Middle East respiratory syndrome coronavirus-encoded ORF8b strongly antagonizes IFN-β promoter activation: its implication for vaccine design

Jeong Yoon Lee, Sojung Bae, and Jinjong Myoung*

Korea Zoonosis Research Institute, Genetic Engineering Research Institute & Department of Bioactive Material Science, College of Natural Science, Jeonbuk National University, Jeonju 54531, Republic of Korea

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Middle East respiratory syndrome coronavirus (MERS-CoV) is a causative agent of severe-to-fatal pneumonia especially in patients with pre-existing conditions, such as smoking and chronic obstructive pulmonary disease (COPD). MERS-CoV transmission continues to be reported in the Saudi Arabian Peninsula since its discovery in 2012. However, it has rarely been epidemic outside the area except one large outbreak in South Korea in May 2015. The genome of the epidemic MERS-CoV isolated from a Korean patient revealed its homology to previously reported strains. MERS-CoV encodes 5 accessory proteins and generally, they do not participate in the genome transcription and replication but rather are involved in viral evasion of the host innate immune responses. Here we report that ORF8b, an accessory protein of MERS-CoV, strongly inhibits both MDA5- and RIG-I-mediated activation of interferon beta promoter activity while downstream signaling molecules were left largely unaffected. Of note, MDA5 protein levels were significantly down-regulated by ORF8b and co-expression of ORF4a and ORF4b. These novel findings will facilitate elucidation of mechanisms of virus-encoded evasion strategies, thus helping design rational antiviral countermeasures against deadly MERS-CoV infection.

Keywords: accessory protein, interferon beta, MERS-CoV, ORF8b

Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV), along with severe acute respiratory syndrome coronavirus (SARS-CoV), belongs to the genus Betacoronavirus in the family Coronaviridae of the order Nidovirales (Chan et al., 2015). Among four genera in the subfamily orthocoronavirinae, only alphacoronaviruses and betacoronaviruses infect mammals, including bats, camels, and humans (Zhou et al., 2018). The first coronavirus isolate was reported in the mid-1960s from the respiratory tracks of patients with common cold (Tyrrell and Bynoe, 1965; Hamre and Procknow, 1966), which was subsequently termed human coronavirus 229E (HCoV 229E) and human coronavirus OC43 (HCoV OC43). Four more human coronaviruses have been isolated and described so far: HCoV NL63 (van der Hoek et al., 2004; Abdul-Rasool and Fielding, 2010), HCoV HKU1 (Woo et al., 2005a; Lau et al., 2006; Vabret et al., 2006), SARS-CoV (Ksiazek et al., 2003; Rota et al., 2003), and MERS-CoV (Corman et al., 2012; Zaki et al., 2012). HCoV 229E and OC43 mostly infect the upper, but rarely the lower, respiratory tracks, thus causing mild, but seldom severe, respiratory diseases, such as common cold (Bradburne et al., 1967; Woo et al., 2005a). On the other hand, HCoV NL63 and HKU1 have been shown to infect not only the upper but also the lower respiratory tracks, thus causing symptoms ranging from mild croup to severe bronchiolitis (Woo et al., 2005b; Abdul-Rasool and Fielding, 2010), of which infections are mostly self-limiting with just few mortality cases reported. The landscape has been widely changing with the emergence of two deadly coronaviruses in humans: SARS-CoV and MERS-CoV. The two newly identified coronaviruses causes severe-to-fatal infection in humans, especially in the presence of preexisting conditions with MERS-CoV cases (Alraddadi et al., 2016; Meyerholz et al., 2016; Nam et al., 2017; Seys et al., 2018). During the SARS-CoV outbreak in 2003, over 8,000 SARS cases were reported in 37 countries resulting in 775 deaths with mortality rate reaching 10% (WHO). Since 2004, no SARS cases have been reported in the human population (Yip et al., 2009; Abdul-Rasool and Fielding, 2010). On the contrary, MERS-CoV is a lingering threat causing sporadic outbreaks since its identification and characterization in 2012. Due to its zoonotic nature of infections from dromedary camels and low-level medical management, MERS-CoV cases are mostly reported in the Arabian Peninsula (van den Brand et al., 2015; Widagdo et al., 2019) with one exceptional outbreak in South Korea in 2015 (Ki, 2015; Lim, 2015; Kim et al., 2017).

The outbreak of MERS-CoV in South Korea is generally considered as a failure of crisis management (Chowell et al., 2015; Fung et al., 2015; Ki, 2015). 28 secondary MERS-CoV infections in a single hospital arose by transmission from a 68-year-old business man (Park et al., 2015) who had returned from the Arabian Peninsula, complaining symptoms similar to those of MERS-CoV infection. MERS-CoV transmission spread to sixteen clinics and hospitals and the chain of transmission via intra- and inter-hospital route...
Table 1. Primers for cloning of MERS-CoV accessory proteins

| Name             | Sequence (5’→3’)                        |
|------------------|-----------------------------------------|
| MERS-ORF3-F      | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF3-R      | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF4a-F     | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF4a-R     | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF4b-R     | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF5-F      | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF5-R      | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF8b-R     | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF8b-F     | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF8b-R     | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF8b-F     | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF8b-R     | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |
| MERS-ORF8b-F     | GGGGAATCATGAGCTTGAATGCAAGACCCACC       |

