Title: Generating a Deep-Mutationally Scanned (DMS) CHIKV E3/E2 Virus Library to Map Virus-Antibody Interactions

Megan M. Stumpf[1], Bennett J. Davenport[1], Tonya Brunetti[1], Mary K. McCarthy[1], Thomas E. Morrison[1]

[1]Dept. of Immunology and Microbiology, University of Colorado Anschutz Medical Campus

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that can cause fever and severe, debilitating acute and chronic joint pain. There is currently no approved vaccine or specific treatment available for CHIKV. Using deep mutational-scanning (DMS) to individually substitute all amino acids at each position in the ectodomain of the E3/E2 glycoproteins of CHIKV, we can evaluate the effects of individual mutations on CHIKV cell entry, egress, and antibody-mediated neutralization. Using a novel CHIKV plasmid encoding a CMV promoter and mKate fluorescent reporter (pCHIKV-CMV-mKate), the E3/E2 ectodomain was mutagenized using NNK forward and reverse primer pools. Fragments were joined in a single joining PCR reaction and reintroduced back into a pCHIKV-CMV-mKate recipient plasmid encoding stop codons in E3 to prevent selection for WT virus. The ligation reaction was electroporated into DH10B Electromax cells and plated on LB-Amp agar plates to obtain 22,000 clones. 40 clones were miniprepped and sequenced by Sangar sequencing to evaluate mutation efficiency. We found that 36/40 (90%) clones contained a new, mutated E3/E2 insert and 32/36 (89%) contained no stop codons. The average number of mutations per productive clone (no stop codons) was 2.0 amino acid mutations/clone. The viral plasmid library was transfected into HEK293 cells to generate the initial virus library passage (p1) and then characterized for titer and mKate expression. P1 was then used to infect HEK293 cells at an MOI of 0.01 FFU/cell and the second passage (p2) harvested and characterized. Percent mKate positive cells for p1 and p2 were 39.6% and 89.7%, respectively. The plasmid library was submitted for deep sequencing using the NovaSeq 6000 platform. The average mutational frequency per codon is 8.0×10^{-3} for the plasmid library and the average number of amino acids represented at each codon is 16.4 [range = 5-21]. These results demonstrate a new method for characterizing viral mutations in an alphavirus, such as CHIKV. This comprehensive virus library can be used to characterize viral escape mutants in response to a variety of E3/E2 targeted antibody, vaccine, or drug challenges and guide decisions for therapies in clinical settings.