



HAL
open science

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

Nolwenn Jouvenet, Caroline Goujon, Arinjay Banerjee

► **To cite this version:**

Nolwenn Jouvenet, Caroline Goujon, Arinjay Banerjee. Clash of the titans: interferons and SARS-CoV-2. Trends in Immunology, 2021, 42 (12), pp.1069-1072. 10.1016/j.it.2021.10.009 . hal-03447813

HAL Id: hal-03447813

<https://hal.science/hal-03447813>

Submitted on 19 Jun 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

2 *Nolwenn Jouvenet^{1,*}, Caroline Goujon^{2,*} and Arinjay Banerjee^{3,4,5,*}*

3 ¹Institut Pasteur, Université de Paris, CNRS UMR3569, Virus Sensing and Signaling Unit,

4 Paris, France

5 ²IRIM, CNRS, Montpellier University, Montpellier 34090, France

6 ³Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatoon, SK

7 S7N 5E3, Canada

8 ⁴Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK S7N

9 5B4, Canada

10 ⁵Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1, Canada

11 *Correspondence: nolwenn.jouvenet@pasteur.fr (NJ), caroline.goujon@irim.cnrs.fr (CG) and

12 arinjay.banerjee@usask.ca (AB)

13

14

15

16

17

18

19

20

21

22

23

24

25

26 **Abstract**

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

31 **Keywords**

32 SARS-CoV-2, innate immunity, interferon, virus-host interaction, interferon stimulated genes

33

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

40

41 **Cellular detection of SARS-CoV-2**

42 The identity of pattern recognition receptors (PRRs; Box 1 and Figure 1) that contribute to the
43 expression of IFN and inflammatory cytokine production in SARS-CoV-2 infected cells is an
44 important question. Silencing the expression of a large set of PRRs [2] or of a selection of
45 retinoic-acid inducible gene I-like receptors (RLRs) [3] revealed that SARS-CoV-2 infection-
46 mediated induction of type I and III IFNs largely depends on melanoma differentiation-
47 associated protein 5 (*MDA5*) expression in lung epithelial Calu-3 cells. Knockdown of other
48 PRRs had little to no impact on type I and III IFN induction or downstream ISG upregulation
49 in these cells [2]. A role of retinoic acid-inducible gene I (RIG-I) in type I IFN induction in
50 SARS-CoV-2 infected Calu-3 cells was also proposed [4], but this remains controversial.

51 Depleting the expression of the laboratory of genetics and physiology 2 (*LGP2*) gene - a
52 known potentiator of MDA5-mediated IFN responses - significantly reduced type I IFN
53 (*IFN β*) mRNA abundance in SARS-CoV-2 infected Calu-3 cells [2]. Collectively, these
54 results highlight that MDA5 is particularly important for inducing type I and III IFN
55 responses against SARS-CoV-2. PRRs other than MDA5 are likely to contribute but remain
56 largely unidentified. Of note, interleukin 6 (*IL6*) induction seems to depend on RIG-I and not
57 MDA5 expression in SARS-CoV-2 infected Calu-3 cells that were depleted for these RLRs
58 using RNA interference (RNAi) [4]. RLR usage may thus trigger the expression of different
59 inflammatory mediators and vary depending on cell type.

60

61 Toll-like receptor (TLR) 3 depletion has no impact on *IFN β* transcript abundance in SARS-
62 CoV-2-infected Calu-3 cells [2]. However, experimental infection of plasmacytoid
63 predendritic cells (pDCs) from healthy patients and from patients with genetic defects
64 demonstrated that IRAK4 and UNC93B1, which are required for signaling downstream of
65 TLRs, are essential for type I and III IFN production by pDCs [5].

66

67 Accumulating data demonstrate that molecular components of SARS-CoV-2 are recognized
68 by both RLRs and TLRs [2,3,5]. Further investigations, ideally in animal models and in
69 human primary cells, are warranted to continue delineating the role of PRRs in SARS-CoV-2-
70 induced innate immune responses. Moreover, it is of utmost interest to precisely identify
71 specific viral replication intermediates that are recognized by PRRs, by, for instance,
72 sequencing viral genomic, sub-genomic or messenger RNA molecules bound to RLRs
73 purified from infected cells. Implications for the recognition of SARS-CoV-2 proteins and
74 nucleic acids by other cellular immune sensors, such as protein kinase R (PKR) and
75 nucleotide-binding and oligomerization domain (NOD)-like receptors remain less studied.

76 Finally, the kinetics of the interactions between viral components and PRRs remain
77 unaddressed. For instance, characterizing the accessibility of viral components to PRRs over
78 the course of SARS-CoV-2 infection will be crucial in understanding the tug-of-war between
79 the virus and host IFN responses and can inform potential therapeutic strategies for COVID-
80 19.

