Inhibition of SARS–CoV-2 by type I and type III interferons

Ulrike Felgenhauer¹, Andreas Schoen¹, Hans Henrik Gad², Rune Hartmann³, Andreas R. Schaubmar³, Klaus Failing⁴, Christian Drosten⁴,⁵, and Friedemann Weber¹,²,⁶,⁷

From the ¹Institute for Virology and the ³Unit for Biomathematics and Data Processing, FB10-Veterinary Medicine, Justus Liebig University, Giessen, Germany, the ²Department for Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark, the ⁴German Centre for Infection Research (DZIF), partner sites Giessen and Charité Berlin, Germany, and the ⁵Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Virology, Berlin, Germany

Edited by Craig E. Cameron

The recently emerged severe acute respiratory syndrome coronavirus-2 (SARS–CoV-2) is the causative agent of the devastating COVID-19 lung disease pandemic. Here, we tested the inhibitory activities of the antiviral interferons of type I (IFN-α) and type III (IFN-λ) against SARS–CoV-2 and compared them with those against SARS–CoV-1, which emerged in 2003. Using two mammalian epithelial cell lines (human Calu-3 and simian Vero E6), we found that both IFNs dose-dependently inhibit SARS–CoV-2. In contrast, SARS–CoV-1 was restricted only by IFN-α in these cell lines. SARS–CoV-2 generally exhibited a broader IFN sensitivity than SARS–CoV-1. Moreover, ruxolitinib, an inhibitor of IFN-triggered Janus kinase/signal transducer and activator of transcription signaling, boosted SARS–CoV-2 replication in the IFN-competent Calu-3 cells. We conclude that SARS–CoV-2 is sensitive to exogenously added IFNs. This finding suggests that type I and especially the less adverse effect–prone type III IFN are good candidates for the management of COVID-19.

The massive pandemic caused by coronavirus SARS–CoV-2 (1, 2) is calling for rapid evaluation of potential therapeutics through repurposing of drugs already in clinical use. Interferons of type I (IFN-α/β) and type III (IFN-λ) constitute an important branch of the mammalian innate immune response. These cytokines are produced by virus-infected cells and are able to establish an antiviral state in target cells by triggering the so-called JAK/STAT signaling pathway (3–5). Both type I and type III IFNs are clinically used or being tested, respectively, against a range of ailments that include viral diseases (6, 7). Previously, we and others have demonstrated the potential of IFNs to inhibit the two related, previously emerged pathogenic coronaviruses SARS–CoV-1 and MERS–CoV (8–15). Here, we investigated the potential of type I and type III IFNs against the newly emerged SARS–CoV-2.

Results

Type I IFN

We tested the effect of type I IFN against SARS–CoV-2 compared with the SARS–CoV-1 from 2003. Two different cell lines were employed, namely the human bronchial epithelial Calu-3 and the primate kidney epithelial Vero E6. The cells were first treated for 16 h with 100, 500, or 1000 units/ml of recombinant human IFN-α(B/D) and then infected with the viruses at a multiplicity of infection (MOI) of 0.01 plaque forming units (PFU)/cell to obtain multistep growth. Virus titers in supernatants were determined 24 h later, when titers are reaching a plateau (see below). The data of three biological replicates are shown in Fig. 1. Because several titers were below the detection limit of our plaque assay, a rank correlation test (Spearman’s exact rank correlation test) was used for statistical dose–response correlation analysis. For SARS–CoV-2 (dark gray bars), statistically significant negative correlation coefficients (CC) were obtained for both cell lines, indicating that viral replication is increasingly inhibited by IFN-α. For SARS–CoV-1 (light gray bars), titers were also affected. However, at least in Vero E6 cells, the reduction of SARS–CoV-1 appears to be weaker than the reduction of SARS–CoV-2 (Fig. 1B), Observations were similar when the input MOI was reduced to 0.001 (Fig. S1), except that titers of SARS–CoV-1 in Calu-3 cells were already very low in the absence of any IFN-α, resulting in a nonsignificant effect of additional IFN. These data may suggest that the potency of IFN to reduce viral titers may be stronger and more consistent against SARS–CoV-2 than against SARS–CoV-1.

