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Superinfection Exclusion Using Host Restricted-live Attenuated Virus In Persistently Infected Mosquitoes As An Arbovirus Control Strategy

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Emergence and re-emergence of arbovirus diseases expose more than 80% of the world's population at risk of infection by mosquito-borne arboviruses such as dengue (DENV) and chikungunya (CHIK) viruses, two medically important viruses which co-circulate. The epizootic transmission and pandemic potential of arboviruses has become exacerbated by climate change, population growth, urbanization and intercontinental travel and trade. Vacunax has created phylogenetically distinct, host restricted, Arbovirus live attenuated vaccine strains (HR-LAV) which grow efficiently in Arthropoda (insect) cells but produces replcation defective non-pathogenic particles in Chordata (mammal) cells. In addition to HR-LAV application as a traditional prophylactic medical intervention, we have developed a superinfection exclusion biocontrol strategy based on the premises that if a mosquito is infected with our HR-LAV, a secondary infection with the pathogenic virus form is reduced or prevented, breaking the transmission cycle of the second pathogenic infection. Unlike other virus mitigation strategies that alter the insect ecosystem, such as pesticides, infection with the bacterium Wolbachia, and the use of genetically modified mosquitoes, our strategy targets the virus, shifting the virus pathogenic genome in the insect forest or urban ecosystem to a non-pathogenic phenotype.

We have tested the superinfection exclusion strategy in-vitro by persistently infecting three well-characterized A. albopictus mosquito cell lines (C7 -10, C6/36, U4.4) with HR-LAV homologous strains of CHIK and DENV virus. We have compared virus yields after secondary exposure of wild type (WT) CHIK and DENV in each HR-LAV persistently infected mosquito cell line by monitoring the release of infectious WT virus into fresh culture fluid during the preceding 24 h at 24, 48, and 72 h times post-infection as measured by plaque assay and RT-qPCR. The WT CHIK and WT DENV secondary infections exhibited markedly reduced virus titers (i.e. 2 to 5 orders of magnitude) in homologous HR-LAV persistently infected mosquito cell lines compared to WT CHIK and WT DENV primary infections in naïve mosquito cells. It has been previously established that alphavirus can interfere with and establish conditions of superinfection exclusion to flavivirus in these Ae. albopictus cells lines.

In nature, arbovirus infections are persistently maintained through the interactions of the vector (mosquito) with the virus reservoir hosts through vertical and horizontal transmission mechanisms (i.e. venereal, maternal, feeding). The persistent infection and superinfection exclusion processes exhibited in tissue culture are directly reproducible in whole mosquitoes, which would suggest that persistently infected HR-LAV mosquitoes can be cultivated as a barrier to the spread of pathogenic arboviruses in the field. Advanced deployment of HR-LAV mosquitoes in war zones where these pathogens are endemic or pose risk can greatly diminish the threat to military personnel.