



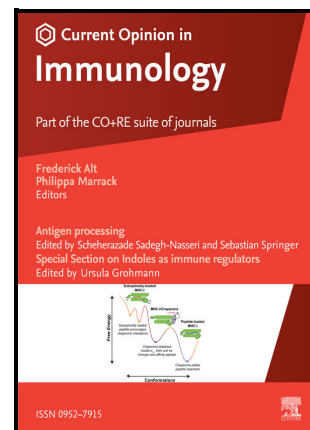
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Insights into pandemic respiratory viruses: manipulation of the antiviral interferon response by SARS-CoV-2 and influenza A virus  
Short title: “IFN antagonism by SARS-CoV-2 and influenza A virus”

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# **Insights into pandemic respiratory viruses: manipulation of the antiviral interferon response by SARS-CoV-2 and influenza A virus**

short title "IFN antagonism by SARS-CoV-2 and influenza A virus".

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## **Abstract**

The outbreak of the COVID-19 pandemic one year after the centennial of the 1918 influenza pandemic reaffirms the catastrophic impact respiratory viruses can have on global health and economy. A key feature of SARS-CoV-2 and influenza A viruses is their remarkable ability to suppress or dysregulate human immune responses. Here, we summarize the growing knowledge about the interplay of SARS-CoV-2 and antiviral innate immunity, with an emphasis on the regulation of type I or III interferon responses that are critically implicated in COVID-19 pathogenesis. Furthermore, we draw parallels to influenza A virus infection and discuss shared innate immune sensing mechanisms and the respective viral countermeasures.

## **Introduction**

Respiratory viruses such as coronaviruses and influenza A viruses (IAV) can cause significant morbidity and mortality in the human population, as evidenced by the ongoing COVID-19 pandemic and the past influenza pandemics in the 20<sup>th</sup> century and in 2009. Early clinical symptoms of upper respiratory infections by SARS-CoV-2 or IAV are mostly indistinguishable, and both viruses can gain access to the lower respiratory tract causing pneumonia and severe respiratory distress [1].

A leading cause of COVID-19 fatality is the cytokine storm syndrome (CSS), which is characterized by the excessive induction of proinflammatory cytokines such as IL-6 and IL-1 $\beta$ . CSS induced by severe influenza infection shares similar immunopathology, though the inflammatory

signatures can vary due to distinct cell tropism and viral virulence factors [2]. Important for the initiation of inflammatory responses is the activation of pattern-recognition receptors (PRRs), which are molecular sentinels at the front line of host defense that detect and respond to invading pathogens. Recognition of pathogen-associated molecular patterns (PAMPs) and/or damage-associated molecular patterns (DAMPs) by innate immune sensors initiates cascades of signaling events that 1) transcriptionally upregulate cytokines and other antiviral factors and 2) can prompt direct antiviral mechanisms such as cell death. Type I and III interferons (IFNs) are important cytokines in antiviral defenses to respiratory viruses (and also other viruses). Key functions of type I and III IFNs are the priming of an antiviral state by inducing IFN-stimulated gene (ISG) expression. Additionally, IFNs also instruct innate and adaptive immune cell functions. Given the essential role of type I and III IFNs in antiviral innate immune responses, nearly all viruses, and in particular successful pandemic viruses such as SARS-CoV-2 and IAV, have evolved effective strategies to thwart or delay induction of IFNs or to antagonize their downstream antiviral functions. In this review, we summarize the recent advances on IFN induction and dysregulation during SARS-CoV-2 infection, and also compare these mechanisms with those during IAV infection. Understanding the common and distinct strategies used by SARS-CoV-2 and IAV to manipulate human IFN responses may provide insights into new antiviral strategies for future pandemics.

