Critical Role of MDA5 in the Interferon Response Induced by Human Metapneumovirus Infection in Dendritic Cells and In Vivo

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Human metapneumovirus (hMPV) is a respiratory paramyxovirus of global clinical relevance. Despite the substantial knowledge generated during the last 10 years about hMPV infection, information regarding the activation of the immune response against this virus remains largely unknown. In this study, we demonstrated that the helicase melanoma differentiation-associated gene 5 (MDA5) is essential to induce the interferon response after hMPV infection in human and mouse dendritic cells as well as in an experimental mouse model of infection. Our findings in vitro and in vivo showed that MDA5 is required for the expression and activation of interferon (IFN) regulatory factors (IRFs). hMPV infection induces activation of IRF-3, and it regulates the expression of IFN-β. However, both IRF-3 and IRF-7 are critical for the production of type I and type III IFNs. In addition, our in vivo studies in hMPV-infected mice indicated that MDA5 alters viral clearance, enhances disease severity and pulmonary inflammation, and regulates the production of cytokines and chemokines in response to hMPV. These findings are relevant for a better understanding of the pathogenesis of hMPV infection.
containing 2 mmol/liter L-glutamine, 10% fetal bovine serum (FBS), 50 μM 2-mercaptoethanol (2-ME), 1,000 IU penicillin-streptomycin, and granulocyte-macrophage colony-stimulating factor (GM-CSF; 100 ng/ml) and interleukin-4 (IL-4; 20 ng/ml).

Establishment of mouse BMDC. Bone marrow-derived DC (BMDC) were generated from the femurs, tibiae, and humeri of mice as previously described (35). Briefly, bone marrow cells were depleted of contaminating erythrocytes by ACK lysis buffer (Gibco) and filtered (100 μm) to remove large aggregates. Cells were cultured at a final concentration of 5 × 10⁵ cells/ml for 3 days in RPMI 1640 medium supplemented with 400 mM L-glutamine, 10% FBS, 2 mM 1,000 IU penicillin-streptomycin, and murine GM-CSF (mGM-CSF; 10 ng/ml; PeproTech). Nonadherent cells were removed, and remaining cells were cultured for an additional 3 days in the presence of 10 ng/ml of mGM-CSF and 10 ng/ml of murine IL-4 (PeproTech). Recovered cells were stained with anti-CD11c, anti-I-A/I-E (major histocompatibility complex II [MHC-II]), anti-CD40, anti-CD80, and anti-CD86 antibodies as previously described (30) and analyzed by flow cytometry using a FACScan flow cytometer (BD Biosciences) and Flowjo software (version 7.6.3; Tree Star).

Infection of dendritic cells. Human moDC and murine BMDC (1 × 10⁶) were plated in 12-multwell plates in a total volume of 1 ml of medium and infected at multiplicities of infection (MOI) of 5 and 3, respectively. At different time points after infection, cells or supernatants were collected for subsequent analysis.

siRNA transfections. The expression levels of MDA5 and RIG-I were knocked down by delivering predesigned small interfering RNAs (siRNAs; On-TargetPlus; Thermo Scientific) by electroporation using an Amaxa Nucleofector device (Lonza), as described elsewhere (36). Briefly, 1 × 10⁶ moDC were transfected with 2 μM siRNA by using the human DC nucleofector kit (Lonza), resuspended in 1 ml of medium, and plated in 12-well plates for 24 h. Prior to infection, transfected cells were counted, and 2.5 × 10⁵ cells/condition were seeded in 500 μl of fresh medium in 24-well plates.

Western blot analysis. MDA5 and RIG-I expression was determined by Western blot assays. Cell pellets were lysed using RIPA buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100) supplemented with a protease inhibitor cocktail (Complete; Roche). Protein cell extracts were electrophoresed in precast polyacrylamide gels (Life Technologies) and transferred to polyvinylidene difluoride membranes (Bio-Rad). Membranes were blocked with 5% nonfat dried milk in Tris-buffered saline solution plus 0.1% Tween 20 overnight at 4°C followed by an additional 2 h at 4°C in the presence of anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH) or anti-Actin (both from Santa Cruz Biotechnology) or anti-MDA5 (Cell Signaling Technology) antibodies. Membranes were washed and incubated for 1 h at room temperature with anti-rabbit IgG–horseradish peroxidase (Santa Cruz Biotechnology). Protein expression was revealed by chemiluminescence (ECL Plus; GE Healthcare Biosciences).