lasted for two months (Oh, 2016), resulting in 186 confirmed cases of MERS-CoV infections with 38 deaths as well as a huge economic loss (estimated, 8.5 billion US). The MERS-CoV genome from the second patient was extracted and fully sequenced (KT029139.1) (Kim et al., 2015). Characterization and analysis of the genome revealed that it has 99.5% to 99.8% similarity to 53 known MERS-CoVs. Like other MERS-CoVs, the genome of Korean isolate encodes 16 non-structural proteins and 4 canonical structural proteins (S, E, N, and M) (Kindler et al., 2016). As characterized in other coronaviruses, MERS-CoV expresses a unique set of subgenomic RNA’s that are translated into accessory proteins, which vary in number and function among coronaviruses (Fehr and Perlman, 2015). MERS-CoV encodes 5 accessory proteins (3a, 4a, 4b, 5, and 8b) while SARS-CoV has 8 of them (3a, 3b, 6, 7a, 7b, 8a, 8b, and 9b), suggesting that accessory proteins vary in number as well as function. Indeed, some accessory proteins have been shown to target a number of host cellular processes, especially those involved in innate immunity: 1) ORF4a is shown to antagonize type I interferon (IFN) responses by employing a few different strategies, blocking melanoma differentiation-associated protein 5 (MDA5) (Niemeyer et al., 2013), interacting with protein activator of the interferon-induced protein kinase (PACT) (Siu et al., 2014), or inhibiting IFN-β as well as interferon-stimulated response element (ISRE), promoter activity (Yang et al., 2013). 2) ORF4b interacts in the cytoplasm as well as in the nucleus with TANK binding kinase 1 (TBK1) and IκB kinase epsilon (IKKε), disrupting optimal activation type I IFN signaling (Matthews et al., 2014; Yang et al., 2015). 3) ORF5 may also interfere with IFN signaling by blocking nuclear localization of interferon regulatory factor 3 (IRF3), the major transcription factor for the activation of IFN-β promoter.

As viruses have been shown to evolve diverse tactics to evade host IFN responses, it is likely that in addition to antagonistic viral proteins described above, MERS-CoV-encoded proteins may encode other mechanisms to counteract IFN-β promoter induction upon infection in the host cell. With an aim to identify novel virus-encoded antagonist(s), we systematically screen MERS-CoV-encoded accessory proteins against each individual signaling molecule involved in the type I IFN induction pathway: MDA5, retinoic acid-inducible gene I (RIG-I), mitochondrial antiviral-signaling protein (MAVS), TBK-1, IKKe, and IRF3.

Table 1. Primers for cloning of immune genes

| Name | Sequence (5’→3’) |
|------|-----------------|
| MDA5-F | AGGTGGTGGCCGGTGGGTCCGAGTAATGCAATGCAAGACCCACC |
| MDA5-R | GGGGAATCATGAGCTTGAATGCAAGACCCACC |
| RIG-I-F | GGGGAATCATGAGCTTGAATGCAAGACCCACC |
| RIG-I-R | GGGGAATCATGAGCTTGAATGCAAGACCCACC |
| MAVS-F | GGGGAATCATGAGCTTGAATGCAAGACCCACC |
| MAVS-R | GGGGAATCATGAGCTTGAATGCAAGACCCACC |
| TBK1-F | AGGTGGTGGCCGGTGCGGCGCTCCGAAGAGCAAGACCCACC |
| TBK1-R | AGGTGGTGGCCGGTGCGGCGCTCCGAAGAGCAAGACCCACC |
| IKKe-F | CGGGGATCATGCCAAGCAGCAGCAAGACCCACC |
| IKKe-R | CTAGTCAGATTAGACATCAGCAGGTTGCTGGACTCTAT |
| IRF3-F | TTGGCGGCGCGGAACCGCAAGCAAGACCCACC |
| IRF3-R | CCCTGAGCTCAGCTCAGCAGCAGCAGCAAGACCCACC |
Plasmid construction

Multiple cloning site (MCS) of an expression vector, pcDNA-3.1-Hygro(+) (Lee et al., 2018), was modified with a linker DNA to generate an HA-tagged Neo-JY4 vector: 5′-GCTAGCTACATGTACCCATACGACGTCCCAGACTACGCTAGCTTTCTGGTGCGGCTCGGAGGTTGGTCGGTGCGGCGGCGGATCCCTGGTGGCGGTGGGTCGGGGCGGCGGAGGTGGCCAGCTCGATGAGTCGAGTGGCGCCACTGGACTAATGGTCCGTACGCTCGACTGTACAGGCCGGCCTCAGGTTAACACGGTGACCCGGGGGCCTCGAGTCTAGAGTTTAAAC-3′. The modified vector, named pcDNA3.1-Hygro-JY4-HAN-GS3 harboring a HA tag and a spacer (3xGGGGS) at the N-terminus of MCS, was used to clone immune genes (see text) by employing sequence and ligation independent cloning (SLIC) (Jeong et al., 2012; Islam et al., 2017). MDA5, RIG-I, MAVS, IKKe, TBK1, and IRF3 were amplified by PCR using Q5 Hot Start High-Fidelity DNA Polymerase (NEB): 98°C for 10 sec, 58°C for 30 sec, 72°C for 30 sec/kb for 30 cycles. Purified PCR products were cloned into the pcDNA3.1-Hygro-JY4-HAN-GS3 vector. All primers for the immune genes are listed in Table 2. Pfu Plus DNA Polymerase (Elpisbio) was utilized to PCR-amplify each MERS accessory gene: 95°C for 20 sec, 58°C for 20 sec, 72°C for 10 sec/kb for 30 cycles. MERS accessory genes were cloned in p3xFLAG-CMV10 vector (Millpore Sigma) by conventional ligation method using restriction enzymes. The sequence of MERS-CoV isolated from a Korean patient was used to construct expression plasmids, which was authorized by Korea Centers for Disease Control (Approval No. 16-RDM-019).

