

NOTES

Antibodies Induced by Dengue Virus Type 1 and 2 Envelope Domain III Recombinant Proteins in Monkeys Neutralize Strains with Different Genotypes[∇]

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In the present work, we evaluated the neutralizing capacity of the antibodies induced by dengue virus type 1 and 2 envelope domain III recombinant proteins in monkeys against strains of different dengue virus type 1 and 2 genotypes. Here we demonstrated that dengue virus type 1 and 2 recombinant proteins induced high titers of neutralizing antibodies against different genotype strains.

An effective humoral immune response is critical for protecting against dengue viruses (DEN) (12–14) and is the essential goal of recombinant subunit vaccines based on envelope (E) domain III. Domain III is thought to mediate interactions between the virus and cellular receptors involved in virus attachment (6, 21). In addition, many of the most potent anti-DEN neutralizing monoclonal antibodies characterized to date recognize this domain (8, 9, 24).

There is some evidence that the antibody response to a DEN genotype does not necessarily neutralize homogenotypic DEN strains. In fact, sera from patients infected with DEN type 2 (DEN 2) or DEN 3 show variations in the neutralizing antibody responses against strains isolated early and late during the same epidemic (1, 26). Preclinical studies have exposed that the primary immune responses induced after infection of mice and monkeys with DEN 2 strains belonging to both Asian and American genotypes show differences in the responses of neutralizing antibodies against the same and different strains of infection (4, 5). Based on monoclonal antibody data, changes of specific amino acids in domain III result in the loss of binding of neutralizing monoclonal antibodies (11, 19, 24). A recent study has also demonstrated that monoclonal antibodies show differentiated neutralizing activities depending on the virulence of the strain (7). Based on the previously reported evidence (1, 4, 5, 7, 11, 19, 24, 26), the humoral immune response induced by a vaccine candidate should be evaluated against strains of different genotypes of each serotype.

Previously, we have reported that recombinant proteins containing domain III of DEN 1 or DEN 2 E proteins fused to the

P64k protein from *Neisseria meningitidis* (PD10 and PD5, respectively) induce neutralizing antibodies and partial protection in immunized monkeys (3, 10). In the context of P64k, amino acid changes in E domain III included in the fusion protein have been involved in the antigenicity and immunogenicity of the resultant molecules in the mouse model (28). In the present work, we evaluate the neutralizing antibody activity in sera collected from *Macaca fascicularis* monkeys immunized with such recombinant proteins against strains of different genotypes.

Sera from *Macaca fascicularis* monkeys previously immunized with DEN 1 or DEN 2 recombinant fusion proteins were evaluated by a plaque reduction neutralization test (PRNT) against DEN 1 or DEN 2 strains belonging to different genotypes of the corresponding serotype (Table 1) (17, 23). The E domain III used for the PD10 or PD5 genetic construction belongs to strain DEN 1 Jamaica or DEN 2 Jamaica, respectively (27). In brief, monkeys were immunized subcutaneously with four doses of PD10 (two animals, 100 µg/dose) or PD5 (three animals, 50 µg/dose) in Freund solution as an adjuvant. Sera were collected 15 days after the last immunization dose (3, 10). Sera collected 60 days after DEN 1 (two monkeys, 10⁶ PFU of the Jamaica strain) or DEN 2 (five monkeys, 10⁴ to 10⁵ PFU of the A15 strain) virus inoculation were also evaluated (3, 4, 10). PRNT was performed with BHK-21 cells as described by Morens et al. with some modifications (2, 20). The serum dilution that resulted in a 50% reduction in the plaque count, as determined by probit analysis, was considered the neutralizing antibody titer. Monkeys were maintained in accordance with Cuban guidelines for the care and use of laboratory animals.

Sera from monkeys immunized with PD10 showed high geometric mean titers (GMT) of neutralizing antibodies against DEN 1 strains of the genotypes studied (IV and V) (Fig. 1).

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TABLE 1. Characteristic of the DEN strains used in this study

DEN serotype	Strain	Genotype ^a	Place, yr of isolation
1	Jamaica ^b	V	Jamaica, 1977
	West Pacific	IV	Nauru, 1974
2	A15	Asian	Cuba, 1981
	Jamaica ^b	Asian	Jamaica, 1982
	I348600	American	Colombia, 1986

^a Genotypes were considered according to the classifications of Laille and Roche (17) and Rico-Hesse (23) for DEN 1 and 2, respectively.

^b DEN 1 and 2 strains used for PD10 and PD5 genetic constructions, respectively (26).

Similarly, the antibodies induced in monkeys infected with DEN 1 neutralized strains of both DEN 1 genotypes. The GMT of neutralizing antibodies in sera from DEN 1-infected monkeys was slightly higher to the DEN 1 Jamaica strain (genotype V), which was the infecting strain, than to the genotype IV strain. However, sera from animals immunized with PD10 showed GMT of neutralizing antibodies to strain DEN 1 West Pacific (genotype IV) sevenfold higher than those to strain DEN 1 Jamaica (genotype V), even when E domain III of PD10 construction belonged to the latter. The DEN 1 E domains III of genotypes IV and V differ by a substitution of Thr for Ile at position 359 in the genotype V strain (17). Thr-359 is a conserved residue among serotypes 1, 2, and 3. DEN 2 Thr-359-Ile mutants have resulted in marked reductions in the binding affinity of monoclonal antibodies (24). It has also been postulated that the specificity of the E domain III epitopes is associated with variations in charge or hydrophobicity at the surface of this domain (25). The substitution of

