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An Immuno-informatics Approach for the Design of Subunit Vaccine for Non-human Homolog Proteins of *A. aegypti* Species

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Abstract

Mosquito borne diseases account for a major fraction of vector-borne diseases that account for significant morbidity amongst the population worldwide. The prevalence rate of mosquitoes in the common habitable area is so high that even after the implementation of several eradication programs, their disease-causing rate is still quite significant. A. aegypti and A. albopictus are two prominent species of genus Aedes, which transmit viruses causing several infectious diseases such as dengue, zika virus, chikungunya, and yellow fever. The spread of Aedes-borne illnesses can be prevented with the use of techniques targeting vectors specifically, human hosts and interaction between humans and vectors. Vector control techniques are primarily used because they provide direct or biological reduction/elimination of vectors while inflicting minimal impact on human hosts. Presently, the availability of specific treatment for these infections is absent, only symptom targeting is the available approach. Therefore, the present study was undertaken to design a multi-epitope subunit vaccine from novel non-human homologs for vector control proteins for Aedes genus using an immuno-informatics approach. Sequences of the six novel targets for Aedes involved in vector specific processes such as host-seeking behaviour, odorant receptors, oocyte formation and neuropeptide activity were obtained from UniProtKB, followed by analysis with in-silico tools. Identification of T-cell and B-cell epitopes was done using NetCTL and IEDB resource server, while AntigenPro and Aller TOP were used to access antigenicity and allergenicity of the epitopes and vaccine construct. The tertiary structure of vaccine construct having 321 amino acid residues was predicted, followed by Protein-protein docking with TLR-4 using ClusPro. The resulting structure obtained showed high binding energy and proper orientation suggesting strong interactions, indicating that the multi-epitope subunit vaccine construct may be able to trigger a significant immunological response.

Keywords: Immuno-informatics approach, multi-epitope subunit vaccine, protein-protein docking, T-cytotoxic and T-helper cells, Aedes mosquito, vector-borne diseases

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INTRODUCTION

Vector-borne diseases (VBDs) concern serious global public health issues and economic consequences. They account for more than 1,000,000 deaths and 1 billion contaminations, respectively worldwide. They are spread by hematophagous arthropods such as mosquitoes, ticks, sand flies, and triatomine bugs, common in tropical and subtropical areas where mosquito-borne illnesses make up a large fraction of the disease load [1].

The most prevalent viral infections spread or carried by Aedes mosquitoes are Dengue, Zika,

Chikungunya, and Yellow Fever, with varying rates of emergence in various regions of the world each year. The bite of the infected vector, which transfers the virus into the body of the receiving organism, shares the same niche. The *Aedes* mosquito has two separate life cycles: enzootic and urban epidemic [2].

The enzootic cycle occurs mostly in human-inhabitable environments, such as rural or wooded regions with wild mosquitoes and non-human primates, whereas the urban cycle occurs in areas with well-developed urbanization, i.e., locations where humans dwell and are inhabited by urban mosquito species. It thrives in thickly populated regions which need dependable water supplies, and proper sanitization and waste disposal [3].

Arthropod-borne disease breakouts are known to cause significant morbidity and mortality and in spite of several programs or precautions implemented by the government, their prevalence amongst the common population is still unmanageable [4]. The currently available vector eradication programs specifically target the vector, its human host, or the interaction between host and vectors emphasizing on removing the vectors with chemical or biological approaches [5]. Prevention strategies include cleaning of any potential larval growth sites, using mosquito repellent, and wearing full-length clothing while going outside that cannot be followed up in every locality or every possible area. Therefore, there is a need to develop a vaccination programme for targeting vectors that are responsible for a number of infectious diseases.

