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Evaluation of single-round infectious, chimeric dengue type 1 virus as an antigen for dengue functional antibody assays

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ABSTRACT

Dengue fever and dengue hemorrhagic fever are endemic throughout tropical and subtropical countries. Four serotypes of dengue viruses (DENV-1 to DENV-4), each with several genotypes including various subclades, are co-distributed in most endemic areas. Infection–neutralizing and -enhancing antibodies are believed to play protective and pathogenic roles, respectively. Measurement of these functional antibodies against a variety of viral strains is thus important for evaluating coverage and safety of dengue vaccine candidates. Although transportation of live virus materials beyond national borders is increasingly limited, this difficulty may be overcome using biotechnology that enables generation of an antibody-assay antigen equivalent to authentic virus based on viral sequence information. A rapid system to produce flavivirus single-round infectious particles (SRIPs) was recently developed using a Japanese encephalitis virus (JEV) subgenomic replicon plasmid. This system allows production of chimeric SRIPs that have surface proteins of other flaviviruses. In the present study, SRIPs of DENV-1 (D1-SRIPs) were evaluated as an antigen for functional antibody assays. Inclusion of the whole mature capsid gene of JEV into the replicon plasmid provided higher D1-SRIP yields than did its exclusion in cases where a DENV-1 surface-protein-expressing plasmid was used for co-transfection of 293T cells with the replicon plasmid. In an assay to measure the balance between neutralizing and enhancing activities, dose (antibody dilution)-dependent activity curves in dengue-immune human sera or mouse monoclonal antibodies obtained using D1-SRIP antigen were equivalent to those obtained using DENV-1 antigen. Similar results were obtained using additional DENV-2 and DENV-3 systems. In a conventional Vero-cell neutralization test, a significant correlation was shown between antibody titers obtained using D1-SRIP and DENV-1 antigens. These results demonstrate the utility of D1-SRIPs as an alternative antigen to authentic DENV-1 in functional antibody assays. SRIP antigens may contribute to dengue vaccine candidate evaluation, understanding of dengue pathogenesis, and development of serodiagnostic systems.

1. Introduction

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are the most globally important mosquito-borne viral diseases [1,2]. The World Health Organization estimates that 50–100 million infections, including 500,000 DHF cases and approximately 12,500 deaths, occur annually [3]. Vaccines and specific antivirals are currently unavailable. DF and DHF are caused by any of four types of dengue viruses (DENVs) generally designated as serotypes DENV-1 to DENV-4. All of these serotypes are currently co-distributed in most tropical and subtropical areas worldwide [4]. Additionally, each of the four serotypes has 4–6 distinct genotypes with subclades that are locally distributed in various areas and countries [5]. Moreover, introduction of foreign DENV strains occurs in many areas, sometimes accompanied by increases in the number of patients or higher proportions of severe cases (DHF) [6–11]. Furthermore, a new DENV serotype genetically and serologically distinct from the current four serotypes has also recently been discovered [12]. Potential thus exists for human exposure to a variety of DENV strains.

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Several dengue vaccine candidates have been developed and are currently being evaluated in clinical trials [13,14]. All of these candidates are able to induce neutralizing antibody in humans. Neutralizing antibody contributes to reduction in viremia levels and is believed to be an important factor in disease protection [15]. Most neutralizing antibody species against DENVs display infection-enhancing activity in sub-neutralizing doses in vitro [16]; there is consequent concern that neutralizing antibody-inducible dengue vaccine may cause antibody-dependent enhancement (ADE) of infection if insufficient neutralizing antibody levels are induced by vaccination [17,18]. ADE, the mechanism most likely responsible for increased viremia levels, is a process in which monocytes are efficiently infected in an Fc gamma receptor (FcγR)-mediated manner [19]. Measurement of vaccine-induced neutralizing and enhancing antibodies is therefore important for dengue vaccine evaluation. Because of their potential human infectivity, various DENV strains are required as antigens in the antibody assays.

Despite these requirements, transportation of live virus materials beyond national borders is increasingly limited owing to current regulations, such as governmental security export control policies [20] as well as access and benefit-sharing restrictions of the Convention on Biological Diversity [21]. These limitations may be overcome using biotechnology that enables the generation of material equivalent to the authentic virus based on viral full-genome nucleotide sequence information. Although the technique to construct an infectious clone of DENV has already been established [22–25], the method is arduous and thus not practical for preparation of various antigens for antibody assays.