81

82 **SARS-CoV-2 infection-mediated induction of IFNs**

83 SARS-CoV-2 infection induces a type I (IFN β) and III (IFN λ) IFN response in Calu-3 cells
84 [2,3,6], primary airway epithelia (either derived from healthy patient biopsies or from induced
85 pluripotent stem cells, iPSC) cultured at the air-liquid interface [2,3], as well as in intestinal
86 organoids [7]. Primary human pDCs can also induce the expression of type I and III (*IFN α*
87 and *IFN- λ*) IFNs upon *ex vivo* exposure to SARS-CoV-2 [5]. Sequencing global RNA
88 extracted from nasopharyngeal swabs from patients with various COVID-19 disease profiles
89 revealed a strong induction of ISGs such as *OASL*, *IFIT2*, and *MX1* [8]. In addition,
90 significantly higher serum levels of type I IFN was detected in sera from patients with
91 mild/moderate COVID-19, relative to patients with severe/critical disease [6,9]. Moreover,
92 the physiological importance of the IFN response in limiting COVID-19 severity has been
93 highlighted by the presence of inborn mutations in genes involved in IFN signaling pathways
94 (e.g. interferon-alpha/beta receptor subunit 1 [*IFNARI*] and interferon regulatory factor 9
95 [*IRF9*]), and by the presence of neutralizing auto-antibodies against type I IFN(s) in 3-5%
96 patients and >10% of critically-ill patients, respectively [10].

97

98 The role of endogenous IFNs (i.e. produced by infected epithelial cells) in the control of
99 SARS-CoV-2 replication *in vitro* is debatable and may depend on the cell type [2,3,7]. It is
100 also worth noting that studies have suggested the ability of SARS-CoV-2 proteins to

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

125

126 **The kinetics of IFN induction vs. modulation by SARS-CoV-2 proteins**

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

136

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

151 processes. Evidently, extensive and robust research is needed to delineate the kinetics of IFN
152 responses during SARS-CoV-2 infection as well as the systemic effect of these cytokines on
153 COVID-19 severity.

154

155 **Concluding Remarks**

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

166

167 **References**

- 168 1 Lucas, C. *et al.* (2020) Longitudinal analyses reveal immunological misfiring in severe
169 COVID-19. *Nature* 584, 463–469
- 170 2 Yin, X. *et al.* (2021) MDA5 Governs the Innate Immune Response to SARS-CoV-2 in
171 Lung Epithelial Cells. *Cell Rep* 34, 108628
- 172 3 Rebendenne, A. *et al.* (2021) SARS-CoV-2 triggers an MDA-5-dependent interferon
173 response which is unable to control replication in lung epithelial cells. *J Virol* DOI:
174 10.1128/JVI.02415-20
- 175 4 Thorne, L.G. *et al.* (2021) SARS-CoV-2 sensing by RIG-I and MDA5 links epithelial
176 infection to macrophage inflammation. *EMBO J* DOI: 10.15252/emboj.2021107826
- 177 5 Onodi, F. *et al.* (2021) SARS-CoV-2 induces human plasmacytoid dendritic cell
178 diversification via UNC93B and IRAK4. *J Exp Med* 218, e20201387
- 179 6 Banerjee, A. *et al.* (2021) Experimental and natural evidence of SARS-CoV-2-
180 infection-induced activation of type I interferon responses. *iScience* 24, 102477
- 181 7 Stanifer, M.L. *et al.* (2020) Critical Role of Type III Interferon in Controlling SARS-
182 CoV-2 Infection in Human Intestinal Epithelial Cells. *Cell Rep* 32, 107863
- 183 8 Cheemarla, N.R. *et al.* (2021) Dynamic innate immune response determines

184 susceptibility to SARS-CoV-2 infection and early replication kinetics. *J Exp Med* 218,
185 e20210583
186 9 Hadjadj, J. *et al.* (2020) Impaired type I interferon activity and inflammatory
187 responses in severe COVID-19 patients. *Science* 369, 718–724
188 10 Beck, D.B. and Aksentijevich, I. (2020) Susceptibility to severe COVID-19. *Science*
189 370, 404–405
190 11 Lokugamage, K.G. *et al.* (2020) Type I Interferon Susceptibility Distinguishes SARS-
191 CoV-2 from SARS-CoV. *J Virol* 94, e01410-20
192 12 Martin-Sancho, L. *et al.* (2021) Functional landscape of SARS-CoV-2 cellular
193 restriction. *Molecular Cell* 81, 2656-2668.e8
194 13 Pfaender, S. *et al.* (2020) LY6E impairs coronavirus fusion and confers immune
195 control of viral disease. *Nat Microbiol* 5, 1330–1339
196 14 Wickenhagen, A. *et al.* (2021) A prenylated dsRNA sensor protects against severe
197 COVID-19. *Science* 0, eabj3624
198 15 Xia, H. *et al.* (2020) Evasion of Type I Interferon by SARS-CoV-2. *Cell Rep* 33,
199 108234
200

