RESEARCH ARTICLE

Large-scale analysis of B-cell epitopes of envelope: Implications for Zika vaccine and immunotherapeutic development [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: Cases of the re-emergence of Zika virus in 2015 were associated with severe neurologic complications, including Gillien-Barre syndrome in adults and congenital Zika syndrome in newborns. The major structural determinant of immunity to the Zika virus is the E protein. Although B-cell epitopes of Zika E protein were recently identified, data regarding epitope variations among Zika strains in pre-epidemic and epidemic periods are lacking.
Methods: Here, we conducted systematic bioinformatics analyses of Zika strains isolated between 1968 and 2017. Multiple sequence alignment of E protein as well as B-cell epitopes annotations were performed. In addition, homology-based approach was utilized to construct three-dimensional structures of monomeric E glycoproteins to annotate epitope variations. Lastly, of *N*-glycosylation patterns and prediction of protein stability upon mutations were also investigated.
Results: Our analyses indicates that epitopes recognized by human mAbs ZIKV-117, ZIKV-15, and ZIKV-119 were highly conserved, suggesting as attractive targets for the development of vaccines and immunotherapeutics directed against diverse Zika strains. In addition, the epitope recognized by ZIKV-E-2A10G6 mAb derived from immunized mice was highly conserved across Zika strains.
Conclusions: Our data provide new insights regarding antigenic similarities between Zika strains circulating worldwide. These data are essential for understanding the impact of evolution on antigenic cross-reactivity between Zika lineages and strains. Further *in-vitro* analyses are needed to determine how mutations could impact the development of vaccines that can effectively neutralize Zika viruses.

Keywords

Antibody, Bioinformatics, Envelope, Epitope, Homology Modeling, Neutralizing Antibodies, Vaccine, Zika

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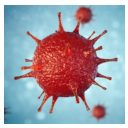

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Introduction

Zika is a positive-sense, enveloped, RNA virus of the *Flaviviridae* family[1], which also includes dengue virus, West Nile virus (WNV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and yellow fever virus (YFV)[2]. Zika was originally discovered in a rhesus monkey in 1947 in Uganda[3], and the first case of spread to humans was reported in 1952[4]. Since that time, the virus has spread globally, with Zika outbreaks reported in Micronesia in 2007 and in the Pacific islands in 2013–2014[5,6]. A recent outbreak that began in Brazil in 2015 eventually spread to countries in North America and the Caribbean[7,8].

The Zika virus genome encodes three structural proteins (capsid [C], premembrane [PrM], and envelope [E]) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5)[2]. Similar to other flaviviruses, the structural proteins and viral genome form virions that assembles as immature particles at the endoplasmic reticulum of infected cells[9]. The immature particles are composed of 60 PrM/E protein heterodimers that protrude from the viral surface[10]. In the Golgi apparatus, PrM is cleaved by furin-like protease[2]. After maturation, pr is released from the host cell, and 90 E protein homodimers rearrange in an antiparallel orientation to form a herringbone-like array[11,12].

The E protein is the major surface glycoprotein of flaviviruses and plays an essential role in virus attachment and fusion. Each E protein monomer consists of three domains: DI, DII, and DIII[13], which undergo major rearrangements during the virus maturation cycle[14,15]. DI is a central beta-barrel domain; DII is a finger-like dimerization domain; and DIII is an immunoglobulin-like domain[10,11]. DI, which connects DII to DIII, is essential for the conformational changes required for viral entry into cells[16]. DII contains a fusion loop (FL) that interacts with the endosomal membrane, whereas DIII contains the receptor-binding site and is thus essential for attachment of virus particles to the host cell[15,16]. DIII also plays an essential role in mediating the fusion of virus particles with the endosomal membrane after endocytosis[17].

The global spread of Zika virus in conjunction with the neurologic consequences of infection have increased the urgency of efforts to develop Zika vaccines and immunotherapeutics. Humoral immunity is the major source of host protection against flaviviruses, in which neutralizing antibodies play an important role in virus clearance[18]. Antibodies generated against the E protein have been shown to block the entry of viruses into host cells[19]. Previous attempts to map the antigenic epitopes of the Zika E protein utilized antibodies specific for other flaviviruses, such as dengue virus[20–22]. Recently, several B-cell antigenic epitopes within an individual E domain were identified in studies of antibodies isolated from Zika-virus infected patients[23–25] and Zika-vaccinated mice[13,26,27].

Although antigenic epitopes of the E protein have been characterized based on maps prepared using Zika-specific antibodies, no systematic analyses of the specificity of Zika monoclonal antibodies (mAbs) for available Zika E protein sequences have been conducted. Such data are particularly important, as RNA viruses exhibit high mutation rates and can generate mutations that enable them to evade the host immune system. Importantly, the structural stability of E protein is a key factor for antibody binding. The amino acids substitutions and subsequent effects on structural stability and antibody binding can be performed by a homology based *in-silico* approach of the three-dimensional (3D) structure of E proteins. Hence, analyzing sequence data to annotate mutations at key residues and subsequent prediction of the effect of those mutations on protein structure stability is essential. Therefore, identifying novel amino acids mutations that are likely to contribute in the immune evasion is important.

In the present study, therefore, we extracted all of the available E protein sequences for Zika isolates obtained from 1968 to 2017 and constructed three-dimensional (3D) structures of E proteins from various Zika strains using homology modeling. We also investigated the patterns and conservation of E protein B-cell epitopes and assessed their structural stability upon mutation.

Methods

Data selection

Complete Zika polypeptide sequences for isolates identified between 1968 and 2017 were obtained from the National Center for Biotechnology Information (NCBI) Zika resource[28]. A total of 409 complete polypeptide sequences were retrieved, and duplicate sequences were removed. Multiple sequence alignment was performed using MUSCLE in the Geneious tool, version 11.0, and the E protein region as extracted[29]. Lastly, MUSCLE alignment was performed for E protein sequences and duplicate E sequences were subsequently removed.

Phylogenetic analysis

A phylogenetic tree for all unique Zika virus E protein sequences was constructed using the maximum-likelihood method with the PhyML tool, version 3.0[30], with 100 bootstrap replications. The tree applied an LG substitutional model to determine the divergence of E protein sequences. Lastly, the phylogenetic tree was edited using the Figtree tool, version 1.4.3.

Homology modeling

The SWISS model[31] server was used to generate 3D structures of the E proteins of Zika isolates identified in 1968, 2007, 2013, 2015, and 2016. Chain A of the E protein structure (PDB: 5GZN) was used as a template for homology modeling. The best homology model was selected based on global model quality estimate (GMQE) and Qmean statistics. Each homologous 3D structure was evaluated using Ramachandran plots prepared with PROCHECK[32]. Hydrogen bonds were added using molprobity[33]. Each model was subjected to energy minimization using the ModRefiner server described by Xu and Zhang[34].

