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Short Communication

Potent Inhibition of SARS-Associated Coronavirus (SCoV) Infection and Replication by Type I Interferons (IFN-α/β) but Not by Type II Interferon (IFN-γ)

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ABSTRACT

We sought to investigate the anti-severe acute respiratory syndrome (SARS)-associated coronavirus (SCoV) activities of type I (α and β) and type II (γ) interferons (IFN) *in vitro*. Type I IFNs protected cells from cytopathic effects (CPE) induced by SCoV, and inhibited viral genomic RNA replication in FRhk-4 cells (measured by quantitative RT-PCR) in a dose-dependent manner. Intracellular viral RNA copies were reduced 50% by IFN-α at a concentration of 25 U/ml and by IFN-β at a concentration of 14 U/ml. IFN-γ had fewer effects on inhibition of viral infection and replication. The type I IFN receptor signaling pathway in host cells is mainly involved in the inhibition of SCoV infection and replication. Type I IFNs could be used as potential agents for anti-SARS treatment.

A NOVEL CORONAVIRUS (SCoV) has been identified as the causative agent of the recent worldwide outbreak of severe acute respiratory syndrome (SARS).[1,2] Coronaviruses are positive-stranded RNA viruses with the largest known viral RNA genomes. SARS remains a threat to public health worldwide, as it may cross-transmit from animal to human. Interferons (IFNs) exhibit potent antiviral activities, and, therefore, they are in regular use for antiviral therapy. IFNs transmit signals to the cell via the receptor complex to induce an antiviral response. The binding affinities and the biologic activities among IFN species are different. The type I (α,β) and the type II (γ) IFNs transmit their signals through different receptors.[3–5] There are several hundred genes transcriptionally regulated by IFNs in response to viral invasion. IFN-β, and not IFN-α or IFN-γ, was reported to exhibit potent anti-SCoV activity in Vero and Caco2 cells challenged with a low dose of SCoV (multiplicity of infection [moi] 0.01).[6] In this study, we investigated the effect of the type I and type II IFNs on inhibition of SCoV infection and replication in FRhk-4 cells challenged with high doses of SCoV (moi 0.05) by measuring the viral genomic RNA copies by quantitative RT-PCR and the viral titers by back-titration.

IFNs α (recombinant IFN-α2a) was a gift from Dr. Bill Clark (PBL Co., Piscataway, NJ). IFN-β (recombinant IFN-β) and IFN-γ (recombinant IFN-γ) were purchased from Sigma Chemical Co. (St. Louis, MO). The biologic activities (units) of the IFNs were determined by inhibition of cytopathic effects (CPE) in Vero cells challenged in the antivesicular stomatitus virus (VSV) assay. To evaluate the anti-SCoV activity of IFNs, fetal rhesus monkey kidney cells (FRhk-4, purchased from ATCC, Rockville, MD) in MEM medium supplemented with 10% fetal bovine serum (FBS) were seeded into 96-well plates (3×10^3 cells per well) and cultured overnight. Cells were incubated for 1 h with various concentrations of different IFNs dissolved in 100 μl MEM medium, then infected with SCoV (moi 0.05) diluted in MEM with 1% FBS. Thirty-six hours after infection with SCoV, the degree of protection against SCoV viral CPE was determined by observing cell morphology under a phase-contrast microscope. Total cellular RNA was extracted

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from cells using QIAamp Virus RNA Mini Kit (Qiagen, Hilden, Germany) as instructed by the manufacturer and was reverse-transcribed using SuperScript (Invitrogen, San Diego CA). The FastStart DNA Master SYBR Green I fluorescence reaction (Roche, Mannheim, Germany) (forward primer 5′-TACACACCTCAGCGTTG-3′; reverse primer 5′-CACGAACGTGACGAAT-3′) was used in the quantitative PCR assay.[6] Plasmids containing the target sequence were used as positive controls. The viral titers were measured by back-titration according to standard protocols.

IFN-α and IFN-β protected cells from viral CPE. Preincubation for 1 h with 128 U/well IFN-α (Fig. 1A, top) and IFN-β (Fig 1A, bottom) protected cells almost completely. The cell morphology was indistinguishable from that of normal, uninfected cells. Marked protection was visible at concentrations as low as 16 U/ml IFN-α and IFN-β (Fig. 1B, C). However, only

