

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

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32 **Abstract:**

33 The ongoing historic outbreak of COVID-19 not only constitutes a global public health crisis, but also carries a
34 devastating social and economic impact. The disease is caused by a newly identified coronavirus, Severe
35 Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2). There is an urgent need to identify antivirals to
36 curtail the COVID-19 pandemic. Herein, we report the remarkable sensitivity of SARS-CoV-2 to recombinant
37 human interferons α and β (IFN α/β). Treatment with IFN- α at a concentration of 50 international units (IU) per
38 milliliter drastically reduces viral titers by 3.4 log or over 4 log, respectively, in Vero cells. The EC₅₀ of IFN- α
39 and IFN- β treatment is 1.35 IU/ml and 0.76 IU/ml, respectively, in Vero cells. These results suggest that SARS-
40 CoV-2 is more sensitive than many other human pathogenic viruses, including SARS-CoV. Overall, our results
41 demonstrate the potent efficacy of human Type I IFN in suppressing SARS-CoV-2 infection, a finding which
42 could inform future treatment options for COVID-19.

43

44 **Introduction**

45 The COVID-19 outbreak started in Wuhan, China in December 2019 and rapidly spread globally, causing over
46 752,000 confirmed cases and 36,000 deaths as of April 1, 2020. The causative agent for the COVID-19
47 disease is a newly identified Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) (1), which is
48 transmitted through aerosol/droplet inhalation or contact. This historic outbreak has caused a public health
49 crisis much more severe than the SARS outbreak, which “only” caused 8,098 infections and 774 deaths
50 between November 2002 and July 2003. The COVID-19 outbreak also has had a devastating social and
51 economic impact worldwide. In March, the World Health Organization has declared COVID-19 a pandemic. In
52 the USA, there are over 200,000 confirmed cases and 4,300 deaths as of April 1, 2020. It is warned by the
53 CDC that the COVID-19 pandemic may claim over 100,000 lives in USA ([https://www.msn.com/en-
54 nz/news/world/us-could-face-200000-coronavirus-deaths-millions-of-cases-fauci-warns/ar-BB11UIQj](https://www.msn.com/en-nz/news/world/us-could-face-200000-coronavirus-deaths-millions-of-cases-fauci-warns/ar-BB11UIQj)).

55 Therefore, there is an urgent need to find treatments for COVID-19. Drugs already approved for the treatment

56 of other diseases may offer the most expedient option for treating COVID-19, and several such drugs are
57 already being tested in clinical trials.

58
59 Type I interferons (IFN- α/β) have broad spectrum antiviral activities against RNA viruses, which act by inducing
60 an antiviral response across a wide range of cell types and mediating adaptive immune response. Humans
61 produce 13 types of IFN- α and a singular IFN- β (2). Type I IFNs ultimately induces a number of interferon-
62 stimulated genes (ISGs) which encode for a variety of antiviral effectors (3). Notably, IFN- β production leads to
63 a positive feedback loop that further stimulates the expression of many of the IFN- α genes (4). Clinically, Type
64 I IFNs have already been approved for use in the treatment of certain cancers, autoimmune disorders, and
65 viral infections (hepatitis B and hepatitis C). We assessed the sensitivity of SARS-CoV-2 to both IFN- α and
66 IFN- β *in vitro*. Herein, we report that type I IFNs exhibited potent anti-SARS-CoV-2 activities in cultured cells,
67 demonstrating the therapeutic potency of type I IFNs for COVID-19.

69 **MATERIALS AND METHODS**

70 **Virus and Cells.**

71 The SARS-CoV-2 (USA-WA1/2020) was obtained from The World Reference Center for Emerging Viruses and
72 Arboviruses (WRCEVA), University of Texas Medical Branch, Galveston, TX. Stock virus was propagated by
73 infecting Vero cells (ATCC CCL-81) at a low multiplicity of infection (MOI) of 0.0025. Three days after infection,
74 supernatants were harvested and centrifuged at 2000 rpm for 5 min to remove cell debris. Stock virus was
75 titrated with a 50% tissue culture infectious dose assay (TCID₅₀) (5). All experiments involving infectious virus
76 were conducted at the University of Texas Medical Branch (Galveston, TX) in approved biosafety level 3
77 laboratories in accordance with institutional health and safety guidelines and federal regulations.

78 **Virus growth curve:**

79 Vero cells were infected by SARS-CoV-2 at MOI 1 or 0.01 for 1 hr. Then inoculum was removed, replaced with
80 media (DMEM+5%FBS) and incubated at 37 °C and 5% CO₂. At different time points after infection,
81 supernatants were harvested. Virus titers were determined by a TCID₅₀ assay on Vero cells.