Mapping of antigenic epitopes

E protein-specific antigenic epitopes of monoclonal antibodies (mAbs) isolated from Zika-virus infected humans and Zika immunized mice were retrieved from the Immune Epitope Database (IEDB)[35], which is a free resource funded by the

National Institute of Allergy and Infectious Diseases devoted to disseminating antigenic epitope data. Linear and conformational B-cell epitopes with positive major histocompatibility complex ligands were selected. B-cell epitope regions mapped with B-cell receptor (BCR)-positive neutralizing antibodies were also selected. Epitopes that mapped with screening peptides and did not elicit an immune response were removed. A total of 7 human and 10 mouse (from mice immunized with E protein) B-cell epitopes were identified. The identified epitopes were annotated against aligned E sequences as well as the 3D structures of monomeric E proteins using the Chimera tool[36]. Potential sites of *N*-glycosylation were predicted using NetNglycan 1.0 server[37]. Potential *N*-glycosylation sites were defined by the sequence Asp/X/Ser/Thr, where X represents any amino acid except Pro. A threshold of >0.5 suggested an *N*-glycosylated residue.

Analysis of mutations on E protein stability

The effect of mutations on the stability of E protein was predicted using the mutation cutoff scanning matrix (mCSM)[38], site-directed mutator (SDM)[39], DUET[40], and I-Mutant 2.0[41] tools. The mCSM is a machine-learning algorithm based on a 3D physiochemical environment, and the data are summarized as a graphical signature. The SDM is a statistical potential energy function based on the propensity of amino acids in wild-type and mutant proteins to assume folded and unfolded conformations. DUET is an integrated computational approach that utilizes both SDM and mCSM to predict the effect of non-synonymous single-nucleotide polymorphisms on protein stability. Lastly, the I-Mutant webserver is a neural network-based tool for predicting mutation-associated free energy changes. The I-Mutant2.0 tool enables prediction of free energy changes under differing conditions of pH, temperature, neighboring residues, and solvent accessibility.

Results

Strain frequencies

Antigenic variations among Zika strains were examined by first obtaining the complete sequences of Zika polypeptides from the NCBI Zika resource[19]. A total of 409 Zika polypeptide sequences were retrieved. Identical polypeptide sequences were removed, resulting in a final total of 257 sequences. Sequences were aligned by MUSCLE using Geneious software, E protein sequences were extracted, and duplicate E protein sequences were removed, resulting in a total of 75 unique sequences (Table 1). Of note, the majority of the 75 unique E protein sequences

Table 1. List of unique E protein sequences and their frequency.

Number	Accession	Sequence Description	Frequency	N-Glycosylation
1	AMR68906	|Homo sapiens|Nigeria|1968/09/09		-
2	ACD75819	|Homo sapiens|Micronesia|2007/06/01		154 NDTG
3	AMR39834	|Homo sapiens|Cambodia|2010	2	154 NDTG
4	AMD61711	|Homo sapiens|Philippines|2012/05/09		154 NDTG
5	ANO46307	|Homo sapiens|French Polynesia|2013/11		154 NDTG
6	ANO46309	|Homo sapiens|French Polynesia|2014/01		154 NDTG
7	AMD61710	|Homo sapiens|Thailand|2014/07/19		154 NDTG
8	AMK49165	|Homo sapiens|Brazil|2015		154 NDTG
9	AMK49164	|Homo sapiens|Brazil|2015		154 NDTG
10	AOC50654	|Homo sapiens|Honduras|2015/01/06		154 NDTG
11	AMX81917	|Homo sapiens|Thailand|2015/01/16		154 NDTG
12	ASB32509	|Homo sapiens|Brazil|2015/05/13	4	154 NDTG
13	ALX35659	|Homo sapiens|Suriname|2015/10/02		154 NDTG
14	AMC13913	|Homo sapiens|Guatemala|2015/11/01		154 NDTG
15	AMD16557	|Homo sapiens|Brazil|2015/11/30		154 NDTG
16	ASU55392	|Homo sapiens|Colombia|2015/12		154 NDTG
17	ASU55393	|Homo sapiens|Colombia|2015/12		154 NDTG
18	ASU55394	|Homo sapiens|Colombia|2015/12		154 NDTG
19	ASU55403	|Homo sapiens|Colombia|2015/12		154 NDTG
20	ASU55399	|Homo sapiens|Colombia|2015/12		154 NDTG
21	ASU55411	|Homo sapiens|Colombia|2015/12		154 NDTG
22	ASU55407	|Homo sapiens|Colombia|2015/12	3	154 NDTG
23	ASU55396	|Homo sapiens|Colombia|2015/12		154 NDTG
24	ASU55398	|Homo sapiens|Colombia|2015/12		154 NDTG
25	ASU55409	|Homo sapiens|Colombia|2015/12		154 NDTG
26	AMQ48982	|Homo sapiens|Brazil|2016/01/29		154 NDTG

