Potentialities of Bioinformaticaly Predicted Linear B-cell Epitopes on Dengue prM Protein

MN Nadugala[1,2], PH Premaratne[1,2] and CL Goonasekara[1,2]
[1]*Institute for Combinatorial Advanced Research and Education (KDU-CARE), General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka*
[2]*Faculty of Medicine, General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka*
[#]mahesha@kdu.ac.lk

***Abstract*—** *Predicting linear B-cell epitopes using bioinformatic tools is attractive as an initial step for screening such epitopes with potential use in Dengue (DENV) diagnosis and therapeutics, for their high speed and low cost. B-cell epitopes on the premembrane protein (prM) of DENV are major targets of inducing humoral immunity. However, B-cell epitopes on prM protein of DENV have not been well characterized. In this study, three tools namely: BepiPred, Ellipro and SVMTrip, were used to predict linear B cell epitopes of dengue prM protein and results were compared. Further, a concise verification against available biochemical-assay positive data was carried out. The prM protein sequences of 50 strains of each of the four DENV serotype with temporal differences and geographical variations were analysed. Predictions yielded 12 epitopes with variable lengths (5–50 aa). Most predicted epitopes, at least partially, overlap with regions shown to generate antibodies or are recognized by natural antibodies in other studies. The predicted epitopes of the three tools further demonstrated good agreement, where the peptide location had been predicted as epitopes by more than one tool. Collectively, results demonstrate the potentiality of computer based tools in predicting truly immunogenic epitopes. In terms of conservancy of predicted epitopes, many showed low conservancy levels among the four serotypes (less than 70%). Such epitopes could contribute to antibody dependent enhancement of secondary dengue infections rather than neutralization as has been documented. Overall, serotype specific conservation seemed to be higher in epitopes from DENV3 and DENV4, but not in the other two serotypes. Out of the predicted epitopes only two epitopes, EP9 and EP10, have conservation higher than 70%, indicating a potential use of them as a universal vaccine candidate. In conclusion, based on the predictions observed in the current study, the bioinformatic approach is found to be a good and positive initial step to screen potential linear epitopes in proteins*.

***Keywords*—** *Dengue, prM epitope, bioinformatic tools.*

I. INTRODUCTION

Dengue disease exists in over 100 countries and is the most common arthropod-borne viral disease of humans (Guzman *et al*., 2010, Mackey and Liang, 2012). It is caused by Dengue virus (DENV), which exists as four closely-related serotypes (DENV1-DENV4). DENV targets susceptible populations residing in tropical and subtropical regions of the globe. An estimated 400 million people worldwide are infected with DENV annually, leading to approximately 100 million cases of dengue and 21,000 deaths (Thomas and Endy, 2011 and Bhatt, 2013)

Viral protein epitopes are fundamental in the pathogenesis of virus infection and therefore in the development of effective therapeutic and diagnostic tools. As such, identification of B-cell epitopes for DENV prM antibodies possibly will provide important information for the understanding of the pathogenesis of DENV infection, there by contributing to the development of dengue vaccine. Though many efforts have been made into mapping the epitopes of Envelope (E) protein in DENV, only few epitopes have been identified on prM structural protein. The glycoprotein shell of the mature DENV virion consists of 180 copies each of the E protein and mature prM protein. Consequently, the precise antigenic structures of prM, their functions in the immune response and infection pathogenesis remain as areas yet to be explored.

The prM protein is a 166-amino-acid protein that acts as a chaperone for correct folding and assembly of E protein. The cleavage of prM to M is required for DENV maturation and infectivity (Mukhopadhyay *et al*., 2005). Many previous studies have established that prM specific antibodies could mediate DENV specific immune response in humans. These prM specific mAbs are highly cross-reactive among four DENV serotypes even at high concentrations, and do not neutralize infection but potently promote Antibody dependent Enhancing (ADE) infection over a broad range of concentration. It has also been suggested that anti-prM antibodies could render

essentially non-infectious immature DENV (imDENV) highly infectious.

This study was accordingly focused to verify the potentiality of computational methods available to accurately predict B-cell epitopes, in place of traditional experimental methods which are expensive in terms of time, cost and effort. Three bioinformatical tools namely: BepiPred, Ellipro and SVMTrip, were used and the results thus obtained were then compared with each other, followed by a concise verification against available biochemical-assay positive data.

