PREDICTION EPITOPE OF NS1 DENGUE FOR VACCINE DEVELOPMENT BY IMMUNOINFORMATIC APPROACHMENT

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Abstract. NS1 protein is a multifunctional protein that can be used as an antigen for vaccine development. This study investigated NS1 protein as a predictor for various immune cell epitopes in human host cells in an immunoinformatic manner. The research started by predicting the epitopes of various immune cells such as T and B cells by including the NS1 protein as input. As a result, the NS1 protein has the potential to be a vaccine candidate that needs to be studied further in the laboratory and in test animals..

INTRODUCTION

Dengue is caused by one of any of four related viruses: Dengue virus 1, 2, 3, and 4 (DENV 1-4). For this reason, a person can be infected with a dengue virus several times in his or her lifetime. Dengue viruses are spread to people through the bites of infected mosquitoes, mainly Aedes aegypti mosquitoes (1). DENV infection has infected nearly 1 billion people with symptoms ranging from mild to severe. Victims of this disease are mostly in tropical countries because of the environment that supports the vector's life (2).

Most prevention methods for DENV infection are to keep the environment clean, no standing water, burying all containers that can hold water and sprinkling larvicides in any water reservoirs such as ponds and etc.(3)(4). This is effective if everyone in the same environment applies it, it's just that sometimes, not everyone obeys this rule. Another prevention method is Dengue vaccination (5)(6). However, the dengue vaccine currently circulating is based on live-attenuated virus (LAV) which still has many drawbacks(7). the vaccine can only be used at the age of over nine years, is more effective in patients who have had a history of dengue (secondary infection) and there are still issues of Antibody Dependent Enhancement (ADE)(8). Therefore, it is necessary to develop another platform-based vaccine that is safer and does not cause side effects and can be used for children, who are the age most vulnerable to severe infections such as ruptured blood vessels(9).

There are currently five platforms for dengue vaccine development, including live attenuated vaccine, viral vectored vaccine, inactivated vaccine, recombinant subunit vaccine, and DNA vaccine(10). They act primarily by increasing the immune responses against DENV E protein and non-structural protein 1 (NS1)(11). Envelope protein (E) is the outermost protein of DENV and is an ideal target for binding by host antibodies(12). The structure of protein E is initially synthesized as a dimer, but low pH induces a conformational change to a trimer. The E protein also provides the first point of contact between the virus and the host cell(13). The mechanism is that protein E interacts with several molecules (ICAM3-grabbing nonintegrin, CD209 and mannose receptors) that mediate the attachment

and entry of the virus into cells. These attachment factors help in concentrating the virus on the cell surface which can increase its access to specific cellular receptors. it is this mechanism that researchers have developed for the dengue vaccine. The drawback is that research on E protein still has cross-reactivity with host cell antibodies.(14–16)

The latest development of current vaccines is with NS1 as a predictor of the binding epitope of either cellular or humoral immunity. According to research by Lebeau et al, 2021, the NS1 glycoprotein has recently emerged as a potential viral antigen target for the development of dengue candidate vaccines. The NS1 protein is a protein that is a virulence factor for DENV and also acts as a regulator of the viral replication process(17). The structure of NS1 was first synthesized by the virus in the form of a monomer, then in the post-translational modification process it underwent glycosylation and formed a dimer(18). The NS1 protein in the dimer form is an active protein that has a function in the DENV virus replication process while in the hexamer form it functions as a virulence factor. The NS1 protein is secreted as a hexameric lipoparticle that is independent of viral replication(19). The secreted NS1 protein can be used for viral RNA replication, virion assembly, or particle release and is present in patient serum. High levels of NS1 in the patient's serum correlate with the severity of the viral infection. Therefore, the amount of NS1 protein during infection is very large and specific in host cells so that NS1-based vaccines represent promising strategies that have the potential to significantly advance dengue vaccine development.(20)

This study has used the NS1 protein as a target protein for prediction of vaccine epitopes. Given that their efficacy could be greatly improved by reducing off-target antibody responses to the DENV NS1 protein, it would be of great interest to use an immunoinformatic approach as an early stage of vaccine development. It is hoped that the peptide sequence as an epitope can be used to become a vaccine in the future.

