21st Century Biochemicals, Marlboro, MA). Additional anti-APOL1 antibodies used in this paper were from Sigma (HPA01885) and from ProteinTech (11486-2-AP).

**Confocal immunofluorescence microscopy.** cRNA-injected oocytes and un.injected oocytes were incubated for 3 days in at 17.5°C in HiK MBS containing gentamicin (10 μg/ml). Ten to twelve oocytes in each experimental group were fixed in 3% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min at room temperature, washed three times with PBS-Tween (PBST), permeabilized with 1% SDS in PBS supplemented with 0.02% Na azide (PBS-azide) for 1–2 min, and washed again three times with PBST.

Fixed, permeabilized oocytes were incubated overnight at 4°C in 0.5 ml PBST containing affinity-purified polyclonal rabbit anti-APOL1 (1:500), rinsed in PBST, and stained with Cy3-conjugated donkey-anti-rabbit secondary Ig (1:1,000) for 90 min at 20°C. Stained oocytes were washed 3× in PBST, 2× in PBS-azide, and postfixed in paraformaldehyde for 10 min. Fixed oocytes were washed 2× in PBST, extensively washed with PBS-azide, and stored in PBS-azide at 4°C until imaged. Cy3-labeled oocytes aligned in uniform orientation along a Plexiglas groove were sequentially imaged through the ×10 objective of a Zeiss LSM510 laser scanning confocal microscope using the 543 nm laser line at 512 × 512 resolution at a uniform setting of 80% intensity, pinhole 54 (1.0 Airy units), detector gain 650, Amp gain 1, zero amp offset.

Polypeptide abundance at or near the oocyte surface was estimated by quantitation of specific fluorescence intensity (FI) at the circumference of one quadrant of an equatorial focal plane image of the oocyte (Image J v. 1.38, National Institutes of Health). Background correction was performed by subtraction from FI of each cRNA-injected oocyte the mean FI value of an equatorial plane quadrant of water-injected oocytes.

**Oocyte lysate immunoblots.** Twenty to twenty-five oocytes previously injected with cRNA (10 ng APOL1 and/or 50 ng MCL1) were placed in MBS in a 1.5 ml centrifuge tube. After 24, 48, or 72 h incubation, MBS was aspirated and replaced with RIPA buffer containing Complete Protease inhibitor (6 μl per oocyte), then vortexed vigorously and immediately stored at −80°C for at least 2 h. The thawed mixture was again vortexed and then centrifuged 20–30 min at 4°C at maximal microfuge speed. The fairly clear infranatant layer between the pellet and foamy debris was withdrawn and subjected to two more cycles of vortexing, 4°C centrifugation, and infranatant harvest. The final, clarified protein extract...
tracts were assayed by BCA for protein and stored at −80°C until use. Twenty micrograms of protein were brought up to 10 μl volume with 4 μl SDS load buffer plus β-mercaptoethanol. The mixture was heated to 97°C for 5 min, cooled, loaded on a 10% polyacrylamide Tris-acetate gel (BIO-RAD), and subjected to SDS-PAGE. Protein was transferred to PVDF membrane (iBlot 2, Life Technologies), washed in TBST, and blocked 1 h with TBST plus 5% powdered milk. The blocked membrane was washed with TBST and incubated overnight at 4°C with rabbit anti-APOL1 (Sigma HPA018885) diluted 1:300 in TBST/5% BSA and then further washed and incubated 1 h with horseradish peroxidase-coupled secondary goat anti-rabbit Ig (Thermo Scientific no. 31460; diluted 1:8,000 in TBST/5% milk). Signal was developed (Supersignal West DURA kit, Life Technologies) and imaged (FluorChem E, Bio-Techne). To control for loading, each membrane was probed with horseradish peroxidase-coupled anti-GAPDH (Genetex, GTX-627408-01 diluted 1:10,000) either simultaneously with the secondary antibody or afterwards, as indicated in the figure legends.

**Immunoblot of mouse serum and HDL.** Mouse experiments were approved by the Institutional Animal Care and Use Committee of Hunter College.

APOL1 expression vectors used in mice were constructed in plasmid pRG997, containing a mammalian ubiquitin promoter, 5' intronic splicing sequences from rabbit β-globin, and a 3' SV40 polyadenylation signal. APOL1 missense and deletion mutants were generated by the Stratagene mutagenesis kit (La Jolla, CA), using primers listed in Table 1, bottom. All plasmids injected into mice were purified with the Qiagen Endotoxin-free Maxiprep Kit.

Approximately 20 g female outbred Swiss Webster mice (Taconic Biosciences, Hudson, NY) underwent tail vein injection (27 g needle with 2–3 ml saline (10% body weight) containing 50 μg (14.5 pmol) of expression plasmid pRG997-APOL1 (5.2 kb). Two days after this hydrodynamic DNA injection, mice were subjected to intraperitoneal injection of 5 × 10^6 Trypanosoma brucei brucei 427 parasites in 200 μl DMEM. Twenty-microtiter tail vein blood samples were taken from infected mice on day 3 postinfection and twice per week thereafter to monitor parasitemia. Mice were euthanized at ~1 × 10^9 parasites/ml (29, 53), and postinfection survival times were recorded.

