Prediction of CD8 and CD4 T cell epitopes in the polyprotein of Zika Virus; an immunoinformatics approach

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Background: Zika virus (ZIKV) is an arbovirus that belongs to family Flaviviridae. The virus has emerged as a global threat and no FDA approved vaccine is available, so an efficient vaccine needs to be designed in order to prevent the infection. Computationally designed vaccines can be used for broad-spectrum therapeutics as they can evoke response against viral infections. In the current study, we have predicted antigenic promiscuous T cell epitopes from Zika virus polyprotein using a range of immune-informatics tools and servers.

Methods: A total of 238 polyprotein sequences derived from 238 complete genomes were retrieved using NIAID Virus Pathogen Resource and multiple aligned. Using a consensus sequence, the promiscuous CD8-T cell epitopes were predicted from Propred I and CTLPred and their binding affinities were determined by NetMHC4.0. CD4-T cell epitopes were predicted using ProPred and the binding affinities were determined by MHCPred. Antigenicity score and Immunogenicity score was determined from Vaxijen 2.0 and IEDB immunogenicity tool. Homology was found by pBLAST.

Results: Among 78 predicted HLA-I binding epitopes, 19 highly antigenic, immunogenic and high-affinity epitopes are prioritized among which 15 are novel vaccine candidates. However, 66 strong HLA-II interacting T cell epitopes are pooled out from 70 predicted epitopes. Among the shortlisted CD4-T cell epitopes 56 epitopes are novel.

Conclusion: Epitope-based vaccines are robust and promising candidates against bacterial and viral infections. The predicted epitopes can serve as potential vaccine candidates. Our study shows promising epitopes that can be used to generate stimulate active immune responses in the majority of the human population around the world, However, our results need validation through experimental studies for confirmation.

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Abstract:

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35 **Keywords:** Zika virus; CD8 T cell epitopes; CD4 T cell epitopes; Vaccine; Antigenicity;

36 Immunogenicity

Background

Zika virus (ZIKV) family Flaviviridae that has almost 53 known viruses that are single-stranded positive polarity RNA viruses (Huhtamo et al., 2009; Malone et al., 2016). A mosquito-borne flavivirus is a large group of viruses that are distinguished phylogenetically on the basis of disease transmission and hemorrhagic complications mediated by Aedes genus of mosquito (Gaunt et al., 2001). Previously, seven groups of mosquito-borne flavivirus were recognized, namely Dengue, Aroa, Kokobera, Japanese encephalitis, Yellow fever, Spondweni, and Ntaya (Mayo et al., 2003). Transmission of ZIKV has been associated with mosquitoes mainly by Aedes genus mosquitoes, and *Aedes aegypti* species is the major vector of its infection in Asia (Boorman & Porterfield, 1956; Malone et al., 2016).

The RNA of ZIKV is single-stranded and serves as mRNA that has one open reading frame (ORF), the translated polyprotein of which further cleaves to form a membrane, capsid, envelope and seven non-structural (NS) proteins that mainly include enzymes necessary for viral replication (Kuno & Chang, 2007). ZIKV can cause serious health threatening issues to the life of newborns so an efficient vaccine or antiviral drugs need to be designed that can target structural as well as non-structural proteins of ZIKV and help in preventing the infection (Chambers et al., 1990; Leyssen, De Clercq & Neyts, 2000). No FDA approved treatment or vaccine is available for the cure or prevention of this viral infection. Initially, there was no indication of ZIKV's involvement in disease progression, however, serosurvey data suggested that 6.1% of ZIKV antibodies were present in the residents of Uganda which revealed frequent manifestation of infection (Dick, Kitchen & Haddow, 1952). For about 70 years from the first isolation, ZIKV remained in obscurity, then within the span of one year it emerged as a global threat and spread from Pacific Island to Brazil and later throughout the states of America (Fauci & Morens, 2016). WHO (World Health Organization) professed ZIKV infection as a global

emergency to public well-being due to the alarming situation it has caused around the globe (Gulland, 2016). Pakistan and other South Asian countries are at a risk of ZIKV infection as antibodies were suspected in the healthy individuals; the seroprevelance of ZIKV antibodies in Pakistan has been reported to be 2.3% (Posen et al., 2016).

Historically, empirical vaccine formulation was the most trusted and widespread method, but due to the emergence of viruses having greater genetic instability and antigenic variance, computational tools are of great importance in designing efficient vaccine candidates (Soria-Guerra et al., 2015). Computationally designed vaccines can be used for broad-spectrum therapeutics as they can evoke better and increased immune response as compared to the normal viral infection. Typically, in viral infection patients use to have an exposure to a limited number of antigenic and immunogenic components of the pathogens (Sette & Fikes, 2003). So, by employing strategy of Reverse vaccinology antigenic and immunogenic regions of the whole pathogen can be identified. The computationally predicted T cell epitopes from full-length polyprotein can be used to generate a strong cell-mediated as well as a humoral response against the whole pathogen. Epitope-based vaccines designed using computational biology are more potent and safe and evoke a greater immune response. The natural infection may not necessarily recognize all the epitopes, so the need of the hour is to design an epitope-based vaccine to have greater and more specific T cell response against the MHC-epitope complex (Sette & Fikes, 2003). Antigen Presenting Cells (APC) showing MHC-epitope complexes activates a repertoire of T cells that recognize these complexes and induce immune responses accordingly (Chen et al., 2000).