201

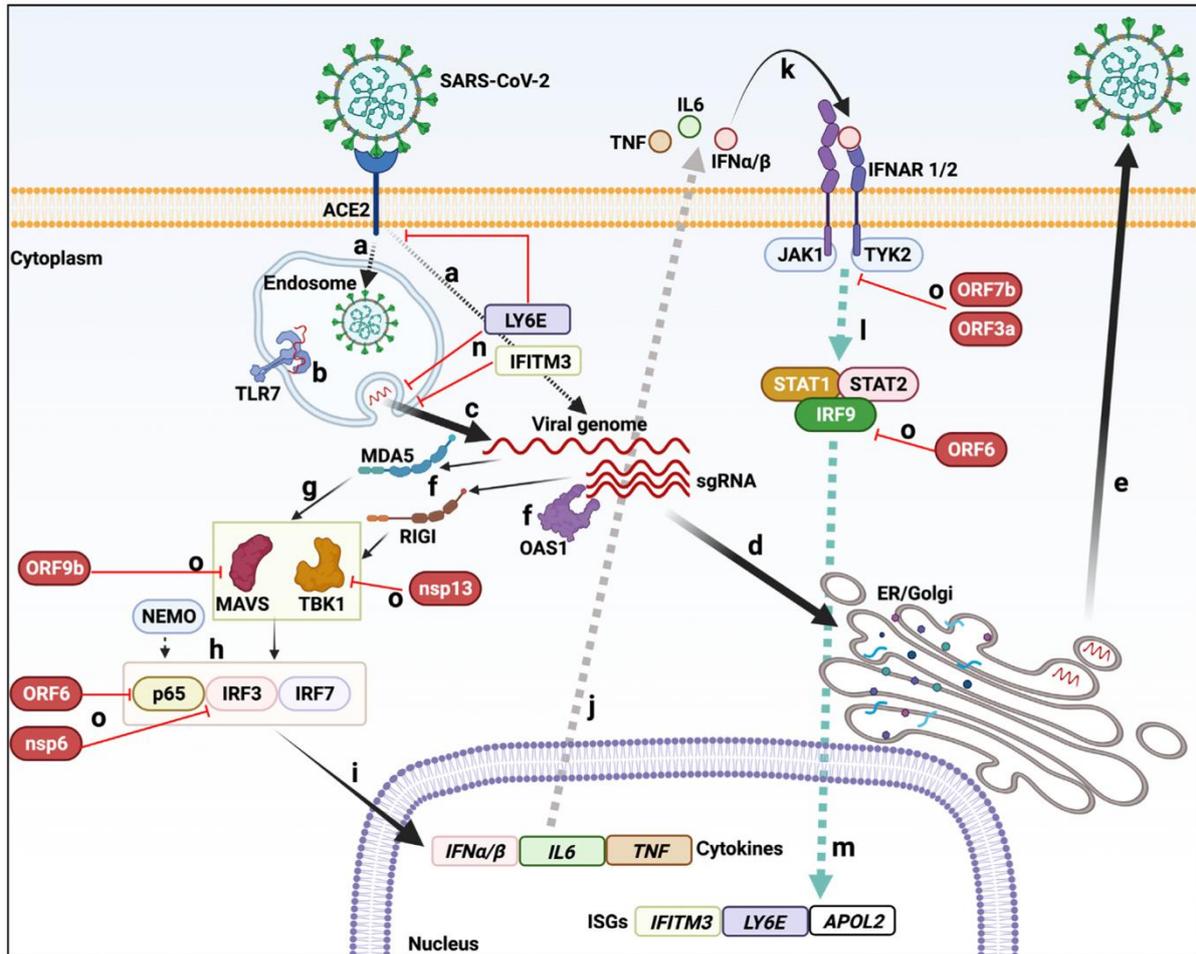
202

203 **Acknowledgements**

204 SARS-CoV-2 studies in NJ laboratory are funded by the ‘Urgence COVID-19’ fundraising
205 campaign of Institut Pasteur and the Agence Nationale de la Recherche. CG is supported by
206 the INSERM, CNRS and Montpellier university, and SARS-CoV-2 studies in CG laboratory
207 are funded by the ERC Starting Grant ANTIViR, the ANR RA-COVID CRISPR-TARGET-
208 CoV and the Fondation CNRS. The Vaccine and Infectious Disease Organization (VIDO)
209 receives operational funding for its CL3 facility (InterVac) from the Canada Foundation for
210 Innovation through the Major Science Initiatives. VIDO also receives operational funding
211 from the Government of Saskatchewan through Innovation Saskatchewan and the Ministry of
212 Agriculture. This article is published with the permission of the Director of VIDO, journal
213 series no. 952.

