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Review

Type I and Type III Interferons – Induction, Signaling, Evasion, and Application to Combat COVID-19

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Coronavirus disease 2019 (COVID-19) is a global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Without approved antiviral therapeutics or vaccines to this ongoing global threat, type I and type III interferons (IFNs) are currently being evaluated for their efficacy. Both the role of IFNs and the use of recombinant IFNs in two related, highly pathogenic coronaviruses, SARS-CoV and MERS-CoV, have been controversial in terms of their protective effects in the host. In this review, we describe the recent progress in our understanding of both type I and type III IFN-mediated innate antiviral responses against human coronaviruses and discuss the potential use of IFNs as a treatment strategy for COVID-19.

Coronavirus disease 2019 (COVID-19) first appeared in Wuhan, China in December 2019 and has since rapidly spread across the world (Zhu et al., 2020). On March 11th, the World Health Organization declared the COVID-19 outbreak as a pandemic. As of April 10th, the global death toll has surpassed 100,000. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Human coronavirus (HCoV) includes the two other highly pathogenic viruses, SARS-CoV and MERS-CoV, which were responsible for epidemics in the past two decades, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS) (Fung and Liu, 2019). There is no approved antiviral drug or vaccine to date for COVID-19.

The interferon (IFN) response constitutes the major first line of defense against viruses. Recognition of viral infections by innate immune sensors activates type I and type III IFN response. Type I IFNs (IFN-α, IFN-β, IFN-ε, IFN-κ, IFN-ω in humans) bind to the ubiquitously expressed type I IFN receptor (IFNAR) in an autocrine and paracrine manner. This activates a powerful antiviral defense program of hundreds of interferon-stimulated genes (ISGs), which have the capacity to interfere with every step of viral replication (Schoggins and Rice, 2011). Type III IFNs (IFN-λ) bind to the type III IFN receptor (IFNLR), preferentially expressed on epithelial cells and certain myeloid cells (Kotenko et al., 2019). Whereas type I and III IFNs induce a similar ISG signature, type I IFN signaling leads to a more rapid induction and decline of ISG expression (Lazear et al., 2019).

Type I and type III IFNs establish the cellular state of viral resistance, as well as activate the adaptive immune responses to viruses (Ivashkiv and Donlin, 2014). Successful viral pathogens therefore have evolved mechanisms to escape both immune recognition and suppress the functions of IFNs and ISGs. Many viral proteins are dedicated to modulating the host IFN response. These mechanisms have been extensively investigated for SARS-CoV and MERS-CoV (Lim et al., 2016; Nelemans and Kikkert, 2019; Totura and Baric, 2012). Both viral and host factors determine the outcome of IFN signaling. Type I IFN signaling in particular can be deleterious through its systemic, pro-inflammatory effects (Ivashkiv and Donlin, 2014). Whether the IFN response has a protective or pathogenic effect in SARS and MERS seems to be dependent on the context in which IFN signaling is induced.

Recombinant and pegylated IFN-α and IFN-β have been used in treatment of various diseases including multiple sclerosis and viral hepatitis (Lazear et al., 2019). Recombinant IFN-λs, although not yet approved for any indication, are in clinical trials for viral hepatitis. There is a worldwide interest in repurposing existing antivirals for COVID-19. In this regard, the biology of IFNs in coronavirus infection needs to be thoroughly examined in order to implement rational treatment strategies and safely evaluate their clinical efficacy in COVID-19. In this review, we first describe the IFN-mediated antiviral response in coronavirus infection, focusing on the innate recognition of and immune evasion by SARS-CoV and MERS-CoV. Furthermore, we examine the role of type I and type III IFN response in SARS and MERS and speculate on the promise and challenges of using IFNs as a therapeutic agent in COVID-19.

Innate Recognition of Coronavirus Infection

The innate immune system recognizes invading pathogens by sensing their pathogen-associated molecular patterns (PAMPs) with various pattern recognition receptors (PRRs). Viral PAMPs are often distinct molecular signatures not found in host cells, such as unique nucleic acid structures of the viral genome or viral replication intermediates (Iwasaki, 2012). RNA viral recognition occurs mainly in the endosomal or cytosolic compartment by two different classes of PRRs, Toll-like receptors (TLRs), and RIG-I-like receptors (RLRs), respectively (Figure 1). Whereas most host cells are equipped with the cytosolic RLRs, endosomal TLRs are mostly expressed in innate immune cells.

