

## Potent Antiviral Activities of Type I Interferons to SARS-CoV-2 Infection

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## 29 **Abstract:**

30 The historical outbreak of COVID-19 disease not only constitutes a global public health crisis, but also has a  
31 devastating social and economic impact. The disease is caused by a newly identified coronavirus, Severe  
32 Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2). There is an urgent need to identify antivirals to  
33 curtail the COVID-19 pandemic. Herein, we report the remarkable sensitivity of SARS-CoV-2 to recombinant  
34 human interferons  $\alpha$  and  $\beta$  (IFN $\alpha/\beta$ ). Treatment with IFN- $\alpha$  or IFN- $\beta$  at a concentration of 50 international units  
35 (IU) per milliliter drastically reduce viral titers by 3.4 log or 4.5 log, respectively in Vero cells. The EC<sub>50</sub> of IFN- $\alpha$   
36 and IFN- $\beta$  treatment is 1.35 IU/ml and 0.76 IU/ml, respectively, in Vero cells. These results suggested that  
37 SARS-CoV-2 is more sensitive to many other human pathogenic viruses, including the SARS-CoV. Overall,  
38 our results demonstrate the potent efficacy of human Type I IFN in suppressing SARS-CoV-2 replication, a  
39 finding which could inform future treatment options for COVID-19.

## 41 **Introduction**

42 The COVID-19 outbreak has started from the Wuhan Province, China since December 2019 and rapidly  
43 spread globally, causing over 752,000 confirmed cases and 36,000 deaths as of April 1, 2020. The causative  
44 agent for the COVID-19 disease is a newly identified Severe Acute Respiratory Syndrome coronavirus 2  
45 (SARS-CoV-2) (1), which is transmitted through aerosol/airdrop inhalation or contact. The historical outbreak  
46 causes a public health crisis much severe than the SARS outbreak, which caused 8,098 infections and 774  
47 deaths between November 2002 and July 2003. The COVID-19 disease also has a devastating social and  
48 economic impact worldwide. WHO has declared COVID-19 disease a pandemic. In USA, there is over 200,000  
49 confirmed cases and 4,300 deaths as of April 1, 2020. It is estimated by CDC that the COVID-19 pandemic  
50 may claim 200,000 to 1.7 million lives in USA. Treatments are urgently needed. Due to the high rate of new  
51 infections reported each day, drugs already approved for clinical use for other disease may offer the most  
52 expedient option for treating COVID-19, and several such drugs are already being tested in clinical trials.  
53  
54 Type I interferons (IFN- $\alpha/\beta$ ) are cytokines that play a pivotal role in inducing an antiviral response across a  
55 wide range of cell types. Humans produce 13 types of IFN- $\alpha$  and a singular IFN- $\beta$  (2). Each Type I IFN  
56 ultimately induces a number of interferon-stimulated genes (ISGs) which encode for a variety of antiviral

57 effectors. Notably, IFN- $\beta$  production leads to a positive feedback loop that further stimulates the expression of  
58 many of the IFN- $\alpha$  genes (3). Clinically, Type I IFNs have already approved for use in the treatment of certain  
59 cancers, autoimmune disorders, and viral infections. We assessed the sensitivity of SARS-CoV-2 to both IFN-  
60  $\alpha$  and IFN- $\beta$ . Herein, we report that type I IFNs exhibited potent anti-SARS-CoV-2 activities in cultured cells,  
61 demonstrating the therapeutic potency of type I IFNs for COVID-19.

## 62

## 63 MATERIALS AND METHODS

64 **Virus and Cells.** The SARS-CoV-2 (USA-WA1/2020) were obtained from The World Reference Center for  
65 Emerging Viruses and Arboviruses (WRCEVA), University of Texas Medical Branch, Galveston, TX). Stock  
66 virus were propagated by infecting Vero cells (ATCC CCL-81) at a low multiplicity of infection (MOI) 0.0025.  
67 Three days after infection, supernatants were harvested and centrifuged at 2000 rpm for 5 min to remove cell  
68 debris. Stock virus was titrated with a 50% tissue culture infectious dose assay (TCID<sub>50</sub>) (4). All experiments  
69 involving infectious virus were conducted at the University of Texas Medical Branch (Galveston, TX) in  
70 approved biosafety level 3 laboratories in accordance with institutional health and safety guidelines and federal  
71 regulations.

72 **Virus growth curve.** Vero cells were infected by SARS-CoV-2 at MOI 1 or 0.01 for 1 hr. Then inoculum was  
73 removed, replaced with media (DMEM+5%FBS) and incubated at 37°C and 5% CO<sub>2</sub>. At different time points  
74 after infection, supernatants were harvested and virus titers were determined by a TCID<sub>50</sub> assay on Vero cells.

75 **Virus sensitivity to IFN treatment.** Vero cells (2x10<sup>4</sup>/well) were seeded into 48-well plates for 24 h and  
76 treated with human IFN- $\beta$ 1a (mammalian, cat# 11415, PBL) and IFN- $\alpha$  (Universal Type I alpha A/D (Bg III),  
77 PBL, cat# 11200-1) at different concentrations for 16 h. Cells were then infected with SARS-CoV-2 at an MOI  
78 of 0.01 TCID<sub>50</sub>/cell. IFNs were supplemented after virus infection. Supernatants were collected at 22 hr post  
79 infection and assayed for virus titers.

