Published in final edited form as:
Virology. 2014 January 20; 449: 174–180. doi:10.1016/j.virol.2013.11.015.

Rift Valley fever virus infection induces activation of the NLRP3 inflammasome

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Abstract

Inflammasome activation is gaining recognition as an important mechanism for protection during viral infection. Here, we investigate whether Rift Valley fever virus, a negative-strand RNA virus, can induce inflammasome responses and IL-1β processing in immune cells. We have determined that RVFV induces NLRP3 inflammasome activation in murine dendritic cells, and that this process is dependent upon ASC and caspase-1. Furthermore, absence of the cellular RNA helicase adaptor protein MAVS/IPS-1 significantly reduces extracellular IL-1β during infection. Finally, direct imaging using confocal microscopy shows that the MAVS protein co-localizes with NLRP3 in the cytoplasm of RVFV infected cells.

Keywords

inflammasome; NLRP3; ASC; caspase-1; Rift Valley fever virus; virus; IL-1β; dendritic cells; murine

Introduction

The inflammasome is a large multi-protein complex that can assemble in response to viral, fungal, or bacterial pathogens. The active inflammasome leads to auto-catalytic cleavage of cysteine protease caspase-1, which in turn processes pro-IL-1β and pro-IL-18 into their mature biologically active forms (1–3). In addition to processing its specific substrates, caspase-1 also has a role in the export of pro-IL-1α and in mediating cell death through pyroptosis (4, 5). Mature IL-18 is secreted as a result of inflammasome activation and results in elevated NK and NK-T cell cytotoxic activity as well as IFN-γ secretion by T cells (6, 7). Secreted mature IL-1α and IL-1β act as pyrogens to induce fever (8). While inflammasome activation during infection is often thought of as protective, disregulated IL-1β production in the absence of pathogens can be harmful. For instance, a hallmark of

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autoinflammatory disease is the potential for resolution with an IL-1β blockade (9–11). Thus, activation of inflammasome signaling is carefully regulated.

Activation of the inflammasome is a complex process requiring two signals. The first step involves initiation of NF-κB mediated signaling through pattern recognition receptors, such as Toll-like receptors (TLRs), and results in accumulation of pro-IL-1β and increased expression of inflammasome components (12). TNF-α has also been shown to serve as a first signal through mechanisms independent of TLRs (13). The second signal leads to assembly of the inflammasome in the cytoplasm and activation of caspase-1. Even after activation of the inflammasome has occurred, further regulation of IL-1β and IL-1α signaling can be achieved by secretion of the IL-1 receptor antagonist (IL-1RA) which prevents binding of IL-1α and IL-1β to their shared receptor, IL-1R1 (9).

Classical inflammasomes are found within the NOD-like receptor (NLR) family and include NLRPs (containing leucine rich repeat and pyrin domain containing proteins) and NLRCs (containing leucine rich repeat and caspase recruitment domain containing proteins). RNA viruses such as respiratory syncytial virus, encephalomyocarditis virus, influenza, and rabies virus have been shown to induce activation of the NLRP3 inflammasome (14–17). Additionally, inflammasomes can be found outside of the NLR family. Absent in melanoma 2 (AIM2) can serve as a pattern recognition receptor and an inflammasome. The AIM2 protein, like other members of the Hin200 family, contains a DNA binding domain (18). Viruses with DNA genomes such as vaccinia and mouse cytomegalovirus have been shown to activate the AIM2 inflammasome leading to caspase-1 activation (19). Retinoic acid-inducible gene 1 (RIG-I) has been suggested to act as an inflammasome in concert with the adaptor protein ASC (apoptosis-associated speck-like protein containing C-terminal caspase recruitment domain) in response to vesicular stomatitis virus (VSV) (20). However, this report is controversial as another group demonstrated that IL-1β was not present without priming during VSV infection and that the NLRP3 inflammasome was primarily responsible for IL-1β processing (21).

Rift Valley fever virus (RVFV) is a negative-strand RNA virus of the family *Bunyaviridae*. The virus causes high mortality in young livestock and will induce infected pregnant livestock to abort. In humans, RVFV can cause a multitude of disease manifestations ranging from febrile illness to hemorrhagic fever and death. In our previous studies, we observed a low level of IL-1β in the serum of mice that had been infected with attenuated RVFV strain rMP-12 via the intranasal route (22). In addition, goats infected subcutaneously with the virulent ZH501 strain of RVFV are reported to have elevated IL-1β in their serum (23). Human serum collected from patients during the 2000–2001 RVFV outbreak in Saudi Arabia was analyzed for pro-inflammatory and suppressive cytokines, including inflammasome related cytokines. Levels of IL-1RA were elevated overall and relative to IL-1α levels in fatal versus non-fatal cases (24). These studies led us to question whether RVFV could induce inflammasome activation during infection.