Mice and infection protocol. Animal care and use were conducted in accordance with the National Institutes of Health and Louisiana State University institutional guidelines. Control C57BL/6j mice (wild type [WT]) used in this work were purchased from Harlan Laboratories. MDA5−/−mice were generated by Tadatsugu Taniguchi (University of Tokyo, Japan) (40) and generously transferred by Michael Diamond (Washington University, St. Louis, MO) (41). Under light anesthesia, 8- to 10-week-old sex-matched mice were infected intranasally (i.n.) with 50 μl of hMPV diluted in phosphate-buffered saline (PBS; final administered dose, 1 × 10⁶ PFU) (28). As mock treatment, mice were inoculated with an equivalent volume of PBS (here referred to as the mock group).

Broncholaveral lavage, histology, and lung tissue collection. Mice were sacrificed by an intraperitoneal injection of ketamine and xylazine and exsanguinated via the femoral vessels as previously reported (27, 30, 41). To collect a broncholaveral lavage (BAL) sample, the lungs were flushed twice with ice-cold sterile PBS (1 ml) as previously described (27, 41). Cell-free supernatants were stored at −75°C until analysis. For histological analysis, lungs of mice were perfused and fixed in 10% buffered formalin and embedded in paraffin. Multiple 4-μm-thick sections were stained with hematoxylin and eosin (H&E). Lung sections were visualized by bright-field microscopy using the high-throughput slide scanner NanoZoomer with a 20× objective and the NanoZoomer digital pathology view software (both from Hamamatsu Photonics, Hamamatsu City, Japan). For gene expression experiments, lung tissue was collected, snap-frozen in liquid nitrogen, and stored at −75°C until analysis.

Measurement of cytokines and IFNs. Cell-free supernatants from moDC were tested for the production of human IFN-α, β, ω, γ, and λ by using the VeriPlex enzyme-linked immunosorbent assay (human IFN multiplex ELISA; PBL Interferon Source), according to the manufacturer’s instructions. Production levels of IFN-α, IFN-β, and IFN-λ (IL-28A/IL-28B) by BMDC and in BAL samples from mice were determined by ELISA (PBL Interferon Source), following the manufacturer’s protocol. Levels of cytokines and chemokines in BAL fluid were determined with the Milliplex MAP 32-Mouse Plex cytokine detection system (Millipore), according to the manufacturer’s instructions. The range of the sensitivities of the assays was 3.2 to 10,000 pg/ml.

Determination of IRF-3 activation by ELISA. Activated mouse and human IRF-3 were measured in cell nuclear extracts by using a modified ELISA (42–44), according to the manufacturer’s instructions (TransAM; Active Motif). Briefly, DNA binding activity of activated IRF-3 was measured using a 96-well plate on which oligonucleotides containing the IRF-3 consensus binding sites were immobilized. The activated forms of IRF-3 in nuclear cell extracts bind to the oligonucleotides, and the amount bound is measured using antibodies specific to IRF-3. Nuclear extracts from moDC or BMDC were obtained by treating cells with a phosphatase inhibitor cocktail (PhosSTOP; Roche) prior to harvesting the cells. Cell pellets were treated with an hypotonic buffer (1.5 mM MgCl₂, 10 mM KCl, 0.2 mM PMSF, and 0.5 mM dithiothreitol) on ice for 15 min, followed by vortexing 10 s in the presence of 0.5% (final concentration) Nonidet P 40 substitute (Fluka). After centrifugation, the remaining pellet was resuspended in the lysis buffer provided in the assay kit. Supernatants containing the nuclear fraction were stored at −75°C until analysis. Protein concentrations were determined by a colorimetric assay (Bio-Rad) and normalized across all samples prior to the ELISAs.

Real-time quantitative reverse transcription-PCR (qRT-PCR). The first-strand cDNA was synthesized from RNA using the Maxima First Strand cDNA synthesis kit (Fermentas) according to the manufacturer’s instructions. cDNA fragments of interest were amplified using the FastStart Universal probe master (Roche). Predesigned TaqMan assay primers and probes were used (Integrated DNA Technologies). All assays for MDA5, RIG-I, IFNβ, IFN-3, IFN-5, IFN-7, ISG56/IFIT-1, ISG54/IFIT-2, ISG75/IFIT-3, MX1, hMPV N, and GAPDH were run on the 7900HT fast real-time PCR system following the manufacturer’s suggested cycling parameters (Applied Biosystems). The comparative cycle threshold (Cₜ) method (∆∆Cₜ) was used to quantitate the expression of target genes that were normalized to the endogenous reference (GAPDH) expression levels of transcripts from uninfected control cells.