82 **Virus sensitivity to IFN treatment (infectious virus reduction assay):**

83 Vero cells (2×10^4 /well) were seeded into 48-well plates for 24 h and treated with human IFN- β 1a (mammalian,
84 cat# 11415, PBL) and IFN- α (Universal Type I alpha A/D (Bg III), PBL, cat# 11200-1) at different
85 concentrations for 16 h. Cells were then infected with SARS-CoV-2 at an MOI of 0.01 TCID₅₀/cell. IFNs were
86 supplemented after virus infection. Supernatants were collected at 22 hr post infection and assayed for virus
87 titers.

88 **Virus sensitivity to IFN treatment (CPE inhibition assay)**

89 Vero cells grown on 96-well plates (2×10^4 /well) were treated with 2-fold serial diluted human IFN- β 1a or IFN- α
90 for 16 h (250 IU/ml to 0.49 IU/ml). Cells were then infected with SARS-CoV-2 at an MOI of 0.01 TCID₅₀/cell or
91 Vesicular stomatitis virus (VSV, Indiana strain) at MOI 0.1 PFU/cell for 1 hr. The inoculums were removed and
92 replaced with fresh media. As controls, cells were mock-infected, or infected without IFN treatment. All
93 experiments were performed in quadruplicates. For VSV samples, the supernatants were aspirated at 12 hpi.
94 The monolayers were washed with PBS for three times to remove dead cells, fixed with 10% formaldehyde,
95 and stained with crystal violet for cytopathic effect (CPE) observation. For SARS-CoV-2 samples, CPE was
96 observed at 72 hpi.

97

98 **Results**

99 The growth kinetics of the newly identified SARS-CoV-2 in cultured cells had not been characterized. Thus, we
100 first examined the growth kinetics of SARS-CoV-2 in Vero cells. Vero cells were infected at either a low MOI
101 (MOI=0.01) or high MOI (MOI=1). Supernatant was collected every 8-16 hours. At both conditions, viral titers
102 peaked at approximately 24 hours post-infection (hpi) and remained stable until 40 hours post-infection before
103 declining (Fig. 1). The peak virus titer was 5.5×10^6 TCID₅₀/ml at MOI 0.01 and 3.75×10^5 TCID₅₀/ml at MOI 1,
104 indicating that viral replication was more efficient at a low MOI (MOI=0.01) than a high MOI (MOI=1).
105 Additionally, virus infection caused strong cytopathic effect (CPE), which was evident at 48 hpi, much later than
106 the peak of virus production (at 40 hpi).

107

108 Next, we examined the effect of recombinant human IFN- α and IFN- β treatment on viral infection. Vero cells
109 were pre-treated with different concentrations of IFN- α or IFN- β ranging from 50-1000 international units (IU)
110 per milliliter for 16 hours. After 1 hour of infection with SARS-CoV-2 (MOI 0.01), media containing IFN was

111 returned, and cells were incubated for a further 22 hours. Supernatants were then collected, and viral titers
112 were determined via TCID₅₀ assay. The result indicated that IFN- α treatment potently inhibited SARS-CoV-2
113 infection. Virus titers were not detectable except at the lowest concentration tested (50 IU/ml), at which the viral
114 titers were drastically reduced by 4 logs of magnitude (Fig. 2). For IFN- β , the virus titers were below the
115 detection limit at all concentrations tested (50 u/ml-1000u/ml), indicating more potent anti-SARS-CoV-2 activity
116 than IFN- α . Consistently, no CPE was observable under microscopic examination in all IFN-treated samples.

117
118 We next tested the antiviral efficacy of IFN- α and IFN- β at lower concentrations (1-50 IU/ml). Both IFN- α and
119 IFN- β dose-dependently inhibited virus infection at these lower concentrations (Fig. 3). IFN- α exhibited anti-
120 SARS-CoV-2 activity at a concentration as low as 5 IU/ml, resulting in a significant reduction of viral titer by
121 over 1 log ($P < 0.01$). With increasing IFN- α concentrations, the virus titers steadily decreased. Treatment with
122 IFN- α at 50 IU/ml drastically reduces viral titers by 3.4 log. Treatment with 1 IU/ml of IFN- β resulted in a
123 moderate (approximately 70%) but significant decrease in virus titer ($P < 0.05$, Student t test). Infectious virus
124 was nearly undetectable upon treatment with 10, 25, and 50 IU/ml of IFN- β . The EC₅₀ of IFN- α and IFN- β
125 treatment is 1.35 IU/ml and 0.76 IU/ml, respectively. Taken together, these results indicate that treatment with
126 low concentrations of both IFN- α and IFN- β significantly inhibited viral infection, with IFN- β being slightly more
127 effective than IFN- α .