In this study, we have taken an increased number of polyproteins available in databases than previous studies to reveal new antigenic and immunogenic conserved epitopes using multiple

bioinformatics tools and software to predict efficient subunit vaccine candidates against ZIKV infection (Dar et al., 2016).

Methods

Retrieval of sequences

A total of 238 ZIKV polyprotein sequences derived from 238 complete genomes were retrieved from the NIAID Virus Pathogen Resource (Pickett et al., 2012). The source of the sequences was human, and all the geographical regions of the World were targeted for the acquisition of these sequences. The sequences were multiple-aligned using default parameters on the CLUSTALW server available at www.genome.jp/tools/clustalw/.

Exploration of CD8-T cell epitopes

The consensus sequence derived from multiple alignment was then submitted to ProPred I (available at http://crdd.osdd.net/raghava/propred1/) and CTLPred (available at http://www.imtech.res.in/raghava/ctlpred/index.html) (Singh & Raghava, 2003; Bhasin & Raghava, 2004).

CTLPred ANN (Artificial Neural Network) and SVM (Support Vector Machine) methods using consensus approach were used for the prediction of HA-I interacting T cell epitopes (Bhasin & Raghava, 2004). The default cutoff score for SVM and ANN (0.36 and 0.51 respectively) was used. The best accuracy obtained by consensus method is 77.6%, which is greater than the individual as well as combined approach, hence the consensus approach was followed for the prediction of epitopes.

Lastly, the consensus polyprotein sequence of ZIKV was submitted to ProPred I server (Singh & Raghava, 2003). The default threshold of 4% was applied, immunoproteasome and proteasome filters were kept at 5% cutoff value to increase the chances of determining CD8T cell-specific epitopes. The default threshold value provides a compromise between the sensitivity and

specificity of the server. ProPred I determines the affinity of peptides to bind with 47 HLA-I alleles.

The projected peptide sequences were analyzed for their antigenicity using Vaxijen server 2.0 (Doytchinova & Flower, 2007). The threshold was selected at 0.5 and the antigenic epitopes were taken for further analysis. The epitopes were evaluated for their immunogenic property using immunogenicity tool of IEDB (http://tools.iedb.org/immunogenicity/) (Calis et al., 2013). The predicted epitopes from both CTLPred and ProPred I were then shortlisted based on their antigenicity and immunogenicity.

Prediction of MHC-I binding affinity

NetMHC 4.0, HLA supertype representatives were used to check the binding with predicted 9mer epitopes. NetMHC 4.0 uses ANN to determine the binding of the epitope with alleles (Nielsen et al., 2003; Andreatta & Nielsen, 2015). Default 0.5% threshold rank for strong binders while 2% rank for weak binders. Strong MHC binding epitopes were shortlisted. The HLA super-type representative chosen for the study were from HLA-A superfamily (HLA-A0201, A0101, A2402, A0301, A2601), and HLA-B superfamily alleles (HLA-B2705, B0702, B3901, B0801, B4001, B5801, B1501).

Analysis of HLA class II binding T cell epitopes

The consensus polyprotein sequence of ZIKV was submitted to ProPred server (Singh & Raghava, 2001). ProPred was used at a 3% threshold to predict epitopes for a total of 51 HLA-II alleles. ProPred enables the prediction of binding potential of epitopes for 51 HLA class II alleles. Like ProPred I server, the default cutoff value provides a compromise between the sensitivity and specificity of the server. The projected peptide sequences were analyzed for their antigenicity using Vaxijen server 2.0 (Doytchinova & Flower, 2007). The threshold was selected at 0.5 and the antigenic epitopes were taken for further analysis.

Analysis of MHC-II binding affinity

Prediction of MHC-II interacting epitopes was performed using MHCPred (Guan et al., 2003a,b; Hattotuwagama et al., 2004). "Amino acids and interaction" model was chosen to calculate numeric values of affinity in -log IC50(M) and IC50. Epitopes having -log IC50 > 6.4 were prioritized and further analyzed.

Identification of homology with human proteome

The peptide sequences were checked for their similarity against the human proteome by performing protein BLAST (accessible at https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine their harmless use as potential vaccine candidates.

Results

Exploration of CD8 T cell epitopes

A total of 78 MHC-I binding MHC-I binding epitopes are stipulated in Table S1. All the predicted epitopes were antigenic, immunogenic and lie in the structural and non-structural region of viral polyprotein.

Prediction of MHC-I binding affinity

Immunogenicity of potential epitope depends upon its binding affinity with MHC molecule. Based on this, 19 epitopes out of 78 predicted epitopes were pooled out. The prioritized epitopes were predicted to have a strong interaction with HLA alleles. The shortlisted epitopes can be seen in Table 1 along with their antigenicity, immunogenicity and affinity score.

In NS2A region out of 6 prioritized epitopes, RLVDPINVV NS2A$^{1353\text{-}61}$ has maximum antigenicity, however, 10 HLA alleles were predicted to bind with it. Meanwhile, VPRTDNITL NS2A$^{1290\text{-}1298}$ has a maximum number of interacting HLA alleles so it can target greater HLA population. In NS3 only one epitope has been prioritized i.e. VPNYNLYIM NS3$^{1778\text{-}1786}$ that has good antigenicity and immunogenicity score. Epitope TPLTLIVAI of NS4B$^{2374\text{-}82}$ has been

predicted by all of the servers and has good antigenicity, immunogenicity, binding affinity as well as promiscuity. All the prioritized epitopes are strong MHC binders as can be seen in Table 1.