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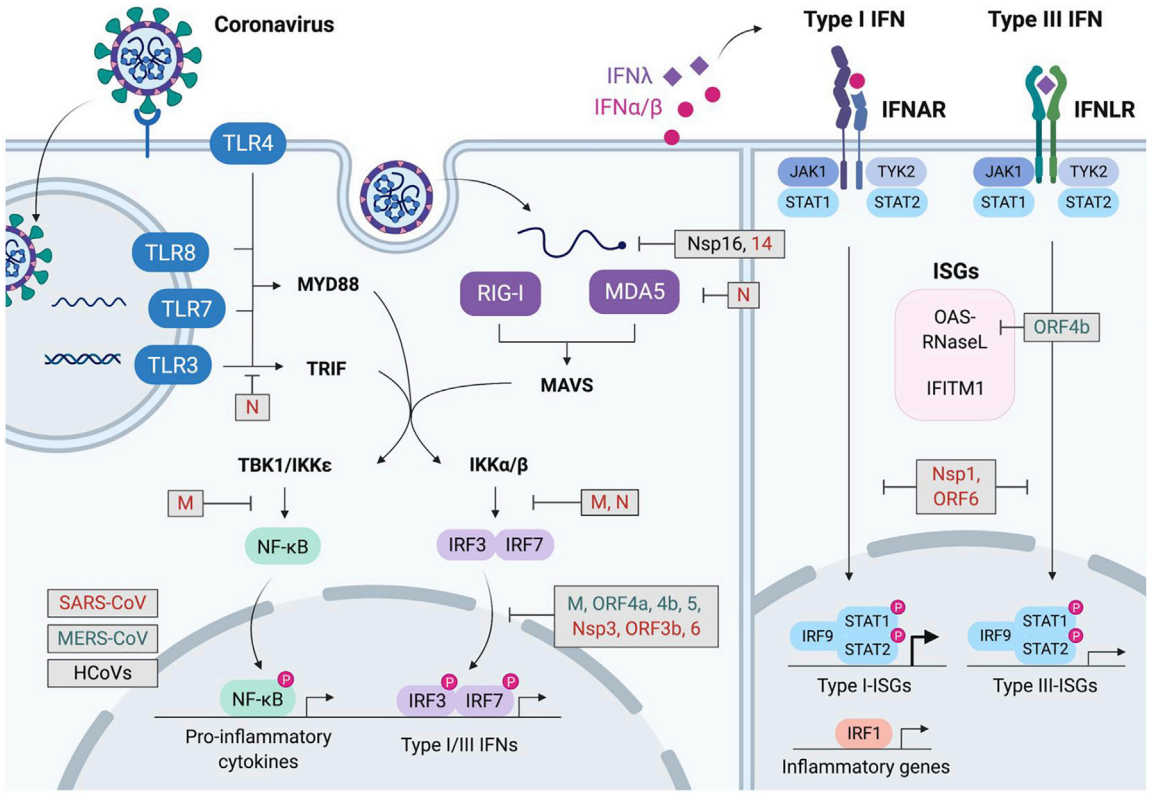

Figure 1. Innate Recognition, Interferon Signaling, and Immune Evasion by Coronaviruses

Upon sensing of coronaviruses by various pathogen recognition receptors, TLRs (TLR3, TLR4, TLR7, TLR8; blue) and RLRs (RIG-I, MDA5; purple), transcription factors nuclear factor-κB (NF-κB) and interferon regulatory factor 3 and 7 (IRF3, IRF7) stimulate the production of pro-inflammatory cytokines and type I and III interferons (IFNs), respectively. IFNs are secreted in an autocrine and paracrine manner to induce expression of interferon-stimulated genes (ISGs) via the JAK-STAT signaling pathway. While type I and III IFNs induce a similar set of ISGs, type I IFN signaling activates a stronger and faster ISG response, in addition to expression of pro-inflammatory cytokines and chemokines. Many viral proteins encoded by coronaviruses (red: SARS-CoV, green: MERS-CoV, black: multiple human coronaviruses) antagonize various steps of this antiviral response.

Additionally, certain ISGs, like OAS and IFIT family proteins, can also directly recognize and execute their function on viral RNA (Schoggins and Rice, 2011).

The role of various PRRs in coronavirus infection has largely been elucidated by genetic studies that revealed increased susceptibility to the infection in the absence of specific PRRs and their signaling pathways. Since coronaviruses replicate in the cytoplasm, their replication intermediates and replicated viral genomes can be recognized by cytosolic RNA sensors, RIG-I and MDA5. Both RIG-I and MDA5 are involved in sensing of murine coronavirus mouse hepatitis virus (MHV) infection; in their absence, IFN induction by MHV is abrogated (Li et al., 2010). It is likely that SARS-CoV-2 is also sensed by these RLRs. RNA sensing TLRs, TLR3, TLR7, and TLR8, are located in the endosomal membrane and detect double-stranded RNA (dsRNA; TLR3) and single-stranded RNA (ssRNA; TLR7 and TLR8). TLR7 in particular plays a critical role in sensing of coronaviruses including SARS-CoV, MERS-CoV, and MHV and is required for IFN-α production by plasmacytoid dendritic cells in these infections (Cervantes-Barragan et al., 2007; Scheuplein et al., 2015). Additionally, TLR4, which is expressed on the surface of innate immune cells, can recognize viral glycoproteins such as the respiratory syncytial virus fusion protein (Kurt-Jones et al., 2000). TLR4-deficient mice are more susceptible to both SARS-CoV and MHV infections (Khanolkar et al., 2009; Totura et al., 2015).