II. MATERIALS AND METHOS

A. Retrieving the protein sequences

The prM protein sequences from 200 variants belong to all 4 serotypes of DENV (DENV1, DENV2, DENV3 and DENV4) were retrieved from National Center for Biotechnology information (NCBI) (http://www.ncbi.nlm.nih.gov/).

The data set includes isolates with temporal differences (isolates from 1963-2011) and geographical variation (representing seven continents) to best represent a wide range of DENV strains. The sequence format utilized for saving downloaded protein sequences in each DENV serotype was FASTA format. All the selected sequences were compared to find mutational and conservative regions by using Clustal W software.

B. B -cell epitope prediction

B-cell epitope prediction of the retrieved protein sequences described on Section A, was carried out by computational B-cell epitope prediction methods. Selection of the appropriate prediction tools used in this analysis was conducted by screening currently available free computational methods.

Selected prediction tools were; BepiPred, Ellipro and SVMTrip. Using the selected computational methods, B-cell epitopes were predicted as the following example;

For DENV1 of prM protein, 50 sequences were uploaded for each of the 03 computational tools. Results of the each tool were combined to obtain the final list of epitopes predicted for DENV1 prM.

C. Prediction of epitope conservancy

Conservancy pattern of the predicted epitopes was determined by using the Epitope Conservancy Analysis tool developed by IEDB (www.iedb.org). In addition to overall conservancy measurement among four serotypes, conservancy within serotype was also measured.

D. Visualization of conservation

The level of overall conservation was visualized using WebLogo 3.0, in which letter size is proportional to the level of conservation of each amino acid in the epitope sequence.

E. Construction of phylogentic tree for each epitope

The multiple sequence alignments (MSA) were generated to each epitope from the retrieved protein sequences; this MSA formed the basis for construction of phylogenic tree. The phylogenetic trees were used to elucidate the evolutionary distance among variant within an epitope. Neighbour-joining method on MEGA6 was used for the construction of phylogenetic trees.

F. Homology Mapping *of epitopes*

Mapping of linear epitopes to 3D structures of proteins was done using homology mapping tool available in IEDB.

III. RESULTS AND DISCUSSION

Multiple sequence alignment (MSA) of prM protein of 200 isolates (50 each from the 4 serotype) was generated on MEGA6. A partially conserved region (65-117 a.a) was found distinctive on all 200 isolates on the WebLogo as shown in the Figure 1.

In total twelve epitopes with variable lengths (5–50 a.a) were predicted: four epitopes by BepiPred, six by Ellipro and two others by SVMTriP (Table 1). Detail composition of amino acid arrangement is demonstrated in WebLogo results (Table 2).

The overall conservancy was more than 50% in most of the epitopes (except EP3, EP5, EP6, EP7 and EP11) but less than 75%. At the same time it was noted that DENV 3 and 4 isolates are genetically more similar within the serotype than isolates of DENV1 and DENV2. Because of same reason DENV3 and 4 isolates had yielded epitopes with higher serotypic conservancy (>80). Further, during phylogenetic analysis neither specific phylogenetic patterns nor geographical variation patterns were observed within the predicted epitope sequences (Data not shown).

The mapping carried out establishes that epitopes 8, 9 and 10 are located in the partially conserved region of prM. It is worthy to note that EP9 and EP10 showed an overall conservancy of over 70% and a serotypic conservancy higher than 85% within each of the four serotype. This result suggests EP9 and EP10 may have a potential use as universal vaccine candidates.

Table 1. Characteristics of epitopes identified by bioinformatics

Epitope (EP) ID	#Epitope sequence *sequence position	Epitope length (a.a)	Method of Prediction (Score)	Percentage Conservancy within the Serotype (Minimum %)				Percentage Conservancy among four serotypes
				DENV1	DENV2	DENV3	DENV4	
EP1	FHLTTRDGE *1-9	9	Ellipro (0.6)	88	77	88	88	55
EP2	TRDGEPH *5-10	7	BepiPred (0.4-1.1)	85	85	85	85	57
EP3	GKQERGKS *15-22	8	Ellipro (0.5)	87	37	87	87	25
EP4	KTAEG *26-30	5	Ellipro (0.5)	80	60	100	100	60
EP5	IAMDL *37-41	5	Ellipro (0.5)	100	40	100	80	40
EP6	LLTETEPEDID *55-65	11	Ellipro (0.6)	36	81	81	90	18
EP7	LLTETEPEDID *55-65	11	BepiPred (0.-1.7)	36	81	81	90	18
EP8	TCTQTGEHRREK *79-90	12	BepiPred (0.5-1.2)	91	75	91	91	58
EP9	VALAPHVGMGLETRTETWMS *93-112	20	SVMTrip (1.0)	95	100	95	90	75
EP10	ETWMSSEGAWK *108-118	11	BepiPred (0.4-0.8)	90	90	100	100	72
EP11	GAWKHAQRVETWILRHPGFTIL ALFLAHTIGTSGTQKVVIFILLML VPS *115-165	50	Ellipro (0.7)	86	80	94	94	46
EP12	ALRHPGFTILALFLAHTIGT *127-146	20	SVMTriP (0.8)	90	95	85	90	50

