Low type I interferon response in COVID-19 patients: Interferon response may be a potential treatment for COVID-19

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Abstract. Interferons (IFN) are antiviral cytokines that mitigate the effects of invading viruses early on during the infection process. SARS-CoV and MERS induce weak IFN responses; hence, the clinical trials which included recombinant IFN accompanied with other antiviral drugs exhibited improved results in terms of shortening the duration of illness. The aim of the present study was to evaluate the type I IFN response in COVID-19 patients to determine whether it is sufficient to eliminate or reduce the severity of the infection, and whether it can be recommended as a potential therapy. Total RNA samples were converted to cDNA and used as templates to evaluate the gene expression levels of IFN regulatory factor (IRF)3 and IFN-β in COVID-19 patients or control. The results showed that IRF3 gene expression was upregulated ~250-fold compared with the negative samples. In contrast, IFN-β expression increased slightly in COVID-19 patients. Consistent with other coronaviruses, such as SARS-CoV and MERS, COVID-19 infection does not induce an efficient IFN response to reduce the severity of the virus. This may be attributed to an incomplete response of IRF3 in activating the IFN-β promoter in the infected patients. The results suggest IFN-β or α may be used as potential treatments.

Introduction

The new coronavirus, termed COVID-19, emerged in Wuhan, China in late 2019. Although the newly emerged virus has a mutation in the sequence of the spike protein, its binding affinity for the angiotensin-converting enzyme 2 (ACE2) receptor is identical to that of the severe acute respiratory syndrome (SARS-CoV)-1 (1). The cytokine storm is as a group of inflammatory responses, which includes interleukin (IL)-I, IL-2, IL-6, IL-10 and interferon (IFN)-γ (2), is a serious complication of COVID-19 infection (3-6).

Different types of vaccines are being developed to assist in limiting the spread of the virus, and reduce mortality rates going forward, some of which have been approved by the regulatory bodies of several countries, and are being widely distributed. Various companies are currently developing a vaccine introducing mRNA to produce viral proteins, specifically the spike protein, by the host cells. A more stable DNA vaccine is another option to prevent infection with SARS-CoV-2 using adenovirus plasmids encoding the SARS-CoV-2 spike protein (7,8). Another alternative is to use other viral proteins, usually by recombinant DNA. The inactivated whole SARS-CoV-2 vaccine is also a candidate being assessed in preclinical trials. However, using a SARS-CoV-2 live attenuated vaccine carries potential risks, such as the reactivation or the virulence of SARS-CoV-2 in immunocompromised patients (9).

IFNs were named initially due to their role in interfering with viral infections. Influenza-infected chick cells mounted antiviral resistance states by producing secreted cytokines, which were later termed IFNs (10). IFNs are cytokines that are implicated in antiviral responses, immune induction and regulating cell division (11). The gene expression of type I IFN is primarily regulated at the transcriptional stage, and in the absence of stimulators, such as double-stranded RNA, IFNs are not translated. The lack of the IFN-β gene through gene knockout makes mice vulnerable to infection with viruses, such as vaccinia virus and blocks the IFN-α response (12).

To induce the innate immune response during viral infection, it is essential to stimulate the IFN response. The absence of IFN regulatory factor (IRF)3 or defective IRF7 function decreases the gene expression of IFN-α/β, making mice more sensitive to viral infection (13). IRF3 modulates the innate antiviral response that is triggered by the invading virus. IRF3 is primarily modified by hyperphosphorylation when the virus begins replication (14).

IRF3 and IRF7 are the most common regulators of IFN-β. They replace IRF2, serving a key role in type I IFN responses (15-17). IRF3 and IRF7 have specific binding

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properties that allow them to bind to the type I IFN promoters, and their ratio to the bound elements modulates the IFN type I response during viral infection (18). However, IRF3 degradation has been reported to repress IFN-β rather than the activation of the transcriptional repressors. The lack of transcriptionally active IRF3 abolishes the activation of IFN-β to the Sendai virus in mouse embryonic fibroblasts (19).

