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SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon

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SARS-CoV is a pathogenic coronavirus that emerged from a zoonotic reservoir, leading to global dissemination of the virus. The association SARS-CoV with aberrant cytokine, chemokine, and Interferon Stimulated Gene (ISG) responses in patients provided evidence that SARS-CoV pathogenesis is at least partially controlled by innate immune signaling. Utilizing models for SARS-CoV infection, key components of innate immune signaling pathways have been identified as protective factors against SARS-CoV disease, including STAT1 and MyD88. Gene transcription signatures unique to SARS-CoV disease states have been identified, but host factors that regulate exacerbated disease phenotypes still remain largely undetermined. SARS-CoV encodes several proteins that modulate innate immune signaling through the antagonism of the induction of Interferon and by avoidance of ISG effector functions.

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SARS-CoV: the first viral pandemic of the new millenium

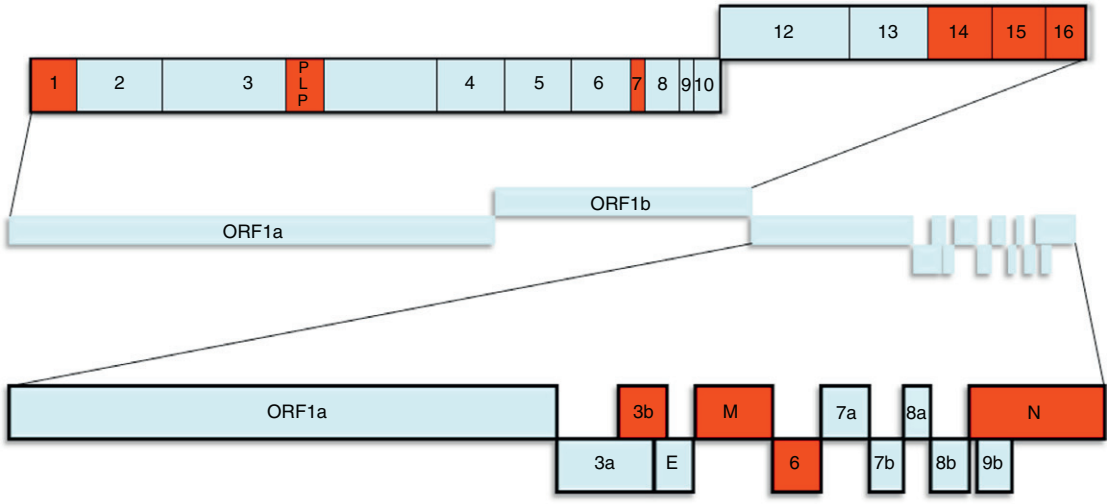
In 2002 the first viral pandemic of the millennium emerged from the Guangdong province in Southern China. Severe Acute Respiratory Syndrome (SARS) presented as initial 'flu-like' symptoms (cough, sore throat, and fever) that could progress to atypical pneumonia in patients with severe SARS disease [1,2]. A rapid response from scientists identified a novel coronavirus as the causative agent of SARS, named SARS-Coronavirus (SARS-CoV, Figure 1a) and angiotensin converting enzyme 2 (ACE2) as the viral receptor [3]. Despite identification of the virus, the disease spread from China to other Southeast Asia countries, becoming a global threat with significant outbreaks reported in Singapore, Hong Kong, Taiwan, and Canada [4]. At the end of the epidemic, 774 of the 8096 confirmed cases resulted in death (a mortality rate of 9.6%) [5]. By July of 2003 the virus was controlled by public health measures, but no vaccines or antivirals are currently approved for the treatment of SARS-CoV should the virus re-emerge [6,7].

SARS disease in patients with poor outcome was marked by the progression to Acute Respiratory Distress Syndrome (ARDS): approximately 25% of SARS cases were diagnosed with ARDS and the ARDS-associated mortality rate exceeded 50%[8]. Elderly SARS patients had a poor prognosis with mortality rates of 50% in patients over 65 year of age [9]. In SARS patients with ARDS, the acute phase characterized by pulmonary edema, severe hypoxia, and the accumulation of inflammatory cells in the lungs could progress to ARDS late phase fibrosis, organizing pneumonia, systemic inflammation responses, and multiple organ failure [10,11]. Consistent with ARDS progression, the primary targets of SARS-CoV infection are ciliated cells of the airway epithelium and alveolar Type II pneumocytes [12,13]. ARDS is also associated with the induction of inflammatory cytokines including IL-1, IL-6, IL-8, CXCL-10, and TNFα, many of which were highly expressed in the lungs of SARS patients [14,15]. In many viral infections the antiviral cytokine Interferon (IFN) acts not only to control viral infections, but also to program the adaptive immune response to promote viral clearance [16]. However, in patients with severe SARS disease, aberrant IFN, Interferon Stimulated Genes (ISGs), and cytokine responses were observed compared to healthy individuals providing evidence that SARS is an innate immune regulated disease [17,18].

