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Innate immune responses to rotavirus infection in macrophages depend on MAVS but involve neither the NLRP3 inflammasome nor JNK and p38 signaling pathways

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ABSTRACT

Rotavirus infection is a major cause of life-threatening infantile gastroenteritis. The innate immune system provides an immediate mechanism of suppressing viral replication and is necessary for an effective adaptive immune response. Innate immunity involves host recognition of viral infection and establishment of a powerful antiviral state through the expression of pro-inflammatory cytokines such as type-1 interferon (IFN). Macrophages, the front-line cells of innate immunity, produce IFN and other cytokines in response to viral infection. However, the role of macrophages during rotavirus infection is not well defined. We demonstrate here that RRV rotavirus triggers the production of proinflammatory cytokines from mouse bone marrow-derived macrophages. IFN and antiviral cytokine production was abolished in rotavirus-infected MAVS (−/−) macrophages. This indicates that rotavirus triggers innate immunity in macrophages through RIG-I and/or MDA5 viral recognition, and MAVS signaling is essential for cytokine responses in macrophages. Rotavirus induced IFN expression in both wild type and MDA5 (−/−) macrophages, showing that MDA5 is not essential for IFN secretion following infection, and RIG-I and MDA5 may act redundantly in promoting rotavirus recognition. Interestingly, rotavirus neither stimulated mitogen-activated protein kinases p38 and JNK nor activated the NLRP3 inflammasome, demonstrating that these components might not be involved in innate responses to rotavirus infection in macrophages. Our results indicate that rotavirus elicits intracellular signaling in macrophages, resulting in the induction of IFN and antiviral cytokines, and advance our understanding of the involvement of these cells in innate responses against rotavirus.

1. Introduction

Initiation of the innate immune response against viruses involves recognition of viral infection by host pattern-recognition receptors (PRRs). PRRs trigger intracellular signaling cascades leading to the induction of type I interferon (IFN), including IFN-α and IFN-β, which mediates effective innate and adaptative immune responses through the establishment of a powerful antiviral state (Randall and Goodbourn, 2008; Thompson et al., 2011). RNA viruses can trigger the IFN response through interactions with different cellular PRRs, including the cytoplasmic RNA helicases retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) and the transmembrane toll-like receptors (TLRs) (Jensen and Thomsen, 2012). Once stimulated, RIG-I and MDA5 signal through the adaptor protein mitochondrial antiviral signaling (MAVS), while TLRs use either the TIR-domain-containing adaptor-inducing IFN-β (TRIF) or the myeloid differentiation primary responses 88 (MyD88) pathways.

Abbreviations: AP-1, activator protein-1; BMM, bone marrow-derived macrophage; DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; FCFU, fluorescent cell forming units; FITC, fluorescein isothiocyanate; HIV, human immunodeficiency virus; IFN, interferon; IRFs, IFN regulatory factors; IRRV, inactivated rhesus monkey rotavirus; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MAVS, adaptor protein mitochondrial antiviral signaling; MDA5, melanoma differentiation-associated gene 5; MOI, multiplicity of infection; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor κB; NLR, nucleotide-binding domain and leucine rich repeat; NLRP3, NLR containing family pyrin domain containing 3; NSP, non-structural protein; PI, post-infection; PRRs, pattern recognition receptors; RIG-I, retinoic acid-inducible gene I; RRV, Rhesus monkey rotavirus; STAT, transducer and activator of transcription; TLRs, toll-like receptors; TRAF, tumor necrosis factor receptor-associated factor; TRIF, TIR-domain-containing adaptor-inducing IFN-β.

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Almost all TLR signaling occurs through MyD88, although TLR3 utilizes TRIF and TLR4 recruits either MyD88 or TRIF. These signaling cascades culminate in the activation of numerous transcription factors, including the IFN regulatory factors (IRFs) and nuclear factor κB (NF-κB). In the nucleus these factors mediate the transcription of genes encoding type I IFN and other proinflammatory cytokines. IFN secreted from infected cells binds to specific cell-surface receptors on uninfected cells and activates intracellular signaling, resulting in the expression of a wide range of antiviral genes and resistance to viral infection (Randall and Goodbourn, 2008; Thompson et al., 2011).

Another important cellular process following cellular detection of many viruses is the activation of mitogen-activated protein kinase (MAPK) signaling pathways. These pathways are responsible for the phosphorylation of a large group of transcriptional activators, including the activator protein-1 (AP-1) (Shaulian and Karin, 2002). AP-1 activation occurs through c-Jun N-terminal kinase (JNK) and p38 MAPK pathways. Together with IRF3 and NF-κB, AP-1 directly regulates the expression of a wide variety of pro-inflammatory and immunomodulatory cytokines (Mogensen and Paludan, 2001).

Rotaviruses comprise a genus of the subfamily *Sedoreovirinae* within the *Reoviridae* family. Members of the type species *Rotavirus A* are the leading cause of severe diarrhea in infants and an important cause of hospital admission among young children worldwide (Tate et al., 2012). These non-enveloped viruses contain a segmented double-stranded RNA genome encoding six structural and six non-structural proteins (NSP). Globally, rotaviruses are responsible for approximately 5% of all deaths in children under the age of 5, with most occurring in developing countries (Glass et al., 2014). Previous studies implicated RIG-I and MDA5 as the critical PRRs for rotavirus detection in infected intestinal cells in mice, and in mouse embryonic fibroblasts. Rotavirus-infected cells lacking RIG-I/MDA5 or MAVS demonstrated enhanced viral replication and diminished type I IFN expression. Similar results were observed in MAVS-deficient mice (Sen et al., 2011; Broquet et al., 2011). It has also been shown that the AP-1 transcription factor is activated during rotavirus infection through kinases JNK/p38, enhancing the expression of the immune cell-attracting chemokine IL-8 (Holloway and Coulson, 2006). A role for RIG-I and/or MDA5 receptors in AP-1 activation following rotavirus infection is not established.