Results

IL-1β production in response to RVFV is dose dependent and requires viral replication

In order to assess whether RVFV can induce IL-1β responses, conventional dendritic cells (cDCs) were generated from the bone marrow of WT mice and were infected with attenuated strains rMP-12 and NSs del of RVFV at varying MOI. The NSs del strain was generated on the rMP-12 backbone and lacks the gene encoding the NSs virulence factor (25). Dendritic cells had previously been identified to be potent producers of IL-1β in response to many viruses (17, 21). While macrophages have also been shown to support inflammasome activation in response to viruses, we chose to focus our efforts on dendritic

cells (14, 21). Cells were infected under both unprimed and LPS-primed conditions. When administered alone, LPS is known to induce production of pro-IL-1β. In the absence of a secondary signal, pro-IL-1β accumulates within the cell and cannot be processed by caspase-1 into its 17 kDa biologically active form. Addition of ATP to LPS-primed cells is a known inducer of NLRP3 inflammasome activation and served as a positive control for inflammasome activation and IL-1β processing. LPS priming of cells has been a useful tool for other virus infection studies in which ample amounts of pro-IL-1β are not produced in response to the virus alone *in vitro*, yet inflammasome activation and IL-1β processing clearly occurs when enough substrate is present (15).

In the absence of LPS priming, minimal IL-1β was detected in the cell supernatant (Fig. 1A). With priming, IL-1β was detected after infection with both the parent rMP-12 and NSs del strains. The level of extracellular IL-1β from NSs del infected cells was higher than that produced by rMP-12 infected cells and also positively correlated with increasing dose of virus. The elevated production of IL-1β from NSs del infected cells compared to rMP-12 infected cells was expected since the NSs protein of RVFV limits host transcription during infection by preventing assembly of general transcription factor TFIIH (26). The requirement for live viral infection to induce IL-1β production was assessed by infecting or stimulating cDCs with live or UV-inactivated virus. Extracellular levels of IL-1β were significantly reduced in response to UV-inactivated virus (Fig. 1B). Complete inactivation of the virus was verified using plaque assay (Fig. 1C).

Secretion of IL-1β is dependent upon NLRP3, ASC, and caspase-1

Several RNA viruses can initiate formation of the NLRP3 inflammasome complex, which results in caspase-1 activation and cleavage of IL-1β into its mature form (17, 27, 28). We examined whether NLRP3 and adaptor ASC were required for release of IL-1β by cDCs during RVFV infection. Absence of NLRP3 or ASC resulted in negligible amounts of IL-1β in the supernatant from RVFV infected cells (Fig. 2A). Cells from caspase-1 deficient mice (which have also been shown to lack caspase-11) (29) also showed reduced IL-1β responses compared to WT cells.

We next verified whether NLRP3 was necessary for processing of pro- IL-1β into mature IL-1β in response to RVFV by Western blot analysis. Levels of pro-IL-1β were clearly detected in the lysate of WT and *Nlrp3* $^{-/-}$ cDCs after RVFV infection. However, mature IL-1β (17 kD) was only detected in the extracellular fraction of WT cDCs that had been infected with RVFV compared to cDCs from *Nlrp3* $^{-/-}$ mice (Fig. 2B).

IL-1β production is dependent upon MAVS signaling during RVFV infection

The pro form of IL-1β is typically generated after an innate immune receptor recognizes viral patterns and signals through the adaptors MyD88/TRIF (for TLRs) or MAVS (for RNA helicases) resulting in NF-κB activation and induction of the IL-1β gene. In order to determine the impact of TLR signaling through MyD88 and TRIF on RVFV induced IL-1β, we examined pro-IL-1β in the lysate of unprimed RVFV infected cells. We did not use priming for these studies, since LPS would drive production of pro-IL-1β through the TLR pathway, thus confounding the interpretation. We observed that even in the absence of priming, pro- IL-1β could be detected in NSs del infected cDCs from *Myd88/Trif* $^{-/-}$ mice (Fig. 3). Presence of pro-IL-1β in the absence of TLR signaling suggests that other mechanisms may be responsible for initiating signal 1 during inflammasome activation, potentially including other PRR pathways. Levels of mature IL-1β were low to undetectable in the supernatant of NSs del treated MyD88/TRIF deficient cDCs and absent in the supernatants of NSs del infected WT cDCs (data not shown). This is presumably due to the minimal amounts of pro-IL-1β present as initial substrate.

Next, we examined the dependence of a number of IL-1 and inflammasome related responses in rMP-12 infected cDC on MAVS vs. MyD88/TRIF signaling pathways by using NanoString technology. Upregulation of many of the examined genes was dependent upon the MAVS pathway, including *Il-1β*, *Il-1α*, *Il-1ra*, *caspase-1*, *Nf-κb-1*, *Nf-κb2*, *Nlrp3*, *Nlrp12*, *Aim2*, and *Nos2* (Fig. 4). In contrast, *Il-18*, *Nlrc4* and *Unc93b* were similarly and only modestly induced in WT, *Myd88/Trif* $^{-/-}$ *and Mavs* $^{-/-}$ cells. None of the responses examined were found to be strongly dependent on MyD88/TRIF, indicating a minimal role for this pathway in RVFV induced inflammation.

The impact of MAVS on IL-1β protein production in RVFV infected cDCs was then determined via ELISA. WT and *Mavs* $^{-/-}$ cDCs were infected with rMP-12 or NSs del with or without LPS priming. In the absence of priming, negligible IL-1β responses were observed. WT cells that were pre-primed with LPS and subsequently infected with RVFV had robust IL-1β responses that were significantly reduced in the absence of MAVS (Fig. 5A).

