

## REVIEW

# SARS-CoV-2-mediated evasion strategies for antiviral interferon pathways

Soo-Jin Oh and Ok Sarah Shin\*

BK21 Graduate Program, Department of Biomedical Sciences,  
College of Medicine, Korea University Guro Hospital, Seoul 08308,  
Republic of Korea

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**With global expansion of the COVID-19 pandemic and the emergence of new variants, extensive efforts have been made to develop highly effective antiviral drugs and vaccines against SARS-CoV-2. The interactions of coronaviruses with host antiviral interferon pathways ultimately determine successful viral replication and SARS-CoV-2-induced pathogenesis. Innate immune receptors play an essential role in host defense against SARS-CoV-2 via the induction of IFN production and signaling. Here, we summarize the recent advances in innate immune sensing mechanisms of SARS-CoV-2 and various strategies by which SARS-CoV-2 antagonizes antiviral innate immune signaling pathways, with a particular focus on mechanisms utilized by multiple SARS-CoV-2 proteins to evade interferon induction and signaling in host cell. Understanding the underlying immune evasion mechanisms of SARS-CoV-2 is essential for the improvement of vaccines and therapeutic strategies.**

**Keywords:** SARS-CoV-2, COVID-19, interferon, immunity

## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent behind coronavirus disease 2019 (COVID-19), has become one of the gravest public health challenges of the 21st century (Li *et al.*, 2020a; Zhou *et al.*, 2020). SARS-CoV-2 has rapidly spread across 200 countries worldwide with more than 250 million confirmed cases, including over 5 million deaths as of November 2021, according to World Health Organization (COVID-19) dashboard. COVID-19 patients exhibit broad range of clinical manifestations, ranging from mild symptoms such as headache, dry cough, and flu-like symptoms to severe symptoms such as critical pneumonia, septic shock, multiple organ dysfunction,

and even death (Chen *et al.*, 2020b; Huang *et al.*, 2020; Wu and McGoogan, 2020).

Coronaviridae family of viruses contain non-segmented, positive sense single-stranded RNA genome that encodes proteins for viral replication, translation, and virion assembly (Perlman and Netland, 2009). SARS-CoV-2 genome is approximately 30,000 nucleotides in length. Several studies have revealed that SARS-CoV-2 genome shares 82% and 50% sequence identity with SARS-CoV and MERS-CoV genomes respectively (Krishnamoorthy *et al.*, 2020; Lu *et al.*, 2020; Zhou *et al.*, 2020). The genome of SARS-CoV-2 encodes 4 structural proteins, 16 non-structural proteins (NSPs) and 6 accessory proteins.

Structural proteins consist of spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins that are involved in viral pathogenesis, viral RNA genome replication, and virion assembly (Cui *et al.*, 2019; Zhu *et al.*, 2020). Among the structural proteins, S protein interacts with the host cell membrane receptor angiotensin converting enzyme 2 (ACE2) and is essential for viral entry into the host cells. Subsequently, the host protease TMPRSS2 cleaves S protein/ACE2 receptor complex to facilitate complete viral entry into the host cell (Hoffmann *et al.*, 2020). Finally, SARS-CoV-2 gain access to host replication and translation machinery to duplicate and express their genes and proteins respectively.

NSPs are produced as cleavage products of two polyproteins encoded by the open reading frame (ORF) 1a and ORF1b, which form approximately 70% of the viral genome from the 5' end. NSPs play a key role in viral replication and gene expression through the formation of replication and transcription complexes (RTCs). Viral RTCs consist of RNA-dependent RNA polymerase (translated from NSP12), methyltransferases (translated from NSP14 and NSP16) to edit viral RNA, co-factors (translated from NSP7 and NSP8), and helicase (translated from NSP13) (Hartenian *et al.*, 2020). Additionally, there are several NSPs that exhibit protease and exoribonuclease activities for facilitating viral gene expression and disruption of the host cell signaling pathways, respectively (Perlman and Netland, 2009).

Meanwhile, accessory proteins of SARS-CoV-2 are considered as major virulence factors that counteract the host immune system, regulate cell death, and induce autophagy to provide a site for viral replication. For example, ORF3a can manipulate host responses, modulating autophagy, cell death, and immune response; while another accessory proteins such as ORF6, ORF7, and ORF8, can mitigate type I/III IFN responses or inflammatory responses (Ren *et al.*, 2020; Xia *et*

\*For correspondence. E-mail: oshin@korea.ac.kr; Tel.: +82-2-2626-3280; Fax: +82-2-2626-1962  
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**Table 1. Virological and immunological role of SARS-CoV-2-encoded proteins**