Crystal structures of Envelope, NS3 and NS5 were available and were retrieved from Protein DataBank (Berman et al., 2006; Sirohi et al., 2016; Tian et al., 2016; Upadhyay et al., 2017). Modeled structures of NS2A and NS2B were obtained from Gupta et al (Gupta et al., 2016). HLA-I epitopes that were found in models were visualized and colored in chimera, and are shown in Fig. 1 (Pettersen et al., 2004).

Identification of host homology

All the prioritized epitopes do not have a putative conserved domain in humans. All the promiscuous epitope

Prediction of CD8 T cell epitopes

A total of seventy HLA-II binding antigenic T cell epitopes were projected in the various regions of ZIKV proteins as outlined in Table S2. Antigenicity score, as well as amino acid position of each epitope, was predicted.

Prediction of MHC-II binding affinity

Among 70 MHC-II binding epitopes, only 66 epitopes have a strong binding affinity with major MHC-II alleles. IC50 and -log IC50 of each shortlisted epitope is illustrated in table 2.

In the Protein C, Protein C^{50-58} was predicted to bind 18 MHC class II alleles with high antigenic score i.e. 1.0423. In PrM region, Epitope $PrM^{277-285}$ was projected to bind 38 HLA-II alleles. $E^{677-685}$, with antigenicity score 2.6489, was found to be the most antigenic HLA class II binding epitope according to our analysis. However, it was predicted to bind 9 HLA-II alleles. Comparatively, $E^{784-792}$ was found to bind 16 HLA-II alleles with good antigenic score i.e. 0.5846. In the NS1 region, epitope $NS1^{1006-1014}$ was projected to bind 16 HLA-II alleles with antigenicity

score 1.0212. Highly antigenic epitope NS1$^{1117\text{-}1125}$ with antigenicity value 2.3636 was predicted to bind 10 alleles.

Among seven NS2A epitopes, NS2A$^{1161\text{-}1169}$ and NS2A$^{1352\text{-}1360}$ were projected to bind 40 HLA class II alleles each, with good antigenic scores i.e. 0.8218 and 0.9703, respectively. A total of four antigenic peptides were mapped into the NS2B protein as can be seen in the table. In NS3 region, NS3$^{1597\text{-}1605}$ was projected to bind 34 HLA-II alleles with 0.5392 antigenicity score. Amongst six NS4A epitopes, NS4A$^{2189\text{-}2197}$ and NS4A$^{2231\text{-}2239}$ were predicted to bind a maximum number of HLA-II alleles i.e. 41 and 44, respectively. Especially, the promiscuous epitope NS4A$^{2231\text{-}2239}$ was found to bind to the highest number of HLA-II alleles amongst all the predicted ZIKV epitopes.

Peptide 2k$^{2255\text{-}2263}$ was projected to bind 35 HLA class II alleles with 1.2518 antigenicity score. Out of the seven projected epitopes in NS4B region, NS4B$^{2442\text{-}2452}$ was predicted to bind 43 HLA-II alleles with 0.703 antigenic score. Amongst NS5, epitope NS5$^{3142\text{-}3150}$ was projected to bind 26 alleles with good antigenicity score i.e. 0.6478.

All the prioritized epitopes had >6.3 -log IC50 value which reveals their strong interaction with HLA-II major alleles.

Crystal structures of PrM, Envelope, NS1, NS3, and NS5 were available and were retrieved from Protein DataBank (Berman et al., 2006; Brown et al., 2016; Jain et al., 2016; Sirohi et al., 2016; Upadhyay et al., 2017). Modeled structures of NS2A, NS2B, NS4A, were obtained from Gupta et al (Gupta et al., 2016). However, due to the unavailability of high-quality crystal structure or homology model, the structure of NS4B was modeled by Phyre 2 (Kelley et al., 2015). HLA-II prioritized epitopes were visualized and colored in chimera, and are shown in Fig. 2 (Pettersen et al., 2004).

Identification of homology with human proteome

All the shortlisted epitopes were found to be non-homologous with *Homo sapiens* (taxid 9609). No putative conserved domain of any epitope was predicted in Humans. The predicted epitopes are safe to use as vaccine candidates.

Discussion

Vaccine development is a long-term high-priced process that lasts for years (Leroux-Roels et al., 2011). employing modern computational vaccinology, we have developed insight into ZIKV and utilized the information to screen antigenic as well as immunogenic T cell epitopes. These epitopes can be used in a vaccine to combat epidemic infection of ZIKV. The main objective of our analysis was to evaluate T-cell (both MHC-I and MHC-II interacting T cells) epitopes using bioinformatics tools to evoke cell-mediated as well as humoral response using the available genomes at the time of writing. A similar study was conducted in 2013 in which only T cell epitopes from 54 available genomes were taken into account (Dar et al., 2016). However, the present study is more focused and comprehensive based on 238 genomes.

