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Forum

Clash of the titans: interferons and SARS-CoV-2

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Interferons are our first line of defense against invading viruses. However, viruses encode effector proteins that can modulate human interferon responses. In this forum article, we highlight important discoveries and discuss outstanding questions that will enable us to better understand the nuances of this evolutionary battle between interferons and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Disease severity in coronavirus disease 2019 (COVID-19) patients is driven by a dysregulated immune response, which includes delayed induction of antiviral interferon (IFN) responses, along with exaggerated proinflammatory responses [1]. Despite an increasing number of studies investigating the interactions between SARS-CoV-2 proteins and IFNs, the impact of timing and duration of the IFN response on SARS-CoV-2 replication and COVID-19 severity remains elusive.

Cellular detection of SARS-CoV-2

To identify mammalian pattern recognition receptors (PRRs; Box 1 and Figure 1) that contribute to the expression of IFN and inflammatory cytokine production in SARS-CoV-2 infected cells, several approaches have been undertaken. Silencing the expression of a large set of PRRs [2] or of a selection of retinoic acid-inducible gene I-like receptors (RLRs) [3] revealed that SARS-CoV-2 infection-mediated induction of type I and III IFNs largely depends on melanoma differentiation-associated protein 5 (*MDA5*) expression in lung epithelial Calu-3 cells. Knockdown of other PRRs had little to no impact on type I and III IFN induction or downstream IFN-stimulated gene (ISG) upregulation in these cells [2]. A role of retinoic acid-inducible gene I (RIG-I) in type I IFN induction in SARS-CoV-2 infected Calu-3 cells was also proposed [4], but this remains controversial. Depleting the expression of the laboratory of genetics and physiology 2 (*LGP2*) gene, a known potentiator of MDA5-mediated IFN responses, significantly reduced type I IFN (*IFNβ*) mRNA abundance in SARS-CoV-2 infected Calu-3 cells [2]. Collectively, these results highlight the concept that MDA5 is particularly important for inducing type I and III IFN responses against SARS-CoV-2. PRRs other than MDA5 are likely to contribute to the initiation of host cell responses against SARS-CoV-2 infection but remain largely unidentified. Of note, interleukin 6 (*IL6*) induction seems to depend on RIG-I and not MDA5 expression in SARS-CoV-2 infected Calu-3 cells that were depleted for these RLRs using RNA interference (RNAi) [4]. RLR usage may thus trigger the expression of different inflammatory mediators and vary depending on cell type.

Toll-like receptor (TLR) 3 depletion has no impact on *IFNβ* transcript abundance in SARS-CoV-2 infected Calu-3 cells [2]. However, SARS-CoV-2 infection of plasmacytoid predendritic cells (pDCs) from healthy donors and from patients with genetic defects in IRAK4 and UNC93B1 *ex vivo*, demonstrated that these proteins (required for signaling downstream of TLRs) were essential for type I and III IFN production by pDCs [5]. Accumulating data demonstrate that molecular components of SARS-CoV-2 are recognized by both RLRs and TLRs [2,3,5]. Further investigations, ideally in animal models and in human primary cells, are warranted to continue delineating the role of PRRs in SARS-CoV-2-induced innate immune responses. Moreover, it is of utmost interest to precisely identify specific viral replication intermediates that are recognized by PRRs, via, for instance, sequencing viral genomic, subgenomic, or mRNA molecules bound to RLRs purified from infected cells. The implications for the recognition of SARS-CoV-2 proteins and nucleic acids by other cellular immune sensors, such as protein kinase R (PKR) and nucleotide-binding and oligomerization domain (NOD)-like receptors remain, however, less studied. Finally, the kinetics of the interactions between viral

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Box 1. Pattern recognition receptors (PRRs) in antiviral immunity

