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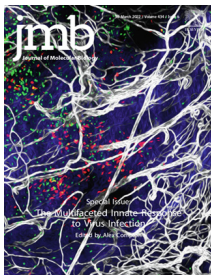

Interferon Control of Human Coronavirus Infection and Viral Evasion: Mechanistic Insights and Implications for Antiviral Drug and Vaccine Development

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Abstract

Recognition of viral infections by various pattern recognition receptors (PRRs) activates an inflammatory cytokine response that inhibits viral replication and orchestrates the activation of adaptive immune responses to control the viral infection. The broadly active innate immune response puts a strong selective pressure on viruses and drives the selection of variants with increased capabilities to subvert the induction and function of antiviral cytokines. This revolutionary process dynamically shapes the host ranges, cell tropism and pathogenesis of viruses. Recent studies on the innate immune responses to the infection of human coronaviruses (HCoV), particularly SARS-CoV-2, revealed that HCoV infections can be sensed by endosomal toll-like receptors and/or cytoplasmic RIG-I-like receptors in various cell types. However, the profiles of inflammatory cytokines and transcriptome response induced by a specific HCoV are usually cell type specific and determined by the virus-specific mechanisms of subverting the induction and function of interferons and inflammatory cytokines as well as the genetic trait of the host genes of innate immune pathways. We review herein the recent literatures on the innate immune responses and their roles in the pathogenesis of HCoV infections with emphasis on the pathobiological roles and therapeutic effects of type I interferons in HCoV infections and their antiviral mechanisms. The knowledge on the mechanism of innate immune control of HCoV infections and viral evasions should facilitate the development of therapeutics for induction of immune resolution of HCoV infections and vaccines for efficient control of COVID-19 pandemics and other HCoV infections.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has led to an unprecedented global public health crisis. SARS-CoV-2 belongs to the family of *Coronaviridae,* which is comprised of a diverse

group of enveloped positive-sense single-stranded RNA viruses with broad host ranges in vertebrates[1,2] (Figure 1). While four human coronaviruses (HCoVs), including HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1, cause mild upper respiratory tract infections, three coronaviruses (CoVs) have breached species barriers within the last 20 years to infect humans. These zoonotic CoVs have caused severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and COVID-19, with fatality rates of 10%, 36%, and 0.1 to 2%, respectively.[3,4] Despite the availability of several effective SARS-CoV-2 vaccines, SARS-CoV-2 has undergone rapid evolution with the selection of variants with increased infectivity and resistance to vaccine-induced immunity and thus, become the dominant lineages.[5–9]

As illustrated in Figure 1, viral infections are promptly sensed by infected cells to mount an innate immune response leading to the transcriptional activation of genes for the production of intracellular antiviral proteins and secretion of cytokines, most prominently, the type I and type III interferons (IFN). While the antiviral proteins limit viral replication in infected cells, the IFNs induce hundreds of IFN-stimulated genes that establish an antiviral state to protect uninfected cells from oncoming infections.[10] Clinical studies indicate that type I interferons (IFN-I) play critical roles in the control and pathogenesis of HCoVs, including SARS-CoV-2. This notion is supported by the findings that mutations rendering the gene products incapable of producing or responding to IFN-I are associated with life-threatening COVID-19 pneumonia.[11,12] In addition, autoantibodies capable of neutralizing IFN-I were found to dampen ISG induction in patients and are associated with severe COVID-19.[13,14] Here we review the recent progress on innate immune response in the control and pathogenesis of HCoV infections with emphasis on the pathobiological role and therapeutic efficacy of type I IFNs and their antiviral

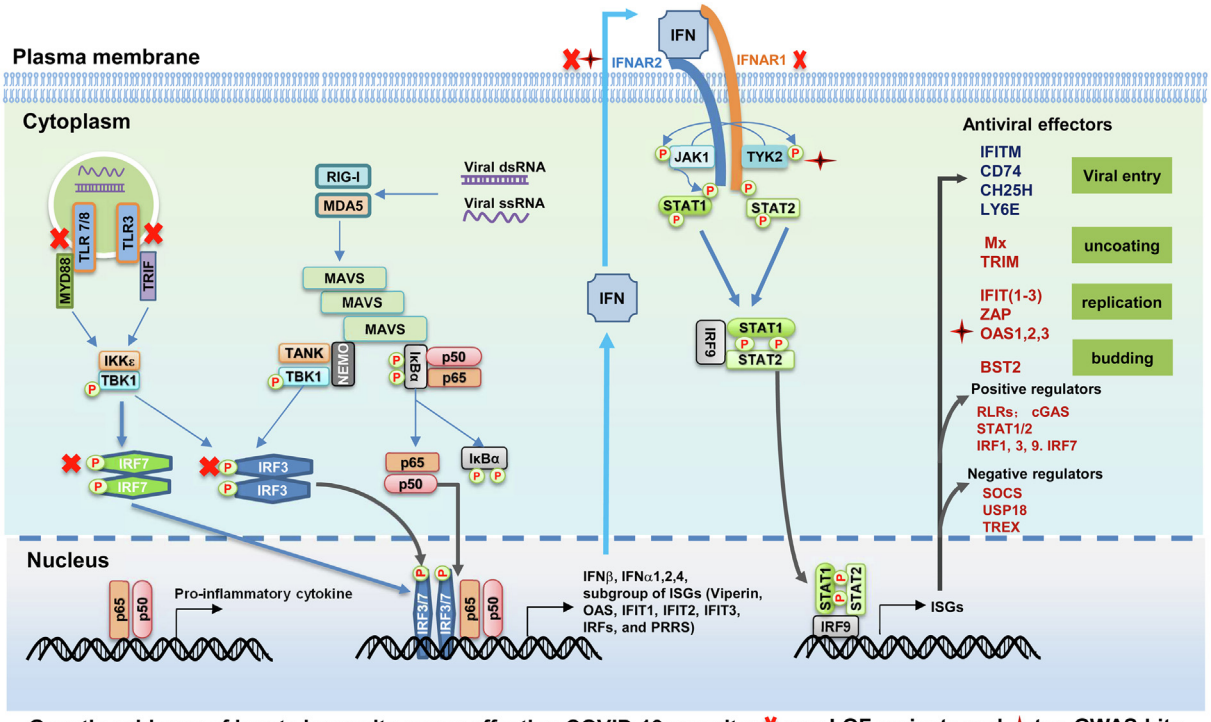

Figure 1. Mechanism of innate immune activation and control of viral infection. Recognition of viral RNA by cytoplasmic RIG-I-like receptors or endosomal TLRs activates signaling cascades leading to the production of IFNs, inflammatory cytokines and chemokines. IFNs bind to their specific receptors to activate JAK-STAT signaling pathway to induce the expression of hundreds of ISGs. While many of ISGs encode proteins that regulate innate immune response, some of them directly inhibit distinct steps of viral replication. Genetic evidence of innate immunity genes affecting COVID-19 severity was revealed in a GWAS report with hits in IFNAR2, TYK2 and OAS1 (RAF, risk allele frequency in the UK Biobank) as well as in studies that discovered at least 3.5% of patients with life-threatening COVID-19 carry rare loss-of-function (LOF) genetic variants in innate immunity genes (TLR3, TLR7, IRF7, IRF3, IFNAR1 and IFNAR2). These genes impair the type I and II IFN response to SARS-COVID-2 and are marked with a red cross. See text for further interpretation.

mechanisms against HCoVs. Better understanding of HCoV interaction with the IFN-mediated innate immunity should facilitate the discovery and development of novel therapeutic agents for the treatment of COVID-19 and vaccines to control the COVID-19 pandemic.

