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Host cell-intrinsic innate immune recognition of SARS-CoV-2

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged at the end of 2019 and caused the pandemic of coronavirus disease 2019 (COVID-19). Basic and clinical investigations indicate that severe forms of COVID-19 are due in part to dysregulated immune responses to virus infection. The innate immune system is the first line of host defense against most virus infections, with pathogen recognition receptors detecting SARS-CoV-2 RNA and protein components and initiating pro-inflammatory and antiviral responses. Notwithstanding this response, SARS-CoV-2 proteins evade, inhibit, and skew innate immune signaling early in infection. In this review, we highlight the components of cell-based recognition of SARS-CoV-2 infection and the mechanisms employed by the virus to modulate these innate immune host defense pathways.

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Introduction

The SARS-CoV-2 pandemic has caused hundreds of millions of infections and resulted in over 5 million deaths as of November 2021 [1]. Multiple vaccines have been developed, approved, and deployed, yet vaccine hesitancy, inadequate global distribution, and emergence of resistant and more transmissible SARS-CoV-2 variants have sustained its spread [2]. SARS-CoV-2 causes a range of clinical syndromes in humans including asymptomatic infection, mild to moderate upper respiratory infection, pneumonia, acute respiratory distress syndrome, hyperinflammatory disease, and long-term neurological/cognitive dysfunction [3]. The risk of developing severe and fatal COVID-19 depends on age, comorbidities, and genetic or acquired factors [4]. While most individuals infected with SARS-CoV-2 recover, many develop chronic debilitating health conditions [5].

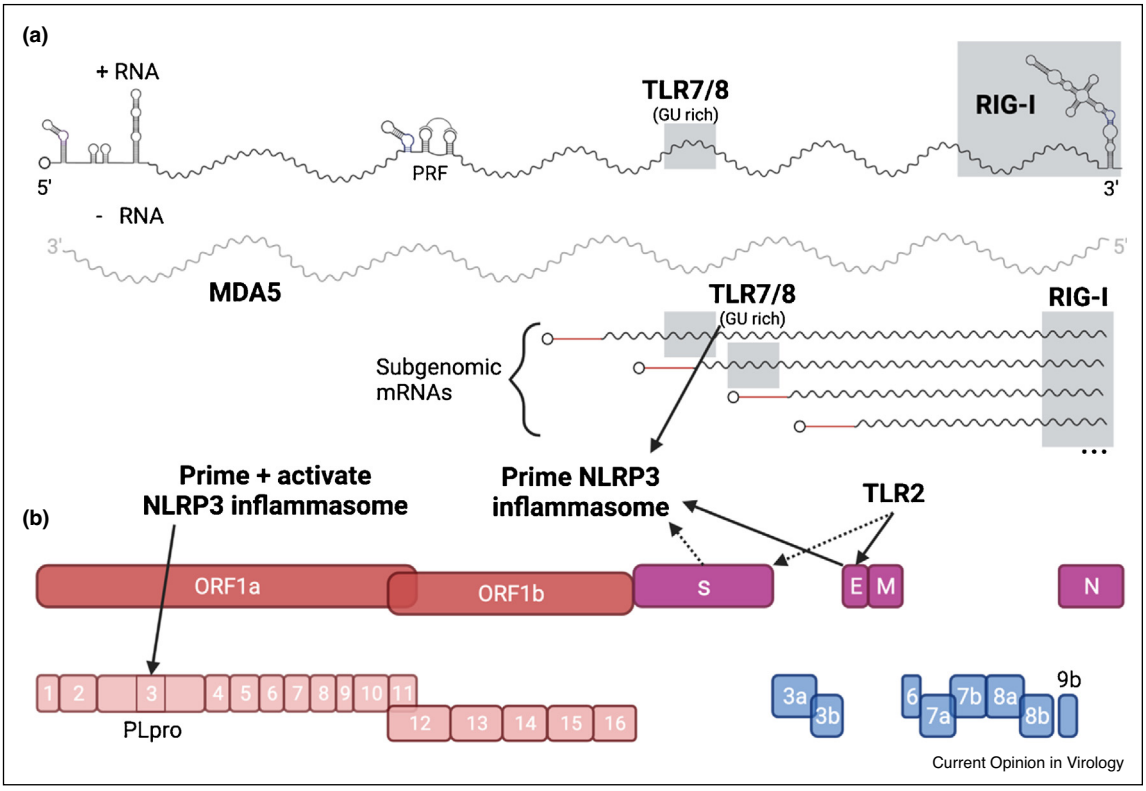
SARS-CoV-2 is a positive-sense single-stranded (ss)RNA Betacoronavirus in the *Coronaviridae* family. The ~30 kb capped and methylated RNA genome encodes 4 major structural proteins and 16 nonstructural proteins (Nsps) that mediate virus replication, infection, and pathogenesis (Figure 1). Additional accessory proteins encoded at the 3′ end of the genome have structural or immune evasion roles and vary among individual coronaviruses (CoVs). SARS-CoV-2 uses its spike (S) protein to infect cells [6]. The primary receptor for SARS-CoV-2 on human cells is ACE2, although other receptors exist [7–11]. Proteolytic cleavage of S by the serine protease TMPRSS2 on the cell surface or cathepsins in the endosome triggers conformational changes in S and fusion with the host plasma or endosomal membrane [8,12–14]. Replication of the viral genome occurs in endoplasmic reticulum (ER)-associated replication complexes and generates full-length genomic RNA and nested subgenomic RNAs from which structural and accessory proteins are translated. Virion assembly and budding occurs along the ER-to-Golgi network, and new virions are released by exocytosis [6].