The prioritized epitope AMILGGFSM of NS2A$^{1189\text{-}1197}$, VPRTDNITL of NS2A$^{1290\text{-}1298}$, SYGWNIVRL of NS5$^{2638\text{-}2646}$, WYMWLGARF of NS5$^{2996\text{-}3004}$ were also reported in the literature (Lund et al., 2011; Sidney et al., 2011; Michael Rasmussen; Mikkel Nors Harndahl.; Anne Bregnballe Kristensen; Ida Kallehauge Nielsen; Kasper W Jorgensen; Anette Stryhn; Morten Nielsen; Sören Buus Buus, 2014; Dar et al., 2016; Huang et al., 2017; Wen et al., 2017) among which AMILGGFSM of NS2A$^{1189\text{-}1197}$ and WYMWLGARF of NS5$^{2996\text{-}3004}$ were found to be experimentally verified (Lund et al., 2011; Sidney et al., 2011; Michael Rasmussen; Mikkel Nors Harndahl.; Anne Bregnballe Kristensen; Ida Kallehauge Nielsen; Kasper W Jorgensen; Anette Stryhn; Morten Nielsen; Sören Buus Buus, 2014; Huang et al., 2017). The experimentally

verified epitopes lie in the non-structural proteins that are crucial for viral replication (Bollati et al., 2010). Our study revealed 19 prioritized HLA-I binding T cell epitopes that lie in structural as well as non-structural proteins and can prove to be good vaccine candidates. Out of these 19, a total of 15 epitopes are novel and are not reported in the literature. All the shortlisted epitopes lie in the surface exposed region (as can be seen in Fig 1 and 2). Some of the epitopes predicted in our study proved their efficacy after experimental evaluation, these epitopes are indicated in Table 3 along with the prediction method. All the experimentally analyzed epitopes except SNRDFVEGM and DTAWDFGSV of $E^{297-305}$ and $E^{716-724}$ respectively, were reported in more than one viruses. Based on this we can hypothesize that novel prioritized epitope predicted in our study can also turn out to be efficient vaccine targets in experimental scrutinization and can be used to generate broad-spectrum immune response.

In case of HLA-II epitopes, VVVLGSQEG of $E^{547-555}$, VREDYSLEC of $NS1^{967-975}$, VKGKEAVHS of $NS1^{984-992}$, VQLLAVPPG, VILAPTRVV, LMCHATFTS, WLAYQVASA of $NS3^{1599-1607}$, $NS3^{1724-1732}$, $NS3^{1764-1772}$ and $NS3^{2048-2056}$ respectively, FGMVTLGAS of region $NS4A^{2204-2212}$, and lastly IKSVSTTSQ of $NS5^{2572-2580}$ and LIGHRPRTT of $NS5^{3374-3382}$ were also reported in literature which revealed the promiscuity of these epitopes. Most of the matching epitopes lie in the zone of NS3 serine protease which is involved in viral replication (Bollati et al., 2010). Moreover, VVYGTCHHK of $PrM^{198-206}$ is predicted to bind both MHC-I and MHC-II alleles. The epitope can elicit both cell-mediated and humoral responses.

The epitopes analyzed and shortlisted in our study were found to be conserved. They do not bear homology to human proteins, so the epitope-based vaccine is not expected to create any autoimmune or harmful inflammatory responses in the human body. The epitope targeting vaccine is an efficient, promising and widely used strategy to prevent viral and bacterial infections. Epitope-based vaccine developmental studies using *in silico* approaches are indicated

in many studies (Dikhit et al., 2016; Alam et al., 2016). Vaccine development studies against Flaviviruses are less because of the natural immunity of animal models toward Flaviviruses. Hence, the computational prediction of epitopes is an effective method to analyze and report vaccine candidate for Flaviviruses. The antigenic and highly conserved epitopes highlighted in our study can be used to generate a multi-epitope vaccine construct that can elicit a more pronounced response than the individual epitopes.

The work presented here is purely computational and it needs *in vitro* studies for verification of results and that is the only limitation of our study. Although wet lab validation studies are considered more accurate and reliable, computational approaches have provided sound basis to proceed further (Brusic & Petrovsky, 2005; Vivona et al., 2008; Patronov & Doytchinova, 2013).

Conclusion

Peptide based vaccines are safe and promising candidates against viral infection. The current study revealed some novel antigenic and promising vaccine candidates from the consensus sequence of 238 genomes of ZIKV. The MHC-I and MHC-II interacting T cell epitopes prioritized in our study can be used to elicit cell mediated and humoral immune response or both. The shortlisted epitopes present in more than one Flaviviruses can be used to construct broad-spectrum vaccine candidate. We believe that methodology followed in this study can be extended to target viruses.

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Declaration

Authors' contributions

AA, TZ, and HAD designed the study. TZ, HAD, MTSK, SAT carried out systemic methodology, analyzed and interpreted the data. TZ, HAD, MTSK, SAT helped in drafting the manuscript. AA, RZP contributed to critical revision of the manuscript.

Competing interests

The authors declare no conflict of interest.

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Figure 1

Visualization of HLA-I predicted epitopes in ZIKV proteins.

All the predicted epitopes were highlighted in the 3D structure of proteins (A) Epitopes DTAWDFGSV, LEHGGCVTV in Envelope crystal structure are shown. (B) Epitopes LLVSFIFRA is highlighted in the modeled structure of NS2A. (C) Epitopes SEVLTAVGL are indicated in the modeled structure of NS2B. (F) Epitopes VPNYNLYIM is shown in the crystal structure of NS3. (I) Epitopes SYGWNIVRL, RSNAALGAI, WYMWLGARF, and ARFLEFEAL are highlighted in the NS5 crystal structure.

