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Continuous dengue type 1 virus genotype shifts followed by co-circulation, clade shifts and subsequent disappearance in Surabaya, Indonesia, 2008–2013

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ABSTRACT

Four serotypes of dengue virus (DENV-1 to DENV-4) and their genotypes are distributed in tropical and subtropical regions. Indonesia has been recently suggested as the origin of some dengue virus genotypes. In Surabaya, the second biggest city of Indonesia, we previously reported a shift of the predominantly circulating serotype from DENV-2 to DENV-1 in November 2008, followed by a genotype shift of DENV-1 from genotype IV (GIV) to genotype I (GI) in September 2009, based on nucleotide sequences in the envelope protein coding region. Since then, GI strains had predominantly circulated until December 2010. In this report, we investigated further DENV-1 transitions in Surabaya during 2011–2013 in order to comprehend dengue dynamics during 2008–2013 in more detail. From January 2011 through December 2011, only GIV strains were isolated, indicating that a genotype shift again took place from GI to GIV. In January 2012, GI and GIV strains started co-circulating, which continued until June 2013. To further investigate this phenomenon, analysis was performed at a clade level. GI and GIV strains isolated in Surabaya formed four and three distinct clades, respectively. Concomitant with co-circulation, new clade strains appeared in both genotypes. In contrast, some previously circulating clades were not isolated during co-circulation, indicating clade shifts. Among our Surabaya isolates, nucleotide and amino acid differences in the E region were, respectively, 1.0–2.3% and 0.2–1.0% for GI isolates and 2.0–6.3% and 0.0–1.8% for GIV isolates. Several characteristic amino acid substitutions in the envelope ectodomain were observed in some clades. After July 2013, DENV-1 strains were not isolated and were replaced with DENV-2. This study showed that continuous shifts of more than one genotype resulted in their co-circulation and subsequent disappearance and suggested the relevance of clade replacement to genotype co-circulation and disappearance in Surabaya.

Abbreviations: DENV, dengue virus; DF, dengue fever; DHF, dengue hemorrhagic fever; E, envelope; G, genotype; ADE, antibody-dependent enhancement; MAb, monoclonal antibody; NJ, neighbor-joining; MCMC, Markov Chain Monte Carlo; BEAST, Bayesian Evolutionary Analysis by Sampling Trees; TMRCA, the most recent common ancestor; HPD, highest posterior density; C, clade.

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1. Introduction

Infection with any of four types of dengue virus (generally designated serotypes; DENV-1, DENV-2, DENV-3 and DENV-4) causes globally important arthropod-borne diseases, dengue fever (DF) and dengue hemorrhagic fever (DHF) (Halstead, 2007); or dengue and severe dengue recently classified by World Health Organization (WHO, 2012). Since its primary isolation in Japan in 1943 (Kimura and Hotta, 1944; Hotta, 1952), more than 10,000 DENV strains had been registered in GenBank as of 3rd March 2014. Phylogenetic analysis of nucleotide sequences in the envelope (E) coding region demonstrated that each serotype is further divided into several genotypes (Weaver and Vasilakis, 2009). For DENV-1, there are five genotypes, genotypes I–V (GI–GV), distributed in Southeast Asia, China and the Middle East for GI; Thailand (limited to 1950s–1960s) and Indonesia (limited to 2012) for GII; Malaysia for GIII (sylvatic strain); Southeast Asia, China, the Western Pacific islands, Australia, the Indian Ocean and Central America for GIV; and the Americas, West Africa and Asia for GV (Chen and Vasilakis, 2011; Villabona-Arenas and Zanotto, 2013; Fahri et al., 2013). Recently, three genotypes, GI, GIV, and GV, have accounted for most DENV-1 infections in humans.

DENVs are maintained in a natural transmission cycle between amplifier humans and vector mosquitoes in urban areas (Weaver, 2005). Anthropophilic mosquitoes, *Aedes aegypti* and *Aedes albopictus*, are the principal and secondary vectors, respectively. Many factors are involved in transmission efficiency and thus viral dynamics in a given area (Chen and Vasilakis, 2011; Andraud et al., 2012). Population size and the density of human and mosquito hosts are principally important for contact frequency between the hosts. In addition, vector competence is a major determinant of virus transmission from mosquitoes to humans, whereas viremia levels are the most critical factor for transmission from humans to mosquitoes. The viremia level depends on levels of infection-neutralizing or -enhancing antibodies in the circulation of the human host. Specifically, neutralizing antibody suppresses viremia, while enhancing antibody (serotype cross-reactive, non- or sub-neutralizing antibody) is thought to increase viremia levels. The enhancing antibody is the cause of antibody-dependent enhancement (ADE) of infection, a hypothetical mechanism behind increased disease severity from DF to DHF (Halstead and O'Rourke, 1977). Additionally, the viremia level correlates with disease severity (Vaughn et al., 2000). It is important that neutralizing antibody may show enhancing activity at sub-neutralizing doses (Pierson et al., 2007; Rothman, 2011).