Transfection and luciferase reporter assay

HEK293T cells (4 × 10^5 cells/well) were seeded in a 6-well plate a day before transfection as previously described (Kang et al., 2018; Kim and Myoung, 2018; Park et al., 2019). Briefly, mixtures, containing 500 ng of Interferon (IFN)-β-luc, 100 ng of β-gal expressing plasmid as the internal control, 500 ng of immune gene stimulating plasmid and 1,000 ng of MERS accessory gene encoding or empty plasmid, were transfected using PEI transfection reagent as ratio of 1:2 (DNA: PEI) in 200 μl of opti-MEM (Thermo Fisher Scientific). At 24 h post-transfection, the transfected cells were lysed using 1 × Reporter Assay Lysis Buffer of the Luciferase Assay System (Promega) with 1 × Protease Inhibitor (Millipore Sigma). Lysates were incubated on ice for 10 min and centrifuged at 150,000 rpm, 4°C for 15 min. The supernatant was transferred to a new tube. 25 μl of sample was mixed with 25 μl of Assay Substrate in the Luciferase Assay System and Beta-Glo Assay System (Promega), and the luciferase or β-gal intensities were measured on GloMax 96 Microplate Luminometer (Promega). Firefly luciferase activity was normalized by β-gal activity, and fold induction of luciferase gene, in the presence or absence of MERS-CoV accessory protein, was calculated.
Western blotting

Protein amount was quantified by Pierce BCA Protein Assay Kit (Thermo Fisher Scientific) as described before (Cho and Myoung, 2015; Ha et al., 2016; Kang et al., 2016). In brief, 15 μg of protein was loaded and separated on a SDS-PAGE gel and subsequently transferred to a NC blotting membrane (GE Healthcare Life Sciences). Primary antibodies were incubated at 4°C overnight while secondary antibodies for 1 h at RT. For statistical analysis, paired two-tailed Student’s t-test was performed. Difference between means was considered significant when P-value was < 0.05.

Results

ORF8b strongly antagonizes IFN-β promoter activation induced by both MDA5 and RIG-1

The MERS-CoV genome encodes 5 accessory proteins. Although some studies have been conducted (Niemen et al., 2013; Siu et al., 2014), detailed mechanisms of how MERS-CoV-encoded accessory proteins interrupt type I IFN induction remain still elusive. A full panel of MERS-CoV accessory genes were cloned into an expression vector (for details, see 'Materials and Methods'). ORF8b was included in this study although it has been neglected in the previous screening (Niemen et al., 2013; Siu et al., 2014). Accessory proteins were expressed more or less comparably with albeit lower expression in ORF3 and ORF5. Codon optimization of ORF5 and lysate preparation with sonication did not improve soluble levels of those two proteins. MERS-CoV ORF’s were expressed either individually or in combination (ORF4 and ORF4b) to investigate if those two proteins function in the same pathways or they interact synergistically. To examine whether MERS-CoV accessory proteins inhibit RIG-I-like receptors (RLR)-mediated activation of IFN signaling, HEK293T cells were transfected with either MDA5 (Fig. 1, left panel) or RIG-I (Fig. 1, right panel) together with a panel of accessory genes. As expected, ORF4a inhibited MDA5-mediated (Niemen et al., 2013; Niemen et al., 2013; Siu et al., 2014), but not RIG-I-mediated induction of IFN-β signaling. In addition, ORF4b cooperated with ORF4a in the inhibition of MDA5-mediated IFN signaling induction in an additive manner (Fig. 1A). A surprise comes with ORF8b. ORF8b suppressed over 80% the activation of IFN signaling induced by both MDA5 and RIG-I (Fig. 1A and B). Furthermore, expression of ORF8b or co-expression of ORF4a and ORF4b led to significant reduction in protein levels of MDA5, but not RIG-I, suggesting for a differential mechanism(s) involved in MERS-CoV protein-mediated inhibition of those two cellular helicases. Taken together, 3 MERS-encoded proteins (ORF4a, ORF4b, and ORF8b) efficiently block IFN induction via blockade of the RLR’s.