Upon activation, IRF3 molecules translocate to the nucleus after phosphorylation and bind to the ornithine cyclodeaminase or P300 to form complexes in the IFN sensitive response element region (20). Most RNA viruses elicit a type I IFN response in toll-like receptors (TLRs) or independent mechanisms (cytosolic recognition system) through retinoic acid-inducible genes (RIG-1), which sense the viral RNA molecules (21). Moreover, RIG-1 is central to the stimulation of the type I IFN response to RNA virus infection via activation of IRF3 through kinases in fibroblasts and dendritic cells (22). Synthetic or natural dsRNAs are differentially recognised by RIG-1 and melanoma differentiation-associated protein 5 (MAD5), as the former induces production of IFNs to paramyxoviruses, the influenza virus and Japanese encephalitis virus, whereas picornavirus is detected by MAD5 (23). As a coronavirus model, the mouse hepatitis virus antagonises the type I IFN through the Nucleocapsid protein (24). Previous outbreaks with SARS and MERS revealed that the virus does not increase the expression of IFN-β or its promoter activity. Therefore, treatments with recombinant interferons were used to boost the effects of antiviral drugs (25,26). Infection with respiratory viruses activates the TLR signalling pathways, and eventually leads to the induction of the type I IFN response. The SARS-CoV-2 infection stimulates the TLR downstream pathway to produce mature-IL-1β. An increase in IL-1β causes lung fibrosis and fever. The virus is more highly infectious in adults than children, which may be explained by the high expression levels of aryl hydrocarbon receptors in children compared with the relatively lower expression levels in adults (27).

The present study evaluated the gene expression of IFN and IRF3 in COVID-19-infected patients compared with the control, suggesting a mechanism for the induction of IFNs, and highlighting IFNs as a therapeutic option for treating COVID-19 patients in clinical trials.

Materials and methods

Sample collection, RNA extraction and reverse transcription quantitative PCR. RNA samples were collected from 30 patients suspected of infection with COVID-19 between February and April 2020 at the Public Health Laboratory in Basrah, Iraq. The age range of the patients was 25-55 years old, whereas that of the non-COVID-19 infected individuals was 28-60 years old. The infected patients included 8 females and 12 males whereas the non-infected individuals consisted of were 3 females and 7 males.

Infection was diagnosed using a LightMix SarbecoV E-gene plus EAV control (cat. no. 40-0776-96). The control samples were negative for COVID-19 and were diagnosed with either the common cold or influenza. The present study was approved by the Public Health Department, Basrah Health Directorate (approval no. F112020). All patients provided signed consent to participate in the present study.

The RNA from nasal swabs (total RNA) was extracted using an easy spin™ total RNA extraction kit (Intron; cat. no. 17221) according to the manufacturer's protocol, and used to evaluate the gene expression of IFN-β and IRF3 in COVID-19-infected or uninfected samples using gene-specific primers. An intron HiSenScript™ (RH-)RT-PCR PreMix transcription kit was used to reverse transcribe the RNA, according to the manufacturer's protocol. Quantitative PCR was performed using SYBR-Green MasterMix, according to the manufacturer's protocol (Bioneer; cat. no. K-6210). The reaction consisted of a mixture of 10 μl SYBR-Green, 3 μl cDNA template, 1 μl each of both the forward and reverse primers against IFN-β (forward, CAACTTGCTTGGATTCCTACAAAG and reverse primer, TATTCAAGCCTCCCATTCAATTG); IRF3 (forward, CGGAAAGAAGTGTTGCGGTTAG and reverse primer, TTTGCCATTGGTGTCAGGAGAG); and β-actin (forward, CCTGGCACCCAGCACAAT and reverse primer, GCCGATCCACACGGAGATCT), and 5 μl free deionised diethylpyrocarbonate D.W. to a final volume of 20 μl. The sequences of the primers are based on previous studies (28,29).

The thermocycling conditions were: Initial denaturation at 94˚C for 5 min; followed by 40 cycles of 15 sec of denaturation at 95˚C, annealing at 58˚C for 30 sec, and an extension step at 72˚C for 45 sec; with a final extension at 72˚C for 5 min. The products were subjected to dissociation curve analysis. Using the $2^{-\Delta\Delta Cq}$ method for analysis of mRNA expression, data were normalised to β-actin, which was used as a housekeeping gene (30).

Statistical analysis. Data are presented as the mean ± standard deviation of three technical repeats per patient. All data were analysed using a Student's t-test. Statistical analysis was performed using Microsoft Excel (Office 365; Microsoft Corporation). $P<0.05$ was considered to indicate a statistically significant difference.