Elucidation of innate immune pathogenesis mechanisms through models of SARS-CoV infection

Initial models of SARS-CoV innate immune pathogenesis were viral infection of cell lines including Vero E6, Caco-2, and Huh-7 cells, as well as PBMCs; however, these systems may not yield relevant biological information consistent with SARS-CoV infection of pneumocytes, because they are not derived from lung tissues [19–21]. Human Airway Epithelial Cultures (HAEs) are primary cell lines of pseudostratified mucocilliary epithelium that replicate the morphological and physiological characteristics of human airways. HAEs can be infected with SARS-CoV, are derived directly from normal lung tissues,

Figure 1



SARS-CoV Protein	Innate Immune Function	References
nsp1	Antagonizes IFN; inactivates host translation; degrades host mRNA; blocks phosphorylation of STAT1	[73-75]
PLP	Antagonizes IFN; interacts with STING; blocks IRF Phosphorylation; blocks NF- κB signaling	[76-78]
nsp7	Antagonizes IFN by undescribed mechanism	[77]
nsp14	Guanine-N7-methyltransferase activity part of viral RNA capping machinery for evading host detection	[68,70]
nsp15	Antagonizes IFN by undescribed mechanism	[77]
nsp16	2'-O-methlytransferase activity modifies the cap of viral RNAs for evading host detection	[68,69,71]
ORF3b	Antagonizes IFN signaling induced by MAVS/RIG-I	[80,86]
Membrane	Antagonizes IFN signaling through kinases TBK1/IKKε	[82]
ORF6	Antagonizes IFN by blocking nuclear import of STAT1	[80,83,87-89]
Nucleocapsid	Antagonizes IFN by unknown mechanism	[79,80]

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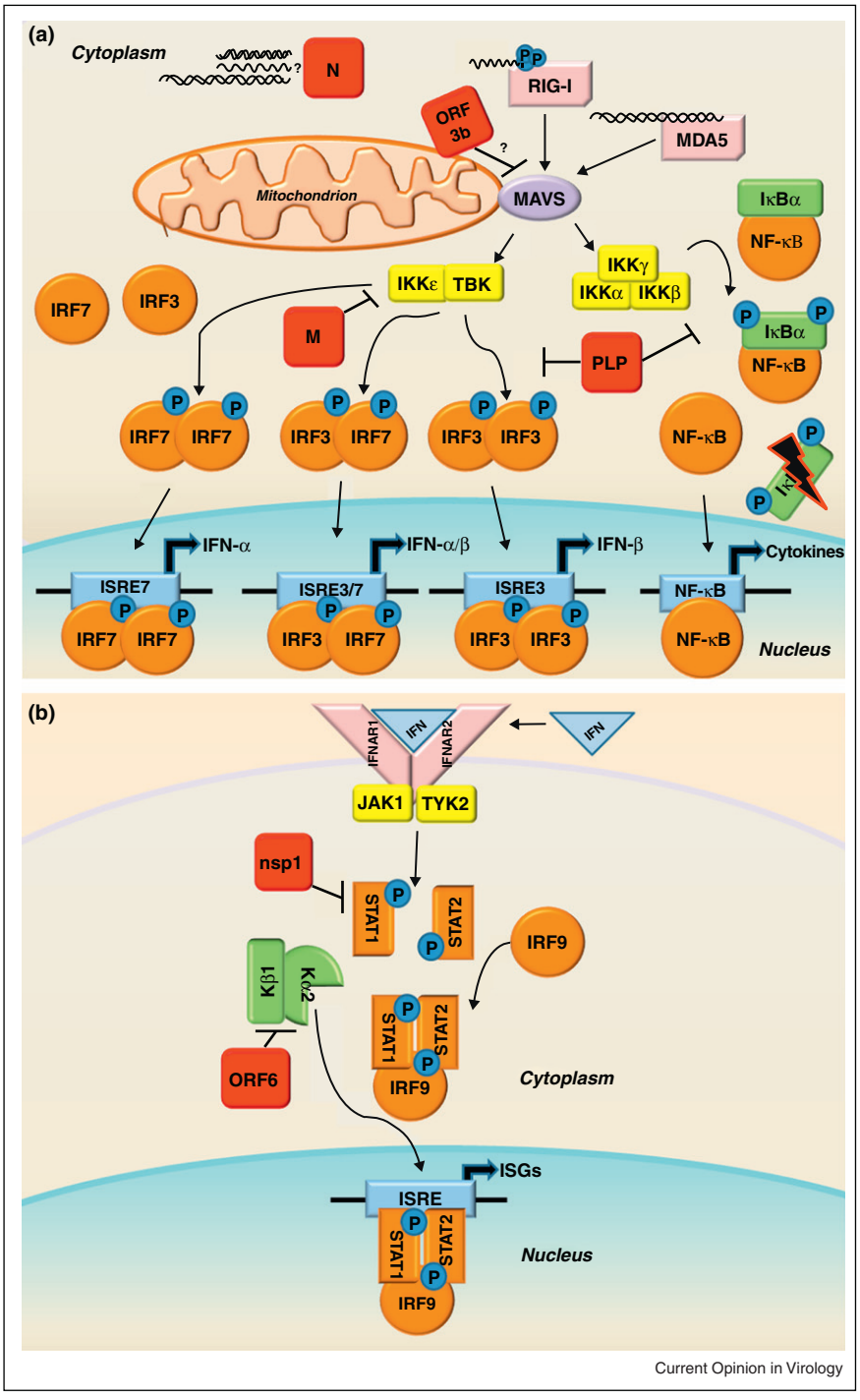
The SARS-CoV genome and functions of SARS-CoV innate immune antagonists. **(a)** The typical coronavirus genome size is quite large in comparison to many other positive-sense RNA viruses; within the SARS-CoV genome of 29.7 kB at least ten genes with potential functions that modulate innate immunity have been characterized (highlighted here in red). Like other members of the viral family *Coronaviridae*, SARS-CoV has a positive-sense, single-stranded RNA genome that is amenable to manipulation using reverse genetic techniques [90]. In SARS-CoV the first open reading frame (ORF) encodes the 16 nonstructural proteins that make up the viral replicase, while the ensuing ORFs encode four structural proteins that compose the virion, as well as eight accessory proteins. The SARS-CoV accessory proteins share no homology to the accessory proteins of other human coronaviruses, and while dispensable for replication *in vitro*, encode functions that probably impact viral pathogenesis *in vivo* [91]. While SARS-CoV was a novel virus not previously recognized before the 2002 outbreak, other coronaviruses have been associated with disease in humans. Coronaviruses known to infect humans include HCoV-HKU1, HCoV-OC43, HCoV-229E, and HCoV-NL63, which also cause respiratory infections but are generally much less severe than SARS [92]. **(b)** Transcription and subsequent signaling of Interferon is vital for activating the antiviral response in host cells. Because of this, many viruses (including SARS-CoV) encode proteins that antagonize the IFN response to viral infection. Of the SARS-CoV viral proteins listed here, eight have been identified as Interferon antagonists and two have been implicated in the viral RNA capping machinery.