When exposed to viruses, most mammalian cells produce cytokines, including interferons (IFNs). Three classes of IFN have been identified, designated types I to III, and are classified according to the receptor complex they signal through. In contrast to types I and III IFNs, type II IFNs are not secreted by virus-infected fibroblasts, epithelial, or endothelial cells, but mainly by natural killer and T cells. IFN production is initiated when PRRs recognize specific viral products, such as viral nucleic acids or viral proteins. These PRRs can be membrane-associated, such as Toll-like receptor (TLR), or cytosolic, such as retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs). Three RLR members, expressed in most tissues, have been identified: RIG-I, melanoma differentiation-associated protein 5 (MDA5), and the laboratory of genetics and physiology 2 (LGP2). The human TLR family contains ten TLRs (TLR1–10) and several of them, including TLR2, 3, 4, 7, 8, and 9 are implicated in the early interplay of host cells and invading viruses. PRR activation by viral components enables interaction with adaptor proteins and the recruitment of signaling complexes that stimulate the rapid expression of inflammatory cytokine production and IFNs. Upon their secretion, IFNs bind to their receptors, in an autocrine or paracrine manner, to activate a signaling pathway that ultimately triggers the expression of hundreds of IFN-stimulated genes (ISGs), whose products have antiviral properties, effectively establishing an antiviral state in infected and surrounding cells. Other cytokines can boost the development of a more long-term antigen-specific adaptive immunity that is pivotal for pathogen clearance and immune memory. Of note, a prolonged uncontrolled cytokine response, or 'cytokine storm', can drive cell death and subsequent tissue dysfunction.
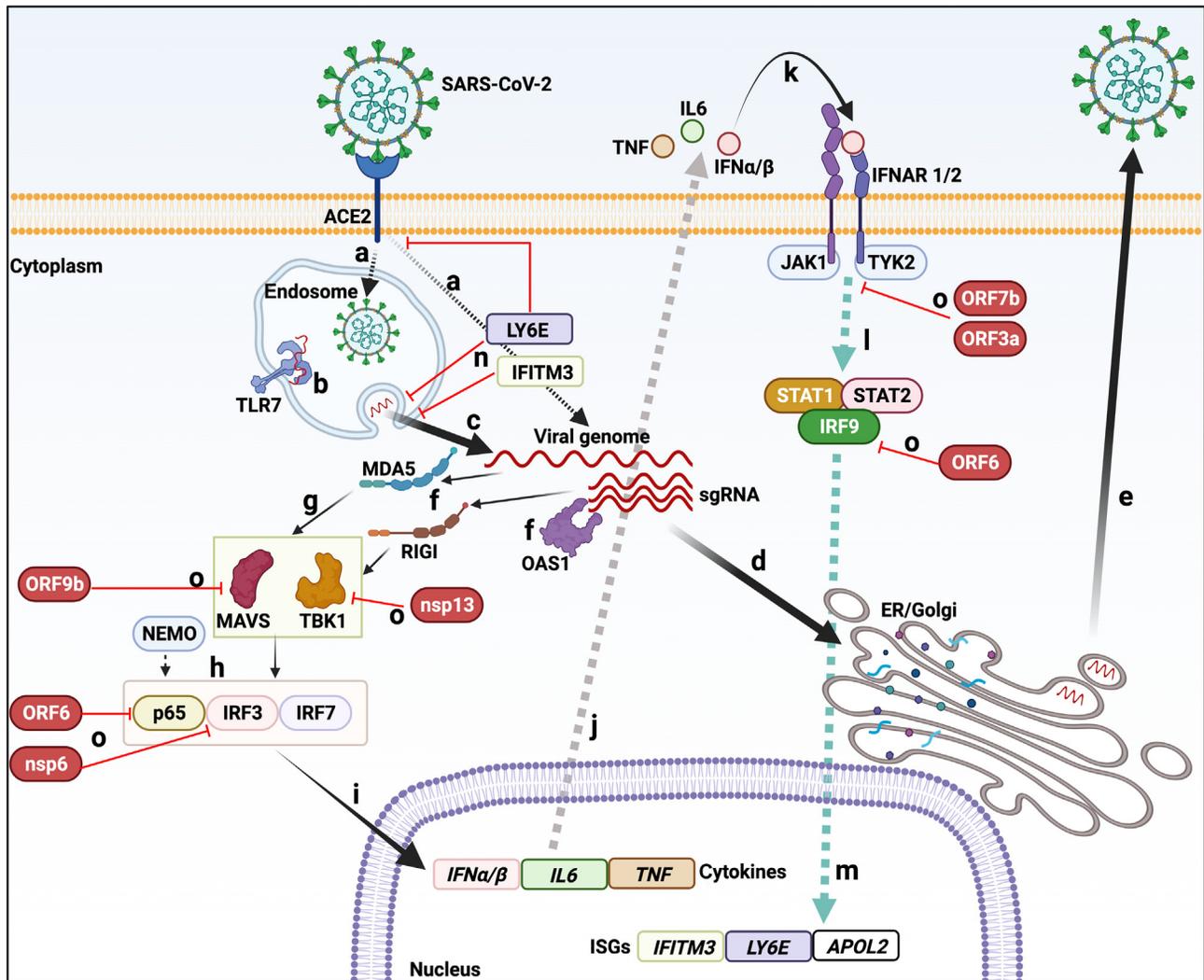
Figure 1. Dynamic interactions between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins and type I interferon (IFN) responses. The SARS-CoV-2 spike protein interacts with the cellular receptor angiotensin-converting enzyme 2 (ACE2) to allow virus entry into human epithelial cells of the respiratory system. The virus can use two routes of cell entry: either fusion at the plasma membrane or internalization within an endosome (a). Once internalized within an endosome, viral components, such as genomic RNA, can be detected by endosomal Toll-like receptor 7 (TLR7) (b). Following release of viral RNA into the cytoplasm (c), the genomic RNA undergoes replication, transcription, and translation (d) to produce progeny virions (e). During the process of replication and transcription of viral RNA, cellular pattern recognition receptors (PRRs), such as melanoma differentiation-associated protein 5 (MDA5), retinoic acid-inducible gene I (RIGI), and 2′-5′-oligoadenylate synthetase 1 (OAS1) detect viral RNA (f). Cellular adaptor proteins, such as mitochondrial antiviral-signaling protein (MAVS) and TANK binding kinase I (TBK1) (g) mediate activation signals from PRRs to activate key transcription factors, such as p65, interferon regulatory factor 3 (IRF3), and IRF7 (h). Upon activation, these transcription factors translocate to the nucleus to induce the expression of cytokines such as type I interferons (*IFNα/β*), interleukin 6 (*IL6*), and tumor necrosis factor (*TNF*) (i). Secreted cytokines such as type I IFNs (j) carry out their effector functions in an autocrine or paracrine manner (k). Type I IFNs interact with their cognate receptors (IFNAR1/2) to activate downstream signaling cascades (l) via transcription factors signal transducer and activator of transcription 1 (STAT1), STAT2, and IRF9, to induce the expression of interferon-stimulated genes (ISGs) (m). ISGs act on various stages of virus replication to inhibit virus propagation (n). However, SARS-CoV-2 has evolved multiple proteins that can inhibit the host antiviral response, some of which are highlighted here (o) [15]. Figure created with BioRender.com. Abbreviation: ER, endoplasmic reticulum.