Role of IFN response in HCoV pathogenesis

HCoVs induce innate immune response via activation of multiple intracellular pattern recognition receptors

Viral infection of cells is sensed by pattern recognition receptors (PRRs) that bind unique structural motifs of viral components, *i.e.,* pathogen associated molecular patterns (PAMPs).[15] Similar to other RNA viruses, HCoV genomic RNA and its replication intermediates can be detected by the cytoplasmic RNA receptors, such as retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), or endosomal RNA sensors, including toll-like receptor (TLR) 3, 7 and 8[16,17] (Figure 1). PRR recognition of viral RNA activates signaling cascades, leading to the activation of downstream transcription factors, including interferon regulator factor (IRFs) and nuclear factor κB (NF-κB). Transcriptional activation of IRF3/7 and NF-κB coordinately induces the expression of type I and type III IFNs to restrict viral replication as well as inflammatory cytokines and chemokines to recruit specific subsets of leukocytes and facilitate the activation of adaptive immune responses for the resolution of viral infections.[16–18]

RIG-I and MDA5 are ubiquitously expressed in various cell types and have been shown to be responsible for SARS-CoV-2 induction of IFN response in airway epithelial cells among other cell types.[19–21] Two viral RNA fragments spanning nt24001–25000 and nt27001–28000 of SARS-CoV-2 genome significantly stimulated type I IFN production upon transfection into HEK293 cells, suggesting the viral RNA structural motifs or PAMPs recognized by RIG-I and/or MDA5 are likely located in these regions.[22] SARS-CoV-2 RNAs in infected cells have the methylated adenosine bases at the nitrogen 6 position (m^6A). Recent studies revealed that depletion of host cell m^6A methyltransferase METTL3 decreased levels of m^6A in SARS-CoV-2 RNA, but increased RIG-I binding and induction of IFN-I and other inflammatory cytokines.[23] In addition, Yamanda et al. suggested RIG-I can also bind the 3′ non-translational region of SARS-CoV-2 RNA genome in human lung epithelial cells *via* its helicase domain to directly inhibit viral RNA replication independently of type I/III IFN production.[24]

Unlike RIG-I-like receptors, TLR7 and TLR8 are primarily expressed in dendritic cells (DCs), B lymphocytes and monocytes/macrophages. DCs play a critical role in viral immunity as they connect the innate immune response to the adaptive immune response when presented with viral antigens.[25] While plasmacytoid DCs (pDCs) are the primary producers of IFN-I in response to viral infections,[26] conventional DCs (cDCs) recognize various pathogens to produce inflammatory cytokines and activate T lymphocytes.[27] In an *in vitro* human peripheral blood mononuclear cell (PBMC)-based experimental system, SARS-CoV-2 activates a robust TLR7/8-dependent response leading to the production of type I and III IFNs as well as inflammatory cytokines in the absence of productive viral replication. Type I IFNs are produced by pDCs in response to SARS-CoV-2 infection *via* a neuropilin-1-dependent, but ACE-2 independent mechanism.[28] Based on the structural features of known TLR7/8 ligands,[29,30] a total of 491 GU-rich RNA fragments have been identified as putative immunostimulatory ligands within the SARS-CoV-2 genomic RNA and two of them were synthesized and validated as direct activators of endosomal TLR7/8 in a MyD88-depenent manner in DCs.[31]

HCoVs subvert the induction of innate immune response and signal transduction of IFNs via multiple mechanisms

The broadly active antiviral response induced by PRR recognition of the viral pathogens puts strong selective pressure on viruses. This evolutionary process drives the selection of the variant viruses that have higher capabilities to evade or counteract the innate antiviral immune response. Similar to other viruses, HCoVs encode multiple proteins to subvert the innate immune response (Figure 2).[32] Approximately 10 SARS-CoV and SARS-CoV-2 proteins, including membrane protein (M), nucleocapsid protein (N), and non-structural protein (Nsp) 10, 14, 15 and 16, were found to play a role in counteracting the production of IFNs and induction of ISGs (reviewed in.[33] SARS-CoV-2 Nsp5, the main protease, targets RIG-I and MAVS proteins for degradation.[34] ISG15 conjugation (ISGylation) of MDA5 at the CARD domain promotes its oligomerization and triggers the activation of IFN and inflammatory cytokine response to the infection of CoVs and other RNA viruses. This ISG15-dependent MDA5 activation can be antagonized by SARS-CoV-2 papain-like protease catalyzed de-ISGylation.[35] SARS-CoV and MERS-CoV endonuclease (EndoU, Nsp15) efficiently prevents the early activation of double-stranded RNA (dsRNA) sensors, such as MDA5, 2′-5′-oligoadenylate synthase (OAS) and double-stranded RNA-dependent protein kinase (PKR). EndoU localizes at the site of viral RNA synthesis within the RNA replication complex, suggesting that CoVs have evolved a viral RNA decay pathway to evade early innate and intrinsic antiviral host cell responses.[36]

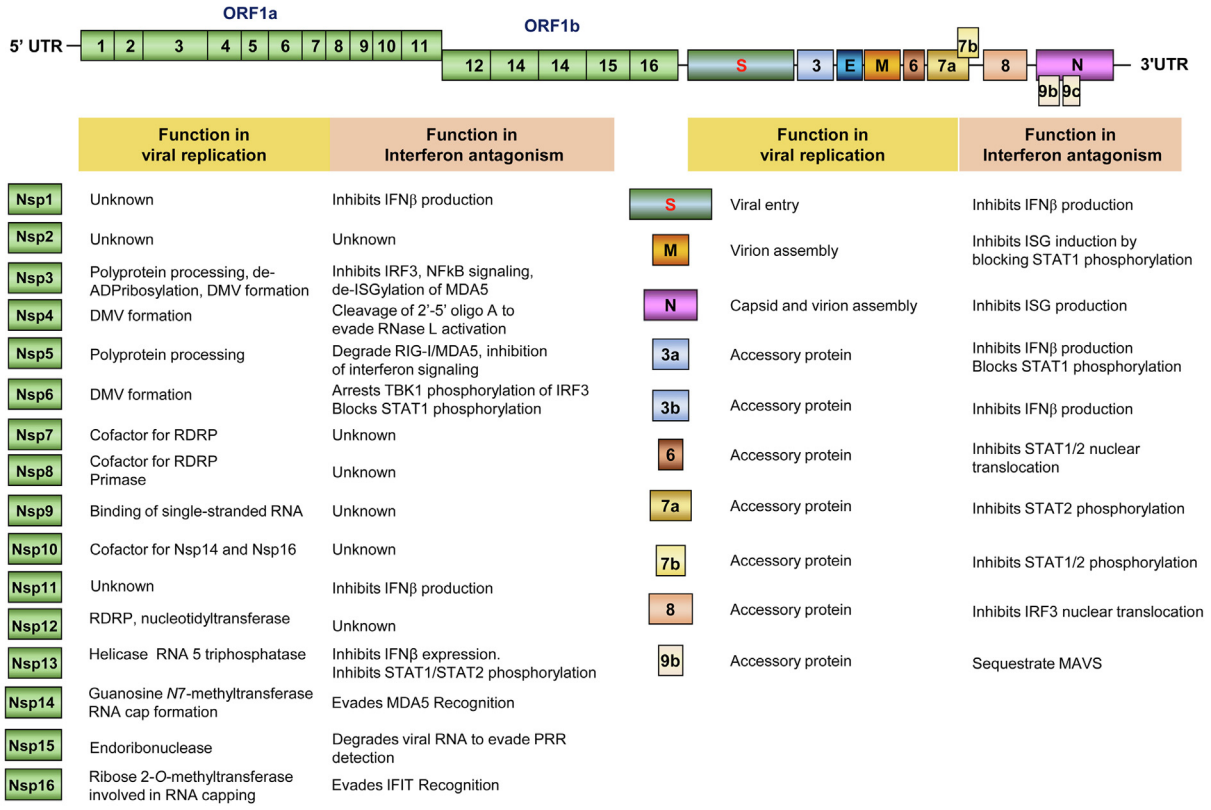

Figure 2. SARS-CoV-2 genome structure and functions of viral proteins in viral replication and evasion of innate immune response. SARS-CoV-2 has a single-stranded, positive sense RNA with a cap at its 5′ end and a poly (A) tail of 30–60 nt in length at the 3′ end. The genome encodes 16 non-structural proteins (Nsp1 to Nsp16) and 4 structural proteins (S, spike; E, envelope; M, membrane and N, nucleocapsid) as well as eight accessory proteins. Their functions in viral replication and evasion of innate immune response are listed.