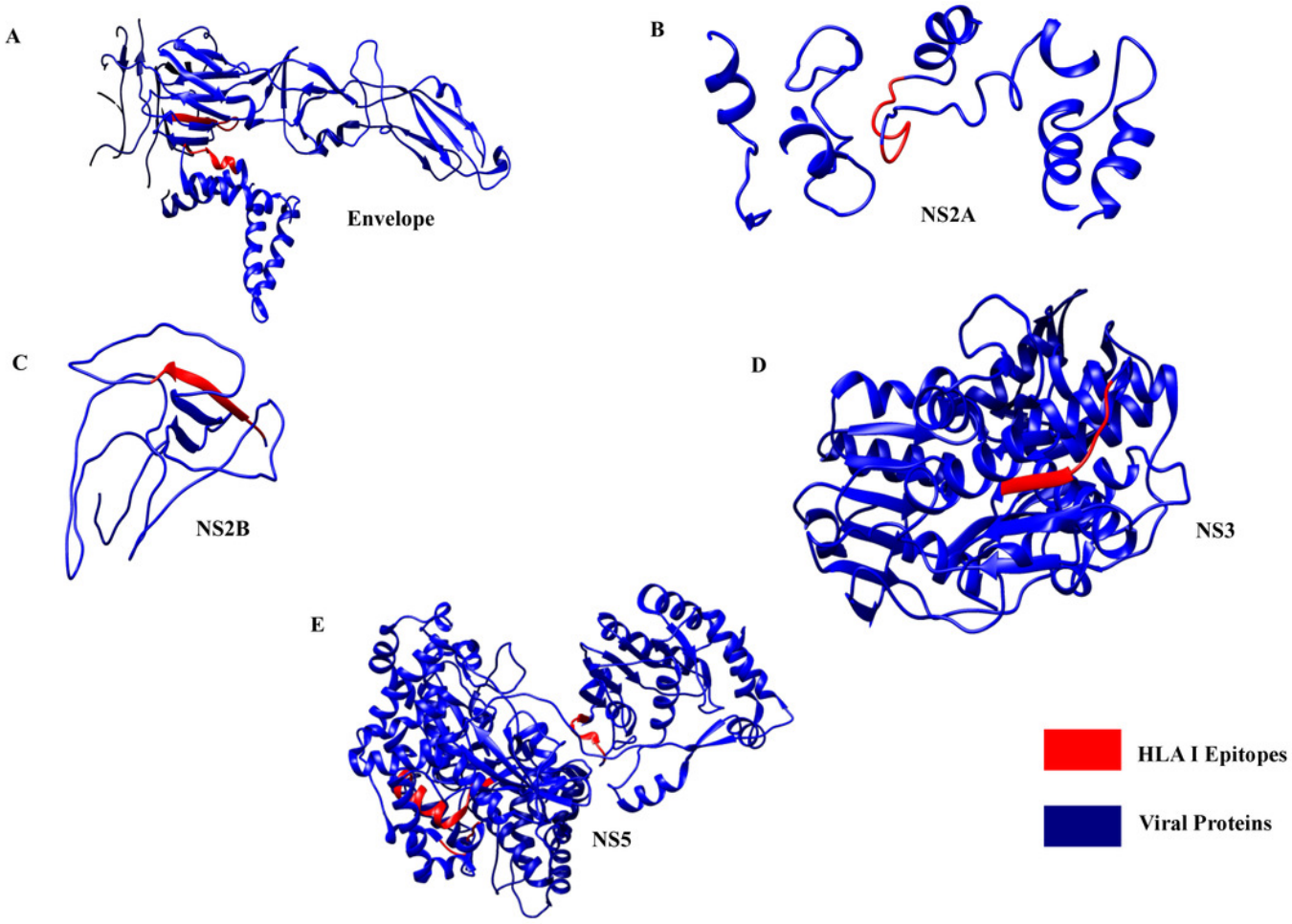

Figure 2

Visualization of HLA-II predicted epitopes in ZIKV proteins

All the predicted epitopes were highlighted in the 3D structure of proteins (A) Epitopes LLGSSTSQK, VIYLVMILL, IYLVMILLI, YLVMILLIA in the crystal structure of PrM. (B) Epitopes YYLTMNNKH and VLIFLSTAV are highlighted in the crystal structure of Envelope. (C) Epitopes FVRAAKTNN, VREDYSLEC, LKRAHLIEM, and FRAKDGCWY are shown in the crystal structure of the NS1 protein. (D) Epitopes VILLMVQEG, VVGLLLLTR, VGLLLLTRS, LLLLTRSGK are shown in the modeled structure of NS2A. (E) Epitopes VGLLIVSYV, VVSGKSVDM, ICGMNPIAI, and YVYVKTGKR in NS2B model are highlighted. (F) Epitopes VILAPTRVV, LMCHATFTS, FVPSVRNGN, VIQLSRKTF, IYLQDGLIA, YLQDGLIAS, VELMKRGDL, and WLAYQVASA in NS3 crystal structure are indicated. (G) Epitopes FVLMRNKGI, MRNKGIGKM, FGMVTLGAS, VVFLLLVVL, and VFLLLVVLI in NS4A are highlighted in model of NS4A. (H) Epitopes IVAIILLVA, IILLVAHYM, MYLIPGLQA, YMYLIPGLQ, VLLIAVAVS, LLIAVAVSS and LIAVAVSSA are shown in modeled NS4B protein. (I) Epitopes IVRLKSGVD, IKSVSTTSQ, ISRQDQRGS, IRNMEAEEV, WSIRETACL, LLYFHRRDL, FHRRDLRLM, and LIGHRPRTT are highlighted in NS5 crystal structure.

