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Expression of enhancing-activity-free neutralizing antibody against dengue type 1 virus in plasmid-inoculated mice

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ABSTRACT

Background: Most candidate dengue vaccines currently under development induce neutralizing antibodies, which are considered important for immunoprotection. However, the concomitant induction of infection-enhancing antibodies is an unavoidable concern. In contrast, a neutralizing antibody developed for passive immunotherapy has been engineered to eliminate its enhancing activity. Therefore, a strategy for the long-term expression of enhancing-activity-free neutralizing antibodies may resolve this concern.
Methods: A mouse monoclonal antibody, 7F4, of the IgG3 subclass and with no detectable enhancing activity, was selected as the model neutralizing antibody to evaluate the potential of this strategy. Equal amounts of commercial vector (pFUSE)-based plasmids containing 7F4 heavy (H)- or light (L)-chain variable region genes were mixed and used for the cotransfection of 293T cells and co-delivery into ICR and BALB/c mice. The recombinant plasmids were designed to express IgG2b or IgG3 subclass antibodies (p7F4G2b or p7F4G3, respectively).
Results: 293T cells transfected with 2 μg of p7F4G2b or p7F4G3 produced approximately 15,000 or 800 ng/ml IgG in the culture fluids, respectively. The dose is expressed as the total amount of H- and L-chain plasmids. Neutralizing antibody was detected dose-dependently in ICR mice inoculated with 50–200 μg of p7F4G2b. A 1:2 dilution of sera from ICR and BALB/c mice inoculated with 100 μg of p7F4G3 showed average plaque reduction levels of >70% on day 3 and >90% on days 5–9. BALB/c mice maintained detectable neutralizing antibody for at least 3 months. The neutralizing antibody expressed by p7F4G3 in mice showed no enhancing activity.
Discussion: Although the expression of neutralizing antibodies from immunoglobulin genes is a type of passive immunization, its durability can be utilized as a dengue vaccine strategy. This "proof-of-concept" study using a mouse model demonstrates that the enhancing-activity-free characteristic of this strategy augurs well for dengue vaccine development, although further improvement is required.

1. Introduction

Dengue fever and dengue hemorrhagic fever are global threats that must be resolved urgently [1,2]. These diseases are caused by four serotypes of dengue virus (DENV-1 to DENV-4) [3], and vaccination is a cost-effective measure for controlling dengue diseases [4,5]. Currently, six advanced-stage vaccine strategies have been developed and evaluated in clinical trials [6,7]. These candidate vaccines successfully induce neutralizing antibodies in the recipients. Although the immunological correlate for protection has not yet been determined, neutralizing antibodies are considered the most important protective factor, effectively reducing viremia in infected individuals [7,8], and viremia is generally accepted to determine disease severity [9].

One obstacle hindering the development of a dengue vaccine is the infection enhancement phenomenon, characteristic of DENV infections [10,11]. Enhancing antibodies are the most probable factor increasing viremia, as proposed in the antibody-dependent

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infection enhancement theory [12]. Enhancing antibodies are non-neutralizing, cross-reactive antibodies induced by the primary infection with a single viral serotype, and increase the number of infected monocytes/macrophages in an Fc-gamma receptor (FcγR)-mediated manner during secondary infection with a different serotype. Because most monoclonal antibodies (MAb) with neutralizing activity also show enhancing activity at subneutralizing concentrations [13], there is concern that vaccines that induce neutralizing antibodies will also induce enhancing antibodies [10,11].

The polyclonal antibody status of the circulation includes many antibody species. Some display neutralizing activity and others enhancing activity. The currently developed assay systems that use FcγR-expressing or -bearing cells to measure the balance between neutralizing and enhancing antibodies usually estimate lower neutralizing activities than conventional neutralization tests in Vero cells [14–16]. This may explain the protective efficacy (approximately 60%) of a dengue tetravalent chimeric candidate vaccine in an efficacy trial, even though it induced antibodies against all serotypes in Vero cell neutralization tests [17–19]: the concomitant induction of enhancing antibodies may reduce a vaccine's capacity to induce protective antibodies. Therefore, an ideal dengue vaccine should only induce neutralizing antibodies.

The neutralizing antibodies developed for passive immunotherapeutic use have been engineered to eliminate their enhancing activity [20–22]. The positions and amino acid sequences responsible for binding to FcγR have been identified [23,24]. However, the effective period in which passively transferred neutralizing antibodies persist is too short to protect against natural infections. Recently, a technique was developed to deliver the genes responsible for antibody expression into the host, allowing the long-term production of detectable antibodies [25,26]. Therefore, a theoretical solution to the induction of enhancing antibodies by the current neutralizing-antibody-inducing dengue vaccines is the long-term expression of neutralizing antibodies without enhancing activity.