SARS-CoV-2 infection also disrupts the function of IFNAR1, JAK and Tyk2 proteins to block IFN signal transduction and induction of ISGs (reviewed in 17,32). Through the unbiased screen of SARS-CoV-2 proteins for inhibition of IFN response, many were identified to inhibit IFN-I response through distinct mechanisms.[37,38] For example, Nsp6 inhibits IRF3 phosphorylation by binding to TBK1; NSP13 interacts with and impedes TBK1 phosphorylation; and ORF6 inhibits IRF3 nuclear translocation *via* sequestration of karyopherin α 2 (KPNA2). Interestingly, the Nsp1 and Nsp6 of SARS-CoV-2 interrupt IFN-I signaling more efficiently than those of SARS-CoV and MERS-CoV.[37]

The unique interactions between a specific virus and its host cells at various molecular levels lead to the virus- and cell type-specific transcriptome responses. This phenomenon is highlighted by the transcriptome analysis of SARS-CoV-2 infection. In comparison to other respiratory viruses, such as SARS-CoV, Influenza A virus (IAV), respiratory syncytial virus and parainfluenza virus 3, SARS-CoV-2 infection induces a low and delayed type I and type III IFN response, but increased levels of chemokines and inflammatory cytokines, such as IL-6 and IL-1RA.[39] A single cell RNA sequencing analysis of SARS-CoV-2 tropism and antiviral response in primary human airway epithelium revealed that the virus predominantly infects ciliated cells and induction of IFN response within infected cells was low and heterogeneous, while heavily infected secretory cells expressed abundant IL-6.[40] Furthermore, SARS-CoV-2 induction of IFN and inflammatory cytokines is tissue specific and associated with clinical conditions. For instance, high levels of IFN-III and modest levels of IFN-I were induced in the cells of the upper airways of patients with high viral burden and mild symptoms, whereas patients experiencing severe COVID-19 symptoms had elevated expression of IFN-I and IFN-III in the cells of the lower airway.[41]

Innate immune response plays critical roles in HCoV pathogenesis

The correlation between the severity of COVID-19 symptoms and the profiles of IFN and inflammatory cytokine responses indicates the important role of PRR-mediated innate immune response in the SARS-CoV-2 pathogenesis. Specifically, a clinical study with a cohort of COVID-19 patients with varying symptom

severities revealed that patients with severe clinical conditions were associated with highly impaired type I interferon responses. This impairment was characterized by undetectable IFN-β levels, low IFN-α production and persistent viral load in their blood, and strong inflammatory responses with increased TNFα and IL-6 productions.[42] Additionally, a longitudinal analysis of the natural killer (NK) cells from 205 COVID-19 patients exhibited early elevation of IFN-I in plasma with increased NK cell expression of ISGs and genes involved in IFN-I signaling. This trend was associated with early severe disease, whereas upregulation of TNF-α-induced genes in NK cells was indicative of moderate symptoms. These findings suggest the persistent dysregulation of innate immune responses in NK cells are associated with the severity of COVID-19 manifestation.[43]

Interestingly, genetic studies in mice argue the protective role of IFN response in CoV infection. For instance, genetic ablation of IFN-I receptor (IFNAR) protected mice from lethal infection by SARS-CoV.[41] In addition, blockade of IFN signaling with an anti-IFNAR2 antibody attenuated the overactive inflammation in SARS-CoV-2 infected MISTRG6-hACE2 mice.[44] However, accumulating evidence supports a protective role of IFN response in SARS-CoV-2 infection in humans. As illustrated in Figure 1, it was reported recently by several research groups that inborn genetic defects at the loci involved in the TLR3- and IRF7-dependent induction of IFN-I are enriched in patients with life-threatening COVID-19 pneumonia.[11,45–47] Additionally, a genome-wide association study revealed several gene variants encoding IFN-induced antiviral proteins (*OAS1*, *OAS2* and *OAS3*) or components involved in IFN signal transduction, such as interferon receptor gene *IFNAR2* and tyrosine kinase 2 (*TYK2*), are associated with the increased risk of suffering from severe COVID-19 symptoms.[48] It was estimated that the rare loss-of-function variants in human genes involved in recognizing viral infections and stimulating interferon production or antiviral signaling may account for as many as 3.5% of severe life-threatening COVID-19.[11] In support of these genetic findings, higher titers of neutralizing autoantibodies targeting IFN-I were found in 10% of patients with severe COVID-19 pneumonia, however, were not detected in asymptomatic patients or those with mild symptoms.[13] The prevalence of these autoantibodies targeting IFN-I was significantly increased in patients over the age of 70 and involved in approximately one-fifth of COVID-19 fatalities.[49] These findings thus imply the critical role of IFN response in the control and pathogenesis of SARS-CoV-2 infection. The discrepancy observed between human genetic studies and mice models implies the critical role of species-specific virus-host interaction in the pathogenesis of SARS-CoV-2 infection.

In comparison with the highly pathogenic CoVs, the innate immunity response and its role in the pathogenesis of the common cold HCoVs remains largely unknown. HCoV-229E was found to induce more potent IFN-I response than SARS-CoV does[50] and double transgenic *hAPN* $^{+/+}$*Stat1*$^{-/-}$ mice gain susceptibility to HCoV-229E infection,[51] suggesting that IFN-I and STAT1 pathway play an essential role in the control of HCoV-229E infection. Apparently, the pathobiological role of IFN response in the infection of common cold HCoVs deserves further investigation.

Antiviral effects and therapeutic efficacy of IFNs to HCoV infections

Despite the IFN evasion and antagonistic strategy utilized by CoVs, most HCoVs are sensitive to IFN-I and IFN-III in cultured cells (Table 1). However, HCoV-OC43 is enhanced by all three types of IFNs through the induction of IFITM2/3 to facilitate its cellular entry.[52] Although replication levels of SARS-CoV and SARS-CoV-2 are comparable in infected cells in culture, SARS-CoV-2 is more sensitive to IFN-I.[53] IFN-α treatment was found to reduce viral load and ameliorate pathogenesis by SARS-CoV and MERS-CoV in cynomolgus macaques and rhesus macaques, respectively.[54,55] Moreover, a type III IFN, IFN-λ1a, effectively suppressed the replication of a mouse-adapted SARS-CoV-2 in mice.[56] Hoagland et al. found when IFN-α was administered to hamsters intranasally prophylactically or at earlier times (one day) post infection, it reduced viral load and SARS-CoV-2 pathogenesis through the elevation of ISG expression.[57] However, intranasal administration of IFN-α to hamsters after onset of symptoms did not improve clinical outcomes of SARS-CoV-2 infection, but was rather associated with signs of more severe disease.[58]

Similar to the results obtained from animal models infected with SARS-CoV-2, Wang et al. found in a retrospective clinical study that IFN-α2b treatment of COVID-19 patients was only beneficial if the treatment started within 5 days of post-hospital admission, whereas IFN treatment administered later resulted in a slower recovery and extended hospital stay.[59] Likewise, patients with MERS-CoV infection gained beneficial outcomes with earlier treatment regimens of IFN-β.[60] However, the clinical efficacy of pegylated IFN-λ for COVID-19 remains controversial. Peg-IFN-λ-1a did not ameliorate symptoms or reduce SARS-CoV-2 viral shedding time in outpatients with mild to moderate COVID-19.[61,62] However, another study showed peg-IFN-λ accelerated viral clearance in COVID-19 outpatients with a high baseline of viral load.[62] Collectively, these results indicate that administration of IFN-α may not always be ben-

Table 1 Susceptibility of human coronavirus to interferon treatment.

HCoV	Type of Study	IFN	Efficacy	References
SARS-CoV-2	*In vitro*	IFNβ, IFNλ1	Inhibition in primary human bronchial epithelial cells.	211,212
		IFNγ	No effect in primary human bronchial epithelial cells.	211
		IFNβ, IFNγ, IFNλ1	Inhibition of viral replication in Calu-3 cells.	211
		IFN-α, IFN-λ	Inhibition of viral replication in human Calu-3 and simian Vero E6 cells.	213
	Animal model of hamster	IFN-I (IFN-α A/D)	Intranasal administration of IFN-I limits SARS-CoV-2 replication and inflammation in hamsters.	57
	Retrospective Observational Study	IFN-α2b	Early administration of IFN-α2b was associated with the reduction of in-hospital mortality in comparison to patients that did not receive IFN-α2b treatment.	59
	Clinical trial (phase 2)	IFN-β1b	Early treatment with triple combination of interferon IFNβ-1b, lopinavir-ritonavir, and ribavirin was associated with reduced duration of viral shedding compared to the lopinavir-ritonavir treatment group.	214
		Peg- IFN-λ1	Acceleration of viral decline and clearance in COVID-19 outpatients.	62
	Randomized Controlled Trial	Peg IFN-λ1a	Neither reduced duration of viral shedding nor improved symptoms.	61
MERS-CoV	*In vitro*	IFN-β	Inhibition of viral replication in Calu-3 cells.	215
		IFN-β	Inhibition of viral replication in Vero E6 cells.	216
	Animal models	IFN-α2b	Treatment of MERS-CoV infected rhesus macaques with IFN-α2b and ribavirin reduces viral replication, moderates the host response, and improves clinical outcomes.	55
		IFN-β1b	IFN-β1b improved clinical, radiological, and pathological outcomes while reducing the viral load of the infected Common Marmoset.	217
		Combination of lopinavir, ritonavir and IFN-β (LPV/RTV-IFNb)	Therapeutic LPV/RTV-IFN-β improves pulmonary function but does not reduce viral replication or severe lung pathology in human DPP4 mouse model.	215
	Observational Study	Ribavirin and/or rIFN-α2a, rIFN-α2b, or rIFN-β1a	RBV/rIFN (RBV and/or rIFN-α2a, rIFN-α2b, or rIFN-β1a) therapy was commonly used in critically ill MERS patients but was not associated with reduction in 90-day mortality or faster MERS-CoV RNA clearance.	218
	Retrospective cohort study	Ribavirin and IFN-α2a	In patients with severe MERS-CoV infection, ribavirin and interferon alfa-2a therapy is associated with significantly improved survival at 14 days, but not at 28 days.	219
	Randomized Controlled Trial	IFN-β1b	Early treatment of IFN-β1b and lopinavir-ritonavir led to lower mortality in comparison to the placebo treatment.	60
SARS-CoV	*In vitro*	IFN-α	IFN lead to a modest reduction in viral titer in Calu-3 cells.	53
		IFN alfacon 1	Inhibit viral yield in Calu-3 cell line.	220
		IFN-α, IFN-β	IFN-α and IFN-β protected cells from CPE and reduced viral replication in Vero, Caco2 and Frhk-4 cells.	221–223
	Animal model	IFN-α	IFN-α significantly reduces viral replication and excretion in cynomolgus macaques.	54