Viral protein	Virological function	Immunological function	Target pathway	Reference
<b>N</b>	Virion structure, RNA packaging	Inhibition of TRIM25-RIG-I and TBK1-IRF3 interaction Disruption of TBK1, IRF3, and STAT1/2 activation Suppression of MAVS oligomerization	RLR activation IFN production	Chen <i>et al.</i> (2020a) Lei <i>et al.</i> (2020) Mu <i>et al.</i> (2020) Oh and Shin (2021)
<b>M</b>	Virion structure	Inhibition of RIG-I-MAVS, MAVS-TBK1, and TRAF3-TBK1 interaction Disruption of IRF3 activation Suppression of MAVS oligomerization	RLR activation IFN production	Lei <i>et al.</i> (2020) Xia <i>et al.</i> (2020) Zheng <i>et al.</i> (2020) Fu <i>et al.</i> (2021)
<b>NSP1</b>	Non-structural protein	Cleavage of host mRNAs Inhibition of host protein translation and IFN response	IFN activation production	Banerjee <i>et al.</i> (2020) Schubert <i>et al.</i> (2020) Thoms <i>et al.</i> (2020) Vazquez <i>et al.</i> (2021)
<b>NSP3</b>	PLpro	Inhibition of MDA5 activation Proteolysis of IRF3 de-ISGylation and de-ubiquitination of ISG15	RLR activation IFN production	Klemm <i>et al.</i> (2020) Shin <i>et al.</i> (2020) Liu <i>et al.</i> (2021) Moustaqil <i>et al.</i> (2021)
<b>NSP5</b>	3CLpro	Cleavage of NLRP12 and TAB1 Disruption of IRF3 nuclear translocation	IFN activation & production Inflammation	Moustaqil <i>et al.</i> (2021)
<b>NSP6</b>	DMV formation	Restricts host autophagosome expansion Disruption of IRF3 and STAT1/2 activation	IFN activation & production	Xia <i>et al.</i> (2020)
<b>NSP7/8</b>	RNA primase	Suppression of K63-ubiquitination of MDA5 Attenuation of host translational system	RLR activation IFN activation & production	Banerjee <i>et al.</i> (2020) Yang <i>et al.</i> (2020)
<b>NSP12</b>	RdRp	Disruption of IRF3 and STAT1 activation	IFN production signaling	Wang <i>et al.</i> (2021b)
<b>NSP13</b>	NTPase, Helicase	Disruption of TBK1, IRF3, and STAT1/2 activation Interference with USP13-mediated ubiquitination	IFN production & signaling	Xia <i>et al.</i> (2020) Yuen <i>et al.</i> (2020) Guo <i>et al.</i> (2021) Vazquez <i>et al.</i> (2021)
<b>NSP14</b>	ExoN, Guanine-N7-MTase	Prevention of RIG-I mediated signaling and IRF3 activation Suppression of host translational system Downregulation of ISG15 and IFNAR1 expression	IFN activation & production ISG expression	Yuen <i>et al.</i> (2020) Hayn <i>et al.</i> (2021) Hsu <i>et al.</i> (2021)
<b>NSP15</b>	NendoU	Disruption of IRF3 activation	IFN activation	Yuen <i>et al.</i> (2020)
<b>NSP16</b>	2'-O-MTase, Viral mRNA capping	Prevention of MDA5-mediated signaling and IRF3 activation Inhibition of mRNA splicing system Downregulation of ISG expression	IFN production ISG expression	Banerjee <i>et al.</i> (2020)
<b>ORF3b</b>	Accessory protein	Disruption of IRF3 activation	IFN activation & production	Konno <i>et al.</i> (2020)
<b>ORF6</b>	Accessory protein	Disruption of IRF3/7 and STAT1/2 Interruption of nucleocytoplasmic transport system Downregulation of <i>IFNAR1</i> expression	IFN production & signaling ISG expression	Li <i>et al.</i> (2020b) Miorin <i>et al.</i> (2020) Addetia <i>et al.</i> (2021) Kato <i>et al.</i> (2021)
<b>ORF7a</b>	Accessory protein	Inhibition of STAT1 phosphorylation	IFN signaling	Xia <i>et al.</i> (2020)
<b>ORF7b</b>	Accessory protein	Disruption of STAT1 and STAT2 phosphorylation	IFN signaling	Xia <i>et al.</i> (2020)
<b>ORF8</b>	Accessory protein	Disruption of IRF3 activation Induction of cytokine storm	IFN activation Inflammation	Li <i>et al.</i> (2020b) Lin <i>et al.</i> (2021) Rashid <i>et al.</i> (2021)
<b>ORF9b</b>	Accessory protein	Inhibition of MAVS/TRAF3/TRAF6 complex	RLR activation IFN activation production	Han <i>et al.</i> (2021) Wu <i>et al.</i> (2021)

Pro, protease; DMV, double membrane vesicle; RLRs, RIG-I-like receptors; RdRp, RNA-dependent RNA polymerase; NTPase, Nucleoside-triphosphatase; ExoN, Exonuclease; MTase, Methyltransferase; NendoU, Nidoviral uridylylate-specific endoribonuclease; IFN, interferon;

*al.*, 2020; Miao *et al.*, 2021).

In this review, we highlight the recently described role of antiviral interferon (IFN) pathways in limiting SARS-CoV-2 infection, as well as the molecular mechanisms by which the virus evades the IFN response. We will also comprehensively review recent work showing the modulation of the IFN pathway by SARS-CoV-2-encoded proteins (Table 1). A detailed understanding of the mechanisms by which SARS-CoV-2 suppresses host antiviral pathways may provide novel

avenues for the development of antiviral drugs against SARS-CoV-2.