Vaccinomics combines systems biology with immunogenetics and genomics to advance our basic understanding of vaccines and their development. This existing insight will be used to overcome the challenges in developing effective vaccines to protect against infections that have the largest current health impact amongst the population. In recent years, immuno-informatics approaches have been used to design multi-epitope subunit vaccines against vector components that are known to have several immunogenic and antigenic proteins which are responsible for enhanced infectivity [2, 6, 7]. Therefore, the present study was undertaken to identify antigenic T_H and T_C cell epitopes from non-human homolog proteins of *Aedes* genus identified in our previous study, to impede the transmission of disease-causing pathogen design a multi epitope-subunit vaccine [8].

METHODOLOGY

Retrieval of Immunogenic Protein Sequences

Sequences of the 12 non-human homologous proteins of the *Aedes* genus [8] were retrieved from UniProt Knowledgebase [9] in FASTA format.

Vaxigen Assessment of the Potential Target Proteins

Analysis of antigenicity for retrieved proteins was done using Vaxigen having a favourable threshold value of ≥0.4, while sequences having a threshold of ≤0.4 were discarded as they had non-antigenic nature [10].

T_C-cell Epitope Prediction and MHC-I Allele Analysis

Using the NetCTL-1.2 server, TC-cell epitopes were predicted based on a threshold value of 0.75 for a combinatorial score, where prediction is dependent on the combined score of TAP transport efficiency scores, MHC-I binding, and proteasomal C-terminal cleavage [11]. Using the MHC-I binding tool of the IEDB server (Immune Epitope Database), the immunogenicity score of the anticipated epitopes was examined [12].

T_h-cell Epitope Prediction

The prediction of T_H-cell epitopes was done using the MHC-II tool of IEDB resource. Utilizing a network-based method called SMM (stabilized matrix base method), on the basis of the percentile rank (<1) of peptides that bind to MHC-I molecules was used to evaluate anticipated epitopes [13].

IFN-gamma Induced Epitope Analysis

Prediction of T_H-cell epitopes for their potential to elicit IFN-gamma production was done using the INfepitope server, whose predictions are based upon models involving hybrid, motif-based, and SVM (Support Vector Machine) approaches [14].

Allergenicity and Antigenicity Assessment

Aller TOP server was used in predicting the allergenicity of T_C, T_H-cell epitopes, and the vaccine construct. It predicts the sequences as either non-allergen or allergen based on the concerning physiochemical properties [15]. On the other hand, Antigen Pro was used to analyse the antigenicity score of the designed vaccine that uses an alignment-free, sequence-based pathogen-independent approach [16].

Prediction, Refinement, and Validation of Tertiary Structure of Vaccine Construct

The prediction of the tertiary structure of the vaccine construct was done using the online server I-TASSER. It is based on the hierarchical approach for structure and function prediction of the protein sequence and uses multiple threading approaches for identifying structural templates from PDB [17]. This was followed by refinement of the predicted structure using Galaxy Refine which performs sequential structure perturbation and subsequent overall structural relaxation based upon a molecular dynamic's simulation approach [18]. Finally, the quality parameters of the structure were evaluated by generating Ramachandran plot using Rampage server [19].

Docking Studies

To access the binding affinity and binding pose of the multi-subunit epitope vaccine construct and TLR-4 (PDB ID: 4G84), protein-protein docking was performed using ClusPro server [20].

IN-SILICO MULTI-EPITOPE SUBUNIT VACCINE DESIGN

The sequences of 12 non-human homologous proteins of *Aedes* genus reported were retrieved from UniProt (Table 1). These proteins were found to be mostly involved in *Aedes* specific pathways and mechanisms such as host-seeking behaviour, odorant receptors, pigmentation, oocyte formation, and neuropeptide activity [8].