Table 1 (*continued*)

HCoV	Type of Study	IFN	Efficacy	References
	Retrospective cohort study	IFN alfacon 1 plus corticosteroids	The combination of Interferon alfacon-1 plus corticosteroids was associated with reduced disease-associated impaired oxygen saturation, more rapid resolution of radiographic lung abnormalities, and lower levels of creatine kinase.	224
HCoV-NL63	Clinical trial (Single case)	IFN-α2b	Treatment of IFN-α2b eliminated NL63 infection in a leukemia patient.	225
HCoV-OC43	*In vitro*	IFN-α, IFNγ, IFNλ	Enhanced viral infection in cells via the induction of IFITM2/3.	52

eficial for all the patients, but may be useful for subsets of patients who bear a high viral load and receive it in the early stage of infection.

Systematic identification of IFN-induced cellular proteins that restrict HCoV infection.

IFNs control viral infections by inducing the expression of IFN-stimulated genes (ISGs) that coordinately restrict viral replication.[63] The ISGs that modulate viral replication can be identified by systematic gain-of-function or loss-of-function genetic screens.[64–66] Currently, four independent gain-of-function genetic screens were performed to identify ISGs inhibiting the infection of SARS-CoV-2 and HCoV-229E.

Martin-Sancho et al. screened 399 ISGs with sequence validated full-length cDNA clones in HEK293 cells through the co-transfection of V5-epitope tagged individual ISG in the lentiviral expression vector, pLX304, with plasmids expressing human ACE2 and TMPRSS2.[67] A total of 65 ISGs were identified to significantly inhibit SARS-CoV-2 infection while 38 ISGs were further validated in HEK293-ACE2-derived cell lines stably expressing a V5-tagged individual ISG. Eight ISGs, including MyD88, STAT1, STAT2, DDX60, ELF1, REC8, TRIM21 and CLEC4D, were identified as upstream signaling components and regulators of PRR/IFN pathways which execute their antiviral activity through the transcriptional activation of other cellular proteins. The remaining ISGs were comprised of well characterized IFN-induced direct-acting antiviral effector proteins (IFITM2, IFITM3, BST2, Viperin and IFIT) as well as unfamiliar ISGs that inhibit cellular entry (UBD and FAM46C) and viral RNA synthesis (SPATS2L, DNAJC6, RGSS2, LOC152225, ZBP1, B4GALT5). A highly enriched cluster of endoplasmic reticulum (ER)/Golgi-resident ISGs inhibits virion assembly/egress (ERLIN1, APOL2, HSPA8, *etc*). While IFITM2, IFITM3, ZBP1 and IFIT1 were demonstrated to restrict multiple RNA viral infections, eight ISGs, including ER/Golgi-resident proteins NAPA, APOL2, and ERLIN1, were found to inhibit the replication of both SARS-CoV and SARS-CoV-2, but did not have this effect on the other twenty RNA viruses tested. These results imply that regulation of the membrane composition at sites relevant for viral replication or trafficking is a key target for IFN-induced innate immune control of SARS-CoV-2 infection (Figure 2).

However, ectopic expression of more than 500 arrayed individual ISGs in ACE2-expressing and interferon regulatory factor 3 (IRF3)–deficient A549 cells (A549-Npro-ACE2) identified only six ISGs that inhibit SARS-CoV-2 infection/replication without inducing substantial toxicity or inducing interferon-stimulated response element (ISRE) expression[68]. While OAS1 efficiently inhibited

SARS-CoV-2 infection of A549-ACE2 cells irrespective of serine protease transmembrane serine protease 2 (TMPRSS2) expression status, other five ISGs, including NCOA7, UNC93B1, CCARB2, ANKF1 and ZBTB42, only inhibited SARS-CoV-2 infection in the absence of TMPRSS2 expression, suggesting TMPRSS2-mediated SARS-CoV-2 entry evades the restriction of these antiviral factors.[69,70]

By inducible CRISPR activation (CRISPRa) screen of pooled 414 ISGs for identification of individual ISGs that confer resistance to the cytopathic effects of SARS-CoV-2 in A549 lung adenocarcinoma cells, Oded Danziger et al. identified several previously known SARS-CoV-2 restriction factors, such as LY6E, CD74, IFITM1 and OAS1, as well as new candidate ISGs with putative antiviral activity to SARS-CoV-2, such as ERLIN1, ADPRHL2 and CTSS.[71] In comparison to a cDNA over-expression screen, CRISPRa activates the transcription of ISG gene from its endogenous promoter enabling expression of multiple gene isoforms in physiologically relevant levels. This approach offers clear advantage for the identification of ISGs with only one of its isoforms restricting SARS-CoV-2 infection, such as CD74.[72]

Through the screening of more than 350 human ISG cDNAs in human hepatoma cells (Huh7), Pfaender at al. identified 33 ISGs that inhibited HCoV-229E infection by more than 20% at 24 h post infection.[73] Some broad-spectrum antiviral effectors aside from the PRR/IFN signaling modulators, such as IFITM2, IFITM3, BST2, IFIT1, OAS2, OAS3, and LY6E, were identified to inhibit HCoV-229E infection. Several ISGs that inhibit SARS-CoV-2, including APOL2, RAB27A, FAM46A and FAM46C, also inhibit HCoV-229E. However, ISGs that potently inhibit HCoV-229E, such as SQLE, SLC1A1, and STARD5, were not identified to inhibit SARS-CoV-2. This finding suggests that there are overlapping but distinct antiviral networks induced by IFNs to restrict different HCoVs.

ISGs that inhibit distinct steps of HCoV replication

As summarized in Table 2 and illustrated in Figure 3, many IFN-induced proteins have been identified to directly inhibit specific steps of HCoV replication. The antiviral properties and modes of action of well-studied ISGs as well as viral strategies to evade their actions will be further discussed.

Multiple IFN-induced proteins inhibit HCoV entry into host cells

Interferon-induced transmembrane proteins (IFITMs). The human IFITM family has five members, including IFITM1, IFITM2, IFITM3, IFITM5 and IFITM10. However, only IFITM1, IFITM2, and IFITM3 are IFN-inducible and inhibit the cellular entry of a broad spectrum of enveloped viruses.[74,75] IFITMs inhibit the entry of multiple HCoVs, including HCoV-NL63, 229E, MERS-CoV, SARS-CoV, SARS-CoV-2,[76–79] while IFITM2 and IFITM3 facilitate the entry of HCoV-OC43.[52,78] As potent viral restriction factors, IFITM genes evolve in vertebrates under the selective pressure of microorganism infection.[80] Thus, a dozen of single-nucleotide polymorphisms (SNPs) have been identified in the human population, some of which associate with severity and prognosis of viral infection (reviewed in 81). SNP rs12252 and rs34481144, two well-studied IFITM3 SNPs associated with severe outcomes of IAV infection, have also be reported to associate with COVID-19 mortality or increased risk of hospitalization.[82–84]

IFITM proteins are widely expressed in human tissues and cell types. IFITM1 primarily localizes in the cellular plasma membrane and early endosomes,[85] whereas IFITM2 and IFITM3 mainly co-localize with Rab7, CD63, and lysosome-associated membrane protein 1 (LAMP1) in late endosomes and lysosomes.[86,87] The cellular entry of HCoVs requires sequential protease processing of the viral spike glycoprotein to trigger membrane fusion. This processing takes place in either the plasma membrane or the endolysosomal membranes, depending on the structural property of the viral spike protein and the subcellular distribution of cellular protease that cleave viral spike protein. Accumulating evidence supports the notion that the presence of IFITM proteins at the site of viral membrane fusion is essential for their restriction of virus entry. Thus, the entry route of CoVs will largely determine their susceptibility to the different IFITMs. Although SARS-CoV-2 utilizes both early endosomes and lysosomes to fulfill infectious entry,[88] its infection is mainlyrestricted by IFITM2 and the removal of the furin cleavage site in the spike protein renders SARS-CoV-2 entry predominant via late endosomes and makes the infection more sensitive to IFITM2 restriction.[89] Moreover, the depletion of IFITM2 attenuates the antiviral effect of IFN-I to SARS-CoV-2 infection.[89] Interestingly, SARS-CoV-2 utilizes S1/S2 furin cleavage sites and TMPRSS2 to escape the endosomal IFITM2 and IFITM3 restriction, which contribute to SARS-CoV-2 transmission in ferrets.[70] However, a recent study reported that the endogenous expression but not artificial overexpression of IFITMs aids SARS-CoV-2 infection in human lung cells.[90] The discrepancy of those studies is not clear and deserves further investigation. It is important to point out that IFITMs can be incorporated into the viral envelope and IFITM proteins on both viral and cellular membranes can restrict the infectious entry of various enveloped viruses. The restriction occurs through the blockade of viral fusion, but not the endocytosis of virions into the cells.[91–94]

Table 2 ISGs directly-inhibiting coronavirus replication.