Downstream adaptor protein molecules for TLRs, MyD88 (for TLR4, TLR7, TLR8) and TRIF (for TLR3, TLR4), are required for protection against coronavirus infections, indicating the essential role of innate sensing in host immunity. Mice deficient in MyD88 challenged with mouse-adapted SARS-CoV are unable to control viral replication and succumb to infection (Sheahan et al., 2008). Mice deficient in TRIF are also highly susceptible to SARS-CoV, with morbidity comparable to the MyD88-deficient mice (Totura et al., 2015). In MERS-CoV infection, lack of MyD88 signaling results in delayed viral clearance and increased lung pathology (Zhao et al., 2014). By contrast, this study did not find a requirement for MAVS signaling, which is downstream of RLR sensing. Consistently, another study showed that *Tlr7*$^{-/-}$ mice, but not *Mavs*$^{-/-}$ mice, have reduced IFN expression compared with wild-type mice (Channappanavar et al., 2019). This suggests that TLR7-MyD88 is the primary pathway of innate immune sensing in MERS-CoV infection.

Innate viral recognition triggers a signaling cascade leading to both NF-κB-mediated induction of pro-inflammatory cytokines (e.g., IL-1, IL-6, TNF-α) and IRF3 and IRF7-mediated induction of type I and type III IFNs (IFN-I and IFN-III) (Figure 1).

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Transcriptome profiling of various cell types revealed that SARS-CoV-2 infection elicits very low IFN-I or IFN-III and limited ISG response, while inducing chemokine and pro-inflammatory cytokine genes (Blanco-Melo et al., 2020). In addition to viral intrinsic suppression of IFN response, age of the host dictates the cytokine profiles. In a macaque model of SARS-CoV infection, aged macaques had more lung pathology and higher expression of pro-inflammatory cytokines but lower expression of IFN-Is compared to younger macaques (Smits et al., 2010). These results are consistent with older human monocytes having defective IFN-I and IFN-III production while maintaining intact pro-inflammatory cytokines in response to influenza A virus (IAV) infection (Molony et al., 2017). The defect in IFN induction in older monocytes was due to proteolytic degradation of TRAF3, a key signaling molecule downstream of many PRRs required for IFN transcription. These collectively suggest that the imbalance between pro-inflammatory versus IFN response in aging may have important disease implications for COVID-19 pathogenesis.

Modulation of Innate Antiviral Response by Coronavirus

Type I and type III IFNs induce hundreds of antiviral effectors, or ISGs, to achieve a cell-intrinsic state of viral resistance. Despite this powerful host antiviral strategy, coronaviruses remain highly pathogenic, at least in part due to the various viral mechanisms to evade and suppress the IFN response. Indeed, a more robust IFN-I response is induced in mild HCoV-229E infection compared to SARS-CoV and MERS-CoV infections (Lim et al., 2016). Coronaviruses can interfere with any of the following processes in innate antiviral immunity: (1) innate sensing, (2) IFN production, (3) IFN signaling, and (4) ISG effector function (Figure 1).

First, HCoVs encode viral proteins dedicated to evading innate recognition by PRRs. SARS-CoV and other coronaviruses replicate in the interior of double membrane vesicles to prevent RLR activation by dsRNA replication intermediates (Stertz et al., 2007). RLRs use the 5′ cap to distinguish viral RNA from host mRNA. SARS-CoV nonstructural protein 14 (nsp14) has guanine-N7-methyltransferase activity that can mimic this cap structure on the viral RNA (Chen et al., 2009). Nsp16 of SARS-CoV further modifies this cap with its 2'-O-methyl-transferase activity, allowing the virus to efficiently evade recognition by MDA5 (Daffis et al., 2010). SARS-CoV with a mutated nsp16 displays reduced virulence that is dependent on MDA5 sensing (Menachery et al., 2014b). Mutating nsp16 also attenuates virulence in MERS-CoV and reduces disease severity in infected mice (Menachery et al., 2017). Thus, nsp16 is critical to alter the innate antiviral response in SARS and MERS. Nsp16 of SARS-CoV-2 and of SARS-CoV share 92% amino acid sequence homology, suggesting this evasion strategy is likely retained in the novel virus (Lokugamage et al., 2020).

Second, HCoVs inhibit IFN-I and IFN-III production. The membrane (M) protein of SARS-CoV directly interacts with the innate sensors and the signaling molecules to sequester them in membrane-associated cytoplasmic compartments (Siu et al., 2009). MERS-CoV M protein inhibits nuclear translocation of IRF3, the transcription factor for IFN genes (Yang et al., 2013). SARS-CoV nucleocapsid (N) protein also interferes with IRF3 function (Kopecky-Bromberg et al., 2007). SARS-CoV nsp3 protein, in addition to its papain-like protease (PLP) activity, blocks phosphorylation and nuclear translocation of IRF3 (Devaraj et al., 2007). This step is further inhibited by the accessory proteins of SARS-CoV, ORF3b and 6, and those of MERS-CoV, ORF 4a, 4b, and 5 (Lu et al., 2011; Yang et al., 2013). These mechanisms may collectively contribute to the low IFN-I and IFN-III induction in cells infected with SARS-CoV-2 (Blanco-Melo et al., 2020).