Many steps of the CoV replication cycle co-opt host factors, reorganize subcellular structures, and generate pathogen associated molecular patterns (PAMPs). The cell-intrinsic host defense system uses multiple strategies to detect and restrict propagation of pathogens including SARS-CoV-2. Here, we review how host cellular sensors detect and induce an antiviral immune response to SARS-CoV-2 infection and describe some of the purported viral evasion strategies used to counteract these defenses. While substantial progress has been made eludcidating this host–pathogen interface, further research is needed to define immunomodulatory drug targets and their mechanisms of action, including ones currently being tested in humans [15–19].

Recognition by RIG-I-like-receptors

RIG-I like receptors (RLRs) are cytosolic pathogen recognition receptors (PRRs) that detect non-self RNA PAMPs. After RNA PAMP recognition, RIG-I and

Figure 1



SARS-CoV-2 replication components and PRR triggers.
(a) SARS-CoV-2 single-stranded RNA genome is capped and methylated. RNA structures of the 5′-UTR, 3′-UTR, and programmed ribosome frameshift (PRF) element are based off predicted structures in Ref. [83]. SARS-CoV-2 replication generates negative sense RNA intermediates and multiple subgenomic mRNAs. **(b)** SARS-CoV-2 nonstructural proteins (red) are translated as polyproteins and processed to indivual components. Structural proteins (purple) and accessory ORFs (blue) are translated from subgenomic mRNAs. Components that induce PRR pathways are labeled with PRR or indicated with solid arrows. Dashed arrows represent PRR induction after prior SARS-CoV-2 infection. Shaded regions are approximate. Created with BioRender.com.

MDA5, the two most prominent RLR members, associate at the mitochondrial membrane with the shared adaptor protein MAVS and trigger a TBK1-IRF3 signaling cascade that induces a type I and III interferon (IFN) response [20,21]. Which RLR primarily responds depends on the particular RNA virus and cell type [20,22]. Whereas CoVs can be detected by both RIG-I and MDA5, certain CoVs have evolved strategies to prevent RLR detection or downstream signaling pathways [22,23]. SARS-CoV-2 can be recognized by both RIG-I and MDA5, although the primary RLR sensor is cell type-dependent and influenced by post-transcriptional RNA modifications [7,24,25•,26–30].