ISG	Activity	Mechanism	References
ISGs inhibit the cellular entry of HCoVs			
IFITM1, IFITM2 and IFITM3	Inhibits cellular entry of all the known HCoVs except for HCoV-OC43, which uses human IFITM2 and IFITM3 as entry factors to facilitate its infection of target cells.	Inhibition or promotion of the fusion of viral envelopes with cellular membranes.	52,78,86,226–228
LY6E	Inhibits cellular entry of all the known HCoVs.	Interrupts the fusion of the viral envelope and cellular membranes as well as impairs syncytia formation.	73,76
CH25H	Inhibits cellular entry of SARS-CoV-1, SARS-CoV-2 and MERS-CoV.	Inhibits the fusion of viral envelopes and cellular plasma membranes through the reduction of cholesterol in the plasma membrane.	132,133
GILT	Inhibits cellular entry of SARS-CoV-1.	Inhibition of SARS-CoV-1 entry in lysosomes *via* disruption of the stability and activity of cathepsin L.	121
CD74	Inhibits the cellular entry of SARS-CoV-1 and SARS-CoV-2.	Inhibition of cathepsin L-mediated cleavage of viral spike proteins.	72
FAM46C, UBD, CLEC4D	Inhibit viral entry mediated by spike protein of SARS-CoV-2.	To be determined.	67
ISGs that bind viral RNA and inhibit viral replication			
IFIT1, IFIT3 and IFIT5	Inhibit SARS-CoV-2 RNA replication.	Prevention of active viral RNA replication by sequestering the single-stranded 5′-ppp or 2′-O-unmethylated RNA.	67
IFIT2	Inhibits MHV RNA replication.		167,229
ZAP	Restricts SARS-CoV-2 replication.	Binds CpG dinucleotides in viral RNA to mark it for degradation	142–144
OAS1-RNase L	Restricts SARS-CoV-2 replication.	Synthesize 2′-5′oligo (A) that actives RNase L to degrade viral and cellular RNA.	19
SPATS2L, DNAJC6, RGSS2, LOC152225, ZBP1 B4GALT5		To be determined.	67
ER-Golgi-associated proteins that inhibit virion assembly and egress			
Viperin	Inhibits PEDV replication.	Restricts viral replication and/or assembly via interactions with N proteins.	230
BST2	Inhibits the release of HCoV-229E and SARS-CoV-2 virion particles.	Tethers virions on the cellular surfaces or intracellular membranes.	67,193,202
HSPA8, CNP, Rab27a, ERLIN1	Inhibits late-stage SARS-CoV-2 replication.	To be determined.	67

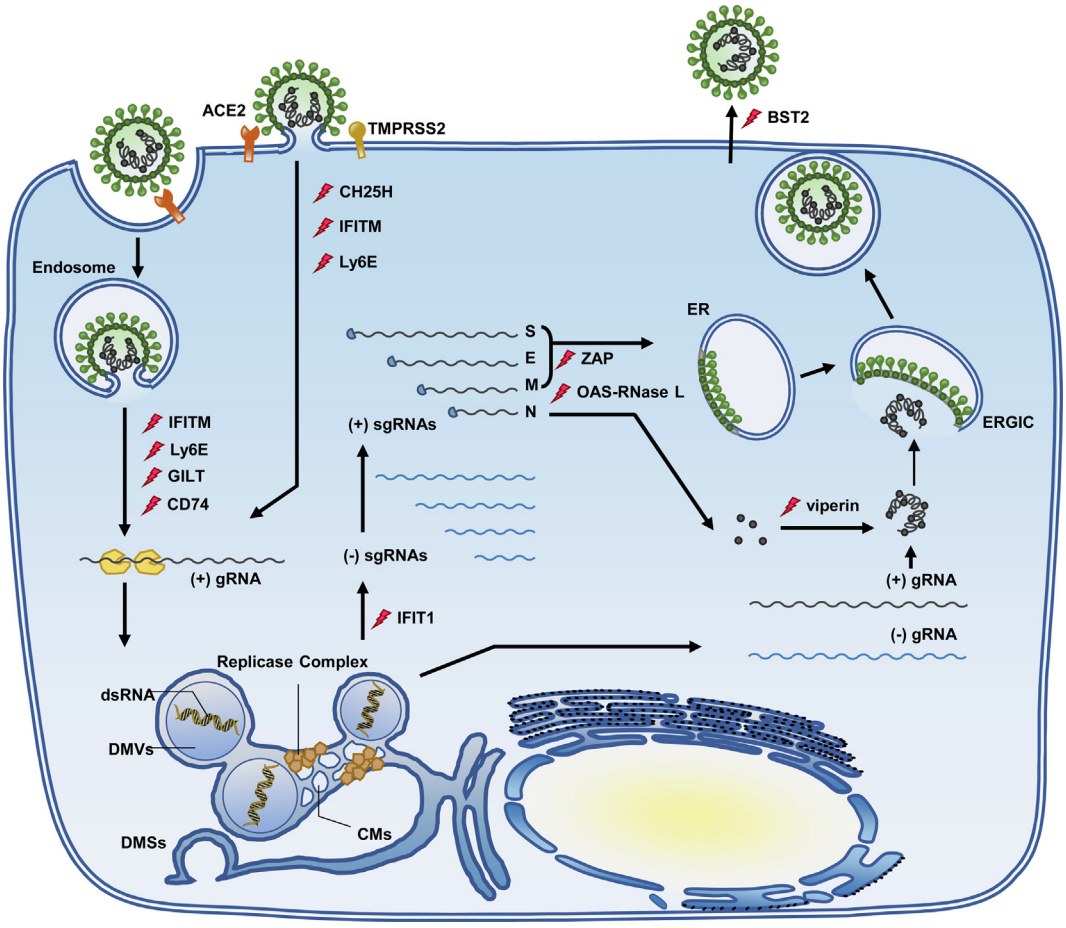

Figure 3. IFN-induced antiviral proteins inhibit distinct steps of CoV replication. Systematic genetic screens identified more than 30 ISGs that directly inhibit SARS-CoV-2 and/or HCoV-229E replication. The replication steps inhibited by some of these ISGs are illustrated.

IFITMs belong to the CD225 transmembrane protein superfamily which share the CD225 domain and play important roles in many cellular events through the regulation of membrane fusion, such as neurotransmission, immunity and metabolism.[95,96] As illustrated in Figure 4, the CD225 domain is defined by two hydrophobic regions separated by a cytoplasmic intracellular loop. One of the hydrophobic regions is a transmembrane helix and the other is an intramembrane helix. Recent studies revealed that the intramembrane helix of IFITMs is amphipathic and required for antiviral activity.[97] It was reported recently that the amphipathic helix spanning amino acid residues 59–68 partitions into lipid-disordered domains at the site of IAV fusion to block the fusion pore formation by inducing negative membrane curvature and increasing the membrane lipid order and stiffness.[98] Furthermore, a ^{91}GxxxG^{95} motif in the CD225 domain of IFITM3 drives its oligomerization, which is essential for its antiviral functions.[99] In addition to directly modulating membrane physical property at the site of membrane fusion, IFITMs may also inhibit viral entry through the regulation of endosomal trafficking. IFITM3 inhibition of the cellular entry of reovirus, a naked capsid virus, seems to support this notion.[100] Moreover, site-specific fluorophore tagging and live-cell imaging studies revealed that IFITM3 on endocytic vesicles engages with and promotes the trafficking of incoming virus particles to lysosomes in a S-palmitoylation dependent manner.[101] S-palmitoylation of IFITMs, a conserved post-translational modification for all the IFITM proteins, may assist IFITM conformation alteration, association with specific membrane domains or interaction with other proteins and is required for restriction of all the viruses examined.[102,103] It is thus likely that the S-palmitoylation of IFITMs functions in both inhibition of membrane fusion and direction of incoming virions to lysosomes for degradation.[104]