### **IFN dysregulation and its role in COVID-19 pathogenesis**

The important role of type I/III IFNs in COVID-19 pathogenesis has been recognized at the onset of the pandemic. Early studies indicated that SARS-CoV-2 infection impaired IFN production but induced an overzealous proinflammatory response in vitro and in patient peripheral blood, which likely accounted for the CSS observed in severe disease [3-5]. Longitudinal and single-cell transcriptomics studies however found elevated and sustained expression of type I/III IFNs and/or ISGs in bronchoalveolar lavage fluid or peripheral blood in severe COVID-19 compared to mild cases [6-9]. Interestingly, whereas patients with IAV infection mount robust type I/III IFN and proinflammatory responses from illness onset on and regardless of disease severity, COVID-19 patients did not produce

IFNs until days 7-10 after symptom onset [10]. This delayed IFN production combined with prolonged inflammation distinguishes COVID-19 from other known respiratory viral infections and likely underlies the unique pathogenicity of SARS-CoV-2 [7,10].

The identification of neutralizing autoantibodies against type I IFNs in a proportion of critically ill COVID-19 patients confirms the importance of IFN-mediated immunity in SARS-CoV-2 pathogenesis [11]. The presence of preexisting autoantibodies does not appear to predispose these patients to severe viral infections other than SARS-CoV-2, indicating a key role for an effective early type I IFN response in influencing COVID-19 progression. In accord, the IFN/ISG levels in the nasal mucosa and blood during early SARS-CoV-2 infection coincided with viral loads in mild illness, but not with those in critically ill patients harboring anti-IFN autoantibodies [11].

Mounting evidence shows efficient early SARS-CoV-2 replication in the nasopharynx despite induction of type I/III IFNs and/or ISGs [12,13]. The inability of the initial wave of antiviral IFN responses to efficiently restrict SARS-CoV-2 has also been corroborated in studies using human airway and intestinal epithelial cell cultures and organoids [13-15], which showed that robust viral replication precedes peaks of IFN and ISG induction. This delayed antiviral IFN response can drive immunopathology via pulmonary recruitment and activation of proinflammatory immune cells and lung injury [9,16]. This phenomenon has also been observed in mouse models of SARS-CoV, MERS-CoV, and IAV infection [17]. On the other hand, early IFN/ISG responses elicited by co-infection with rhinovirus or IAV can substantially block SARS-CoV-2 replication [13,18], indicating that delaying IFN responses represents a major strategy of immune evasion by SARS-CoV-2.

### **Innate immune sensing of SARS-CoV-2 and IAV**

Various families of PRRs mediate the detection of respiratory viral infections and the ensuing induction of type I/III IFNs and proinflammatory cytokines. Once activated by viral or host-derived ligands, these sensors trigger signaling through distinct adaptor proteins and a set of common transcription factors, such as IFN-regulatory factors (IRFs) and nuclear factor kappa B (NF- $\kappa$ B) [19].

SARS-CoV-2, a member of the family *Coronaviridae*, contains a positive-sense RNA genome of ~30 kb and replicates via a mechanism of continuous and discontinuous RNA synthesis that produces long double-stranded RNA (dsRNA) species during infection. In contrast, replication of the segmented negative-sense RNA genome of IAV (an orthomyxovirus) does not generate detectable amounts of long dsRNA. As such, coronaviruses and IAV are generally perceived as virus prototypes that are sensed respectively by MDA5 and RIG-I, two founding members of the RIG-I-like receptor (RLR) family that typically detect cytoplasmic ‘nonself’ dsRNA with length preference. While RIG-I binds preferentially blunt-ended short dsRNA bearing a 5' triphosphate moiety such as the IAV (sub)genomic panhandle structure, MDA5 associates with complex long dsRNA whose precise characteristics remain still elusive (Figure 1) [20]. Several studies have demonstrated that type I/III IFN induction by SARS-CoV-2 relies on MDA5, with RIG-I contributing little to this process [21,22]. However, RIG-I was found to exert IFN-independent antiviral activity by competing with the viral polymerase for binding to the 3' untranslated region (UTR) of the SARS-CoV-2 genomic RNA [23]. Of note, similar mechanisms of signaling-independent virus restriction by RIG-I have also been reported for other viruses including IAV [24]. Interestingly, in contrast to RIG-I, MDA5 preferentially binds negative-strand SARS-CoV-2 RNA during infection [23], suggesting that active viral replication is required for triggering MDA5 activation and that both MDA5 and RIG-I may contribute to SARS-CoV-2 restriction in a temporal manner.

