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Journal of Antivirals & Antiretrovirals

Editorial

Insights of RNA Viruses

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DESCRIPTION

Recoding viral genomes by numerous synonymous but suboptimal substitutions provides live attenuated vaccine candidates. These vaccine candidates should have a low risk of deattenuation because of the many changes involved. However, their genetic stability under selective pressure is largely unknown. We evaluated phenotypic reversion of deoptimized human respiratory syncytial virus (RSV) vaccine candidates in the context of strong selective pressure. Codon pair deoptimized (CPD) versions of RSV were attenuated and temperature-sensitive. During serial passage at progressively increasing temperature, a CPD RSV containing 2,692 synonymous mutations in 9 of 11 ORFs did not lose temperature sensitivity, remained genetically stable, and was restricted at temperatures of 34°C/35°C and above. However, a CPD RSV containing 1,378 synonymous mutations solely in the polymerase L ORF quickly lost substantial attenuation. Comprehensive sequence analysis of virus populations identified many different potentially deattenuating mutations in the L ORF as well as, surprisingly, many appearing in other ORFs. Phenotypic analysis revealed that either of two competing mutations in the virus transcription antitermination factor M2-1, outside of the CPD area, substantially reversed defective transcription of the CPD L gene and substantially restored virus fitness in vitro and in case of one of these two mutations, also in vivo. Paradoxically, the introduction into Min L of one mutation each in the M2-1, N, P, and L proteins resulted in a virus with increased attenuation in vivo but increased immunogenicity. Thus, in addition to providing insights on the adaptability of genome-scale deoptimized RNA viruses, stability studies can yield improved synthetic RNA virus vaccine candidates.

Attenuated viruses have numerous applications, in particular in the context of live viral vaccines. However, purposefully designing attenuated viruses remains challenging, in particular if the attenuation is meant to be resistant to rapid evolutionary recovery. Here we develop and analyze a new attenuation method, promoter ablation, using an established viral model, bacteriophage T7. Ablation of promoters of the two most highly expressed T7 proteins (scaffold and capsid) led to major reductions in transcript abundance of the affected genes, with the effect of the double knockout approximately additive of the effects of single knockouts. Fitness reduction was moderate and also approximately additive; fitness recovery on extended adaptation was partial and did not restore the promoters. The fitness effect of promoter knockouts combined with a previously tested codon deoptimization of the capsid gene was less than additive, as anticipated from their competing mechanisms of action. In one design, the engineering created an unintended consequence that led to further attenuation, the effect of which was studied and understood in hindsight. Overall, the mechanisms and effects of genome engineering on attenuation behaved in a predictable manner. Therefore, this work suggests that the rational design of viral attenuation methods is becoming feasible.

Importance Live viral vaccines rely on attenuated viruses that can successfully infect their host but have reduced fitness or virulence. Such attenuated viruses were originally developed through trial and error, typically by adaptation of the wild-type virus to novel conditions. That method was haphazard, with no way of controlling the degree of attenuation or the number of attenuating mutations or preventing evolutionary reversion. Synthetic biology now enables rational design and engineering of viral attenuation, but rational design must be informed by biological principles to achieve stable, quantitative attenuation. This work shows that in a model system for viral attenuation, bacteriophage T7, attenuation can be obtained from rational design principles, and multiple different attenuation approaches can be combined for enhanced overall effect.

CONCLUSION

Live viral vaccines are in wide use and have been immensely effective. A classic example, the Sabin oral polio vaccine (OPV), is largely responsible for eradicating polio in the majority of the world. Most live vaccines have been developed as “attenuated” or genetically weakened versions of their wild-type counterparts. Use of attenuated vaccines has a long history, and out of necessity in an era before genetic engineering, methods of achieving attenuation were empirical, adapting the wild-type virus to novel conditions in the hope that growth was retarded in the original host. Despite many successes, this method was haphazard, often failing to attenuate or producing unstable attenuations that quickly evolved back to high virulence. The most dramatic example of vaccine reversion, that of OPV, resulted in many vaccine-derived cases of poliomyelitis and circulation of vaccine-derived polioviruses

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Received: December 08, 2020; **Accepted:** December 22, 2020; **Published:** December 29, 2020

Citation: Khan H (2020) Editorial Note for RNA viruses. J Antivir Antiretrovir. S13:e002