Z Proteins of New World Arenaviruses Bind RIG-I and Interfere with Type I Interferon Induction

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The retinoic acid-inducible gene I product (RIG-I) is a cellular sensor of RNA virus infection that regulates the cellular beta interferon (IFN-β) response. The nucleoproteins (NP) of arenaviruses are reported to antagonize the IFN response by inhibiting interferon regulatory factor 3 (IRF-3). Here, we demonstrate that the Z proteins of four New World (NW) arenaviruses, Guanarito virus (GTOV), Junin virus (JUNV), Machupo virus (MAVC), and Sabia virus (SABV), bind to RIG-I, resulting in downregulation of the IFN-β response. We show that expression of the four NW arenavirus Z proteins inhibits IFN-β mRNA induction in A549 cells in response to RNA bearing 5' phosphates (5'pppRNA). NW arenavirus Z proteins interact with RIG-I in communoprecipitation studies and colocalize with RIG-I. Furthermore, expression of Z proteins interferes with the interaction between RIG-I and MAVS. Z expression also impedes the nuclear factor kappa light chain enhancer of activated B cells (NF-κB) and IRF-3 activation. Our results indicate that NW arenavirus Z proteins, but not Z protein of the Old World (OW) arenavirus lymphocytic choriomeningitis virus (LCMV) or Lassa virus, bind to RIG-I and inhibit downstream activation of the RIG-I signaling pathway, preventing the transcriptional induction of IFN-β.

The innate immune system recognizes virus infection and, as a first-line defense, induces antiviral responses by producing type I interferons (alpha and beta interferon [IFN-α/β]), which have antiviral, antiproliferative, and immunomodulatory functions. Events that trigger the antiviral innate immune response include (i) detection of the invading virus by immune system receptors and (ii) activation of protein signaling cascades that regulate the synthesis of IFNs. The innate immune system is activated through pattern recognition receptors (PRR) that recognize conserved microbial molecular structures. Toll-like receptors (TLRs) 3, 7, 8, and 9 and retinoic acid-inducible gene I (RIG-I)-like helicases (RLHs) are the two major receptor systems for detecting viruses. These systems localize to different compartments within the cell and recognize different ligands; whereas TLRs recognize viral nucleic acids present either in the extracellular environment or in endosomes, RLHs detect viral RNA in the cytoplasm (reviewed in reference 34).

RIG-I and another RLH, the melanoma differentiation-associated gene 5 product (MDA5), are intracellular sensors of viral RNA. RIG-I and MDA5 are involved in two caspase-recruiting domains (CARD) at their N terminus, a DExD/H-box helicase domain, and a regulatory domain (RD) at their C terminus. RNA binding requires intact helicase domains and RDs (32). After binding of RNA, the CARDs relay signals to the downstream CARD-containing mediator MAVS (for “mitochondrial antiviral signaling”; also known as VISA [virus-induced signaling adapter], IPS-1 [beta interferon promoter stimulator], or CARDIF [CARD adaptor inducing IFN-β]) (15, 23, 35, 39). Once activated, MAVS triggers activation of two protein complexes, TBK1:IKKe (TANK-binding kinase 1:IkB kinase epsilon) and IKKα-IKKβ (IkB kinase α-IkB kinase β), involved in the activation of IRF-3 and NF-κB transcription factors, respectively. IRF-3 and NF-κB translocate into the nucleus and assemble into a stereospecific enhancosome complex that binds the promoter of IFN-β, resulting in its transcriptional activation (reviewed in references 10 and 31).

Although MDA5 and RIG-I share similar structural architectures and their signaling pathways converge at the MAVS adaptor level, gene knockout studies indicate that the two proteins respond to distinct RNA species. RIG-I recognizes in vitro-transcribed RNA and has specifically been shown to respond to vesicular stomatitis virus (VSV), Newcastle disease virus (NDV), and influenza A virus (FLUAV). In contrast, MDA5 recognizes poly(I:C), a synthetic double-stranded RNA (dsRNA) analog, and is essential for the antiviral response to the picornavirus encephalomyocarditis virus (EMCV) (14). RIG-I, but not MDA5, recognizes RNA bearing 5’ phosphates (11, 30).