To further investigate the potential differences between the viruses, we repeated the experiment three times more with the intermediate dose of 100 units/ml and analyzed the data statistically after pooling them with the previous three replicates. Two-way ANOVA was used to simultaneously evaluate the influence of both IFN-α and virus species on virus reduction. This analysis (Fig. 2, A and B) showed again that (i) both viruses are reduced by IFN (comparison of 0 versus 100 units/ml IFN-α, p(IFN)) and (ii) there are differences between the SARS–CoV species (comparison of the virus experiments, p(virus)). Moreover, the “interaction” p value showed that, at least in Vero cells, the degree of IFN sensitivity depends on the virus species, again indicating that SARS–CoV-2 is more IFN-sensitive than SARS–CoV-1.

Type III IFN

The primary tropism of coronaviruses typically involves epithelia of the respiratory and gastrointestinal tracts (16). On such mucosal barriers, type III IFNs rather than type I IFNs are...
the predominant antiviral cytokine (4, 5). Although the IFN induction as well as signaling and up-regulation of IFN-stimulated genes (ISGs) are very similar, type III IFNs engage a different receptor that is restricted to epithelial cells, and generate a weaker but longer-lasting antiviral response (5, 17). IFN-λ was previously shown to have activity against coronaviruses (11, 18, 19) and proposed as potential COVID-19 treatment (20). Hence, we compared the sensitivity of the two SARS–coronaviruses also to recombinant human IFN-λ. As shown in Fig. 3A, pretreatment with 10 or 100 ng/ml IFN-λ exhibited only in Vero E6 cells a dose-dependent inhibitory effect on SARS–CoV-2. For SARS–CoV-1, by contrast, no significant inhibition was noted in any of the cell lines. To further investigate the difference between the viruses, we repeated the IFN-λ experiment three times more with the intermediate dose of 10 ng/ml and analyzed the data after pooling with the previous 10 ng/ml IFN-λ experiment (Fig. 3B). Conventional statistical analysis (one-tailed Student’s t test, because none of the values was below the detection limit) again revealed a significant impact of IFN-λ on SARS–CoV-2 and the lack of an effect for SARS–CoV-1. Our data thus show that IFN-λ can inhibit SARS–CoV-2 but not SARS–CoV-1.

Blocking JAK/STAT signaling by ruxolitinib

A recent study on the host cell interactome of SARS–CoV-2 identified a number of human proteins for which Food and Drug Administration–approved drugs are available (21). Ruxolitinib, a compound known to target the type I and type III IFN-triggered JAK/STAT signaling pathway (22), was among the proposed inhibitors of virus–host cell interactions (21). Because virus inhibition by an IFN inhibitor seems counterintuitive, we aimed to clarify the influence of this compound on SARS–CoV-2 replication. Cells were pretreated with 1 μM ruxolitinib for 16 h and infected at the two different MOIs, and titers were measured 24 or 48 h later. As shown in Fig. 4, with this setting titers in nontreated controls are already reaching a plateau at the 24-h time point. In Calu-3 cells, ruxolitinib had a
clear boosting effect on SARS-CoV-2 replication, mostly at 48 h postinfection, and at both MOI 0.01 and 0.001 (Fig. 4A and Fig. S2A). By contrast, in Vero E6 there was neither a positive nor a negative effect discernible (Fig. 4B and Fig. S2B). Of note, Calu-3 cells are capable of inducing IFN in response to virus infection, whereas Vero cells are not (15). Our data thus indicate that (i) if anything, ruxolitinib is an enhancer rather than an inhibitor of SARS-CoV-2 multiplication, and (ii) the boosting effect is most likely due to inhibition of the antiviral JAK/STAT signaling pathway, because it is not present in the IFN induction–deficient Vero E6 cells.