**Morphological assessment of the toxic effects of APOL1 expression.** Mouse experiments were immediately prior to parasite injection, as previously described (35). HDL or serum was diluted 1:40 in Laemmli running buffer and subjected to SDS-PAGE on 10% precast gels (Bio-Rad). Overnight protein transfer to PVDF membrane (4°C, 30 V) was followed by immunodetection using anti-human APOL1 (ProteinTech 11486-2-AP, 1:10,000), anti-mouse APOA1 (Abcam ab20453, 1:10,000), and secondary antibody (anti-rabbit TrueBlot-HRP; Rockland 18-8816-33, 1:2,500).

**Statistics.** Ordinal data scoring oocyte morphological toxicity [with integral values of 0 (unaffected), 1 (discolored), and 2 (oozing/blebbing)] as previously described (6, 12, 51), were subjected to the integral values of 0 (unaffected), 1 (discolored), and 2 (oozing/blebbing). In the histograms of Figs. 1–3 and 7, morphological categories of green, yellow, and red were given respective ordinal scores of 0, 1, and 2 for statistical analysis. Comparison of APOL1-injected oocytes with uninjected oocytes demonstrated APOL1-associated toxic morphological changes (Fig. 1, A–C).

As a putative BH3-only protein (58, 60, 63), APOL1-induced toxicity might be expected to be rescued or attenuated by coexpression of an anti-apoptotic BCL2 family member (38, 43, 62). Expression of the pro-apoptotic protein BCL-XS was previously shown to be toxic to Xenopus oocytes, but coexpression of the anti-apoptotic BCL-XL rescued viability (5). In view of the varied BH3-binding specificities of different BCL2-family members (7), additional anti-apoptotic BCL2 family members were individually coexpressed with APOL1 and assessed for ability to attenuate APOL1-associated morphological toxicity (Fig. 1A).

Oocytes were coinjected with 10 ng APOL1 cRNA and 50 ng cRNA encoding the indicated anti-apoptotic BCL2 family proteins (Fig. 1, A, D–H). The degree of rescue of APOL1-associated toxicity fell into three distinct groups. BCL2 alone failed to rescue APOL1-associated toxicity or had minimal effect (compare Fig. 1, B and C, with F). Coexpression with APOL1 of BCLXL or BCL2A1 resulted in morphological changes of intermediate severity that significantly differed from those of APOL1 alone as well as from those of uninjected oocytes (Fig. 1, D and H). Coexpression of MCL1 or BCLW almost completely rescued APOL1-associated toxicity and differed from oocytes expressing APOL1 alone but not from uninjected oocytes (Fig. 1, E and G). The observed rescue or amelioration of APOL1-induced oocyte toxicity by coexpression of selected BCL2 family proteins is consistent with the hypothesis that the putative BH3 domain of APOL1 might itself be the effector of cell death associated with APOL1 expression. However, coexpressed human MCL1 rescued APOL1 toxicity even in the presence of the BH3 domain-dependent MCL1 inhibitor, obatoclax (1 μM, extra- and intracellular; not shown), suggesting other possible mechanisms of MCL1 action.

**APOL1 BH3 domain deletion prevents, whereas Ala(9) substitution of BH3 minimally reduces, oocyte toxicity without preventing rescue by MCL1.** If the BH3 domain serves an important role in either the toxicity of APOL1 or the interaction with anti-apoptotic BCL2 family proteins, then mutation of the BH3 domain should result either in loss of APOL1-associated toxicity, or in loss of rescue from this toxicity by anti-apoptotic BCL2 family proteins. To test these hypotheses, mutants targeting the nine core amino acid residues of the putative BH3 domain (58, 60, 63) of APOL1
were constructed (Fig. 2A). *Xenopus* oocytes injected with deletion mutant APOL1.DΔBH3 cRNA or with unmutated APOL1 (APOL1 WT) were compared with uninjected oocytes, as described in Fig. 1. Oocytes expressing APOL1.DΔBH3 indeed exhibited no APOL1-associated morphological toxicity (Fig. 2, B–E). Initially, these results appeared to support the previously suggested hypothesis (60) that the APOL1 BH3 domain is the effector of cell death. However, deletion of core BH3 domain residues from the APOL1 pore-forming domain may destabilize larger regions of the polypeptide. Therefore, all nine BH3 residues of APOL1 were substituted with helix-preserving Ala residues [APOL1.9AlaBH3 (Fig. 2A)]. Indeed, APOL1 toxicity was not eliminated by APOL1.9AlaBH3, but only minimally attenuated (Fig. 2, F–J). If the function of the nominal BH3 domain of APOL1 is to interact with BCL2 family proteins, then the APOL1.9AlaBH3 mutant should not be rescued by MCL1 coexpression. However, MCL1 coinjection rescued APOL1.9AlaBH3 as completely as it did APOL1 WT (Fig. 2, F–J).

Conserved core residues of the APOL1 putative BH3 domain are not required for APOL1-associated oocyte toxicity. The toxicity associated with oocyte expression of APOL1.9AlaBH3 failed to support the hypothesis that the putative BH3 domain is the effector of APOL1-associated cell death. The role of the APOL1 BH3 domain was further examined by investigating less extensive missense substitutions in more highly conserved BH3 domain residues (Fig. 3A). APOL1 residue D163 was proposed as an important contributor to APOL1-associated cellular toxicity (60), but oocyte expression of APOL1 D163A produced morphological toxicity indistinguishable from that associated with WT APOL1 expression (Fig. 3, B–E). Combined Ala substitution of three highly conserved BH3 domain residues (L158, D163, and V165) (14) in mutant APOL1.3Ala slightly reduced morphological toxicity but remained nearly indistinguishable from that of WT APOL1 (Fig. 3, F–I).