Although RIG-I recognizes the 5′ end of non-capped RNAs, other RNA PAMP motifs such as polyU sequences or short hairpin structures contribute to RIG-I binding [20]. Indeed, RIG-I detects the 3′ end of positive sense SARS-CoV-2 RNA (Figure 1), as was observed with other unrelated RNA viruses [25•,26,29,31]. Recognition of the SARS-CoV-2 3′ end may be inhibited by *N*-6-methyladenosine (m^6A) post-transcriptional modification [24]. The m^6A modification reduces binding of RIG-I to synthetic RNAs, and viral RNA mutated to be m^6A deficient engage RIG-I more efficiently [32,33], induce a more robust IFN-β response, and are less pathogenic *in vivo* [33]. The SARS-CoV-2 genome has multiple m^6A residues within the *N* gene, and viral RNAs lacking these modified nucleotides immunoprecipitate with RIG-I more efficiently and induce greater expression of inflammatory genes in Caco-2 cells [24]. Further investigation using replication competent genomic SARS-CoV-2 RNA with mutated m^6A sites is needed to corroborate these findings.

An MDA5-mediated antiviral IFN response against SARS-CoV-2 requires viral replication [7,25•,26]. The SARS-CoV-2 negative strand RNA is likely the primary target of MDA5 recognition, as the negative strand RNA is more abundant than positive strand RNA in RNA

immunoprecipitation experiments with MDA5 (Figure 1) [25•]. Furthermore, SARS-CoV-2 infection fails to induce IFNs and IFN-stimulated genes (ISG) in the presence of the RNA-dependent RNA polymerase inhibitor Remdesivir [26]. The negative stand RNA may form long hairpin structures providing the double stranded RNA motif typically recognized by MDA5. However, a specific motif or region of the SARS-CoV-2 viral RNA recognized by MDA5 has not yet been defined.

Recognition by Toll-like-receptors

Toll-like receptors (TLRs) are a family of integral membrane PRRs. The receptor domains of TLRs are located on the cell surface or in intracellular vesicles (e.g. endosomes) with the signaling domain on the cytosolic face of the membrane [34]. While there are twelve TLR family members, only some contribute to virus recognition: TLR3, TLR7, TLR8, and TLR9 recognize viral nucleic acids, and TLR2 and TLR4 detect viral glycoproteins [34]. Upon PAMP recognition, TLRs dimerize and recruit the adaptor proteins MyD88 or TRIF, which triggers a signaling cascade that activates MAP kinases and NF-κB to induce transcription of proinflammatory genes. TLR signaling can also promote nuclear translocation of the transcription factors IRF-3 and IRF-7, which directly stimulate type I IFN and ISG expression [34].

In patients diagnosed with severe COVID-19, transcripts levels for *TLR1*, *TLR2*, *TLR4*, *TLR5*, *TLR8* and *TLR9*, along with the adaptor protein transcripts *MYD88* and *TRIF* are elevated compared to healthy patients [35,36]. A separate study reported lower levels of TLR3 expression in the peripheral blood of COVID-19 patients [37], and mutations in the *TLR3* gene were associated with severe disease [38]. Although TLR7 was elevated in patients with moderate but not severe COVID-19, other clinical reports suggest that individuals with *TLR7* genetic mutations, particularly males since *TLR7* is encoded on the X-chromosome, are at increased risk of severe COVID-19 [39–42].

TLR2 and TLR7/8 have been evaluated for their roles in the host response to SARS-CoV-2 infection [36,43,44]. SARS-CoV-2 E protein induces TLR2 signaling and induction of pro-inflammatory cytokines (Figure 1) [36]. IL-6 levels, among other cytokines, are reduced in $Tlr2^{-/-}$ mice compared to wild-type (WT) mice after exogenous administration of the SARS-CoV-2 E protein. Furthermore, therapeutic administration of a TLR2 inhibitor reduced cytokine production and partially protected against SARS-CoV-2 induced disease in mice [36]. There is one report of SARS-CoV-2 S protein inducing TLR2 signaling [45]. Macrophages derived from patients previously infected with SARS-CoV-2 produce IL-1β when stimulated with SARS-CoV-2 S protein. TLR2 likely mediates this response, because IL-1β production is reduced when macrophages are pretreated with TLR2 inhibitors or blocking antibodies before S protein stimulation [45].