MAVS- and TBK1-induced activation of IFN-β promoter is not perturbed by MERS-CoV accessory proteins

Next, it was tested if MERS-CoV accessory proteins are also involved in the inhibition of downstream innate immune molecules, namely MAVS and TBK-1 (Fig. 2). As shown, none of MERS-CoV accessory proteins perturb, if any, MAVS- (Fig. 2A) and TBK-1-mediated (Fig. 2B) induction of IFN-β promoter activity. Thus, it appears that the two cellular RNA helicases (MDA5 and RIG-I) are a major target of the accessory protein.

![Fig. 2. MAVS- and TBK-1-induced activation of IFN-β signaling were not perturbed by accessory proteins of MERS-CoV. MERS-CoV accessory genes were co-transfected into HEK-293T cells with MAVS (A) or TBK-1 (B) together with IFN-β-luc and β-gal expression constructs. Firefly luciferase activities and Western blots are shown at the top and bottom panels, respectively. The arrow heads indicate each MERS-CoV accessory protein with 3XFLAG at the N terminus. Data represent the Mean ± SD. *P < 0.05.](image-url)
IKKe- and IRF3-mediated activation of IFN-β signaling was left little changed by MERS-CoV-encoded accessory proteins

IKKe, a down-stream signaling molecule of IKKe, is a key transcription factor in the induction of IFN-β (Grandvaux et al., 2002; Honda et al., 2006; Honda and Taniguchi, 2006; Liu et al., 2015). To examine whether MERS-CoV accessory proteins regulate IKKe or IRF3-mediated upregulation of IFN-β, HEK293T cells were co-transfected with IKKe or full-length IRF3 (Fig. 3A) together with individual accessory gene. A marginal, but significant reduction in IFN-β promoter activation, induced by IKKe, was detected by ORF5 (Fig. 3A, upper panel) while the protein level of IKKe was not changed (Fig. 3A, lower panel). On the other hand, IFN-β promoter activation, induced by IRF3, was largely unchanged by accessory proteins of MERS-CoV.

Discussion

Type I IFN responses are a first line of defense against invading viruses (Kang and Myoung, 2017a, 2017b; Kang et al., 2018; Kim and Myoung, 2018; Banerjee et al., 2019). There are 16 different type I IFN’s that are known to be expressed in humans (Kindler et al., 2016): IFN-α (13 subtypes), IFN-β, IFN-κ, and IFN-ω (1 type each). Upon viral infection, ligation of viral pathogen-associated molecular patterns (PAMP) with cellular pattern recognition receptors (PRR) initiate a series of activational cascades in cells (Fig. 4). For example, double-stranded RNA (dsRNA) molecules, a byproduct of viral replication and transcription in a cell (Weber et al., 2006; Zielecki et al., 2013), are generally recognized by the RNA helicases (MDA5 and RIG-I), and/or protein kinase R (PKR) in the cytoplasm as well as by toll-like receptor 3 (TLR3) in the endosome. It is now well-known that structurally and
chemically distinctive RNA’s are recognized by the two cellular helicases (Akira et al., 2006; Medzhitov, 2007): RIG-I senses long or short dsRNA molecules with di- or tri-phosphates at the 5’ end (Gobau et al., 2014) while single-stranded RNAs (ssRNAs) with particular features can also be recognized if the ssRNAs are 3’ phosphorylated or polyU/UC-rich (Malathi et al., 2007). On the other hand, types of RNA that MDA5 binds to include long RNA’s with higher-order structures (Runge et al., 2014) as well as some ssRNA’s (negative-sense RNA and hypomethylated 5’ capped mRNA molecules) (Luthra et al., 2011; Zust et al., 2011). Upon recognition of these RNA’s, RIG-I and MDA5 undergo conformational changes, including oligomerization, and the mitochondrial membrane chaperone 14-3-3ε recruits the oligomers to mitochondria, where caspase activation and recruitment domains (CARD) of the RNA helicases interact with MAVS (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005), leading to induction of its aggregation and activation. Activated MAVS, in turn, triggers activation of TBK-1 and IKKe (Fitzgerald et al., 2003; Hacker and Karin, 2006; Gatot et al., 2007; Chau et al., 2008; Clement et al., 2008), and subsequently phosphorylation and dimerization of IRF3 (Grandvaux et al., 2002; Honda and Taniguchi, 2006; Liu et al., 2015). Dimerized IRF3 translocates into the nucleus, stimulating transcription of IFN-β (Fig. 4).

MERS-CoV, like SARS-CoV, is generally considered to have evolved from bat coronaviruses (Li et al., 2005; Hayman, 2016; Goldstein and Weiss, 2017; Maxmen, 2017). Comprehensive genomic and functional analyses of those two viruses have revealed a few interesting distinctions between them (Kindler et al., 2016): 1) SARS-CoV encodes more number of accessory proteins at the 3’ end of the genome compared to MERS-CoV, 2) More number of SARS-CoV-encoded genes are known to inhibit IFN signaling than those of MERS-CoV, 3) MERS-CoV is more sensitive to IFN-mediated inhibition than SARS-CoV (Zielecki et al., 2013). These results suggest that MERS-CoV might have evolved less means to evade the innate immune responses mediated by IFN. However, exact magnitude or multitude of MERS-CoV-encoded antagonistic mechanisms remain to be elucidated.