Number	Accession	Sequence Description	Frequency	N-Glycosylation
27	AMQ48986	|Homo sapiens|USA|2016/02/02		154 NDTG
28	AMK79469	|Homo sapiens|China|2016/02/06		154 NDTG
29	APC60216	|Homo sapiens|Mexico|2016/03/03		154 NDTG
30	AOY08538	|Homo sapiens|Brazil|2016/03/21		154 NDTG
31	AOX49265	|Homo sapiens|Italy|2016/04	2	154 NDTG
32	AQS26698	|Homo sapiens|South Korea|2016/04		-
33	AOY08534	|Homo sapiens|Brazil|2016/04/05	167	154 NDTG
34	ARB07987	|Homo sapiens|Dominican Republic|2016/04/07		154 NDTG
35	ARB07941	|Homo sapiens|Brazil|2016/04/14		-
36	ARB07968	|Homo sapiens|Brazil|2016/04/15		154 NDTG
37	ARB07988	|Homo sapiens|Dominican Republic|2016/04/18		154 NDTG
38	AQS26816	|Homo sapiens|Brazil|2016/04/24		154 NDTG
39	APG56499	|Homo sapiens|Taiwan|2016/05		154 NDTG
40	ATG29278	|Homo sapiens|Honduras|2016/05/10		154 NDTG
41	ARB07930	|Homo sapiens|Honduras|2016/05/13		154 NDTG
42	ATG29285	|Homo sapiens|Mexico|2016/05/17		154 NDTG
43	ART29823	|Homo sapiens|Russia|2016/05/31		154 NDTG
44	ARB07996	|Homo sapiens|Dominican Republic|2016/06/06		154 NDTG
45	ARB07964	|Homo sapiens|Honduras|2016/06/07		154 NDTG
46	ARB07960	|Homo sapiens|Honduras|2016/06/10		154 NDTG
47	ARB07931	|Homo sapiens|Jamaica|2016/06/13		154 NDTG
48	AOY08535	|Homo sapiens|Dominican Republic|2016/06/14		154 NDTG
49	APB03018	|Homo sapiens|USA|2016/06/21		154 NDTG
50	ARB07974	|Homo sapiens|Puerto Rico|2016/06/26		154 NDTG
51	ATG29297	|Homo sapiens|Mexico|2016/07/05		154 NDTG
52	ATG29280	|Homo sapiens|Mexico|2016/07/06		154 NDTG
53	ATG29301	|Homo sapiens|Mexico|2016/07/07		154 NDTG
54	ARB07936	|Homo sapiens|Jamaica|2016/07/10		154 NDTG
55	ATG29267	|Homo sapiens|Guatemala|2016/07/21	2	154 NDTG
56	APO39231	|Homo sapiens|USA|2016/07/29		154 NDTG
57	AQX32984	|Homo sapiens|Honduras|2016/08/26		154 NDTG
58	APH11519	|Homo sapiens|Singapore|2016/08/27		154 NDTG
59	APH11522	|Homo sapiens|Singapore|2016/08/27		154 NDTG
60	APH11526	|Homo sapiens|Singapore|2016/08/27		154 NDTG
61	APH11536	|Homo sapiens|Singapore|2016/08/28		154 NDTG
62	APH11556	|Homo sapiens|Singapore|2016/08/28		154 NDTG
63	APH11539	|Homo sapiens|Singapore|2016/08/29		154 NDTG
64	APH11534	|Homo sapiens|Singapore|2016/08/30		154 NDTG
65	APH11611	|Homo sapiens|Thailand|2016/08/30		154 NDTG
66	APH11493	|Homo sapiens|Singapore|2016/09/04		154 NDTG
67	APH11502	|Homo sapiens|Singapore|2016/09/07	4	154 NDTG
68	APH11511	|Homo sapiens|Singapore|2016/09/13		154 NDTG
69	APH11588	|Homo sapiens|Singapore|2016/09/15		154 NDTG
70	APO39241	|Homo sapiens|USA|2016/10/03		154 NDTG
71	AQX32988	|Homo sapiens|Venezuela|2016/10/19		154 NDTG
72	ARK18853	|Homo sapiens|China|2016/11/01	5	154 NDTG
73	APO08504	|Homo sapiens|China|2016/11/03		154 NDTG
74	BAX00477	|Homo sapiens|Japan|2016/11/22		154 NDTG
75	ASU55416	|Homo sapiens|Colombia|2016/12	2	154 NDTG

were represented strains isolated in 2015 and 2016. Sequences from isolates collected in 2017 did not harbor any unique mutations in comparison to sequences from isolates collected in previous years; thus, 2017 sequences were removed after duplicate E protein sequences were removed.

Phylogeny

A phylogenetic tree of unique E protein sequences was constructed using the maximum-likelihood function in PhyML software, with 100 bootstrap replications (Figure 1). Zika strain accession numbers shown in the phylogenetic tree denote the year of isolation. Notably, E protein sequences from isolates collected in 1968, 2007, 2010, and 2012 clustered in one group, indicating that the associated strains are closely related (Figure 1). However, sequences from strains isolated in 2013 and 2014 exhibited divergence from the sequences of strains isolated in previous years (Figure 1).

Antigenic epitopes

A number of recent reports describe the isolation of mAbs specific for Zika E protein[23–28]. These antibodies bind preferentially to epitopes located in DII and DIII of the E glycoprotein (Table 2). A total of 7 neutralizing mAbs have been isolated from Zika-virus infected humans, and all of these mAbs bind to discontinuous epitopes of E protein. Of note, ZIKV-117 and ZIKV-19 mAbs recognize epitopes located in DII (Table 2), whereas ZIKV-12 and ZIKV-15 mAbs recognize epitopes located in the FL region, and mAbs ZIKV-Z006, and ZIKV-116 as well as the ZKA 190 mAb recognize epitopes located in DIII. Significant overlap between antigenic epitopes specific for ZIKV-Z006 and ZIKV-116 was observed, as three residues in the epitope recognized by the ZIKV-116 mAb are shared with the epitope recognized by ZIKV-Z006 (Table 2). In addition, the epitope region recognized by the ZKA 190 mAb overlapped with that of the mAb specific for ZIKV-Z006 in two amino acids residues.

A total of 10 B-cell epitopes of Zika E protein were found to elicit humoral antibody responses in vaccinated mice (Table 2). Five of these epitopes were shown to be linear and elicited the production of neutralizing antibodies (Table 2). An additional five discontinuous epitopes have been characterized based on antibodies obtained from vaccinated mice (Table 2). The majority of those epitopes are bound to DIII domain of E.

To identify amino acid substitution mutations occurring in B-cell epitopes, we aligned the sequences of 75 unique E protein

