Can we design a subunit vaccine against dengue that will work against all serotypes?

Cecilia P. Mikita[1] and Eduardo A. Padlan*[2]

[1]Walter Reed National Military Medical Center, Department of Medicine,
Allergy/Immunology/Immunizations Service
4954 North Palmer Road, Bethesda, MD 20889-5600, USA
[2]4006 Simms Drive, Kensington, MD 20895-1336, USA

Dengue virus infection is one of the most common mosquito-borne illnesses worldwide, with nearly four billion people living in at risk areas throughout the world. Reported cases to World Health Organization continue to rise exponentially with an estimated 50 – 100 million symptomatic cases, mostly in Asia. Most clinical cases present with a self-limited viral illness, but some experience life-threatening severe disease. Four serotypes of dengue virus exist; infection by one serotype confers long lasting homotypic immunity, but does not confer heterotypic immunity to another serotype. Subsequent infection from a different dengue serotype can lead to the development of severe dengue. Antibody-dependent enhancement of dengue virus infection has been implicated in the development of severe disease. There have been various efforts to develop vaccines against dengue, including subunit vaccines that use only the viral envelope glycoprotein, or portions thereof. Those efforts are hampered by the existence of the different serotypes which could result in antibody-dependent enhancement, if the antibodies raised by the vaccine are cross-reactive, but do not neutralize all serotypes. We analyzed the structures and sequences of the viral envelope glycoprotein of the dengue serotypes and have located a putative epitope that is shared by the various serotypes. Judicious amino acid replacements to enhance the antigenicity of this epitope relative to the rest of the molecule could produce a possible universal subunit vaccine against dengue without antibody-dependent enhancement. We also explored the possibility that such a vaccine might also work against other flaviviruses, specifically Zika and West Nile viruses.

*Corresponding author
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INTRODUCTION

Dengue is a significant global health problem affecting more than 120 countries worldwide, and over half of the world's population is at risk (Halstead 1970, Halstead and O'Rourke 1977, Halstead 1979, Halstead 1988, Halstead 2003, Bhatt et al. 2013). Approximately 5% of dengue clinical cases develop severe dengue and a small percentage of cases are fatal. Dengue virus is a single-stranded RNA flavivirus with four known infectious serotypes, but patients do not develop long-term cross-protective immunity after infection with other serotypes. After a secondary heterotypic infection, there is a broadly neutralizing antibody response, and subsequent dengue infection is usually not severe.

Severe dengue is associated with a secondary heterotypic infection, younger age, longer duration between infections, and certain serotypes (Halstead 1970, Halstead and O'Rourke 1977, Halstead 1979, Halstead 1988, Halstead 2003, Bhatt et al. 2013, Guzmán et al. 2002, Alvarez et al. 2006). Antibody-dependent enhancement (ADE) of infection has been hypothesized (Halstead and O'Rourke 1977) as a mechanism to explain severe dengue disease in the course of a secondary infection and in infants with primary infections. In this model, ADE is caused by the presence of non-neutralizing, cross-reactive antibodies that had been produced during the first infection, or acquired passively at birth (Halstead 1979).

Among the various mechanisms proposed for the existence of ADE is the presence of common epitopes on the envelope glycoprotein (ENV) of the different virus serotypes, that are away from the epitope(s) of neutralizing antibodies and which

elicit non-neutralizing antibodies (Halstead and O'Rourke 1977, Halstead 1988, Halstead 2003). In an earlier study (Mikita and Padlan 2016), we did a structure-sequence analysis and were able to show the existence of such epitopes. We have continued our analysis and here we show the existence of a putative epitope that for the most part is common to all four serotypes. We believe the location of this putative epitope could help in designing a subunit vaccine that would be effective against all four serotypes.

Analysis of the structure and variation of the envelope glycoprotein of dengue virus

X-ray crystallographic structures of the ENV of the different serotypes and of antibody-ENV complexes are available in the Protein Data Bank (PDB). ENV sequences of the different serotypes can be downloaded from the National Center for Biotechnology Information (NCBI) database. Prior to infection, ENV exists as a dimer (Figure 1) on the surface of the virus, as shown by electron-microscopy (Kuhn et al. 2002). We will concern ourselves only with dimeric ENV structures.