Figure 2



(a) RLR family of innate immune receptors induce Type I interferon. The family of RIG-I Like Receptors (RLRs) contains three cytosolic RNA helicases that recognize non-self RNA species resulting from viral replication [93]. The two signaling sensors within the RLR family are retinoic acid-inducible gene I (RIG-I) and melanoma differentiation associated factor 5 (MDA5). The third RLR, laboratory of genetics and physiology 2 (LGP2, not shown), facilitates recognition of viral PAMPs by RIG-I and MDA5, but is dispensable for their signaling [94]. RIG-I recognizes primarily 5′ppp-RNA molecules with secondary motifs of dsRNA or ssRNA of short length [95,96]. MDA5 recognizes longer dsRNA motifs than RIG-I [97]. Following binding of viral RNAs, RIG-I and MDA5 interact with the mitochondrial membrane bound adaptor molecule MAVS (mitochondrial antiviral signaling protein, also referred to as IPS-1, VISA, or CARDIF) to transduce the signal via complexes of kinases: the IKKε/TBK1 complex and the IKKα/IKKβ/IKKγ complex. The IKKε/TBK1 kinases phosphorylate the transcription factors IRF3 and IRF7, which then form homodimers or heterodimers. Upon dimerization, the transcription factors enter the nucleus to initiate transcription of Type I IFNs (IFN-α and IFN-β). While IRF3 is nearly ubiquitously expressed in cells, IRF7 is an ISG typically expressed at low levels, so it is thought that IRF3 mediates transcription of the majority of early IFN expression.

and contain the relevant epithelial cell types within human airways for SARS-CoV infection, but HAEs are difficult to procure and are highly heterogeneous [13,22]. Recently, the 2B4 cell line derived from a clonally selected Calu-3 cell population with high expression of ACE2 (the SARS-CoV receptor) was developed that forms differentiated pseudostratified columnar epithelia highly permissible to SARS-CoV infection. SARS-CoV infection of 2B4 cells provides data on innate immune responses within a biologically relevant and easily replicated *in vitro* system [23••].

Small animal models of SARS-CoV infection have benefits into the elucidation of innate immune pathogenesis beyond cell culture systems due to their ability to model the interaction of lung epithelium and immune cell types within an infected organism. While hamsters and ferrets have been considered for use as small animal models of SARS-CoV infection, a robust mouse model has been more vigorously pursued because of the relative ease of genetic manipulation of the host, as well as greater availability of immunological reagents [24–26]. SARS-CoV epidemic isolates replicate in young mice but do not cause clinical disease, limiting the use of these models for pathogenesis studies [27,28]. SARS-CoV infected aged mice (12 months) exhibit minor clinical illness, but do not address pathogenic mechanisms associated with SARS disease in senescent or non-senescent populations [29–31]. Infections using the mouse coronavirus MHV-1 have also been proposed as models for SARS-CoV infection [32]. Recently, mouse adapted SARS coronaviruses (MA-SARS-CoV) have been developed by serial passage through the lungs of mice yielding several different MA-SARS-CoV strains [33,34]. Infection of 6-10 week old mice with SARS-CoV adapted by 15 serial passages (MA15-SARS-CoV) causes morbidity and mortality, viral replication in the lungs, and lung pathology associated with mild SARS disease [33,34]. In addition, MA-SARS-CoV infections of aged mice exhibit exacerbated SARS disease that mimics the age-dependent and ARDS phenotypes seen in humans [35,36•]. Currently, studies are underway to determine the response of recombinant inbred lines of mice (known as the Collaborative Cross) to MA15-SARS-CoV infection, utilizing Genome Wide Associate Studies to map quantitative trait loci that contribute to *in vivo* phenotypes (e.g. weight loss or lung pathology) [37•]. These studies offer an unbiased approach to determining the contributions of many different genes to the complex trait of SARS-CoV disease, and could identify novel host factors involved in SARS-CoV pathogenesis.