Many viruses have evolved viral products that antagonize the interferon response at different levels. The RIG-I/MAVS pathway appears to be targeted by different viruses to achieve inhibition of the IFN-β system. For example, the nonstructural protein 3/4a (NS3/4A) protease of hepatitis C virus (HCV) cleaves MAVS and abrogates antiviral signaling (18, 20, 23). Another example is NS1 of FLUAV, which interacts with RIG-I to inhibit the RIG-I/MAVS pathway (9, 11, 24, 25, 30). More recently, the nonstructural protein NS2 of human respiratory syncytial virus (RSV) has been shown to antagonize the activation of IFN-β transcription by interacting with RIG-I (19).

Arenaviruses are enveloped single-stranded RNA viruses with bisegmented genomes, comprising a larger (L) and a smaller (S) segment. Although classified as negative-strand RNA viruses, they employ an ambisense coding strategy. The S
segment encodes, in the opposite orientation, an nucleoprotein (NP) and a glycoprotein precursor (GPC). The L segment encodes an RNA-dependent RNA polymerase (L) and a small RING finger protein (Z). The Z protein functions as the arenaviral counterpart of the matrix protein found in other negative-strand RNA viruses. Z is associated with membranes (29, 36, 37); it has also been found in the cytoplasm (33) and the nucleus (1), interacting with cellular proteins such as ribosomal protein P0 (2), eukaryotic translation initiation factor eIF4E (3), promyelocytic leukemia protein PML (1), and the proline-rich homeodomain protein (6).

Arenaviruses are divided into New World (NW) and Old World (OW) complexes based on serologic, geographic, and genetic relationships. Some arenaviruses, including Lassa virus (LASV) and Lujo virus (LUV) from the OW complex and Guanarito virus (GTOV), Junin virus (JNVU), Machupo virus (MADV), Sabia virus (SADV), and Chapare virus (CHPV) from the NW complex, can cause hemorrhagic fevers in humans. Evasion of the host immune response may contribute to their pathogenicity. The NWs of the OW arenavirus lymphocytic choriomeningitis virus (LCMV) and NW arenaviruses JUNV and MADV but not Tacaribe virus (TCRV) have been found to block the innate IFN response by inhibiting IRF-3 (21). To our knowledge, Z has not been implicated in immune evasion. Here, we show that the Z proteins of GTOV, JUNV, MACV, and SADV, but not that of LCMV or Lassa virus (LASV), interact with RIG-I to curb the activation of the IFN-β response.

**MATERIALS AND METHODS**

**Cells, viruses, and antibodies.** 293A and A549 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (In-vitrogen, Carlsbad, CA) and 100 μg/ml penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO). Sendai virus (SeV) strain Cantell (VR007) was purchased from the ATCC (Manassas, VA). Rabbit antisera against Z of arenaviruses GTOV, JUNV, MADV, and SADV were generated by immunizing rabbits with recombinant His6-GTOV Z, His6-JNVU Z, His6-MADV Z, and His6-SADV Z, respectively. The respective six-histidine (His6)-tagged proteins were expressed in prokaryotic systems (pDEST 17 Gateway vector; Invitrogen) and purified by affinity chromatography using 1-ml HisTrap columns (GE Healthcare Life Sciences, Piscataway, NJ) equilibrated with denaturing binding buffer (20 mM Tris-Cl, pH 8, 6 M guanidine, 0.5 M NaCl, 20 mM imidazole), washed with 5 column volumes (CV) of denaturing binding buffer and 5 CV of denaturing wash buffer (20 mM Tris-Cl, pH 8, 6 M urea, 0.5 M NaCl, 20 mM imidazole), and eluted in denaturing elution buffer (20 mM Tris-Cl, pH 8, 6 M urea, 0.5 M NaCl, 0.5 M imidazole) with a linear gradient over 20 CV. The following additional antibodies: goat polyclonal antibodies against RIG-I (SC-48932, rabbit polyclonal antibodies against IFR-3 (SC-9082; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit polyclonal antibodies against phospho-IRF-3 (Ser396) (ab299; Abcam, Cambridge, MA), rabbit polyclonal antibodies against MAVS (A310-243A; Bethyl Laboratories, Montgomery, TX), mouse monoclonal antibody against V5 (R060-25; Invitrogen), horseradish peroxidase (HRP)-coupled goat anti-rabbit IgG or goat anti-mouse IgG antibodies or rabbit anti-goat IgG antibodies (Bio-Rad, Hercules, CA), and Cy2-coupled goat anti-rabbit immunoglobulin (Ig) (Jackson ImmunoResearch, West Grove, PA).