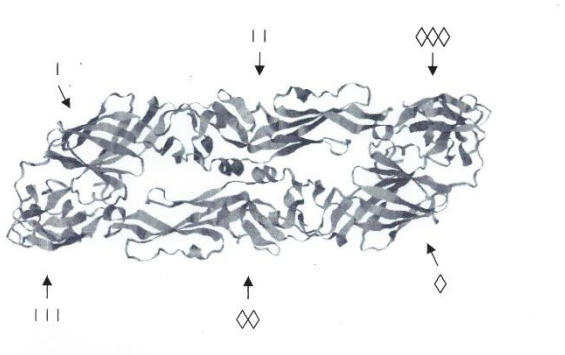

Figure 1: A ribbon diagram of the ENV dimer in PDB Entry 1OAN is shown with the three domains indicated in the two molecules constituting the dimer. In the dimer, the two molecules are side-by-side in an anti-parallel fashion. The domains are comprised by residues: 1-51, 134-192, and 282-295 (domain I); 52-133 and 193-281 (domain II); and 296-394 (domain III). (Adapted from FIG. 1 of Modis et al. 2003)

There is no known structure for the whole dimeric ENV. The best available structures extend only to residue 394 in the case of serotype 2, or to 392 in the case of serotype 3, to which we have limited our analysis. The high-resolution dimeric ENV structures that we analyzed are those for serotype 2 (identified by its PDB Entry code 1OAN (Modis et al. 2003)) and for serotype 3 (PDB Entry 1UZG (Modis et al. 2005)). The dimeric ENV structure for serotype 4 (PDB Entry 3UAJ (Cockburn et al. 2012b)) is missing several residues and will not be considered here. No dimeric ENV structure is available for serotype 1 at the time of this writing.

Each monomer in dimeric ENV consists of three domains, referred to as domains I, II and III (Figure 1). The two ENV monomers in the structure are in close association. Domain III, which consists of residues 296-394 (Modis et al. 2003), is believed to contain the receptor-binding region of the molecule (Crill and Roehrig 2001) and subunit vaccines containing only domain III are under development. Structures of complexes of domain III from the different serotypes with neutralizing antibodies have been studied by X-ray crystallography (Midgley et al. 2012, Cockburn et al. 2012a, Austin et al. 2012, Lok et al. 2008) and are available in the PDB. As identified by their PDB codes, those X-ray structures are: 3UZQ and 4AL8 (serotype 1), 3UZX (serotype 2), 3UZE and 4ALA (serotype 3), and 3UYP and 4AM0 (serotype 4) [Midgley et al. 2012 for 4ALA, 4AL8 and 4AM0; Cockburn et al. 2012a for 3UYP, 3UZE, 3UZQ and 3UZX].

The use of the isolated domain III in vaccines could be impaired by the close association of the two ENV molecules in the dimer. Indeed, the epitope of a neutralizing antibody against an isolated domain III has been found to include residues that are normally buried between the two monomers, leading to the suggestion that a conformational change in the ENV structure must accompany antibody binding (Midgley et al. 2012).

ENV sequences for the four dengue serotypes were retrieved from the NCBI database. Only those sequences in which all 495 residues, or 493 in the case of serotype 3, were identified were included in the comparisons; those which had insertions or deletions were excluded. The total number of ENV sequences compared was 3,900 for serotype 1, 3,450 for serotype 2, 2,101 for serotype 3, and 969 for serotype 4. There has been a reported serotype 5 (Normille 2013), but the six sequences that we retrieved for serotype 5 were identical and differ from a representative serotype 4 ENV sequence at only 22 positions; those differences do not affect our conclusions and no further mention of this serotype will be made here.

Calculation of antigenicities and identification of putative epitopes

The method developed by one of us (Padlan 2008) was used for the characterization of putative epitopes on ENV. Briefly, the method identifies all the residues within a certain radius of each alpha-carbon position in a molecule and assigns an antigenicity value for that location based on the summed reactivities of those residues weighted by their exposure to solvent. The reactivities that we used were those based on the values compiled by De Gentz et al. (2002). We calculated the exposures to solvent by the method of Connolly (1983).

The close association of the two monomers in the ENV dimer (Figure 1) could result in the involvement of some residues in one monomer with an epitope centered in the other. We therefore chose to base our calculations on dimeric ENV instead of an isolated monomer, or fragment thereof. Using the 1OAN dimer (serotype 2) as the basis of our antigenicity calculations, we calculated the antigenicities of putative epitopes centered at all the alpha-carbon positions in the dimer. Since domain III is believed to contain the receptor-binding site of the molecule and would be a good target for neutralizing antibodies, we concentrated our attention on putative epitopes centered in this domain.

In view of the great variety of antigenic structures and of antibody combining sites, the exact extent and shape of epitopes cannot be guessed. We could, nonetheless, make estimates from available structures of antibody-antigen complexes. There are several structures in PDB of serotype-specific, neutralizing antibodies bound to domain III of the ENV of the four serotypes and we examined those. The size (simply defined here as the radius of the circle that encloses all the residues) of the epitopes in those complexes are: 13.9 A (Angstrom units) in 3UZQ, 12.8 A in 4AL8, 10.8 A in 3UZV, 14.2 A in 3UZE, 13.1 A in 4ALA, 14.1 A in 3UYP, and 12.1 A in 4AM0. In our calculations, we chose a radius of 17 A, significantly larger than the extent of any of these observed epitopes, as the size of our putative epitopes in order to include as many potentially contributing epitope residues as possible.