The use of primate models of SARS-CoV infection is typically limited due to ethical concerns and expense. However, infection of nonhuman primates with SARS-CoV is a model more relevant to humans for testing of drug treatments and vaccines. SARS-CoV replicates in the lungs of primate species, including African green monkeys, cynomolgus macaques, and rhesus macaques [38]. Infection of cynomolgus macaques with SARS-CoV replicates aspects of the human disease, including lung pathology of diffuse alveolar damage (DAD) found in humans [39]. Additionally, a comparison of SARS-CoV infection of young adult cynomolgus macaques to aged cynomolgus macaques found age-dependent susceptibility to SARS disease resembling the same trend in humans [40••]. More recently, it has been shown that SARS-CoV causes increased severity of disease in African green monkeys compared to cynomolgus macaques, and that the increased lung injury is probably associated with differential innate immune signaling [41•].

Host antiviral innate immune detection and response to SARS-CoV infection

Innate immune signaling is the earliest differentiation of pathogens from cellular molecules that alerts host cells to the presence of invading viruses. Pattern Recognition Receptors (PRRs), such as the RIG-I-Like Receptors (RLRs, Figure 2a) and Toll-Like Receptors (TLRs, Figure 3a and b) recognize Pathogen Associated Molecular Patterns (PAMPs) from viral components or replication intermediates, resulting in signaling cascades that initiate an antiviral state in cells as a result of infection [42,43]. PRRs are distributed on plasma membranes, endosomal

(Figure 2 Legend Continued) The IKKα/IKKβ/IKKγ kinases phosphorylate IκBα, targeting this repressor protein of NF-κB for degradation. Activation of NF-κB leads to transcription of proinflammatory cytokines, and NF-kB mediated transcription has also been linked to the pathogenesis of ARDS [46]. SARS-CoV encodes proteins that antagonize RLR family signaling, shown here in red.**(b)** Interferon signals through the JAK-STAT pathway to induce interferon stimulated genes. The secretion of IFN-α and IFN-β molecules from an infected cell leads to an autocrine and paracrine signaling through the IFNαβ Receptor (composed of the IFNAR1 and IFNAR2 subunits) resulting in the activation of the JAK-STAT pathway. The JAK/TYK2 kinases phosphorylate the transcription factors STAT1 and STAT2, which form heterodimers complexed with IRF9. The STAT complex translocates to the nucleus leading to the transcription of Interferon Stimulated Genes (ISGs) that establish an antiviral state in the cell. Because neighboring cells can receive IFN stimulation before infection, it is a crucial pathway to preventing viral spread in the host. SARS-CoV also encodes proteins that antagonize the JAK-STAT pathway, shown here in red. Mice deficient in STAT1 showed an increased susceptibility to SARS-CoV infection [98]. Although there were no differences in mice deficient in IFN receptors, STAT1−/− mice showed increased weight loss, viral titer, and lung pathology compared to wild type over the course of MA15-SARS-CoV infection, demonstrating that STAT has important IFN independent role in SARS-CoV infection [80]. Severe lung pathology in STAT1−/− mice infected with MA15-SARS-CoV was associated with the infiltration of immune cells and fibrotic lung response. The STAT1−/− dependent prolonged expression of inflammatory cytokines (IL-1, IL-6, IL-10, IL-12, and TNFα) and chemokines (CCL2, CCL3, CCL4, CCL7, and CCL20), could be a transcriptional regime responsible for fibrotic phenotypes within the lungs. Additionally, ISG responses were significantly lower in STAT1−/− mice compared to wild type or IFNAR−/− mice, leading to the conclusion that STAT1 dependent, IFNAR1 independent ISG expression was protective in these mice [80]. It remains unclear how STAT1 controls ISGs independent of IFNAR expression, or which ISGs have important potential roles in SARS-CoV pathogenesis.

membranes, and within the cytosol of host cells to ensure maximal detection of viral PAMPs including nucleic acid motifs, carbohydrate moieties, glycoproteins, lipoproteins or other small molecules present within the viral life cycle, but absent from normal cellular components.