TLR7 and 8 bind guanosine/uridine (GU) rich ssRNA [34]. Analysis of the SARS-CoV-2 genome highlights regions that could be recognized by TLR7 and 8, including motifs in the spike protein gene [44,46]. Synthetically generated SARS-CoV-2-specific GU rich RNAs act as TLR8 ligands in myeloid cells and were more pro-inflammatory than ligands produced from SARS-CoV-1 or HIV-1 RNA40, the latter a TLR8 ligand standard [44,46,47]. Although confirmatory evidence is required in cells and animal models of infection, the TLR8 response exhibited by SARS-CoV-2 synthetic RNAs and the likely abundance of ssRNA produced during SARS-CoV-2 infection could contribute to the pathological inflammation observed in some COVID-19 patients.

Inflammasome activation and recognition by NOD-like-receptors

Activation of the NLRP3 inflammasome requires two signals: (i) upregulation of NLRP3 expression after induction of a TLR/NF-κB pathway; and (ii) a trigger to assemble NLRP3 into the inflammasome complex, which can be a viral PAMP. After assembly of the NLRP3 inflammasome complex, pro-caspase-1 is cleaved into active caspase-1, which in turn cleaves pro-IL-1β and pro-IL-18 to their bioactive and secreted forms. Activation of the NLRP3 inflammasome also can lead to pyroptosis, a pro-inflammatory form of programmed cell death [48].

NLRP3 is upregulated and inflammasome formation is induced by multiple SARS-CoV-2 PAMPs, including GU-rich RNAs, E, and ORF3a proteins (Figure 1) [36,46,49]. In macrophages, synthetic GU-rich RNAs corresponding to SARS-CoV-2 sequences activate the NLRP3 inflammasome resulting in IL-1β production without induction of pyroptosis [46]. Activation of TLR2 by SARS-CoV-2 E protein in macrophages resulted in increased levels of *NLRP3* mRNA and cleaved caspase-1 [36]. The viroporin ORF3a of SARS-CoV-2 both primes and activates the NLRP3 inflammasome through K^+ efflux [49].

Although SARS-CoV-2 S protein fails to prime or activate the NLRP3 inflammasome in macrophages isolated from healthy mice and humans [37,46], one group reported that it can prime the NLRP3 inflammasome in cells derived from patients with prior SARS-CoV-2 or tuberculosis infection [36,45]. Macrophages isolated from previously infected individuals had distinct transcriptional signatures with epigenetic markers associated with innate immune memory. It is hypothesized that SARS-CoV-2 exposure leaves myeloid cells in a pre-primed state for assembling a functional NLRP3 inflammasome, and this can last several weeks after infection is cleared [45].

Like NLPR3, NOD1 is a member of the NOD-like-receptor (NLR) family but does not oligomerize to form an inflammasome. Instead, NOD1 activation leads to expression of pro-inflammatory cytokines and type I IFNs via NF-κB and IRF-3 or IRF-7 signaling. While usually described in the context of bacterial infection, NOD1 also serves as a pro-inflammatory mediator during viral infections [50]. Indeed, silencing of *NOD1* expression in Calu-3 cells reduced the amount of IFN-β mRNA induced following SARS-CoV-2 infection and resulted in greater infection [26]. More studies are needed to define the role and mechanism of action for NLRs in the context of host defense against SARS-CoV-2 infection.

Activation of cGAS-STING signaling

An innate immune response also can be mounted by cytosolic sensors of damage associated molecular patterns (DAMPs). Cytosolic DNA, either from a pathogen, the nucleus, or the mitochondria, serves as a potent DAMPs during microbial infections. The cGAS-STING signaling pathway detects cytosolic DNA and produces type I IFNs [51]. This pathway occurs in two steps: (i) cGAS detects cytosolic DNA and produces the cyclic dinucleotide cGAMP; and (ii) cGAMP binds the mitochondrial associated receptor STING, which initiates proinflammatory and type I IFN gene expression. For RNA virus infections that cause mitochondrial damage, leakage of mitochondrial DNA into the cytoplasm can activate the cGAS-STING pathway [51–54]. There are no reports that SARS-CoV-2 is directly detected by cGAS-STING, which is not surprising given that SARS-CoV-2 does not produce DNA intermediates during infection [55].