As found for APOL1 WT, the morphological toxicity associated with expression of APOL1 D163A and with APOL1.3Ala was completely rescued by coexpression of MCL1 (data not shown). However, attempts in lysates of cotransfected human embryonic kidney (HEK)-293 cells to demonstrate physical interaction of APOL1 WT with epitope-tagged MCL1 were unsuccessful under the conditions tested (not shown). Thus the putative BH3 domain of APOL1 is not an essential contributor to the toxic morphological effects of APOL1 expression in *Xenopus* oocytes. Initial tests of multiple pharmacological inhibitors failed to...
Integrity of the putative BH3 domain does not contribute to APOL1 protection of mice from lethal infection by trypanosoma brucei. Since APOL1 is found only in the genomes of humans and a small group of higher primates, tests of APOL1 function in experimental animals require heterologous gene expression. Hydrodynamic gene delivery by intravenous injection of naked expression plasmid DNA has proven to be a powerful tool for transient APOL1 expression in mice (29, 53). As shown in Fig. 4A, T. b. brucei-infected mice were completely protected from death during the 30 day observation period by hydrodynamic gene delivery of APOL1 WT (G0) cDNA. Whereas hydrodynamic injection with APOL1ΔBH3 cDNA led to death of all mice by postinfection day 11, mice injected with cDNAs encoding less extensive missense substitutions of the BH3 domain of APOL1, including mutants L158A, R159A, D163A, and triple mutant L158A/R159A/D163A, were completely protected against the lethal effects of trypanosomal infection. These results were indistinguishable from those with WT APOL1 (Fig. 4A). Heterologous human APOL1 expression was confirmed by immunoblot of serum from each mouse subjected to hydrodynamic gene delivery (Fig. 4B). The lack of protection by injected APOL1ΔBH3 did not reflect diminished or absent association with HDL. As shown in Fig. 4C, immunoblot detection of APOL1 and APOA1 in purified HDL fractions from serum (Fig. 4C) was consistent with normal in vivo assembly of TLF1. Thus, the inability of APOL1ΔBH3 to kill Xenopus oocytes, African trypanosomes, and cancer cell lines (60) reflects loss of the lytic activity of the deletion mutant, likely due to APOL1 misfolding. However, neither the trypanolytic activity of APOL1 nor the Xenopus oocyte toxicity associated with APOL1 expression requires integrity of APOL1’s nominal BH3 domain sequence itself.

APOL1-induced oocyte toxicity is accompanied by, but not caused by, elevated oocyte Cl− flux. The reported association of APOL1’s trypanocidal effects with elevated trypanosomal Cl− flux (44) prompted examination of APOL1’s effects on oocyte Cl− flux. Only morphologically intact oocytes (see Fig. 1) were selected for these initial studies, to minimize nonspecific plasma membrane “leak”. The magnitude of Cl− flux in morphologically intact oocytes correlated with the injected amount of APOL1 cRNA, as well as with the proportion of oocytes exhibiting morphological toxicity (Fig. 5A). Blockade of oocyte Cl− flux did not reflect nonspecific “leak”, since fluxes were blocked by the Cl− transport inhibitors DIDS (30, 44) and its noncovalently reactive analog (21) DNDS at 200 μM or 500 μM (Fig. 5B), and (not shown) by the Ca2+-activated Cl− channel inhibitor, tannic acid (50 μM). However, inhibition of Cl− flux by the nontoxic, reversible Cl− transport antagonists DNDS (Fig. 5C) and tannic acid (not shown) did not rescue APOL1-associated morphological toxicity measured after 72 h. APOL1-mediated trypanolysis was previously reported to be rescued by substitution of extracellular Cl− with gluconate during periods of 1–2 h (34, 44). However, prolonged exposure of APOL1 cRNA-injected oocytes to extracellular solutions containing reduced or (nominally) zero [Cl−] failed to rescue morphological toxicity of oocytes (Fig. 5D).

APOL1 expression in oocytes elicits Ca2+ flux and ion currents. APOL1-induced increases in cation permeability have been described previously (34, 52). Xenopus oocytes expressing APOL1 exhibited increased uptake of 45Ca2+ in the presence of extracellular Na+ that was further increased upon Na+ substitution with the impermeant cation N-methyl-D-aspartate (Fig. 6A). The influx of 45Ca2+ was inhibited by extracellular La3+ and by Gd3+ (both at 1 mM), but not by DIDS (Fig. 6B), or by the low-specificity transient receptor potential vanilloid 4 cation channel inhibitor, ruthenium red (100 nM, not shown). These data are consistent with a nonspecific cation flux pathway activated by APOL1 expression, accompanied by secondarily activated oocyte Cl− permeability pathways.

