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Genetic and phenotypic characterization of sylvatic dengue virus type 4 strains

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ABSTRACT

Four serotypes of dengue virus (DENV 1–4) currently circulate between humans and domestic/peridomestic *Aedes* mosquitoes, resulting in 100 million infections per year. All four serotypes emerged, independently, from sylvatic progenitors transmitted among non-human primates by arboreal *Aedes* mosquitoes. This study investigated the genetic and phenotypic changes associated with emergence of human DENV-4 from its sylvatic ancestors. Analysis of complete genomes of 3 sylvatic and 4 human strains revealed high conservation of both the 5′- and 3′-untranslated regions but considerable divergence within the open reading frame. Additionally, the two ecotypes did not differ significantly in replication dynamics in cultured human liver (Huh-7), monkey kidney (Vero) or mosquito (C6/36) cells, although significant inter-strain variation within ecotypes was detected. These findings are in partial agreement with previous studies of DENV-2, where human strains produced a larger number of progeny than sylvatic strains in human liver cells but not in monkey or mosquito cells.

Introduction

There is growing concern about the potential emergence of new pathogens, particularly arthropod-borne viruses (arboviruses), from animal reservoirs into humans (Weaver and Reisen, 2010; Wilder-Smith and Gubler, 2008). To gain insight into this process of emergence, it is particularly instructive to study viruses that have completed the trajectory from an enzootic into a human reservoir, such as the four serotypes of dengue virus (DENV-1–4, genus *Flavivirus*, family *Flaviviridae*). These viruses originated in a sylvatic cycle between nonhuman primates, and possibly other enzootic hosts, and arboreal *Aedes* (*Ae.*) mosquitoes. Each serotype emerged independently into a human transmission cycle, wherein humans now serve as the exclusive reservoir and amplification hosts for the endemic/epidemic lineages (Vasilakis et al., 2011). In this human cycle, DENV-1,-2,-3, and -4 are transmitted by domestic and peridomestic *Aedes* mosquitoes, primarily *Ae. aegypti aegypti* and *Ae. albopictus* (Halstead, 1997; Halstead et al., 1964; Rosen et al., 1954; Sabin, 1952; Simmons et al., 1931). The human DENV cycle is presently found in nearly all urban and peri-urban environments throughout the tropics and subtropics. In recent decades, DENV transmission among humans has intensified due to increased travel, uncontrolled urbanization and lack of effective and sustainable vector control programs (Guzman et al., 2010). By current estimates, DENV infects approximately 100 million people each year in over 100 countries.

Unlike the ancestors of many other human viruses, the ancestral sylvatic cycle of DENV remains extant and has been documented in two foci: one in West Africa involving arboreal *Aedes spp.* (e.g. *Ae. furcifer, Ae. luteocephalus)* and primates including patas monkeys (*Erythrocebus patas*), African green monkeys (*Chlorocebus sabaeus*), and Guinea baboons (*Papio papio*) (Cordellier et al., 1983, Diallo et al., 2003, 2005; Hervy et al., 1984; Rodhain, 1991; Saluzzo et al., 1986a; Vasilakis et al., 2008c) and the other in peninsular Malaysia involving *Ae. niveus s. l.* and primates including cynomolgus macaques (*Macaca fascicularis*), pig-tailed macaques (*Macaca nemestrina*) and silvered leaf monkeys (*Presbytis cristata*) (Rudnick, 1986). The continued circulation of sylvatic DENV provides an opportunity to perform comparative studies to elucidate the virus attributes that promote arboviral emergence. However these sylvatic viruses also pose a considerable threat, because they may retain the capacity to re-emerge even as efforts to control circulation of human dengue intensify (Vasilakis et al., 2011), in a manner analogous to urban yellow fever.

Although sylvatic and human DENV strains show substantial genetic differences, our previous studies of DENV-2 demonstrated that such differences do not constitute an adaptive barrier to emergence into the human transmission cycle. Specifically sylvatic DENV-2 showed no detectable deficit relative to human DENV-2 in replication kinetics in

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