Table 2: For the dengue ENV, the most common amino acid residue observed at a particular position is shown with the residue symbol; only the residues that constitute 10% or more of the total number at that position are shown. The number of sequences that were compared is 3,900 for serotype 1, 3,450 for serotype 2, 2,101 for serotype 3, and 969 for serotype 4. The sequence numbering is for serotypes 1, 2, and 4; that for serotype 3 is two less after position 156. At position 8 in serotype 1, S represents 60.2% of the residues observed at that position, N represents 39.1%. At position 339 in serotype 1, T represents 86.1% of the residues observed at that position, S represents 10.8%. At position 35 in serotype 4, T represents 65.7% of the residues observed at that position, V represents 34.3%.

The solvent accessibilities of the individual residues were calculated using the computer algorithms developed by Connolly (1983); those algorithms are incorporated in the program DSSP (Kabsch and Sander 1983) (accessible, for example, in http://bioweb.pasteur.fr/seqanal/interfaces/dssp-simple.html). The fractional solvent accessibilities given here were estimated by dividing the solvent accessibility obtained from DSSP by the total surface area of the amino acid (obtained from http://prowl.rockefeller.edu/aainfo/volume.htm).

The most common amino acids at the residue positions in the His317 epitope

Position	Serotype				Accessibility		
	1	2	3	4	(in 1OAN dimer)	Zika	WNV
2	R3855	R3319	R1960	R 946	0.27	R	N
6	I3863	I3293	V1945	V 942	0.26	V	M
7	G3870	S3317	G1958	G 947	0.57	S	S
8	S2349/N1523	N3321	N1960	N 943	0.66	N	N
10	D3867	D3316	D1960	D 945	0.58	D	D
11	F3876	F3320	F1960	F 947	0.39	F	F
12	V3876	V3322	V1960	V 947	0.50	V	L
13	E3878	E3324	E1960	E 947	0.60	E	E
29	S3878	S3409	G1960	G 929	0.20	G	S
31	V3862	V3408	V1960	V 948	0.08	V	V
34	M3874	M3410	M1959	M 939	0.27	M	M
312	V3667	I3445	V2099	M 969	0.23	P	P
314	E3899	E3444	E2099	E 969	0.39	E	D
316	Q3898	Q3448	Q2101	Q 969	0.28	L	G
317	H3892	H3447	H2101	H 969	0.36	H	H
339	T3359/S420	I3447	T2101	I 968	0.09	M	S
350	R3899	R3445	R2100	R 969	0.42	R	R
351	L3794	L3447	L2097	I 637/V 332	0.38	L	L
370	E3898	E3447	E2098	E 968	0.35	D	E
372	P3899	P3449	P2101	P 969	0.37	P	P
373	F3846	F3443	F2101	F 966	0.71	F	F
375	E3900	D3448	E2101	D 967	0.54	D	D
391	W3899	W3445	W2101	W 968	0.28	W	W
392	F3896	F3357	Y2098	F 968	0.67	H	H
393	K3891	K3437	K2065	R 965	0.18	R	K
394	K3761	K3441	K2096	K 961	0.74	S	S
Contributions from the other molecule in the dimer:							
75	P3878	P3413	P1963	P 949	0.26	P	P
97	V3879	V3419	V1960	V 951	0.19	V	V
98	D3880	D3413	D1962	D 951	0.25	D	D
99	R3897	R3416	R1963	R 952	0.23	R	R
101	W3896	W3417	W1963	W 951	0.07	W	W
107	L3896	L3413	L1963	L 952	0.90	L	L
110	K3899	K3417	K1962	K 952	0.43	K	K
244	H3897	H3444	H2099	H 955	0.60	H	H
245	A3899	A3443	A2096	A 954	0.80	A	A

Comparison of sequences and identification of a common epitope

For the four serotypes, we identified the sequence with the most commonly occurring residue at each position, as well as the frequency of all the residues occurring at that position. The sequences of the four serotypes are sufficiently similar so that they are readily aligned. This allowed us to readily identify the putative epitopes with residues that are the most common among the four serotypes.

We found that the putative epitope centered at His317 in domain III of the 1OAN dimer contains the greatest number of residues that are most common among the four serotypes (Table 1). Of the 34 solvent-accessible (with fractional accessibility values greater than 0.00) residues in the His317 epitope, 26 of the most common residues are identical in the four serotypes, 6 are structurally very similar (V vs I at position 6, V vs I vs M at position 312, L vs I/V at position 351, I vs T/S at position 339, E vs D at position 375, F vs Y at position 392, and K vs R at position 393), and only 2 are structurally dissimilar (S vs G at positions 7 and 29). The dissimilarity at some locations may be weighted down by the low accessibility to solvent, e.g., the residues at position 339 (only 9% exposed to solvent in the 1OAN dimer). This high degree of overall similarity leads us to predict that an antibody directed against the His317 epitope of

one serotype will probably bind to the corresponding epitope in the other serotypes.