Results

The RNAs of the COVID-19 positive and negative samples were extracted, reverse transcribed, used as the templates and mixed with IRF3 or IFN-β specific primers to assess their relative expression by qPCR. IRF3 gene expression was significantly ($P<0.05$) upregulated in the COVID-19-infected patients by ~250-fold compared with the control (uninfected samples) (Fig. 1A and B). Interestingly, IFN-β relative expression was ~1.5-fold higher in the COVID-19-infected samples compared with the control samples ($P<0.05$) (Fig. 2A and B). The $2^{-\Delta\Delta Cq}$ analysis was used to detect the relative expression after subtracting the β-actin value from each sample, and the control was normalised to 1, to express the results as the fold change.

Discussion

Studies have shown that IRF7 is expressed at a very low level physiologically, and requires activation of a type I interferon response for its induction (31,32). Both MERS and SARS trigger a low level of interferon response (33,34). IRF3 is a key regulator of type I IFN, which triggers the host response against the invading viruses. IRF3 also implicated in unwanted

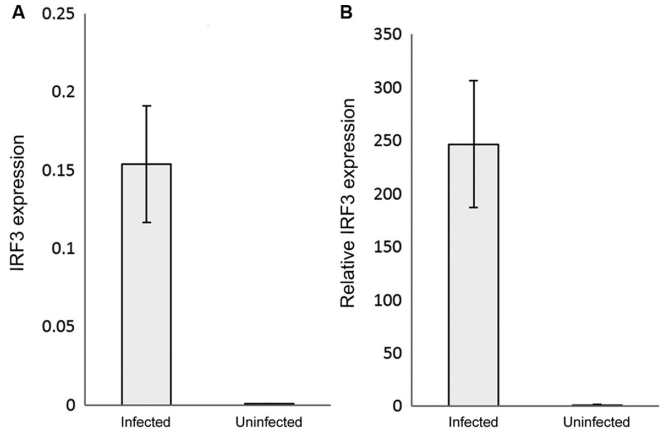

Figure 1. IRF3 gene expression in COVID-19 infected individuals compared with uninfected controls. (A) IRF3 mRNA expression was quantified by reverse transcription-quantitative PCR. (B) Relative IRF3 mRNA expression levels. The results were normalised to the control values, and are presented as the mean ± standard deviation of the three technical replicates. β-actin was used as the internal control. The fluorescence was detected using SYBR-Green as the intercalating dye. IRF, interferon regulatory factor-3.

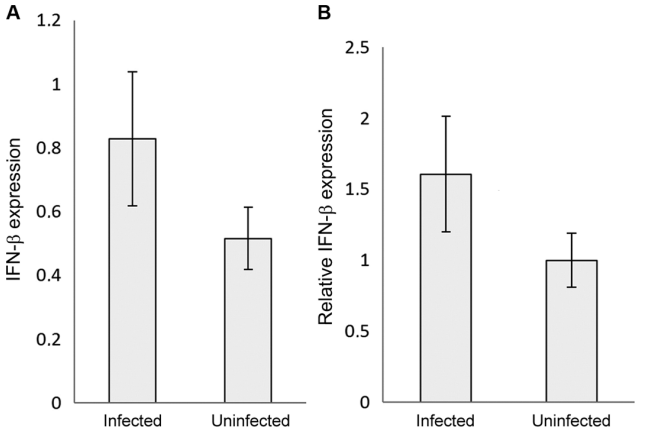

Figure 2. (A) IFN-β gene expression in COVID-19 infected individuals compared with uninfected controls. IFN-β mRNA expression was quantified by reverse transcription-quantitative PCR. (B) Relative IFN-β mRNA expression levels. The results were normalised to the control values, and are presented as the mean ± standard deviation of the three technical replicates. β-actin was used as the internal control. The fluorescence was detected using SYBR-Green as the intercalating dye. IFB-β, interferon-β.

inflammatory responses and septic shock response (35-37). Thus, in the present study, the effects of COVID-19 on an innate immune response were determined.