### SARS-CoV-2 Recognition by RIG-I-like Receptors (RLRs) and Toll-like receptors (TLRs)

To defend against SARS-CoV-2, innate immune responses are activated to produce antiviral factors, especially type I

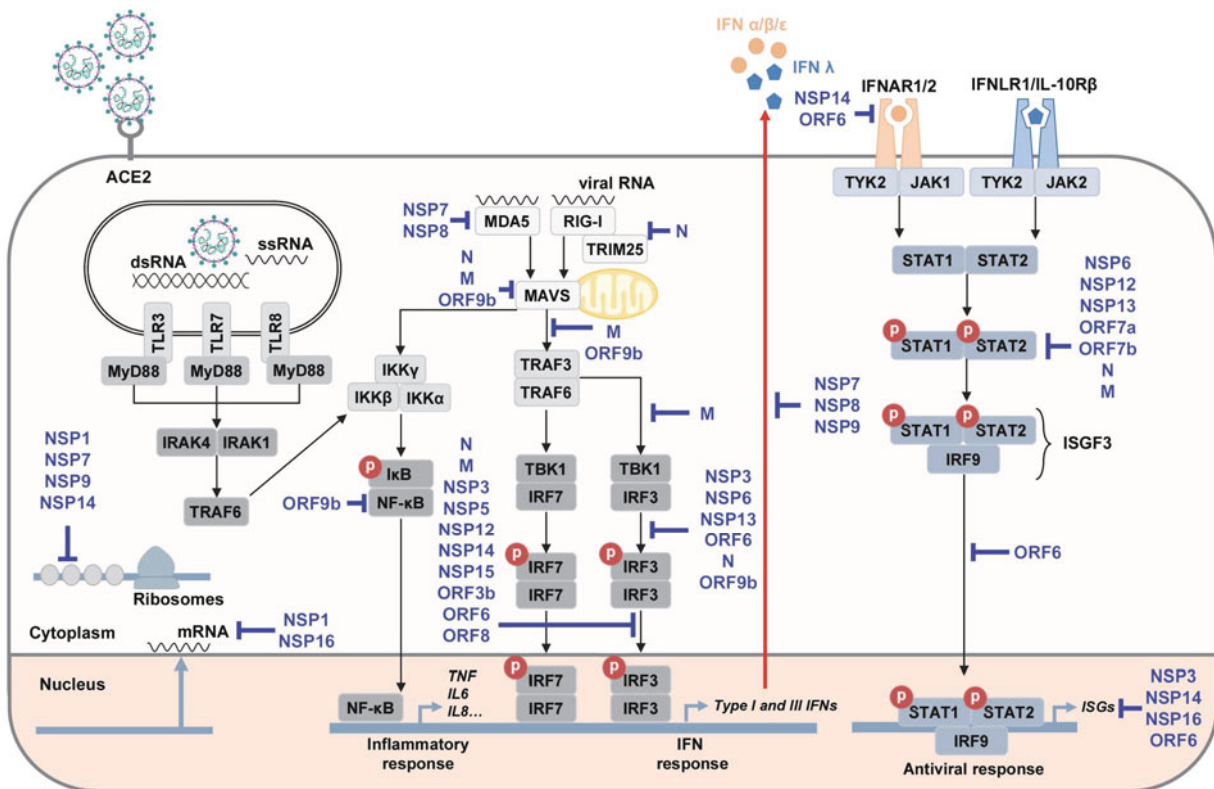
and III IFN, which are rapidly produced by immune or epithelial cells. For IFN production, pattern recognition receptor (PRR) signaling pathways are activated by conserved pathogen-associated molecular patterns (PAMPs) that initiate a cascade of innate immune responses (Fitzgerald and Kagan, 2020). Among PRR signaling pathways, RLR and TLR pathways are highly important for the recognition of viral components and further lead to antiviral immune response, as depicted in Fig. 1.

RLRs, such as retinoic acid inducible gene-I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), are typically expressed in most cell types and sense viral RNA to elicit IFN response (Rehwinkel and Gack, 2020). While RIG-I recognizes viral RNA with 5'-triphosphate or short dsRNA sequences, MDA5 senses long dsRNA with no end specificity (Reikine *et al.*, 2014). Once the ATP-dependent DExD/H box RNA helicase domain of RLR proteins binds to viral RNA, these sensors interact with the N-terminal caspase activation and recruitment domains (CARDs) of mitochondrial antiviral signaling protein (MAVS) and coordinates downstream signaling pathways by recruiting ubiquitin ligases, such as TNF receptor associated factors (TRAFs), TANK-binding kinase 1 (TBK1), IKK- $\epsilon$  and IKK- $\alpha/\beta/\gamma$  complex. As a consequence, several transcriptional factors such as IRF3, IRF7 and NF- $\kappa$ B get phosphorylated and translocated to nucleus, thereby triggering the expression of IFNs or pro-

inflammatory cytokines. IFNs are produced and released in both autocrine and paracrine ways and promote hundreds of interferon stimulated genes (ISGs), which are capable of interfering with every steps of viral replication (Schneider *et al.*, 2014).

