

6-12-2022

Influenza A virus-derived defective interfering particles for antiviral treatment

Sascha Y. Kupke

M D. Hein

Y Genzel

L Pelz

P Marichal-Gallardo

See next page for additional authors

Authors

Sascha Y. Kupke, M D. Hein, Y Genzel, L Pelz, P Marichal-Gallardo, N Bdeir, S Pöhlmann, U Rand, L Cicin-Sain, F Alnaji, C Brooke, M Winkler, L Cicin-Sain, D Bruder, K Schughart, and U Reichl

INFLUENZA A VIRUS-DERIVED DEFECTIVE INTERFERING PARTICLES FOR ANTIVIRAL TREATMENT

SY Kupke, Max Planck Institute for Dynamics of Complex Technical Systems, GER
kupke@mpi-magdeburg.mpg.de

MD Hein; Y Genzel; L Pelz; P Marichal-Gallardo; Y Genzel, Max Planck Institute for Dynamics of Complex
Technical Systems, GER

N Bdeir; S Pöhlmann, German Primate Center - Leibniz Institute for Primate Research, GER
U Rand; L Cicin-Sain, Helmholtz Centre for Infection Research, GER

F Alnaji; C Brooke; C Brooke, University of Illinois at Urbana-Champaign, USA

M Winkler, German Primate Center - Leibniz Institute for Primate Research, GER

L Cicin-Sain; D Bruder; K Schughart, Helmholtz Centre for Infection Research, GER

U Reichl, Max Planck Institute for Dynamics of Complex Technical Systems; Otto von Guericke University, GER

Key Words: Influenza A virus, antiviral, defective interfering particle, genetic engineering, SARS-CoV-2

Here, we report on genetically engineered, propagation-incompetent influenza A virus (IAV) particles, so-called defective interfering particles (DIPs) that have been suggested as a promising novel antiviral agent. Typically, IAV DIPs harbor a large internal deletion in one of their eight genomic viral RNA (vRNA) segments. Further, DIPs are capable of hijacking cellular and viral resources upon co-infection with fully infectious standard virus (STV), resulting in an antiviral effect. Besides this replication interference, DIP infection also stimulates innate immunity, adding to the antiviral efficacy.

So far, DIPs were produced in embryonated chicken eggs. To improve scalability and flexibility of processes as well as to increase product quality, we established a cell culture-based DIP production system [1,2]. This includes the development of a genetically engineered virus-cell propagation system that allows production of DIPs without the need to add infectious STV to complement missing gene functions of DIPs. Specifically, the MDCK suspension cell line generated expresses the PB2 protein [2], encoded by segment 1 (S1) of IAV, which is not expressed by "DI244" - a prototypic, well-characterized DIP harboring a deletion in S1. Using this cell culture-based production process in batch [2,3] and perfusion mode [4] at laboratory scale, we show that we can achieve very high DI244 titers of up to $2.6E+11$ DIPs/mL. Infections of mice demonstrated that intranasal administration of the produced DI244 material resulted in no apparent toxic effects and in a full rescue of mice co-treated with an otherwise lethal dose of IAV [2].

Further, we screened for DIPs with improved efficacy than DI244. For instance, we conducted semi-continuous propagation DIPs and STVs in shake flasks for 21 d [5]. Thus, DIPs were exposed to alternating selection pressures for accumulation of highly interfering DIPs. By next-generation sequencing, we identified DIPs that accumulated to high fractions toward the end of cultivation. Using reverse genetics, corresponding purely clonal candidate DIPs were reconstituted that indeed showed a superior *in vitro* interfering efficacy than DI244 [5]. In addition, we recently discovered a yet-unrecognized novel type of DIP using our platform for single-cell analysis of virus replication [6]. Instead of a large internal deletion, the novel DIP (termed "OP7") showed a significant number of nucleotide substitutions in the genomic S7 vRNA [7]. Intriguingly, OP7 showed a superior *in vitro* and *in vivo* interfering efficacy than conventional IAV DIPs, e.g. DI244 [2,8,9]. Next, we set up a high-yield cell culture-based production process for OP7 in batch mode [8]. As with DI244, administration of the produced OP7 resulted in no toxic effects and a full rescue upon lethal STV challenge in mice [8].

Finally, we recently demonstrated that IAV DIPs even exert an antiviral effect against SARS-CoV-2 replication, as shown by *in vitro* experiments using human lung cells [9]. Here, our results suggest the unspecific stimulation of innate immunity by IAV DIPs as a major contributor. Exploring the antiviral effect of IAV DIPs against yellow fever, Zika, and respiratory syncytial virus (RSV) replication is the subject of ongoing studies.

Overall, we propose IAV DIPs as an effective antiviral agent for treatment of the influenza disease and COVID-19, and potentially also for suppressing the replication of other respiratory interferon-sensitive viruses. Our current studies focus on the elucidation of the antiviral mechanism of DIPs, the construction of genetically engineered virus-cell propagation systems for production of DIPs with superior efficacy, and the development of a GMP production process for DIPs aiming toward the clinic.

[1] Bdeir et al. (2019) PLoS ONE

[2] Hein et al. (2021) BMC Biology

[3] Marichal-Gallardo et al. (2016) J Chromatogr A

[4] Hein et al. (2021) Appl Microb Biotech

[5] Pelz et al. (2021) J Virol

[6] Heldt and Kupke et al. (2015) Nature Commun

[7] Kupke et al. (2019) J Virol

[8] Hein et al. (2020) Appl Microb Biotech

[9] Rand and Kupke et al. (2021) Cells

Patents (pending):

1) EP18159908

2) EP21157812.5