Third, HCoV viral proteins block IFNAR and IFNLR signaling. SARS-CoV nsp1 inhibits phosphorylation of STAT1, the transcription factor for ISGs (Wathelet et al., 2007). SARS-CoV accessory proteins ORF3b and 6 block transcription of ISGs (Kopecky-Bromberg et al., 2007). Specifically, ORF6 inhibits translocation of STAT1 to the nucleus (Frieman et al., 2007).

Lastly, HCoVs can directly suppress ISG effector functions. IFN-induced 2',5′-oligoadenylate synthetase (OAS)-ribonuclease L (RNase L) pathway degrades viral RNA in the cytosol. MERS-CoV accessory protein ORF4b has 2'-5′-phosphodiesterase activity that degrades the product of OAS to prevent activation of RNase L (Thornbrough et al., 2016).

Exogenous IFN may be sufficient to overcome HCoV suppression of the IFN response. Whereas most proteins of SARS-CoV and SARS-CoV-2 share greater than 90% amino acid identity, nsp3, ORF3b, ORF6—all of which antagonize IFN—have relatively low sequence homology (Lokugamage et al., 2020). ORF3b of SARS-CoV-2 contains a premature stop codon that results in a truncated protein. ORF6b, in addition to having only 69% homology with the SARS-CoV protein, is missing two amino acids at the C-terminal critical for the protein function (Frieman et al., 2007). This may explain the enhanced susceptibility of SARS-CoV-2 to IFNs (Lokugamage et al., 2020). Conversely, the truncated ORF3b of SARS-CoV-2 suppresses IFN induction more efficiently than that of SARS-CoV, which may contribute to the poor IFN response reported in COVID-19 patients (Konno et al., 2020).

The Role of IFNs during Coronavirus Infections

Clinical studies have reported lack of IFN response in SARS patients in spite of robust cytokine and chemokine productions, consistent with *in vitro* observations that SARS-CoV infection does not induce significant IFN-I production (Chen and Subbarao, 2007; Reghunathan et al., 2005). Serum analysis of COVID-19 patients showed a similar dynamic; pro-inflammatory cytokines and chemokines were strongly elevated without detectable levels of type I and III IFNs (Blanco-Melo et al., 2020). Other studies suggest that rather than its complete absence, the IFN response may be delayed. Comparison of transcriptome of SARS-CoV-infected cells across multiple time points revealed that expression of IFNs lags that of pro-inflammatory cytokines (Yoshikawa et al., 2010). Induction of IFN-β and ISGs in SARS-CoV and MERS-CoV infection was delayed compared with IAV infection (Menachery et al., 2014a). In a mouse model of SARS-CoV infection, IFN-I was not detectable in the lung until several hours after the peak in viral load (Channappanavar et al., 2016).

Paradoxically, elevated IFNs correlate with worse disease. In a cohort of clinically well-described SARS patients, high levels of IFN-α and ISGs correlated with disease severity (Cameron et al., 2007). In patients who developed severe hypoxemia, high levels of IFN-induced chemokines and IFNAR1 persisted even after the resolution of acute illness. Similarly, IFN-α levels

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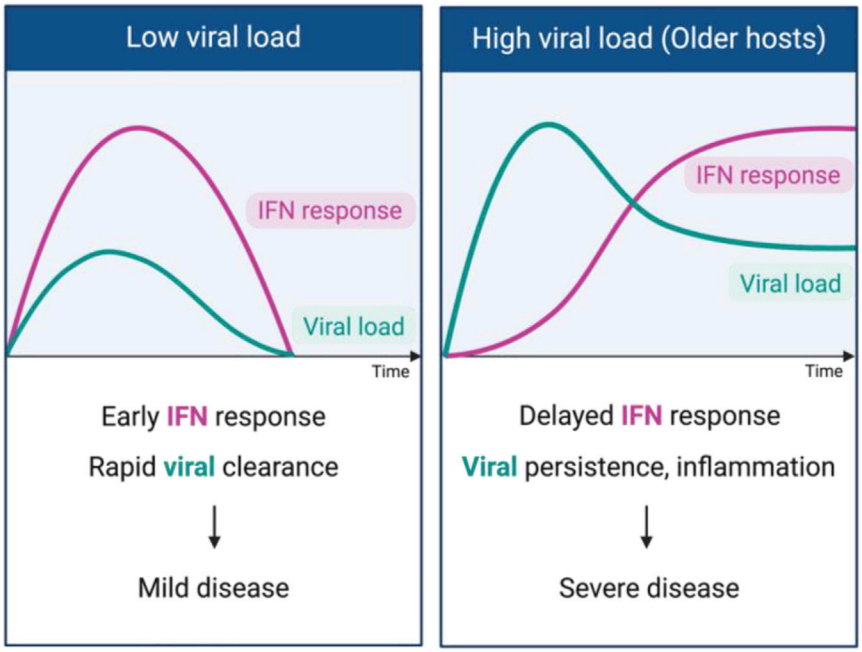