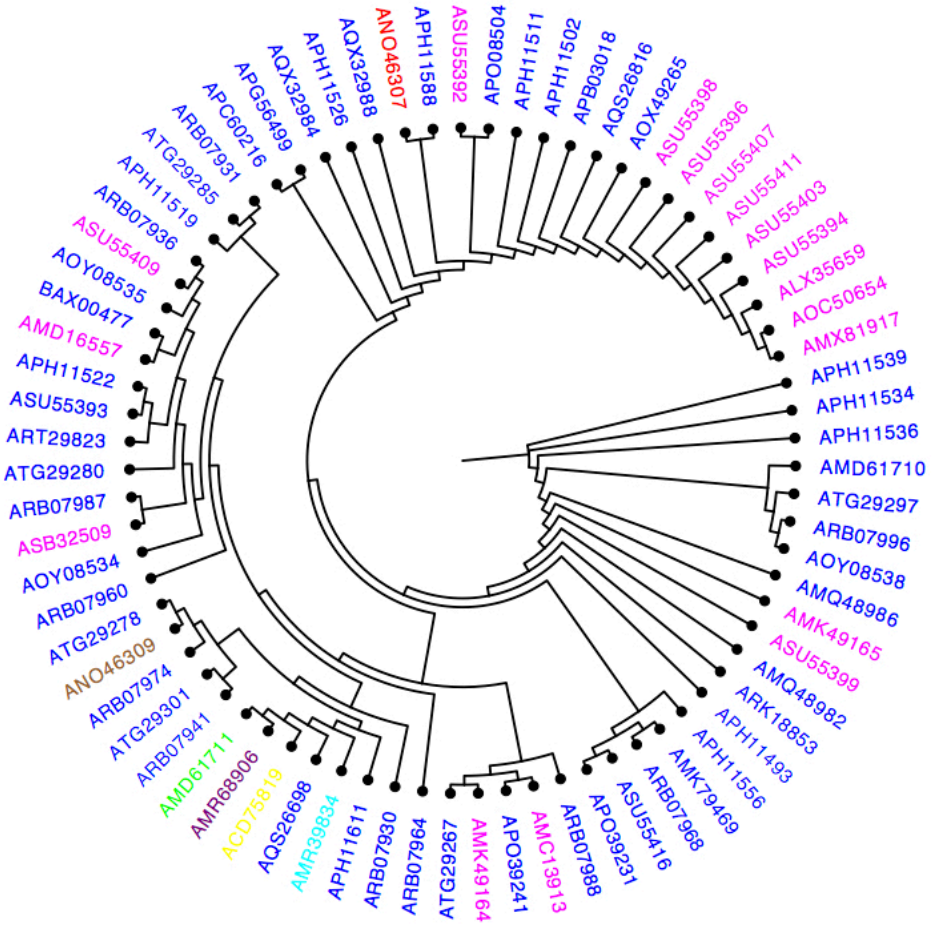

Figure 1. Phylogenic tree of the unique E protein sequences between the years 1968–2017. Year 1968 colored (purple), 2007 (yellow), 2010 (cyan), 2012 (green), 2013(red), 2014 (brown), 2015(magenta), and 2016 (blue).

Table 2. List of B cell epitopes of Zika E protein mapped with neutralizing antibodies from infected humans and vaccinated mice.

	IEDB ID	Epitope	E Domain	mAB	References
Human Epitopes	605975	D67, Q89, K118	DII	ZIKV-117	23
	605976	T309, E393, K394	DIII	ZIKV-116	23
	605977	W101	FL	ZIKV-15	23
	605978	W101, F108	FL	ZIKV-12	23
	605979	W217, F218, D220, P222	DII	ZIKV-19	23
	628722	Y305, S306, L307, T309, A310, A311, G334, T335, D336, K340, Q350, T351, L352, V391, G392, E393, K394, K395	DIII	ZIKV-Z006	24
	733913	Y305, A333, T335, E370, N371	DIII	ZKA 190	25
Mouse Epitopes *Discontinuous*	540699	T76, Q77, W101, G102, G104, C105, G106, L107, F108	DII	ZIKV-E-2A10G6	13
	558355	K301, T315, K316, I317, P318, A319, E320, T321, L322, T327, E329, N362, V364, I365, T366, E367, S372, K373, M374, M375, E377	DIII (C-C' loop)	ZV-48	26
	558356	L307, K340, P342, A343, Q344, V347, D348, Q350, T351, L352, T353, P354, L358, D384, Y386	DIII (LR)	ZV-54	26
	558357	L307, K340, P342, A343, Q344, V347, D348, Q350, T351, L352, T353, P354, V355, L358, V391	DIII (C-C' loop)	ZV-64	26
	558358	T309, A310, A311, F312, T313, F314, Q331, Y332, A333, G334, T335, D336, G337, S368, E370, N371, E393, K394, K395, I396, T397	DIII (LR)	ZV-67	26
Mouse Epitopes *Linear*	745460	D247, A248, H249, A250, K251, R252, Q253, T254, V255, V256, V257, L258, G259, S260, Q261, E262, G263, A264, V265	DII	NA	27
	745469	G383, D384, S385, Y386, I387, V388, I389, G390, V391, G392, D393, K394	DIII	NA	27
	745482	L300, K301, G302, V303, S304, Y305, S306, L307, C308, T309, A310, A311, F312, T313, F314, T315, K316, V317, P318, A319, E320, T321	DIII	NA	27
	753507	L352, T353, P354, V355, G356, R357, L358, I359, T360, A361, N362, P363, V364, I365, T366, E367	DIII	NA	27
	745501	T427, A428, W429, D430, F431, G432, S433, V434, G435, G436, V437, F438, N439, S440, L441, G442, K443	DIII	NA	27

amino acids sequences among the 422 pre-epidemic and epidemic Zika strains identified. The sequence of Zika/Nigeria/9/9/1968 was used as a reference, and the E protein sequences were mapped against all of the mAbs from Zika infected humans or vaccinated mice. We then annotated the mutations in Zika E glycoprotein at predefined B-cell epitopes. Only nine Zika strains were found to carry mutations in the predefined B-cell epitopes recognized by mAbs from Zika-virus infected humans. Of note, two amino acids substitutions (R335T) and (D393E) appeared in 2007 (Figure 2A). These amino acids substitutions were retained in all subsequent strains from 2007 to 2016. Interestingly, no unique mutations were observed in predefined B-cell epitopes for isolates collected between 2008 and 2014. However, in 2015, two additional substitutions of alanine residues for threonine residues appeared at amino acid positions 309 and 333 (Figure 2A). These mutations were not retained in subsequently isolated Zika strains, with the sequences quickly reverting to those of previously isolated strains. The greatest number of amino acid substitution mutations occurred in 2016; the majority of the mutations identified in 2016 involved several deletions in specific regions of the predefined B-cell epitopes (Figure 2A). Of note, the Dominican Republic/6/6/2016 strain exhibited significant deletions in B-cell epitopes recognized by ZIKV-Z006 mAb (Figure 2A). Surprisingly, 3 B-cell epitopes were completely conserved in the pre-epidemic and epidemic strains and indeed have not changed for nearly 50 years (Figure 2A). These epitopes are recognized by the mAbs ZIKV-117, ZIKV-15, and ZIKV-19. Thus, as these mAbs could

exhibit cross-reactivity against a wide range of Zika strains, they have potential for use in the development of vaccines and immunotherapeutics targeting Zika (Figure 2A).