RIG-I like receptor signaling

The RIG-I Like Receptors are cytoplasmic sensors that detect viral RNA PAMPs in a wide range of cell types (Figure 2a). The RLRs RIG-I and MDA5 are ISGs that are transcribed during SARS-CoV infection *in vitro* [23••]. MHV, another coronavirus, is recognized by MDA5 in brain macrophages and microglial cells, and by RIG-I and MDA5 in oligodendrocyte cells [44,45•]. Although it is not known whether SARS-CoV is recognized by RLRs, MHV and SARS-CoV are likely to have similar replication intermediates (putative RLR ligands), so it is likely that SARS-CoV could be detected by the same sensors. RLR signaling leads to the activation of several transcription factors: IRF3, IRF7, and NF-κB. IRF3and IRF7 initiate transcription of Type I IFNs (IFN-α and IFN-β), important for an antiviral response. NF-κB mediated transcription of proinflammatory cytokines has been linked to the pathogenesis of ARDS [46]. *In vitro* SARS-CoV infections have demonstrated that the expression of NF-κB generated transcripts, such as IL-6 and IL-8, happens as early as 12 h post infection, while IRF3/IRF7 transcription of Type I IFNs is delayed until 48 h post infection [23••]. Similarly, in the macaque model of age-dependent SARS-CoV pathogenesis NF-κB induced genes are more highly expressed in aged macaques that have significantly increased lung injury compared to young adult macaques where higher expression of IFNs was observed [40••]. While the correlation of severe SARS-CoV disease with different transcriptional regimes is promising, the key to finding determinants of increased SARS-CoV pathogenesis may be how innate immune sensing mechanisms initiate transcription at critical junctures during infection and which types of innate immune sensing are protective.

Toll-Like Receptor signaling

The Toll-Like Receptor family of membrane bound sensors also recognizes viral PAMPs, although no TLR has been directly implicated in the recognition of SARS-CoV. On the surface of cells, TLR1, TLR2, TLR4, and TLR6 have been implicated in the recognition of PAMPs from other viruses (Figure 3a), while the endosomal receptors TLR3, TLR7, TLR8, and TLR9 detect viral nucleic acid PAMPs (Figure 3b). TLR4 recognizes viral glycoproteins of RSV, is expressed on the surface of lung epithelium, is a potential entry co-factor for respiratory viruses, and has been identified as a protective host factor against MHV-1 in a respiratory model of SARS disease [47–49]. Transcription of TLRs increased in mice following infection with MA15-SARS-CoV and in human dendritic cells infected with SARS-CoV [50••,51]. Additionally, the activation of TLR3 has protective effects in a mouse model of SARS-CoV infection [52]. While there are many TLRs that recognize viral PAMPs, they signal through the common adaptor molecule MyD88, with the exception of TLR3 that uses the adaptor TRIF (Figure 3a and b). Infection of MyD88$^{-/-}$ mice established a protective role for TLR adaptors in MA15-SARS-CoV infection: while wild-type mice experienced transient weight loss, from which they recovered after 7 days, MyD88$^{-/-}$ mice lost significantly more weight, and all of the MyD88$^{-/-}$ mice died by day 6 post-infection [53]. Additionally, higher viral loads, severe lung pathology and differences in cytokines and chemokines were observed in MyD88$^{-/-}$ mice compared to wild-type mice [53]. Currently, studies are in progress to determine the roles of other TLR adaptor proteins (TRIF, MAL, and TRAM) in SARS-CoV infections, as well as what TLR(s) or TLR ligand(s) contribute to the protective role of MyD88.

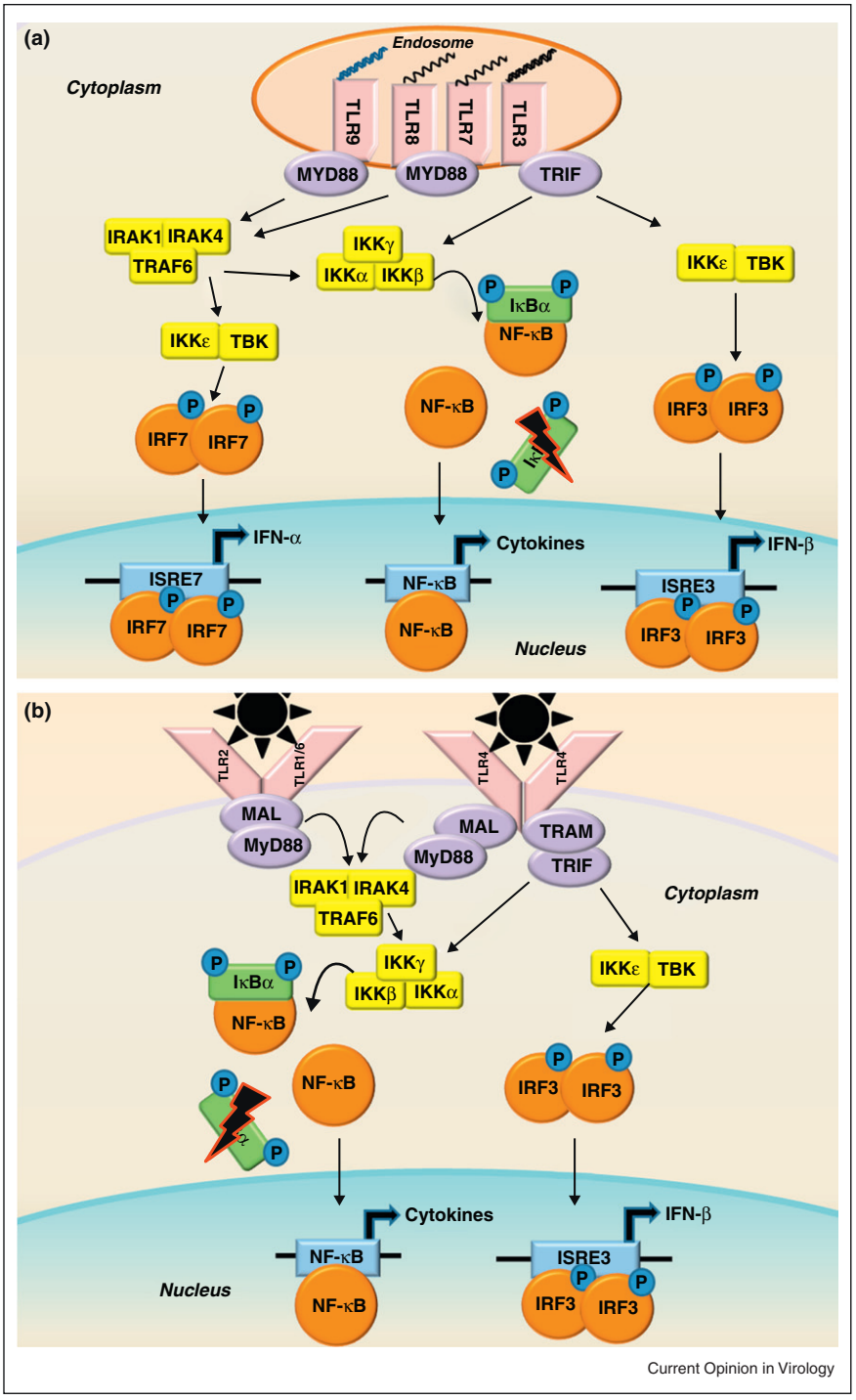
Innate immune signaling effector molecules and SARS-CoV pathogenesis