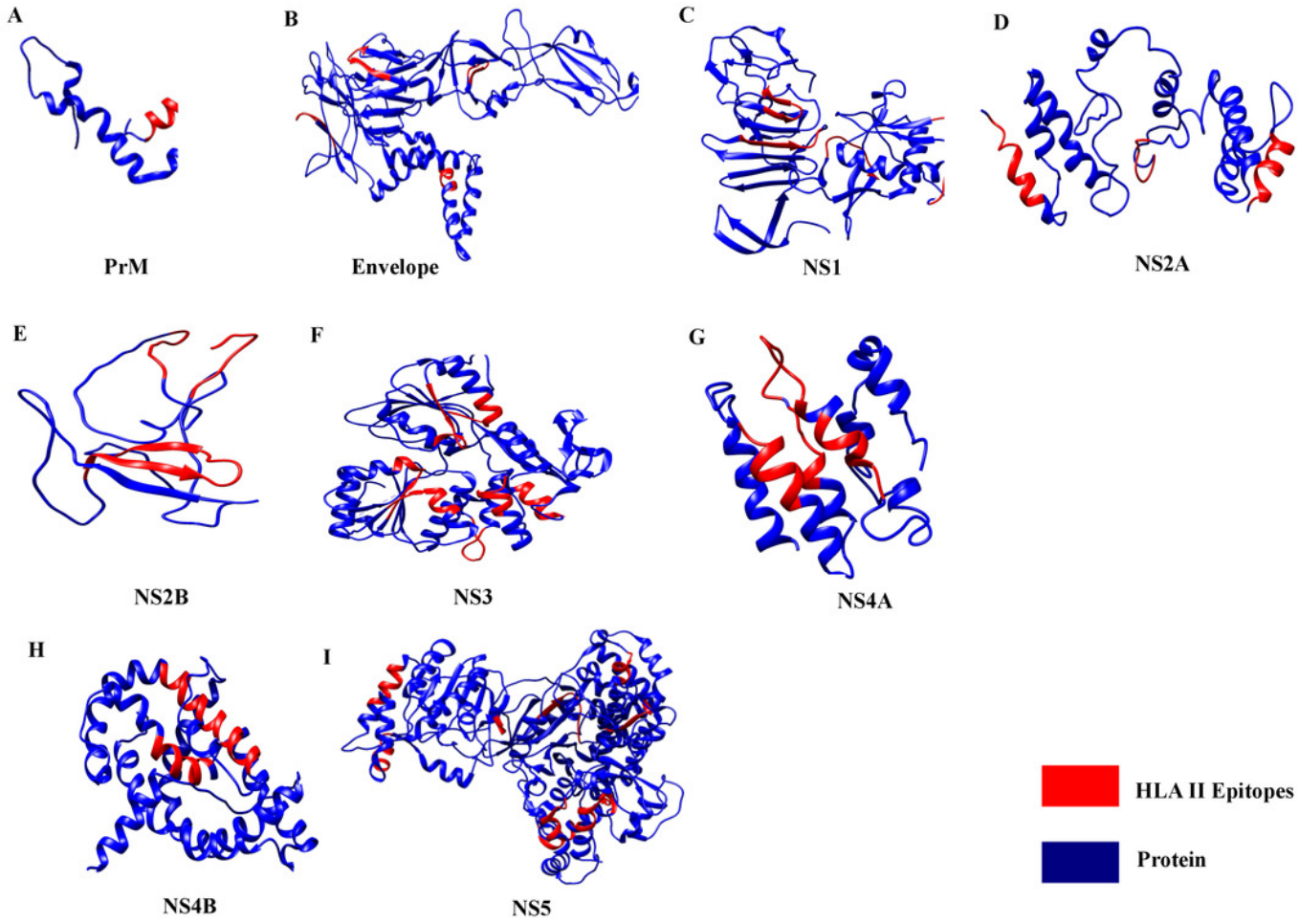

Table 1(on next page)

The prioritized HLA class I-specific CD8 T-cell binding epitopes of the Zika virus polyprotein.

Epitopes having a strong interaction with HLA-I superfamily alleles are prioritized. All the epitopes have good immunogenicity and antigenicity.

Part of Polyprotein	Aa position	Epitope sequence	No. of MHC alleles	Antigenicity	Immunogenicity	Predicted by ProPred I	Predicted by CTL Pred	HLA allele having maximum affinity	1-log50k(aff)	Affinity(nM)
PrM	198	VVYGTCHHK	5	0.6814	0.06615		✓ ü	HLA A0301	0.675	33.79
Envelope	315	LEHGGCVTV	8	0.585	0.08437		✓ ü	HLA B4001	0.722	20.26
	716	DTAWDFGSV	2	2.0217	0.29933		✓ ü	HLA A2601	0.722	20.26
NS2A	1189	AMILGGFSM	6	0.7422	0.09908	✓ ü		HLA -B1501	0.677	32.86
	1235	LLVSFIFRA	9	0.7976	0.21525		✓ ü	HLA A0201	0.746	15.65
	1290	VPRTDNITL	13	0.6454	0.20637	✓ ü		HLA B0702	0.73	18.49
	1299	AILAALTPL	11	0.5738	0.08971	✓ ü		HLA A0201	0.694	27.28