Conversely, none of 10 mouse B-cell epitopes were completely conserved among all Zika strains (Figure 2B, Figure 2C). Of note, the number of Zika strains exhibiting variations in the amino acid sequence at predefined antigenic epitopes of the E protein was higher in mAbs characterized in vaccinated mice than in mAbs characterized in infected humans (Figure 2B, Figure 2C). In addition, both the linear and discontinuous epitopes of vaccinated mice exhibited variations beginning in 2014 and continuing in subsequent years (Figure 2B, Figure 2C). The majority of mAbs in vaccinated mice recognized the DIII of E protein, and these epitopes exhibited higher rates of sequence variation than did the mAbs recognizing DII (Figure 2B, Figure 2C). Of note, the ZIKV-E-2A10G6 mAb binds to a highly conserved discontinuous epitope in which a single amino acid deletion was observed in Zika strain ATG29285|*Homo sapiens*|Mexico|17/05/2016. Remarkably, discontinuous epitopes bound the ZV-67 mAb exhibited the highest degree of variation in amino acid sequence among all the Zika B-cell epitopes examined (Figure 2B). Sequence variations were also observed in all of the Zika E protein linear epitopes (Figure 2C).

E protein homology modeling

The recently solved E protein structure of Zika enabled us to construct a homology model of various E protein sequences. Amino acid substitutions in B-cell epitopes were also annotated on the 3D structure of the E protein. The majority of mutations in the E protein were found to be located within DIII, whereas the B-cell epitopes in DII were highly conserved (Figure 3). Zika strain *Homo sapiens*/French Polynesia/11/13 did not harbor any additional mutations in B-cell epitopes compared with Zika strain *Homo sapiens*/Micronesia/01/06/07 (Supplementary Figure 1).

It is known that *N*-glycosylation can mask antigenic epitopes. However, as the glycosylation site in the Zika E protein is located remotely from the predefined B-cell epitopes, glycosylation

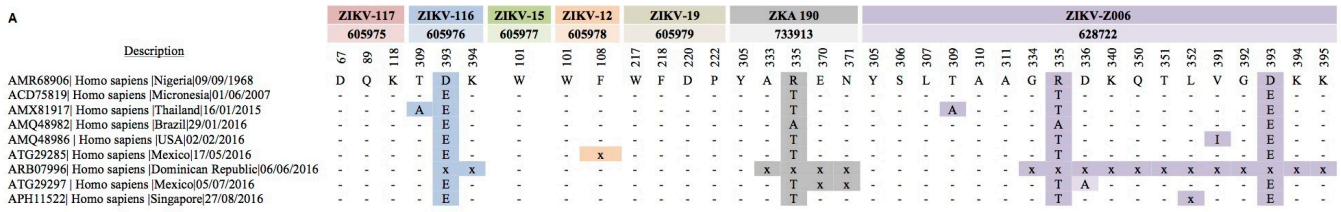

Figure 2A. B-cell epitopes alignments mapped with human anti-E monoclonal antibodies. A total of 9 Zika strains are variable at predefined B-cell epitopes during 1968–2017.

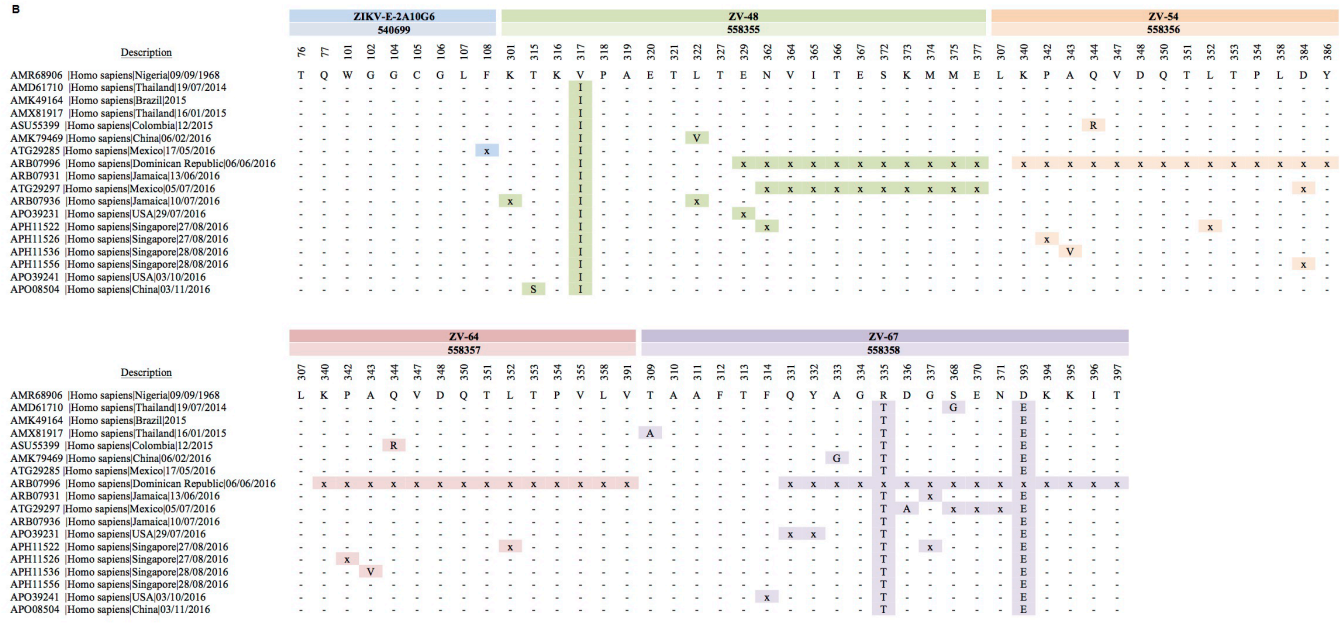

Figure 2B. Discontinuous B-cell epitopes alignments mapped with mice anti-E monoclonal antibodies. A total of 18 Zika strains were variable at predefined B-cell epitopes during 1968–2017.