A novel system to generate flavivirus single-round infectious particles (SRIPs) has recently been established [26]. This method exploits a Japanese encephalitis virus (JEV) subgenomic replicon plasmid lacking coding regions of capsid (C), pre-membrane (prM), and envelope (E) structural proteins. SRIPs are produced by co-transfection of this replicon plasmid with a plasmid expressing JEV structural proteins into 293T cells. As a DNA-based production system, this method facilitates simple and rapid antigen generation. Most importantly, chimeric SRIPs have also been produced using a plasmid expressing structural proteins of other flaviviruses (e.g., dengue, yellow fever, and tick-borne encephalitis viruses), although production levels of chimeric SRIPs derived from DENVs have been much lower than those derived from JEV and other flaviviruses. The successful production of flavivirus SRIPs in this system suggests its potential utility for functional antibody assays, as SRIP surface antigens can be theoretically designed based on prM and E coding region nucleotide sequences.

The purpose of the present study was to evaluate the utility of DENV-1 SRIPs (D1-SRIPs) as an antigen for neutralizing and enhancing antibody assays. Results of assays using dengue-immune human serum samples or mouse monoclonal antibodies (MAbs) against DENV-1 demonstrated that antibody levels obtained using D1-SRIP antigen were equivalent to those obtained using authentic DENV-1 antigen. These results indicate that D1-SRIPs can serve as an alternative functional antibody assay antigen to DENV-1. The use of DENV antigens in the form of SRIPs for neutralizing and enhancing antibody assays may thus be suitable for dengue vaccine candidate evaluation.

2. Materials and methods

2.1. Cells

Human embryonic kidney 293T cells (CRL-3216; American Type Culture Collection [ATCC], Manassas, VA) were cultivated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Vero, C6/36, and K562 cells and their culture media have been described previously [27]. All cells were cultivated in a humidified atmosphere of 5% CO_2–95% air at 37 °C, except for C6/36 cells, which were cultivated at 28 °C.

2.2. Viruses

The Mochizuki strain of DENV-1, New Guinea C (NGC) strain of DENV-2, and H87 strain of DENV-3 were used [28]. Culture fluids harvested from infected C6/36 cells were used as viral antigens in neutralization tests and in an assay to measure the balance between neutralizing and enhancing antibodies.

2.3. Antibodies

Human serum samples previously collected from general patients aged 29–71 years in Indonesia during 1999–2001 and stored at −20 °C [29] were used as antibody specimens for evaluating SRIP antigen. Each of these sera had detectable neutralizing antibody titers against all four DENV serotypes (Supplementary Table 1). As a negative control, we used a human serum sample collected from a residence in a non-dengue-endemic country (Japan) that showed no detectable neutralizing activities against any DENV serotypes. Heat inactivation of sera was performed at 56 °C for 30 min. The use of human serum samples was approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University. MAbs specific for DENV-1 (D1-IV-7F4) or crossreactive to all DENV serotypes (D1-III-9B1, D1-IV-3B8, D1-V-3H12, D1-V-8E8 [30], and JE-10B4 [31]) and D1-4G2 (E-specific, flavivirus group-crossreactive; HB-112, ATCC) in an ascites form were also used for evaluating SRIP antigen. Mouse MAb JE-2D5, specific for JEV non-structural protein 1 (NS1) [32], was used for immunostaining.

2.4. Plasmids

Plasmids pCMV-JErep, pCAG-JEC [26], pcD1ME, pcD2ME, and pcD3ME [28] have been described previously (Fig. 1A). Briefly, pCMV-JErep is a JEV replicon plasmid designed to transcribe viral RNA in transfected cells, and is the full genome of JEV Nakayama strain (GenBank no. EF571853) except lacking 2238 nucleotides (positions 150–2387) corresponding to main portions of C and E and the entire prM gene. pCAG-JEC is an expression plasmid for JEV (Nakayama) mature C, consisting of 105 amino acids, while pcD1ME, pcD2ME, and pcD3ME are expression plasmids for prM and E of DENV-1 (Mochizuki), DENV-2 (NGC), and DENV-3 (H87), respectively. In this study, a portion of C in pCMV-JErep was replaced by the full JEV mature-C region to construct the new replicon plasmid pCMV-JErep-fullC, which was consequently lacking 1971 nucleotides (positions 438–2408) corresponding to a portion of C not responsible for synthesis of mature C, the full prM and a major portion of E (Fig. 1A).

2.5. Preparation of SRIPs

293T cells in a 6-well-plate well were co-transfected with 1 μg each of two plasmids, pCMV-JErep-fullC and pcD1ME (Set II of Fig. 1A), using Lipofectamine LTX and Plus reagent (Invitrogen, Gaithersburg, MD) following the manufacturer's instructions. Culture fluids harvested on days 3–7 served as D1-SRIP antigen in neutralization tests using Vero cells and in an assay to measure the balance between neutralizing and enhancing antibodies using K562 cells. For titration of D1-SRIPs on K562 cells, serial dilutions of D1-SRIPs (50 μl/well) prepared in 96-well poly-L-lysine-coated plates were mixed with 5×10^4 semi-adherent K562 cells (50 μl/well). The mixture was incubated at 37 °C for 2 days, followed by fixation and immunochemical staining (see below). Infective titers