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Original article

Dengue virus infection-enhancing antibody activities against Indonesian strains in inhabitants of central Thailand

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Abstract

Dengue virus (DENV) infection-enhancing antibodies are a hypothetic factor to increase the dengue disease severity. In this study, we investigated the enhancing antibodies against Indonesian strains of DENV-1−4 in 50 healthy inhabitants of central Thailand (Bangkok and Uthai Thani). Indonesia and Thailand have seen the highest dengue incidence in Southeast Asia. The infection history of each subject was estimated by comparing his/her neutralizing antibody titers against prototype DENV-1−4 strains. To resolve the difficulty in obtaining foreign live viruses for use as assay antigens, we used a recombinant system to prepare single-round infectious dengue viral particles based on viral sequence information. Irrespective of the previously infecting serotype(s), most serum samples showed significantly higher enhancement titers against Indonesian DENV-2 strains than against Thai DENV-2 strains, whereas the opposite effect was observed for the DENV-3 strains. Equivalent enhancing activities were observed against both DENV-1 and DENV-4. These results suggest that the genotype has an impact on enhancing antibody activities against DENV-2 and DENV-3, because the predominant circulating genotypes of each serotype differ between Indonesia and Thailand.

Keywords: Dengue; Antibody-dependent enhancement; Viral antigens; Recombinant proteins; Indonesia; Thailand

1. Introduction

Dengue fever (DF) and its more severe form, dengue hemorrhagic fever (DHF), are the most important mosquito-borne viral diseases in the world [1,2]. The annual dengue infection rate was recently estimated to be 390 million worldwide, with approximately 2.5 billion people at risk of infection [3,4]. These diseases are caused by the four serotypes of dengue virus (DENV-1 to DENV-4), which are distributed in tropical and subtropical areas [5]. DENV-1−4 belong to the genus *Flavivirus* in the family *Flaviviridae* [6]. The envelope (E) protein,

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premembrane (prM) protein, and the mature form of prM (M) are the virion surface proteins most relevant to antibody induction. These surface proteins are antigenically cross-reactive among the four serotypes [7]. Each serotype has four to six genotypes [8], and their distributions differ with continent and country [9].

Antibody-dependent enhancement (ADE) of infection is one of the hypothetical mechanisms underlying the increased disease severity from DF to DHF [10]. A secondary infection with the serotype responsible for the primary infection is protective, whereas a secondary infection with a different serotype produces 15–80 times more cases of DHF than are produced by the primary infection [11]. Therefore, a preexisting heterotypic immune factor(s) may cause DHF. Although various immune-associated factors have been proposed, their involvement is still contentious [2,12]. However, most researchers agree that higher viremia levels increase disease severity [13]. Higher viremia can be explained by the ADE hypothesis. Briefly, a preexisting non-neutralizing cross-reactive antibody (the "enhancing antibody") increases the number of infected monocytes/macrophages in an FcγR-mediated manner [14].

Epidemiological evidence supporting the ADE hypothesis has been reported in Cuba, where the first dengue epidemic (DENV-1) in 1977–1979 caused DF in patients, but the first introduction of DENV-2 in 1981 caused DHF for the first time in the Americas. The second introduction of DENV-2 in 1997 caused DHF only in patients older than 15 years [15,16]. Similar DHF outbreaks after the sequential introduction of different DENV serotypes have been reported in other countries [17,18]. However, even now, when all four serotypes are circulating in all tropical countries, increased numbers of DHF patients and/or increased total numbers of DF/DHF patients have been reported after the introduction of a new DENV genotype or clade strain [19–22]. Although the patients' pre-existing antibody status might affect the numbers of DF/DHF patients, there have been few studies of the presence in local populations of enhancing antibodies against foreign DENV strains. We previously reported that most inhabitants of the Philippines and Indonesia carry enhancing antibodies against DENV, but the enhancing antibody assays were performed with laboratory DENV strains [23].

A potential difficulty in investigating enhancing antibodies against currently circulating DENV strains is that the transport of live infectious materials and even their genes beyond national borders is increasingly limited by regulations, such as government export control policies [24] and the restrictions on access imposed by the Convention on Biological Diversity [25]. However, recombinant technologies can resolve this problem. Recently, we developed a simple, rapid system to produce single-round infectious particles (SRIPs) that carry DENV surface proteins by cotransfecting mammalian cells with a Japanese encephalitis virus (JEV) subgenomic replicon plasmid and a plasmid expressing the DENV prM and E genes [26]. The SRIPs were successfully evaluated in functional antibody assays as an alternative antigen to authentic DENVs [27].

In this study, we examined the inhabitants of central Thailand for enhancing antibodies against DENV-1–4 strains currently circulating in Indonesia. We selected Indonesia and Thailand because they are the most strongly DENV-endemic countries in Southeast Asia [28], and the probability that a domestic strain from one country will be introduced into the other is high.

2. Materials and methods

2.1. Serum samples

Serum samples were collected from healthy inhabitants aged 17–38 years (with their informed consent) in metropolitan Bangkok (28 samples) and Uthai Thani Province (22 samples), both in central Thailand. The sera were heat-inactivated at 56 °C for 30 min. The study protocol was approved by the Ethical Committee of the Faulty of Tropical Medicine, Mahidol University, Thailand.

2.2. Preparation of SRIPs

Human embryonic kidney 293T cells in six-well plates were cotransfected with 1 μg of two plasmids, as previously described [27]. pCMV–JErep-fullC contained the JEV genes encoding the whole mature capsid and all the nonstructural proteins. The other plasmid expressed DENV prM/E. Twelve plasmids containing the prM/E genes of Indonesian or Thai DENV-1–4 strains were constructed based precisely on the nucleotide sequences of full-length prM/E genes registered in GenBank (Table 1). The culture media were harvested on days 3–7 and used as SRIP antigens.

2.3. Neutralization tests

Conventional Vero-cell neutralization tests were performed with DENV-1 (Mochizuki strain), DENV-2 (New Guinea C [NGC] strain), DENV-3 (H87 strain), and DENV-4 (H241 strain), as described previously [29]. The culture media of infected Vero cells were used as the assay antigens. Neutralizing activity was expressed as the percentage reduction in the plaque number compared with the control (no antibody). The antibody dilution causing a 75% reduction in plaques was calculated with the FORECAST function in Microsoft Excel and expressed as PRNT75.

2.4. Enhancing antibody assay

The balance between neutralizing and enhancing activities was measured using semiadherent K562 human erythroleukemia cells, as described previously [30], with minor modifications. Briefly, serial 2-fold dilutions of serum samples (36 μl/well) prepared in 96-well poly-L-lysine-coated plates were mixed with the SRIP antigens in the presence of 5% rabbit complement. The infectious unit of SRIPs was adjusted to achieve a multiplicity of infection of 0.001. The SRIP–antibody mixtures were incubated at 37 °C for 2 h, mixed with K562 cells, and then incubated at 37 °C for 3 days. After fixation and immunostaining with a monoclonal antibody (JE-2D5) against the JEV nonstructural 1 protein, the number of

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