**Plasmids, transfections, and 5′pppRNA.** pCAGGS plasmids expressing the Z proteins of GTOV, JUNV, MADV, SADV, or LASV (pCAGGS-ZG, -ZJ, -ZM, -ZS, or -ZL, respectively) were generated by insertion of the respective open reading frame (ORF) between the Nol and Xhol sites; pCAGGS-GFP was generated by the insertion of the GFP ORF (pCAGGS-LCMV) and then the GFP ORF was excised by restriction with XhoI and XbaI. The ORF was then inserted into the plasmid pENTR 1A (Invitrogen) between the KpnI and XhoI sites. In order to generate N-terminal and C-terminal V5-tagged versions, the insert was subsequently recombined into pCDNA3.1/nV5-DEST and pCDNA3.2c/V5-DEST vectors (Invitrogen) by using Gateway LR clonase enzyme mix (Invitrogen). The RIG-I ORF was also cloned into pDEST 17 (Invitrogen) and His-tagged recombinant protein expressed and purified as described above for arenavirus Z proteins. pUNO1-RIG-I-GFP was a generous gift from Joari De Miranda (Columbia University). pUNO1-MAVS was purchased from Invitrogen. Transfections were performed with either calcium phosphate or Lipofectamine 2000 (Invitrogen), according to the manufacturer’s instructions. 5′-Triphosphate RNA (5′pppRNA) was generated from an 80-bp Xenopus elongation factor 1α sequence cloned into pTFI by in vitro transcription using a T7 MEGashortscript kit and purified using a MEGAClear kit (Ambion, Austin, TX).

**qPCR.** The cell monolayer was washed twice with ice-cold phosphate-buffered saline (PBS; Sigma-Aldrich) and RNA extracted using an RNeasy kit (Qiagen, Hilden, Germany). Total RNA was quantified by UV spectrometry. cDNA was synthesized from 0.5 μg of RNA in a 10-μl reaction volume by using TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA). Human IFN-β-specific PCR primers and a probe labeled at the 5′ end with the fluorescent reporter dye 6-carboxy-tetramethylrhodamine (TAMRA) were purchased from Applied Biosystems. Results were normalized to β-actin gene expression levels in the same sample replicate by using intron/exon-spanning human β-actin-specific PCR primers and a probe labeled with a VIC reporter and a Black Hole dark quencher dye (Applied Biosystems). Each 25-μl quantitative real-time reverse transcription-PCR (qPCR) amplification reaction mixture contained 5 μl of the cDNA template, 12.5 μl universal master mix (Applied Biosystems), and 1.25 μl of IFN-β and β-actin primers (at 200 nM each) and probes (at 300 nM). The assay was performed with a model 7700 sequence detector system (Applied Biosystems) with a cycling profile of 50°C for 2 min, 95°C for 10 min, and 45 cycles of 95°C for 15 s, 60°C for 1 min.

**Immunoprecipitation.** Cultures of 293A cells in 6-well plates were transfected with various combinations of plasmids. The total DNA concentration was kept constant by adding empty-vector DNA. Thirty-six hours after transfection, the cells were washed with PBS, trypsinized, collected by centrifugation, and then lysed in 150 μl of nondenaturing lysis buffer (20 mM Tris-Cl, pH 8, 137 mM NaCl, 10% glycerol, 1% Nonidet P-40, 2 mM EDTA, complete protease inhibitor cocktail [Roche Applied Science, Indianapolis, IN]). The lysates were subjected to centrifugation for 20 min at 10,000 × g at 4°C and the clarified supernatants preadsorbed by incubation with 10 μl of protein A-Sepharose (Sigma-Aldrich) for 20 min at 4°C. For each immunoprecipitation, a 100-μl aliquot of lysate was incubated with 1 μg of monoclonal antibody or 5 μl of polyclonal antiserum overnight at 4°C on a rotator. Antibody-antigen complexes were precipitated by adding 50 μl of a 1:1 slurry of protein A-Sepharose beads in lysis buffer for 4 h at 4°C, followed by centrifugation for 1 min at 10,000 × g. The beads were washed four times with 1 ml of lysis buffer and then subjected to Western blot (WB) analysis. In the case of immunoprecipitation with purified protein, 200 ng of protein were combined with 5 μl polyclonal sera against Z proteins.

**Native PAGE.** Native polyacrylamide gel electrophoresis (PAGE) was performed using ReadyGel (Bio-Rad). The polyacrylamide gel was run with 25 μl of Tris and 192 mM glycine, pH 8.4, with and without 1% deoxycholate (DOC) in the cathode and anode chambers, respectively, for 30 min at 40 mA. Samples in native sample buffer (10 μg protein, 62.5 mM Tris-Cl, pH 6.8, 15% glycerol, and 1% DOC) were size fractionated by electrophoresis for 60 min at 25 mA and transferred to nitrocellulose membranes for WB analysis (12).