E protein is the major surface protein of DENV virions and thus the main target of neutralizing and enhancing antibodies (Heinz and Stiasny, 2012). The E protein structure consists of three domains, I, II and III (Modis et al., 2003). Domain III is believed to be responsible for attachment to host cells (Crill and Roehrig, 2001), and studies using mouse monoclonal antibodies (MAbs) indicated that most neutralizing antibodies target domain III (Shrestha et al., 2010; Sukupolvi-Petty et al., 2010; Brien et al., 2010; Sukupolvi-Petty et al., 2013). However, human antibodies binding to domain III have been shown not to play a significant role in neutralization (Wahala and Silva, 2011; Williams et al., 2012). From the aspect of serotype specificity/cross-reactivity, serotype-specific neutralizing epitopes are mainly contained in domain III, whereas other domains are relevant to cross reactive epitopes (Lai et al., 2008). On the other hand, recent studies using dengue-immune human sera reported that domains I and II also contain serotype-specific, strong neutralizing epitopes against DENV, especially in the hinge region between these domains (de Alwis et al., 2012; Messer et al., 2014).

In Indonesia, more than 100,000 cases of DF/DHF have been reported annually (Setiati et al., 2006). Currently, all four serotypes and their genotypes circulate in Indonesia (Fahri et al., 2013; Suwandono et al., 2006). In addition, Indonesia is proposed as a source of some genotypes (e.g. DENV-1 GIV, DENV-3 GI, DENV-4 GII) (Villabona-Arenas and Zanotto, 2011, 2013; Araújo et al., 2009). Previously, we reported the displacement of the circulating dengue serotype from DENV-2 to DENV-1 with a subsequent genotype shift from GIV to GI during 2008–2010 in Surabaya, the second biggest city of Indonesia (Yamanaka et al., 2011).

In the present study, we investigated further DENV transition in Surabaya during 2011–2013. Analysis was performed along with the data of 2008–2010 (Yamanaka et al., 2011) so as to comprehend the DENV dynamics in more detail during 2008–2013. In January 2011, a genotype shit of DENV-1 again took place, from GI to GIV. Interestingly, in January 2012, GI and GIV strains started co-circulating, which continued until June 2013. In clade-level analysis, co-circulation coincided with the appearance and disappearance of some clade strains in both genotypes. After July 2013, DENV-1 was not isolated and was replaced with DENV-2. The clade shift may be relevant to the co-circulation of GI and GIV and subsequent disappearance of DENV-1 in Surabaya.

2. Materials and methods

2.1. Patient samples

Blood samples were obtained in 2011–2013 from patients clinically diagnosed as DF or DHF at Dr. Soetomo Hospital and Soerya Maternal and Child Health Hospital, major medical facilities covering Surabaya, East Java, Indonesia. Collected blood was subjected to virus isolation. This study was approved by the Ethics Committees of Airlangga University (Ethics Committee Approval Number: 24-934/UN3.14/PPd/2013) as well as Kobe University Graduate School of Medicine (Ethics Committee Approval Number: 784). Blood was collected after receiving signed consent from the patients or their parents.

2.2. Virus isolation

Serum specimens diluted 1:10 with culture medium were inoculated into a 96-well plate seeded with Vero cells (Yamanaka et al., 2011). The cultures were incubated at 37 °C in 5% CO_2–95% air for seven days. After three blind passages, cells were subjected to immunostaining with flavivirus group cross-reactive MAb (D1-4G2; American Type Culture Collection, Manassas, VA) to examine the presence of viral antigens as described previously (Konishi et al., 2010).

2.3. RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from immunostaining-positive Vero cells using TRIzol Reagent (Invitrogen, Carlsbad, CA), and then RT-PCR was carried out in order to determine the virus serotype using a primer set described by Lanciotti et al. (1992). RT-PCR-positive samples were then subjected to another RT-PCR for sequence analysis using gene-specific sense primer designed in the pre-membrane protein coding region [5′-GGGAGAGTTATGTG AGG-3′; corresponding to nucleotides 559–575 of the Mochizuki strain (GenBank accession number AB074760)] and antisense primer designed in the non-structural protein 1 coding region (5′-CCCAGCTTTTCCACGAG-3′: nucleotides 2774–2758). RT-PCR products (2216 nt) were purified using illustra ExoProStar (GE Healthcare, Little Chalfont, UK) and then directly sequenced using the Big Dye v.1.1 terminator (Applied Biosystems, Foster City, CA) and ABI PRISM 310 Genetic Analyzer (Applied Biosystems).