METHODS

Multiple sequence alignment
Prior to predicting the epitopes of the reference sequences, multiple sequence alignment (MSA) was performed on the NS1 genome sequences of the four DENV serotypes (DENV 1, DENV 2, DENV 3, and DENV 4) using the MAFFT version 7 server (https://mafft. cbrc.jp/alignment/server/) online version alignment menu options. All strategies and parameters used in advanced settings are left at default. This step was carried out to obtain the MSA results from the entire NS1 genome in advance to speed up the alignment process with the reference sequence.(21)

Consensus sequence construction
Consensus sequence construction was built from the results of MSA amino acid sequences from NS1 DENV using the EMBOSS Consensus Sequences server (https://www.ebi.ac.uk/Tools/msa/emboss_cons/) from EMBL-EBI. Parameter settings are selected in default mode, namely by using the BLOSUM62 matrix. BLOSUM (Blocks Substitution Matrix) itself is a matrix substitution in protein sequence alignment that is used to provide an assessment of local alignment between evolutionarily divergent protein sequences. Meanwhile, BLOSUM62 is a matrix that is constructed using sequences that have ≥62% similarity (identity) which will be grouped into clusters.

T cell epitope prediction
Prediction of cytotoxic T cell (CTL) epitopes was performed using the NET CTL 1.2 web server (https://services.healthtech.dtu.dk/service.php?NetCTL-1.2). The server predicted CTL epitopes with amino acid length of 9 mer against 12 MHC I supertypes (A1, A2, A3, A24, A26, B7, B8, B27, B39, B44, B58 and B62). CTL epitope prediction was performed by setting the default epitope identification threshold, the C terminal cleavage pe weights and transporter weights associated with epitope processing efficiency were 0.75, 0.15, and 0.05, respectively. Epitope prediction was carried out based on the C-terminal cleavage proteosomal approach, TAP transport binding efficiency and binding affinity with MHC class I. Helper T cell (HTL) epitope prediction was performed using the NetMHCII 2.3 server (https://services.healthtech.dtu.dk/ service.php?NetMHCII-2.3). The server predicts HTL epitope with amino acid length of 15 mer against HLA-DR, HLA-DQ and HLA-DP. The prediction used threshold values for strong and weak HLA binding of 2% and 10%.(22)

Antigenicity, allergenicity and toxicity prediction

From the prediction results of the CTL and HTL epitopes, further screening was carried out for antigenicity, allergy and toxicity properties. The antigenicity test used the Vaxijen v2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). The server can predict bacterial, viral and tumor antigens with an accuracy of 70% to 89% (Doytchinova & Flower, 2007). The antigenicity analysis selected the virus as the target and the antigenicity threshold was 0.4. Allergenicity testing was also carried out on the predicted epitopes using the AllerTop server (https://www.ddg-pharmfac.net/AllerTOP/index.html) (Dimitrov et al., 2014). The ToxinPred server was used to predict the toxicity properties of the epitope. The predictions used the SVM (Swiss-Prot) method (Gupta et al., 2013). The ability of HTL epitopes to induce cytokines in the immune system is different, so it is necessary to predict the epitopes that can induce cytokines. Prediction of epitopes that can induce IL-4 and IFN was carried out using IL4Pred servers (https://webs.iiitd.edu.in/raghava/il4pred/design.php) and IFNepitope (https://webs.iiitd.edu.in /raghava/ifnepitope/design.php). In predicting HTL epitopes that can induce IL4, the method used is based on SVM with a threshold of 0.2 (Dhanda, Gupta, et al., 2013). Meanwhile, the prediction of HTL epitopes that can induce IFN uses SVM-based methods and models of IFN versus other cytokines (23)

B cell epitope prediction

Induction of the humoral immune system is important to produce a rapid defense system against pathogenic infections and tumors. B cells are lyphocytes that play an important role in inducing the humoral immune system, so B cell epitope prediction is needed. Linear B cell prediction is carried out using the BCPREDS server (http://ailab-projects1.ist.psu.edu:8080/bcpred/predict.html). While the prediction of discounted B cells is used by the Ellipro server (http://tools.iedb.org/ellipro/).