Oocytes previously injected with 5 ng APOL1 cRNA and lacking morphological toxicity (as in shown in Fig. 1A) were subjected to two-electrode voltage clamp (TEVC) measurements of whole cell currents in the absence and subsequent presence of inhibitors. Both La3+ and Gd3+ significantly inhibited the APOL1-associated currents at holding potentials of −100 mV (Fig. 6, C and D). In addition, TEVC currents were significantly inhibited by tannic acid (50 μM) and by DIDS (200 μM) and activated by bath addition of 10 mM Ca2+ (data not shown). The wide spectrum of modest pharmacological inhibition, variedly elevated current magnitudes, and variably depolarized reversal potentials together suggest that APOL1 expression in oocytes induces a mix of cation and anion currents.

Elevated extracellular [K+] attenuates APOL1-associated morphological toxicity while increasing ion fluxes in morphologically intact oocytes. Mammalian cell susceptibility to bacterial ionophore toxins can be minimized or prevented by...
exposure to elevated extracellular [K⁺] (17). We therefore tested the effect of substituting extracellular Na⁺ with K⁺ during the 72 h post-cRNA injection (17, 34, 52). Not only was the treatment (HiK MBS) well tolerated by uninjected oocytes, but APOL1-associated morphological toxicity in oocytes was greatly reduced by incubation in HiK MBS (Fig. 7, A–F). In contrast, 72 h incubations of APOL1-expressing oocytes in bath solutions of either reduced Ca²⁺ or reduced Na⁺ (NMDG substitution) were without comparable protective effects (not shown). APOL1-expressing oocytes incubated in HiK MBS also exhibited significantly higher uptakes of ⁴⁵Ca²⁺ (Fig. 7G) and of ³⁶Cl⁻ uptake (Fig. 7H) compared with APOL1-expressing oocytes maintained in MBS without added K⁺.

Coexpression of MCL1 inhibits APOL1-associated Ca²⁺ uptake and currents. Since MCL1 coexpression rescued APOL1-associated oocyte toxicity, the effect of MCL1 coexpression on APOL1-associated ion transport was examined. As shown in Fig. 8, expression of MCL1 alone had little or no effect on ⁴⁵Ca²⁺ uptake or on whole oocyte current measured by two microelectrode voltage clamp. The large increase in both ⁴⁵Ca²⁺ uptake and whole oocyte current associated with APOL1 expression was almost com-

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**Fig. 2.** APOL1 BH3 domain deletion (APOL1.ΔBH3) prevents APOL1-associated oocyte toxicity, whereas BH3 Ala(9) substitution (APOL1.9AlaBH3) only minimally reduces oocyte toxicity but does not inhibit rescue of toxicity by MCL1. A: aligned BH3 domain sequences of wild-type (WT) APOL1 and of BH3 mutants, bracketed by amino acid sequence positions (numbers). Mutated residues are highlighted in blue. B: representative oocytes imaged at the indicated times post-injection with cRNA (10 ng) encoding APOL1 or APOL1.ΔBH3, compared with uninjected oocytes (bottom row). C–E: oocyte morphology category histograms at 24, 48, and 72 h post-injection of cRNA (10 ng) encoding APOL1 (C) or APOL1.ΔBH3 (D), compared with uninjected oocytes (E), n = 20 in each group (10 oocytes from each of 2 frogs). F: representative oocytes imaged at the indicated times post-injection with cRNA (10 ng) encoding APOL1 WT or APOL1.9AlaBH3, without or with coinjected MCL1 cRNA (50 ng), and compared with uninjected oocytes or oocytes injected with MCL1 alone (bottom). G–L: oocyte morphology histograms at 24, 48, and 72 h post-injection of the indicated cRNAs. n = 20 in each group (10 oocytes from each of 2 frogs). Statistical differences among groups at 72 h was determined as described for Fig. 1. *P < 0.05 vs. oocytes expressing APOL1 alone. †P < 0.05 vs. uninjected oocytes.
Completely attenuated by MCL1 coexpression after only 24 h in HiK MBS post-cRNA injection, and greatly reduced after 48 and 72 h. Thus the time-dependent increase in 45Ca2+ flux in APOL1-expressing oocytes maintained in HiK MBS parallels the increased morphological toxicity observed in APOL1-expressing oocytes maintained in MBS (Figs. 1, 3, and 7). Moreover, the attenuation of 45Ca2+ flux and whole-oocyte current by MCL1 coexpression mirrors the rescue by MCL1 of APOL1-associated morphological toxicity. Coexpression with APOL1 of BCL-XL similarly attenuated 45Ca2+ flux and whole cell currents in oocytes (data not shown).