Figure 2. Protective and Pathogenic Roles of Type I IFNs during Coronavirus Infection
Both viral and host factors can affect the timing of IFN response. When the initial viral burden is low (left), IFNs can be induced early and clear the infection effectively. High viral load (right) may strongly suppress the IFN response due to viral evasion mechanisms, causing its delayed induction. Alternatively, IFN induction may be compromised in older hosts. When the IFN response is insufficient to control initial viral replication, late onset IFN could lead to inflammation and lung injury.

were more frequently elevated in the severe MERS patient group than in the mild group, and correlated with viral RNA copies (Kim et al., 2016). In a small COVID-19 patient cohort, levels of IFN-α and ISGs were associated with viral load as well as disease severity (Wei et al., 2020). These studies indicate that severe infections lead to high IFN signatures but fail to bring down viral load.

The role of IFN signaling in SARS mouse models depends on the genetic background. In C57BL/6 or 129 mice that develop mild SARS, IFN signaling contributes to protection by enhancing viral clearance (Frieman et al., 2010; Mahlakõiv et al., 2012). *Stat1*$^{-/-}$ 129 mice succumb to SARS-CoV infection owing to impaired viral control and severe pathology (Frieman et al., 2010). Although *Ifnar1*$^{-/-}$ 129 mice have disease severity comparable to the wild-type, their viral control is impaired. Similar results were obtained in the C57BL/6 background; *Stat1*$^{-/-}$ mice are highly susceptible to SARS-CoV infection, and *Ifnar1*$^{-/-}$ mice have higher viral titers (Mahlakõiv et al., 2012; Mordstein et al., 2010). Conversely, in BALB/c mice which develop lethal SARS-CoV disease, IFN-I signaling is detrimental, largely by promoting infiltration of inflammatory monocytes and macrophages to lung tissues (Channappanavar et al., 2016). *Ifnar1*$^{-/-}$ BALB/c mice show mild symptoms and 100% survival rate. In this severe SARS model (BALB/c), IFN-I induction is delayed relative to viral replication (Channappanavar et al., 2016). IFN-I delivery prior to the peak viral load in these mice enhanced viral control and conferred complete protection from the disease. In contrast, administration after the viral peak failed to achieve the same effects (Channappanavar et al., 2016). This demonstrates the importance of early IFNs in restricting viral replication.

In MERS, IFN-I signaling protects mice from disease and death. *Ifnar1*$^{-/-}$ mice have worse clinical and histopathological outcomes compared to wild-type mice after MERS-CoV challenge (Zhao et al., 2014). Blocking IFNAR1 also increases viral load and mortality (Channappanavar et al., 2019). Notably, during MERS-CoV infection, there is no delay in induction of IFN-I response relative to viral replication, which may account for the different impact of IFNs in SARS and MERS. Findings from exogenous IFN-I administration further support the protective role of early IFN-I in MERS. Prophylactic administration of IFN-β in mice accelerated viral clearance without causing weight loss or inflammation (Zhao et al., 2014). Early IFN-β treatment before viral peak was also protective, whereas late treatment resulted in increased inflammation and lethal pneumonia (Channappanavar et al., 2019). These studies all together underscore the importance of timing of IFN-I induction relative to viral replication as a key determinant of the response outcome, with early IFN-I induction or administration conferring protection (Figure 2). In contrast, delayed IFN-I response not only fails to control virus but can also cause inflammation and tissue damage. Therefore, the host may benefit from IFN-I supplementation early in the disease course, particularly when IFN-I expression is delayed or reduced due to viral suppression of IFN response or older age of the host.

It is important to consider host species differences in coronavirus pathogenesis. Animal models do not recapitulate the full spectrum of human disease caused by SARS-CoV and MERS-CoV. This is due to multiple differences in hosts, including host restriction factors and the expression of the receptors for viral entry (ACE2 for SARS-CoV, DPP4 for MERS-CoV) (Sutton and Subbarao, 2015). While expression of human ACE2 renders mice more permissive to SARS-CoV infection, human SARS disease is not accurately reproduced by any of the mouse models. Non-human primate species are susceptible to SARS-CoV and MERS-CoV infections to varying degrees, but do not consistently reproduce the disease severity and mortality seen in patients (Sutton and Subbarao, 2015). Moreover, several differences in IFN response have been described between laboratory animals and humans. In mice and non-human primates, IFN response appears to be generally dysregulated or delayed during SARS-CoV infection, whereas clinical studies have more often than not reported a lack of IFN response (Channappanavar et al., 2016; Reghunathan et al., 2005; Smits et al., 2010). The ISG repertoire also varies among vertebrate species; whereas a subset of ISGs is shared, many ISGs are species-specific (Shaw et al., 2017). Of particular relevance is the finding that

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ACE2 is induced by IFNs in humans but to a lesser extent in mice (Ziegler et al., 2020). These differences highlight the significance of host-specific factors in determining IFN response and disease outcomes. Strong research focus should be on investigating the role and kinetics of IFNs in COVID-19 patients throughout disease progression.