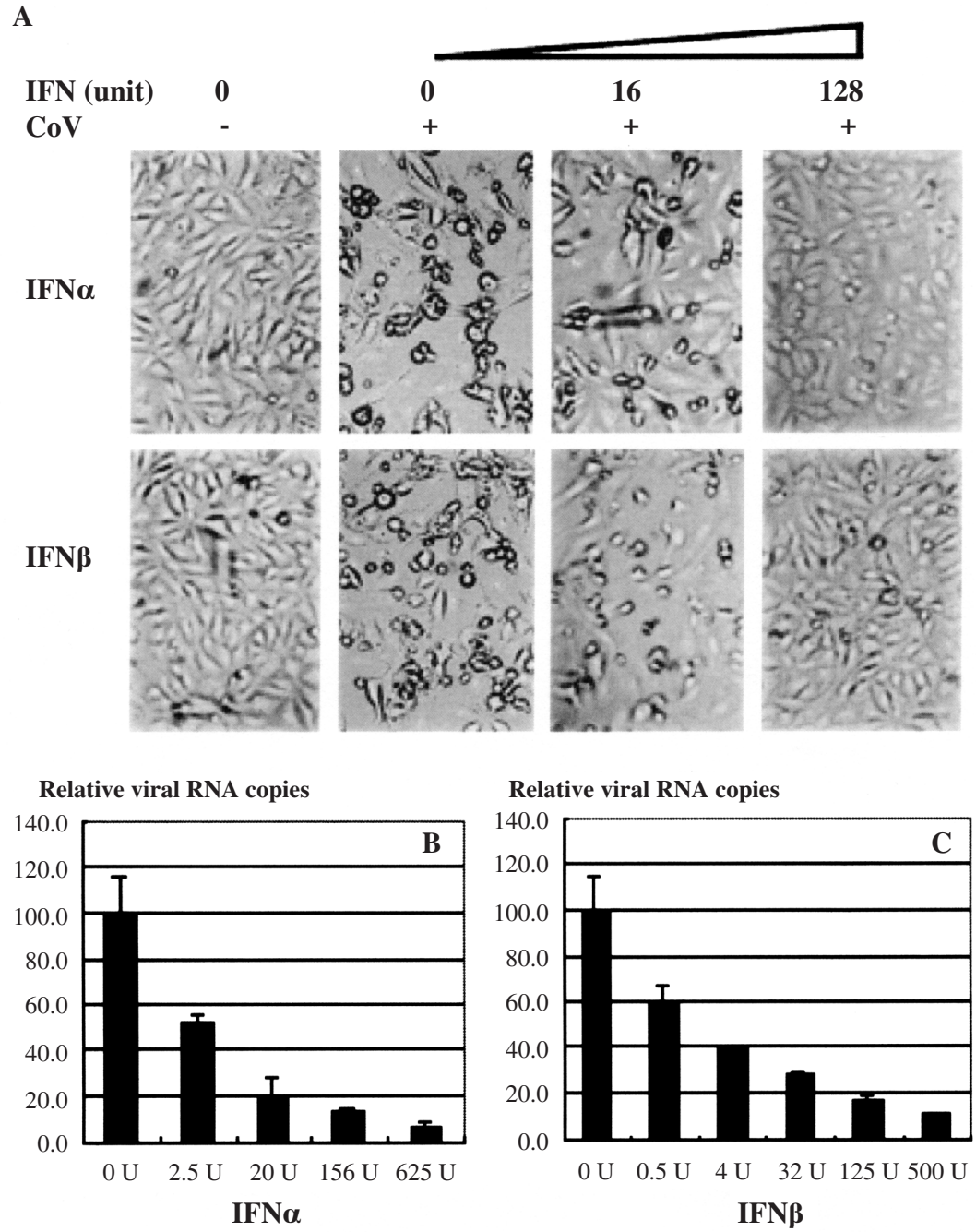

FIG. 1. Inhibition of SCoV-mediated CPE and intracellular viral RNA accumulation by IFNs. The FRhk-4 cells were pretreated with various concentrations of different IFNs as indicated for 1 h and infected with SCoV. (**A**) CPE under phase-contrast microscopy. ×400. (**Top**) IFN-α treated. (**Bottom**) IFN-β treated. Real-time RT-PCR was employed to quantify the viral RNA of SCoV after the cells were treated with IFN and challenged with SCoV. (**B** and **C**) Reduction of intracellular viral RNA copies.

weak protection was observed using IFN-γ at a concentration of 1000 U/ml (data not shown). To further quantify the effects of IFNs on inhibition of SCoV infection and replication in FRhk-4 cells, we measured SCoV intracellular viral RNA copies by quantitative real-time PCR using total cellular RNA as the template and viral titer by back-titration.[6,7] Our results showed that both IFN-α and IFN-β dose-dependently reduced SCoV viral RNA copies in cells and infectious titers in the conditioned medium. Approximately 50% reduction of intracellular viral RNA and viral titers was observed at IFN-α and IFN-β concentrations as low as 25 U/ml and 10 U/ml respectively, and 90% elimination of viral RNA or 98% of viral titers was seen at about 3000 U/ml and 1800 U/ml, respectively. IFN-γ did not exhibit significant antiviral activity. These results were, in general, consistent with the results conducted on Caco-2 cells,[7] although the concentrations of IFNs were miscalculated.[8]

Our results indicate that type I IFNs are much more effective in inhibiting SCoV infection and replication than are type II IFNs. There is a wide variety of type I IFN subspecies, each of which may have different antiviral activities and specificities.[3–5] For example, Cinatl et al.[7] reported that only IFN-β exhibited potent anti-SCoV activity in Vero cells and Caco2 cells. In fact, it has been show that pegylated IFN-α protected type 1 pneumocytes against SCoV in infection in macaques.[9] It will be important to identify the most potent IFN subspecies and conduct preclinical and clinical trials, as SARS is recurring in China.

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