Multiple studies reported that the RLR pathway is activated during SARS-CoV-2 infection for anti-viral responses *in vitro* (Kouwaki *et al.*, 2021; Sampaio *et al.*, 2021; Thorne *et al.*, 2021; Wu *et al.*, 2021; Yamada *et al.*, 2021; Yin *et al.*, 2021). For example, Thorne *et al.* (2021) have reported that RNA sensing of SARS-CoV-2 in lung epithelium can contribute to robust macrophage activation, cytokine production and tissue inflammation. Meanwhile, both Yin and Sampaio *et al.* reported that SARS-CoV-2 is sensed by MDA5, NOD1, and LGP2, but not RIG-I and MAVS; while IRF3, IRF5, and NF- $\kappa$ B/p65 act as the major mediators of IFN response induction (Sampaio *et al.*, 2021; Yin *et al.*, 2021).

In addition to RLRs, TLRs are also considered critical components of host defense against SARS-CoV-2. TLRs comprise of ten members in human and are expressed in the cell membrane or endosomes. Each TLR recognizes different PAMPs, including lipopolysaccharide (recognized by TLR4), peptidoglycan (recognized by TLR2), flagellin (recognized by TLR5), and CpG DNA (recognized by TLR9). The TLR pathway involves activation of downstream adaptor molecules and divides into MyD88- and TRIF-dependent pathways (Fitz-



**Fig. 1.** SARS-CoV-2 proteins antagonize multiple steps of antiviral response. The multiple host factors in the antiviral signaling cascade are targeted by SARS-CoV-2 proteins. During SARS-CoV-2 infection, viral recognition by host innate immune sensors such as RLRs and TLRs triggers an antiviral signaling cascade to elicit the production of type I or type III IFN as well as proinflammatory cytokines. To combat against host defense mechanisms, SARS-CoV-2 have evolved multiple strategies to avoid host recognition by interfering with the function of antiviral proteins using various viral proteins. Indicated in blue are sites at which the SARS-CoV-2 interferes with host innate immune sensing pathways, as explained in the text.

gerald and Kagan, 2020). All TLRs except TLR3 use MyD88-dependent pathway, whereas TLR3 as well as TLR4 induces TRIF-dependent pathway, which activates TBK1 and IKK- $\epsilon$  to phosphorylate IRF3 or IRF7 to propagate IFN responses. Among TLRs, TLR3 and TLR7/TLR8 heterodimer can recognize dsRNA or ssRNA from viruses, respectively and therefore, is likely to be emphasized in the restriction of SARS-CoV-2.

So far, several TLRs have been shown to be involved in pathogenesis of SARS-CoV-2 infection. As an example, Zheng *et al.* (2021) has indicated that TLR2 is important for SARS-CoV-2 recognition. E protein of SARS-CoV-2 is able to stimulate TLR2/MyD88 signaling pathway and blocking TLR2 signaling *in vivo* resulted in protection against SARS-CoV-2 infection. Additionally, TLR4 was shown to directly interact with the S protein of SARS-CoV-2 (Zhao *et al.*, 2021a) and the S1 domain of the S protein was shown to activate NF- $\kappa$ B pathway, which is dependent on TLR4 in macrophages (Shirato and Kizaki, 2021). Given that TLR3 is essential for protection against SARS-CoV by elevating the production of pro-inflammatory cytokines and IFNs in mice (Totura *et al.*, 2015), it is highly likely that TLR3 may contribute to protection against SARS-CoV-2 infection (Iwasaki and Yang, 2020). In fact, SARS-CoV-2 infection increased TLR3-mediated NF- $\kappa$ B signaling pathway and TLR3 antagonist, famotidine, which is an approved drug for peptic ulcers and gastroesophageal reflux disease, mitigates SARS-CoV-2-induced inflammation (Mukherjee *et al.*, 2021). Lastly, TLR7/8 was also demonstrated in SARS-CoV-2 ssRNA recognition, subsequently leading to dendritic cell activation and T cell inflammatory response (Salvi *et al.*, 2021).

### The Role of IFNs in SARS-CoV-2 Infection

IFNs are critical components of host defense against viruses. IFNs are secreted from virus-infected cells, bind to IFN receptors on bystander cells in an autocrine or paracrine manner and strongly induce ISGs for an antiviral response (Park and Iwasaki, 2020). Type I (IFN- $\alpha$ , IFN- $\beta$ , and IFN- $\epsilon$ ) and III (IFN- $\lambda$ ) IFNs can activate receptor associated tyrosine kinase TYK2 and JAK1, subsequently phosphorylating signal transducer and activator of transcription proteins (STATs), such as STAT1 and STAT2. Together with IRF9, STAT1, STAT2 form IFN-stimulated gene factor 3 (ISGF3) complex, which translocate to the nucleus upon phosphorylation and activate the interferon stimulatory response element (ISRE) promoter to express ISGs. So far, there are hundreds of ISGs discovered, and many ISGs exert antiviral functions by inhibiting viral gene expression and eliminating viral components.