components and PRRs remain unaddressed. For instance, characterizing the accessibility of viral components to PRRs over the course of SARS-CoV-2 infection will be crucial in understanding the tug-of-war between virus infection and host IFN responses and can inform potential therapeutic strategies against COVID-19.

SARS-CoV-2 infection-mediated induction of IFNs

SARS-CoV-2 infection induces a type I (*IFNβ*) and III (*IFNλ*) IFN response in Calu-3 cells [2,3,6], primary airway epithelia (either derived from healthy donor biopsies or from iPSC) cultured at the air–liquid interface [2,3], as well as in intestinal organoids [7]. Primary human pDCs can also induce the expression of type I and III (*IFNα* and *IFN-λ1*) IFNs upon *ex vivo* exposure to SARS-CoV-2 [5]. Sequencing global RNA extracted from nasopharyngeal swabs from patients with various COVID-19 disease profiles revealed a strong induction of ISGs such as *OASL*, *IFIT2*, and *MX1* [8]. In addition, significantly higher serum concentrations of type I IFN were detected in patients with mild/moderate COVID-19 relative to patients with severe/critical disease [6,9]. Moreover, the physiological importance of the IFN response in limiting COVID-19 severity has been highlighted by the presence of inborn mutations in genes involved in IFN signaling pathways [e.g., interferon-alpha/beta receptor subunit 1 (*IFNAR1*) and interferon regulatory factor 9 (*IRF9*)] and by the presence of neutralizing auto-antibodies against type I IFN(s) in 3–5% patients and >10% of critically ill patients, respectively [10].