LY6E. LY6E (Lymphocyte antigen 6 complex, locus E), also referred to as TSA-1 (Thymic Shared Antigen-1), SCA-2 (Stem Cell Antigen-2) or RIG-E (Retinoic Acid Inducible Gene E), is a

member of Ly-6/uPAR protein family (Lymphocyte antigen-6/urokinase-type plasminogen activator receptor).[105] LY6E was initially found to be expressed in immature $CD4^-$ $CD8^-$ thymocytes[106] and functions in T cell development and activation.[107] However, LY6E is also found to be an IFN-inducible protein[108] and undergoes active transcription in various organs such as the liver, uterus, spleen, ovary, lung, and brain.[105] As a GPI-anchored protein, LY6E is enriched in the lipid-raft microdomain of the plasma membrane and endosomes[109] and functions in modulating the infectious entry of multiple enveloped viruses.[79,110] Specifically, LY6E differentially regulates HIV-1 entry in a CD4-dependant manner[111,112] and enhances the infectivity of multiple enveloped RNA viruses, including Yellow Fever Virus (YFV), Dengue Virus (DENV), West Nile Virus (WNV), Chikungunya virus (CHIKV), OYANG virus (ONV) and influenza A virus (IAV/PR8).[109] LY6E also serves as the receptor for the endogenous retroviral envelope in mice, Syncytin-A (SynA). This interaction is essential for syncytiotrophoblast formation during the development of the murine placenta.[113]

LY6E potently restricts the cellular entry of multiple CoVs, including mouse hepatitis virus (MHV) and all the HCoVs examined,[73,76] through inhibition of viral spike protein-induced membrane fusion.[73] Its critical role in the control of CoVs infection is demonstrated by the observation that mice lacking LY6E in their immune cells were highly susceptible to MHV infection.[73] Interestingly, unlike IFITMs, amphotericin B treatment does not attenuate the restriction of HCoVs by LY6E, suggesting LY6E inhibits CoV spike protein induced membrane fusion *via* a distinct mechanism.[76]

CD74. CD74 (also designated as the invariant chain protein, Ii) is primarily expressed in antigen-presenting cells as an MHC class II chaperone. In macrophages, CD74 serves as a receptor for migration inhibitory factors (MIF) and is expressed on macrophages that participate in inflammatory responses.[114] CD74 is a type II transmembrane protein and its intracellular domain (ICD) is essential for the activation of NFκB and other signaling pathways upon MIF binding. In humans, four CD74 isoforms (p31, p35, p41 and p43) are expressed due to alternative splicing. As shown in Figure 5, these isoforms differ in the presence or absence of N-terminal ER-retention signal and internal thyroglobulin domain.[115]

CD74 is an IFN-inducible protein. The p41 isoform specifically inhibited the endosomal entry of Ebola virus (EBOV) and HCoVs, including SARS-CoV-2.[72] This isoform contains the thyroglobulin domain, lacks ER retention signal, and normally accumulates in endosomes. The CD74 thyroglobulin domain inhibits cathepsins and when its cathepsin L binding site is mutated it completely abolishes its ability to restrict viral entry.[72,116] Accordingly, apart from its roles in antigen presentation and inflammation, CD74 functions as a crucial restriction factor which inhibits endolysosomal entry of viruses through the inhibition of cathepsin cleavage of viral envelope proteins.

GILT. GILT (Gamma-interferon-inducible lysosomal thiol reductase, also referred to as IFI30) is a soluble thiol reductase responsible for the reduction of protein disulfide bonds in specific conditions. This enzyme is abundantly expressed in the lysosomes of professional antigen-presenting cells (APCs), such as macrophages, dendritic cells and B lymphocytes, but can be induced by IFN-γ in other cell types.[117,118] The role of GILT in adaptive immunity has been well characterized. Specifically, GILT promotes the MHC class II-restricted presentation of exogenous antigens containing disulfide bonds. GILT can also facilitate the MHC class I-restricted recognition of such antigens by $CD8^+$ T cells *via* cross presentation.[119,120]

GILT is synthesized as a 35-kDa precursor and is directed to the endocytic pathway *via* the mannose-6 phosphate receptor (M6PR). The enzyme is transported into the lysosomes which will provide the optimal acidic pH for its thiol reductase activity.[118,120] Mature GILT utilizes two cysteine residues in the ^{72}CXXC^{75} reductase motif to reduce the disulfide bonds of endocytosed antigens, thus,

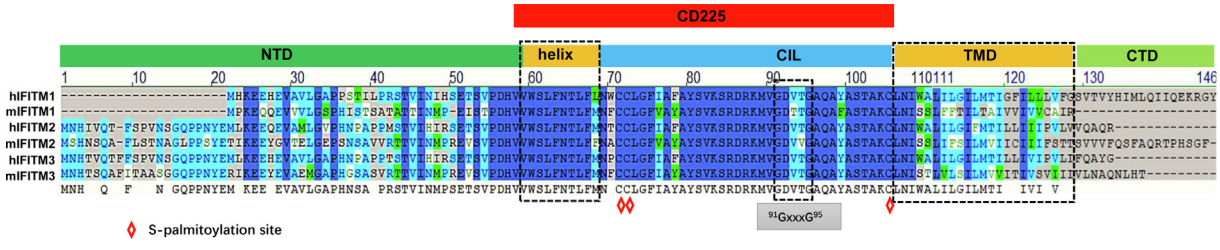

Figure 4. Domain structure and critical motifs of interferon-induced transmembrane (IFITM) proteins. (A) Membrane topology of IFITM proteins. (B) Domain structure of human and mouse IFITM proteins. NTD, N-terminal domain; CTD, C-terminal domain, Helix, amphipathic alpha helix; CIL, conserved intracellular loop; TMD, Transmembrane domain. CD225 domain contains amphipathic alpha helix and CIL domains. The oligomerization motif and cysteines for palmitoylation are indicated.

facilitating antigen processing and presentation by MHC-II and MHC-I.[119,120]

Recent studies revealed that GILT only inhibited the cellular entry of SARS-CoV, EBOV, and LASV, all of which enter host cells through the lysosome.[121] Furthermore, GILT mutations that impair the thiol reductase activity or disrupt N-linked glycosylation, an essential post-translational modification for lysosomal localization, largely compromised its restriction of viral entry. Additionally, the induction of GILT expression reduced the level and activity of cathepsin L, the lysosomal endopeptidase enzyme required for the lysosomal entry of susceptible RNA viruses. These results suggest that GILT is a novel antiviral ISG that specifically inhibits the entry of selected enveloped RNA viruses in lysosomes through the inhibition of cathepsin L activity.

CH25H. Cholesterol-25-hydroxylase (CH25H), a conserved antiviral ISG in mammalian species, contributes to the regulation of cholesterol biosynthesis. CH25H encodes an ER-associated enzyme that converts cholesterol to a soluble antiviral factor, 25-hydroxycholesterol (25HC).[122] The expression of CH25H and 25HC treatment is known to broadly inhibit the multiple enveloped viral infections.[79,123–126] Studies have shown that CH25H-knockout mice exhibited increased susceptibility to murine gamma herpesvirus 68 (MHV68) infection.[124] Through the oxidation of cholesterol, 25HC becomes a soluble oxysterol capable of inhibiting the entry of many enveloped viruses through the disruption of membrane fusion, due to the alteration of the membrane's hydrophobicity or cholesterol composition.[125] In support of its antiviral effects, the significant restriction of HIV replication and rescued T-cell depletion upon the administration of 25HC has been demonstrated in mice models.[124] Administration of 25HC also reduced viremia and protected mice and rhesus macaques from ZIKV infection.[125]

In addition to directly interfering with membrane fusion, CH25H abrogates LASV G1 glycoprotein N-glycan maturation, thus suppressing the production of infectious virions.[127] CH25H inhibited HCV infection at post entry stages by suppressing the maturation of SREBPs which are critical transcription factors for host lipid biosynthesis.[128] Another study demonstrated that 25HC inhibited HCV infection through blocking the formation of the membranous web which serves as a viral RNA replication organelle.[129] Interestingly, CH25H is involved in B cell migration through the generation of 7α,25-OHC, a chemokine that directs immune cell migration.[130,131]

CH25H and 25HC have demonstrated the ability to inhibit cellular entry of many CoVs, including SARS-CoV,[132,133] SARS-CoV-2,[132,133] MERS-CoV,[132] porcine epidemic diarrhea virus (PEDV),[134] and Porcine deltacoronavirus (PDCoV).[135] Of note, a potent antiviral effect of 25HC was observed in mouse model by using a mice-adapted SARS-CoV-2 strain MASCp6.[136] Mechanistically, CH25H inhibits CoV cellular entry by interfering with S protein-mediated membrane fusion.[132,133] Particularly, internalized 25HC accumulates in the late endosomes and lysosomes to inhibit membrane