Predict cross reactions

The predicted epitopes that have been obtained, are predicted to be similar to human protein data to avoid cross-reactivity when immunization is carried out later. The prediction of the similarity of the peptide sequences was carried out using the Protein Information Resource (PIR) server (https://research.bioinformatics.udel.edu/peptidematch/batchpeptidematch.jsp).

RESULT AND DISCUSSION

1. Construction of the consensus sequence

In this study, the approach used for the development of this vaccine candidate was an amino acid sequence consensus-based approach. Thus, the sequence of each protein as a result of translation from aligned nucleotides is constructed to form a consensus sequence. Consensus sequence construction is done by aligning and comparing the entire sequence, then selecting the amino acid residue that appears the most from each sequence or position in the sequence. The development of this consensus sequence is expected to effectively describe genetic profiles and conserved epitope information that can potentially induce cross-reactions in cellular immune responses.

2. T cell epitope prediction

Prediction of T cell epitope is done by mapping the target protein sequence to the HLA allele of the world's population. It is known that T cells can only recognize antigens that have formed complexes with HLA that will be presented by APC (antigen presenting cells). The HLA molecule itself is very polymorphic, especially in the position or peptide binding region, known as the peptide-binding region, so that each MHC allele has different binding specificity. Each population in a certain area or ethnicity usually shows a different frequency of allele types. Therefore, in this study, the vaccine was aimed at all alleles in the world so that the epitope contained in the vaccine has high binding specificity for the alleles that are owned by the majority of the world's population. In this study, the selection of epitopes was epitope that could be recognized by at least three MHC class I and MHC class II alleles. Prediction of class I HLA epitopes was done for 12 supertypes and for HTL epitopes it was done for 54 HLA alleles. Four epitope

sequences were obtained which were predicted to have good affinity for more than two class I HLA alleles used in the study. The peptide sequences that are predicted to have the potential to bind to HLA class I indicate that these sequences are able to form complexes with HLA class I which are expressed by immune cells and other body cells to then be presented to naïve CD8+ T cells. The introduction of the epitope to naïve CD8+ T cells serves to activate and induce the differentiation of naïve cells into effector CD8 T cells, in the form of cytotoxic T cells (CTL), so that the peptide sequence with this ability is called the CD8+ T epitope or CTL epitope.

This research also predicts helper T cell epitopes against 54 HLA alleles in the world. Activation of CD4 T cells into T-helper cells (HTL) is crucial because these cells function in regulating the work of other immune cells that play a role in the immune response to viral infections, including the process of priming naïve CD8 T cells, cell formation and maintenance. T CD8 memory. From the prediction of epitopes, antigenicity, allergenicity, and toxicity, 1 CTL epitope and 8 HTL epitopes were obtained as potential antigens.(24–26)

Table 1 Prediction of CTL epitopes

Epitope	MHC I allele supertype	antigenicity	toxicity	allergenicity	Cross reactions
KAVHADMGY	A26, B58, B62	1.4126	non toxic	allergens	no
NVHTWTEQY	A1, A26, B62	-1.1528	non toxic	allergens	no
ITNELNHIL	B39, B58, B62	0.1374	non toxic	allergens	no
FTTNIWLKL	A1, A26, B39	-0.1743	non toxic	allergens	no

Table 2 HTL epitope predictions

Epitope	MHC II allele supertype	antigenicity	toxicity	allergenicity	IL-4 Inducers	IFN-γ	Cross reactions
GEDGCWYGMEIRPIK	DRB1 0901, DPA10103-DPB10402, DQA10201-DQB10402, DQA10303-DQB10402, DQA10501-DQB10402	0939	Non Toxic	Non Allergen	inducer	Positive	No
CWYGMEIRPIKEKEE	DRB1 0901, DQA10201-DQB10402, DQA10501-DQB10402	1.2107	Non Toxic	Non Allergen	inducer	Positive	No
ELKYSWKTWGKAKII	DRB1 1101, DRB5 0101, DQA10201-DQB10402, DQA10601-DQB10402	0.6891	Non Toxic	Non Allergen	inducer	Positive	No
EKAVHADMGYWIESE	DRB3 0101, DQA10301-DQB10302, DQA10401-DQB10402	0.4949	Non Toxic	Non Allergen	inducer	Positive	No
KAVHADMGYWIESEK	DRB3 0101, DQA10101-DQB10501, DQA10104-DQB10503, DQA10301-DQB10302, DQA10401-DQB10402	0.5547	Non Toxic	Non Allergen	inducer	Positive	No