To shed light on viral and cellular determinants of IFN evasion by MERS-CoV, a full panel of accessory genes of MERS-CoV (van Boheemen et al., 2012) were cloned into an expression vector: ORF3, ORF4a, ORF4b, ORF5, and ORF8b. Either in combination or individually, these genes were transfected into HEK293T cells together with each molecule that is involved in an IFN signaling: MDA5 and RIG-1 (RNA helicases), MAVS, TBK-1, and IKKe (cytoplasmic signaling molecules), or IRF3 (a key transcription factor). The striking finding of current study is that ORF8b was identified as a strong antagonist of two dsRNA sensors (Fig. 1): MDA5 and RIG-1 with or without perturbation of protein levels in the cells, respectively (Fig. 1A vs B). Currently, ORF8b-interacting host proteins are being sought. Identity of host interacting partner(s) will hint on the molecular mechanism(s) of ORF8b-mediated suppression. In addition, ORF4a seems to robustly down-regulate MDA5-mediated activation of IFN signaling. This is in line with a previously published study led by Niemeyer et al. (2013). ORF4a has a dsRNA-binding motif (Niemeyer et al., 2013; Siu et al., 2014; Comar et al., 2019), thus antagonizing IFN signaling like several virus-encoded proteins, such as influenza A virus NS1 (Chan et al., 2018), herpes simplex virus 1 Us11 (Kew et al., 2013), paramyxovirus V (Motz et al., 2013), and Ebola virus VP35 proteins (Cardenas et al., 2006; Prins et al., 2009, 2010). However, IFN-inhibiting function of ORF4a may be controversial. Siu et al. (2014) reported that ORF4a could not inhibit MDA5-mediated initiation of IFN signaling activation, but did suppress an upstream signaling molecule, PACT. PACT binds to dsRNA and recruit it to MDA5 or RIG-1. The authors claimed that ORF4a interrupt dsRNA-PACT interaction with the RLR’s (Kok et al., 2011; Ho et al., 2016; Lui et al., 2017). Furthermore, when a recombinant MERS-CoV with ORF4a deletion infects cells, IFN induction was only marginally reduced (Comar et al., 2019). One may envision that after all ORF4a may play a minor role in viral antagonism of IFN signaling and other viral antagonist(s) may exist. In this regard, identification of ORF8b as a virus-encoded antagonist will help elucidate magnitude and multitude of virus-encoded mechanisms of IFN evasion. Or simply, viral dsRNA might not be exposed to antiviral sensors (PACT, MDA5 or RIG-1) (Versteeg et al., 2007; Zhou and Perlman, 2007) as MERS-CoV induces massive membrane re-organization for the formation of double-membrane vesicles for viral transcription and replication (Gosert et al., 2002; Lundin et al., 2014). Elucidation of exact mechanisms, involved in ORF4a antagonism of IFN signaling, awaits further scrutiny.

ORF4b seems to marginally inhibit both MDA5- and RIG-1-induced IFN signaling (Fig. 1) and also to cooperate with ORF4a to further down-regulate MDA5-mediated signaling (Fig. 1A). To our knowledge, this is the first evidence that ORF4a and ORF4b inhibits IFN signaling in an additive manner. As ORF4b harbors nuclear localizing signal (NLS) (Niemeyer et al., 2013; Comar et al., 2019), it is tempting to postulate that it may interact with one or more of transcription factors that are involved in IFN induction. Taken all together, here we report that ORF8b of MERS-CoV is a potent antagonist of both MDA5- and RIG-mediated activation of IFN signaling (schematic summary in Fig. 4), building up ever-growing list of MERS-CoV evasion strategies against the host innate immune responses. Delineation of molecular mechanisms ORF8b will likely pave way to develop effective protective and/or therapeutic antiviral measures.

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References

Abdul-Rasool, S. and Fielding, B.C. 2010. Understanding human coronavirus HCoV-NL63. Open Virol. J. 4, 76–84.
Akira, S., Uematsu, S., and Takeuchi, O. 2006. Pathogen recognition and innate immunity. Cell 124, 783–801.
Alraddadi, B.M., Watson, J.T., Almarashi, A., Abedi, G.R., Turki-
stani, A., Sadrani, M., Housa, A., Almazroa, M.A., Alraihan, N., Banjar, A., et al. 2016. Risk factors for primary Middle East respiratory syndrome coronavirus infection in humans, Saudi Arabia, 2014. Emerg. Infect. Dis. 22, 49–55.

Banerjee, A., Falzarano, D., Rapin, N., Lew, J., and Misra, V. 2019. Interferon regulatory factor 3-mediated signaling limits Middle East respiratory syndrome (MERS) coronavirus propagation in cells from an insectivorous bat. Viruses 11, 152.

Bradburne, A.F., Bynoe, M.L., and Tyrrell, D.A. 1967. Effects of a “new” human respiratory virus in volunteers. Br. Med. J. 3, 767–769.

Cardenas, W.B., Loo, Y.M., Gale, M.Jr., Hartman, A.L., Kimberly, C.R., Martinez-Sobrido, L., Saphire, E.O., and Basler, C.F. 2006. Ebola virus VP35 protein binds double-stranded RNA and inhibits alpha/beta interferon induction produced by RIG-I signaling. J. Virol. 80, 5168–5178.