## 80

## 81 Results

82 The growth kinetics of the newly identified SARS-CoV-2 in cultured cells remained to be characterized. Thus,  
83 we first examined the growth kinetics of SARS-CoV-2 in Vero cells. Vero cells were infected at either a low  
84 MOI (MOI=0.01) or high MOI (MOI=1). Supernatant was collected every 8-16 hours. At both conditions, viral

85 titers peaked at around 24 hours post-infection (hpi) and remained stable until 40 hours post-infection before  
86 declining (Fig. 1). The peak virus titer was  $5.5 \times 10^6$  TCID<sub>50</sub>/ml at MOI 0.01 and  $3.75 \times 10^5$  TCID<sub>50</sub>/ml at MOI,  
87 indicating that viral replication was more efficient at low MOI (MOI=0.01) than high MOI (MOI=1). Virus  
88 infection also caused strong cytotoxic effect (CPE), which was more evident at 48 hpi and later than the peak  
89 of virus production (at 40 hpi).

90  
91 Next, we examined the effect of recombinant human IFN- $\alpha$  and IFN- $\beta$  treatment on viral infection. Vero cells  
92 were pre-treated with different concentrations of IFN- $\alpha$  or IFN- $\beta$  ranging from 50-1000 international units (IU)  
93 per milliliter for 16 hours prior to infection. After 1 hour of infection with SARS-CoV-2 (MOI 0.01), media  
94 containing IFN was returned, and cells were incubated for a further 22 hours. Supernatants were then  
95 collected, and viral titers were determined via TCID<sub>50</sub> assay. The result indicated a potent inhibition of SARS-  
96 CoV-2 infection by IFN- $\alpha$  treatment. Virus titers were not detectable except at the lowest concentration tested  
97 (50 IU/ml), at which IFN- $\alpha$  drastically reduced viral titers by 4-logs of magnitude (Fig. 2). IFN- $\beta$  exhibited more  
98 potent anti-SARS-CoV-2 activity than IFN- $\alpha$ : the virus titer was below the detection limit at all concentrations  
99 tested (50 u/ml-1000u/ml). Consistently, no CPE was observable under microscope examination in all IFN-  
100 treated samples.

101  
102 We next tested the antiviral efficacy of IFN- $\alpha$  and IFN- $\beta$  at lower concentrations ranging from 1-50 IU/ml. For  
103 both IFNs, a dose-dependent effect was clearly observed using these lower concentrations (Fig. 3). For IFN- $\alpha$ ,  
104 the anti-SARS-CoV-2 activity could be noticed as low as 5 IU/ml, at which the virus titer was significantly  
105 reduced by over 1 log ( $P < 0.01$ ). With increasing IFN- $\alpha$  concentrations, the virus titers steadily decreased. For  
106 IFN- $\beta$ , treatment with 1 IU/ml of IFN- $\beta$  resulted in a moderate (approximately 70%) but significant decrease in  
107 virus titer ( $P < 0.05$ , Student t test). Virus levels were nearly undetectable upon treatment with 10, 25, and 50  
108 IU/ml of IFN- $\beta$ . The EC<sub>50</sub> of IFN- $\alpha$  and IFN- $\beta$  treatment is 1.35 IU/ml and 0.76 IU/ml, respectively. Taken  
109 together, these results indicated that treatment with low concentrations of both IFN- $\alpha$  and IFN- $\beta$  significantly  
110 inhibited viral replication, with IFN- $\beta$  being slightly more effective than IFN- $\alpha$ .

## 111 112 **Discussion**

113 Our data clearly demonstrated that SARS-CoV-2 is highly sensitive to both IFN- $\alpha$  and IFN- $\beta$  treatment in  
114 cultured cells. The experiment was performed in the IFN- $\alpha/\beta$  gene-defective Vero cells. It is plausible that the  
115 anti-SARS-CoV-2 efficacy of exogenous IFN treatment is more potent in IFN-competent cells, as IFN- $\beta$  is  
116 known to mediate the expression of other subtypes of Type I IFNs. Our data may also explain, at least in part,  
117 that approximately 80% of patients actually only developed mild symptoms and recovered (5). It is possible  
118 that many infected people developed IFN- $\alpha$  and IFN- $\beta$ -mediated innate immune response upon SARS-CoV-2  
119 infection, which limits virus infection during initial stage. Later on, adaptive immune response, such as antibody  
120 production, may eventually help the recovery from the COVID-19 disease.

