BRIEF REPORT

Interferon system I e III protects patients with cystic fibrosis from SARS-CoV2 infection

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Doi
10.56164/PediatrRespirJ.2023.32

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ABSTRACT

Cystic fibrosis (CF) is an autosomal recessive disease caused by a deficiency of expression of a chloride channel. Children with CF seem to be more protected than the general population from SARS-CoV2 infection. The interferon system (IFN) plays an important role in the susceptibility and resolution of infections. The aim of this study was to evaluate the interferon system response in patients with CF compared to a control group. The expression levels of genes coding for IFN types I and III and of some interferon-induced genes in the respiratory mucosa of CF patients compared to a control group were analyzed.

16 patients affected by CF and 15 healthy controls, aged between 2 and 18 years were enrolled. The expression of genes coding for IFN types I and III and of some interferon-induced genes ISG15 and ISG56 was studied in nasal swab cells.

We found an increase expression of the following genes in cases compared to controls: IFN-β ($p = 0.002$), IFN-ε ($p = 0.002$) and ISG56 ($p = 0.001$).

This study suggests that in patients with CF the innate immune response is more active than in the healthy population and it may explain why these patients are less exposed to SARS-CoV2 infection.

IMPACT STATEMENT: CF patients' innate immune response is more active than in the healthy population; it may explain why these patients are less exposed to SARS-CoV2 infection.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease caused by a deficiency of expression of a chloride channel, a cystic fibrosis transmembrane conductance regulator (CFTR), expressed in several human tissues (1).

Subjects with CF have a particular predisposition to respiratory infections (2). Measures to prevent the transmission of pathogens among people with CF are recommended: isolation of patients, frequent hand hygiene, use of personal protective equipment and cleaning of medical devices. These control measures, adopted in this historical period but already known to this class of subjects, may have contributed to saving them from coronavirus disease (3, 4).

The immune system enables organisms to fight infections and eliminate threats from the cell (5, 6). Interferons (IFNs) are a class of soluble proteins with a pleiotropic effect that can regulate many biological activities: cell cycle, cell

ABBREVIATIONS LIST

CF: Cystic Fibrosis; CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; IFNs: Interferons; ACE2: Angiotensin-Converting Enzyme 2; SARS-CoV2: Severe Acute Respiratory Syndrome CoronaVirus; TMPRSS2: Transmembrane Serine Protease 2; NPW: Nasopharyngeal Washing.

KEY WORDS

Cystic fibrosis; immune system; interferon system; COVID-19; SARS-CoV-2.

differentiation, innate and adaptive immunity, and angiogenesis (7).

IFNs are divided into three groups: type I IFN (IFN-α, IFN-β, IFN-ε, IFN-ω, IFN-κ) with an antiviral activity (8); type II IFN (IFN-γ) who is a potent macrophage activator (9); type III IFN (IFN-λ1, IFN-λ2, IFN-λ3 and IFN-λ4) with an antiviral activity limited to epithelial tissues (10).

During viral infection, the initial host response is mediated by IFN-α and IFN-β. Type I IFNs are also responsible for the development of the adaptive immune response following infection through the upregulation of cytokines, chemokines and intermediary molecules that induce the activation of the immune system, or directly on dendritic cells (DC), natural killer cells (NK) and lymphocytes. IFN type III (IFN-λ) signal through a heterodimeric receptor consisting of two subunits, IFNλR1 (or IL-28Ra) which forms the ligand binding chain and a second subunit shared with the interleukin IL-10 receptor which forms an accessory chain, and they encode proteins (ISG15, ISG56) that mediate antiviral activities (11).

Several studies have shown that IFN I can upregulate the expression of mRNA and angiotensin-converting enzyme 2 (ACE2), a mediator protein of the Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV2) (12, 13). In fact, to infect the cells of the respiratory system, the virus uses a protein present on the capsid, the SARS-CoV2 Spike (S) protein, which binds to the ACE2 enzyme; the virus also employs two proteases' cells, furin and transmembrane serine protease 2 (TMPRSS2) (14). Oropharyngeal cells from CF patients have been shown to have reduced expression levels of ACE2, furin and TMPRSS2.