128
129 In addition, we compared the IFN sensitivity of SARS-CoV-2 with that of Vesicular stomatitis virus (VSV), an
130 IFN-sensitive RNA virus. IFN- α or IFN- β were 2-fold serially diluted (250 IU/ml to 0.49 IU/ml) and added to
131 Vero cells for 16 hr. Then cells were infected by VSV (MOI 0.1) or SARS-CoV-2 (MOI 0.01). CPE were
132 observed at 12 hpi for VSV and 72 hpi for SARS-CoV-2. In VSV-infected cells, IFN- α and IFN- β both inhibited
133 CPE development at a concentration of 31.25 IU/ml, while at 15.6 IU/ml the CPE was not discernable from that
134 of IFN-untreated samples. For SARS-CoV2, the lowest concentration that IFN- β or IFN- α inhibited CPE was
135 31.25 IU/ml and 62.5 IU/ml, respectively. The CPE inhibition data suggests that the IFN sensitivity of SARS-
136 CoV-2 is comparable to that of VSV.

137 138 Discussion

139 Our data clearly demonstrate that SARS-CoV-2 is highly sensitive to both IFN- α and IFN- β treatment in
140 cultured cells, which is comparable to the IFN-sensitive VSV. Our discovery reveals a weakness of the new
141 coronavirus, which may be informative to antiviral development. The experiment was performed in the IFN- α/β
142 gene-defective Vero cells (6). It is plausible that in IFN-competent cells the efficacy of exogenous IFN- β
143 treatment against SARS-CoV-2 infection is more potent, as IFN- β upregulates other subtypes of Type I IFN
144 expression and augments the IFN-mediated antiviral response (4). Our data may provide an explanation, at
145 least in part, to the observation that approximately 80% of patients actually develop mild symptoms and
146 recover (7). It is possible that many of them are able to mount IFN- α/β -mediated innate immune response upon
147 SARS-CoV-2 infection, which helps to limit virus infection/dissemination at an early stage of disease. At a later
148 stage, the adaptive immune response (antibody etc.) may eventually help patients recover from the COVID-19
149 disease.

150
151 Compared to SARS-CoV-2, it seems that SARS-CoV is relatively less sensitive to IFN treatment *in vitro* (8, 9).
152 One study reported that the EC₅₀ of IFN- β for SARS-CoV is 95 or 105 IU/ml depending on virus strains (10).
153 Many other highly pathogenic viruses are also resistant to exogenous IFN treatment. For Ebola virus, it has
154 been reported that treatment with exogenous IFN- α does not affect viral replication and infectious virus
155 production in cultured cells (11), probably as a result of antagonism of the IFN response by viral protein. Junin
156 virus, an arenavirus that causes Argentine Hemorrhagic Fever, is likewise insensitive to IFN treatment. When
157 treated with a high concentration of human IFN- α , β or γ (1000 U/ml), the titers of JUNV were reduced by less
158 than 1-log in Vero cells. Further work is warranted to characterize the IFN response during SARS-CoV-2
159 infection to better understand the underlying mechanism behind its IFN sensitivity.

160
161 *In vitro*, we have demonstrated that SARS-CoV-2 replication is inhibited by IFN- α and IFN- β at concentrations
162 that are clinically achievable in patients. Recombinant IFN- α s, Roferon-A and Intron-A, which have been
163 approved for hepatitis B and C treatment, can reach concentrations of up to 330 IU/ml and 204 IU/ml,
164 respectively, in serum (12). Recombinant IFN- β drugs, Betaferon and Rebif, which have been approved for the
165 treatment of multiple sclerosis, can reach concentrations of 40 IU/ml and 4.1 IU/ml, respectively, in serum (12).

166 Therefore, some of these drugs may have the potential to be repurposed for the treatment of COVID-19 either
167 alone or in combination with other antiviral therapies.

168

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176

177 **Figure legend:**

178 **Figure 1:** Vero cells were infected by SARS-CoV-2 at MOI 1 or 0.01 for 1 hr. At different time points after
179 infection, virus titers were determined by a TCID₅₀ assay on Vero cells. The average of triplicates and Standard
180 deviation are shown. Dotted line indicates the detection limit.

181

182 **Figure 2:** Vero cells were pretreated with human IFN- α or IFN- β (0, 50, 125, 250, 500, 1000 IU/ml) for 16
183 hours, and then infected with SARS-CoV2 for 1 hour at an MOI of 0.01. Viral inoculums were removed and
184 replaced with fresh media containing listed concentrations of IFN- α or IFN- β . Media was collected at 22 hpi
185 and titers were determined via TCID₅₀ assay on Vero cells. The average of triplicates and Standard deviation
186 are shown. Dotted line indicates the detection limit.

187

188 **Figure 3:** Vero cells were pretreated with human IFN- α or IFN- β (0, 1, 5, 10, 25, 50 U/ml) for 16 hours and
189 then infected with SARS-CoV-2 at an MOI of 0.01. Viral inoculums were removed and replaced with fresh
190 media containing listed concentrations of IFN- α or IFN- β . Virus titers at 22 hpi were determined via TCID₅₀
191 assay. The average of triplicates and Standard deviation are shown. Dotted line indicates the detection limit. (*,
192 P<0.05; **, P<0.01; n.s. not significant, one tail Student T test)

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