121  
122 Compared to SARS-CoV-2, it seems SARS-CoV is not relatively less sensitive to IFN treatment *in vitro* (6, 7).  
123 One study reported that the EC<sub>50</sub> of IFN- $\beta$  for SARS-CoV is 95 or 105 IU/ml depending on virus strain (8).  
124 Many other highly pathogenic viruses are also resistant to exogenous IFN treatment. Treatment of Ebola-  
125 infected cells with exogenous IFN- $\alpha$  does not lead to any reduction in viral replication (9), which is  
126 hypothesized to be a result of viral protein antagonism of the IFN response. Also, arenavirus Junín virus, which  
127 causes Argentine Hemorrhagic Fever, is also found to be insensitive to IFN treatment. In Vero cells, the titers  
128 of JUNV were reduced by less than 1-log when treated with a high concentration of human IFN- $\alpha$ ,  $\beta$  or  $\gamma$  (1000  
129 U/ml) (10). By contrast, the remarkable sensitivity of SARS-CoV-2 to IFN treatment reveals a weakness of this  
130 virus, which may be informative to antiviral development. Further work is needed to characterize the IFN  
131 response during SARS-CoV-2 infection and any possible viral interference.

132  
133 *In vitro*, we have demonstrated that SARS-CoV-2 replication is inhibited by clinically-achievable doses of both  
134 IFN- $\alpha$  and IFN- $\beta$ . Thus, the use of IFN may be a viable treatment option for COVID-19. Serum levels of  
135 Roferon-A and Intron-A, two recombinant IFN- $\alpha$  drugs already approved for the treatment of hepatitis B and C,  
136 can reach up to 330 IU/ml and 204 IU/ml, respectively (11). Betaferon and Rebif, two recombinant IFN- $\beta$  drugs  
137 approved for use in the treatment of multiple sclerosis, can reach respective concentrations of 40 IU/ml and 4.1  
138 IU/ml in the serum (11). Some of these drugs may therefore have the potential to be repurposed for the  
139 treatment of COVID-19 either alone or in combination with other antiviral therapies.

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148

149 **Figure legend:**

150 **Figure 1:** Vero cells were infected by SARS-CoV-2 at MOI 1 or 0.01 for 1 hr. At different time points after  
151 infection, supernatants were harvested and virus titers were determined by a TCID<sub>50</sub> assay on Vero cells. The  
152 average of triplicates and Standard deviation are shown. Dotted line indicates the detection limit.

153

154 **Figure 2:** Vero cells were pretreated with human IFN- $\alpha$  or IFN- $\beta$  (0, 50, 125, 250, 500, 1000 IU/ml) for 16  
155 hours. Cells were then infected with SARS-CoV2 for 1 hour at an MOI of 0.01. Viral inoculums were removed  
156 and replaced with fresh media containing listed concentrations of IFN- $\alpha$  or IFN- $\beta$ . Media was collected at 22  
157 hpi and titers were determined via TCID<sub>50</sub> assay on Vero cells. The average of triplicates and Standard  
158 deviation are shown. Dotted line indicates the detection limit.

159

160 **Figure 3:** Vero cells were pretreated with human IFN- $\alpha$  or IFN- $\beta$  (0, 1, 5, 10, 25, 50 U/ml) for 16 hours and  
161 then infected with SARS-CoV2 at an MOI of 0.01. Viral inoculums were removed and replaced with fresh  
162 media containing listed concentrations of IFN- $\alpha$  or IFN- $\beta$ . Media was collected at 22 hpi and virus titers were  
163 determined via TCID<sub>50</sub> assay. The average of triplicates and Standard deviation are shown. Dotted line  
164 indicates the detection limit. (\*, P<0.05; \*\*, P<0.01; n.s. not significant, one tail Student T test)

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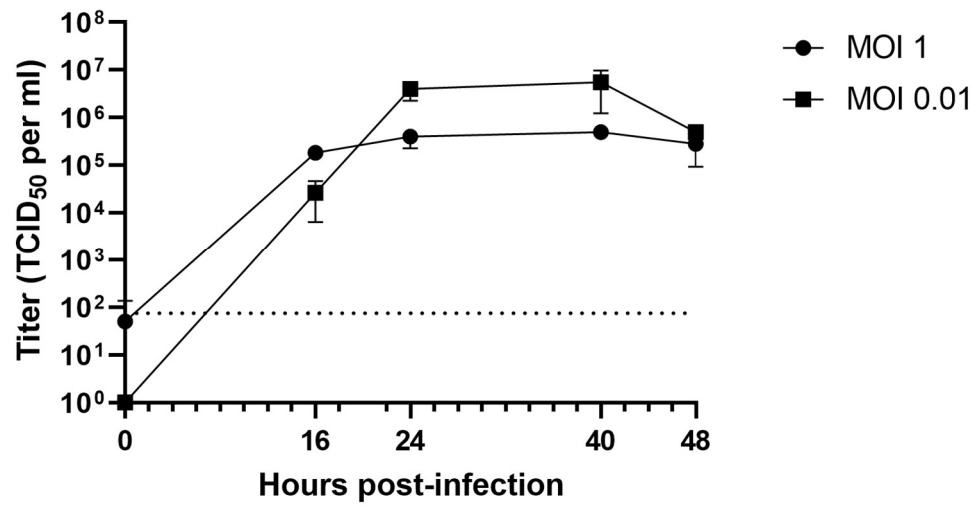
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