Rotavirus effectively subverts host antiviral innate immunity by several strategies (Arnold et al., 2013; Holloway and Coulson, 2013). Rotavirus NSP1 antagonizes IFN production by inducing IRF degradation by the proteasome (Graff et al., 2002; Barro and Patton, 2007). NSP1 also blocks NF-κB action through degradation of β-TrCP, a protein required for IκB degradation and the subsequent release and nuclear translocation of NF-κB (Graff et al., 2009; Di Fiore et al., 2015; Morelli et al., 2015). Rotavirus also interferes with IFN responses by targeting proteins that mediate IFN signals, such as the transducer and activator of transcription (STAT) 1 and STAT2. Rotavirus prevents IFN-induced STAT1 and STAT2 nuclear accumulation, blocking their ability to act as transcriptional enhancers of antiviral genes. However, the mechanism employed by rotavirus to inhibit STAT nuclear translocation is unknown (Holloway et al., 2009, 2014). IFN-mediated STAT1 activation is inhibited at later times during rotavirus infection of intestinal epithelial cells, an effect attributed to NSP1 (Sen et al., 2014).

Macrophages are specialized mediators of innate immunity and initiate an antimicrobial response in the presence of pathogens. In response to viral infection, macrophages play an important role in promoting virus elimination and suppressing viral spread (Karupiah et al., 1996; Kumagai et al., 2007). PRRs expressed in macrophages include cytoplasmic receptors (such as RIG-I and MDA5), endosomal TLRs (such as TLR3, TLR7/8 and TLR9) and TLRs expressed at the cell surface (such as TLR2 and TLR4) (Mercer and Greber, 2013). Downstream signaling from these PRRs in virus-infected macrophages stimulates production of type I IFN and a range of inflammatory cytokines that are important for innate and adaptive immunity (Smith et al., 2011; Mercer and Greber, 2013). Macrophages can also secrete particular inflammatory cytokines via activation of a large multiprotein complex known as the inflammasome (Schroder and Tschopp, 2010; Chen and Ichinohe, 2015). One of the best-characterized inflammasomes consists of nucleotide-binding domain and leucine rich repeat containing family (NLR) and pyrin domain containing 3 (NLRP3). NLRP3 recognizes a wide range of stimuli including viral RNA. RNA viruses, including influenza, Sendai and rabies, stimulate the NLRP3 inflammasome (Kanneganti, 2010; Chen and Ichinohe, 2015). Although the exact mechanism involved in initiating inflammasome aggregation is still unclear, inflammasome activation considerably increases antiviral responses (Allen et al., 2009; Lawrence et al., 2013). Through recruitment and activation of caspase-1, inflammasome complexes induce the maturation of pro-inflammatory cytokines IL-1β and IL-18. These cytokines trigger innate immune responses against viral infection by modulating local and systemic responses such as fever, leucocyte migration to the infected area and activation and polarization of T-cell responses (Schroder and Tschopp, 2010).

Relatively few studies have explored the interaction between rotavirus and macrophages. Infection of macrophages by rotavirus in mouse tissues, including Peyer's patches, mesenteric lymph nodes, pancreas and thymus has been demonstrated and rotavirus gene expression was detected in rat lung macrophages (Brown and Offit, 1998; Crawford et al., 2006; Graham et al., 2007; Webster et al., 2013). Rotavirus-infected macrophage cell lines secrete cytokines (Mohanty et al., 2010). However, the mechanisms involved in rotavirus recognition in infected macrophages and the processes that mediate cytokine production are largely unknown. Using immortalized mouse bone marrow-derived macrophages (BMM) as a model, we show here that innate responses to rotavirus in macrophages involve RIG-I and/or MDA-5 viral recognition and are dependent on the MAVS adaptor, which is required for the stimulation of IRF3 and NF-κB-dependent cytokine production. We found that rotavirus replication was essential for NF-κB-mediated cytokine expression but partially dispensable for IFN-β expression. In contrast, neither JNK/p38 kinases nor the inflammasome complex was triggered by rotavirus infection, indicating that these components may not be important for antiviral responses to rotavirus in macrophages.

2. Materials and methods

2.1. Cell lines and virus

Immortalized BMM generated from wild type (wt), MAVS (−/−) and MDA5 (−/−) C57BL/6 mice as previously described (Hornung et al., 2008) were provided by Douglas Golenbock (University of Massachusetts Medical School, Maryland, USA; Halle et al., 2008). These BMM were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (Bovogen Biologicals), 2 mM L-glutamine (Gibco), 20 mM HEPES (MP Biomedicals), 50 U/ml penicillin and 50 μg/ml streptomycin (Invitrogen). The Rhesus monkey rotavirus strain RRV was propagated in MA104 African green monkey epithelial cells, purified using glycerol gradient ultracentrifugation and the infectious titre determined by indirect immunofluorescence as before (Holloway and Coulson, 2006). Purified RRV was inactivated by psoralen and UV treatment to produce IRRV, and the inactivation verified as previously described (Groene and Shaw, 1992; Halasz et al., 2008).