We previously generated various monoclonal antibodies (MAbs) in mice immunized with the Mochizuki strain of DENV-1 [27]. Among these, the 7F4 antibody, an IgG3 subclass antibody, showed high neutralizing activity but no detectable enhancing activity. Specifically, 50% plaque reduction was achieved at approximately 10 ng/ml 7F4 in a conventional neutralization test using Vero cells, whereas no enhancing activity was seen, even at low concentrations (0.01–1 ng/ml) in our enhancing antibody assay system using FcγR-bearing cells, such as K562, U937, or HL-60 cells [16]. The target epitope of 7F4 is located in domain II of the envelope (E) protein of DENV-1 [27].

In this study, we used 7F4 as the model antibody to evaluate neutralizing antibody expression in mice. Mice were co-inoculated with plasmids containing the 7F4 heavy (H)- or light (L)-chain variable region gene and their serum neutralizing antibody levels were monitored. Mice co-inoculated with 100 μg of plasmids produced detectable serum neutralizing antibody 3 days after inoculation, which was maintained for at least 3 months. This rapid and long-term appearance of the expressed antibody in mouse sera suggests a potential antibody expression strategy against dengue, using a neutralizing antibody with no enhancing activity.

2. Materials and methods

2.1. Antibodies

MAb 7F4, generated in a mouse immunized with the DENV-1 Mochizuki strain, has been described [27].

2.2. Plasmids

Antibody expression vector (pFUSE)-based plasmids containing the gene encoding the variable region of MAb 7F4 and the constant region of the H chain (IgG2b or IgG3) or L chain (κ) were constructed previously [27] (Fig. 1A). Here, a mixture of equal amounts of plasmids expressing the H (IgG2b or IgG3) and L chains was designated 'p7F4G2b' or 'p7F4G3′, respectively. p7F4G3 plasmids in which the original signal sequence gene derived from the 7F4 hybridoma was replaced with that of interleukin 2 (IL2ss) were constructed for both the H- and L-chain-expressing plasmids (the mixture was designated 'p7F4-IL2ss'; Fig. 1A), using the KOD-Plus-Mutagenesis Kit (Toyobo, Osaka, Japan). The nucleotide sequence of the IL2ss gene was 5′-ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTT GTCACGAATTCG-3′. Both the H- and L-chain genes contained in p7F4G3 were cloned into a single plasmid vector (pIRES; Clontech, Mountain View, CA) using the *Nhe*I/*Mlu*I sites downstream from the cytomegalovirus promotor and the *Xba*I/*Not*I sites downstream from the internal ribosome entry site in the order: H- and L-chain genes (pIRES7F4HL) or L- and H-chain genes (pIRES7F4LH; Fig. 1A). The proper insertion of the variable and/or constant region genes was confirmed with sequencing.

2.3. In vitro expression

Human embryonic kidney 293T cells [28] in six-well plates were cotransfected with 2 μg of pFUSE-based plasmids containing the H- or L-chain gene (1 μg each) or the pIRES-based plasmid, using Lipofectamine® LTX with PLUS™ Reagent (Invitrogen, Gaithersburg, MD), according to the manufacturer's instructions. After the cells were incubated at 37 °C for 1–5 days, the culture fluids were harvested to measure the antibody yields.

2.4. Mouse experiment

Groups of 10–12 6-week-old male ICR or BALB/c mice (25–30 g bodyweight; National Laboratory Animal Center, Mahidol University, Bangkok, Thailand) were given a single dose (50–200 μg: 0.5–2 mg/ml) of p7F4G2b or p7F4G3 by intratibial inoculation with an electroporator (NEPA21; Nepa Gene, Chiba, Japan) with a poring pulse (voltage, 100 V; pulse interval, 50 ms; pulse length, 30 ms; number of pulses, 3; 10% decay rate with + polarity) and a transfer pulse (voltage, 20 V; pulse interval, 50 ms; pulse length, 50 ms; number of pulses, 5; 40% decay rate with ± polarity). The dose refers to the total amount of H- and L-chain plasmids. Retroorbital blood was collected from each mouse 3 days before inoculation and 3, 5, 7, and 9 days after inoculation. The mice were bled at half-monthly intervals for 3 months. Sera from the ICR mice were isolated from the blood and examined individually in antibody assays. Sera from the BALB/c mice were pooled before the assay, unless otherwise stated. All animal experiments were conducted according to the Guidelines for Animal Experimentation at the Faculty of Tropical Medicine of Mahidol University and the Research Institute for Microbial Diseases of Osaka University.

2.5. Enzyme-linked immunosorbent assay (ELISA) for antibody against DENV-1

Antibody levels in mouse sera were measured with a conventional ELISA, essentially as previously described [29]. Briefly, microplates sensitized with rabbit hyperimmune serum against DENV-1 were incubated sequentially with the corresponding DENV-1 antigen, mouse serum samples diluted 1:30, alkaline-phosphatase-conjugated anti-mouse IgG, and *p*-nitrophenyl phosphate.

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