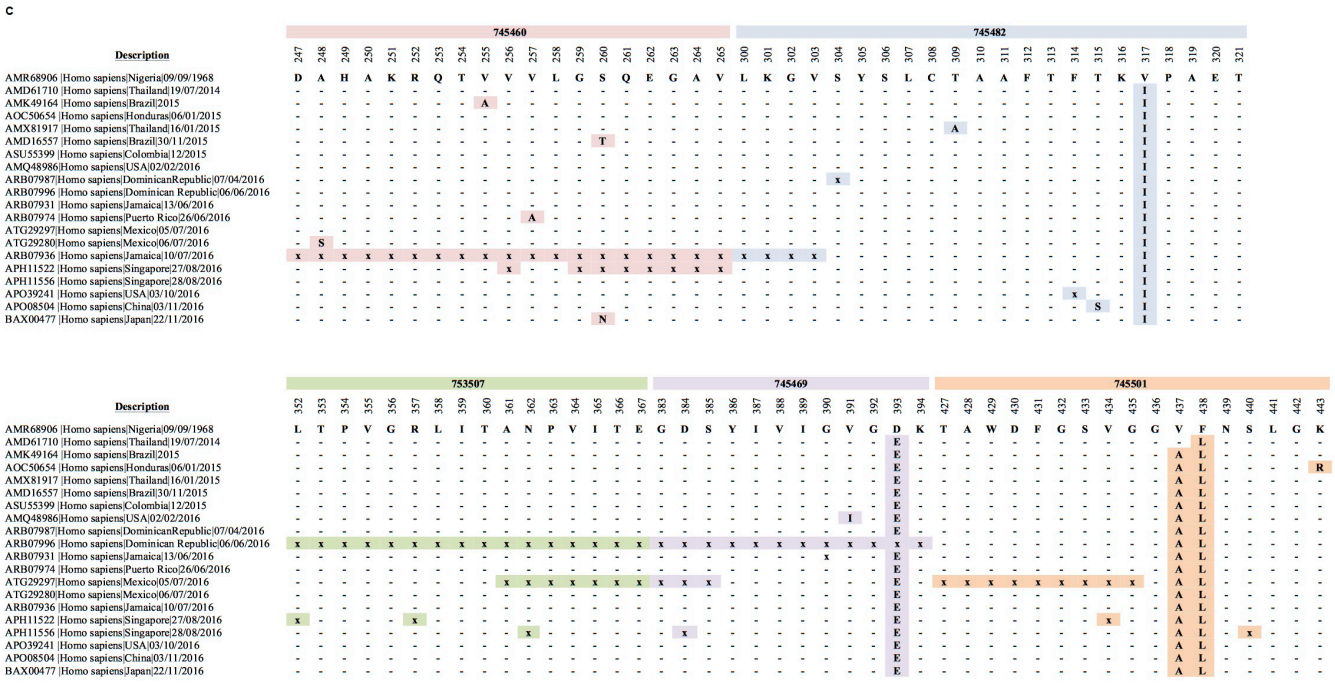

Figure 2C. Linear B-cell linear alignments mapped with mice anti-E antibodies. A total of 20 Zika strains were variable at predefined B-cell epitopes during 1968–2017.

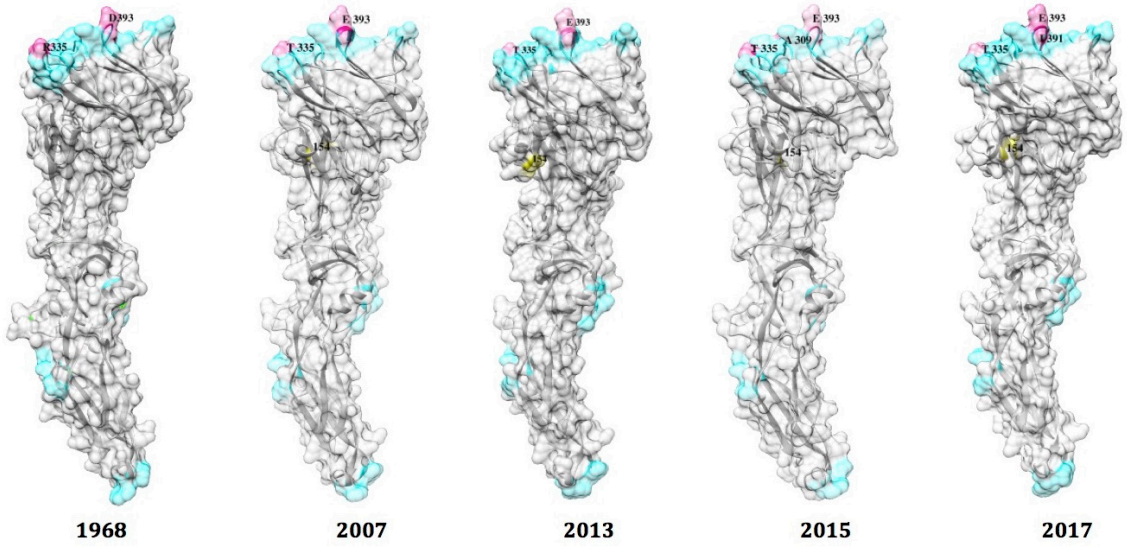

Figure 3. Comparison of B-cell epitopes of monomeric E between the years 1968, 2007, 2010, 2015, and 2016. Homology models of E were built from PDB: 5GZN chain A from strains of following E sequences: AMR68906| Homo sapiens |Nigeria|09/09/1968, ACD75819| Homo sapiens |Micronesia|01/06/2007, ANO46307| Homo sapiens/French Polynesia/11/2013, AMX81917/Homo sapiens/Thailand/16/01/2015, and AMQ48986 | Homo sapiens |USA|02/02/2016. Color blue color represents amino acids conservation at B-cell epitopes and color pink represents amino acids substitutions. Yellow label represent N-glycosylation potential of E.

does not mask the B-cell epitope epitopes (Figure 3). This is consistent with reports of glycosylation in E protein of WNV and JEV[18]. A recent report demonstrated that glycosylation at 154 is critical for Zika infection of both mammalian and mosquito hosts[17]. Our analyses indicate that antigenic changes occur less frequently in Zika strains (Figure 3) and suggest that highly effective neutralizing Zika vaccines and immunotherapies for treating infections with known Zika strains are possible.

Consequently, monitoring antigenic changes in E proteins over time would be useful for evaluating the cross-neutralizing potential of Zika vaccines against newly mutated strains.

Mutational stability

Mutation stability was carried out to predict the effects of non-synonymous variants on the stability of E protein and antibody binding. Here, we analyzed the stability of monomeric E protein upon substitutional mutations. To investigate the effect of amino acids substitutions at antigenic epitopes on the stability of E protein and antibody binding of E, 4 prediction tools for mutational stability were selected.

We predicted the stability of B-cell epitope mutations using the 3D structure of Zika E proteins. In both the T309A and T335A substitutions, a polar threonine residue was substituted with a hydrophobic alanine residue. No change in hydrophobicity with the V391I mutation or change in charge with the D393E were observed. In the R335T mutation, a basic residue was substituted with an aromatic residue. Overall, these suggest that defined substitutions in the E glycoprotein are potentially destabilizing. However, these mutations had moderate destabilizing effect, as the ΔΔG values ranged between -0.3 and -0.7 kcal/mol (Table 3).

Discussion

Attempts to control the spread of Zika virus via mosquito control have met with limited success. Indeed, within the past 3 years, a Zika pandemic occurred. There is a significant gap in knowledge regarding immunogenic cross-reactivity between Zika strains, even six decades after the first human infection was reported. Bioinformatics approaches can play vital roles in identifying rapidly evolving amino acid residues and thereby facilitate precise mapping of key residues that drive antigenic escape in response to the generation of host neutralizing antibodies. In the present study, we evaluated conserved versus rapidly evolving antigenic regions in predefined B-cell epitopes of the Zika E protein in pre-epidemic and epidemic periods.

