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SARS-CoV-2 Omicron variant virus isolates are highly sensitive to interferon treatment

Denisa Bojkova¹, Tamara Rothenburger¹, Sandra Ciesek^{1,2,3}, Mark N. Wass⁴✉, Martin Michaelis⁴✉ and Jindrich Cinatl Jr^{1,5}✉

Dear Editor,

The SARS-CoV-2 Omicron variant (B.1.1.529) causes less severe disease than previous SARS-CoV-2 variants, although immune protection provided by vaccinations and previous infections is reduced against Omicron compared to previous variants¹. In agreement, evidence is emerging that Omicron is inherently less pathogenic than previous SARS-CoV-2 variants. Omicron variant viruses cause less severe disease in animal studies² and appear to display a lower capacity than other variants to replicate in the lower respiratory tract². Additionally, initial clinical data indicated that the Omicron variant causes less severe disease than previous SARS-CoV-2 variants in unvaccinated individuals¹.

We have most recently shown that Omicron variant viruses are less effective at antagonizing the host cell interferon response than Delta variant viruses³, which provides a mechanistic explanation for the reduced clinical severity of Omicron disease in individuals without pre-existing adaptive immunity¹. Omicron virus replication was attenuated relative to Delta virus in interferon-competent Caco-2 and Calu-3 cells, but not in interferon-deficient Vero cells, and Omicron viruses caused enhanced interferon promoter activity compared to Delta viruses³. Additionally, depletion of the pattern recognition receptor MDA5, which plays a critical role in SARS-CoV-2 detection and interferon response initiation⁴, resulted in increased Omicron virus replication in interferon-competent cells³.

The exact molecular reasons for the alleviated interferon response antagonism by Omicron viruses remain to be elucidated. Notably, the Omicron and Delta virus isolates that we investigated (see Supplementary Information) display sequence variants in the viral interferon antagonists nsp3, nsp12, nsp13, nsp14, the membrane (M) protein, the nucleocapsid protein, and ORF3a⁵ (Supplementary Table S1), which may be of relevance.

Here we further show that two SARS-CoV-2 Omicron isolates (Omicron 1, Omicron 2) replicate to lower titers (Fig. 1a) and induce elevated STAT1 phosphorylation (Fig. 1b), a key event during interferon signaling, compared to a Delta isolate (B.1.167.2) in Caco-2 and Calu-3 cells³ (see Supplementary Information).

In A549 cells transduced with ACE2 (SARS-CoV-2 receptor) and TMPRSS2 (mediates SARS-CoV-2 cell entry by cleaving and activating the viral spike protein), the Omicron viruses also displayed alleviated infection capacity compared to the Delta virus (Fig. 1c). This difference largely disappeared upon depletion of either of the pattern recognition receptors MDA5 and RIG-I, both of which mediate the host cell interferon response in virus-infected cells⁶. However, when we compared interferon activity in the supernatants of SARS-CoV-2-infected cells in a HEK-Blue IFN α / β reporter cell assay, the supernatants of Omicron virus-infected RIG-I-knock out cells induced higher interferon promoter activation than the supernatants of Omicron virus-infected MDA5-knock out cells (Fig. 1d). This is in agreement with previous data showing that MDA5 is primarily responsible for virus recognition and the induction of an interferon response in SARS-CoV-2-infected cells^{4,6,7}.

Taken together, these findings further confirm that Omicron viruses are less effective than Delta viruses in antagonizing the host cell interferon response³ and that MDA5 is a major player in SARS-CoV-2 recognition^{4,6}.

Correspondence: Mark N. Wass (M.N.Wass@kent.ac.uk) or Martin Michaelis (M.Michaelis@kent.ac.uk) or Jindrich Cinatl Jr (Cinatl@em.uni-frankfurt.de)

¹Institute for Medical Virology, University Hospital, Goethe University, Frankfurt am Main, Germany

²German Center for Infection Research, DZIF, External partner site, Frankfurt am Main, Germany