Activation of the STING pathway has been reported after SARS-CoV-2 infection of Calu-3 cells, although exogenously expressed SARS-CoV-2 proteins can inhibit cGAS-STING-induced IFN-β promoter activity [56,57]. Some studies assessing the importance of cGAS-STING signaling during SARS-CoV-2 infection focused on small molecule agonists of the pathway as a potential therapy [56,58,59•]. diABZI, an agonist of STING, had antiviral activity against SARS-CoV-2 both in cells and mice [56,58]. The antiviral activity of diABZI depended on JAK1/2 signaling, although another version of the drug, diABZI-4 promoted IFN-independent antiviral effects [56,59•]. Therapeutic administration of diABZI or diABZI-4 protected against SARS-CoV-2 induced disease in mice. Further investigation into the mechanism of cGAS-STING mediated protection by diABZI compounds appears warranted.

ISGs

A major response to IFN signaling is the production of ISGs [21]. ISGs can modulate the infection of many viruses, although the mechanism of action for many of these proteins remains undefined [53,60]. There are a few specific antiviral ISGs that have been investigated in the context of SARS-CoV-2 replication [61,62,63••].

ISG15 has multiple ascribed antiviral functions including ISGylation, the covalent attachment of ISG15 to a target protein [64]. ISG15 is upregulated rapidly in airway epithelial cells after SARS-CoV-2 infection [63••,65•] and appears required for optimal MDA5 signaling [63••]. The SARS-CoV-2 papain like protease (PLpro) of Nsp3 antagonizes MDA5 signaling by removing ISG15 from the MDA5 caspase activation and recruitment domains (CARD), and inhibiting CARD oligomerization and downstream signaling [63••,65•]. PLpro also cleaves ISG15 from other host proteins resulting in elevated levels of free ISG15 in macrophages [65•], which results in increased expression of pro-inflammatory cytokines including IL-1β and IL-6 and reduced antigen presentation [65•].

LY6E is an ISG that restricts infection of SARS-CoV-2 and other CoVs by blocking entry into cells in contrast to phenotypes with unrelated viruses, where its expression enhanced infection [61,62,66–68]. Although data on the role LY6E in SARS-CoV-2 pathogenesis is lacking, studies the distantly related murine hepatitis virus showed that mice lacking LY6E expression in immune cells sustained greater infection in the spleen, weight loss, and fatal disease than in WT, congenic animals [61].

Innate immune evasion strategies of SARS-CoV-2

SARS-CoV-2 has multiple strategies to evade the host innate immune system similar to other pathogenic CoVs (Figure 2) [23,69]. Limiting the immune response by reducing IFN levels is advantageous for SARS-CoV-2, as it appears more susceptible to type I IFN than other pathogenic CoVs [70]. Multiple SARS-CoV-2 proteins reportedly inhibit type I IFN induction, signaling, or both [71•,72,73].