Following detection of virus by the host cells, the production of cytokines, chemokines, and ISGs continues the innate immune response to viral infection by mediating inflammation and cellular antiviral processes. The importance of these effector responses to the prognosis of SARS patients is underscored by the observation that IFN (Type I and Type II IFN), chemokine (CXCL10 and CCL2), and ISG (CIG5, MXA, IFITM1, IFIT3) hyperimmune responses persisted in patients who succumbed to SARS, indicating that differences in expression patterns of innate immune effector molecules may be a determinant of SARS disease outcome [18].

Interferon

Interferons are potent cytokines of critical importance in controlling viral infections and priming adaptive immune responses [54]. Several studies of antiviral treatments tested against SARS-CoV replication show administration of Type I IFN inhibits SARS-CoV growth in cell culture as well as viral replication in cynomolgus macaques and mouse models [39,40••,55–59]. Despite the potential importance of IFNs in controlling SARS-CoV replication, infection of mice deficient in Type I, II, or III IFN receptors showed minimal phenotypic difference in weight loss, viral titer, lung pathology, and mortality from wild-type mice in the MA15-SARS-CoV model [60•]. However, mice deficient in STAT1, a critical transcription factor for IFN signaling (Figure 2b), were significantly more susceptible to MA15-SARS-CoV infection than wild-type or IFNAR1$^{-/-}$ mice [60•]. Transcriptional analysis from these studies showed that ISGs were induced even in the absence of IFNAR1, demonstrating that there may be compensatory mechanisms through other innate immune signaling to protect against severe SARS-CoV disease in the absence of IFN [50••]. In the SARS-CoV infection model, mice deficient in Type I,

Figure 3



Pathogen associated molecular pattern sensing by Toll-Like Receptors. **(a)** In the endosomal compartment, TLRs recognize viral nucleic acid PAMPs: TLR3 recognizes dsRNAs, TLR7/8 recognizes ssRNAs, and TLR9 recognizes CpG DNA motifs. **(b)** On the surface of cells, TLR2 and TLR4 are known to recognize viral glycoproteins [47,99]. TLR2/6 heterodimers help to activate the innate immune response to RSV, though the viral PAMP recognized has not been determined [100]. TLR1/2 heterodimers have been shown to recognize viral glycoproteins, though their potential role in respiratory virus infection has not been determined [99]. While there are many TLRs that recognize viral PAMPs, they signal through common adaptor molecules, including MyD88, MAL, TRAM, and TRIF. The TLR adaptor molecules signal through the IKKε/TBK1 complex and the IKKα/IKKβ/IKKγ complex similarly to RLRs, but can also recruit an IRAK-1/IRAK4/TRAF6 complex capable of activating the transcription factors IRF3, IRF7, and NF-κB. Activation of these transcription factors leads to the transcription of Type I IFNs and proinflammatory cytokines. Due to the considerable crosstalk between TLR and RLR signaling, it is difficult to discriminate between transcriptional products generated by the two sensor families, but it is likely that both play an important role in the innate immune response to SARS-CoV infection.