Cellular RNA helicases RIG-I and melanoma differentiation-associated gene-5 (MDA5) have the potential to recognize RNA viruses and to signal through common adaptor protein MAVS leading to NF-κB activation and pro-IL-1β production. During infection with VSV, , RIG-I has been shown to function as an inflammasome with ASC triggering caspase-1-activation by a mechanism independent of MAVS (20).Therefore, we examined whether RIG-I or MDA5 were required for IL-1β production and processing in response to RVFV. In Fig 5B, we show that primed cDCs from WT, *Rig-I* $^{-/-}$, or *Mda5* $^{-/-}$ mice secreted similar levels of IL-1β in response to rMP-12 or NSs del virus as measured by ELISA. Western blot analysis shows that the mature IL-1β protein was also present in supernatants of primed cDCs from *Rig-I* $^{-/-}$ and *Mda5* $^{-/-}$ mice that were infected with NSs del (Fig. 5C), indicating that processing of IL-1β can occur in the absence of either protein. Finally, caspase-1 activation was evident in the absence of RIG-I or MDA5 in primed cells as indicated by presence of the p10 subunit in the supernatant (Fig. 5C).

MAVS and NLRP3 proteins co-localize during RVFV infection

To determine whether the RNA helicase adaptor protein MAVS molecularly interacts with the NLRP3 inflammasome in response to RVFV, LPS primed BMDC were infected with RVFV at an MOI of 1, stained with antibodies against NLRP3 or MAVS, and imaged by confocal microscopy. In the absence of RVFV infection, no detectable NLRP3 (red) was observed in the cytoplasm of the LPS primed cells (Fig. 6A) and MAVS (green) is seen scattered throughout the cytoplasm of the cells (Fig. 6B). In contrast, after infection with RVFV, NLRP3 is observed to be concentrated in “specks” in the cytoplasm of the cells, consistent with the formation of the multi-protein inflammasome complexes (Fig. 6E). This pattern has also been observed in LPS primed cells stimulated with ATP or other crystalline activators (30). In Fig. 6F, MAVS is observed to be present diffusely throughout the cytoplasm as well as in punctate regions. When the images are overlaid, there is clear co-localization of the punctated MAVS with NLRP3 (Fig. 6 G, inset). These studies demonstrate the punctate formation of the NLRP3 inflammasome after RVFV infection, consistent with current models of the multimeric inflammasome structure (31). Thus, MAVS may also influence inflammasome activation through its interactions with NLRP3.

Discussion

This is the first report of inflammasome activation by RVFV. Our studies demonstrate that IL-1β is produced in response to RVFV, and that the processing of this cytokine is dependent upon the inflammasome NLRP3, ASC, and caspase-1. In these studies, *Casp-1* $^{-/-}$ mice are functionally lacking both caspase-1 and caspase-11 (also known as

caspase-4) (29). Caspase-11 has been shown to be necessary for caspase-1 activation (33). Detection of the mature caspase-1 p10 subunit by western blot in NSs del infected cells verifies a role for active caspase-1 in IL-1β processing. We demonstrate that the common adaptor for RNA helicase signaling, MAVS, is involved in inflammasome activation and molecularly interacts with NLRP3. We observed that viral replication is required for IL-1β processing. This is important to assess since cDCs are capable of phagocytosis and other viruses have been shown to induce IL-1β through replication independent as well as dependent mechanisms (32).

The induction and processing of IL-1β is tightly regulated and requires two distinct signals. First, the induction of the 33 kDa pro-IL-1β and the 45 kDa pro-caspase-1 proteins occur via interactions of pathogens with innate PRRs. In our studies, pro-IL-1β was also produced in response to RVFV in unprimed cDCs from *Myd88/Trif* $^{-/-}$ mice. As all TLRs signal through either MyD88 or TRIF, this data emphasizes that production of pro-IL-1β is TLR independent, and may result from activation through other signaling pathways such as the cellular RNA helicases. Indeed, we observed that the upregulation many inflammasome related genes was dependent upon MAVS and not MyD88/TRIF as determined by NanoString analysis. Specifically, absence of MAVS resulted in decreased production of IL-1β RNA levels in cDCs, lending support to MAVS contribution as a signal 1 during inflammasome activation. Indeed, in cDCs that were infected with RVFV strains, MAVS had a profound effect on secreted IL-1β protein responses. This could in part be due to its initial effects on IL-1β RNA production which may ultimately affect mature IL-1β protein. Despite the pronounced decrease of IL-1β in the absence of MAVS, potential upstream receptors RIG-I and MDA5 did not have as dramatic of an impact on secreted IL-1β when examined individually. There are several reasons why this may occur. As we had previously seen with type I IFN production, RIG-I and MDA5 may compensate for each other, as type I IFN responses were not completely dependent upon either receptor (22). A similar scenario may occur for IL-1β production given the partial decrease in mature IL-1β produced by primed RIG-I or MDA5 deficient cDCs that were infected with RVFV. NOD2 has been shown to signal through adaptor MAVS to lead to type I IFN production in response to respiratory syncytial virus (34). Whether NOD2/MAVS signaling could also lead to accumulation of pro-IL-1β in cell lysates has yet to be determined.