Full list of author information is available at the end of the article

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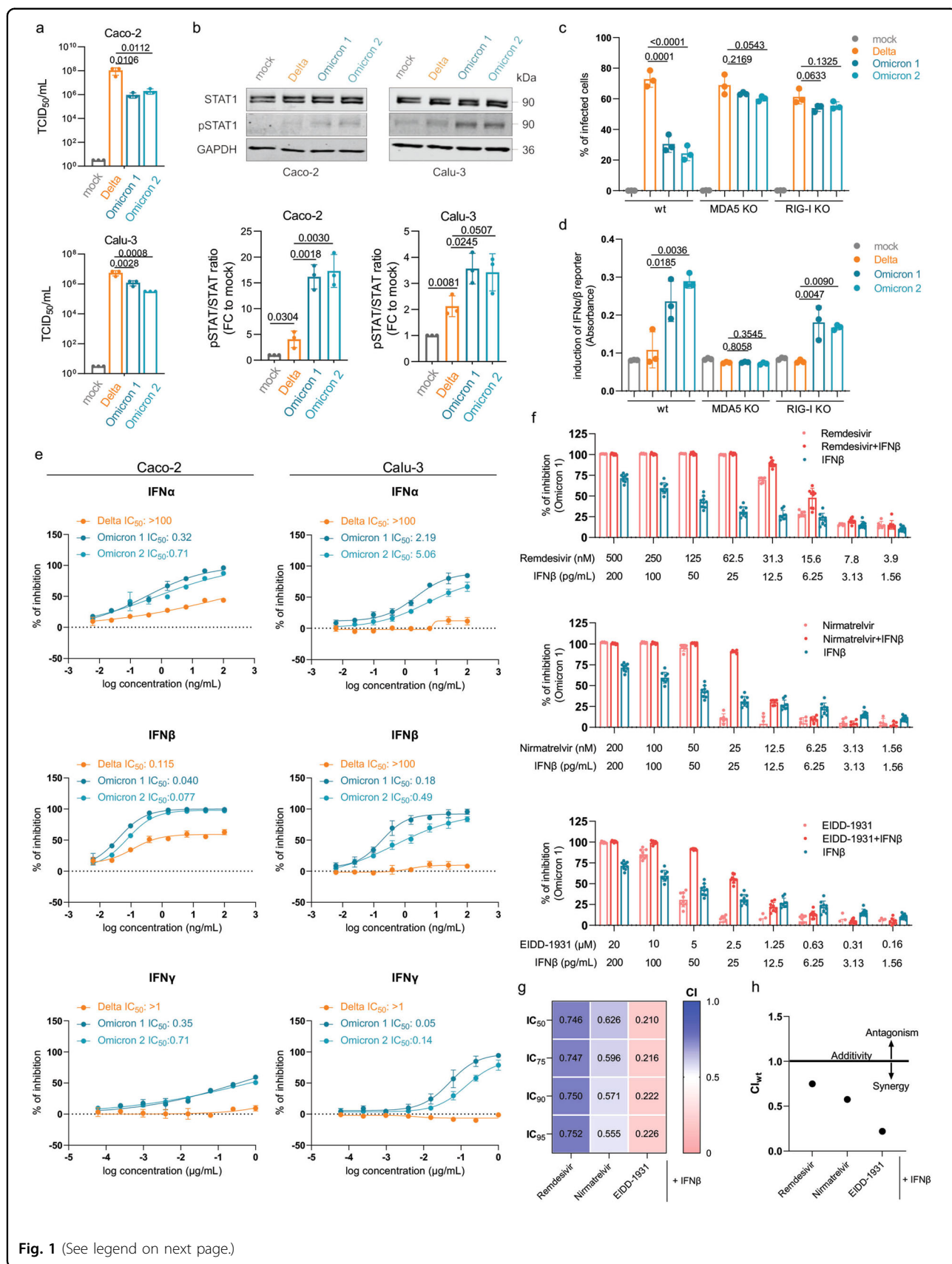


Fig. 1 (See legend on next page.)

(see figure on previous page)