Type I IFNs as a Therapeutic Strategy in COVID-19

Use of recombinant IFN-α or IFN-β as a treatment in SARS, MERS, and now COVID-19 has been a subject of debate (Sallard et al., 2020). IFN-I was identified as a promising therapeutic candidate in SARS and MERS based on numerous *in vitro* and *in vivo* studies (Stockman et al., 2006). In marmosets, treatment of MERS-CoV-infected animals with IFN-β1b reduced viral load and lung pathology (Chan et al., 2015). In a mouse model of MERS-CoV infection, although treatment with IFN-β in combination with lopinavir-ritonavir did not significantly reduce lung pathology, it improved pulmonary function (Sheahan et al., 2020). Administration of IFN-α2b with ribavirin to MERS-CoV-infected macaques resulted in better viral control and clinical outcomes (Falzarano et al., 2013). Animal studies of SARS-CoV infection showed similar efficacy. Prophylactic treatment of macaques with IFN-α prior to SARS-CoV infection greatly reduced viral replication and pulmonary damage (Haagmans et al., 2004).

In contrast, clinical studies of SARS and MERS patients have been less conclusive. In a study of a small number of SARS patients, addition of IFN-α to corticosteroid was associated with better oxygen saturation and quicker resolution of radiographic lung abnormalities (Loutfy et al., 2003). In MERS patients, combination of IFN-α and ribavirin was associated with improved survival at 14 days, but not at 28 days after diagnosis (Omrani et al., 2014). However, the combination therapy was not effective when initiated late in the course of illness (median of 19 days from admission to therapy) (Al-Tawfiq et al., 2014). In another retrospective cohort study, IFN-ribavirin combination was not significantly associated with improved outcome in mortality at 28 days (Arabi et al., 2017). The inconsistent results in human studies may be explained to some degree by the limited number of patients in retrospective studies, drugs used in combinations, and importantly, timing of administration as we have discussed. Moreover, it has been suggested that comorbidities like diabetes affect the response to IFN (Shalhoub et al., 2015). Currently, a randomized clinical trial is ongoing to test the efficacy of IFN-β in combination with lopinavir-ritonavir in MERS patients (Arabi et al., 2020).

Knowledge gained from studies on SARS-CoV and MERS-CoV will be valuable for determining the suitability of IFN-I as a treatment strategy in COVID-19. Two *in vitro* studies have already demonstrated that SARS-CoV-2 has greater sensitivity to IFN-I compared with SARS-CoV (Lokugamage et al., 2020; Mantlo et al., 2020). In these studies, pre-treatment with IFN-α or IFN-β drastically reduced viral titers. These findings suggest that IFN-I may be effective as a prophylactic agent or an early treatment option for SARS-CoV-2. Several efforts are ongoing to test this. The guidelines in China for the treatment of COVID-19 include vapor inhalation of IFN-α, in conjunction with ribavirin (Sallard et al., 2020). This route of delivery has the benefit of targeting IFN-α specifically to the respiratory tract. Several clinical trials to evaluate IFN-I as a single or combination therapy in COVID-19 have been registered across the world. These include the DisCoVeRy trial (NCT04315948, the first clinical trial by the WHO Solidarity consortium) that compares subcutaneous injection of IFN-β1a in combination with lopinavir-ritonavir, lopinavir-ritonavir alone, hydroxychloroquine, or remdesivir, as well as the Phase II trial on inhaled IFN-β1a as a single agent in UK (NCT04385095) (Sallard et al., 2020). In a retrospective study of 77 COVID-19 patients in Wuhan, China treated with nebulized IFN-α2b, arbidol, or a combination of the two, IFN-α2b therapy significantly reduced the duration of detectable virus and inflammatory markers, IL-6 and C-reactive protein (CRP) (Zhou et al., 2020). Another study showed that recombinant IFN-α nasal drops may prevent COVID-19 incidence without adverse effects. In this case series done in Hubei Province, the incidence among the 2944 healthcare workers treated with daily IFN-α for 28 days was zero (Meng et al., 2020). While initial clinical data are encouraging, latest study shows that ACE2 is an ISG, inducible by IFN-α in primary human upper airway cells (Ziegler et al., 2020). Additional results from the ongoing clinical studies, as well as development of animal models, will offer a more instructive answer on the safety and efficacy IFN-I as a therapy in COVID-19.