Integrity of the APOL1 BH3 domain is not required for APOL1-associated oocyte toxicity. A: aligned BH3 domain sequences of WT APOL1 and of BH3 mutants, bracketed by amino acid sequence numbers. Mutated residues are highlighted in blue. B: representative oocytes imaged at the indicated times post-injection with cRNA (10 ng) encoding APOL1 WT or APOL1 D163A, compared with uninjected oocytes (bottom row). C–E: oocyte morphology category histograms at 24, 48, and 72 h post-injection of cRNA encoding APOL1 (C) or APOL1 D163A (D), compared with uninjected oocytes (E). n = 30 in each group (10 oocytes from each of 3 frogs). F: representative oocytes imaged at the indicated times post-injection with cRNA (10 ng) encoding APOL1 WT or APOL1 triple mutant L158A/D163A/V165A (3AlaBH3 compared with uninjected oocytes; bottom). G–I: oocyte morphology histograms at 24, 48, and 72 h post-injection of cRNA encoding the indicated cRNAs. n = 20 in each group (10 oocytes from each of 2 frogs). Statistical differences between groups at 72 h was determined as described in Fig. 1. *P < 0.05 vs. oocytes expressing APOL1 alone. †P < 0.05 vs. uninjected oocytes.
ated between the putative pore forming domain and the C-terminal SRA binding domain were proposed to contribute to pH-sensing (44). Activity of the cation conductance mediated by purified holo-APOL1 incorporated into lipid bilayers required priming by cis-acidification. However, full activation of cation conductance required subsequent alkalizing restoration to cis-neutral pH (52).

Extracellular pH (pHo) sensitivity of APOL1-associated ion transport activity was tested in Xenopus oocytes maintained 72 h in HiK MBS. Acid pHo dramatically suppressed APOL1-associated $^{45}$Ca$^{2+}$/H$^{+}$ influx, whereas alkaline pHo activated it (Fig. 11A). $^{36}$Cl$^{-}$/H$^{+}$ influx was also substantially inhibited by acid pHo (Fig. 11B). APOL1-associated TEVC current was similarly inhibited by acid pHo (Fig. 11, C and D). The effect of acid pHo could not be tested on long-term morphological toxicity of APOL1, since prolonged exposure to acid HiK MBS itself was toxic to both uninjected and APOL1-expressing oocytes (not shown).

**DISCUSSION**

The mechanisms by which APOL1 influences innate immunity and by which APOL1 variants increase risk of renal disease remain little understood. The restricted presence of APOL1 in humans and a few higher primates requires its study in heterologous expression systems. We have shown here that expression of human APOL1 in Xenopus oocytes led to morphological toxicity (physical deterioration) of oocytes representing progression to or toward cell death during the 72 h after cRNA injection. Oocyte morphological toxicity was rescued by coexpression of individual, anti-apoptotic, BCL2 family proteins, with the notable exception of BCL2 itself. APOL1-associated oocyte morphological toxicity was also rescued by maintenance of APOL1-expressing oocytes in bath solutions in which extracellular Na$^+$ was replaced by K$^+$. Although APOL1-associated cell toxicity and death has been considered a reflection of APOL1 activity as an atypical BH3-only protein, our findings demonstrate that sequence integrity of the BH3 domain is required neither for the APOL1-associated toxic morphological phenotype in oocytes, nor for rescue of that phenotype by coexpression of human MCL1 or BCL-XL. Integrity of the BH3 domain of APOL1 is similarly dispensable in a mouse model of APOL1-mediated protection against lethal trypanosome infection. Thus we conclude that, although deletion of nine amino acids from the BH3-like region of the pore-forming domain abrogates APOL1 activity, integrity of that BH3-like sequence is not required for APOL1 activity. APOL1-associated oocyte morphological toxicity was accompanied by increased $^{36}$Cl$^{-}$ permeability blocked by stilbene disulfonates, and increased $^{45}$Ca$^{2+}$ permeability inhibited by Na$^+$, Gd$^{3+}$, and La$^{3+}$. These increased permeabilities of APOL1-expressing oocytes were accompanied by increased TEVC ion currents accompanied by variable depolarization. Fluxes and currents measured in APOL1-expressing oocytes...
maintained in high extracellular $[K^+]_{o}$ were of higher magnitude, possibly reflecting higher proportions of intact oocytes able to survive the mechanical stress of the ion transport assays. Coexpression of MCL1 reduces the magnitude of these fluxes and currents, which are also inhibited by acidic $pH_{o}$. Integrity of the BH3 domain is required neither for APOL1-associated elevation of ion fluxes and current, nor for APOL1 expression at or near the oocyte surface.

Fig. 5. APOL1-induced oocyte toxicity is accompanied by, but not caused by, elevated $Cl^-/H^+$ flux. $A$: correlation between oocyte survival 72 h post-injection of 10 ng APOL1 cRNA (solid bars representing percentage of 37–39 originally injected oocytes later discarded due to morphological changes) and $^{36}Cl^-$ influx into oocytes that remained morphologically intact at 72 h post-injection. Open bars, means ± SE; $n = 9–10$ oocytes/group. $B$: $^{36}Cl^-$ influx into oocytes injected 72 h earlier with 10 ng APOL1 cRNA was sensitive to inhibition by 4,4-diisothiocyanostilbene-2,2-disulfonic acid (DIDS; 200 $\mu M$) and to 4,4-dinitrostilbene-2,2-disulfonic acid (DNDS; 500 $\mu M$). Open bars, means ± SE; $n = 9–10$ oocytes. *$P < 0.05$. $C$: oocyte toxicity is not prevented by pharmacological inhibition of $^{36}Cl^-$ transport. Percentage of oocytes unsuitable for isotopic flux assay (with morphological toxicity) were either injected with 10 ng APOL1 cRNA (hatched bars) or were uninjected oocytes (solid bars) after 72 h incubation in MBS in the absence or presence of DNDS (200 or 500 $\mu M$). $D$: oocyte toxicity is not prevented by prolonged reduction of extracellular $Cl^-$ concentration ($[Cl^-]_{o}$). Percentage of oocytes with morphological toxicity previously were injected with 10 ng APOL1 cRNA (hatched bars) or were uninjected oocytes (solid bars) after 72 h incubation in MBS or in MBS modified to contain either 30 mM $Cl^-$ or 0 mM $Cl^-$.