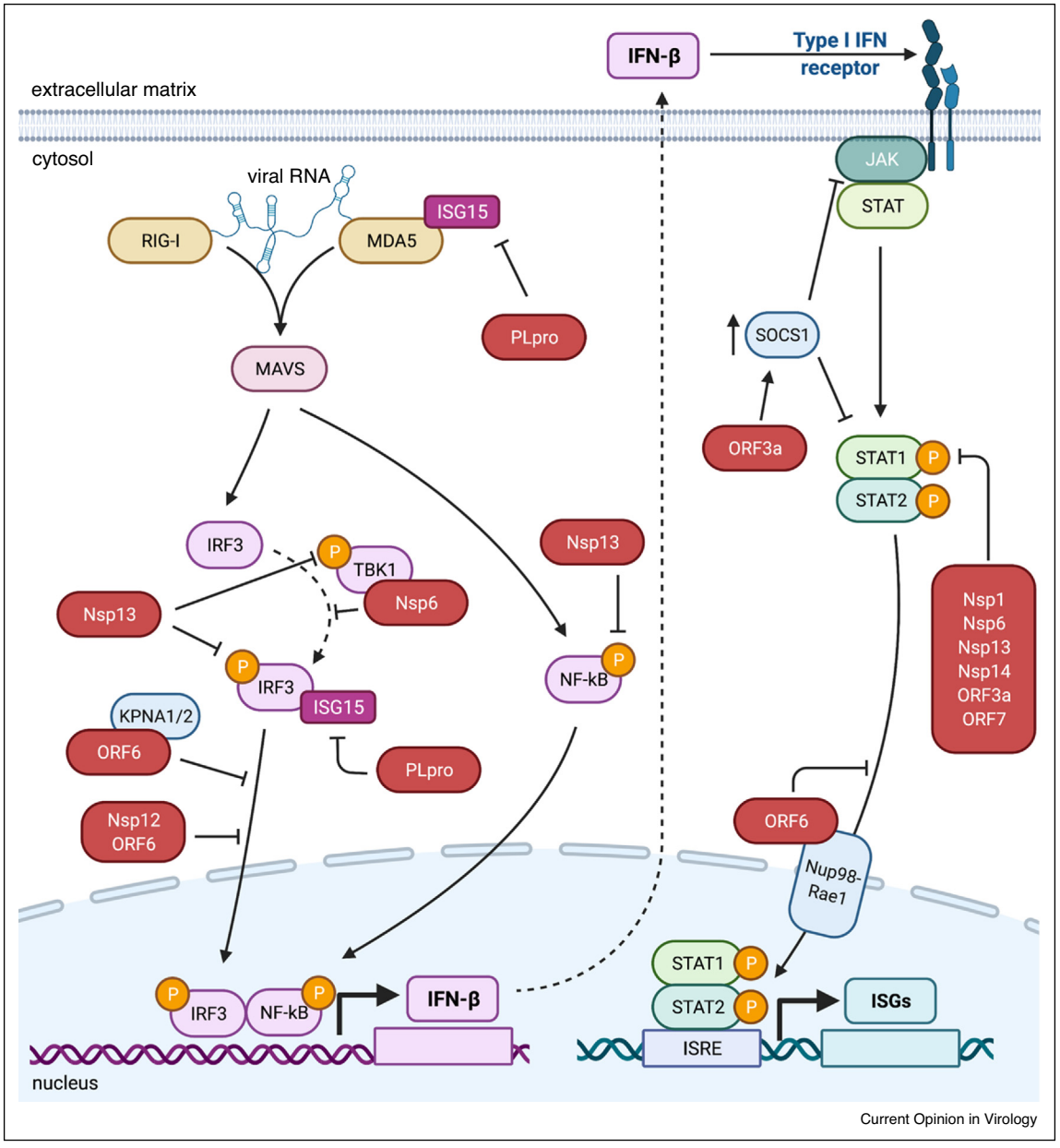
Host translation shut-off

The Nsp1 protein is a potent IFN antagonist that blocks IFN induction and signaling [71•,72,73]. This is achieved by interacting with ribosomes to prevent translation of host mRNAs [71•,72,73–75]. SARS-CoV-2 specific mRNAs evade translation inhibition because of a stem loop structure in the 5′ untranslated region (UTR) of all viral transcripts [75]. Nsp14 also may inhibit cellular translation in a manner that is enhanced by Nsp10 [74].

Host protein cleavage

SARS-CoV-2 PLpro is a protease domain of Nsp3 that cleaves both viral and host proteins. PLpro antagonizes MDA5 signaling through de-ISGylation of MDA5 and IRF3 [65•,76]. While PLpro has some de-ubiquitination activity, it appears more specific for ISG15ylated than ubiquitinated substrates [63••,76,77].

