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Short communication

Differential induction of type I and III interferon genes in the upper respiratory tract of patients with coronavirus disease 2019 (COVID-19)

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ABSTRACT

The natural course of type I and III interferon (IFN) response in the respiratory tract of COVID-19 patients needs to be better defined. We showed that type I/III IFNs, IFN-regulatory factor 7 (IRF7), and IFN stimulated genes (ISGs), are highly expressed in the oropharyngeal cells of SARS-CoV-2 positive patients compared to healthy controls. Notably, the subgroup of critically-ill patients that required invasive mechanical ventilation had a general decrease in expression of IFN/ISG genes. Heterogeneous patterns of IFN-I/III response in the respiratory tract of COVID-19 patients may be associated to COVID-19 severity.

Within the airways—the main entry port of respiratory viruses—innate immune responses represent a critical, first-line response to infection and injury. Reducing virus replication and spread is crucial to maintain the structural and functional lung epithelium integrity until adaptive immune responses will come into play to eliminate the pathogens. Type I interferons (IFN-I) are cytokines produced early during virus infection that are integral for regulating the immune response. Once the IFN-I secreted from infected cells binds to the IFN-I receptor, it upregulates expression of IFN-stimulated genes (ISGs), including IRF7 which in turn may induce the expression of IFNα thus amplifying the IFN-I response. The most recent addition to the IFN family, the type III IFNs or IFNλ1−3 play a major role in antiviral protection of epithelial barriers. IFNλs conferred long-lasting protection in the respiratory tract in a mouse model of influenza viral infection and transmission and they can also block neutrophil-driven inflammation (Lazear et al., 2019).

Of note, a dysregulated and delayed IFN-I expression during respiratory infections caused by severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) (Cameron et al., 2007; Channappanavar et al., 2016) and Middle East respiratory syndrome coronavirus (MERS-CoV) can lead to a severe pathology and disease (Kindler et al., 2016). As for SARS-CoV-2, Chu and colleagues (Chu et al., 2020) have showed that it did not significantly induce types I-III IFN in lung tissues in *ex vivo* experiments. In a concomitant paper, Blanco-Melo (Blanco-Melo et al., 2020) has reported that only low levels of type I and III IFNs are produced during SARS-CoV-2 infection. More recently, a distinct phenotype was observed in severe and critical patients, consisting of low IFN-I levels, which was associated with a persistent blood SARS-CoV-2 viral load and an exacerbated inflammatory response (Hadjadj et al., 2020). On the other hand, strong IFN-I and/or ISGs responses have also been reported in patients with severe coronavirus disease 2019 (COVID-19) (Daamen et al., 2020; Lee and Shin, 2020b; Zhou et al., 2020). Pro-inflammatory roles of IFN-I were shown recently in a mouse model of SARS-CoV-2 supporting the pathogenic role of an inadequate IFN responses in viral infections (Israelow et al., 2020). Also, Lee JS et al., have proposed that IFN-I might have an important role in exacerbating TNF- and IL-1-driven inflammation in the progression to severe COVID-19 (Lee et al., 2020a).

Since a full picture of the IFN response of SARS-CoV2-affected

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Table 1
Expression levels of type I/III IFN and ISGs in SARS-CoV-2 positive patients.

	SARS-CoV-2 positive patients				Healthcare workers n = 29(◆)
	Total positive patients n = 54*(▲)	No oxygen support n = 15(■)	Non-invasive ventilation n = 21	Invasive mechanical ventilation n = 6	
Age median/IQR	58.97/49.51–74.67	57.13/32.07–69.03	66.76/52.30–78.83	78.17/72.40–80.4	43.26/28.03–56.71
Gender M/F	36/18	10/5	12/9	6/0	17/12
IFN-I/III genes**					
IFNα	0.73/0.43–1.21	0.67/0.48–1.05	0.93/0.72–1.89	0.45/0.12–1.30	0.13/0.05–0.71
IFNβ	0.51/0.29–0.93	0.37/0.21–0.54	0.71/0.40–1.53	0.53/0.41–0.87	0.08/0.04–0.39
IFNλ1	0.06/0.03–0.11	0.09/0.03–0.17	0.04/0.02–0.10	0.03/0.01–0.11	0.02/0.007–0.06
IFNλ2	0.72/0.37–1.02	0.55/0.17–0.91	0.75/0.38–1.30	0.91/0.38–1.02	0.05/0.02–0.17
IFNλ3	3.27/1.60–4.50	2.88/2.10–3.99	3.16/1.35–5.34	3.35/1.44–4.80	0.67/0.24–3.02
ISG15	7.21/2.18–45.52	6.60/2.27–18.18	7.98/3.04–51.19	1.85/1.08–4.16	3.86/2.32–6.28
ISG56	0.77/0.31–4.19	0.7/0.29–4.38	0.81/0.43–4.19	0.26/0.12–0.71	0.36/0.25–0.62
IRF7	0.2/0.15–1.53	0.19/0.08–0.57	0.28/0.16–1.33	0.18/0.12–0.23	0.12/0.049–0.26