Based on previous studies, it has been hypothesized that CF patients may have a more active innate immune response than the general population and that this could reflect on the subject's ability to protect themselves from viral infections. We therefore turned our thesis to the analyze the interferon system in patients with CF compared to a control group.

MATERIALS AND METHODS

We enrolled 16 patients with CF (6 males, median age 11.79 (2.4-18.1)) and 15 healthy children (11 males, median age 9.7 (2.13-16.09)) as controls between October 2021 and January 2022. Inclusion criteria were: presence of F508del mutation and clinical stability. Patients with pulmonary exacerbation in the previous three months and patients treated with modulator drugs were excluded. None of controls had respiratory symptoms at the time of collection (3).

Patients and controls underwent a nasopharyngeal washing (NPW) (15) that were transported immediately on ice to the Virology Laboratory for the molecular analyses (16). NPWs were centrifuged, cell pellet resuspended in guanidine isothiocyanate reagent and frozen at 80 °C for gene expression analysis (17). Quantitative gene expression of type I and III interferons (IFNs), their receptors and interferon stimulated genes (ISGs) was measured by Real-Time PCR assay, using TaqMan 5' exonuclease probes in the Light Cycler 480 machine (Roche Diagnostics).

A descriptive analysis was performed. Non-parametric univariate statistics tests were used for discrete variables (X_2 test and Fischer's exact test), while non-parametric tests (Mann-Whitney U test and Kruskal-Wallis) for continuous ones $p \leq 0.05$ were considered statistically significant. Data were analyzed with SPSS version 27.0 (SPSS Inc., Chicago, USA).

The work has been approved by our Ethical Committee and parents' patients gave informed consent.

RESULTS

16 CF patients and 15 controls were enrolled. No statistically significant differences were found for demographic data.

There are some inducible genes with significantly different expression between the two groups (**Table 1**); in particular, our study demonstrated that ISG56, INF-β

Table 1. *Gene expression in the two groups of patients.*

	CF groups	Controls	*p-value*
ISG15 *	1.591 (4.586)	0.901 (0.57)	0.80
ISG56 *	0.264 (0.346)	0.460 (0.147)	**0.010**
IFN - α2 *	2.445 (1.372)	2.158 (0.765)	0.363
IFN – β *	2.099 (0.776)	1.094 (0.746)	**0.002**
IFN – ε *	3.227 (1.532)	1.986 (0.867)	**0.002**
IFN - λ1 *	0.0268 (0.049)	0.0278 (0.0142)	0.980
IFN - λ2 *	0.346 (1.586)	2.121 (0.696)	0.310
IFN - λ3 *	4.408 (4.898)	4.0278 (1.255)	0.367
IFN – ω *	2.889 (4.962)	2,042 (0.875)	0.095

* Target gene levels were calculated by the comparative Ct method (2-ΔCt) with respect to the housekeeping gene β-glucuronidase/GUS.

and IFN-ε were significantly more express in CF patients compared to controls (**Figure 1**). No difference was observed for ISG15, IFN-α2, IFN-λ1, IFN-λ2, IFN-λ3, IFN-ω between the two groups.