Statistical analyses. Statistical analyses were performed with the InStat 3 biostatistics package (GraphPad) using a two-tailed, unpaired Student’s t test. Results are expressed as means ± standard errors of the means.

RESULTS

Expression of MDA5 is necessary for the IFN response in human moDC infected with hMPV. We first analyzed the expression of MDA5 and RIG-I in moDC at 3, 6, 9, 12, and 24 h after infection.
by real-time qRT-PCR using predesigned primers, as previously described (45). Our data indicated that hMPV induced the expression of both MDA5 and RIG-I in moDC as early as 3 h after infection. However, the expression of MDA5 was 3-fold lower than that of RIG-I at all time points tested (Fig. 1A). MDA5 and RIG-I expression was also determined by Western blot assays with anti-MDA5, anti-RIG-I, and anti-GAPDH antibodies. MDA5 and RIG-I were expressed as early as 3 h, with a peak at 9 to 12 h after hMPV infection (Fig. 1B).

In order to investigate the role of MDA5 and RIG-I in the production of type I (α/β/ω), type II (γ), and type III (λ) IFNs after hMPV infection in moDC, the expression levels of MDA5 and RIG-I were knocked down by delivering predesigned siRNAs by electroporation, as described elsewhere (36). After 24 h of in-
Infection, cell-free supernatants were tested for the production of IFN-α, -β, -ω, γ, and ι by human IFN Multiplex assay. As shown in Fig. 1C, after gene silencing, the expression levels of both MDA5 and RIG-I were efficiently knocked down, by 80% and 70%, respectively. The production of IFNs was differentially induced in moDC after hMPV infection: IFN-α (53%), IFN-β (68%), and IFN-ω (41%). Furthermore, the effects of MDA5 on the expression of IFN-related genes was analyzed. We observed a significant reduction of ISG56/IFIT1, ISG54/IFIT2, ISG60/IFIT3, and MX1 (Fig. 1D), confirming the contribution of MDA5 in the IFN response elicited by hMPV in moDC.

Production of IFN-α/β is dependent on MDA5 in mouse DC. To confirm the role of MDA5 in the IFN response by hMPV infection, we used DC from MDA5-deficient mice. BMDC were generated from bone marrow cells cultured for 6 days in the presence of GM-CSF and IL-4 as described above. After flow cytometry analysis, ~87% of the recovered cells were considered BMDC, as indicated by their expression of CD11c (Fig. 2A) and their morphology (Fig. 2B). BMDC express high levels of MHC-II, CD40, CD80, and CD86 and these were overexpressed after hMPV infection (Fig. 2C). Next, BMDC were infected in vitro with hMPV at an MOI of 3. After 24 h of culture, cell-free supernatants were tested for the IFN response by ELISA. Our data showed that the lack of MDA5 resulted in a significant decrease of IFN-α and IFN-β production compared to wild-type mice infected with hMPV (Fig. 3A). In order to determine the effect of MDA5 in local DC, lung conventional DC were isolated by a combination of magnetic isolation and cell sorting as previously described (46) and infected in vitro as for BMDC. As with BMDC, after hMPV infection, the production of IFN-β was abolished in pulmonary DC lacking MDA5 (Fig. 3B).

Effect of hMPV infection on IRF-3 and IRF-7 in moDC. After the activation of the IFN response through the cellular receptors, including MDA5, the induction of IFN-α/β is regulated downstream at the transcriptional level by IRF-3, IRF-5, and IRF-7 to induce IFN gene transcription and protein production (reviewed in references 19 and 20). We therefore analyzed the expression of these IRFs at different time points after hMPV infection in moDC by real-time qRT-PCR. As shown in Fig. 4A, the expression of IRF-3 was modestly but significantly increased, with a peak at 24 h (~3-fold) compared to control cells. IRF-5 was not significantly induced at any time point tested. On the other hand, hMPV significantly induced the expression of IRF-7, particularly at 9 h (43-fold), 12 h (38-fold), and 24 h (57-fold) after infection. To further determine the role of MDA5 in the expression of IRF-7 in response to hMPV, the expression of MDA5 was knocked down in moDC followed by viral infection for 24 h. The expression of IRF-7 was decreased by 65% (Fig. 4B). Furthermore, we determined the role of IRF-7 expression in the IFN-α/β response in moDC. The expression of IRF-7 was efficiently knocked down (87%) (data not shown), and cell supernatants were tested for IFN-α and IFN-β release by ELISA after 24 h of infection. The production of IFN-α was reduced by more than 90% and IFN-β was abolished to levels comparable to uninfected cells (Fig. 4C).
indicating that IRF-7 is required for the induction of IFN-α and IFN-β in human DC after hMPV infection. To confirm whether hMPV infection also induced the activation of IRF-3, we determined the IRF-3 DNA binding activity using an ELISA-based experiment. IRF-3 activation upon hMPV infection was found to be comparable to that of poly(I·C)-treated cells, as the positive control (Fig. 4D).