Besides RLRs, Toll-like receptors (TLRs) and cGAS also play crucial roles in the sensing of SARS-CoV-2 infection (Figure 1). Genetic studies revealed that inborn deficiency in TLR3 and TLR7 signaling underlies defective type I IFN production and severe disease in a group of COVID-19 patients [25,26]. TLR7-mediated type I IFN responses in plasmacytoid dendritic cells reportedly play a critical role in protection against severe COVID-19 [27], while uncontrolled TLR7 activation can lead to CSS in severe influenza [28]. cGAS activation following mitochondrial DNA release during SARS-CoV-2 infection was shown to elicit aberrant IFN production, which causes IFN-mediated immunopathology in severe COVID-19 [29].

Downstream of IFNs, certain ISGs and their effector functions are also implicated in innate restriction and immunomodulation during SARS-CoV-2 infection (Figure 1). A splice variant of 2'-5'-oligoadenylate synthetase 1 (OAS1), which undergoes prenylation in its unique carboxyl-terminus, anchors to SARS-CoV-2 replicative organelles and senses the 5' UTR of viral RNA, which ultimately activates RNase L-mediated restriction [30]. An intronic single nucleotide polymorphism that influences the expression of the prenylated OAS1 isoform was associated with disease severity in a patient cohort [30], making it a potential prognostic marker for severe COVID-19. ISG15, which exists in both a free and a conjugated version (the latter mediating protein ISGylation), was found to be secreted from macrophages infected with SARS-CoV-2 [31]. Extracellular ISG15 then functions 'cytokine-like' by inducing proinflammatory responses in a paracrine manner, likely contributing to hyperinflammation in COVID-19 [31].

#### **IFN antagonism by SARS-CoV-2 and IAV**

Effective viral antagonism of type I/III IFN induction requires the spatiotemporal coordination of multiple viral proteins that target distinct steps in antiviral signal transduction. This sophisticated operation perhaps is mastered best by SARS-CoV-2 encoding ~30 viral proteins, many of which exhibit immunomodulatory activities when ectopically expressed [32]. While extensive research has established a central role for the IAV nonstructural protein 1 (NS1) in innate immune evasion (Figure 1) [33], the strategies used by SARS-CoV-2 proteins to antagonize IFN-mediated antiviral immunity have just begun to be elucidated. Among the nonstructural proteins of SARS-CoV-2 that have immunosuppressive functions, Nsp1 binds to the mRNA channel of the human 40S ribosomal subunit and thereby globally interferes with the translation of host antiviral gene transcripts such as IFNs and ISGs [34,35]. Additionally, Nsp1 induces host mRNA degradation and impedes the nuclear export of host mRNA [36]. Nsp14 and Nsp16 also dysregulate host translation and transcription processes, including splicing, though the precise mechanisms remain elusive [35,37]. It is possible that specific SARS-CoV-2 proteins

contribute temporally to host shutoff during infection, which would mirror the coordinated action of IAV's NS1 and PA-X proteins in mediating host shutoff (Figure 1) [38].