Fig. 1 IFN signaling and therapy during infection with novel SARS-CoV-2 variant Omicron. **a** Caco-2 and Calu-3 cells were infected with SARS-CoV-2 variant Delta (GenBank ID: MZ315141), Omicron 1 (GenBank ID: OL800702), and Omicron 2 (GenBank ID: OL800703) at an MOI of 1. The infectious titer was determined 24 h post infection. Graphs represent means \pm SD of three biological replicates. Statistically significant differences were identified by one-way ANOVA and subsequent Dunnett's test. **b** Immunoblot analysis of total and phosphorylated STAT1 in SARS-CoV-2-infected Caco-2 (left panel) and Calu-3 (right panel) cells 24 h post infection. The protein levels were quantified by ImageJ. Graphs represent means \pm SD of three biological replicates. *P* values were calculated using Student's *t* test. **c** A549-ACE2/TMPRSS2 MDA5/RIG-I-WT (wt), A549-ACE2/TMPRSS2 MDA5 KO (MDA5 KO), and A549-ACE2/TMPRSS2 RIG-I KO (RIG-I KO) cells were infected with Delta, Omicron 1, and Omicron 2 variants at an MOI of 0.01 for 72 h. The number of infected cells was determined by immunofluorescence staining. Graphs represent data of four biological replicates. Statistically significant differences were identified by one-way ANOVA and subsequent Dunnett's test. **d** Production of IFN α / β was measured by incubating supernatants from wt, MDA5-KO, and RIG-I KO cells infected with SARS-CoV-2 variants at an MOI of 0.01 for 48 h using HEK-Blue IFN α / β reporter cells. Graphs displays means \pm SD of three biological replicates. Statistically significant differences were identified by one-way ANOVA and subsequent Dunnett's test. **e** Dose–response curves of IFN α , IFN β , and IFN γ were determined in Caco-2 and Calu-3 cells. All IFNs were added to confluent monolayers and subsequently infected with viral variants at an MOI of 0.01. The inhibition rate was evaluated 24 h (Caco-2) and 48 h (Calu-3) post infection by staining for the spike protein. Graphs depict means \pm SD of three biological replicates. **f** Antiviral effect of IFN β in combination with nirmatrelvir, remdesivir, or EIDD-1931 in Caco-2 cells. Statistically significant differences were identified by one-way ANOVA and subsequent Dunnett's test. **g** Heatmap of combination indexes (CIs) for IC₅₀, IC₇₅, IC₉₀, and IC₉₅. **h** Dots depict weighted average (CI_{wt}) calculated according to the formula: $CI_{wt} = (CI_{50} + 2CI_{75} + 3CI_{90} + 4CI_{95})/10$. $CI_{wt} < 1$ synergism, $CI_{wt} = 1$ additive effects, $CI_{wt} > 1$ antagonism.

Accordingly, elevated MDA5 levels were detected in the upper airways of SARS-CoV-2-infected individuals with mild or asymptomatic disease⁸. Since Delta has been found to display a similar level of interferon antagonism and sensitivity as previous SARS-CoV-2 variants^{9,10}, the reduced interferon antagonism appears to be unique to Omicron.

Most notably, treatment with interferon- α , interferon- β , and interferon- γ revealed that the weaker interferon antagonism by Omicron virus isolates translates into a profoundly increased Omicron sensitivity to interferon treatment (Fig. 1e). Further experiments showed that antiviral interferon- β effects were further increased in combination with nirmatrelvir (the antivirally active agent in paxlovid), remdesivir, and EIDD-1931 (the active metabolite of molnupiravir) (Fig. 1f). Combination experiments using the Chou–Talalay approach¹¹ indicated moderate synergism of interferon- β with remdesivir, synergism with nirmatrelvir, and strong synergism with EIDD-1931 (Fig. 1g, h). So far, clinical studies reported mixed outcomes in COVID-19 patients treated with different interferons^{12–15}. Given the newly discovered substantially increased interferon sensitivity of Omicron viruses, interferons represent a promising option for the treatment of Omicron patients.

In conclusion, we present further evidence that reduced interferon-antagonizing activity explains at least in part why Omicron variant infections are inherently less severe than infections with other SARS-CoV-2 variants. Sequence variations in the SARS-CoV-2 interferon antagonists nsp3, nsp12, nsp13, nsp14, M protein, the nucleocapsid protein, and/or ORF3a may contribute to these differences. Most importantly, this study shows that Omicron variant viruses display enhanced sensitivity to interferon treatment, which makes interferons promising

therapeutic candidates for Omicron patients, in particular in combination with other antiviral agents.

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Author details

¹Institute for Medical Virology, University Hospital, Goethe University, Frankfurt am Main, Germany. ²German Center for Infection Research, DZIF, External partner site, Frankfurt am Main, Germany. ³Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Branch Translational Medicine und Pharmacology, Frankfurt am Main, Germany. ⁴School of Biosciences, University of Kent, Canterbury, UK. ⁵Dr. Petra Joh-Forschungshaus, Frankfurt am Main, Germany

Author contributions

D.B., M.M., and J.C. conceived and designed the study. D.B., T.R., M.N.W., and J.C. performed the experiments. D.B., T.R., S.C., M.N.W., M.M., and J.C. analyzed the data. M.M. wrote the manuscript. D.B., M.N.W., M.M., and J.C. revised the manuscript. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare no competing interests.

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