The Role and Therapeutic Potential of Type III IFNs in Respiratory Infections

Type III IFNs, like type I, are induced upon PRR recognition of PAMPs and signal through the shared JAK-STAT pathway to induce a similar antiviral transcriptional program. Type I and III IFN responses achieve context-specific non-redundant functions through several features. First, IFN-λs bind to IFNLR (IFNLR1/IL10Rβ) expressed preferentially on epithelial cells of the respiratory, gastrointestinal, and reproductive tracts as well as on certain myeloid cell types (Kotenko et al., 2019). This expression pattern, distinct from the ubiquitously expressed-IFNARs, allows local viral control at the site of entry. Second, while type I and III ISG repertoires generally overlap, IFN-III signaling leads to a more sustained expression of ISGs (Lazear et al., 2019). Finally, only IFN-I signaling activates transcription of pro-inflammatory cytokine genes by selective induction of the transcription factor IRF1 (Galani et al., 2017; Forero et al., 2019) (Figure 1). Supporting the unique role of type III IFN response in antiviral defense, IFN-λs are the predominant IFNs produced early during IAV infection by epithelial cells and act on IFNLR on epithelial cells and neutrophils to control viral replication without causing inflammation (Galani et al., 2017).

Several animal studies have investigated the role of type III IFNs in SARS and MERS. During MERS-CoV infection, IFN-λs are produced in a TLR7-dependent manner and correlate with viral replication kinetics (Channappanavar et al., 2019; Scheuplein et al., 2015). Transcriptome analyses of SARS-CoV-infected mice revealed STAT1-dependent but IFNAR-independent ISG induction, raising the possibility that ISGs may be induced by signaling through IFNLR (Zornetzer et al., 2010). In many respiratory tract infections including SARS-CoV and IAV, IFN-λ signaling appears to be protective (Mordstein et al., 2010). *Ifnlr1*$^{-/-}$ mice are unable to control the replication of SARS-CoV. The effect of type I and III IFN signaling is additive; *Ifnar1*$^{-/-}$ *Ifnlr1*$^{-/-}$ double knockout mice have a viral load higher than that in each of the single knockout mice (Mordstein et al., 2010). Even

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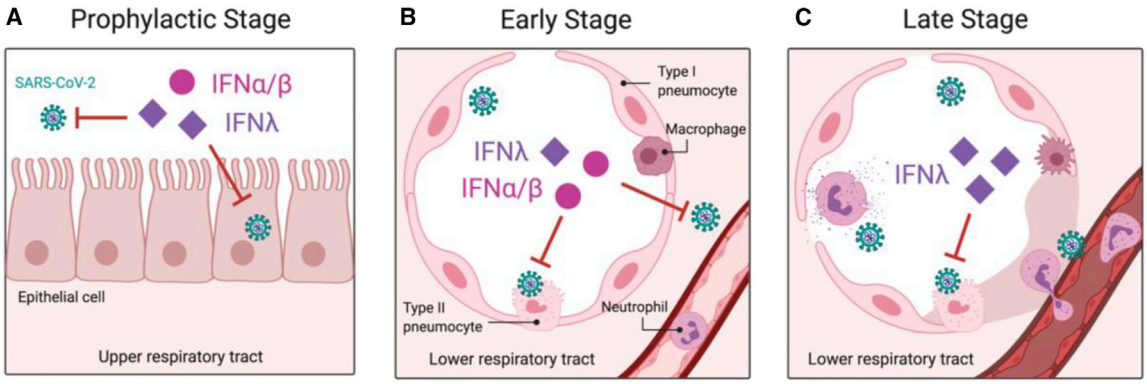

Figure 3. Therapeutic Aims with Type I and Type III IFNs during the Progression of COVID-19

(A) Prophylactic intranasal administration or inhalation of recombinant IFNs, particularly type III IFNs (IFN-λ), can act to restrict viral replication in the upper airway, reducing viral spread to the lungs and transmission.

(B) When initial control fails and virus reaches the lung, the host may benefit from additional IFNs, including the more potent type I IFNs (IFN-α, IFN-β). Given that our natural IFN response may be lacking at this early stage, exogenous IFNs might help control infection and prevent viral dissemination.

(C) In the late stage of the disease, IFNs must be used with caution to not exacerbate inflammation and tissue damage. IFN-λ could continue to activate localized antiviral protection without triggering a systemic response.

though lack of IFN-I and IFN-III signaling strongly impairs viral clearance, these mice do not develop the severe disease observed in SARS-CoV-infected *Stat1*$^{-/-}$ mice, pointing to the contribution of type II IFN in antiviral defense (Mahlakõiv et al., 2012).