Type III, or Type I and Type III IFN receptors had slightly higher levels of viral replication in the lungs [61]. In 2B4 cells expression of Type III IFN was detected 24 h earlier than Type I IFN transcripts, demonstrating a delay in Type I IFN signaling and a potentially protective role of Type III IFN following SARS-CoV infection [23••]. While IFNs continue to be an attractive potential antiviral strategy if SARS were to re-emerge, their role as a protective component of the innate immune response during SARS-CoV infection still needs additional investigation, particularly into protective innate immune mechanisms that occur in the absence of IFN signaling.

Cytokines, Chemokines, and ISGs

Proinflammatory cytokines and chemokines (many of which are ISGs) may be part of a necessary initial immune response to pathogens, but exacerbated expression of these factors is associated with immunopathology and ARDS [46,62]. *In vitro* studies found that SARS-CoV infection initiates a proinflammatory cytokine response at 24 h post infection, but that IFNs and ISGs are delayed in expression until 48 h post infection [23••]. Although the consequences of the timing of these signals are not yet understood, SARS-CoV infection of susceptible aged mice leads to elevated levels of proinflammatory cytokines with ARDS association, including TNFα, IL-6, and IL-1β [30,31]. Chemokine receptors CCR1, CCR2, and CCR5 have protective roles during MA15-SARS-CoV infection in the mouse model as well as SARS-CoV infection of human DCs, indicating the importance of cell recruitment in controlling SARS-CoV infections [51,53]. Transcriptional profiles of ISGs associated with increased SARS-CoV disease have been described in several model systems, but the consequences of ISG signaling responses to SARS-CoV infection has not been characterized [23••,31,40••,41•,50••]. Although it is known that antiviral ISGs of the IFITM family restrict SARS-CoV entry into host cells and several ISGs such as MxA, OAS1, RNaseL, PKR, IFIT, Viperin, and TRIM5α have defined functions in the context of other viral infections, these are only a subset of this large family of molecules, most of which have antiviral properties that are not yet well understood [63••,64]. Additional studies to determine the crucial ISGs that control SARS-CoV infection or contribute to SARS disease could be exploited for the development of antiviral therapies. Extant reagents to study the overexpression of ISGs singly and in combination could determine which ISGs are effective at initiating an antiviral state against SARS-CoV infection [65••].

Modulation of innate immune response by SARS-CoV: evasion of innate immune detection

Evasion of innate immune responses to SARS-CoV infection requires avoidance of detection by cellular PRRs. During the SARS-CoV replication cycle, the segregation of viral dsRNA intermediates in the interior of Double Membrane Vesicles (DMVs) may potentially shield viral PAMPS from recognition by cytosolic PRRs [66,67]. It is unknown whether small viral ssRNA or dsRNA degradation products are also sequestered within DMVs or can be sensed by PRRs. The lack of a 5′cap distinguishes viral mRNAs from other eukaryotic mRNAs, and many viruses (including SARS-CoV) have evolved mechanisms to mimic host capping machinery. *In vitro* capping of SARS-CoV RNAs requires nsp14 and an nsp16/nsp10 complex [68•,69]. The guanine-N7-methyltransferase activity of SARS-CoV nsp14 is the initial step to building an RNA cap that is structurally similar to the RNA cap used by the host, making it more difficult for the host to discriminate viral non-self RNAs from self mRNAs [68•,70]. Additionally, nsp16 of SARS-CoV has been identified as a 2′-O-methlytransferase capable of modifying the cap of viral RNAs, which seems to be of particular import in evading recognition by host PRRs such as MDA5, as well as host ISGs such as IFIT family members IFIT1 and IFIT2 [71,72••].

Strategy of antagonism of innate immune molecules by SARS-CoV: block IFN

To counter innate immune signaling, SARS-CoV encodes eight proteins that antagonize the IFN response to prevent activation of antiviral effectors in host cells (Figures 1b and 2a, b).

Nonstructural proteins

The first nonstructural protein SARS-CoV nsp1 antagonizes Type I IFN by three mechanisms: inactivation of host translational machinery, degradation of host mRNAs, and inhibition of phosphorylation of STAT1 [73,74,75•]. While nsp1 mediates host mRNA degradation, SARS-CoV mRNAs are not susceptible to the cleavage or subsequent degradation [75•]. Part of the third nonstructural protein, SARS-CoV PLP is a papain like protease that antagonizes IFN by blocking phosphorylation IRF3 [76]. PLP prevents IRF3 phosphorylation in cell culture but not with purified components of the signaling pathway, indicating that a direct interaction between PLP and IRF3 does not take place [77]. More recently, findings that PLP interacts with STING resulted in a proposed mechanism of IFN antagonism by disruption of the signaling complex that leads to phosphorylation of IRF3 [78]. PLP also disrupts NF-κB signaling in addition to IRF3 signaling, possibly by a similar mechanism [77]. In addition to SARS-CoV nsp1 and PLP, SARS-CoV nsp7 and nsp15 have both been identified as potential IFN antagonists, but are not well described [77]. The majority of the SARS-CoV nonstructural proteins are required for replication, including those that have been identified as IFN antagonists. Their essential functions in viral replication may be due at least partly to their innate immune modulatory functions.