The role of type I and III IFN response in COVID-19 has been examined by several groups. First, multiple groups have reported that SARS-CoV-2 infection is sensitive to IFN pretreatment or production *in vitro* (Miorin *et al.*, 2020; Hayn *et al.*, 2021; Yin *et al.*, 2021). Moreover, the dysregulated type I and III IFN response strongly contributes to the pathogenesis of COVID-19. Zhang *et al.* (2020) have revealed that patients with genetic mutations in TLR pathways, including TLR3 and IRF7, show severe symptoms including death, as these mutations hamper type I IFN responses. The

same group also reported that autoantibodies against type I IFNs, including IFN- $\omega$  and multiple subsets of IFN- $\alpha$ , were produced in 10% of patients with critical symptoms, in contrast to 0.3% of patients with mild symptoms. Further, the patients who produce autoantibodies also show a lower level of IFN- $\alpha$  production, and the autoantibodies against IFNs can neutralize type I IFN response in such patients (Bastard *et al.*, 2020). While it is certain that IFNs play important role in anti-viral responses, they have also been reported to enhance the expression of ACE2, thereby possibly contributing to the higher infectivity of SARS-CoV-2 (Busnadiago *et al.*, 2020; Ziegler *et al.*, 2020). Despite the contradictory roles of IFNs in SARS-CoV-2 infection, there is no doubt that interventions with IFN responses can play a significant role in restricting SARS-CoV-2 infection and replication.

Multiple groups have reported that a distinct immune phenotype is observed in COVID-19 patients, wherein COVID-19 patients with severe symptoms exhibit defective innate and adaptive immune responses, including hypercytokinemia, T cell loss, dysfunctional NK cells, and shortened memory T cell responses (Yang *et al.*, 2021). In particular, significant imbalances in the secretion of cytokines and chemokines have been detected in patients with severe COVID-19 as compared to that in patients with mild symptoms. Although impaired production of type I IFNs have been suggested to be responsible for detrimental outcomes of COVID-19 (Acharya *et al.*, 2020; Blanco-Melo *et al.*, 2020; Hadjadj *et al.*, 2020), robust IFN responses were detected in severe COVID-19 patients along with elevated production of pro-inflammatory cytokines and chemokines, including TNF- $\alpha$ , IL-6, and IL-1 $\beta$  (Lee *et al.*, 2020). Given that cytokine storm can be worsened by delayed IFN production, the use of IFN in clinical settings should be carefully evaluated; and further studies on the detailed mechanisms of innate immune sensing and the role of IFNs in SARS-CoV-2 infected cells are essential.

### IFN Antagonisms by SARS-CoV-2 Structural Proteins

#### Nucleocapsid (N) protein

N protein encoded by coronaviruses has a function in the packaging of RNA into structural proteins and is involved in viral replication and release. Similar to SARS-CoV (Lu *et al.*, 2011; Hu *et al.*, 2017), multiple groups, including ours, have shown that SARS-CoV-2 N protein can interfere with RLR pathways and thus antagonize IFN production and signaling (Chen *et al.*, 2020a; Lei *et al.*, 2020; Mu *et al.*, 2020; Oh and Shin, 2021). In detail, SARS-CoV-2 N inhibits TRIM25-RIG-I interaction, TBK1-IRF3 interaction and represses the phosphorylation of TBK1 and IRF3, and nuclear translocation of IRF3 induced by poly I:C or RNA viruses (Chen *et al.*, 2020a; Gori Savellini *et al.*, 2021; Oh and Shin, 2021). Another proposed mechanism of IFN antagonism by N protein targets K63-linked polyubiquitination and oligomerization of MAVS (Wang *et al.*, 2021a; Zotta *et al.*, 2021). The dimerization domain of the N protein, which forms small droplets to isolate viral RNA, inhibits MAVS oligomerization and further leads to delayed type I IFN response both *in vivo* and *in vitro*. Interestingly, a dual role of the N pro-

tein in the regulation of IFN response was recently proposed (Zhao *et al.*, 2021b). A low dose of N protein has the function of IFN antagonism; however, a high dose of N protein induces robust type I IFN signaling and inflammation. Along with interfering RLR pathways, the inhibition of JAK/STAT pathway by N protein also has been reported (Mu *et al.*, 2020). N proteins can inhibit phosphorylation of STAT1 and 2 stimulated by IFN- $\beta$  treatment by directly interacting with STAT1 and STAT2. Given that emerging variants of SARS-CoV-2 contain genetic mutations in N proteins (Toyoshima *et al.*, 2020), it will be important to characterize the effect of N mutations on IFN responses.