**Comparison of the cell lines**

Our observations so far suggest that SARS-CoV-2 is consistently more sensitive to IFNs than SARS-CoV-1. Moreover, type I IFN seems to have a more profound effect than type III IFN. To see whether principal differences in signaling or subsequent gene expression could account for these phenomena, we tested the ability of the cell lines to respond to the IFNs. The immunoblot analysis (Fig. 5) shows that Calu-3 cells have a very similar reaction to both types of IFN concerning phosphorylation of STAT1 and STAT2 and expression of the IFN-stimulated MxA and ISG15. Vero E6 cells also responded to IFN-λ as expected (23), but the ISG response was lower than to IFN-α. Moreover, in nontreated Calu-3 cells, there was already a background ISG expression, which was not observed in Vero cells.

Ruxolitinib was in principle able to influence these ISG responses, as expected, but it was more potent against IFN-λ than against IFN-α, and its effects on IFN-stimulated genes were more evident in the Vero E6 compared with the Calu-3 (Fig. 5). Thus, both cell lines are capable to respond to the different types of IFN, but IFN-λ was less potent, which is in agreement with our observations on SARS-CoV sensitivity, as well as with previous studies (5, 17).

**Discussion**

The recently emerged SARS-CoV-2 is responsible for major health crises all over the world. Here, we show that type I and type III IFNs are able to inhibit SARS-CoV-2 replication, with effects that in our hands were consistently more profound than against the SARS-CoV-1 from 2003. It should be noted however, that the differences between the viruses could be due to the cell types used or due to the observed differences in virus replication (which could result in higher production of IFN antagonists). Thus, the question whether SARS-CoV-2 is intrinsically more sensitive to IFNs remains to be solved.

PEGylated IFN-α was the standard of care against chronic infection with hepatitis C virus until the recent introduction of other, directly acting antiviral drugs (24). Although associated with some side effects, IFN-α is well-characterized, has been used to treat millions of patients, is considered safe, and is available.
Ruxolitinib was proposed as a potential treatment against SARS-CoV-2 (21, 40), and a small clinical trial is underway (clinicaltrials.gov, NCT04334044), although case reports were discouraging (41). The replication boost obtained with ruxolitinib on the IFN-competent Calu-3 cells indicates that ruxolitinib is not at all inhibiting SARS-CoV-2 replication. Thus, drugs that interfere with viral host interactors may not necessarily be antiviral but rather boost the infection.

Experimental procedures

Cells and viruses

Calu-3 and Vero E6 cells were cultivated in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) in a 5% CO₂ atmosphere at 37°C. SARS-CoV-2 (strain SARS-CoV-2 /München-1.2/2020/984, p.2) (42) and SARS-CoV-1 (strain SARS-FRA1, p.2) (43) were grown on Vero E6 cells and purified via VivaSpin columns (Sartorius Stedim Biotech). The viruses were titered on Vero E6 cells. Infection experiments were done under biosafety level 3 conditions with enhanced respiratory personal protection equipment. Of note, all cells were tested mycoplasma-negative.

Inhibitor assays

The cells were pretreated for 16 h with the indicated amounts of pan-species IFN-α(B/D) (PBL Assay Science) (44), purified recombinant IFN-λ3 (18, 45), or with 1 μM ruxolitinib (Selleckchem). Infections were performed at a MOI of 0.01 and 0.001. At the indicated times postinfection, cell supernatants were collected and titered by plaque assay on Vero E6 cells.

Immunoblot analysis

The cells were treated for 24 h with the indicated amounts of IFNs or ruxolitinib (added 1 h before IFN) and lysed in T-PER protein extraction reagent (Thermo Fisher) supplemented containing 1× Protease inhibitor mixture (complete, Roche), 1× phosphatase Inhibitor mixture set II (Calbiochem), and sample buffer (35.8 mM Tris-HCl, pH 6.8, 7.15% glycerol, 1.43% SDS, 1.08 mM bromphenol blue). Protein samples were run on 12% acrylamide gels and transferred to polyvinylidene fluoride membranes (Millipore) via semidry blotting. After blocking in TBS with 5% BSA (for detection of phospho-STATs, MxA, and total STAT2) or milk powder (all other detections), primary antibody staining was performed overnight at 4°C. The membranes were washed in TBS with 0.1% Tween 20, stained with secondary antibodies, washed again in TBS with 0.1% Tween 20, and once in TBS. Finally, the membranes were stained with secondary antibodies. Of note, all cells were tested mycoplasma-negative.