Design of a subunit vaccine that could be effective against all dengue serotypes

Antibody epitopes are quite irregular in shape and extent, so that almost certainly there are some residues within the 17 A circle that we have chosen as the extent of the putative His317 epitope, which might not interact with antibody at all. Nevertheless, our results show the existence of a putative epitope in the four serotypes that is the same, or is very similar, in all the residues within 17 A of the alpha-carbon of His317.

A subunit vaccine that directs antibodies to this epitope could be designed using a variety of methods (Fazekas de St. Groth 1977, Jones et al. 1991, Shafferman et al. 1991, Scheelinck et al. 1991, Temoltzin-Palacios and Thomas 1994, Garrity et al. 1997, Cleveland et al. 2000, Selvarajah et al. 2008, Tobin et al. 2008, Padlan 2010). Of particular use is the method proposed by one of us (Padlan 2010) that aims to lower the antigenicity of all but the His317 epitope while maintaining the three-dimensional structure of the molecule. This would enhance the antigenicity of the His317 epitope relative to the rest of the molecule and could produce a possible universal subunit vaccine against dengue. The reduction in antigenicity of the rest of the molecule would also result in de-emphasizing the epitope that probably causes antibody-dependent enhancement Mikita and Padlan 2016).

Various dengue vaccines are under development (see, for example, Durbin and Whitehead 2010). Of these, the one that was developed by Sanofi Pasteur (commercial name Dengvaxia), an attenuated tetravalent vaccine, has passed Phase 3 of clinical trials (Guy et al. 2010) and has been licensed for use in several countries, although limited for use to individuals between 6 and 45 years of age. Potential problems with Dengvaxia has been predicted (Halstead 2017) and, indeed, the use of Dengvaxia in the Philippines is blamed for numerous deaths among children (Arkin 2019). The Dengvaxia fiasco in the Philippines may be due to the difficulty of detecting prior dengue infection that may have mild symptoms.

Our suggestion to develop a dengue vaccine that focuses the antibody response to an epitope common to all four serotypes, while reducing, or conceivably even completely eliminating, the possibility of antibody-dependent enhancement, should be a good one.

Could the vaccine also work against other flaviviruses?

We explored the possibility that a subunit vaccine design proposed above might also work against other flaviviruses, specifically Zika and West Nile viruses.

X-ray crystallographic structures of Zika and West Nile virus ENV have been deposited in PDB as of the time of this writing. However, all of the structures available for the Zika virus ENV had missing residues. Further, all of the crystallographic structures available for the West Nile virus ENV were of relatively low resolution. Instead, we chose simply to use the results of our analysis of the structure of dengue 2 ENV (PDB Entry 1OAN).

We retrieved ENV sequences for Zika and West Nile viruses from the NCBI database. Only those sequences, in which all the 403 residues that correspond to residues 1-394 in dengue 2 ENV were identified, were included in the comparisons; those that had insertions or deletions were excluded. The total number of Zika virus ENV sequences compared was 469, of which 378 were identical to GenBank Entry ANT96596. The total number of West Nile virus ENV sequences compared was 2702, of which 1732 were found to be identical to GenBank Entry ABJ90103. In our analysis, the GenBank entry ANT96596 was chosen to represent the Zika virus ENV and GenBank entry ABJ90103 to represent the West Nile virus ENV. We aligned these sequences with that of GenBank entry ABD17402, which we have found to be among the most common dengue 2 ENV sequences in NCBI. The alignment was done using the NCBI Standard Protein BLAST program.

We have added the most common Zika and West Nile ENV sequences to Table 1. Indicated in Table 1 are the residues that are included in the putative His317 epitope, which we have proposed to be a common epitope among the dengue serotypes. We find that the putative His317 epitope in dengue ENV is mostly present also in Zika and West Nile ENV. This is a possible explanation for the observation that prior exposure to dengue is associated with reduced risk and symptoms of Zika infection (Rodríguez-Barraquer et al. 2019). We would not be surprised if this is also true for West Nile virus infection.

CONFLICTS OF INTEREST

There are no conflicts of interest. The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Army/Navy/Air Force, Department of Defense, or U.S. Government.

CONTRIBUTION OF INDIVIDUAL AUTHORS

C. P. Mikita provided the medical significance of the work and researched the early literature. E. A. Padlan did the sequence analyses.

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