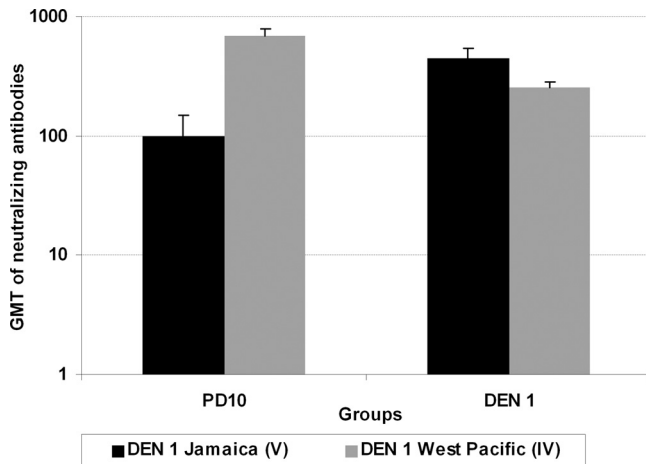


FIG. 1. Titers of neutralizing antibody against DEN 1 strains in sera collected from monkeys immunized with PD10 or inoculated with DEN 1. Monkeys immunized with PD10 ($n = 2$) received four doses of 100 μ g of the recombinant protein, and sera were collected 15 days after the last immunization. Monkeys inoculated with DEN 1 ($n = 2$) received one dose of 10^6 PFU of the strain Jamaica, and sera were collected 60 days later. Titers of neutralizing antibody against DEN 1 strains West Pacific (genotype IV) and Jamaica (genotype V) were evaluated by PRNT in BHK-21 cells and estimated as the higher serum dilution that reduced the number of plaques by 50%. Data represent the reciprocals of the GMT of neutralizing antibodies \pm standard deviations per group.

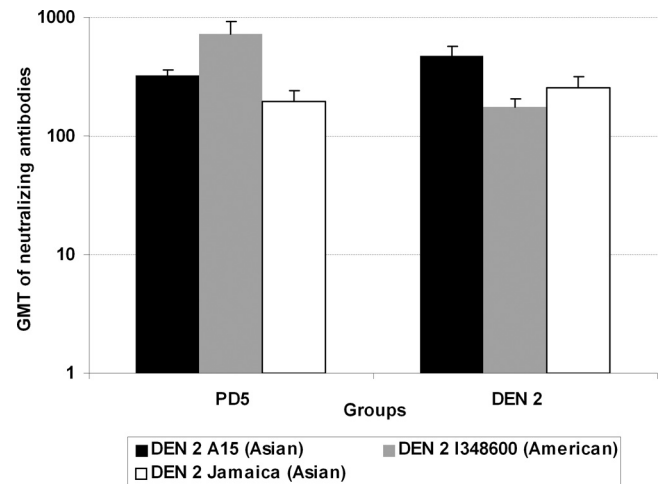


FIG. 2. Titers of neutralizing antibody against DEN 2 strains in sera collected from monkeys immunized with PD5 or inoculated with DEN 2 virus. Monkeys immunized with PD5 ($n = 3$) received four doses of 50 μ g of this recombinant protein, and sera were collected 15 days after the last immunization. Monkeys inoculated with DEN 2 virus ($n = 5$) received one dose of 10^4 to 10^5 PFU of strain A15, and sera were collected 60 days later. Titers of neutralizing antibody against strains DEN 2 A15 (Asian genotype), Jamaica (Asian genotype), and I348600 (American genotype) were evaluated by PRNT in BHK-21 cells and estimated as the higher serum dilution that reduced the number of plaques by 50%. Data represent the reciprocals of the GMT of neutralizing antibodies \pm standard deviations per group.

Thr-359 in DEN 1 genotype IV for Ile-359 in genotype V represents a change from a polar amino acid to a nonpolar one that decreases the binding affinity of neutralizing antibodies directed to epitopes containing this residue.

On the other hand, sera from monkeys immunized with PD5 recombinant protein neutralized the strains of Asian and American DEN 2 genotypes (Fig. 2). Monkeys inoculated with DEN 2 also showed neutralizing antibodies to both genotypes. In this case, the titers of neutralizing antibody to strain DEN 2 A15, which was used for inoculating the animals, were higher than those to the Jamaica and I348600 strains. However, the GMT of neutralizing antibodies in sera from monkeys immunized with PD5 protein were fourfold higher to the American-genotype strain than to the Asian strain DEN 2 Jamaica, which was used for the genetic construction of PD5.

Previous studies of humans and monkeys have shown that DEN 2 American-genotype strains are neutralized more effectively than Asian strains by homologous and heterologous immunization, which has been associated with the lower pathogenicity of the American strains during secondary heterotypic infections (15, 16). There is an amino acid change at position 390 of envelope domain III from Asp in the Asian genotype to Asn in the American genotype. The E-390 residue has a major importance since it is located in a highly hydrophilic region at the outer lateral surface of the E dimer, which has been suggested to contain residues that determine virulence in different flaviviruses (18, 22). It is known that the charge of the residues is significant in the interaction of antigenic sites with antibodies (25). Even when Asp and Asn amino acids have the same hydrophilicity value, perhaps a change from

neutral Asp (Asian genotype) to acidic Asn (American genotype) increases antibody-mediated virus neutralization.

In spite of the fact that some strains appeared to be better neutralized than the vaccine-homologous strain, the results indicated that neutralizing antibodies were elicited against different genotypes. With regard to the crucial role of neutralizing antibodies as the primary correlate of protection for DEN viruses, a broad neutralizing response is desirable for any vaccine candidate. The present results consistently demonstrate the capacity of PD5 and PD10 recombinant proteins to induce such a response for serotypes 1 and 2.

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