Chan, J.F., Lau, S.K., To, K.K., Cheng, V.C., Woo, P.C., and Yuen, K.Y. 2015. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin. Microbiol. Rev. 28, 465–522.

Chan, C.P., Yuen, C.K., Cheung, P.H., Fung, S.Y., Lui, P.Y., Chen, H., Kok, K.H., and Jin, D.Y. 2018. Antiviral activity of double-stranded RNA-binding protein PACT against influenza A virus mediated via suppression of viral RNA polymerase. FASEB J. 32, 4380–4393.

Chau, T.L., Gioia, R., Gatot, J.S., Patrascu, F., Carpentier, I., Chapelle, J.P., O’Neill, L., Beyaert, R., Piette, J., and Chariot, A. 2008. Are the IKKs and IKK-related kinases TBK1 and IKK-epsilon similarly activated? Trends Biochem. Sci. 33, 171–180.

Cho, M. and Myoung, J. 2015. OX40 and 4-1BB downregulate Kaposis’s sarcoma-associated herpesvirus replication in lymphatic endothelial cells, but 4-1BB and not OX40 inhibits viral replication in B-cells. J. Gen. Virol. 96, 3635–3645.

Chowell, G., Abdurizak, F., Lee, S., Lee, J., Jung, E., Nishiura, H., and Viboud, C. 2015. Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. BMC Med. 13, 210.

Clement, J.F., Meloche, S., and Servant, M.J. 2008. The IKK-related kinases: from innate immunity to oncogenesis. Cell Res. 18, 889–899.

Comar, C.E., Goldstein, S.A., Li, Y., Yount, B., Baric, R.S., and Weiss, S.R. 2019. Antagonism of dsRNA-induced innate immune pathways by NS4a and NS4b accessory proteins during MERS coronavirus infection. mBio 10, e00319-19.

Corman, V.M., Eckerle, I., Bleicker, T., Zaki, A., Landt, O., Eschbach-Bludau, M., van Boheemen, S., Gopal, R., Ballhaus, M., Bestebroer, T.M., et al. 2012. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 17, pii:20285.

Fehr, A.R. and Perlman, S. 2015. Coronavirus: an overview of their replication and pathogenesis. Methods Mol. Biol. 1282, 1–23.

Fitzgerald, K.A., McWhirter, S.M., Faia, K.L., Rowe, D.C., Latz, E., Golenbock, D.T., Coyle, A.J., Liao, S.M., and Maniatis, T. 2003. IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nat. Immunol. 4, 491–496.

Fung, I.C., Tse, Z.T., Chan, B.S., and Fu, K.W. 2015. Middle East respiratory syndrome in the Republic of Korea: transparency and communication are key. Western Pac. Surveill. Response J. 6, 1–2.

Gatot, J.S., Gioia, R., Chau, T.L., Patrascu, F., Warnier, M., Close, P., Chapelle, J.P., Muraire, E., Brown, K., Siebenlist, U., et al. 2007. Lipopolysaccharide-mediated interferon regulatory factor 5 activation involves TBK1-IKK epsilon-dependent Lys(63)-linked polyubiquitination and phosphorylation of TANK1-TRAF. J. Biol. Chem. 282, 31131–31146.

Goldstein, S.A. and Weiss, S.R. 2017. Origins and pathogenesis of Middle East respiratory syndrome-associated coronavirus: recent advances. Fl1000Res 6, 1628.

Gosert, R., Kanjanaahalutethai, A., Egger, D., Bienz, K., and Baker, S.C. 2002. RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. J. Virol. 76, 3697–3708.

Goubau, D., Schlee, M., Dedouche, S., Prijusser, A.J., Zillinger, T., Goldeck, M., Schuberth, C., Van der Veen, A.G., Fujimura, T., Rehwinkel, J., et al. 2014. Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5′-diphosphates. Nature 514, 372–375.

Grandvaux, N., Servant, M.J., tenOever, B., Sen, G.C., Balachandran, S., Barber, G.N., Lin, R., and Hiscott, J. 2002. Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes. J. Virol. 76, 5532–5539.

Ha, S., Choi, I.S., Choi, C., and Myoung, J. 2016. Infection models of human norovirus: challenges and recent progress. Arch. Virol. 161, 779–788.

Hacker, H. and Karin, M. 2006. Regulation and function of IKK and IKK-related kinases. Sci. STKE 2006, re13.

Hamre, D. and Procknow, I.J. 1966. A new virus isolated from the human respiratory tract. Proc. Soc. Exp. Biol. Med. 121, 190–193.

Hayman, D.T. 2016. Bats as viral reservoirs. Annu. Rev. Virol. 3, 77–99.

Ho, T.H., Kew, C., Lui, P.Y., Chan, C.P., Satoh, T., Akira, S., Jin, D.Y., and Kok, K.H. 2016. PACT- and RIG-I-dependent activation of type 1 interferon production by a defective interfering RNA derived from measles virus vaccine. J. Virol. 90, 1557–1568.