IRF-3 and IRF-7 regulate the IFN response in hMPV-infected DC. In order to confirm the role of IRF-3 and IRF-7 in hMPV-induced IFN-α/β production, we generated BMDC from IRF-3−/− and IRF-7−/− mice. Cells were infected as described above, and the production of IFN-α and IFN-β was determined by ELISA. We observed that in the lack of either transcription factor, the production of IFN-α and IFN-β was reduced almost to a level of that of the uninfected cells (Fig. 5A), indicating that both IRF-3 and IRF-7 regulate the IFN response during hMPV infection. We next investigated the extent by which the activation of IRF-3 or IRF-7 was regulated by the MDA5 expression. In order to do that, we determined the expression levels of IRF-3, IRF-5, and IRF-7 in hMPV-infected BMDC from MDA5−/− mice. We observed that, whereas the expression of IRF-3 and IRF-5 in BMDC from MDA5−/− mice was not significantly different from that of wild-type cells (Fig. 5B), the induction of IRF-7 expression after hMPV infection was almost ablated in MDA5−/− BMDC (Fig. 5B), indicating that MDA5 regulates the expression of IRF-7. When we examined the activation of IRF-3 by hMPV in BMDC from MDA5−/− mice, we observed that the lack of MDA5 reduced the activation of IRF-3 by ~50% (Fig. 5C). Finally, in order to deter-

FIG 3 Lack of MDA5 alters the production of IFN in response to hMPV infection in mouse DC. (A) BMDC were differentiated from marrow cells cultured in the presence of murine GM-CSF and IL-4 for 6 days. BMDC from MDA5−/− or WT mice were infected with hMPV at an MOI of 3 for 24 h, and production of IFN-α and IFN-β was determined by ELISA (n = 3). (B) Lung conventional DC were isolated by collagenase digestion, and IFN-β production was determined by ELISA. Bar graphs represent means ± standard errors of the means. *, P < 0.05.

FIG 4 Expression and activation of IRF-3 and IRF-7 in moDC infected with hMPV. (A) Human moDC were infected with hMPV at an MOI of 5. After 3, 6, 9, 12, 24, and 48 h of hMPV infection, the expression of IRF-3, IRF-5, and IRF-7 was determined by real-time qRT-PCR (n = 3). (B) MDA5 expression was knocked down in moDC with specific siRNA prior to hMPV infection. Scrambled siRNA was used as a control. Expression of IRF-7 was determined by real-time qRT-PCR (n = 8). (C) moDC were treated with siRNA IRF-7 or scrambled siRNA for 24 h and infected with hMPV at an MOI of 5 for additional 24 h. Production of IFN-α and IFN-β was measured by ELISA (n = 3). (D) IRF-3 activation was measured by DN binding activity in hMPV-infected moDC (n = 3). Bar graphs represent means ± standard errors of the means. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
mine how IRF-3 and IRF-7 could regulate each other after hMPV infection, we determined their expression in BMDC from IRF-deficient mice. As indicated in Fig. 5D, the expression of IRF-7 was significantly reduced (∼70%) in IRF-3−/− cells. On the other hand, the lack of IRF-7 did not have any effect on IRF-3 expression after hMPV infection. Together, these data indicate that MDA5 contributes in part to the activation of IRF-3 and that IRF-3 regulates the expression of IRF-7.