The results showed that the gene expression levels of IRF3 were notably increased by ~250-fold compared with its expression in the virus-free samples. The increase in the IFN-β levels were not consistent with the increase in the expression of its primary regulator. The results agree with a study concerning coronavirus infection and IFN responses; infection with SARS-CoV does not induce IFN-β production or its promoter activity (38). A lower IFN response was detected in the COVID-19-infected lung tissue compared with SARS, which makes the former virus more sensitive to treatment with a type I IFN (22,39). However, in SARS infections, IRF3 is shown to translocate to the nucleus, independent of nay phosphorylation, dimerization or binding to cAMP response element-binding protein (CREB) binding protein. The SARS-CoV virus may block IRF3 hyperphosphorylation-mediated homodimerization CREB after transport of IRF3 to the nucleus (38).

Another hypothesis suggests that coronaviruses use the IFN-inducible transmembrane proteins (IFITM) to enter the cell, and the IFITM structural motifs required for entry inhibit the entry of other viruses. The IFITM theory explains how the virus can invade the lower respiratory tract (40). Coronaviruses, such as SARS, avoid the inhibitory effects of type I IFNs either through induction of double-membrane vesicles to physically hide the viral RNA intermediates from pattern recognition receptors or by expressing open reading frame (ORF)3, ORF6, ORF7, nucleocapsid protein and non-structural RNA binding protein 1, which when combined, abolish the IRF-3-dependent IFN-β pathway (41,42).

Based on the mechanism by which SARS inhibits the IFN response, recombinant IFNs were used to treat SARS-infected patients. The treatment of human corona Erasmus medical centre (HcoV-EMC) human-infected tissues with the type I or III IFN, 1 h post-infection, decreased the replication of the virus (43). *In vitro*, treating SARS-CoV-infected Vero and Caco2 cells with human recombinant IFN-β inhibited viral replication in the Caco2 cells by ~5 times compared with the Vero cells (44). Replication of HcoV-EMC was notably reduced when treated with type I or type III IFN in the human airway epithelium culture (43,44).

A delay in the induction of the type I IFN response enables SARS-CoV to replicate efficiently in mice and augments the accumulation of inflammatory monocyte-macrophages (45). A lack of type I and type III IFN responses in signal transducer and activator transcription-1 knockout mice resulted in uncontrolled SARS-CoV replication with both liver and neurological consequences (46). Treatment of MERS-CoV-infected patients with IFN-α2a improved the survival rates to a maximum of 14 days (43). The type I IFN and TLR3 agonist were the most effective combined drugs for SARS/MERS CoV treatment (26).

Regarding COVID-19 infections, a clinical trial showed that treating hospitalised patients with IFN-α2b, either alone or in combination with arbidol, shortened the time of detectable viral presence in upper respiratory infections and reduced the IL-6 and C-reactive protein levels (47). Addition of IFNs to the national regime of treating COVID-19 patients reduced the 28-day mortality rate (48). The antiviral effect was augmented when Lopinavir-ritonavir was administered to mild or moderate cases of COVID-19 in combination with IFN-β 1b, and the enhancing effect was associated with a reduction in symptoms, the length of stay in hospital and viral shedding (49). In terms of COVID-19 infections and IFN responses, it was revealed that the reduced type I IFN levels in the peripheral blood system increased the expression of IL-6 and tumour necrosis factor (50). A limited type I IFN response was detected concomitantly with a large chemokine response, including production of IL-6, in the transcriptomes of SARS-CoV2 infected cells (51). In contrast, increased type I IFN and interferon stimulatory gene responses were reported in COVID-19 hospitalised patients. Several factors may underlie these contradictory results, such as the individual immune systems of patients, duration between initial infection and when the samples were obtained, and the severity of the infection (52).

Based on the similarities between the results of the present study and previous studies regarding the pattern of IFN responses, it is hypothesized that IFNs may be used as a potential treatment for management of COVID-19 infections. However, the present study has some limitations. The data assessed was done so irrespective of the severity of infections. Additionally, clinical trials will be required to assess both the safety and efficacy of IFN in managing COVID-19 infections.

In conclusion, increases in the gene expression of the key regulator of type I interferon was not shown to be effective and efficient in mounting an interferon response.

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Availability of data and materials

The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

AAS and MHW completed the RNA extraction and SARS-CoV-2 diagnosis. AAA-A and ZWA achieved the gene expression of the target gene and data analysis. The writing of the study was mainly conducted by ZWA. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Health Directorate (approval no. F112020) and according to an application that was made by the authors. All patients provided signed consent to participate in the present study and gave their written consent to publish any corresponding data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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