Fig. 6. APOL1 expression in oocytes elicits $Ca^{2+}/H^+$ flux and currents. $A$: oocytes injected 72 h previously with APOL1 cRNA (10 ng, open bars) or uninjected oocytes (solid bars) were subjected to measurements of $^{45}Ca^{2+}$ influx in the absence or presence of 96 mM bath $Na^+$ (*$P < 0.05$). $B$: $^{45}Ca^{2+}$ uptake by APOL1-expressing oocytes (open bars) is inhibited by 1 mM $Gd^{3+}$ or $La^{3+}$, but not by DIDS (200 $\mu M$). *$P < 0.05$ vs. oocytes expressing APOL1 alone. †$P < 0.05$ vs. uninjected oocytes. $C$: oocytes injected 72 h previously with APOL1 cRNA (5 ng) were subjected to two-electrode voltage clamp measurements of whole oocyte currents in the absence (ND 96, open circles, $n = 5$) and subsequent presence of 1 mM $Gd^{3+}$ (solid circles, $n = 5$) and subsequent presence of 1 mM $La^{3+}$ (solid circles). *$P < 0.05$. Values are means ± SE.
The status of APOL1 expressed in Xenopus oocytes as a BH3-only protein. APOL1 homology with BCL2 family proteins was first noted by Perez-Morga et al. (44), leading soon thereafter to the suggestion that APOL1 is an atypical BH3-only protein with pore-forming properties (58). The BH3 designation was tested experimentally by Wan et al. (60), who showed that APOL1 expression induced macroautophagic cell death in several tumor cell lines, by a mechanism proposed to resemble that mediated by the BH3-only protein Beclin-1 (46). Cell death was reduced or abrogated by deletion of nine core amino acid residues from the putative BH3 domain of APOL1, just as deletion of core BH3 residues from APOL6 had been previously reported to inhibit APOL6-mediated apoptosis in colon cancer cells (33). However, the related putative BH3-only protein APOL2 failed to mediate or increase susceptibility to several inducers of cell death, or to regulate autophagy in the several cell types tested (14).

We have demonstrated here that APOL1 expression greatly accelerates the morphological deterioration of Xenopus oocytes during ex vivo incubation. Deletion of putative BH3 domain core residues from APOL1, as previously tested in cancer cell lines (60), prevented toxic morphological deterioration of oocytes (Fig. 2) without altering APOL1 abundance at or near the oocyte surface (Fig. 9). However, missense substitutions in highly conserved residues of the putative core BH3 domain failed to abolish either the toxic morphological effects of APOL1 expression in Xenopus oocytes (Figs. 1–3) or the protection conferred by APOL1 against lethal trypanosome infection in mice (Fig. 4). Thus APOL1 toxicity and morphological degeneration do not require the conserved core sequence of the putative BH3 domain. APOL1-induced autophagic changes observed in murine embryonic fibroblasts were absent from embryonic fibroblasts lacking ATG5 or ATG7 (60). However, APOL1-associated morphological toxicity in Xenopus oocytes was unattenuated by multiple inhibitors of autophagy, by a caspase inhibitor, or by inhibitors of necroptosis or autosis. Thus the death pathway(s) activated by APOL1 in the Xenopus oocyte remains undefined. Cytotoxicity of APOL1 G0 transiently expressed in HEK-293T cells was similarly unaffected by inhibitors of caspase, of lysosomal function, and of clathrin-mediated endocytosis, and was independent of APOL1-associated impairment of autophagosomal maturation (27).
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surface delivery or increase its endocytosis from the surface, either specifically or by enhanced cell wound repair pathways (1).

APOL1 expression induces increased Cl\(^{-}\) and Ca\(^{2+}\) fluxes, accompanied by increased whole oocyte currents. APOL1-associated morphological toxicity in Xenopus oocytes was accompanied by increased Cl\(^{-}\) and Ca\(^{2+}\) permeabilities, and by increased ion currents (Figs. 5–7). Stilbene disulfonates inhibited elevated Cl\(^{-}\) flux (Fig. 5). La\(^{3+}\) and Gd\(^{3+}\) cations inhibited elevated Ca\(^{2+}\) flux, but DIDS did not (Fig. 6). The APOL1-associated increase in oocyte TEVC current was partially blocked by each of these inhibitors (Figs. 7 and not shown).

Rescue of the morphological toxicity by coexpression of MCL1 or BCL-XL (not shown) was paralleled by reduction in oocyte ion fluxes and currents (Figs. 8 and 9). In contrast, morphological rescue of APOL1-expressing oocytes by high extracellular [K\(^{+}\)] was accompanied by substantially increased Ca\(^{2+}\) and Cl\(^{-}\) fluxes (Fig. 7). The mechanistic relationship between oocyte morphological rescue by MCL-1 coexpression and morphological rescue by oocyte maintenance in HiK medium remains unclear. Apoptosis in cultured cells has been associated with elevated K\(^{+}\) efflux and cell shrinkage (4), although reduction in intracellular [K\(^{+}\)] is not required for apoptosis (3). The protective effect of high extracellular [K\(^{+}\)] on APOL1-associated morphological toxicity may be related to inhibition of excess Na\(^{+}\) entry, similar to the protective effect of bath K\(^{+}\) replacement during staurosporine-induced caspase activation and induction of non-inactivating Na\(^{+}\) channel activity in the Xenopus oocyte (13). Elevated extracellular [K\(^{+}\)] also prolonged survival of primary neurons in culture (41) and rescued autophagy triggered by the ionophore toxin proaerolysin (17).