At least four nonstructural proteins of SARS-CoV-2 target the RLR pathway to antagonize type I/III IFN induction (Figure 1). Being the largest multi-domain coronaviral protein, Nsp3 is a key component of the viral RNA synthesis machinery. In addition, Nsp3 has immunomodulatory activity, which is primarily attributable to its PLpro domain. PLpro is known to enzymatically remove from substrates covalently conjugated polyubiquitin or ISG15, thereby subverting antiviral signaling proteins that require for their activation ubiquitination or ISGylation. ISGylation recently emerged as a key activation mechanism for the initiation of MDA5-mediated antiviral signaling by promoting MDA5 higher-order assemblies. This activation step is counteracted by SARS-CoV-2 via PLpro-mediated de-ISGylation of MDA5 [21]. Downstream of MDA5 and other PRRs, PLpro also removes ISGylation from IRF3, thereby suppressing IFN induction [39]. Moreover, dysregulation of the cellular ratio of free vs. conjugated ISG15 by SARS-CoV-2 PLpro was shown to lead to aberrant macrophage activation and proinflammatory cytokine production [31]. Targeting essential posttranslational modifications in RNA sensors is also well-characterized for IAV NS1 which, however, does not possess catalytic activity itself. NS1 binds to and inhibits the ubiquitin E3 ligases TRIM25 and Riplet to prevent activating K63-linked polyubiquitination of RIG-I, thereby blunting antiviral signaling [40,41]. The SARS-CoV-2 main protease, Nsp5, was found to cleave and inactivate RIG-I and to induce degradation of the mitochondrial antiviral-signaling protein (MAVS), the shared downstream adaptor protein for RIG-I and MDA5 [42]. Several coronaviruses including SARS-CoV-2 also modify viral RNA ligands to evade recognition by MDA5. The viral 2'-O-methyltransferase Nsp16, together with the N7 methyltransferase activity of Nsp14, catalyzes Cap-1 modification of viral RNA to mimic host mRNA, thereby escaping detection by MDA5. In accord, inhibiting viral and cellular methyltransferase activities during SARS-CoV-2 infection elevated antiviral gene expression and restricted viral replication [43]. The endoribonuclease activity of Nsp15 can cleave and limit the accumulation of polyuridine-containing negative-sense viral RNAs



activating MDA5 during mouse hepatitis virus infection [44]; whether an analogous immune escape mechanism is utilized by SARS-CoV-2 requires further exploration.

SARS-CoV-2 accessory and structural proteins, which are expressed from viral subgenomic RNAs, also target key signaling hubs in the IFN pathway (Figure 1). Orf9b interacts with TOM70 at the mitochondrion and disrupts the recruitment of TANK-binding kinase 1 (TBK1) to MAVS [45]. Notably, the IAV PB1-F2 protein similarly targets MAVS at mitochondria to inhibit IFN induction [46]. SARS-CoV-2 Orf6 binds to nuclear pore complexes and blocks the cytoplasmic-to-nuclear translocation of IRF3 and STAT1/2 [47]. Other viral proteins, such as Orf3a, Orf7a/b, M, and N, also appear to interfere with STAT phosphorylation and/or MAVS activation [48]; however, the mechanistic details and physiological relevance of these evasion strategies require further investigation. Interestingly, compared to the ancestral strains, the Alpha variant of SARS-CoV-2 expresses notably higher levels of Orf6, Orf9b and N proteins, which contributes to the enhanced immunosuppression of this variant and provides evolutionary evidence for the importance of these viral proteins in IFN antagonism [49]. Moreover, profound IFN-mediated attenuation of a recombinant mutant SARS-CoV-2 was only seen when Orf3a was removed in addition to deletion of Orf6, Orf7 and Orf8 [50], suggesting a major role of Orf3a in manipulating antiviral IFN responses. Additionally, this study underscores the necessity of evaluating the relative contribution of viral proteins to innate immune evasion through the engineering of recombinant viruses using reverse genetics.