**WB analysis.** Clarified cell lysates were boiled in SDS sample buffer for 5 min (17); protein A-Sepharose beads from immunoprecipitations were boiled in 2× SDS sample buffer. The protein samples were size fractionated by SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad) (38). The membranes were probed with the appropriate primary antibodies. After being washed with PBS containing 0.1% Tween 20, bound antibodies were probed by secondary incubation with HRP-coupled goat anti-rabbit IgG, goat anti-mouse IgG, or rabbit anti-goat IgG antibodies. Proteins were visualized by using an ECL Plus Western blotting detection system (GE Healthcare) and chemiluminescence scanned by a Storm phosphorimager (GE Healthcare).

**ELISA.** IFN-β protein was measured with an enzyme-linked immunofluorescence assay (ELISA) (25- to 2,000-pg/ml range; PBL InterferonSource, Piscataway, NJ).

**Immunofluorescence assay and confocal microscopy.** 293A cells plated on 4-chamber culture slides (Becton Dickinson, Franklin Lakes, NJ) were incubated overnight at 37°C and then transfected with空格vector or a Z expression plasmid. At 24 h posttransfection, the cells were transfected with 5′pppRNA, and 6 h later, they were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature (RT) and then permeabilized with 0.1% Triton X-100 in PBS for 10 min at RT. Culture slides were mounted and images taken using a H2E-RFCA micro-
scope with a 40× oil immersion objective (Olympus, Tokyo, Japan). In colocalization studies, cells were cotransfected with pUNO1-RIG-I-GFP and pCAGGS-ZI, stained with antibodies, and imaged using a scanning confocal ECLIPSE TE2000-U microscope (Nikon, Tokyo, Japan).

RESULTS

NW arenavirus Z proteins inhibit IFN-β induction by 5’pppRNA. During the course of building sequence libraries for a diagnostic viral microarray (13, 27), we found that Z proteins of NW arenaviruses include a domain with a predicted secondary structure folding pattern that resembles structures found in several cellular proteins implicated in innate immunity. Indeed, we found that NW arenavirus Z proteins lead to decreased levels of IFN-β mRNA (Fig. 1). A549 cells were transfected with plasmids, each encoding one of the four NW arenavirus Z proteins (GTOV, JUNV, MACV, and SABV) or two OW arenavirus Z proteins (LCMV and LASV). All six proteins showed comparable signals in Western blots of transfected cell extracts, indicating similar expression levels (not shown). Thirty-six hours after Z transfection, cells were cotransfected with 5’pppRNA for 6 h and then IFN-β levels measured by qPCR. After normalization to the β-actin expression level, the IFN-β expression level was calculated as fold induction with respect to a baseline level measured for cells transfected with empty vector and not stimulated with 5’pppRNA.

FIG. 1. New World arenavirus Z proteins decrease IFN-β induction in A549 cells stimulated with 5’pppRNA. A549 cells (5 × 10⁵) were transfected with 3 μg of pCAGGS-Z or empty vector by use of calcium phosphate. At 36 h posttransfection, cells were transfected with 1 μg of 5’pppRNA by use of Lipofectamine 2000. Six hours later, the cells were harvested and the IFN-β expression level was measured by qPCR, with normalization to the β-actin expression level. Data represent three biological replicates. *, significant differences (P < 0.05; t test).