Figure 4. Effect of the JAK/STAT inhibitor ruxolitinib on SARS-CoV-2 replication. Calu-3 (A) and Vero E6 (B) cells were pretreated with 1 μM ruxolitinib (Rux) and infected with SARS-CoV-2 at an MOI of 0.01, and titers were determined at 24 and 48 h postinfection. Individual titers (dots) and geometric mean values (bars) from three biological replicates are shown. Log-transformed titers were analyzed by unpaired two-tailed Student’s t test. n.s., nonsignificant.
Statistical analyses

The statistical analysis of the data were done by means of the statistical program packages BMDP (46) and StatXact® (version 9.0.0). For the statistical testing of the dose-response effect of IFN (type I and III) against SARS–coronaviruses, the typical regression procedures were not applicable because of several values below the detection limit and some ties in the data. Instead of this, the nonparametric Spearman rank CC was used in the exact version (software StatXact). Because the scientific question was clearly one-sided formed (only PFU reduction under application of IFN), one-sided p values were given.

If only two IFN concentrations were to compare with no data below the detection limit, then the t test for independent samples was used (program BMDP3D). For testing the effect of IFN and virus type simultaneously, the two-way ANOVA (program BMDP7D) was applied especially considering a possible interaction between the two tested factors.

In the parametric statistical analyses as well as the graphical representations, the response variable PFU was logarithmically transformed because of its right skewed statistical distribution. In all cases a statistical significance level of \( \alpha = 0.05 \) was applied.

Data availability

All data presented and discussed are contained within the article.

**Author contributions**—U. F., A. S., R. H., A. R. S., K. F., C. D., and F. W. conceptualization; U. F., C. D., and F. W. validation; U. F., A. S., A. R. S., and K. F. investigation; U. F. and F. W. visualization;
References

1. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses (2020) The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat. Microbiol. 5, 536–544 CrossRef Medline

2. Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., Meng, J., Zhu, Z., Zhang, Z., Wang, J., Sheng, J., Quan, L., Xin, Z., Tan, W., Cheng, G., et al. (2020) Genome composite and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe 27, 325–328 CrossRef Medline

3. Lazear, H. M., Schoggins, J. W., and Diamond, M. S. (2019) Shared and distinct functions of type I and type III interferons. Immunity 50, 907–923 CrossRef Medline

4. Wack, A., Terczyńska-Dyla, E., and Hartmann, R. (2015) Guarding the frontiers: the biology of type III interferons. Nat. Immunol. 16, 802–809 CrossRef Medline

5. Ye, L., Schepf, D., and Staeheli, P. (2019) Interferon-λ orchestrates innate and adaptive mucosal immune responses. Nat. Rev. Immunol. 19, 614–625 CrossRef Medline

6. O’Brien, T. R., Young, H. A., Donnelly, R. P., and Prokunina-Olsson, L. (2019) Interferon lambda: disease impact and therapeutic potential. J. Interferon Cytokine Res. 39, 586–591. CrossRef Medline

7. Snell, L. M., McGaha, T. L., and Brooks, D. G. (2017) Type I interferon in chronic virus infection and cancer. Trends Immunol. 38, 542–557 CrossRef Medline

8. Chan, R. W. Y., Chan, M. C. W., Agnihotram, S., Chan, L. I. Y., Kuok, D. T. T., Fong, J. H. M., Guan, Y., Poon, L. L. M., Baric, R. S., Nicholls, J. M., and Peiris, J. S. M. (2013) Tropism of and innate immune responses to the novel human betacoronavirus lineage C virus in human ex vivo respiratory organ cultures. J. Virol. 87, 6604–6614. CrossRef Medline

9. Cinatl, J., Morgenstern, B., Bauer, G., Chandra, P., Rabenau, H., and Doerr, H. W. (2003) Treatment of SARS with human interferons. Lancet 362, 293–294 CrossRef Medline