Antigenicity Assessment of Potential Target Proteins for Design of Vaccine Construct

All these 12 protein sequences were analysed using VaxiJen and AntigenPro server to predict the sequence with the highest antigenicity. Out of the 12 sequences, 11 sequences were identified as probable antigens having favourable threshold of ≥0.4 and ≥0.7 for VaxiJen and AntigenPro servers respectively [10, 16], while CORZ_AEDAE was returned as a probable non-antigen. The four unreviewed proteins namely A0A6I8TQT6_AEDAE, A0A6I8TNK9_AEDAE, Q177I5_AEDAE, and Q177I2_AEDAE were also not considered for vaccine design, as their role in Aedes is uncertain. Protein Vitellogenin-A1 (VIT1_AEDAE) having 2,169 amino acid residues was also not included in the design of the vaccine construct due to its length, and therefore may be further explored as a potential target for drug design.

Table 1. 12 protein sequences retrieved from UniProt.

S.N.	Protein name	S.N.	Protein name
1	ORCO_AEDAE	7	VIT1_AEDAE
2	BURS_AEDAE	8	CORZ_AEDAE
3	ATP8_AEDAE	9	A0A6I8TQT6_AEDAE
4	SNPF_AEDAE	10	A0A6I8TNK9_AEDAE
5	ALL3_AEDAE	11	Q177I5_AEDAE
6	HPEP_AEDAE	12	Q177I2_AEDAE

Therefore, only 6 proteins, namely ORCO_AEDAE, BURS_AEDAE, ATP8_AEDAE, SNPF_AEDAE, ALL3_AEDAE, and HPEP_AEDAE that were reviewed entries from UniProt having protein lengths of ≤500 amino acid residues were further considered for the design of multi-epitope vaccine construct (Table 1).

IMMUNOLOGICAL ANALYSIS USING *IN-SILICO* TOOLS

T_c-cell Epitope Prediction

In order to anticipate cytotoxic T-lymphocyte epitopes (TC-cells), all 6 of the chosen proteins were evaluated using the NetCTL 1.2 server. TC-cells are able to generate memory cells as well as trigger cell-mediated immune responses against antigens in our bodies [21]. The prediction of MHC class I binding peptides, proteasomal C-terminal cleavage, and TAP transport efficiency are all integrated into the NetCTL server, which has been trained on a dataset of 886 known MHC class I ligands. In order to reliably identify epitopes, a combinatorial score threshold of 0.75 was used to identify 30 TC-cell epitopes (9-mer peptide sequences) [11].

Immunogenic Tc-Cell Epitopes Prediction

All 30 predicted TC-cell epitopes were examined for immunogenicity using the MHC-I binding tool of the IEDB server in order to determine the most immunogenic CTL epitope for constructing the vaccine. Immunogenic epitopes function as possible response activators in the body.

Based on the immunogenicity of TC-cell epitopes, the IEDB server gives positive and negative ratings, and the immunogenicity score must be positive to be significant [22]. Using the AllerTop server, 14 TC-cell epitopes with positive immunogenicity scores were further examined for their allergenicity (Table 2). The non-allergenic TC-cell epitopes were chosen, while allergic epitopes were disregarded since they can result in adverse and perhaps lethal reactions in the body and cannot be exploited in vaccine development [23]. In order to create the multi-epitope subunit vaccination, a total of 10 TC-cell epitopes were acquired (highlighted in Table 2).

Prediction of Helper T-cell Epitopes

Helper T-lymphocytes are a crucial component of the immune system's adaptive limb, which controls essentially all adaptive immune responses [21]. They carry out a variety of tasks, including activating macrophages and B cells to release antibodies and kill microorganisms, respectively [24]. T_H-cells also activate T_C-cells to kill infected target cells and are an integral part of cell-mediated and humoral immunity for generating an effective immune response [23].

The H2-IAb, H2-IAd, and H2-IEd nominated alleles for Homo sapiens were utilized to create 15-mer TH-cell epitope peptides using the MHC-II epitope binding tool on the IEDB server for the six

Table 2. Predicted Cytotoxic T-lymphocyte-specific epitopes obtained with ≥0.75 as the threshold combinatorial score and having positive immunogenicity score.