The role of endogenous IFNs in the control of SARS-CoV-2 replication *in vitro* is debatable and may depend on the cell type [2,3,7]. Noteworthy, studies have suggested that SARS-CoV-2 proteins can shut down host translation, which would also affect the translation of IFN transcripts. Nevertheless, there is strong consensus that SARS-CoV-2 replication is prevented by pre-exposure of various susceptible human cells (including primary airway epithelia or Calu-3 cells) to type I/III IFNs, as assessed by reverse transcription (RT)-qPCR analysis or plaque assays [2,3,7,11]. However, the identity of ISGs responsible for this potent antiviral effect remains to be fully unraveled. A subset of ISGs, including lymphocyte antigen 6 family member E (*LY6E*), apolipoprotein L2 (*APOL2*), and interferon-induced protein with tetratricopeptide repeats 3 (*IFIT3*) can individually limit SARS-CoV-2 replication when ectopically expressed in human cell lines prior to SARS-CoV-2 infection [12,13]. Interferon-induced transmembrane protein 3 (*IFITM3*), when expressed in HEK293T cells, also partially inhibits endosomal-mediated SARS-CoV-2 cell entry [12] (Figure 1). While approaches using ectopic expression of ISGs are useful for identifying antiviral genes of interest, they are not sufficient to ascertain a role for these genes in a physiological context. The importance of ISGs, individually or in combination, should be studied using knockout experiments in physiologically relevant iPSC-derived airway epithelia. An important question to address is whether numerous ISGs have an additive effect in limiting SARS-CoV-2 replication or few ISGs play a major role [e.g., as seen for HIV-1 with MX dynamin like GTPase 2 (*MX2*) and tripartite motif containing 5 alpha (*TRIM5*)]. The importance of studying the impact of ISGs on COVID-19 was recently highlighted by the demonstration that the expression of prenylated isoforms of 2′-5′-oligoadenylate synthetase 1 (*OAS1*) in hospitalized patients was associated with protection from severe COVID-19 [14]. Thus, it will be important to decipher the full landscape of ISGs that might prevent SARS-CoV-2 replication in primary host cells. Understanding their modes of action may indeed pave a way for the development of new candidate antiviral drugs and immunotherapies against COVID-19.

The kinetics of IFN induction versus modulation by SARS-CoV-2 proteins

Multiple studies have now identified SARS-CoV-2 proteins that can inhibit different aspects of human IFN production and signaling (Figure 1) (e.g., [15]). Following virus entry, the SARS-CoV-2 genome undergoes replication and transcription, which stimulates IFN-mediated antiviral responses [2,3]. However, subsequent translation of viral mRNA produces viral proteins that can inhibit IFN mRNA export, protein production, and signaling (Figure 1) [15]. Despite these studies, the timing and kinetics of IFN induction versus inhibition in SARS-CoV-2 infected cells, to our knowledge, remain unknown. Accordingly, the optimal duration and intensity of IFN-mediated responses that are required to restrict SARS-CoV-2 replication also remain elusive.

The timing of IFN responses may play an important role in SARS-CoV-2 pathogenesis. Specifically, an absent, delayed, or weak IFN response in COVID-19 patients correlates with increased immunopathology and disease outcomes [9]. Early induction of IFN responses has been associated with mild or moderate COVID-19, presumably protecting patients from severe disease [8,9]. However, the factors that determine the extent and intensity of early or delayed IFN responses in COVID-19 patients remain to be further investigated. While *in vitro* studies have demonstrated that SARS-CoV-2 proteins, such as NSP1, NSP6, NSP13, or ORF6, for instance [15], can inhibit type I IFN responses when ectopically expressed in human HEK293T cells, an important approach will be to identify the effects of early IFN induction in primary airway epithelial cells before viral proteins can further inhibit IFN production. As SARS-CoV-2 is not as adept as SARS-CoV in blocking IFN signaling [11], perhaps triggering the early production of IFNs in SARS-CoV-2 infected cells might provide a modest protective effect. Time-dependent transcriptomic and proteomic analyses in SARS-CoV-2 infected human cells might be the first step to delineate such dynamic viral and cellular processes. Evidently, extensive and robust research is needed to delineate the kinetics of IFN responses during SARS-CoV-2 infection, as well as the systemic

effect of these cytokines on COVID-19 severity.

Concluding remarks

Accumulating data suggest that SARS-CoV-2 proteins can modulate IFN responses. Data also suggest that SARS-CoV-2 infection induces an MDA5-dependent IFN response in human epithelial cells. These studies raise multiple intriguing questions, namely: (i) how protective are IFNs during early stages of COVID-19? (ii) What are the identities and modes of action of the main ISG effectors *in vivo*? (iii) What amounts of viral proteins are sufficient to inhibit IFN responses in infected cells? (iv) How do the interactions between IFN responses and viral proteins differ in different cell types? (v) How can the kinetics of IFN responses inform the development of more effective candidate therapeutic interventions against COVID-19? Thus, the nuances of SARS-CoV-2–IFN interactions and the protective or detrimental outcomes in COVID-19 patients must remain an area of intense investigation.

Declaration of interests

The authors declare no conflict of interest.

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