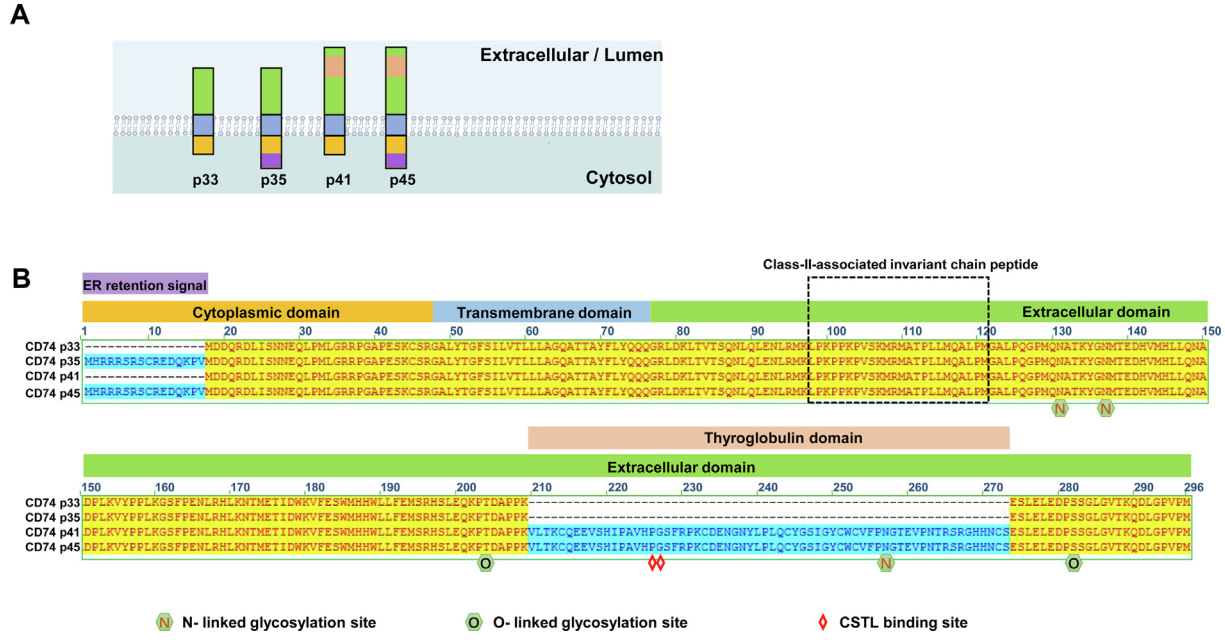

Figure 5. Domain structure and splicing variants of CD74. (A) Membrane topology of CD74 proteins. (B) The topogenic feature and key structure domains or motifs are indicated. The structural features of the four CD74 variants are highlighted.

fusion.[133] Plasma membrane fusion is also restricted by 25HC through the activation of ER-resident acyl-CoA: cholesterol acyltransferase (ACAT) which depletes the accessible cholesterol from the plasma membrane.[132]

IFN-induced cellular proteins targeting HCoV RNA

Zinc finger antiviral protein (ZAP). ZAP is an RNA binding protein containing an N-terminal ~227-aa RNA-binding domain (RBD) with 4 CCCH zinc fingers (ZnF1 to ZnF4), a WWE domain, and a C-terminal poly(ADP-ribose) polymerase domain which is present in the ZAP-L isoform but not in the ZAP-S isoform.[137] ZAP broadly inhibits the infection of various RNA viruses through the detection of specific viral RNAs that have a greater frequency of CG dinucleotides than cellular RNAs. These dinucleotides promote viral RNA degradation via its co-factor, KHNYN, a presumed endonuclease that is important for ZAP antiviral activity against retroviruses[138] or the RNA exosome.[139] Crystal structural analysis of the protein-RNA complex containing the N-terminal 4-zinc finger human ZAP RNA-binding domain (RBD) and the CG dinucleotide-containing RNA revealed the formation of a CG-binding pocket from the four zinc fingers on the ZAP RBD surface specifically accommodating the CG dinucleotide.[140] However, ZAP induces the pregenomic RNA degradation of hepatitis B virus through the recognition of a stem-loop (ε) structure at the 5′ terminal region of the viral RNA in the 4-zinc finger-dependent manner.[141]

Recent studies indicated that ZAP restricts SARS-CoV-2 by targeting CpG dinucleotides in viral RNA sequences.[142–144] Interestingly, SARS-CoV-2 and two closely-related animal CoVs isolated from horseshoe bats, RaTG13 and RmYN02, exhibited remarkable CpG suppression in comparison to other human and bat CoVs.[142] These findings imply the CG depression observed in SARS-CoV-2 genome may be a result of adaptive selection to evade ZAP restriction, which highlights the critical role of ZAP in the control of CoVs infection, thus, shaping the evolution of viruses.[145,146]

2′,5′-Oligoadenylate synthetases (OAS)/RNase L pathway. The 2′,5′-oligoadenylate (2-5A) synthetase (OAS)-RNase L system is a potent IFN-induced antiviral pathway that restricts the infection of a diverse group of viruses. Three human OAS species, OAS1, OAS2, and OAS3, can bind cytosolic dsRNA and be activated to synthesize 2′-5′ oligoadenylates (2-5A).[147] Binding of 2-5A to RNase-L induces its dimerization and activation of nuclease activity. This results in the degradation of both the cellular and viral RNA as well as the consequential inhibition of viral replication and induction of infected cell death.[10] Although OAS proteins are highly IFN-inducible, the basal levels of OAS expression can be detected in many cell types. Interestingly, although all three human OAS proteins synthesize 2-5A upon binding of dsRNA, it was demonstrated that only OAS3 is primarily responsible for the activation of RNase L in cells infected with a diverse group of viruses, including CoVs.[148] The dominant role of OAS3 in viral activation of RNase L may be attributed to its higher binding affinity binding to dsRNA. However, it was reported recently that the induction or overexpression of OAS1, but not OAS2 or OAS3, by CRISPR activation or cDNA transfection significantly protects against SARS-CoV-2 induced cytopathic effects and reduces viral yield. Further mechanistic analysis demonstrated that OAS1 inhibition of SARS-CoV-2 is dependent on its oligoadenylate synthesis activity.[71] A knockout of RNase L in $A549^{ACE2}$ cells increased replication of SARS-CoV-2 and cytopathic effect.[19] Moreover, *OAS1* genetic variants were found to be associated with infection and morbidity of SARS-CoV while the single nucleotide polymorphisms (SNPs) in the *OAS* loci are linked to COVID-19 mortality[48,149,150] (Figure 1). A Neanderthal isoform of OAS1 in individuals of European ancestry (rs4767027-T and rs10774671-G) was associated with reduced COVID-19 susceptibility and severity.[151] OAS1 alternative splicing is regulated by the rs10774671-G allele, which increases the isoform p46. The p46 isoform has a higher enzymatic activity against viruses than the p42 isoform.[152] Mechanistically, it was reported by two independent research groups that OAS1 p46 isoform is prenylated at the terminus, which targets the ER membrane to enhance its accessibility to viral RNA for more efficient activation thus increasing the antiviral activity to viruses that replicate their RNA genome in membranous organelles. These viruses include flaviviruses, piconaviruses and human coronaviruses, such as SARS-CoV-2.[68,153]

As anticipated, CoVs have evolved multiple strategies to counteract OAS-RNase L pathway to facilitate their replication. For example, MHV employs Nsp2, an accessory protein that possesses 2′,5′-phosphodiesterase activity, to cleave 2′,5′-oligoadenylates and prevent RNase L activation.[154,155] MERS-CoV as well as closely-related bat CoVs also encode accessory proteins Nsp4A and Nsp4B with phosphodiesterase activity to inhibit the activation of OAS-RNase L pathway.[156,157] The critical role of OAS-RNase L system in defending CoV infection and pathogenesis is highlighted by the observation that Nsp2 antagonism of this intricate dsRNA sensor pathway is required for MHV replication in the liver and induction of hepatitis.[154] However, the result that OAS1 restricts SARS-CoV-2 replication in A549 cells and protects SARS-CoV-2 infection and pathogenesis in humans suggests that SARS-CoV-2 may not evade this antiviral pathway. Indeed, a recent study

on SARS-CoV-2 activation of innate immune response in the relevant respiratory tract derived cells shows that SARS-CoV-2 activates OAS–RNase L and PKR, while inducing minimal levels of interferon. These phenomena indicate viral SARS-CoV-2 evasion of RIG-I/MDA5 detection in contrast to MERS-CoV infection, which evades the activation of all the three dsRNA detection pathways.[19] It will be interesting to uncover why OAS1, but not OAS2 or OAS3, restricts SARS-CoV-2 infection and whether different CoVs activate distinct OAS species.