*Clinical data were available for 42 out of 54 SARS-CoV-2 positive patients. **Data are indicated as median (interquartile range = IQR). Transcript levels of type I/III IFN and ISGs in SARS-CoV-2 positive patients and in healthcare workers were calculated using $2^{-\Delta Ct}$. (▲) Total SARS-CoV-2 positive patients vs healthcare workers: $p < 0.05$ for all genes. (■) No oxygen support vs non-invasive ventilation vs invasive mechanical ventilation: $p < 0.05$ for ISG15 and ISG56. Multivariate general linear model (GLM) analysis adjusted for age and gender was used to analyze gene expression data (R version 4.0.1).

respiratory tract has not emerged due to the contradictory results obtained (Lee and Shin, 2020b), to give some insights to the immunopathogenesis of the novel coronavirus SARS-CoV-2, we evaluated the upper respiratory tract expression of the genes encoding IFNα, IFNβ, IFNλ1−3, IRF7 and of well known ISGs, such as ISG15 and ISG56.

These analysis were perfomed in 54 hospitalized patients with symptomatic COVID-19 and in SARS-CoV-2 negative healthcare workers (n = 29). Demographic data are reported in Table 1. Clinical data were available for 42 out of 54 SARS-CoV-2 positive patients analyzed. Oropharyngeal swabs were collected from all patients within few hours after the arrival in the COVID-19 emergency rooms of Policlinico Umberto I Hospital in Rome and from healthcare workers during control measures for SARS-CoV-2 infection. The study was approved by the institutional review board (Policlinico Umberto I Hospital, Sapienza, University of Rome) and the Ethics Committee (Sapienza, University of Rome). Briefly, all respiratory samples were divided into two aliquots: one was treated for SARS-CoV-2 detection using RealStar® SARS-CoV-2 RT-PCR Kit 1.0 (Altona diagnostics, Germany); the second was centrifuged at 2000 rpm for 10 min, total RNA was extracted from cell pellet and then stored at -80 C° for gene expression analysis. Gene expression of IFNα, IFNβ, IFNλ1−3, IRF7, ISG15, and ISG56 was evaluated by RT/Real Time PCR as previously reported (Pierangeli et al., 2020). The housekeeping gene β-glucuronidase was used as an internal control. Gene expression values were calculated by the comparative $2^{-\Delta Ct}$ methods (Table 1 and raw data on Online Resource 1). Multivariate general linear model (GLM) analysis was used to analyze gene expression data (R version 4.0.1). Correlations between transcript levels and Ct values (i.e. viral load) were evaluated using Spearman rank correlation.

SARS-CoV-2 patients presented with fever [39/42 (92.86 %)], cough [26/42 (61.91 %)], shortness of breath [14/42 (33.33 %)] and pharyngitis [6/42 (14.29 %)]; chest CT scans showed bilateral patchy shadows or ground glass opacity in 27/42 (64.29 %). SARS-CoV-2 patients were then stratified on the base of oxygen support: 15/42 (35.71 %) who do not required oxygen support, had a median Charlson index of 1 (range 0–4); 21/42 (50 %) that received non-invasive ventilation (NIV), Venturi mask, or 6/42 (14.29 %), supported by invasive mechanical ventilation (IMV) had a median Charlson index of 2 (range 1–5). The time from the onset of COVID-19 symptoms to respiratory samples collection was 2–3 days for SARS-CoV-2 positive patients with mild illness and 4–8 days for those with required oxigen support. All healthcare workers were negative for SARS-CoV-2 (Table 1).