	1353	RLVDPINVV	10	0.7870	0.16886	✓ ü		HLA A0201	0.678	32.48
NS2B	1372	RSWPPSEVL	16	0.5910	0.00283	✓ ü		HLA B5801	0.648	44.96
	1377	SEVLTAVGL	9	0.7064	0.13151	✓ ü		HLA B4001	0.829	6.34
NS3	1778	VPNYNLYIM	14	0.5301	0.05208		✓ ü	HLA B0702	0.568	107.62
Peptide 2k	2251	NQMAIIIMV	11	0.7050	0.24697	✓ ü		HLA B3901	0.576	98.53
								HLA A0201	0.718	21.18
	2374	TPLTLIVAI	16	0.5771	0.20764	✓ ü	✓ ü	HLA B3901	0.592	82.52
	2453	ILSRTAWGW	6	1.1679	0.27975		✓ ü	HLA B5801	0.738	17.11
NS5	2638	SYGWNIVRL	8	1.1058	0.41795	✓ ü		HLA A2402	0.577	97.09
	2925	RSNAALGAI	4	1.0339	0.11639		✓ ü	HLA B5801	0.556	121.41

	2996	WYMWLGARF	9	0.9423	0.25025		✓ ü	HLA A2402	0.801	8.61
	3002	ARFLEFEAL	8	1.0073	0.3419	✓ ü		HLA B3901	0.541	143.16
	3083	RALALAIIK	6	0.7005	0.25188		✓ ü	HLA A0301	0.559	118.33

Table 2 (on next page)

The prioritized HLA class II-specific CD4 T-cell binding epitopes of ZIKV polyprotein.

Epitopes having a strong interaction with HLA-II superfamily alleles are prioritized. All the epitopes have good immunogenicity and antigenicity.

Part of polyprotein	aa position	Epitope sequence	No. of alleles	Antigenicity score	-logIC_{50} (M)	IC_{50} Value (nM)	Alleles' name
Protein C	38	LLGHGPIRM	19	0.9662	6.678	209.89	DRB0101
	50	ILAFLRFTA	18	1.0423	7.035	92.26	DRB0101
	53	FLRFTAIKP	9	0.7263	7.373	42.36	DRB0101
					9.249	0.56	DRB0701
PrM	198	VVYGTCHHK	8	0.6814	6.709	195.43	DRB0401
	267	LLGSSTSQK	11	1.0300	6.739	182.39	DRB0101
	276	VIYLVMILL	9	0.7869	6.539	289.07	DRB0101
	277	IYLVMILLI	38	0.6002	6.778	166.72	DRB0401
					8.73	1.86	DRB0101
	278	YLVMILLIA	31	0.5658	6.664	216.77	DRB0701
					9.027	0.94	DRB010

							1
					6.616	242.1	DRB0401
	492	YYLTMNNKH	11	1.7549	6.927	118.3	DRB0401
					8.606	2.48	DRB0101
	784	VLIFLSTAV	16	0.5846	6.591	256.45	DRB0401
					6.754	176.2	DRB0101
NS1	917	FVRAAKTNN	14	0.8520	6.963	108.89	DRB0401
					8.092	8.09	DRB0101
	965	VREDYSLEC	11	1.6908	6.887	129.72	DRB0401
					6.726	187.93	DRB0101
	1006	LKRAHLIEM	16	1.0212	6.654	221.82	DRB0101
	1054	YRTQMKGPW	9	0.7986	6.555	278.61	DRB0701
					6.651	223.36	DRB0401
					8.109	7.78	DRB010

							1
	1117	FRAKDGCWY	10	2.3636	6.839	144.88	DRB0101
NS2A	1159	VLVILLMVQ	27	0.8091	7.094	80.54	DRB0101
	1161	VILLMVQEG	40	0.8218	6.492	322.11	DRB0401
	1226	IAAFKVRPA	12	1.8641	7.542	28.71	DRB0101
	1269	FKVRPALLV	16	1.3985	7.548	28.31	DRB0701
					6.802	157.76	DRB0401
					8.622	2.39	DRB0101
	1282	WLAIRAMVV	19	0.6740	6.668	214.78	DRB0701
					7.3	50.12	DRB0101
	1298	LAILAALTP	9	0.5735	6.793	161.06	DRB0701
					7.818	15.21	DRB0101
	1347	LGLTAVRLV	11	1.7237	6.696	201.37	DRB0401
					7.228	59.16	DRB010

							1
	1352	VRLVDPINV	40	0.9703	7.034	92.47	DRB0701
					6.766	171.4	DRB0401
	1360	VVGLLLLTR	28	1.3460	6.757	174.98	DRB0101
	1361	VGLLLLTRS	16	1.1066	7.269	53.83	DRB0101
	1363	LLLLTRSGK	19	1.0843	7.259	55.08	DRB0701
					7.42	38.02	DRB0101
NS2B	1407	VGLLIVSYV	11	0.7343	6.862	137.4	DRB0701
					6.845	142.89	DRB0401
					6.528	296.48	DRB0101
	1415	VVSGKSVDM	14	1.4706	7.06	87.1	DRB0401
					6.722	189.67	DRB0101
	1478	ICGMNPIAI	12	0.5728	7.059	87.3	DRB0701
					6.869	135.21	DRB010