**Antiviral response to hMPV infection is impaired in MDA5−/− mice.** To determine the relevance of MDA5 in the antiviral response to hMPV *in vivo*, we determined first the induction of MDA5 expression in the lungs of treated mice. C57BL/6J WT mice were infected i.n. with 10⁷ PFU/mouse of hMPV or mock infected with PBS. As shown in Fig. 6A, hMPV induced significant expression of MDA5 at days 1, 3, and 5 after infection. Next, we determined by real-time qRT-PCR the IFN response at different time points after infection. We observed that IFN-β mRNA was significantly induced in hMPV-infected mice at day 1 after infection (peak) and gradually decreased over time (Fig. 6B). We then examined the role of MDA5 in the IFN response *in vivo*. MDA5−/− and WT mice were infected as indicated above. Based on the mRNA data, BAL samples were collected after 24 h of infection, and IFN production was measured by ELISA. As shown in Fig. 6C to E, in WT mice, the levels of IFN-α (∼8,000 pg/ml), IFN-β (∼600 pg/ml), and IFN-λ (∼1,500 pg/ml) were induced after hMPV infection. In contrast, in MDA5−/− mice, the production of IFN-λ was reduced to comparable levels to mock-infected mice, while the IFN-α and IFN-β production was decreased by ∼90% and ∼70%, respectively. Overall, these results confirm those findings observed in DC and reveal an essential role of MDA5 in the IFN response by hMPV infection *in vivo*. Finally, we determined the expression of the hMPV N gene in infected mice. The assessment was made using primers and a probe set designed to amplify the hMPV N gene transcript. Analysis of the N gene revealed a significant increase in hMPV N expression on day 10, but no differences were found at earlier times. Thus, these data indicate that MDA5 contributes to the viral clearance in hMPV infection (Fig. 6F).

Lack of MDA5 increases disease severity and exacerbates pulmonary inflammation in hMPV-infected mice. In order to assess the role of MDA5 in the regulation of disease severity and inflammation during hMPV infection, body weight loss, histology analysis, and cytokine release levels were determined. WT and MDA5−/− mice were infected i.n. with hMPV (1 × 10⁷ PFU/mouse), monitored daily, and lung tissue and BAL fluid were analyzed at day 7 after infection. Our data show that the absence of...
increased cellular infiltration in the alveolar, perivascular, and peripheral alveolar spaces. Compared to sections obtained from MDA5<sup>−/−</sup> mice infected with hMPV, that response was exacerbated. To define the role of MDA5 in the regulation of the hMPV-induced cytokine response, the levels of cytokines and chemokines were assessed by multiplex analysis in MDA5<sup>−/−</sup> mice and compared to the WT. Mice were infected i.n. with hMPV, and BAL samples were collected at day 7 after infection from each group of mice and assessed for the presence of cytokines by using a multiplex cytokine detection system. Our data indicated that after hMPV infection, MDA5<sup>−/−</sup> mice exhibited an increased release of cytokines and chemokines compared to WT mice (Fig. 7C). The production of CXCL1 (KC, IL-8 homologue), a cytokine that regulates the chemotaxis and function of neutrophils, was increased over two times in MDA5<sup>−/−</sup> mice. A similar effect was observed in the production of proinflammatory cytokines, such as IL-6 and IL-1α, which were 10-fold and 4-fold increased in the deficient mice. G-CSF, a cytokine that stimulates the survival, differentiation, proliferation, and function of neutrophil precursors and mature neutrophils, was also significantly increased in MDA5<sup>−/−</sup> mice. Although induced after hMPV infection, no significant changes were observed in the production of CXCL10 (IP-10), IFN-γ, CCL2 (MCP-1), CCL5 (RANTES), and CCL11 (Eotaxin). Overall, these data indicate that MDA5 regulates the inflammatory response in hMPV-infected mice.

**DISCUSSION**

Different cell types secrete various amounts of IFN after hMPV infection (30, 31, 47). Nonetheless, the cell-specific mechanism(s) for IFN induction by hMPV remains largely uncharacterized. Activation of RIG-I by hMPV has been previously documented in epithelial cells (31, 32). However, to the best of our knowledge, this is the first report to define the role of MDA5 in hMPV infection. In fact, our findings indicate that MDA5, more than RIG-1, is necessary for the production of IFN-α, -β, and -λ by hMPV in human DC, according to the data in moDC treated with siRNA. However, we cannot rule out the possibility that the remaining 30% of expressed RIG-1 in moDC after the silencing experiments could account for the activation of the IFN response in hMPV-infected cells. Our data from mouse DC confirmed the finding that MDA5 is critical for the production of IFN in response to hMPV infection.