10. Falzarano, D., de Wit, E., Martellaro, C., Callison, J., Munster, V. J., and Feldmann, H. (2013) Inhibition of novel β coronavirus replication by a combination of interferon-a2b and ribavirin. Sci. Rep. 3, 1686 CrossRef Medline

11. Kindler, E., Jonsdottir, H. R., Muth, D., Hamming, O. J., Hartmann, R., Rodriguez, R., Gefers, R., Fouchier, R. A., Drosten, C., Muller, M. A., Dijkman, R., and Thiel, V. (2013) Efficient replication of the novel human beta-coronavirus EMC on primary human epithelium highlights its zoonotic potential. mBio 4, e00611-12 CrossRef Medline

12. Kindler, E., Thiel, V., and Weber, F. (2016) Interaction of SARS and MERS coronaviruses with the antiviral interferon response. Adv. Virus Res. 96, 219–243 CrossRef Medline

13. Spiegel, M., Pichlmair, A., Mühlberger, E., Haller, O., and Weber, F. (2004) The antiviral effect of interferon-beta against SARS-coronavirus is not mediated by MxA protein. J. Clin. Virol. 30, 211–213 CrossRef Medline

14. Stroher, U., DiCaro, A., Li, Y., Strong, J. E., Aoki, F., Plummer, F., Jones, S. M., and Feldmann, H. (2004) Severe acute respiratory syndrome coronavirus is inhibited by interferon-alpha. J. Infect. Dis. 189, 1164–1167 CrossRef Medline

15. Zielecki, F., Weber, M., Eickmann, M., Spiegelberg, L., Zaki, A. M., Matrosovich, M., Becker, M., and Weber, F. (2013) Human cell tropism and innate immune system interactions of human respiratory coronavirus EMC compared to those of severe acute respiratory syndrome coronavirus. J. Virol. 87, 5300–5304 CrossRef Medline

16. Hulswit, R. J. G., de Haan, C. A. M., and Bosch, B. J. (2016) Coronavirus spike protein and tropism changes. Adv. Virus Res. 96, 29–57 CrossRef Medline

17. Pervolaraki, K., Rastgou Talemi, S. R., Albrecht, D., Bormann, F., Bamford, C., Mendoza, J. L., Garcia, K. C., McLauchlan, J., Höfer, T., Stanifer, M. L., and Boulant, S. (2018) Differential induction of interferon stimulated genes between type I and type III interferons is independent of interferon receptor abundance. PLoS Pathog. 14, e1007420 CrossRef Medline

18. Hamming, O. J., Terczyńska-Dyla, E., Vieyres, G., Dijkman, R., Jørgensen, S. E., Akhtar, H., Supka, P., Pietschmann, T., Thiel, V., and Hartmann, R. (2013) Interferon lambda 4 signals via the IFN λ receptor. Adv. Virus Res 80, 267–297 CrossRef Medline

19. Mordstein, M., Neugebauer, E., Ditt, V., Jessen, B., Rieger, T., Falcone, V., Sorgeloos, F., Ehl, S., Mayer, D., Kochs, C., Schwemmel, M., Günther, S., Drosten, C., Michiels, T., and Staeheli, P. (2010) Lambda interferon renders epithelial cells of the respiratory and gastrointestinal tracts resistant to viral infections. J. Virol. 84, 5670–5677. CrossRef Medline

20. Prokunina-Olsson, L., Alphonse, N., Dickenson, R. E., Durbin, J. E., Glenn, J. S., Hartmann, R., Kotenko, S. V., Lazear, H. M., O’Brien, T. R., Odendall, C., Onabajo, O. O., Piontkivska, H., Santer, D. M., Reich, N. C., Wack, A., et al. (2020) COVID-19 and emerging viral infections: The case for interferon lambda 1. J. Exp. Med. 217, e20200653 CrossRef Medline