### Membrane (M) protein

M protein is the most abundant structural protein expressed in the envelope of coronaviruses. SARS-CoV-2 M protein can localize in the endoplasmic reticulum (ER) and Golgi and possess the first transmembrane domain (TM1) at the N-terminus to localize in the Golgi (Zheng *et al.*, 2020). SARS-CoV-2 M protein suppresses type I/III IFN response induced by poly I:C or RNA virus infection and interacts with RIG-I, MDA5, MAVS, TRAF3, and TBK1. M protein inhibits RIG-I-MAVS, MAVS-TBK1, TRAF3-TBK1 interaction, and finally, downregulates the phosphorylation and nuclear translocation of IRF3. Meanwhile, TM1-lacking M protein no longer interacts with RIG-I, but still interacts with MAVS and TBK1 (Lei *et al.*, 2020; Xia *et al.*, 2020; Zheng *et al.*, 2020). More recently, one group further reported IFN-antagonizing properties of M protein by inhibition of MAVS aggregation (Fu *et al.*, 2021). Taken together, M protein serves as IFN antagonist via interaction with RLR molecules and inhibits the oligomerization of MAVS, which in turn suppresses the phosphorylation and nuclear translocation of IRF3 and blocks IFN signaling.

## IFN Antagonism by SARS-CoV-2 NSPs

### NSP1

NSP1 is an IFN antagonist (Lei *et al.*, 2020; Xia *et al.*, 2020), which hampers IFN responses, cleaves host mRNAs and suppress protein translation (Banerjee *et al.*, 2020; Schubert *et al.*, 2020; Thoms *et al.*, 2020; Vazquez *et al.*, 2021). In detail, NSP1 downregulates mRNA translation in the host cells by interacting with the 18S ribosomal RNA and 40S ribosomal subunit *in vivo* and *in vitro*. In addition, blocking the host translation system subsequently reduces RIG-I and ISG15 protein expression, resulting in decreased ISRE promoter activity (Thoms *et al.*, 2020). Given that NSP1 interacts with nuclear export receptor NXF1 and shutdowns poly (A) RNA export system, NSP1 offers potential uncovered strategies for interrupting the host translational system to inhibit the IFN response (Zhang *et al.*, 2021b).

### NSP3

NSP3 is a papain-like protease, which contains multiple domains necessary for viral replication, gene transcription, and protein translation. As an immune evasion strategy, NSP3 physically interacts with IRF3 and inhibits IRF3 phospho-

rylation, dimerization, and translocation (Klemm *et al.*, 2020; Shin *et al.*, 2020). In addition to targeting IRF3 activation, NSP3 induces de-ISGylation and de-ubiquitinylation of ISG15, which subsequently attenuates ISG15-dependent activation of MDA5 (Shin *et al.*, 2020; Liu *et al.*, 2021). Similar to other viral proteins with protease activity, which induce cleavage or degradation of host proteins associated with antiviral signaling to evade the host immune response, the papain-like protease activity of NSP3 also shows the proteolysis of IRF3 (Moustaqil *et al.*, 2021). Considering that the NSP3 acts to inhibit host innate immunity via protease activity, it will be essential to develop protease inhibitors particularly targeting NSP3.

### NSP5

Similar to NSP3, NSP5 is a protease, which contributes to viral replication via processing viral polyproteins through 3C-like protease (3CLpro) activity. NSP5 was detected to cleave nucleotide-binding leucine-rich domain containing receptors, NLRP12 and TAB1, which are involved in the inflammatory response (Moustaqil *et al.*, 2021). Along with the downregulation of inflammation, NSP5 also inhibits the nuclear translocation of IRF3 without affecting the phosphorylation of IRF3, resulting in decreased IFN- $\beta$  luciferase activity (Fung *et al.*, 2021).

### NSP6

Previous studies have revealed that SARS-CoV NSP6 can generate ER-derived autophagosomes by forming host double-membrane vesicles (DMV) and facilitating viral replication and translation (Cottam *et al.*, 2011). In regard to this, it will be interesting to see if SARS-CoV-2 NSP6 can also sequester the autophagy pathway to inhibit the formation of the autolysosome. Meanwhile, NSP6 interferes with the host immune response by interacting with TBK1 to inhibit the phosphorylation and nuclear translocation of IRF3 (Xia *et al.*, 2020). In particular, NSP6 inhibits not only the RLR signaling but also the JAK/STAT pathway. Overexpression of NSP6 in cells show lower activation and cytoplasmic localization of STAT1 and STAT2 during IFN- $\alpha$  treatment. Furthermore, NSP6 has also been shown to significantly inhibit STAT1 and STAT2 as compared to that of other coronaviruses such as SARS-CoV, and MERS-CoV (Xia *et al.*, 2020).

### NSP7/8

NSP7 forms heterodimers with NSP8 and plays a role in facilitating the RNA-dependent RNA polymerase encoded by NSP12 (Kasuga *et al.*, 2021). NSP8 directly interacts with MDA5 via the CARD domain and inhibits MDA5-mediated ISRE luciferase activity by suppressing K63-linked poly-ubiquitination of MDA5 (Yang *et al.*, 2020). Intriguingly, NSP7 can interfere with NSP1; which is associated with host translational machinery. Furthermore, NSP8 and NSP9 bind to the signal recognition particle complex, 7SL RNA, to inhibit the host protein trafficking system from secreting proteins (Banerjee *et al.*, 2020). These findings imply that NSP8 and NSP9 can inhibit several cytokines or chemokines that are involved in the innate immune response, including type I/III IFN or pro-inflammatory responses.