The finger-like DII of the Zika E protein contains a FL that is inserted into the endosomal membrane as a result of pH-dependent conformational changes[14]. The FL is located within the beta-sheet structure in the terminal region of DII and contains a highly conserved hydrophobic peptide that triggers the structural changes required for fusion processes under conditions of low pH[15]. Our analysis demonstrated that B-cell epitopes in DII of Zika E protein are highly conserved. mAbs ZIKV-117, ZIKV-15, and ZIKV-19 are bound to the highly conserved region of DII and are therefore attractive candidates in the design of Zika vaccines and immunotherapeutics.

The immunoglobulin-like DIII of the Zika E protein contains receptor-binding sites and plays an essential role in attachment and fusion of the virus to host cells[11,12]. Importantly, DIII reportedly induces the production of type-specific neutralizing antibodies[26], as mAbs isolated from patients infected with either Zika or dengue are highly specific. In the present study, we found that epitopes within E protein DIII vary greatly within Zika strains.

While dengue is considered as a single serotype, it is characterized by four distinct serotypes. The antibody-dependent enhancement (ADE) hypothesis holds that cross-reactive antibodies generated as a result of previous infections with heterologous flaviviruses can enhance the infectivity of other viruses[42,43]. Infection with the same serotype elicits a protective immune response, but re-infection with a different serotype can lead to serious disease[42,43]. Previous studies demonstrated that mAbs isolated from patients infected with dengue virus cross-react with Zika virus[44–47].

Similarly, mAbs isolated from Zika patients directed against E protein DII cross-react with dengue[44,48], indicating possible increased risk of ADE. Furthermore, another study demonstrated that dengue-virus derived mAbs also cross-react with Zika with high potency[20]. Those mAbs are bound to quaternary epitopes, which include the site of interaction of E protein dimer with PrM during virus maturation[20]. In contrast, most mAbs directed against DIII of Zika E protein do not cross-react with dengue[44].

In the present study, we compared mAbs of Zika E protein elicited in cases of Zika-virus infected humans versus mAbs induced by Zika vaccination in mice and identified several conserved epitope footprints. The conserved E protein epitopes could be useful in research aimed at developing vaccines that elicit the production of antibodies that provide protection against Zika strains but do not cross-react with dengue. For example, immunization with a peptide cocktail of antigenic DIII

Table 3. Mutational stability prediction of substituted amino acids at B-cell epitopes of E.

Mutations	mCSM	Effect	SDM	Effect	DUET	Effect	I-Mutant2.0	Effect
T309A	- 0.464 Kcal/mol	Destabilizing	0.12 Kcal/mol	Stabilizing	-0.22 Kcal/mol	Destabilizing	-0.15 Kcal/mol	Decreased stability
T335A	- 0.596 Kcal/mol	Destabilizing	0.12 Kcal/mol	Stabilizing	-0.337 Kcal/mol	Destabilizing	0.33 Kcal/mol	Increased stability
R335T	- 0.304 Kcal/mol	Destabilizing	-0.38 Kcal/mol	Destabilizing	-0.173 Kcal/mol	Destabilizing	NA	NA
V391I	- 0.375 Kcal/mol	Destabilizing	0.08	Stabilizing	-0.022	Destabilizing	-0.25 Kcal/mol	Decreased stability
D393E	- 0.38 Kcal/mol	Destabilizing	0.06 Kcal/mol	Stabilizing	-0.149 Kcal/mol	Destabilizing	NA	NA

epitopes might provide broad protection against a variety of Zika strains yet demonstrate no cross-reactivity with dengue, thus eliminating the possibility of ADE associated with the anti-Zika antibodies.

Data availability

Zika protein sequences data can be found at the NCBI Zika resource.

Zika B-cell antigenic epitopes can be found at Immune Epitope Database (IEDB).

Grant information

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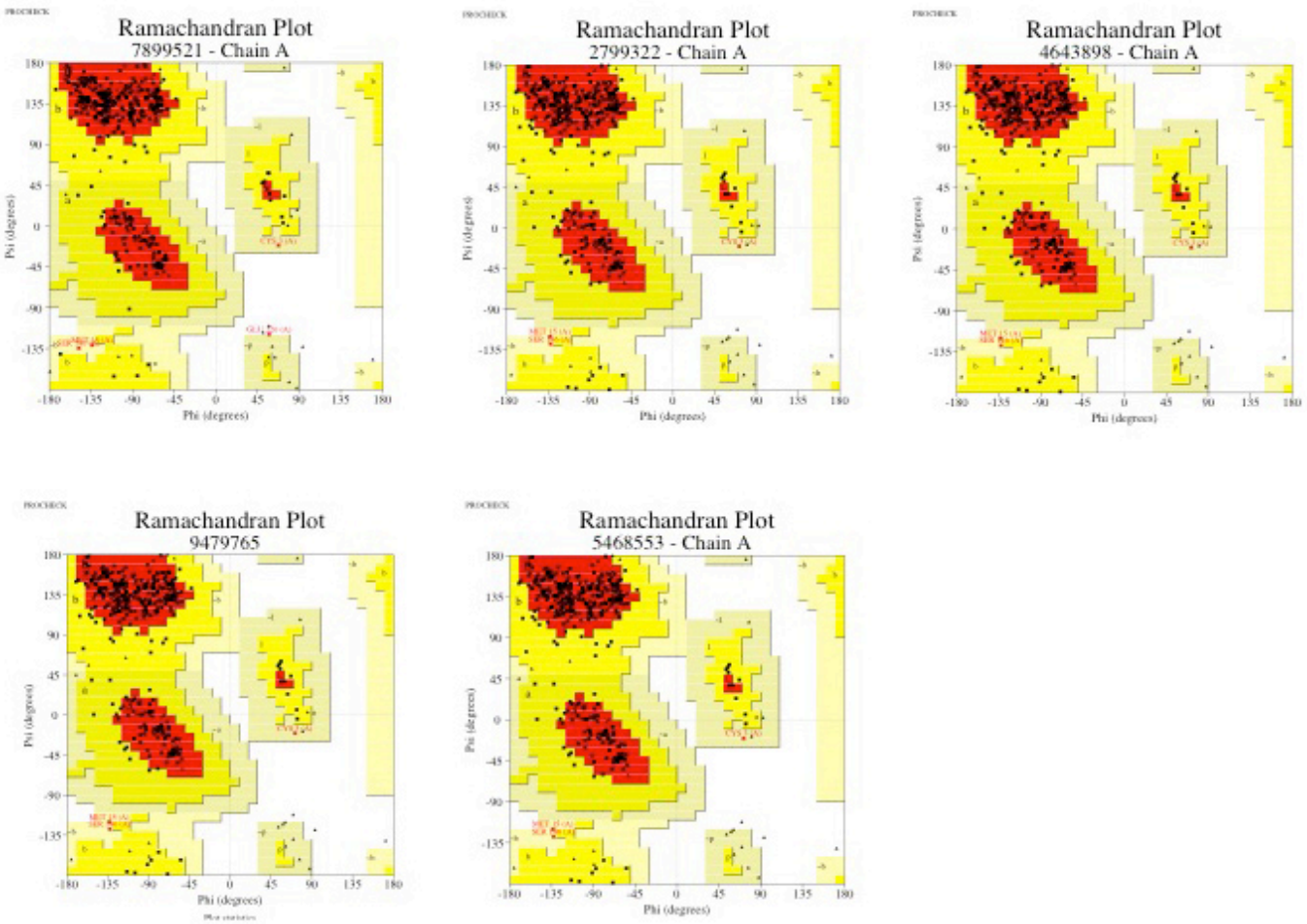
The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgment

We would like to thank Arwa Alghamdi from College of Pharmacy at IAU who assisted in the research.

Supplementary material



Supplementary Figure 1. Ramachandran plot analysis of 3D structure of monomeric E. Homology model for sequences of |Nigeria|09/09/1968, ACD75819| Homo sapiens |Micronesia|01/06/2007, ANO46307| Homo sapiens/French Polynesia/11/2013, AMX81917/ Homo sapiens/Thailand/16/01/2015, and AMQ48986 | Homo sapiens |USA|02/02/2016 were verified.

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Current Peer Review Status: ? ✓

Version 1

Reviewer Report 21 May 2019

https://doi.org/10.5256/f1000research.17985.r42684

✓ **Sunil K Joshi**
Department of Pediatrics, Miller School of Medicine, University of Miami, Miami, FL, USA

Authors described *in-silico* methods to delineate the data which are particularly important due to high mutation rates by RNA viruses which can generate mutations, that enable them to evade the host immune system. Authors emphasized the structural stability of E protein, which is a key factor for antibody binding. The bioinformatic analysis of B cell epitopes of Zika is very important study in terms of developing effective vaccine and may be also in cross-protection against other similar viruses.
In the present study, the authors extracted all of the available E protein sequences for Zika isolates obtained from 1968 to 2017 and constructed three-dimensional (3D) structures of E proteins from various Zika strains using homology modelling. Further the authors investigated the patterns and conservation of E protein B-cell epitopes and assessed their structural stability upon mutation. The authors did meticulous data analysis and discussed very thoroughly.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Innate Immunity, Infectious diseases, Vaccinology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 06 November 2018

https://doi.org/10.5256/f1000research.17985.r39301

?

Meirong Jia
Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, IA, USA

Summary: Zika virus is still a big threat to human health, and E protein is its major structural determinant of immunity. The identification of B-cell epitopes of Zika E protein is crucial for the development of vaccines and immunotherapeutics. Here, the authors conducted systematic bioinformatics analyses of Zika strains isolated between 1968 and 2017, they've found conservative epitopes between Zika strains, which could be attractive targets for treating against diverse Zika strains. The research report is generally sound and the finding is scientifically valuable, which would provide guidance for later research in Zika vaccine development. Thus, I would recommend the indexing of this work in F1000Research.

The followings are some suggestions and comments that might help to make the report more suitable for indexing:

1. In the 'Introduction' part, for the 2nd paragraph, "After maturation, pr is released from the host cell" is that "PrM" being released instead?
2. In 'Table 2', the first row, should "mAB" be "mAb"?
3. In the 'Abstract', for the 'Results' part,
"Our analyses indicate that epitopes recognized by human mAbsZIKV-117, ZIKV-15, and ZIKV-119 Should be "ZIKV-19", please correct it. Also, since there is mutation as shown in 'Fig 2B' other than the strict conservation pattern in 'Fig 2A', please rewrite the claim that
"ZIKV-E-2A10G6 mAb derived from immunized mice was highly conserved across Zika strains".
4. In the 'Results', for E protein homology modelling, the 2nd paragraph, "Glycosylation does not mask the B-cell epitope epitopes" should be "antigenic epitopes?"
5. In the 'Method' part, for 'Mapping of antigenic epitopes', "Potential N-glycosylation sites were defined by the sequence Asp/X/Ser/Thr, where X represents any amino acid except Pro. A threshold of >0.5 suggested an N-glycosylated residue." Please further explain what values >0.5 would suggest an N-glycosylated residue.
6. The authors described in the 'Methods' that "The I-Mutant2.0 tool enables prediction of free energy changes under differing conditions of pH, temperature, neighboring residues, and solvent accessibility." For the data shown in 'Table 3', what conditions did authors apply to conduct the prediction?

7. In the 'Results', the last paragraph, "Overall, these suggest that defined substitutions in the E glycoprotein are potentially destabilizing." Could the authors further discuss what the consequence or effects of destabilization has on virus infection since authors said that the structural stability of E protein is a key factor for antibody binding.
8. In the 'Discussion' part, the authors claimed that "The conserved E protein epitopes could be useful in research aimed at developing vaccines that elicit the production of antibodies that provide protection against Zika strains but do not cross-react with dengue." Yet from the manuscript, it seems that no conserved epitopes in DIII has been identified. If so, authors might need to rewrite the last part of the discussion.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Jun 2019

Iman Almansour, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

We thank Dr.Jia for the careful reading of the manuscript and the constructive remarks.

Response to general comments:
1.Corrections has been made.
2.Corrections has been made.
3.Yes, thank you for pointing this out. Corrections were made.
4.Yes, we are sorry for the unintended mistake. Corrections were made.
5. Explanation has been made.
6.pH 7.0 and temperature at 25 C were applied. The details were added in the method section.
7.In the revised document, we further explained the effect of mutations on protein stability and protein binding (Results section, mutation stability, first paragraph).

8.In the discussion section, we have explained that immunization with a cocktail of antigenic DIII epitopes might provide broad protection against a variety of Zika strains.

Competing Interests: No competing interests were disclosed.

Comments on this article

Version 1

Reader Comment 30 Jan 2019

Million Abraha, University of Bern, Switzerland

I have read the whole article and i find it to be really interesting.

Competing Interests: No competing interests were disclosed.