							1
	1494	YVYVKTGKR	19	1.2996	6.675	211.35	DRB0401
					8.433	3.69	DRB0101
NS3	1597	VQLLAVPPG	34	0.5392	7.125	74.99	DRB0401
	1656	VVIKNGSYV	17	0.6539	6.854	139.96	DRB0701
					6.967	107.89	DRB0401
	1722	VILAPTRVV	18	0.6400	6.883	130.92	DRB0701
					7.094	80.54	DRB0401
					6.996	100.93	DRB0101
	1762	LMCHATFTS	25	0.6299	6.766	171.4	DRB0101
	1784	YIMDEAHFT	9	0.8165	7.183	65.61	DRB0701
					8.866	1.36	DRB0101
	1864	FVPSVRNGN	11	1.4490	6.541	287.74	DRB0401
					7.57	26.92	DRB010

							1
	1885	VIQLSRKTF	10	1.1234	6.628	235.5	DRB070 1
	1999	IYLQDGLIA	11	0.7520	7.687	20.56	DRB010 1
	2000	YLQDGLIA S	11	0.5242	6.784	164.44	DRB040 1
					8.546	2.84	DRB010 1
	2035	VELMKRG DL	9	0.8255	6.558	276.69	DRB040 1
					6.572	267.92	DRB010 1
	2046	WLAYQVAS A	13	0.6893	6.851	140.93	DRB010 1
	2189	FVLMRNK GI	41	1.0925	7.126	74.82	DRB040 1
					7.201	62.95	DRB010 1
	2192	MRNKGIGK M	28	1.0909	6.567	271.02	DRB040 1
					6.544	285.76	DRB010 1
	2202	FGMVTLGA S	13	1.0230	6.552	280.54	DRB070 1
					6.848	141.91	DRB040

							1
					8.584	2.61	DRB010 1
	2230	VVFLLLVV L	38	0.6070	6.707	196.34	DRB070 1
					7.314	48.53	DRB010 1
	2231	VFLLLVVLI	44	0.7194	7.131	73.96	DRB070 1
					6.853	140.28	DRB040 1
					7.149	70.96	DRB010 1
Peptide 2k	2255	IIIMVAVGL	35	1.2518	7.506	31.19	DRB070 1
					7.911	12.27	DRB010 1
	2258	MVAVGLLG L	16	1.5851	6.942	114.29	DRB010 1
	2261	VGLLGLIT A	13	0.7854	6.765	171.79	DRB040 1
NS4B	2379	IVAIILLVA	31	0.6415	7.691	20.37	DRB010 1
	2382	IILLVAHYM	33	0.5467	7.158	69.5	DRB070 1
					8.37	4.27	DRB010

							1
	2390	MYLIPGLQA	21	0.9741	7.09	81.28	DRB0401
					7.626	23.66	DRB0101
	2389	YMYLIPGLQ	23	0.9295	7.951	11.19	DRB0101
	2442	VLLIAVAVS	43	0.7030	6.966	108.14	DRB0401
					6.78	165.96	DRB0101
	2443	LLIAVAVSS	38	0.6199	7.327	47.1	DRB0701
					7.939	11.51	DRB0101
	2444	LIAVAVSSA	11	0.6057	7.362	43.45	DRB0101
NS5	2544	FYSYKKSGI	12	0.9506	8.284	5.2	DRB0101
	2643	IVRLKSGVD	10	0.5177	7.711	19.45	DRB0101
	2750	IKSVSTTSQ	13	0.8471	6.587	258.82	DRB0101
	3102	LRPAEKGKT	13	1.9040	6.854	139.96	DRB0701
					6.818	152.05	DRB010

							1
	3115	ISRQDQRGS	9	1.8141	7.505	31.26	DRB0101
	3142	IRNMEAEEV	26	0.6478	6.639	229.61	DRB0701
					7.549	28.25	DRB0101
	3268	WSIRETACL	9	0.9492	7.474	33.57	DRB0101
	3286	LLYFHRRDL	18	1.7080	7.161	69.02	DRB0101
	3289	FHRRDLRLM	14	1.3147	8.14	7.24	DRB0101
	3372	LIGHRPRTT	12	1.5275	6.814	153.46	DRB0701

Table 3(on next page)

Predicted experimentally verified epitopes.

Experimentally validated epitopes that were also reported predicted in our analysis are listed here.

Region	Epitope sequences	Experimental verification	Predicted by NetMHC 4.0	Predicted by CTLPred	References
$E^{297-305}$	SNRDFVEGM	ZIKV		✓	(Ngono et al., 2017)
$E^{488-496}$	FSDLYYLTM	ZIKV, DENV	✓		(Wen et al., 2017)
$E^{716-724}$	DTAWDFGSV	DENV	✓	✓	(Sidney et al., 2011)
$NS2A^{1290-1298}$	VPRTDNITL	ZIKV, DENV	✓		(Wen et al., 2017)
$NS5^{2996-3004}$	WYMWLGARF	DENV, YFV	✓	✓	(Harndahl et al., 2007; Lund et al., 2011; Sidney et al., 2011; Michael Rasmussen; Mikkel Nors Harndahl.; Anne

					Bregnballe Kristensen; Ida Kallehauge Nielsen; Kasper W Jorgensen; Anette Stryhn; Morten Nielsen; Sören Buus Buus, 2014)