21. Gordon, D. E., Jang, G. M., Bouhaddou, M., Xu, J., Obernier, K., White, K. M., O’Meara, M. J., Rezelj, V. V., Guo, J. Z., Swaney, D. L., Tummino, J. A., Huettenhain, R., Kaake, R. M., Richards, A. L., Tutucuoglu, B., et al. (2020) A SARS–CoV-2 protein interaction map reveals targets for drug repurposing. Nature, in press Medline

22. Davis, M. I., Hunt, J. P., Herrgard, S., Ciceri, P., Wodicka, L. M., Pallares, G., Hocker, M., Treiber, D. K., and Zarrinkar, P. P. (2011) Comprehensive analysis of kinase inhibitor selectivity. Nat. Biotechnol. 29, 1046–1051 CrossRef Medline

23. Stoltz, M., and Klingström, J. (2010) Alpha/Beta interferon (IFN-alpha/ beta)–independent induction of IFN-lambda 1 (interleukin-29) in response to Hantaan virus infection. J. Virol. 84, 5790–5797. CrossRef Medline

24. Fried, M. W., Shiffman, M. L., Reddy, K. R., Smith, C., Marinós, G., Gonzáles, F. L., Jr., Häussinger, D., Diago, M., Carosi, G., Dumeaux, D., Craxi, A., Lin, A., Hoffman, J., and Yu, J. (2002) Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N. Engl. J. Med. 347, 975–982 CrossRef Medline

25. Muir, A. J., Arora, S., Everson, G., Flišák, R., George, J., Ghalib, R., Gordon, S. C., Gray, T., Greenboom, S., Hassanein, T., Hillson, J., Horga, M. A., Jacobson, I. M., Jeffers, L., Kowdley, K. V., et al. (2014) A randomized phase 2b study of peginterferon lambda-1a for the treatment of chronic HCV infection. J. Hepatol. 61, 1238–1246 CrossRef Medline
ACCELERATED COMMUNICATION: SARS–CoV-2 and antiviral interferons