MAVS may instead have effects on IL-1β release that are independent of generation of pro-IL- 1β, i.e. affecting signal 2 and caspase-1 activation. Recently, MAVS was shown to directly interact with NLRP3 and to influence IL-1β secretion for many known activators of NLRP3 (35). Similarly, in RVFV infected dendritic cells we observed the molecular interaction of NLRP3 with MAVS by confocal microscopy. It is also possible that MAVS could affect signal 2 and IL- 1β processing through other mechanisms unrelated to MAVS/NLRP3 binding. As observed in our NanoString studies, *Nos2* levels of RNA were reduced in *Mavs* $^{-/-}$ cells compared to wild- type cells. MAVS is a mitochondrial associated protein and thus may affect reactive oxygen species production which would in turn affect signal 2 through indirect mechanisms.

Although RIG-I has been shown to have potential to serve as an inflammasome during viral infection, we did not see dependence upon RIG-I for IL-1β processing in primed cDC. We also did not observe residual IL-1β in cell supernatant of NLRP3 deficient cells, indicating IL-1β processing is primarily driven through the NLRP3 inflammasome. In this study, we have demonstrated that RVFV induces NLRP3 inflammasome activation leading to the processing and release of IL-1β. The impact of inflammasome activation during RVFV infection should be further investigated using *in vivo* studies to determine whether this will protect against severe morbidity and mortality.

Materials and methods

Mice

C57BL/6 mice were obtained from Jackson Laboratories. *Myd88* $^{-/-}$ or *Trif* $^{-/-}$ mice were generated by Shizuo Akira (Osaka University, Osaka, Japan) and were subsequently used to produce *Myd88/Trif* double knockout mice. *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ and *Nlrc4*$^{-/-}$ mice were generated by Millenium Pharmaceuticals. *Casp1*$^{-/-}$ mice were generated by R. Flavell (Yale University). *Rig-I* $^{-/-}$ mice were provided by Michael Gale, Jr. (University of Washington). *Mda5* $^{-/-}$ mice were provided by Marco Colonna (Washington University). *Mavs* $^{-/-}$ mice were generated by Zhijian Chen (University of Texas Southwestern). Since the MAVS mouse strain is not fully backcrossed onto C57BL/6, wild-type controls from the same generation were used in these experiments. Mice were housed in filter-top micro-isolator cages in ventilated racks. Conditions for animal experiments were approved by the Institutional Animal Care and Use Committee at Case Western Reserve University.

Cells, viruses, reagents

Attenuated rMP-12 and NSs del strains of RVFV were a gift from Shinji Makino (UTMB, Galveston, TX) and were previously described (22). Viral infection of cells was carried out under BSL-2 conditions. Samples were inactivated with 2-4J UV light to cross-link RNA (Stratagene).

Bone marrow-derived cDCs were generated as previously described (22). Purity of cDC populations generated by this method were greater than 80% and were confirmed with flow cytometry as previously described (22). Cells were harvested between days 8–10 of maturation. For ELISA analysis of cytokines, 10^5 cells were plated per well in a 96 well plate. Cells were stimulated with tri-phosphate (ppp) RNA (Invivogen) for 8 hours, 1 ug Ultrapure *E. coli* K12 LPS (Invivogen) for 4.5 hours, rMP-12 and NSs del at MOI 1 or as otherwise designated for 6 hours. Primed cells were first stimulated with LPS for 4 hrs after which the supernatant was removed and replaced with 5 mM ATP (Sigma Aldrich) for 30 minutes, or rMP-12 and NSs del strains for 6 hours.

For western blot analysis, cDCs were plated in 6 well plates with 4 million cells per well and were stimulated with control ligands or infected with virus as described above. For these samples, LPS was administered at 500 ng/mL.

Cytokine responses

R&D Systems DuoSet ELISA kit was used to assess IL-1β present in cell supernatant (DY401). The assay was run according to the manufacturer's protocol.

Plaque assay

Vero E6 cells were plated in 6 well plates and were near 90% confluency at the time of infection. Live or UV inactivated virus sample was diluted in 1X αMEM media (Sigma Chemicals) with sodium bicarbonate and 2% FBS (Atlanta Biologicals). After 1 hr adsorption, the inoculum was removed and replaced with 1:1 dilution of 1% agarose (Promega) and 2X αMEM with 4% FBS. After incubating for 3 days at 37°C, cells were fixed with 10% formaldehyde in PBS and stained with 1% crystal violet 20% ethanol solution.

Western blotting

Cell lysates were prepared on ice in RIPA buffer with 1 mM DTT and a protease inhibitor cocktail with EDTA (Thermo Scientific). Supernatants were precipitated using 10% sodium

deoxycholate and 100% trichloroacetic acid (TCA). Samples were boiled for 5 min in Laemmli buffer and were subjected to SDS-PAGE using 4% bis-acrylamide stacking and 15% resolving gels. Proteins were transferred to PVDF membrane and 4% non-fat dried milk was used for blocking and antibody dilutions. Blots were stripped with Restore stripping buffer (Thermo Scientific) and were re-probed with anti-β-actin antibody A-15 (Santa Cruz sc-69879) and detected with secondary goat anti-mouse IgG –HRP (sc2005). Mouse IL-1β antibody was obtained from R&D (BAF410) and was detected with bovine anti-goat IgG-HRP (sc-2378). Pro- and mature caspase-1 p10 (M-20) was purchased from Santa Cruz (sc-514) and were detected with secondary antibody goat anti-rabbit IgG-HRP (sc2004).