Chronic exposure to high extracellular [K\(^{+}\)] should maintain or elevate cytosolic [K\(^{+}\)] while preventing potentially pathological elevation of cytosolic [Na\(^{+}\)]. However, substitution of extracellular Na\(^{+}\) or Cl\(^{-}\) with impermeant cations or anions over 24–72 h failed to rescue the APOL1 morphological phenotype in Xenopus oocytes. In the absence of prominent voltage-gated Ca\(^{2+}\) channels in the oocyte, depolarization may serve to minimize electrogenic Ca\(^{2+}\) entry through nonspecific cation channels, preventing elevation of cytosolic Ca\(^{2+}\) and, secondarily, Ca\(^{2+}\)-induced Ca\(^{2+}\) release from intracellular stores, or Ca\(^{2+}\) release controlled by other signals. Previously defined interactions of BCL2 family proteins with intracellular Ca\(^{2+}\) release pathways (22, 23, 42, 50) do not explain the BH3-independent rescue by BCL2 family members of APOL1-induced changes in oocyte morphology and Ca\(^{2+}\) flux. Further experiments will be needed to explore these pathways in APOL1-expressing oocytes and other cell types.

APOL1-associated oocyte permeabilities and the intrinsic ion channel activity of APOL1. Recombinant APOL1 WT was expressed at or near the surface of Xenopus oocytes (Fig. 9), associated with DIDS-sensitive Cl\(^{-}\) fluxes (Fig. 5), trivalent cation-sensitive Ca\(^{2+}\) fluxes, and increased TEVC currents partially blocked by DIDS and by trivalent ions, likely representing mixed anion and cation currents (Figs. 6–9, 11). The data are consistent with APOL1-induced Cl\(^{-}\) permeability being secondary to primary elevation of Ca\(^{2+}\) or cation permeability in the oocyte. However, the relationship between these oocyte cation and anion permeabilities and the previously

Rescue of APOL1 morphological toxicity by anti-apoptotic BCL2 proteins. Despite the apparent lack of importance of the putative BH3 domain to oocyte morphological toxicity, coexpression of some human anti-apoptotic BCL2 family members rescued APOL1-associated toxicity in an apparently BH3-independent manner. Rescue was exhibited by MCL-1, BCL-X\(_{L}\), BCLW, and BCL2A1, but not by BCL2 itself. The distinct behavior of BCL2 overexpression might reflect the lack of intrinsic anti-apoptotic activity in oocytes [recombinant xBcl2 fails to regulate in vitro caspase activity and poly(ADP-ribose)polymerase activation in egg lysates], or the lack of endogenous xBcl2 expression in egg and ovary of X. laevis (54). In contrast, anti-apoptotic xMcl-1, xBcl-X\(_{L}\), xBcl-W, and xBcl-B/xBcl2A10 were easily detected in Xenopus egg and ovary. However, xMcl-1 could not be coimmunoprecipitated with its functional pro-apoptotic partner, xBid (54), consistent with our unsuccessful attempts to coimmunoprecipitate MCL1 with APOL1. Steady-state expression of APOL1 at or near the oocyte surface was decreased by MCL1 coexpression, without change in total APOL1 content of the oocyte (Fig. 10). Oocyte morphological rescue from APOL1-associated toxicity by the anti-apoptotic BCL2 family members might occur by direct interaction of insufficient affinity for detection by pulldown, or by induction of an alternate prosurvival pathway. Thus coexpressed BCL2 family members may decrease APOL1

Fig. 8. Coexpression of MCL1 inhibits APOL1-associated Ca\(^{2+}\) uptake and currents. A: injection of 50 ng MCL1 cRNA together with 10 ng APOL1 cRNA greatly reduces APOL1-associated *Ca\(^{2+}\)* uptake at the indicated times post-injection. n = 20 in each group (10 oocytes from each of 2 frogs). B: injection of 50 ng MCL1 cRNA together with 10 ng APOL1 cRNA also greatly reduces APOL1-associated two-electrode voltage clamp current at the indicated times post-injection. Values are means ± SE. *P < 0.05.

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reported pore-forming activities of APOL1 remains incompletely defined.

Different preparations of recombinant APOL1 have yielded distinct types of complex ion currents upon reconstitution into planar lipid bilayers. Recombinant His-tagged APOL1 fragment aa 60–235 (encompassing the colicin-like domain and the putative BH3 domain) was expressed in E. coli and purified in the dialyzable detergent CHAPS. Fusion of recombinant protein into asolectin bilayers separating cis- and trans-solutions of symmetric pH 6 exhibited complex currents of 3.2:1 anion-to-cation selectivity. However, this construct lacking the APOL1 C-terminal putative coiled-coil domain itself lacked trypanolytic activity (44).