The upper and lower respiratory tract may have different requirements for IFN-λ. As opposed to in the lung (lower respiratory tract) where the activities of IFN-α and -λ overlap, only IFN-λ offers critical protection in the upper respiratory tract in mice (Klinkhammer et al., 2018). When viruses were delivered specifically to the upper airway at a lower dose to closely mimic a natural respiratory viral infection, IFN-λ was required for preventing IAV spread to the lung. Moreover, *Ifnlr1*$^{-/-}$ mice shed more infectious viral particles and caused more frequent virus transmission to naive contacts than wild-type or *Ifnar1*$^{-/-}$ mice (Klinkhammer et al., 2018). Whereas prophylactic intranasal administration of either IFN-α or IFN-λ blocked IAV replication in the lung, only IFN-λ conferred long-lasting antiviral protection in the upper airway and limited contact virus transmission (Klinkhammer et al., 2018).

Unlike type I IFNs which are already widely used in clinic, type III IFNs are not yet approved for any indication. Nevertheless, the unique qualities of type III IFN response—focused, long-lasting, and non-inflammatory—make IFN-λ an attractive intervention strategy in COVID-19. Importantly, IFN-λ administration has been shown to offer effective therapeutic effects without appreciable immunopathology in mice challenged with IAV (Davidson et al., 2016; Galani et al., 2017; Kim et al., 2017). IFN-λ was as protective as IFN-α when adminsterered prophylactically, and more protective than IFN-β when administered simultaneously with IAV (Davidson et al., 2016; Kim et al., 2017). When administered after the onset of symptoms, IFN-λ2 was protective whereas IFN-α4 exacerbated the disease by promoting pro-inflammatory cytokine secretion and immune cell infiltration (Davidson et al., 2016). Recent study on SARS-CoV-2 shows that knocking out IFNLR1 in human intestinal epithelial cells impairs the ability to control viral replication, even more so than IFNAR1, and that SARS-CoV-2 is sensitive to pre-treatment with either IFN-β or IFN-λ (Stanifer et al., 2020). In a newly developed mouse model of SARS-CoV-2 infection, both prophylactic and therapeutic application of pegylated IFN-λ1 reduced viral replication (Dinnon et al., 2020). Therefore, clinical use of IFN-λ in COVID-19 holds promise, and clinical trials are under way (NCT04343976, NCT04331899). For both maximal efficacy and minimal toxicity, we envision an intervention strategy that draws upon the strengths of both type I and III IFN response (Figure 3). Type III IFNs may help to achieve a sustained antiviral state that limits viral spread in the upper airway as well as the lung. Type I IFNs, which are more potent but also more inflammatory, should be restricted to the early phase to facilitate viral clearance and prevent systemic inflammation.

Concluding Remarks

In order to harness the IFN-mediated innate immune response as an antiviral therapy in COVID-19, we highlight a few research questions that have implications for therapy design and implementation. How can we make current IFN-I therapy more effective without adverse effects? The natural course of IFN-I signaling during SARS-CoV-2 infection needs to be defined. If we can understand the different kinetics of IFN-I secretion in mildly symptomatic and severe COVID-19 patients relative to the kinetics of viral replication, we may be able to identify the window of therapeutic opportunity. Based on the large existing body of work available, some of which we review here, early administration prior to viral peak or prophylactic treatment may offer maximal protection without appreciable pathology. We suggest several efforts that will help establish the feasibility as well as increase the versatility of IFN-I therapy. First, the prophylactic effects of IFNs that have been reported should be validated by randomized clinical trials. The intervention should be tested on healthcare workers and other individuals at risk for SARS-CoV-2 infection. Second, in order to administer IFN in the early stage of infection, robust public health measures including testing and contact tracing need to be established to rapidly identify those who have been exposed before symptom onset. Furthermore, investigating cellular targets that can limit or

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reverse IFN-I-associated inflammation will be valuable for potential therapeutic application with IFN-Is. Possible mechanisms include inhibiting inflammatory genes downstream of IFN-I signaling and promoting negative feedback of the IFN response. Finally, identifying host factors that lead to delayed or reduced IFN-I induction may instruct us on patient groups that may particularly benefit or should be refrained from IFN-I treatment. In addition to host age, genetic polymorphisms may affect IFN outcomes. For example, single nucleotide polymorphism (SNP) near the *IL28B* gene (encoding IFN-λ3) is associated with enhanced response to pegylated IFN-α hepatitis C treatment (Ge et al., 2009).

How can we expand the treatment strategies that work by augmenting our natural antiviral response? One possibility is the use of synthetic PRR agonists to increase the induction of IFN response. Notably, poly(I:C), a double-strand RNA that can activate RLRs and TLR3, provides protection in two different mouse models of SARS-CoV infection (Kumaki et al., 2017; Zhao et al., 2012). We have discussed in this review another promising target, the type III IFNs, as both a preventive and a therapeutic measure in COVID-19. Delineating spatiotemporally distinct roles of type I and III IFN response in SARS-CoV-2 infection will teach us when to preferentially use one or synergize the two responses. The IFN response is a complex host defense strategy that, with accurate understanding of its biology, can be translated into safe and effective antiviral therapies.

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