NanoString gene expression analysis

Cells were plated at 10^6 cells per well in 6 well plates and were mock infected or infected with rMP-12 at MOI 5. After 6 hrs, total RNA was extracted using an RNeasy Mini kit (Qiagen). 100 ng of total RNA was hybridized to a custom mouse-gene expression CodeSet and analyzed on an nCounter Digital Analyzer (NanoString Technologies). Counts were normalized to internal spike-in and endogenous housekeeping controls according to the manufacturer's protocol.

Confocal Microscopy

cDCs were primed with LPS for 4 h and then infected with the rMP-12 strain at an MOI of 1, or left uninfected for 6 h. Cells were washed with PBS, fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 before staining with anti-NLRP3 antibody (Adipogen) and anti-MAVS antibody (Cell Signaling). The cells were then visualized with confocal fluorescence microscopy on a Leica SP2 AOBS confocal laser scanning microscope, as described previously {Charrel-Dennis, 2008 #4696; Hornung, 2008 #4712.

Statistical analysis

Data were analyzed using commercial software (GraphPad). ELISA data from at least three independent experiments were averaged and graphed as means ± standard deviation. Significance was determined using Student's independent t-test and is defined as *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$.

Acknowledgments

We thank Michael Gale, Jr. (University of Washington, Seattle, Washington) for providing us with the *Rig-I* $^{-/-}$ mice, Marco Colonna (Washington University, St. Louis, MO) for the *Mda5* $^{-/-}$ mice, Zhijian Chen (UT Southwestern, Dallas, TX) for the *Mavs* $^{-/-}$ mice, and Richard Flavell (Yale, New Haven, CT) for the *Casp-1* $^{-/-}$ mice. We also thank Shinji Makino and Tetsuro Ikegami (University of Texas Medical Branch, Galveston, TX) for providing the attenuated RVFV strains. This work was supported by NIH grant R21AI083693 (A.G.H.), R21AI079617 (A.G.H), U54AI057160 to the Midwest Regional Center of Excellence for Biodefense and Emerging Infectious Disease Research (A.G.H). M.E.E was supported in part by NIH Training grant T32AI089474.

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Research Highlights

- Rift Valley fever virus induces IL-1β responses.
- RVFV activates the NLRP/ASC/Caspase-1 inflammasome.
- The RNA helicase adaptor protein MAVS co-localizes with NLRP3 in the cytoplasm of RVFV infected cells.