The rationale behind this approach is that conserved epitopes constitute the regions in DENV proteins with minimal amino acid differences among different DENV serotypes/variants, and therefore will cause the least if not no variability, in immune response against different dengue viral serotypes/strains. Thus, such epitopes with neutralizing immunogenicity will be excellent candidates for vaccine development.

Predictions done by BepiPred and Ellipro tools converged to an identical location of 55-65 for EP6 and EP7. Interestingly this peptide stretch has shown a higher serotypic conservancy within DENV2 (81%), DENV3 (81%) and DENV4 (90%) while showing lower serotypic conservancy in DENV1 (36%).

A previous study on DENV infected mice and humans using prM protein of DENV2 too had established that production of specific antibodies against the region 57-71, indicating the importance of EP6 and EP7 during natural infection (Song *et al.,* 2013). This evidence reinforces the potentiality of the computer based predictions.

Figure 1. Partially conserved region (65-117) on WebLogo

*Logo depicts amino acid composition of all 200 isolate at each position within the epitope sequence whereas the height of symbols within the stack reflects the relative frequency of the corresponding amino acid at that position.

Table 2. Conservation depicted epitopes on Weblogo

Epitope ID	WebLogo results*
EP1	
EP2	
EP3	
EP4	
EP5	
EP6	
EP7	
EP8	
EP9	
EP10	
EP11	
EP12	

EP3 and EP4 did fall on peptide sequence from 19-34. A separate previous study again have concluded that peptide sequence from 19-34 could elicit high titer antibodies in Balb/c mice, and this epitope could react with sera from DENV2 infected patients, suggesting that specific antibodies against epitope were elicited in both. DENV infected mice and human.

In addition, that study had demonstrated that anti sera showed limited neutralizing activity but significant antibody dependant enhancement (ADE) activity toward standard DENV serotypes and immature DENV (imDENV).

Hence, it seems responsible to hypothesize that peptide sequence 19-34 as an infection enhancing epitope (Luo *et al*., 2015). In addition EP3 includes the amino acid residues 14 to 18, infection enhancing epitope (Luo *et al*., 2013). The less overall conservancy observed with EP3 in the current study too suggest that this region is probably infection enhancing rather than neutralizing.

These findings may provide significant implications for future vaccine design and facilitate understanding the pathogenesis of DENV infection.

In the study done by Song *et al* (2013) positive epitopes on prM have been predicted bioinformaticaly in regions of 6-10 and 79-86 which are represented by EP2 (6-10) and by EP8 (79-86) respectively in the present study.

In 2002 Cardosa *et. al* has proven that antibodies against the prM protein of Dengue viruses do not react with Japanese encephalitis virus (JEV) prM. It is conceivable that prM is a useful viral protein to investigate as a tool in differentiating antibody responses to different flaviviruses and thus provide a method to investigate specific flavivirus seroepidemiology.

IV. CONCLUTIONS

This study accordingly concludes that:

1. Bioinformatic tools can be considered as a good initial step to screen potential linear B-cell epitopes in proteins with diagnostics or therapeutic interests.
2. Most epitopes of prM showed high serotypic conservancy but low overall conservancy, suggesting more of an infection enhancing role rather than neutralizing role.
3. EP3 and EP4 are potential infection enhancing epitopes.
4. EP9 and EP10 are potential vaccine candidates subjected to establishment of a high potency of neutralization during the laboratory tests to follow.

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BIOGROPHIES OF AUTHORS

Ms. Mahesha Nadugala is a PhD candidate of KDU working on dengue epitope screening as targets for dengue vaccine development, under the supervision of Dr. Charitha Goonasekara and Dr. Prassad Premaratne.

Dr. Charitha Goonasekara is a Senior Lecturer at the Faculty of Medicine, KDU and an affiliated member of KDU-CARE.

Dr. Prasad Premaratne is a Senior Lecturer at the Faculty of Medicine, KDU and an affiliated member of KDU-CARE.