In contrast, purified hetero-oligomeric human TLF1 contained, in addition to APOL1, pore-forming cathelicidin (18–20), haptoglobin-related protein, and at least five additional protein components. Fusion of TLF1 into bilayers of whole trypanosome lipids separating cis- and trans-solutions of symmetric pH 6 exhibited complex currents of 3.2:1 anion-to-cation selectivity. However, this construct lacking the APOL1 C-terminal putative coiled-coil domain itself lacked trypanolytic activity (44).

In contrast, purified hetero-oligomeric human TLF1 contained, in addition to APOL1, pore-forming cathelicidin (18–20), haptoglobin-related protein, and at least five additional protein components. Fusion of TLF1 into bilayers of whole trypanosome lipids separating solutions of cis pH 5.5 and trans pH 7.4, yielded complex currents with cation-to-anion selectivity of 4.8:1 (34). Most recently, recombinant His-tagged human APOL1 holoprotein was extracted from E. coli particulate fraction in Zwittergent 3–14, gel filtration-purified to apparent homogeneity, then fused into planar bilayers of asolectin depleted of nonpolar lipids. The resulting channels were ideally cation-selective, but required priming by cis compartment acid pH prior to dramatic activation by alkalinizing restoration of neutral pH (52). Interestingly, the acidic priming itself induced small currents similar in magnitude to those observed in the TLF1 experiments recorded at cis-acidic pH (34), but the restoration of neutral or alkaline pH produced currents of ~100-fold greater magnitude (52).

The Cl⁻ and Ca²⁺ fluxes and whole cell currents of APOL1-expressing oocytes measured at neutral or alkaline pH were similarly reduced at more acid pH values. The regulatory patterns of APOL1-associated function in whole oocytes complement those of recombinant APOL1 subdomains and intact proteins, and of APOL1-containing TLF1 studied in lipid bilayers. The ion transport manifestations of APOL1 expression in Xenopus oocytes likely integrate, in ways yet to be determined, intrinsic APOL1 activities with APOL1’s secondary effects on endogenous ion channels and/or transporters.

APOL1 phenotypes in Xenopus oocytes and APOL1-associated kidney disease. Elevated renal disease risk is associated with homozygosity or compound heterozygosity for the G1 or G2 missense variants of APOL1. The genetic absence of APOL1 discovered in a single patient (59) has not been associated with evident renal insufficiency (25). Our studies of the toxic effects of APOL1 expression in Xenopus oocytes were performed with APOL1 G0 transcript 1 (39) (referred to as APOL1 WT). Under the conditions tested, oocyte expression of APOL1 disease risk variants G1 or G2...
led to morphological deterioration indistinguishable from that of G0, as well as to comparably complete rescue by coexpressed MCL1 and BCL-XL (data not shown). This variant-independent toxicity of APOL1 observed in Xenopus oocytes under the conditions tested here differs from the increased cell death observed in HEK-293 cells transiently transfected with APOL1 risk variants G1 and G2 (39) or in immortalized human podocytes transduced by lentiviral G1 or G2 (30), in which these risk variants sensitized cell responses to a diverse range of pharmacological and viral stressors.

Fig. 10. MCL1 coexpression with APOL1 is associated with reduced steady-state levels of APOL1 at or near the oocyte surface. A: immunoblot of APOL1 in detergent lysates (20 mg protein per lane) from oocytes expressing APOL1 in the absence or presence of coexpressed MCL1. GAPDH reprobing of the same blot confirmed equal loading of lanes, a result supported by Ponceau red stain of the blot pretransfer (not shown). One of three similar experiments with indistinguishable results is shown. B: representative median intensity images of whole mount confocal immunofluorescence micrographs of uninjected oocytes or oocytes injected 72 h previously with cRNAs encoding APOL1, MCL1, or both, as indicated. C: normalized fluorescence intensity of APOL1 in 26–27 oocytes expressing the indicated cRNAs. Values are means ± SE. *P < 0.05 vs. oocytes expressing APOL1 alone; ANOVA with Tukey’s t-test.

Fig. 11. APOL1-associated ion flux and current are inhibited by acidic extracellular pH. A and B: extracellular protons inhibit 45Ca2+ uptake (n = 20; A) and 36Cl− uptake (n = 10–20; B) by oocytes injected 72 h earlier with 10 ng APOL1 cRNA and subsequently maintained in HiK MBS. *P < 0.05. C and D: extracellular protons inhibit the increased two-electrode voltage clamp current in oocytes maintained 72 h in HiK MBS after injection with 10 ng APOL1 cRNA (n = 11; C), but inhibit only minimally the smaller currents recorded in uninjected oocytes (n = 7; D). Values are means ± SE. *P < 0.05 by Mann-Whitney test. **P < 0.001 by t-test.
Thus the Xenopus oocyte is useful for study of APOL1 toxicity, but conditions remain to be defined for which the renal disease-associated G1 and G2 risk variants are reliably more toxic than the G0 variant. We speculate that Xenopus oocytes may lack protein(s) that confer on cultured mammalian kidney cells discrimination of the G1 and G2 variants of APOL1 from G0. In this context, oocyte studies may provide both an important control and a useful contrast for studies in cultured cells.

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