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LONG-TERM PROPAGATION OF INFLUENZA A VIRUS AND ITS DEFECTIVE INTERFERING PARTICLES: ANALYZING DYNAMIC COMPETITION TO SELECT ANTIVIRAL CANDIDATES

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Influenza A virus (IAV) is a major threat to human health. A potential antiviral therapy against influenza disease could be the intranasal application of defective interfering particles (DIPs) [1,2]. During intracellular IAV replication, these IAV DIPs are randomly generated. They contain at least one defective interfering (DI) RNA, typically a genome segment with a large internal deletion, rendering them replication-incompetent. During co-infection with infectious standard virus (STV), DIPs impede STV replication via a growth advantage [3] and by stimulation of the innate immune response [4]. In this work, we profiled the propagation competition between a variety of DIPs for selection toward accumulation of highly interfering DIPs in Madin-Darby canine kidney (MDCK) suspension cell culture.

To that end, we propagated IAV and its DIPs over 21 days using a small-scale two-stage cultivation system. Illumina-based next-generation sequencing (NGS) in combination with a lately reported bioinformatic pipeline was utilized to detect and quantify specific deletion junctions within the virus population [5]. During long-term propagation, both the infectious and total virus titers oscillated periodically, a characteristic pattern of DIP and STV interplay [6]. NGS analysis revealed that the highest *de novo* formation and accumulation of DI vRNAs occurred on the polymerase-encoding segments. Moreover, we identified an accumulation of short DI vRNAs with an optimum fragment length underlining their replication advantage. Deletion junctions were usually situated near both vRNA ends. For efficient DI vRNA propagation, retaining the incorporation signal but not the entire bundling signal was required. Few DI vRNAs propagated to high levels toward the end of cultivation, while the level of others declined. Reverse genetics was utilized to generate purely clonal DIPs derived from DI vRNAs of segment 1 that showed the highest increase in accumulation during cultivation. For this, genetically engineered adherent and suspension MDCK cells complementing the defect in segment 1 were employed [7,2]. During co-infection with STV, these DIPs displayed a higher interfering efficacy relative to DIPs derived from less competitive DI vRNAs.

Overall, this study provides new insights into the dynamics of DIP formation and the characteristics of strongly interfering DIPs. Moreover, it enabled the identification of highly potent DIPs for effective therapy during influenza virus infection. Future investigations will comprise animal experiments to assess the antiviral effect *in vivo* and to study the therapeutic mechanisms in more detail. The platform technology applied here could also be transferred to other viruses exploring further possible roles and applications of DIPs in vaccinology.

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