FIG. 2. New World arenavirus Z proteins interact with RIG-I in coimmunoprecipitation experiments. (A) 293A cells (10⁶) were cotransfected with 0.5 μg of pcDNA3.1-V5-RIG-I (N terminally V5 tagged [N-V5-RIG-I] or C terminally V5 tagged [C-V5-RIG-I]) and with 0.5 μg of pCAGGS-Z (ZG, GTOV Z; ZJ, JUNV Z; ZM, MACV Z; ZS, SABV Z), pCAGGS-HA-NS1, or pCAGGS-GFP. Thirty-six hours later, cells were lysed and clarified supernatants subjected to immunoprecipitation assays using anti-V5 antibody (IP:V5). Z, NS1, and GFP were detected after Western blotting using anti-Z (WB:Z), anti-hemagglutinin (anti-HA) (WB:HA), and anti-GFP (WB:GFP) antibodies, respectively. “Input” represents untreated lysate prior to precipitation subjected to Western blot analysis. (B) 293A cells (10⁶) were cotransfected with 0.5 μg of pCAGGS-Z (ZG, GTOV Z; ZJ, JUNV Z; ZM, MACV Z; ZS, SABV Z) and 0.5 μg of pcDNA3.1/nV5-RIG-I (N terminally V5 tagged [N-V5-RIG-I]) or 0.5 μg of pcDNA3.2/cV5-RIG-I (C terminally V5 tagged [C-V5-RIG-I]) and then immunoprecipitated with antisera to the respective Z protein (IP:Z) and analyzed by Western blotting with anti-V5 antibody (WB:V5).
All four NW arenavirus Z proteins reduced IFN-β mRNA induction by ~60% (P < 0.05). In contrast, LCMV and LASV Z proteins had no significant effect on IFN-β mRNA induction (Fig. 1), consistent with an earlier report on a lack of inhibition of the type I interferon response by OW LCMV Z protein (22). No significant effect on IFN-β mRNA levels in comparison to those for untreated cells was found in cells transfected with vector alone or with Z expression constructs in the absence of 5'pppRNA stimulation; comparable results were obtained in transfection experiments of 293A cells (not shown). A comparable ~50% reduction of interferon induction was also measured with all four NW arenavirus Z proteins by ELISA (not shown). These results suggested that NW arenavirus Z proteins, but not LCMV or LASV Z protein, interfere with the transcriptional activation of IFN-β.

**The Z proteins of NW arenaviruses interact with RIG-I.** Since NW arenavirus Z proteins inhibited IFN-β production in cells transfected with 5’pppRNA, a ligand of RIG-I, we tested whether NW arenavirus Z proteins interact with RIG-I. 293A cells were cotransfected with a V5-tagged RIG-I expression plasmid and one of the four arenaviral Z expression plasmids. After 36 h, interaction of RIG-I (N and C terminally V5 tagged) with GTOV, JUNV, MACV, or SABV Z proteins was assessed in coimmunoprecipitation assays using anti-V5 antibody (Fig. 2A). FLUAV NS1 and GFP-expressing plasmids were used as positive and negative controls, respectively, for

**FIG. 3.** New World arenavirus Z proteins bind RIG-I. (A) Purified recombinant Z and RIG-I were incubated in nondenaturing lysis buffer and subjected to immunoprecipitation assays using anti-Z antibody. RIG-I was detected by Western blotting using anti-RIG-I antibody. (B) 293A cells were transfected with pUNO1-RIG-I-GFP (I) or pCAGGS-ZJ (II) or cotransfected with both plasmids (III to V). Proteins were visualized by confocal immunofluorescence microscopy. (I, III) RIG-I-GFP (green). (II, IV) Staining with the ZJ antibody (anti-Z [α Junin Z]) (red). (V) Overlay (yellow).

**FIG. 4.** JUNV Z protein blocks complex formation between RIG-I and MAVS. 293A cells (1.5 × 10⁶) were triple transfected with 0.5 μg of pUNO1-MAVS, 0.5 μg of pcDNA3.1nV5-RIG-I, and 0.5 μg of pCAGGS-ZJ or transfected with vector without Z instead of pCAGGS-ZJ. pcAGGS-GFP was used as a negative control. Amounts of transfected DNA were adjusted with empty pCAGGS plasmid. At 36 h posttransfection, cells were lysed and clarified supernatants subjected to immunoprecipitation assays using anti-MAVS antibody (IP:MAVS). RIG-I, Z, and GFP were detected by Western blotting using the respective antibodies (WB:MAVS, WB:V5, WB:Z, and WB:GFP).
RIG-I interaction. In a complementary experiment, we performed coimmunoprecipitation assays using four antisera specific for each of the four NW arenavirus Z proteins. RIG-I was coimmunoprecipitated with GTOV, JUNV, MACV, and SABV Z proteins (Fig. 2B). No interaction was observed between OW arenavirus LCMV or LASV Z protein and RIG-I (not shown). MDA5, although structurally similar to RIG-I, did not interact with the Z proteins of GTOV, JUNV, MACV, SABV, or LCMV (not shown).