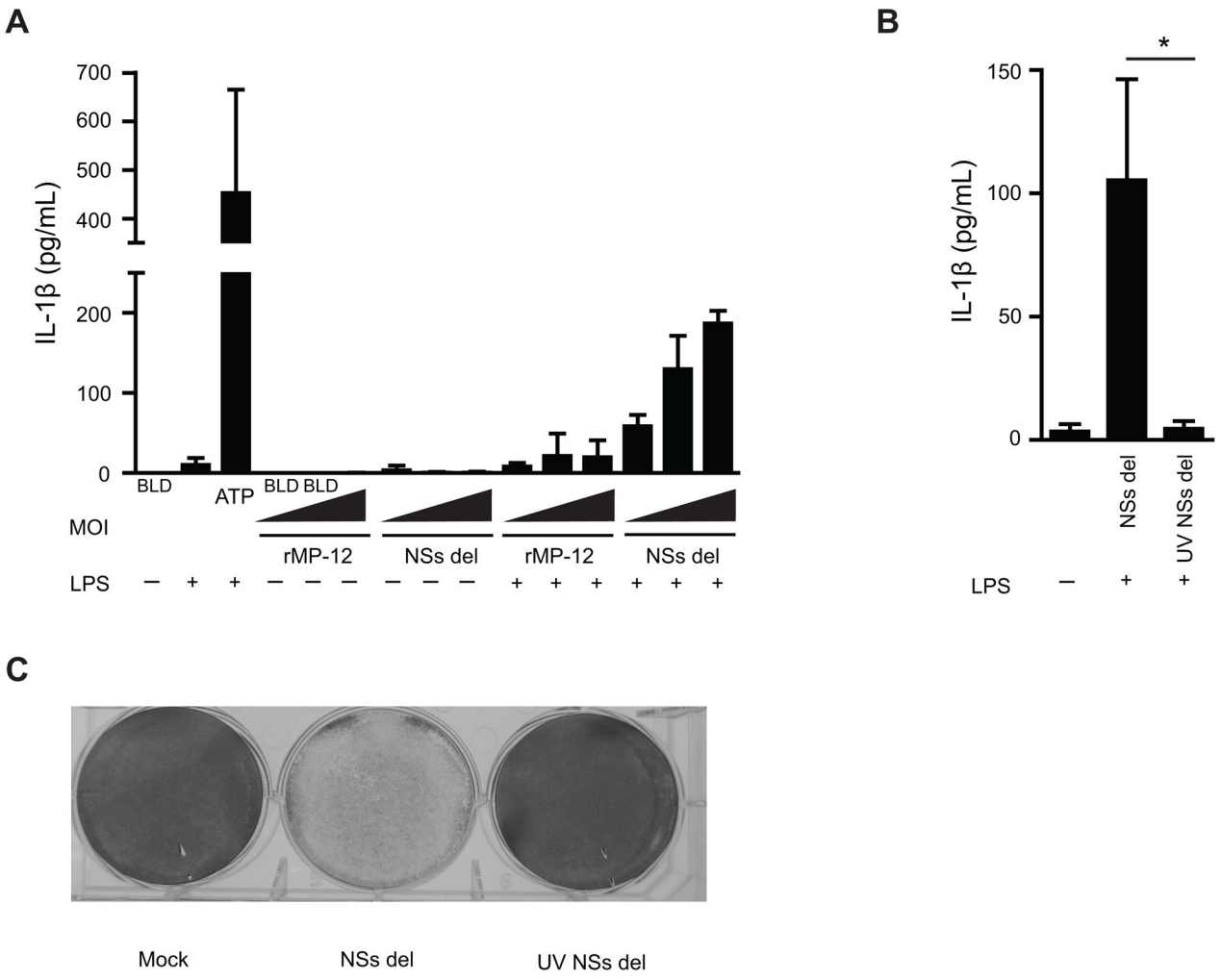

Figure 1. RVFV infection induces IL-1β release from cDCs

Bone marrow-derived cDCs were infected with rMP-12 or NSs del RVFV strains for 6 h. In some cases, cells were first primed with LPS for 4 h prior to infection (as designated). A) cDCs were infected with rMP-12 or NSs del at an MOI of 0.3, 1, or 3 and IL-1β in the supernatant was detected by ELISA. B) Dependence of IL-1β production on viral replication was assessed by treating cDCs with NSs del or UV inactivated NSs del at an MOI of 1. A and B) The mean level of IL-1β ± the standard deviation from three experiments is shown. Significance: *, $P \leq 0.05$. C) Efficiency of UV inactivation was verified by plaque assay. Inactivation was verified for each sample of UV inactivated NSs del virus that was used in panel B.