IRF-3 and IRF-7 are master transcriptional factors that regulate type I IFN gene induction and innate immune defenses after virus infection. Activation of IRF-7 is known to be preceded by activation of IRF-3 (40, 48). However, depending on the cell type, IRF-7 can also activate the initial phase of IFN-α and IFN-β gene expression (21, 49). After virus infection, type I IFN induction is known to occur in a two-step model that is modulated by IRF-3 and IRF-7 (30, 51). In the first step, viral recognition by pattern recognition receptors induces the activation of IRF-3, which further stimulates the production of IFN-β and IFN-α. In the second step, the produced IFN activates the IFN-α/β receptor in a paracrine and autocrine manner to activate the IFN pathway-stimulating antiviral genes, which limit viral replication in the infected host. Based on the present observations, induction of IFN-α/β by hMPV in myeloid DC is regulated by the expression of both IRF-3 and IRF-7. Moreover, IRF-3 controls the expression of IRF-7. However, the lack of IRF-7 alone prevented the production of IFN-α and IFN-β in hMPV-infected cells, suggesting that IRF-3 alone is not sufficient to promote the robust IFN response.
induced by hMPV. These data represent novel evidence of the critical role of IRF-7 in hMPV infection. Future work is aimed to define the role of IRF-7 in hMPV infection in vivo.

To our knowledge, we present here the first set of in vivo data defining the role of MDA5 in hMPV infection. We have previously reported robust induction of IFN-α and IFN-β in BALB/c mice after hMPV infection (27, 28). In the present work, we observed that hMPV also induced high levels of IFN-α, IFN-β, and IFN-λ in C57BL/6 mice. Moreover, the production of IFNs was impaired in MDA5−/− mice. In agreement with these data, the lack of MDA5 has been reported to decrease the gene expression of IFN-α, IFN-β, and IFN-λ in mice infected with other respiratory viruses, such as Sendai virus (SeV), a negative-stranded RNA parvovirus also known as murine parainfluenza virus type 1 (52), and rhinovirus (RV) (39). The protective effect of IFN-α and IFN-λ in hMPV infection has been previously documented (27, 53). IFN-λ or interleukin-29 and -28A/B triggers similar gene expression profiles in responsive cells as IFN-α/β, suggesting that both types of IFN have similar antiviral functions (54) and, based on the present observations, their induction after hMPV infection depends on the expression of MDA5. We also observed a delayed viral clearance in hMPV-infected MDA5−/− mice. In line with our
data, enhanced viral replication of SeV, RV, murine norovirus 1 (MNV-1), and Theiler’s virus has been reported in MDA5−/− mice (38, 39, 52, 55).

In order to investigate the role of MDA5 in hMPV-induced disease, we determined a series of parameters, including body weight loss, lung histology analysis, viral antigen expression, and production of cytokines in MDA5−/− mice. Body weight loss is a parameter to monitor the severity of the disease after hMPV infection (30, 41). In this study, the body weight loss was enhanced in MDA5−/− mice at the recovery phase of the disease, indicating that MDA5 expression exerts a regulatory role in hMPV-induced illness. These data are consistent with studies reported in MDA5−/− mice infected with SeV, in which deficient mice did not recover baseline body weight as WT mice did (52). Pulmonary inflammation represents a critical host response to control viral infections in the lung. However, an exacerbated inflammatory response may result in a severe lung disease. In this work, we showed that airway inflammation was increased in MDA5−/− mice compared with that of WT mice infected with hMPV. Similar to the findings reported in this work, studies in an experimental model of SeV infection indicated that the lack of MDA5 exacerbated the infiltration of cells into the lungs of infected animals (52). However, the data reported here provide the first evidence demonstrating that MDA5 plays a critical role in controlling pulmonary inflammation during a human respiratory paramyxovirus infection.

Production of several inflammatory mediators is induced in infants and mice infected with hMPV (28, 56). We found that the cytokine response to hMPV infection at day 7 was altered in MDA5−/− mice. In the absence of MDA5, hMPV induced higher levels of CXCL1, IL-6, IL-1α, and G-CSF, which could potentially account for the enhanced pulmonary inflammation observed in the airways of MDA5−/− mice. In agreement with our findings, in the model of SeV, the lack of MDA5 also altered the expression of the cytokine profile in the lungs of infected mice, increasing the expression of IL-6 at day 2 and 5 after infection.

In conclusion, our data highlight the relevance of the MDA5 helicase pathway in hMPV infection. The results presented herein indicate that MDA5 plays a central role in the regulation of the antiviral and inflammatory responses against hMPV infection. Future studies aimed to define the role of this helicase in shaping the adaptive immune response to hMPV are warranted. These findings provide a better understanding of the nature of virus-host cell interactions that lead to the activation of the antiviral immunity to hMPV and will be critical for the development of novel therapies for hMPV infection.

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