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Original article

Differences in the replicative capacities of clinical isolates of dengue virus in C6/36 cells and in urban populations of *Aedes aegypti* from Colombia, South America

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ABSTRACT

Dengue, the most prevalent arboviral disease worldwide, is caused by any of the four dengue virus (DENV) serotypes that co-circulate constantly in hyperendemic areas such as Medellin (Colombia), and these serotypes are transmitted by mosquitoes of the genus *Aedes*. In this study, we evaluated the replicative capacity of strains isolated in Medellin between 2003 and 2007 in C6/36 cells and in colonies of *Aedes aegypti* collected during 2010–2011 from high or low-incidence areas within the same city. The phylogenetic analysis grouped isolates according to the predominant genotypes found in the Americas, and the *in vitro* characterization showed differences in the morphological changes induced by the isolates of each of the isolated serotypes compared to the reference serotypes. *In vitro* replicative capacity studies demonstrated that genomic copy number increased at four days post-infection and that cell viability decreased significantly compared to the control for all serotypes. The largest number of genomic copies in C6/36 was produced by DENV-2, followed by DENV-1 and DENV-4; DENV-3 produced the smallest number of genomic copies and had the smallest negative effect on cell viability. Finally, differences in the *in vivo* replication of intercolonial serotypes between the Rockefeller colony and the field colonies and among the intracolonial serotypes were found. The replication of DENV-2 at 7 and 14 days in both high- and low-incidence colonies was higher than that of the other serotypes, and replication of DENV-3 in the mosquito colonies was the most stable on the days evaluated. Our results support

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the notion that replication and, possibly, DENV transmission and severity depend on many factors, including serotype and vector characteristics.

Introduction

Dengue, a disease that is endemic in many countries, is classified as the main viral disease transmitted by arthropods (arbovirosis). Although it has been reported that 50–200 million cases of dengue occur annually, [1] recent studies have suggested that this number is underestimated and that the most likely number of cases per year is approximately 400 million, of which symptomatic cases represent only 96 million, with the remainder of cases being asymptomatic.[2] In addition, the World Health Organization has estimated that in the past five decades the number of cases of dengue has increased 30-fold. Thus, it is urgent that existing control strategies be implemented and improved to achieve a significant reduction in the number of cases in the coming years.[3] These cases are distributed in at least 125 countries that are located in tropical and subtropical regions, with Southeast Asia and the Western Pacific being the regions with the highest incidence, followed by the region of the Americas (primarily Latin America).[1]

The etiological agent of this disease is the dengue virus (DENV), of which four serotypes (DENV-1 to DENV-4) belonging to the urban viral cycle have been identified.[4] This virus belongs to the family *Flaviviridae* and the genus *Flavivirus* and has a positive-sense RNA genome[5] that encodes a single polyprotein that is cleaved by proteases of cellular and viral origin, resulting in the production of three structural proteins (C, M and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3A, NS4B, NS4B and NS5).[6] Based on the variability of their genomes, specific genotypes have been identified within each serotype. Five DENV-1, six DENV-2, five DENV-3, and five DENV-4 genotypes are currently known.[7]

Colombia, a country located in the northern part of South America, is one of the Latin American countries most affected by dengue. The first epidemics of dengue in Colombia were reported in the 1950s with the presence of DENV-1, DENV-2, and DENV-4 serotypes; these serotypes have spread throughout the country during the following decades. Their re-emergence occurred in the early nineteenth century with the introduction of DENV-3. In Colombia, the disease is classified as endemic and even hyperendemic in some cities, presenting up to 150,000 cases in certain years (such as in 2010).[8]

The DENV is transmitted by the bite of infected mosquitoes of the genus *Aedes* (Diptera: Culicidae), with *Aedes aegypti* being the most important species in urban areas. Although the vector is mainly distributed in regions located below 1800 m.a.s.l., in Colombia it has been detected in regions above that altitude.[9] Since the re-emergence of serotype 3 in Colombia in 2002, the four serotypes of the DENV have been constantly co-circulating. Approximately 80% of the cases reported between 2009 and 2010 were associated with serotypes 1 and 2, and the remaining 20% were associated with serotypes 3 and 4.[8] Although there is insufficient evidence to determine the association between serotype and disease severity in Colombia, only serotype 3 has not been associated with cases of severe dengue.[8] In addition to the problem of endemicity, the recent emergence of *Aedes albopictus*, a species that is considered a more competent vector for DENV infection,[10] in urban areas as well as the recent description of different lineages of *A. aegypti*,[11] which is considered the most efficient species in the transmission of DENV, aggravate the problem in the country.

The dynamics of dengue transmission involve a complex set of factors related to the virus. These factors include its vertebrate and invertebrate hosts, which under certain spatio-temporal conditions allow the vector to transmit the virus.[12] Several approaches, both *in vitro* (using mammalian or mosquito cell lines) and *in vivo* (using mosquito colonies), have been used to evaluate factors that may positively or negatively affect viral transmission; one of these factors is viral replication.

In vitro, the C6/36 cell line, which was derived from *A. albopictus* larvae, is widely used to study the virus-cell interaction. This cell line is highly susceptible to infection by all four serotypes and is easy to grow and maintain.[13] Differences have been reported in the ability of viral serotypes to enter and replicate in C6/36 cells. For example, two polypeptides of molecular weights 80 and 67 kDa[14] and two glycoproteins of 40 and 45 kDa[15] that are present in the membranes of these cells are used by several strains of DENV-2 for their entry and subsequent replication. In contrast, several strains of DENV-3 and DENV-4 use only one laminin-binding protein of 37–67 kDa molecular weight for this process.[16] These results indicate that although the line is highly susceptible to infection, there are differences in the viral replication process dependent on the infectious serotype.

In vivo, colonies of *A. aegypti* adapted to laboratory conditions, such as the Rockefeller colony (ROCK).[17] have been used to study the replication and the transmission capacity of DENV in the vector. This transmission capacity is related to extrinsic or ecological factors, such as climatic conditions, the abundance of vertebrate hosts and vector population densities, as well as intrinsic factors, such as the genetic basis of the vector, which is also known as vector competence.[18,19] In turn, replication of the virus in a given vector is used as an indirect measure of the ability of that vector to transmit the virus. For example, it has been reported that genetic variability among mosquito populations is an important factor in susceptibility to infection and competence for DENV transmission, with differences in susceptibility to infection of mosquito populations from different geographical regions[20] and among laboratory populations of *A. aegypti* compared to field populations.[21] These facts show that the differences between *A. aegypti* populations from different countries or continents or even within the same country may be associated with differ-

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