214

215



217

218 **Figure 1. Dynamic interactions between SARS-CoV-2 proteins and type I IFN response.**

219 The SARS-CoV-2 spike protein interacts with human cellular receptor, angiotensin-
 220 converting enzyme 2 (ACE2) to allow virus entry into human epithelial cells of the
 221 respiratory system. The virus can use two routes of cell entry: either fusion at the plasma
 222 membrane or internalization within an endosome (a). Once internalized within an endosome,
 223 viral components, such as genomic RNA, can be detected by endosomal toll-like receptor 7
 224 (TLR7) (b). Following release of viral RNA into the cytoplasm (c), the genomic RNA
 225 undergoes replication, transcription, and translation (d) to produce progeny virions (e). During
 226 the process of replication and transcription of viral RNA, cellular pattern recognition

227 receptors (PRRs) such as melanoma differentiation-associated protein 5 (MDA5) and retinoic
228 acid-inducible gene I (RIGI) detect viral RNA (f) and mediate this signal through cellular
229 adaptor proteins, such as mitochondrial antiviral-signaling protein (MAVS) and TANK
230 binding kinase I (TBK1) (g) to activate key transcription factors, such as p65, interferon
231 regulatory factor 3 (IRF3) and IRF7 (h). Upon activation, these transcription factors
232 translocate to the nucleus to induce the expression of cytokines such as type I interferons
233 (*IFN α/β*), interleukin 6 (*IL6*) and tumor necrosis factor (*TNF*) (i). Secreted cytokines, such as
234 type I IFNs (j) carry out their effector functions in an autocrine (acting on the same cell) or
235 paracrine (acting on neighboring cells) manner (k). Type I IFNs interact with their cognate
236 receptors (IFNAR1/2) to activate downstream signaling cascades (l) via transcription factors
237 signal transducer and activator of transcription 1 (STAT1), STAT2 and IRF9 to induce the
238 expression of interferon stimulated genes (ISGs) (m). ISGs act on various stages of virus
239 replication to inhibit virus propagation (n). However, SARS-CoV-2 has evolved multiple
240 proteins that can inhibit the host antiviral response, some of which are highlighted here
241 (o)[15]. Figure created with BioRender.com.

242 **Box 1. Pattern recognition receptors in antiviral immunity**

243 When exposed to viruses, most mammalian cells produce cytokines, including interferons
244 (IFNs). Three classes of IFN have been identified, designated types I to III, and are classified
245 according to the receptor complex they signal through. In contrast to types I and III IFNs, type
246 II IFNs are not secreted by virus-infected structural cells (e.g. epithelial cells) but mainly by
247 natural killer and T cells. IFN production is initiated when pattern recognition receptors
248 (PRRs) recognize specific viral products, such as viral nucleic acids or viral proteins. These
249 PRRs can be membrane-associated, such as Toll-like receptor (TLR), or cytosolic, such as
250 retinoic acid inducible gene I (RIG-I)-like receptors (RLRs). Three RLR members, which are
251 expressed in most tissues, have been identified: RIG-I, melanoma differentiation-associated

252 protein 5 (MDA5), and the laboratory of genetics and physiology 2 (LGP2). The human TLR
253 family contains 10 TLRs (TLR1-10) and several of them, including TLR2, -3, -4, -7, -8 and -9
254 are implicated in the early interplay of host cells and invading viruses. PRR activation by viral
255 components enables interaction with adaptor proteins and the recruitment of signaling
256 complexes that stimulate the rapid expression of inflammatory cytokine production and IFNs.
257 Upon their secretion, IFN bind to their receptors, in an autocrine or paracrine manner, to
258 activate a signaling pathway that ultimately triggers the expression of hundreds of IFN-
259 stimulated genes (ISGs), whose products have antiviral properties, effectively establishing an
260 antiviral state in infected and surrounding cells. Other cytokines will boost the development
261 of a more long-term antigen-specific adaptive immunity that is pivotal for pathogen clearance
262 and immune memory. Of note, a prolonged uncontrolled cytokine response, also called
263 ‘cytokine storm’, can drive cell death and subsequent tissue dysfunction.
264