### **Concluding remarks**

The sophisticated immunomodulatory abilities of SARS-CoV-2 and IAV, combined with their capacity to adapt to new host species, allow these viruses to cause outbreaks or pandemics. Despite the global efforts over the past two and a half years in understanding COVID-19 pathogenesis, the mechanisms underlying the protective vs. pathogenic role of type I/III IFNs have just begun to be elucidated. More detailed insights into the strategies by which individual SARS-CoV-2 proteins rewire antiviral IFN responses are warranted to develop interventions that may help restore the protective

functions of IFNs. Deciphering the specific interactions of SARS-CoV-2 with host innate immune proteins will also guide the rational design of live-attenuated vaccines that may mediate effective and long-lasting immunity.

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## Figure legend

### Figure 1. Induction and antagonism of type I/III interferon responses by SARS-CoV-2 and IAV.

Type I/III IFN induction by SARS-CoV-2 relies primarily on MDA5, which senses long dsRNA species. MDA5 then undergoes a series of posttranslational modifications (PTMs) including ISGylation in its caspase activation and recruitment domains (CARDs), oligomerizes, and translocates to the mitochondrion where it interacts and activates MAVS. MAVS recruits downstream signaling molecules such as TBK1/IKK $\epsilon$  and the IKK $\alpha/\beta/\gamma$  complex that subsequently activate transcription factors including IRF3 and NF- $\kappa$ B. Upon translocation from the cytoplasm to the nucleus, these transcription factors drive the expression of type I/III IFNs and proinflammatory cytokines which, once secreted, prompt autocrine and paracrine signaling in infected and bystander cells, respectively. Specifically, type I/III IFNs engage cognate IFN receptors that signal through the JAK-STAT axis to upregulate ISGs. Besides MDA5, the cGAS-STING pathway (via released mitochondrial DNA (mtDNA)), as well as TLR3 and TLR7, are also implicated in SARS-CoV-2 sensing possibly in a cell-type-specific manner, though the mechanistic details require further investigation. These sensing pathways activate similar downstream kinases and transcription factors leading to type I/III IFN gene expression. A prenylated OAS1 isoform, which anchors to the endoplasmic reticulum (ER)-derived double-membrane vesicles (DMVs) where SARS-CoV-2 replication takes place, restricts virus replication by activating RNase L. RIG-I exerts an IFN-independent restriction mechanism by competing with the SARS-CoV-2 polymerase for binding to the 3' UTR of the viral genome (not depicted). Likewise, during IAV infection, RIG-I binds to the genomic RNA panhandle region associated with the IAV polymerase, which can impede viral replication in an IFN-independent manner (not illustrated). In many cell types (except plasmacytoid dendritic cells for example), IFN induction by IAV is primarily or exclusively dependent on RIG-I. Upon recognition of the IAV (sub)genomic panhandle structure, RIG-I undergoes conformational changes and PTMs such as activating K63-linked polyubiquitination in its CARDs and C-terminal domain (CTD) by TRIM25 and Riplet, respectively. RIG-I then activates an analogous signaling pathway as MDA5 to induce antiviral immunity. To evade immune surveillance, IAV uses the NS1 protein as the primary IFN antagonist. NS1