IFIT1 inhibits viral protein translation

There are 4 members of the interferon-induced proteins with tetratricopeptide repeats (IFITs) family found humans which include: IFIT1 (ISG56), IFIT2 (ISG54), IFIT3 (ISG60) and IFIT5 (ISG58).[158,159] The expression of IFITs can be induced by the activation of a IFN regulatory factor (IRF) family of transcription factors in viral infection and by dsRNA recognition independently of IFN production. IFIT proteins contain multiple copies of tetratricopeptide repeats (TPR), a conserved motif composed of 34 amino acid residues, which adopt a helix–turn–helix structure.[158,159] The TPRs of IFITs coalesce into distinct super helical subdomains that form clamp-shaped structures.[160–162]

IFITs execute their antiviral function by specifically interacting with a range of cellular and viral RNAs and proteins. For instance, IFIT1 and IFIT2 bind to the multisubunit eukaryotic translation initiation factor 3 (eIF3) which inhibits viral and cellular mRNA translation initiation.[163,164] IFIT1 can bind to the 5′ ends of the mRNA lacking the ribose 2′-*O* methylation on the first cap-proximal nucleotide, a hallmark of some viral mRNAs but not cellular mRNAs. This binding selectively inhibits the translation of viral mRNA.[165–167] In addition, IFIT1 also specifically recognizes and sequesters the viral RNA replication intermediates with uncapped 5′-ppp termini to inhibit viral RNA replication.[160,168] Therefore, IFIT1 is a unique pattern recognition receptor and antiviral effector molecule,[169] whereas, other human IFIT members do not have a preferable binding affinity to those viral RNA molecular patterns.[165,170]

IFIT1 has been shown to inhibit the replication of RNA viruses which produce RNA with 5′-ppp, such as VSV and Rift valley fever virus.[170] IFIT1 also inhibits HCV[171] and parainfluenza virus 3 and 5.[172,173] Four groups of viruses, including CoVs, flaviviruses, reoviruses, and poxviruses, encode enzymes to catalyze the methylation at the 2′-*O*-ribose of the first viral RNA nucleotide for evasion of recognition by IFIT1.[174] SARS-CoV and SARS-CoV-2 Nsp10/Nsp16 heterodimer forms a 2′-*O* methyltransferase.[175,176] Mutant CoVs lacking 2′-*O* methyltransferase activity were observed to be more vulnerable to antiviral action of IFN and IFIT than their wild-type counterparts.[177] Furthermore, attenuated vaccines with mutations at Nsp10 or/and Nsp16 have been developed due to the observation that targeting 2′-O-MTase activity will reduce viral proliferation and virulence.[178–180]

BST-2 inhibits the release of HCoV virions

Bone marrow stromal cell antigen 2 (BST-2), also referred to as CD317 or tetherin, is a type II transmembrane protein with a short intracellular N-terminal domain, an extracellular coil-coil domain and a glycosyl phosphatidyl inositol (GPI) membrane anchor at its C terminus. The GPI membrane anchor directs BST-2 association with cholesterol-enriched lipid rafts in the cellular membrane. BST2 traffics through the ER and Golgi and localizes in the plasma membrane and endosomes.[181,182] BST2 blocks the release of many enveloped viruses from infected cells, including members of the following families: *Retroviridae* (HIV-1),[183,184] *Arenaviridae* (Lassa virus),[185] *Paramyxoviridae* (Nipah and Hendra virus),[186,187] *Orthomyxoviidae* (Influenza A virus),[188,189] *Rhabdoviridae* (vesicular stomatitis virus),[94] *Filoviridae* (Ebola and Marburg viruses),[183] and *Hepadnaviridae* (Hepatitis B virus).[190] Accumulating evidence suggests BST-2 is incorporated into budding viral particles and through coil-coil domain mediated homo-oligomerization with other BST-2 molecules in the cellular membranes, viral particles become 'tethered' to the cell surface or intracellular membranes.[191] Unlike HIV and many other viruses that bud from the plasma membrane, CoVs bud in the ER-Golgi apparatus intermediate compartment (ERGIC) and are transported to the plasma membrane inside vesicles.[192] However, it has been shown that BST2 also restricts the replication of HCoV-229E and SARS-CoV-2 by tethering progeny virions on the cellular surface and intracellular membranes.[67,193] This observation suggests that BST-2 can also restrict viruses that bud in the ERGIC and are then released from the cell via vesicle fusion.

Viruses have evolved many strategies to evade BST2 restriction by inducing its degradation or directing it away from its site of action on the cellular surface. For instance, primate lentiviruses employ at least three accessory proteins including Vpu, Nef, and Env, to counteract tetherin.[194,195] HIV-1′s Vpu interacts with BST2 via its transmembrane domains and directs BST2 to the lysosome and/or the proteasome for degradation.[196–198] HIV-2 and some SIV strains utilize their Env and Nef proteins, respectively, to downregulate BST2 expression on the cellular surface through sequestrating it to endocytic compartments.[199,201] SARS-CoV ORF7a selectively binds to unglycosylated BST2 to prevent its glycosylation, which is required for restriction of SARS-CoV release from infected cells. However, the interaction between SARS-CoV-2 ORF7a and BST2 does not depend on BST2 glycosylation status.[67,202] In addition, both

SARS-CoV and SARS-CoV-2 spike proteins down-regulate BST2 to facilitate viral spread.[203,204] However, BST-2 can be hijacked by human cytomegalovirus to enhance cellular entry. In other studies, murine BST2 was found to promote measles virus infection in brains of permissive mice and in primary neuron cultures.[205,206]

Perspectives

The activation and evasion of the innate immune response are the key determinants of viral host range and cell tropism and thus, effect cross-species transmission and pathogenesis. In addition to antiviral drugs that directly target viral proteins or RNA for specific inhibition of viral replication, drugs that disrupt the viral evasion of host innate immune response should facilitate immune control and resolution of viral infections.[207] Furthermore, mutant viruses that fail to counteract the innate immune restriction could be candidates for attenuated vaccines, which could induce more efficient and durable protection from HCoV infection and/or diseases.[208,209] Therefore, having a better understanding of the molecular mechanism of innate immune control and HCoV evasion should facilitate the discovery and development of novel antiviral drugs and vaccines for the treatment of CoV diseases and prevention of CoV infections. These developments will particularly be important for future zoonotic CoV epidemics in humans.[210]

While recent intensive investigation on the innate immune response to SARS-CoV-2 infection uncovered important features of the virus-host cell interactions and provided many mechanistic insights, some important questions on the role and mechanisms of innate immune response in SARS-CoV-2 pathogenesis have yet to be answered. *First*, the innate immune response of specific cell types, such as immune cells (NK cells and pDCs) and distinct cell types in the lung epithelium, to CoV pathogenesis should be further investigated. *Second*, it appears that CoVs can activate all the three families of known cytoplasmic dsRNA sensors, *i.e.*, RIG-I-like receptors, PKR, and OAS. The mechanisms underlying the activation of these dsRNA receptors and their contributions to CoV pathogenesis and control should be dissected. *Third*, it is rather surprising that OAS3 is primarily responsible for RNase L activation in the infection of CoVs and several other RNA viruses.[19] However, forward genetic screen and population genetics studies demonstrated that OAS1 restricts SARS-CoV-2 infection and plays a critical role in SARS-CoV-2 pathogenesis.[71] Therefore, further investigation on the mechanisms of activation and biological function of the distinct members of the OAS family in the infection and pathogenesis of CoVs and other viruses is warranted. *Finally*, it is important to apply our understanding on the innate immune restriction and viral evasion of PRR- and IFN-mediated antiviral immune responses in the discovery and development of therapeutic and prophylactic medicines to control the infection of CoVs and other viruses. Particularly, the development of small molecular antiviral agents disrupting the key interaction between the viral and cellular proteins for the evasion of IFN restriction of viral replication hold great promise in the treatment of coronavirus diseases.

CRediT authorship contribution statement

Xuesen Zhao: Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition, Supervision. **Danying Chen:** Writing – original draft, Visualization. **Xinglin Li:** Writing – original draft, Visualization. **Lauren Griffith:** Writing – review & editing. **Jinhong Chang:** Writing – review & editing, Funding acquisition. **Ping An:** Writing – review & editing, Visualization. **Ju-Tao Guo:** Conceptualization, Writing – review & editing, Visualization, Funding acquisition, Supervision.

DATA AVAILABILITY

No data was used for the research described in the article.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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