### NSP12

NSP12 is an RNA-dependent RNA polymerase that requires the NSP7-NSP9 heterodimer as a processivity factor responsible for replication and methylation. NSP12 has been shown to dampen the RIG-I-, MDA5-, MAVS-, and IRF3-5D-mediated IFN- $\beta$  and ISRE luciferase activity (Wang *et al.*, 2021b). In addition, NSP12 inhibits Sendai virus-induced phosphorylation of STAT1. On the other hand, Li *et al.* (2021) reported that NSP12 does not affect IRF3 phosphorylation, although it does prevent IRF3 nuclear translocation and inhibit the JAK/STAT pathway to evade the host antiviral response.

### NSP13

NSP13 possesses NTPase and helicase activity and was first suggested as a potent IFN antagonist by Yuen *et al.* (2020). The mechanisms of IFN suppression by NSP13 have been reported by several groups. In particular, NSP13 has been shown to inhibit TBK1 and IRF3 phosphorylation by interacting with TBK1 and interfere with the JAK/STAT pathway (Xia *et al.*, 2020; Vazquez *et al.*, 2021). Furthermore, Guo *et al.* (2021) have reported that NSP13 also interferes with the host deubiquitinase USP13 to stabilize itself and attenuate host IFN responses.

### NSP14

NSP14 possess exonuclease and methyltransferase activity for viral gene modification. In addition to being a viral replisome, NSP14 is another inhibitor of IRF3 nuclear translocation that disrupts RIG-I-mediated IFN- $\beta$  production but not IFN- $\beta$ -induced ISRE promoter activities (Yuen *et al.*, 2020). NSP14 interferes with the host translational system as well as the RLR pathway to manipulate the host immune system, similar to NSP1. Also, NSP14 associates with NSP10 to block the host translational machinery to attenuate ISG expression in NSP14-expressing VeroE6 cells. Thus, the catalytic activity of NSP14 contributes to the inhibition of host protein translation (Hsu *et al.*, 2021). Together with the manipulation of the translational system, NSP14 can repress IFNAR1 protein expression by lysosomal degradation (Hayn *et al.*, 2021).

### NSP15

NSP15 is a nidoviral uridylyate-specific endoribonuclease that processes viral RNA in its mature form. Yuen *et al.* (2020) have demonstrated NSP15's ability to antagonize IFN production by inhibition of IRF3 translocation, but the specific mechanisms by which NSP15 mediates IFN inhibition remain to be fully examined.

### NSP16

Similar to NSP13, 14, and 15, NSP16 is an RNA-modifying enzyme that methylates the 2'-O position to modify the viral genome in association with NSP10. NSP16 is involved in interrupting mRNA splicing mechanisms. NSP16 has been shown to bind with U1 and U2, which are components of the spliceosome, to suppress host mRNA splicing and thereby result in decreased ISG expression (Banerjee *et al.*, 2020).

## IFN Antagonisms by SARS-CoV-2 Accessory Proteins

### ORF3

ORF3a was first identified as a pro-apoptotic factor (Ren *et al.*, 2020), whereas ORF3b robustly reduces IFN- $\beta$  promoter activity to inhibit type I IFN response by inhibiting IRF3 translocation during Sendai virus infection (Lei *et al.*, 2020) and the antiviral effect is influenced by C-terminal length of the protein (Konno *et al.*, 2020). Moreover, the patients infected the SARS-CoV-2 strains with ORF3b variants developed more severe symptoms as compared to the other patients infected with the originally identified SARS-CoV-2. These findings thus implicate the IFN-suppressive activity of ORF3b as an important virulence factor in the pathogenesis of SARS-CoV-2 (Konno *et al.*, 2020).

### ORF6

SARS-CoV-2 ORF6 is known to antagonize IFN responses (Yuen *et al.*, 2020; Kimura *et al.*, 2021), whereas SARS-CoV ORF6 was shown to disrupt the nuclear translocation of IRF3 and STAT1 (Frieman *et al.*, 2007; Kopecky-Bromberg *et al.*, 2007). Consistent with this, SARS-CoV-2 ORF6 can also antagonize IFN responses by disrupting the nucleocytoplasmic transport system (Miorin *et al.*, 2020; Addetia *et al.*, 2021; Kato *et al.*, 2021). Specifically, Miorin *et al.* (2020) have reported that SARS-CoV-2-infected VeroE6 cells show the localization of STAT1 and STAT2 in the cytoplasm, not in the nucleus, as a result of ORF6-NUP98-RAE1 complex formation and especially the ORF6-NUP98 interaction. Similarly, the association of ORF6, NUP98, and RAE1 has been shown to disrupt the nuclear translocation of IRF3 (Kimura *et al.*, 2021) and the association strength of the ORF6-NUP98-RAE1 complex is stronger than that of SARS-CoV-1 ORF6 (Addetia *et al.*, 2021). Furthermore, Kato *et al.* (2021) have reported that SARS-CoV-2 ORF6 induces mislocalization of RAE1. Together with the interference from NUP98-RAE1 complex, ORF6 also binds with karyopherin  $\alpha 2$ , which is an important factor for the nuclear localization of IRF3, IRF7, and STAT1, to block the nuclear translocation of IRF3 (Xia *et al.*, 2020). Furthermore, ORF6 has the potential to disrupt the JAK/STAT pathway as ORF6 represses the expression of *IFNAR1* (Hayn *et al.*, 2021). Taken together, the studies suggest that ORF6 exploits IFN pathways via inhibition of IRF3 and STAT nuclear translocation.