NW arenavirus Z proteins bind to and colocalize with RIG-I. In order to determine whether the interactions between NW arenavirus Z proteins and RIG-I are direct, we performed coimmunoprecipitation assays using purified recombinant proteins. Incubation of purified recombinant His6-tagged RIG-I with purified recombinant His6-tagged Z proteins of GTOV, JUNV, and MACV and their cognate anti-Z antisera resulted in coprecipitation of RIG-I (Fig. 3A). Experiments with Z protein of SABV yielded no visible band. Whether this reflects a difference in the avidity of the anti-Z antiserum or a bona fide failure of direct interaction between SABV Z protein and RIG-I is unknown. We also examined the cellular localization of NW arenavirus Z proteins and RIG-I in 293A cells by confocal immunofluorescence microscopy. Consistent with earlier work, staining of JUNV Z/RIG-I-GFP double-transfected cells with anti-Z antibody indicated that Z protein is membrane bound (4, 7, 29, 36, 37) but also present in the cytoplasm (1, 2, 33), with a distribution similar to that observed for RIG-I (Fig. 3B).

NW arenavirus Z proteins interfere with the interaction between RIG-I and MAVS. The finding that NW arenavirus Z proteins bind RIG-I and decrease IFN-β mRNA induction in response to the RIG-I ligand 5'pppRNA suggests that Z pro-
teins may deregulate RIG-I signaling to downstream adapters. MAVS acts downstream of RIG-I in signal transmission through complex formation with activated RIG-I. To investigate whether Z proteins abrogate the interaction between RIG-I and MAVS, we conducted coimmunoprecipitation analyses. 293A cells were cotransfected with MAVS and N-terminally V5-tagged RIG-I expression vector as well as with a JUNV Z expression vector or vector without Z (control). Whereas MAVS-binding to RIG-I was readily demonstrated in the absence of Z protein, no interaction was seen in the presence of JUNV Z protein (Fig. 4). These results are consistent with JUNV Z protein preventing the RIG-I/MAVS complex formation.

New World arenavirus Z proteins impede NF-κB and IRF-3 activity. RIG-I signaling activates transcription factors NF-κB and IRF-3 via MAVS binding. We examined the activity of endogenous NF-κB and IRF-3 in 293A cells expressing JUNV Z. Twenty-four hours after transfection, cells were mock infected or infected with SeV for 16 h. Cell lysates were subjected to SDS-PAGE or native PAGE. The presence of JUNV Z protein blocked the activation of NF-κB. The active form of NF-κB, p52, was not detected in lysates from SeV-infected cells expressing JUNV Z protein (Fig. 5A). Phosphorylation of IRF-3 at Ser396 was observed only in SeV-infected cells when JUNV Z protein was absent; uninfected cells and infected cells that express JUNV Z protein showed no IRF-3 phosphorylation (Fig. 5B). IRF-3 dimer formation is a consequent of IRF-3 phosphorylation and enables nuclear translocation. As anticipated, IRF-3 dimerization was reduced in SeV-infected cells expressing JUNV Z protein (Fig. 5C). Similarly, nuclear translocation of IRF-3 was blocked by the expression of JUNV Z protein (Fig. 5D).

DISCUSSION

Given the central role of RIG-I in virus sensing and IFN induction, both RNA and DNA viruses have devised gene products that target the RIG-I signaling pathway to inhibit IFN production by infected cells. Inhibition of RIG-I signaling is achieved by targeting different steps of the pathway. In many instances, viruses express redundant IFN-antagonizing activities. For example, the severe acute respiratory syndrome coronavirus (SARS-CoV) encodes three proteins with IFN antagonism activity, the N, ORF 3b, and ORF 6 products (16). In arenaviruses, the NP antagonizes IFN by inhibiting IRF-3 (21). Inhibition of RIG-I signaling is achieved by targeting different steps of the pathway. In many instances, viruses express redundant IFN-antagonizing activities. For example, the severe acute respiratory syndrome coronavirus (SARS-CoV) encodes three proteins with IFN antagonism activity, the N, ORF 3b, and ORF 6 products (16). In arenaviruses, the NP antagonizes IFN by inhibiting IRF-3 (21). Our data reveal an additional strategy whereby NW arenaviruses evade the host innate immune response. In a mechanism that may be specific to NW arenaviruses, Z proteins inhibit host cell IFN mRNA induction via direct binding of Z to RIG-I. This interaction disrupts the necessary complex formation between RIG-I and MAVS, impeding downstream IRF-3 and NF-κB activation and resulting in decreased IFN-β induction.

The precise mechanism by which NW arenavirus Z proteins inhibit RIG-I activity remains to be determined. However, several recent studies provided more-detailed insights into the potential mechanism: (i) studies by Cui et al. (5) show that the C-terminal RD of RIG-I binds viral RNA in a 5′-triphosphate-dependent manner and activates the RIG-I ATPase through RNA-dependent dimerization, (ii) RIG-I requires ubiquitina-

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