Honda, K., Takaoka, A., and Taniguchi, T. 2006. Type I interferon [corrected] gene induction by the interferon regulatory factor family of transcription factors. Immunity 25, 349–360.

Honda, K. and Taniguchi, T. 2006. IRF8: master regulators of signalling by toll-like receptors and cytosolic pattern-recognition receptors. Nat. Rev. Immunol. 6, 644–658.

Islam, M.N., Lee, K.W., Yim, H.S., Lee, S.H., Jung, H.C., Lee, J.H., and Jeong, J.Y. 2017. Optimizing T4 DNA polymerase conditions enhances the efficiency of one-step sequence- and ligation-independent cloning. Biotechniques 63, 125–130.

Jeong, J.Y., Yim, H.S., Ryu, J.Y., Lee, H.S., Lee, J.H., Seen, D.S., and Kang, S.G. 2012. One-step sequence- and ligation-independent cloning as a rapid and versatile cloning method for functional genomics studies. Appl. Environ. Microbiol. 78, 5440–5443.

Kang, S., Choi, C., Choi, I., Han, K.N., Rho, S.W., Choi, J., Kwon, J., Park, M.K., Kim, S.J., and Myoung, J. 2018. Hepatitis E virus methyltransferase inhibits type I interferon induction by targeting RIG-I-1. Microbiol. Biotechnol. 28, 1557–1562.

Kang, S. and Myoung, J. 2017a. Host innate immunity against hepatitis E virus and viral evasion mechanisms. J. Microbiol. Biotechnol. 27, 1727–1735.

Kang, S. and Myoung, J. 2017b. Primary lymphocyte infection models for KSHV and its putative tumorigenesis mechanisms in B cell lymphomas. J. Microbiol. 55, 319–329.

Kang, H.S., Myoung, J., So, E.Y., Bahk, Y.Y., and Kim, B.S. 2016. Transgenic expression of non-structural genes of Theliers’s virus suppresses initial viral replication and pathogenesis of demyelination. J. Neuroinflammation 13, 133.

Kawai, T., Takahashi, K., Sato, S., Coban, C., Kumar, H., Kato, H., Ishii, K.J., Takeuchi, O., and Akira, S. 2005. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat. Immunol. 6, 981–988.

Kew, C., Lui, P.Y., Chan, C.P., Liu, X., Au, S.W., Mohr, I., Jin, D.Y., and Kok, K.H. 2013. Suppression of PACT-induced type I interferon production by herpes simplex virus 1 Us11 protein. J. Virol. 87, 13141–13149.

Kim, M. 2015. 2015 MERS outbreak in Korea: hospital-to-hospital transmission. Epidemiol. Health 37, e2015033.

Kim, Y.J., Cho, Y.J., Kim, D.W., Yang, J.S., Kim, H., Park, S., Han, Y.W., Yun, M.R., Lee, H.S., Kim, A.R., et al. 2015. Complete genome sequence of Middle East respiratory syndrome coronavi-
A novel coronavirus associated with severe acute respiratory syndrome. Nature. 2003, 423, 314–319.

Matthews, K.L., Coleman, C.M., van der Meer, Y., Snijder, E.J., and Malathi, K., Dong, B., Gale, M.Jr., and Silverman, R.H. 2013. Middle East respiratory syndrome coronavirus accessory protein 4a is a type I interferon antagonist. J. Virol. 87, 12489–12495.

Oh, M.D. 2016. The Korean Middle East respiratory syndrome coronavirus outbreak and our responsibility to the global scientific community. Infect. Chemother. 48, 145–146.

Park, M.K., Cho, H., Roh, S.W., Kim, S.J., and Myoung, J. 2019. Cell type-specific interferon-gamma-mediated antagonism of KSHV lytic replication. Sci. Rep. 9, 2372.

Park, Y.S., Lee, C., Kim, K.M., Kim, S.W., Lee, K.J., Ahn, J., and Ki, M. 2015. The first case of the 2015 Korean Middle East respiratory syndrome outbreak. Epidemiol. Health 37, e2015049.

Prins, K.C., Cardenas, W.B., and Basler, C.F. 2009. Ebola virus protein VP35 impairs the function of interferon regulatory factor-3, activating kinases IKKepsilon and TBK-1. J. Virol. 83, 3069–3077.

Prins, K.C., Delpeut, S., Leung, D.W., Reynard, O., Volchkova, V.A., Reid, S.P., Ramanan, P., Cardenas, W.B., Amarasinghe, G.K., Volchkov, V.E., et al. 2010. Mutations abrogating VP35 interaction with double-stranded RNA render Ebola virus avirulent in guinea pigs. J. Virol. 84, 3004–3015.

Rota, P.A., Oberste, M.S., Monroe, S.S., Nix, W.A., Campagnoli, R., Incenoge, J.P., Penaranda, S., Bankamp, B., Maher, K., Chen, M.H., et al. 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300, 1394–1399.

Runge, S., Sparrer, K.M., Lassig, C., Hembach, K., Baum, A., Garcia-Sastre, A., Soding, J., Conzelmann, K.K., and Hopfner, K.P. 2014. In vivo ligands of MDA5 and RIG-I in measles virus-infected cells. PLoS Pathog. 10, e1004081.