We found that IFNα, IFNβ, IFNλ1−3, IRF7, and ISGs mRNA levels in oropharyngeal swabs were significantly increased in SARS−COV-2 infected patients compared to those detected in healthcare workers, suggesting an induction of the IFN-I/III transcriptome in COVID-19 patients (SARS−COV-2 vs healthcare donors: $p < 0.05$ for all genes, Table 1). In agreement, an increase of genes important for the IFN related innate immune responses have been found in both the COVID-19 lung and airway compartments (Daamen et al., 2020). In this regard, SARS-CoV-2 threshold cycle (Ct) values obtained with the Altona diagnostics procedure (a low Ct value reflects a high target RNA concentration, i.e. viral load) negatively correlated to ISG15, ISG56 and IRF7 mRNAs levels (ISG15 r=-0.3066, p = 0.0405; ISG56 r=-0.3672, p = 0.0182; IRF7 r=-0.3733, p = 0.0192), suggesting an intrinsic capacity of the virus to induce ISGs responses. In agreement, SARS-CoV-2 infected patients have been also shown to mount strong expression of antiviral ISGs during acute illness (Liou et al., 2020).

Interestingly, however, the small subgroup of IMV patients showed a general decrease in the expression of some IFN genes with a remarkably lower level of ISG15 and ISG56 (Table 1). This finding confirms that an heterogeneous pattern of IFN response might exist in COVID-19 patients (Blanco-Melo et al., 2020). Indeed, critically-ill patients have been shown to exhibit impaired IFNα response (Trouillet-Assant et al., 2020) and no IFN-I or IFN-III were detected in post mortem lung samples from COVID-19 patients (Blanco-Melo et al., 2020).

The higher sensitivity of SARS-CoV-2 to IFN-I as compared to SARS-CoV-1 (Clementi et al., 2020; Sallard et al., 2020) apparently suggests that the new CoV might be susceptible to ISG-mediated antiviral activities. However, here high ISGs levels seemed to be ineffective in controlling disease severity suggesting that SARS−COV-2 may interfere with ISGs' antiviral activities. In line with these findings, a large-scale gain-of-function analysis evaluating the impact of ISGs on SARS−COV-2 replication has recently shown that only a limited subset of ISGs control viral infection (Martin-Sancho et al., 2020).

Since antiviral immunity needs to be finely tuned during respiratory viral infections, through the induction of effective antiviral immunity in the respiratory tract that limits viral replication without compromising host fitness, our study indicated that IFN-I/III are expressed in the respiratory tract of most COVID-19 patients, although a general decrease of IFN genes (IFNα, IFNβ, IFNλ1 and ISGs), might be associated to the development of immunopathology and more severe disease.

We acknowledge limitations of this study, related to the small number of SARS−COV-2 infected patients included and the lack of measurement of IFN pathways in lower lung samples.

Moreover, the sampling dates of severe cases were different from those of mild cases although we did not found any relationships between gene expression, the Ct data used as a proxy of viral load values and the time of oropharyngeal swabs collection from the onset of COVID-19 symptoms (data not shown).

Viral and host factors should be explored to understand the causes of defects in IFN-I/III production and antiviral actions in some patients with COVID-19. Indeed, IFNα negative COVID-19 patients more

frequently experienced fatal outcome (Trouillet-Assant et al., 2020); moreover, autoantibodies against IFN-I (Bastard et al., 2020) and genetic defects at Toll-like receptor 3– and IRF7–dependent IFN immunity (Zhang et al., 2020) have been detected in severe SARS-CoV-2 infections highlighting the multifactorial design of a tailored immune-therapy for COVID-19 patients.

Author contributions

Conceptualization, C.S.; SARS-COV-2 diagnosis data: C.B., A.V., G. O., L.M., D.D.C., and O.T.; gene expression analysis, F.F., C.B., A.V., G. O., M.S.; statistical analysis: A.S.; data curation, M.G., A.S., C.B., F.F. and G.C.; writing—original draft preparation C.S., C.B. and F.F.; writing—review and editing, C.S., G.d.E. and A.P.; editing C.M.M. and G.A.; visualization, supervision and project administration, C.S. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics approval

The study was approved by the institutional review board (Policlinico Umberto I Hospital, Sapienza, University of Rome) and the Ethics Committee (Sapienza, University of Rome) and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Availability of data and material

Data available within the article and its supplementary materials.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2020.198283.

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