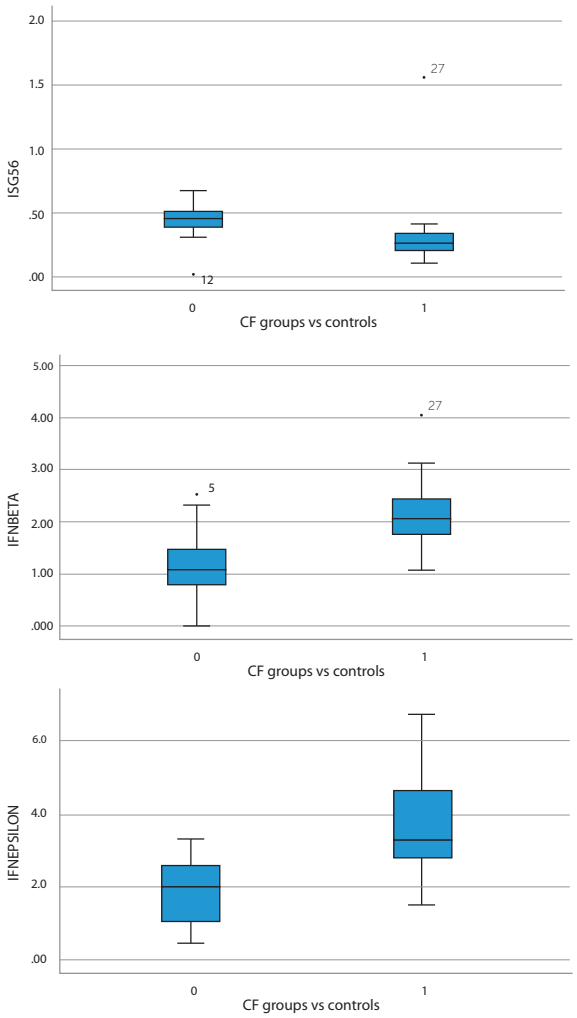

***Figure 1**. ISG56, IFN-β, IFN-ε expression levels in the two patient groups.*

DISCUSSION

We evaluated the innate immune response of the interferon system, in particular the expression levels of the genes encoding type I and type III IFN and two induced genes (ISG15 and ISG56), in patients with cystic fibrosis compared to a control group.

The finding of a lower incidence of SARS-CoV2 infection and a reduction in the severity of the infection compared to the general population (4) led us to ask whether a different modulation of immunity could intervene on this factor.

From the analyses it was found that CF patients, compared to controls, show a different activation of the IFN system, in particular IFN-β and IFN-ε and one of the genes induced by it, ISG56. Gibbert *et al*. showed that type I interferons were the first type to be activated in the initial stages of the viral infection, unlike type III IFNs which are more active in the resolution phase of a viral infection (18).

The results of this study show how IFN-β and IFN-ε are more expressed than in controls. This aspect could in part contribute to limiting the spread of the virus in this category of patients, as SARS-CoV-2 is highly sensitive to the antiviral action of type I IFN, upregulation of IFN response in the airways of CF patients could be involved in mediating protection against SARSCoV-2 infection.

In a previous study conducted by our group, we studied the expression of ACE2 receptors in oropharyngeal cells in pediatric patients with CF by comparing with a control group (19).

Our results are consistent with the previous study, therefore an increase in type I IFN expression could contribute to a reduction of infections in these patients.

The main methodological limitation of this study is due to the limited number of patients enrolled. In part, this is attributable to the narrow and selective inclusion criteria adopted, and in particular to the selection of patients on the basis of the mutation they carry. We only selected patients who had F508del (to make the sample more homogeneous) and only patients who didn't use therapy with CFTR gene modulators because the effects on the immune system are not yet fully known. These patients would have started therapy soon or the parents refused the new drugs.

This study suggests that in patients with CF the innate immune response is more active than in the healthy population. This evidence may, in part, explain why the incidence and severity of SARSCOV-2 infection in CF patients is low.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There were no institutional or private fundings for this article.

Author contributions

Conceptualization, methodology and writing the original draft: AF; conceptualization and methodology: RN; data curation: LP, MG, FB; formal analysis: AP; investigation and resources: PT and GC; supervision validation: FM. All the Authors have read and agreed to the published version of the manuscript.

Ethical approval

Human studies and subjects

The work has been approved by our Ethical Committee and parents' patients gave informed consent.

Animal studies

N/A.

Data sharing and data accessibility

The data underlying this article can be shared just before a reasonable request to the Corresponding Author.

Publication ethics

Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

All the data correspond to the real.

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