binds to TRIM25 and Riplet, thereby inhibiting the K63-linked polyubiquitination of RIG-I in the cytoplasm. NS1 also localizes to the nucleus where it blocks polyadenylation and nuclear export of cellular mRNAs via binding to the cellular cleavage and polyadenylation factor 30 (CPSF30). This host shutoff strategy is believed to act in concert with another host shutoff mechanism carried out by IAV PA-X protein, which selectively degrades host RNA polymerase II (Pol II) transcripts via its endonucleolytic activity. The IAV PB1-F2 protein binds to MAVS at the mitochondrion and suppresses MAVS activation by decreasing the mitochondrial membrane potential. Like IAV, SARS-CoV-2 targets critical PTMs of innate sensors and downstream signaling molecules to antagonize IFN responses. The PLpro domain of Nsp3 actively removes conjugated ISG15 from MDA5 and IRF3 to suppress their activation. Furthermore, extracellular secretion of free ISG15 prompted by PLpro's de-ISGylating activity can exacerbate proinflammatory cytokine responses. Nsp5 cleaves and disables RIG-I and also induces MAVS degradation. Nsp1 plugs the mRNA entry tunnel of the 40S ribosomal subunit to shut off host protein translation. Nsp14 and Nsp16 also reportedly disturb host translation and transcription processes, though the precise mechanisms are still unknown. Nsp14 and Nsp16 catalyze Cap-1 modification of viral RNA to mimic host mRNA and escape recognition by MDA5. Nsp15 of murine hepatitis virus and likely also SARS-CoV-2 cleaves and limits the accumulation of viral dsRNA to evade MDA5 sensing. Orf9b competes with the chaperone protein Hsp90 for binding to TOM70 and thereby impairs the recruitment of TBK1 and IRF3 (both associated with Hsp90) to the TOM70-MAVS complex. Orf6 interacts with the nuclear pore complex Nup98-Rae1 and impedes the nuclear import of IRF3 and STAT1/2. Orf3a, Orf7a/b, M, and N also reportedly dampen STAT1/2 and/or MAVS activation; the underlying mechanisms, however, require further investigation. SARS-CoV-2 proteins inhibiting IFN/ISG responses are depicted in pink. IAV proteins blocking innate immune signaling are illustrated in orange. 'Ub' indicates ubiquitin.

### **Conflicts of Interest**

The authors declare no conflicts of interest.



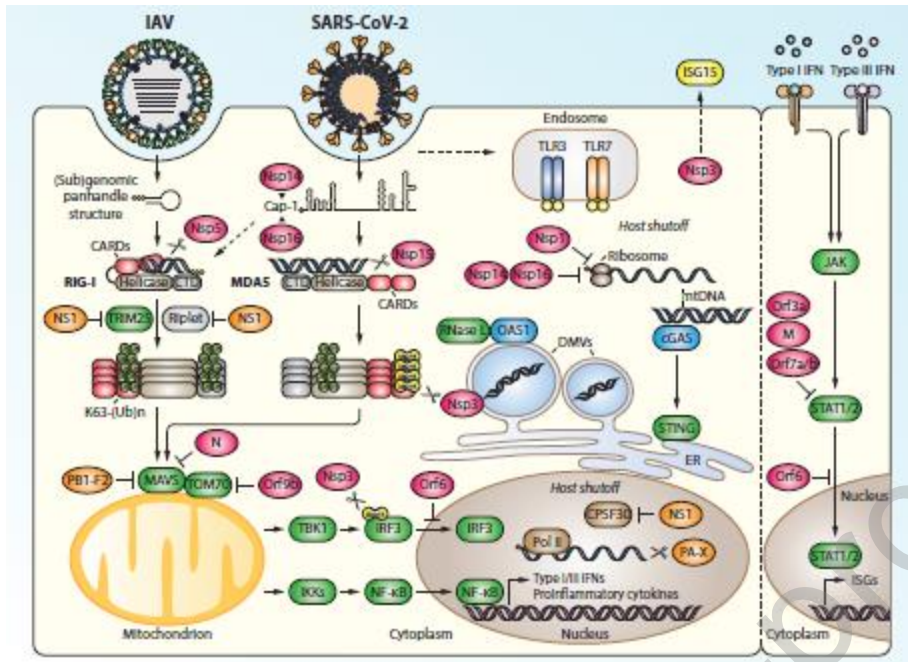


Fig 1

### Highlights

- SARS-CoV-2 and influenza A virus dysregulate human innate immune responses.
- Severe COVID-19 and severe influenza exhibit distinct cytokine patterns.
- Delayed IFN production along with sustained inflammation drives severe COVID-19.
- MDA5 and RIG-I are key innate immune sensors of SARS-CoV-2 and influenza A virus.
- SARS-CoV-2 and influenza A virus have evolved effective interferon antagonisms.