Structural proteins

In addition to functioning as components of the SARS-CoV virion two structural proteins antagonize IFN

signaling. The Nucleocapsid (N) protein of SARS Co-V is capable of blocking Type I IFN when induced by Sendai virus or polyI:C, but not upstream signaling components such as RIG-I, MDA5, MAVS, IKKε, TBK1 or TRIF, indicating that N exerts its effects before these signaling mediators [79•,80]. Additional studies of MHV Nucleocapsid identified IFN antagonism activity through RNaseL mediated host translation shutoff, but this has not yet been shown with SARS-CoV N [81]. The SARS-CoV Membrane (M) protein blocks transcription of IFN-β when stimulated by dsRNA as well as components of the RIG-I signaling pathway including RIG-I, MAVS, IKKε, and TBK1, but not the transcription factor IRF3, suggesting that the block in signaling is prior to IRF3 initiation of transcription [82]. SARS-CoV M also co-immunoprecipitated with RIG-I, IKKε, and TBK1, suggesting that SARS-CoV M interacts with a complex formed by these proteins as a mechanism for disrupting IFN-β transcription. SARS-CoV M was not identified as an IFN antagonist by the Venezuelan Equine Encephalitis Virus Replicon or Newcastle Disease Virus-GFP screens, demonstrating the need for multiple approaches to identify all of the IFN antagonist proteins within the SARS-CoV genome [80,83]. Structural components of the SARS-CoV virion acting as antagonists of IFN may be important for blocking innate immune responses immediately upon introduction of the virion into the cell; however, the temporal nature of antagonism of IFN signaling by SARS-CoV is not yet well understood.

Accessory proteins

SARS-CoV encodes eight accessory proteins that share no homology with proteins from other human coronaviruses and are dispensable for viral replication [84,85]. SARS-CoV ORF3b protein was identified as an antagonist of Type I IFN capable of inhibiting RIG-I and MAVS mediated induction of IFN-β by the transcription factors IRF3 and NF-κB [80,86]. However, ORF3b does not inhibit TNFα mediated activation of NF-κB transcription, leading to speculation that the disruption of NF-κB signaling is specific for induction by the RLRs [86]. SARS-CoV ORF3b temporally distributes to the mitochondrial outer membrane, indicating that the mechanism of IFN antagonism may involve MAVS, also located on the mitochondria [86]. SARS-CoV ORF6 protein antagonizes IFN by inhibiting signaling of the JAK-STAT pathway downstream of IFNAR by blocking nuclear translocation of the transcription factor STAT1 [80,83,87]. ORF6 binds to karyopherin-α2, and tethers karyopherin-β1 on internal membranes, disrupting formation of the complex of proteins associated with the nuclear import of STAT1 [87]. The C-terminus of ORF6 interferes with proteins with NLS-signals, disrupting the classical nuclear import pathway [88,89]. The disruption of nuclear transport is specific to a nuclear import pathway, indicating that there are potentially many other transcription factors that modulate innate immunity that could be affected by SARS-CoV ORF6.

Many of the SARS-CoV gene products that modulate IFN signaling have been identified by overexpression in cell culture using individual viral components, a system that may not accurately reflect innate immune signaling that occurs during SARS-CoV infection *in vivo*. Additional studies are needed to elucidate IFN antagonism by these proteins in the context of infection, particularly because SARS-CoV proteins can form large complexes during viral infection, and the role of these complexes in potentially modulating innate immune responses is not yet known. Because of the complicated replication scheme utilized by coronaviruses like SARS-CoV, some viral proteins may be temporally expressed at different levels during viral infection or compartmentalized in different areas of the cell, which are factors that still need to be investigated in the context of how viral proteins affect innate immune signaling.

SARS-CoV pathogenesis: innate immune factors still at large

SARS-CoV is a highly pathogenic respiratory virus where the mechanisms of severe disease are largely mediated by innate immune pathways. Excellent models exist for studying SARS-CoV pathogenesis that replicate findings from the SARS outbreak in humans: cell lines for studying *in vitro* responses in human lung epithelial cells, mouse models of fibrosis and GWAS mapping of traits, as well as primate models of comparative species infection and age-dependent phenotypes. Due to the development of these models, SARS-CoV is uniquely suited for a systems biology based platform to compare respiratory virus infection in multiple relevant model systems as an unbiased approach to identify novel host modulators of innate immunity in the context of viral infections. In addition, SARS-CoV encodes many proteins that antagonize the host's Interferon response, but questions remain about the effects of these antagonists of viral pathogenesis during SARS-CoV infection *in vivo*. Of the currently well-described innate immune signaling pathways, there is evidence to support that RLR and TLR sensors detect and respond to SARS-CoV infection, but no mechanism or SARS-CoV ligand for these receptors has been determined. Unique gene transcription signatures associated with defined temporal expression of proinflammatory cytokines and ISGs in models of severe SARS-CoV disease have been described, but few of these genes have been evaluated for their role in SARS pathogenesis or the host antiviral response to SARS-CoV, which could help identify novel immunomodulatory therapies in the event of SARS-CoV re-emergence. In future studies, SARS-CoV could be particularly useful as a comparative model for Influenzavirus or RSV infection to evaluate common targets for antiviral strategies as well as

unique mechanisms of innate immune pathogenesis across multiple virus families with similar tropisms.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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