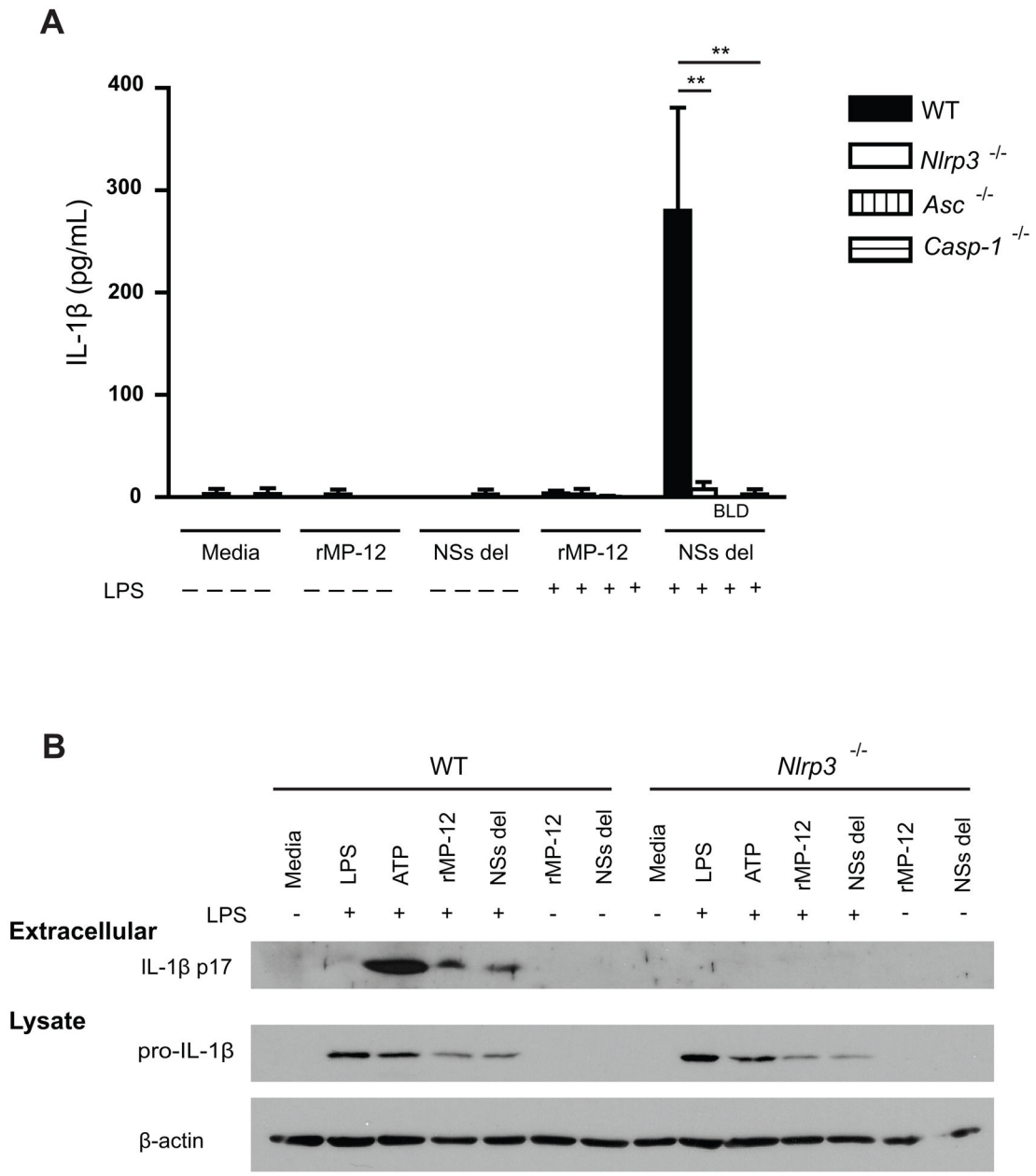

Figure 2. IL-1β production is dependent upon NLRP3, ASC, and caspase-1

A) Bone marrow-derived cDCs from WT, *Nlrp3* $^{-/-}$, *ASC* $^{-/-}$, or *caspase-1* $^{-/-}$ mice were stimulated with rMP-12 and NSs del with and without LPS priming. IL-1β from the supernatant was detected by ELISA. The mean level of IL-1β ± the standard deviation from three experiments is shown. Significance: **, $P \leq 0.01$. BLD denotes the value was below the limit of detection. B) Supernatants and lysates from stimulated or infected cDCs were subjected to western blot. Mature IL-1β was detected in the supernatant, and pro-IL-1β and β-actin levels were determined in cell lysate. Data shown is representative of three independent experiments.

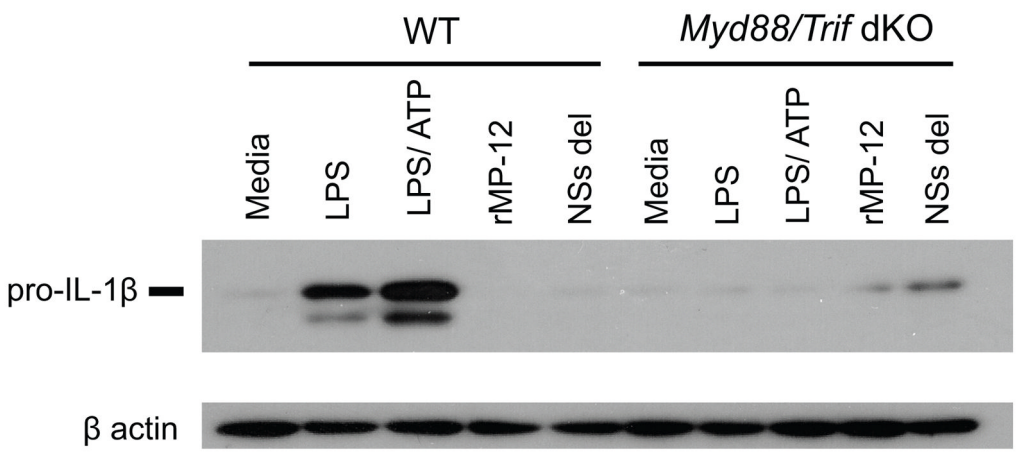

Figure 3. Lack of TLR signaling does not hinder production of pro-IL-1β

cDCs from WT and *Myd88/Trif* $^{-/-}$ mice were stimulated with LPS, LPS/ATP, or rMP-12 and NSs del (6 h). Presence of pro-IL-1β was determined via western blot assay. Data shown is representative of three independent experiments.

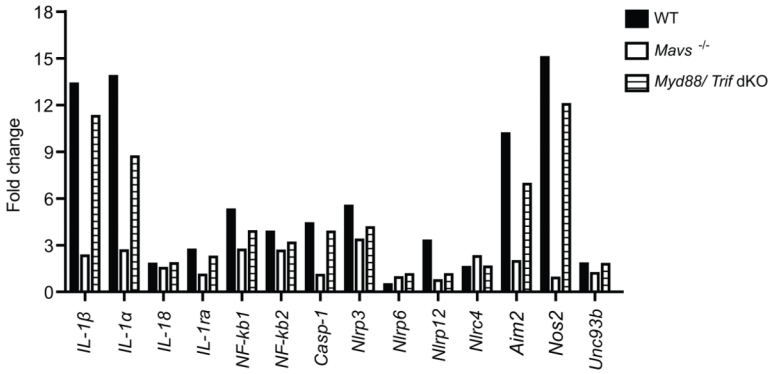

Figure 4. Dependence of inflammasome related genes on MAVS or MyD88/TRIF pathways

Bone marrow-derived cDCs from WT, *Mavs* $^{-/-}$, or *Myd88/Trif* $^{-/-}$ mice were infected with rMP-12 for 6 h as single samples. Total RNA was isolated and analyzed using NanoString technology. Fold change is relative to uninfected cells of the same strain and normalized to endogenous housekeeping controls.