| | 1,093 | Non Toxic | Non Allergen | inducer | Positive | No |

| PMELKYSWK TWGKAK | DRB50101, DQA10201-DQB10402, DQA10601-DQB10402 | | | | | |

3. Linear B cell epitope prediction

B cell epitope prediction was carried out on target protein consensus sequences (NS1) to obtain peptide sequences with a certain length that can be recognized by B cell receptors so that later vaccine candidates prepared from these sequences are able to induce humoral immune responses. B cell epitope basically can be linear (continuous) and discontinuous or conformational (discontinuous). B cell linear epitoprediction was performed using the Bepipred IEDB server (http://tools.iedb.org/bcell/). The server predicts the amino acid sequence that can be recognized by B cell receptors (BCR). The results of the B cell eptop prediction are in the following table.(27–34)

Table 3 B-cell epitope prediction

No.	Start	end	Peptides	Length
1	7	12	NWKGKE	6
2	26	41	HTWTEQYKFQPDSPKR	16
3	50	53	EEGV	4
4	92	129	VKGILEQGKRALRPQPMELKYSWKTWGKAKIITAEVQN	38
5	139	149	TPECPNEQRAW	11
6	173	177	YTQVC	5
7	226	240	SHTLWSNGVLESEMI	15
8	246	270	AGPISQHNYRPGYHTQTAGPWHLGK	25
9	276	279	DYCE	4
10	283	283	V	1
11	287	316	EDCGNRGPSLRTTTVSGKLIHEWCCRSCTL	30
12	322	322	R	1
13	336	346	IKEKEENMVKS	11

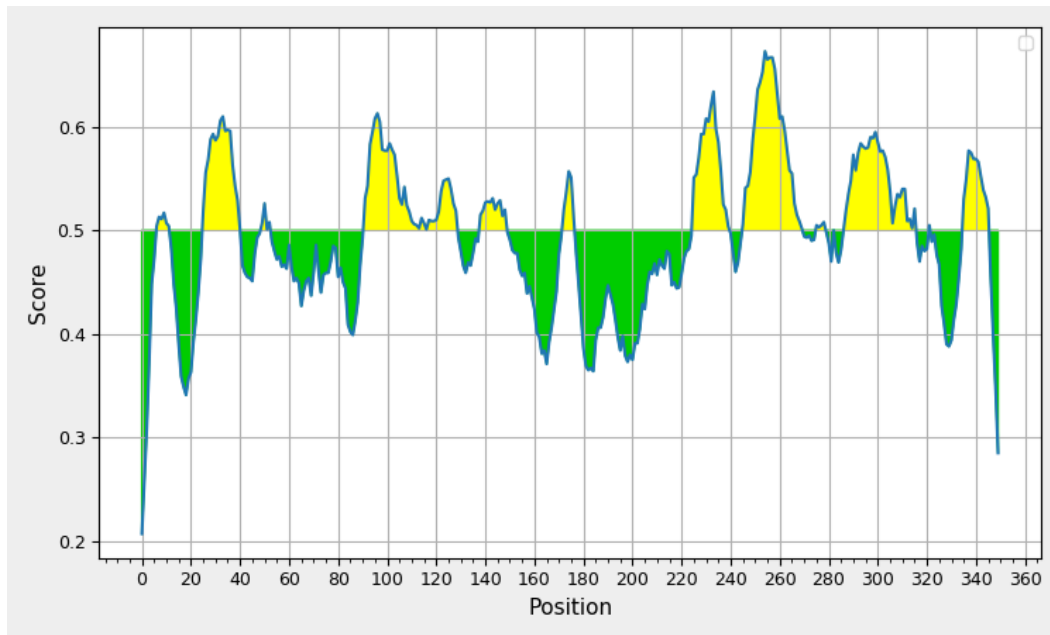*The red one overlaps with the HTL epitopes

Figure 1 candidate vaccine from NS1 epitope.

The yellow area is a potential epitope with a threshold of 0.5

CONCLUSION

NS1 protein has potential as an epitope platform vaccine candidate. however, in vitro and in vivo test studies must be carried out

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