26. Lokugamage, K. G., Hage, A., Schindewolf, C., Rajksbaum, R., and Menghery, V. D. (2020) SARS–CoV-2 is sensitive to type I interferon pretreatment. bioRxiv CrossRef
27. Mantlo, E. K., Bukreyeva, N., Maruyama, J., Paessler, S., and Huang, C. (2020) Potent antiviral activities of type I interferons to SARS–CoV-2 infection. bioRxiv CrossRef
28. Stanifer, M. L., Kee, C., Cortese, M., Triana, S., Mukhenhirn, M., Kraeußlich, H.-G., Bartschenger, T., and Boullant, S. (2020) Critical role of type III interferon in controlling SARS–CoV-2 infection, replication and spread in primary human intestinal epithelial cells. bioRxiv CrossRef
29. Channappanavar, R., Fehr, A. R., Vijay, R., Mack, M., Zhao, J., Meyerholz, D. K., and Perlman, S. (2016) Dysregulated type I interferon and inflammatory monocyte–macrophage responses cause lethal pneumonia in SARS–CoV-2–infected mice. Cell Host Microbe 19, 181–193 CrossRef Medline
30. Frieman, M. B., Chen, J., Morrison, T. E., Whitmore, A., Funkhouser, W., Ward, J. M., Lamirande, E. W., Roberts, A., Heise, M., Subbarao, K., and Baric, R. S. (2010) SARS–CoV pathogenesis is regulated by a STAT1 dependent but a type I, II and III interferon receptor independent mechanism. PLoS Pathog 6, e1000849 CrossRef Medline
31. Haagmans, B. L., Kuiken, T., Martin, B. E., Fouche, R. A., Rimmelzwaan, G. F., van Amerongen, G., van Riel, D., de Jong, T., Murakami, S., Chan, K. H., Tashiro, M., and Osterhaus, A. D. (2004) Pegylated interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. Nat. Med. 10, 290–293 CrossRef Medline
32. Mahlakõiv, T., Ritz, D., Mordstein, M., DeDiego, M. L., Enjuanes, L., Müller, M. A., Drosten, C., and Staelens, P. (2012) Combined action of type I and type III interferon restricts initial replication of severe acute respiratory syndrome coronavirus in the lung but fails to inhibit systemic virus spread. J. Gen. Virol. 93, 2601–2605 CrossRef Medline
33. Mordstein, M., Kochs, G., Dumoutier, L., Renaud, J. C., Paludan, S. R., Klucher, K., and Staelens, P. (2008) Interferon-lambda contributes to innate immunity of mice against influenza A virus but not against hepatotropic viruses. PLoS Pathog 4, e1000151 CrossRef Medline
34. Arabi, Y. M., Shalhoub, S., Mandourah, Y., Al-Hameed, F., Al-Omari, A., Al Qasim, E., Jose, J., Alraddadi, B., Almotairi, A., Al Khatib, K., Abdulmomen, A., Qusmaa, I., Sindi, A. A., Mady, A., Solaiman, O., et al. (2020) Ribavirin and interferon therapy for critically ill patients with middle east respiratory syndrome: a multicenter observational study. Clin. Infect. Dis. 70, 1837–1844 CrossRef Medline
35. Loutfy, M. R., Blatt, L. M., Siminovich, K. A., Ward, S., Wolff, B., Lho, H., Pham, D. H., Deif, H., LaMere, E. A., Chang, M., Kain, K. C., Farcas, G. A., Ferguson, P., Latchford, M., Levy, G., et al. (2003) Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. JAMA 290, 3222–3228 CrossRef Medline
36. Omrani, A. S., Saad, M. M., Baig, K., Bahlool, A., Abdul-Matin, M., Aladipooros, A. Y., Almakhlaifi, G. A., Albarakka, M. M., Memish, Z. A., and Albarakka, A. M. (2014) Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study. Lancet Infect. Dis. 14, 1090–1095 CrossRef Medline
37. Strayer, D. R., Dickey, R., and Carter, W. A. (2014) Sensitivity of SARS/ MERS CoV to interferons and other drugs based on achievable serum concentrations in humans. Infect. Disord. Drug Targets 14, 37–43 CrossRef Medline
38. Stockman, L. J., Bellamy, R., and Garner, P. (2006) SARS: systematic review of treatment effects. PloS Med. 3, e343 CrossRef Medline
39. Davidson, S., Maini, M. K., and Wack, A. (2015) Disease-promoting effects of type I interferons in viral, bacterial, and coinfections. J. Interferon Cytokine Res. 35, 252–264 CrossRef Medline
40. Rothe, C., Schunk, M., Sörmann, P., Bretzel, G., Froeschl, G., Wallrauch, C., Zimmer, T., Thiel, V., Janke, C., Guggemos, W., Selmaier, M., Drosten, C., Vollmar, P., Zwirglmaier, K., Zange, S., et al. (2020) Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N. Engl. J. Med. 382, 970–971 CrossRef Medline
41. Rehle, C., Schunk, M., Sörmann, P., Brezel, G., Froeschl, G., Wallrauch, C., Zimmer, T., Thiel, V., Janke, C., Guggemos, W., Selmaier, M., Drosten, C., Vollmar, P., Zwirglmaier, K., and Selmaier, M. (2020) Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N. Engl. J. Med. 382, 970–971 CrossRef Medline
42. Horisberger, M. A., and de Staritzky, K. (1987) Identification of a novel coronavirus in patients with severe acute respiratory syndrome. J. Infect. Dis. 157, 945–970 CrossRef Medline
43. Ferguson, N. M., Lenton, M., Leung, G., Ng, C., and Ng, Y. (2020) Antiviral interferons to SARS–CoV-2 infection: a retrospective cohort study. BioRxiv 2020.03.11.037920. CrossRef Medline
44. Al Qasim, E., Jose, J., Alraddadi, B., Almotairi, A., Al Khatib, K., Abdulmomen, A., Qusmaa, I., Sindi, A. A., Mady, A., Solaiman, O., et al. (2020) Dysregulated type I interferon and inflammation of type III interferon in controlling SARS–CoV-2 infection. bioRxiv 2020.03.11.037920. CrossRef Medline