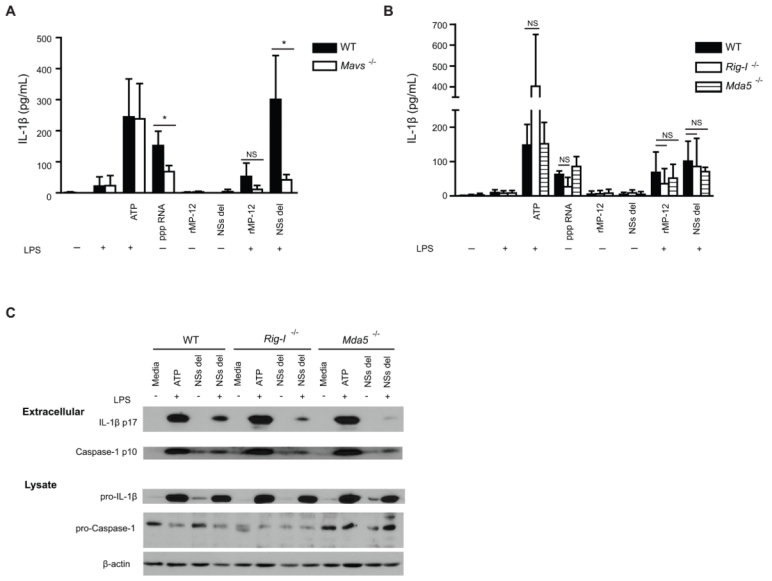

Figure 5. IL-1β responses to RVFV are dependent on MAVS

Differentiated cDCs from WT and A) *Mavs* $^{-/-}$, or B) *Rig-I* $^{-/-}$ or *Mda5* $^{-/-}$ mice were primed with LPS for 4 hrs, or were infected without priming with rMP-12 and NSs del strains for 6 hours. Supernatant was analyzed for IL-1β protein by ELISA. The mean level of IL-1β ± the standard deviation from three experiments is shown. Significance: *, $P \leq 0.05$. NS: not significant, $P > 0.05$. C) Mature IL-1β and caspase-1 p10 protein were detected in the supernatant of cDCs from WT, *Rig-I* $^{-/-}$ or *Mda5* $^{-/-}$ mice. Pro-IL-1β and pro-caspase-1 were detected in the lysate. Data shown is representative of three independent experiments.

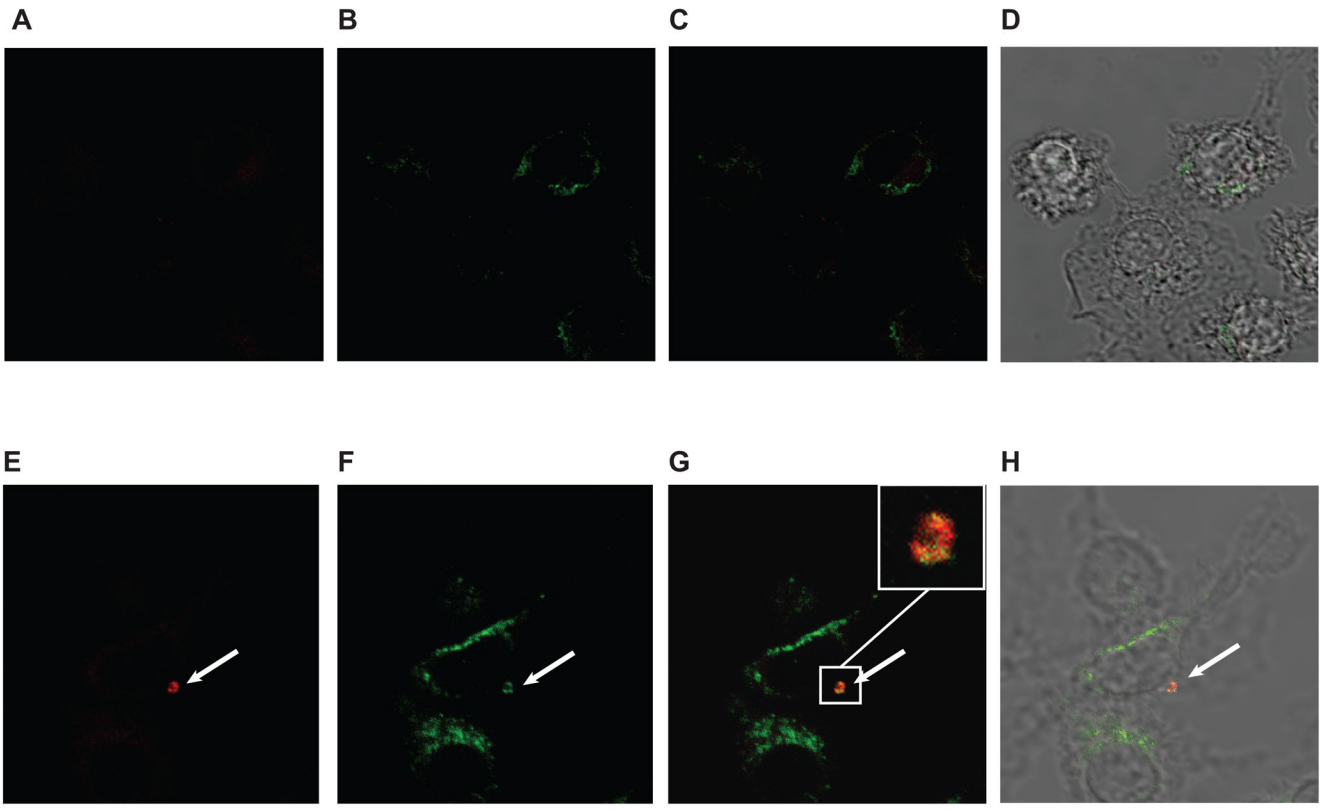

Figure 6. MAVS and NLRP3 molecularly interact in the cytoplasm of RVFV infected dendritic cells

cDC from WT mice were primed with LPS for 4 hrs. After washing with PBS, cells were fixed, permeabilized and stained with monoclonal antibodies specific for A) NLRP3 or B) MAVS. Images were overlayed C). Lightfield imaging of the same cells shown in D). Primed cDC were infected with rMP-12 at MOI 1 for 6 hours and processed as above. E) NLRP3 protein shown in red forming punctate staining; F) MAVS shown in green; G) overlay showing co-localization of NLRP3 and MAVS (arrow); D) Lightfield imaging of the same cells showing overlay of NLRP3 (green) and MAVS (red).