### ORF7

ORF7 is divided into ORF7a and ORF7b depending on translation initiation sites; both ORFs can abrogate the JAK/STAT pathway to evade the host immune response (Xia *et al.*, 2020). Both ORF7a and ORF7b have been shown to strongly inhibit ISRE luciferase activity induced by recombinant IFN- $\alpha$  treatment as compared to unstimulated conditions. While ORF7a has been shown to inhibit STAT1 phosphorylation, ORF7b has been demonstrated to inhibit both STAT1 and STAT2 (Cao *et al.*, 2021).

## ORF8

SARS-CoV-2 ORF8 is an accessory protein that encodes ER import sequences to facilitate ER localization and shows very low sequence identity as compared to that of SARS-CoV. The function of IFN antagonism by ORF8 was first proposed by Li *et al.* (2020b) suggesting that ORF8 attenuates the promoter activities of IFN- $\beta$ , ISRE, and NF- $\kappa$ B and the expression of ISGs. Furthermore, ORF8 inhibits the nuclear translocation of IRF3 to suppress the *IFN- $\beta$*  gene expression (Rashid *et al.*, 2021). Another group, Zhang *et al.* (2021a), reported that ORF8 is capable of modulating adaptive immune response by autophagy-mediated MHC-I degradation and downregulation, however, the detailed mechanisms by which ORF8 hijacks IFN responses have not been fully elucidated. Given that ORF8 of SARS-CoV-2 has been proposed to play a role in ER stress response, unfolded protein response (UPR), and autophagy process by interacting host proteins involved in these pathways, further studies on the immunological effect of ORF8 will be necessary (Gordon *et al.*, 2020).

## ORF9b

ORF9b is expressed rapidly during SARS-CoV-2 infection and strongly co-localizes with mitochondria as well as with ER and Golgi in ORF9b-transfected HeLa cells (Han *et al.*, 2021). In SARS-CoV, ORF9b attenuates type I IFN response by targeting MAVS/TRAF3/TRAF6 complex that are expressed on the mitochondria (Shi *et al.*, 2014). Similarly, SARS-CoV-2-ORF9b also targets the mitochondrial protein, TOM70, to repress the antiviral signaling (Jiang *et al.*, 2020). Wu *et al.* (2021) reported that ORF9b attenuates both type I IFN and inflammatory response by targeting RIG-I-MAVS signaling pathways. The proposed mechanisms of IFN antagonism by ORF9b involve the suppression of NF- $\kappa$ B activation and K63-linked polyubiquitination of NEMO. Overexpression of ORF9b suppresses DNA sensing pathways by interacting with RIG-I, MDA5, MAVS, TBK1, STING, and TRIF (Han *et al.*, 2021). As ORF9b is expressed rapidly during SARS-CoV-2 infection, IFN antagonism of ORF9b is attributed to be one of the causes of delayed immune responses in COVID-19 patients.

## Concluding Remarks

Viral evasion of host innate immunity is necessary for successful virus replication and thus, it is essential to characterize mechanical insights into how SARS-CoV-2 antagonizes antiviral interferon response. What makes SARS-CoV-2 highly pathogenic and deadly in certain population? It is very likely that SARS-CoV-2's ability to circumvent and escape antiviral immunity may contribute to failure of appropriate and timely activation of host antiviral defense mechanisms and instead lead to successful viral replication. Extensive studies in the past two years have provided insights into how each viral factors of SARS-CoV-2 may avoid, subvert or interfere with antiviral innate immune signaling pathways. However, many questions still remained unanswered, including which genes are required for transmission to a new host despite the robust immune response; Are there a functional redundancy

for multiple virulent factors? Additionally, are there tissue- or cell type specificity of how these virulence factors may work? Are there functions of viral factors modulated by its genetic context and is there any evidence for cooperativity? To date, although we have accumulating evidences to show each viral factor's function and structure by ectopic expression, further studies involving the construction of virus mutants and *in vivo* functional analysis of viral factors in terms of transmission and pathogenesis are yet to begin. Functional analysis of viral factors in the context of virus infection *in vitro* and *in vivo* should be further investigated to understand better about the complex interaction between viruses and host.

Despite massive efforts to control the COVID-19 pandemic, we are still facing multiple challenges concerning the long-term efficacy of vaccines as well as their effectiveness against present and future variants. Therefore, it is essential to have a better understanding of the interplay between the immune system and the virus in addition to the cells or molecules that drive disease pathology to develop proper therapeutic interventions. As millions of people will be suffering from long-term effects of COVID-19 even after the pandemic is controlled by vaccination, we must continue to improve our understanding of the underlying pathogenesis of SARS-CoV-2 to better equip ourselves against such threats in the future.

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## Conflict of Interest

The authors state no conflicts of interest.

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