

Thesis Title Studies on the Attenuation of Unclassed Dengue
1-4 Viruses: Biological Markers for Virulent
and Avirulent of Unclassed Viruses Serially
Passaged in Primary Kidney Cells.

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ABSTRACT

Parent strains of dengue (DEN) 1, 2, 3 and 4 serotypes isolated from human sera were passaged in tissue cultures and Toxorhynchites amboinensis mosquitoes and then serially passaged in primary kidney cells. DEN-1, DEN-2 and DEN-4 viruses were passaged in primary dog kidney (PDK) cells, while primary green monkey kidney (PGMK) cells were used for DEN-3 virus. Attempts to adapt DEN-3 virus to PDK cells failed. DEN-1 virus was serially transferred to passage 43, DEN-2 virus to passage 53, DEN-3 virus to 50 and DEN-4 virus to 60. Biological markers were studied at every ten passage levels. At the thirtieth passage, all four dengue serotypes exhibited homogenous small plaque size and failed to produce cytopathic effect in LLC-MK2 cells and were temperature sensitive. DEN-2 and DEN-4 retained ability to replicate in human peripheral blood mononuclear cells until passages 53 and 60, respectively. In contrast, DEN-1 and DEN-3 lost this property between passages 20 to 40. Increase in passage levels of DEN-1, DEN-2 and DEN-3 resulted in gradual lengthening of the mean interval to the day of death in suckling mice. DEN-1 at PDK passage 43, DEN-2 at PDK passage 53 and DEN-3 at PGMK passage 50 did not kill suckling mice following intracerebral inoculation. Passaged DEN-4 did not significantly alter in mouse neurovirulence as compared with the parent virus. Rhesus monkeys inoculated with DEN-1 PDK 43, DEN-2 PDK 53 and DEN-4 PDK 48 developed low level of viremia or no detectable viremia. All of them developed neutralizing antibody responses. The graduated change in biological properties noted provided a range of potential vaccine candidate for evaluation in human beings.

The aim of this research was to identify biological markers that could be used as indices for degrees of attenuation as far as human virulence was concerned. Scoring of biological markers of dengue passaged variants had been accomplished by observation of changes in biological characteristics under controlled laboratory conditions. Each biological marker had been analyzed in correlation with clinical trial results in man wherever possible. It was found that variants with biological properties which differed from the dominant proportion of the "wild" virus population were identified and were demonstrated to decrease virulence for man. In this study, it appeared to us that plaque size morphology was the most beneficial marker to define the appropriate level of attenuation correlating with the less of the ability to cause disease in man.