Figure 2



SARS-CoV-2 inhibition of IFN induction and signaling.
Key components of IFN induction and signaling are shown with solid arrows denoting signaling pathway. SARS-CoV-2 proteins (red) that are repotyed to inhibit different steps in the pathway. Direct interacting partners of SARS-CoV-2 proteins are shown as overlapping. SARS-CoV-2 proteins that inhibit phosphorylation steps are shown with flat head arrows toward phosphorylation symbols (yellow circles). Proteins that inhibit translocation events are shown with flat arrows towards translocation path. Created with BioRender.com.

Inhibition of phosphorylation

Multiple SARS-CoV-2 proteins reportedly reduce phosphorylation of PRR and IFN signaling pathway intermediates. Nsp1, Nsp6, Nsp13, Nsp14, ORF3a, ORF7a, and ORF7b all have been reported to prevent or reduce phosphorylation of STAT1 and 2 [71•,78]. Nsp6 also directly binds to TBK1 to block phosphorylation of IRF3 [71•]. Nsp13 also inhibits the phosphorylation of IRF3, in addition to TBK1 and NF-κB, although the mechanism remains uncharacterized [73].

Preventing transcription factor translocation

Because many innate immune signaling pathways converge on transcription factors, they are commonly

targeted by viral proteins. SARS-CoV-2 proteins Nsp12 and ORF6 prevent the nuclear translocation of IRF3, and Nsp13 prevents translocation of NF-κB [72,73,79,80]. ORF6 also prevents translocation of STAT1 into the nucleus [71•,72,81]. The C-terminal domain of ORF6 is required to prevent translocation of both proteins [71•,72,73], however the direct binding partners to prevent the translocation of STAT1 and IRF3 differ. ORF6 binds the nuclear importin KPNA2 and possibly KPNA1 to block translocation of IRF3, whereas ORF6 interacts with the Nup98-Rae1 nuclear pore complex to disrupt nuclear import of STAT1 [71•,81].

Regulating host protein expression

ORF3a can antagonize IFN signaling by promoting JAK2 ubiquitination and degradation, and upregulating the negative regulator SOCS1 at both the transcript and protein level [82]. Silencing of SOCS1 alleviated the ORF3a inhibition of STAT1 phosphorylation and JAK2 degradation [82].

Despite intensive study on the mechanisms of antagonism and evasion of IFN induction and signaling by SARS-CoV-2 proteins, many questions remain. To date, all of the studies have used ectopic expression systems in cells, and usually a single SARS-CoV-2 protein is expressed at a time. Several of these findings must be corroborated with infectious virus in primary cells. Moreover, exploring synergistic relationships between evasion proteins (e.g. Nsp10 and Nsp14 [74]) may explain how SARS-CoV-2 infection evades host defense responses in specific cell types.

Conclusions

Much has been learned about the interface between cell-intrinsic immunity and and SARS-CoV-2 infection in the past 22 months. The rapidity of discovery has been aided by decades of research on other CoVs before the pandemic. Nonetheless, many questions remain, the answers of which could impact the development of drugs against SARS-CoV-2 including those targeting innate immunity. While detection of SARS-CoV-2 by RLRs, NLRs, TLRs, the inflammasome, and responses by the cGAS-STING pathway all contribute to the initial response to infection, multiple SARS-CoV-2 proteins antagonize IFN induction and subsequent signaling, which likely delays the control of virus replication and spread. Beyond the early host defense response, an overexuberant innate immune reaction in the later stages of COVID-19 likely contributes to more severe disease. Indeed, limiting the innate immune response with the JAK inhibitor, baricitinib in combination with antiviral treatment improved the clinical status of hospitalized COVID-19 patients [18,19]. A better understanding of the mechanisms of innate immune restriction of SARS-CoV-2 and the viral pathways of evasion could facilitate deployment of targeted therapies that restrict SARS-CoV-2 replication more rapidly without causing pathological inflammation.

Conflict of interest statement

M.S.D. is a consultant for Inbios, Vir Biotechnology, and Carnival Corporation, and on the Scientific Advisory Boards of Moderna and Immunome. The Diamond laboratory has received unrelated funding support in sponsored research agreements from Vir Biotechnology, Moderna, and Emergent BioSolutions.

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