INFLUENZA A VIRUS OP7 DEFECTIVE INTERFERING PARTICLES: CELL CULTURE-BASED PRODUCTION AND ANTIVIRAL EFFICACY IN VIVO

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Seasonal influenza A virus (IAV) infections constitute a serious hazard to public health. Besides vaccination and common antiviral strategies, defective interfering particles (DIPs) have been proposed as an option for prevention and treatment of disease [1,2]. Conventional DIPs (cDIPs) are naturally arising viral mutants that contain a large internal deletion in one of their eight viral RNA (vRNA) segments. Further, DIPs impede infectious standard virus (STV) propagation in a co-infection scenario. Due to the deleted protein, cDIPs are deficient in propagation. For helper virus-free and purely clonal production of cDIPs, a system based on reverse genetics and complementation of the missing protein through expression by Madin-Darby canine kidney (MDCK) suspension cells has been established [3,2]. Previously, we identified the novel DIP “OP7”. Instead of internal deletions, OP7 carries multiple point mutations within its genome [4]. As the defect is unknown, OP7 had to be produced upon coinfection with STVs for complementation (helper virus infection) in the past. To inactivate infectious STVs, UV inactivation was required; yet, that lowered the antiviral efficacy [5]. Nevertheless, OP7 exhibited an antiviral efficacy higher than for cDIPs both *in vitro* and *in vivo* [5,2].

In this study, we developed a cell culture-based propagation system for OP7 that does not require STV coinfection. To this end, we adapted the previously established reverse genetics system of cDIPs to generate a novel chimeric OP7 DIP. Here, the defective interfering (DI) vRNA of segment 1 and the seven full length vRNA-encoding plasmids were co-transfected with a ninth plasmid encoding for mutated OP7 DI vRNA. After rescue of the construct, OP7 production in shake flasks in batch mode with complete medium exchange before infection was dependent on the multiplicity of infection (MOI). Production at the optimal MOI of 1E-4 yielded in a high IAV titer of 2.6 $\log_{10}$(HAU/100 µL). For applicability in large scale production, changing to a 1:2 dilution of the medium prior to infection instead of a full medium exchange (centrifugation step) was evaluated. Surprisingly, this resulted in a strong reduction of yields probably due to inhibitory ammonium concentrations. By decreasing the temperature from 37°C to 32°C at time of infection, this effect could be mitigated and virus yields increased to 3.2 $\log_{10}$(HAU/100 µL) at a MOI of 1E-4. In a next step, this optimized batch process was transferred to a 1 L stirred tank bioreactor with slightly reduced virus yield (2.9 $\log_{10}$(HAU/100 µL)). For process intensification, a perfusion culture with an alternating tangential flow filtration (ATF) system connected to a hollow fiber membrane (polyethersulfone, 0.2 µm pore size) was established. The perfusion rate was controlled via a capacitance probe and cell infection was done at 24.9 × 10^6 cells/mL. By maintaining the cell-specific virus yield from the batch process, a very high HA titer of 4.0 $\log_{10}$(HAU/100 µL) was obtained. The OP7 material purified by steric exclusion chromatography was tested in a mouse infection model. Intranasal OP7 delivery only was well tolerated (no body weight loss, no negative effect on lung integrity and survival of all mice). Moreover, OP7 administration mediated full protection from virus-induced morbidity and mortality after lethal IAV challenge.

In sum, we present a powerful approach for manufacturing highly effective DIPs for use as IAV antivirals. Currently, a production process following good manufacturing practice standards is established for initiation of clinical studies.

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