Seth, R.B., Sun, L., Ea, C.K., and Chen, Z.J. 2005. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell 122, 669–682.

Seys, I.J.M., Widagdo, W., Verhamme, F.M., Kleinjan, A., Janssens, W., Joos, G.F., Bracke, K.R., Haagmans, B.L., and Brusselle, G.G. 2018. DPP4, the Middle East respiratory syndrome coronavirus receptor, is upregulated in lungs of smokers and chronic obstructive pulmonary disease patients. Clin. Infect. Dis. 66, 45–53.

Siu, K.L., Yeung, M.L., Kik, K.H., Yuen, K.S., Lee, K.H., Chan, K.P., Tse, H., Woo, P.C., Yuen, K.Y., et al. 2014. Middle East respiratory syndrome coronavirus receptor, is upregulated in lungs of smokers and chronic obstructive pulmonary disease patients. Clin. Infect. Dis. 66, 45–53.

van Boheemen, S., de Graaf, M., Lauber, C., Bestebroer, T.M., Raj, V.S., Zaki, A.M., Osterhaus, A.D.M.E., Haagmans, B.L., Gorbalenya, A.E., Snijder, E.J., et al. 2012. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. mBio 3, e00473-12.

van den Brand, J.M., Smits, S.L., and Haagmans, B.L. 2015. Patho-
Antagonism of type I IFN by ORF8b 811

 genesis of Middle East respiratory syndrome coronavirus. J. Pathol. 235, 175–184.

 van der Hoeck, L., Pyrc, K., Jebbink, M.F., Vermeulen-Oost, W., Berkhout, R.J., Wolthers, K.C., Wertheim-van Dillen, P.M., Kaandorp, J., Spaargaren, J., and Berkhout, B. 2004. Identification of a new human coronavirus. Nat. Med. 10, 368–373.

 Versteeg, G.A., Bredenbeck, P.J., van den Worm, S.H., and Spaan, W.J. 2007. Group 2 coronaviruses prevent immediate early interferon induction by protection of viral RNA from host cell recognition. Virology 361, 18–26.

 Weber, F., Wagner, V., Rasmussen, S.B., Hartmann, R., and Paludan, S.R. 2006. Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. J. Virol. 80, 5059–5064.

 Widagdo, W., Sooksawasdi Na Ayudhya, S., Hundle, G.B., and Haagmans, B.L. 2019. Host determinants of MERS-CoV transmission and pathogenesis. Viruses 11, 280.

 Woo, P.C., Lau, S.K., Chu, C.M., Chan, K.H., Tsoi, H.W., Huang, Y., Wong, B.H., Poon, R.W., Cai, J.J., Luk, W.K., et al. 2005a. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J. Virol. 79, 884–895.

 Woo, P.C., Lau, S.K., Tsoi, H.W., Huang, Y., Poon, R.W., Chu, C.M., Lee, R.A., Luk, W.K., Wong, G.K., Wong, B.H., et al. 2005b. Clinical and molecular epidemiological features of coronavirus HKU1-associated community-acquired pneumonia. J. Infect. Dis. 192, 1898–1907.

 Yang, Y., Ye, F., Zhu, N., Wang, W., Deng, Y., Zhao, Z., and Tan, W. 2015. Middle East respiratory syndrome coronavirus ORF 4b protein inhibits type I interferon production through both cytoplasmic and nuclear targets. Sci. Rep. 5, 17554.

 Yang, Y., Zhang, L., Geng, H., Deng, Y., Huang, B., Guo, Y., Zhao, Z., and Tan, W. 2013. The structural and accessory proteins M, ORF 4a, ORF 4b, and ORF 5 of Middle East respiratory syndrome coronavirus (MERS-CoV) are potent interferon antagonists. Protein Cell 4, 951–961.

 Yip, C.W., Hon, C.C., Shi, M., Lam, T.T., Chow, K.Y., Zeng, F., and Leung, F.C. 2009. Phylogenetic perspectives on the epidemiology and origins of SARS and SARS-like coronaviruses. Infect. Genet. Evol. 9, 1185–1196.

 Zaki, A.M., van Boheemen, S., Bestebroer, T.M., Osterhaus, A.D.M.E., and Fouchier, R.A.M. 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N. Engl. J. Med. 367, 1814–1820.

 Zhou, P., Fan, H., Lan, T., Yang, X.L., Shi, W.F., Zhang, W., Zhu, Y., Zhang, Y.W., Xie, Q.M., Mani, S., et al. 2018. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. Nature 556, 255–258.

 Zhou, H. and Perlman, S. 2007. Mouse hepatitis virus does not induce Beta interferon synthesis and does not inhibit its induction by double-stranded RNA. J. Virol. 81, 568–574.

 Zielecki, F., Weber, M., Eickmann, M., Spiegelberg, L., Zaki, A.M., Matrosovich, M., Becker, S., and Weber, F. 2013. Human cell tropism and innate immune system interactions of human respiratory coronavirus EMC compared to those of severe acute respiratory syndrome coronavirus. J. Virol. 87, 5300–5304.

 Zust, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Baraet, W., Diamond, M.S., et al. 2011. Ribose 2’-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. Nat. Immunol. 12, 137–143.