Dengue Type Four Viruses with E-Glu345Lys Adaptive Mutation from MRC-5 Cells Induce Low Viremia but Elicit Potent Neutralizing Antibodies in Rhesus Monkeys

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Background/Objective

Knowledge of virulence and immunogenicity is important for development of live-attenuated dengue vaccines. We previously reported that an infectious clone-derived dengue type 4 virus (DENV-4) passaged in MRC-5 cells acquired a Glu345Lys (E-E345K) substitution in the E protein domain III (E-DIII). The same cloned DENV-4 was found to yield a single E-Glu327Gly (E-E327G) mutation after passage in FRhL cells and cause the loss of immunogenicity in rhesus monkeys.

Method

Here, we used site-directed mutagenesis to generate the E-E345K and E-E327G mutants from DENV-4 and DENV-4△30 infectious clones and propagated in Vero or MRC-5 cells.

Result

The E-E345K mutations were consistently presented in viruses recovered from MRC-5 cells, but not Vero cells. Recombinant E-DIII proteins of E345K and E327G increased heparin binding correlated with the reduced infectivity by heparin treatment in cell cultures. Different from the E-E327G mutant viruses to lose the immunogencity in rhesus monkeys, the E-E345K mutant viruses were able to induce neutralizing antibodies in rhesus monkeys with an almost a 10-fold lower level of viremia as compared to the wild type virus. Monkeys immunized with the E-E345K mutant virus were completely protected with no detectable viremia after live virus challenges with the wild type DENV-4.

Conclusion

These results suggest that the E-E345K mutant virus propagated in MRC-5 cells may have potential for the use in live-attenuated DENV vaccine development.