S.N.	Peptide	S.N.	Peptide
1.	VVCLAWAVY	8.	ELSATLDTY
2.	LVSAIGETY	9.	SLDLFASVL
3.	LTANTITTL	10.	ESDARYHSI
4.	LTVIGYLVY	11.	TANTITTLF
5.	FFSFIYQAY	12.	SVLGAVVTY
6.	FSFIYQAYF	13.	VAVNSEHFY
7.	ESNDDIQHY	14.	VMFCSWLL

Table 3. Predicted helper T-lymphocyte-specific epitopes based on their percentile rank (<1), IFN score, and SMM score.

Allele	Peptide	Percentile Rank (<1)	IFN score	SMM Score< (500)
H2-IEd	SAIKYWVERHKHVVR	0.14	0.1746	312.00
H2-IEd	RSAIKYWVERHKHVV	0.27	0.0274	273.00
H2-IAd	VMQQKAIRAPQLRLR	0.54	0.3120	118.00

Aedes genus proteins. Only 2 proteins, i.e., (ORCO_AEDAE and SNPF_AEDAE) gave 11 15-mer peptide sequences of T_H-cell epitopes which adhered to the peptide selection criteria of percentile rank (<1) and SSM score >500 (stabilized matrix base method) which denotes higher affinity of that peptide. Only three of the 11 TH-cell epitopes analysed using the IFNepitope server had a positive IFN score, indicating their effectiveness in eliciting IFN-gamma production by activating TH type immune response [14]. These three epitopes were also shown to be non-allergens and acceptable for vaccine formulation (Table 3).

Vaccine Construct Design

Because they play a significant part in the generation of cytokines, toll-like receptors (TLRs) are essential for innate immunity against viral infection [25]. In order to link the TH and TC-cell epitopes discovered in this work together, the TLR4 agonist (PDB ID: 4G8A) was chosen as an adjuvant [26]. In order to simulate the vaccine design to function as an independent immunogen and produce larger antibody titers than those of a single immunogen, linkers are crucial. 10 9-mer TC-cell epitopes and three 15-mer TH-cell epitopes were joined with the adjuvant and three linkers, EAAAK, GGGS, and GPGPG, to create a 321 amino acid residue multi-epitope subunit vaccine construct in the current work. In order to connect the TC and TH epitopes, GGGS and GPGPG linkers, respectively, were inserted at the intra-epitope location. For a multi-epitope subunit vaccination to be efficacious, it must contain both TH and TC-cell epitopes [27].

Antigenicity and Allergenicity and Antigenicity Assessment of Designed Vaccine

A vaccine administered to a human host must have immunogenic properties and be able to elicit a sizable humoral immune response, which in turn causes the development of memory cells against the harmful epitopes. The vaccine construct had an antigenicity probability of 0.649, determined using ANTIGENpro server which denotes a good antigenic probability to trigger an effective immune response [16, 22]. Furthermore, the vaccine construct was found to be non-allergic in nature on analysis with AllerTOP online server [15] and therefore would be safe for human use.

Tertiary Structure Prediction, Refinement, and Validation

The tertiary structure of a 321 amino acid residue multi-epitope vaccine construct, which was modelled as a single domain with 1% disorder, was predicted using the I-TASSER system. I-TASSER server returns 5 predicted structures having C-scores of –4.47, –3.54, –4.13, –4.74, and –3.81. The server determines each model's C-score based on the importance of threading template alignments and the convergence parameters used in simulations of structure assembly. The C-score normally falls between –5 and 2, with a greater C-score denoting a model with higher confidence and a lower C-score denoting the opposite [17]. All these five structures were refined using GalaxyRefine to increase the number of amino acid residues in the Rama-favoured residues, and following refinement, they had ≥85% Rama-favoured residues.

The structure with the most favourable C-score of –3.54 contained 72% helical, 5% beta-strands, and 25% coils, and following refinement using GalaxyRefine, on plotting Ramachandran plot it showed 92.5% residues in the allowed region (Figure 1(a and b)).

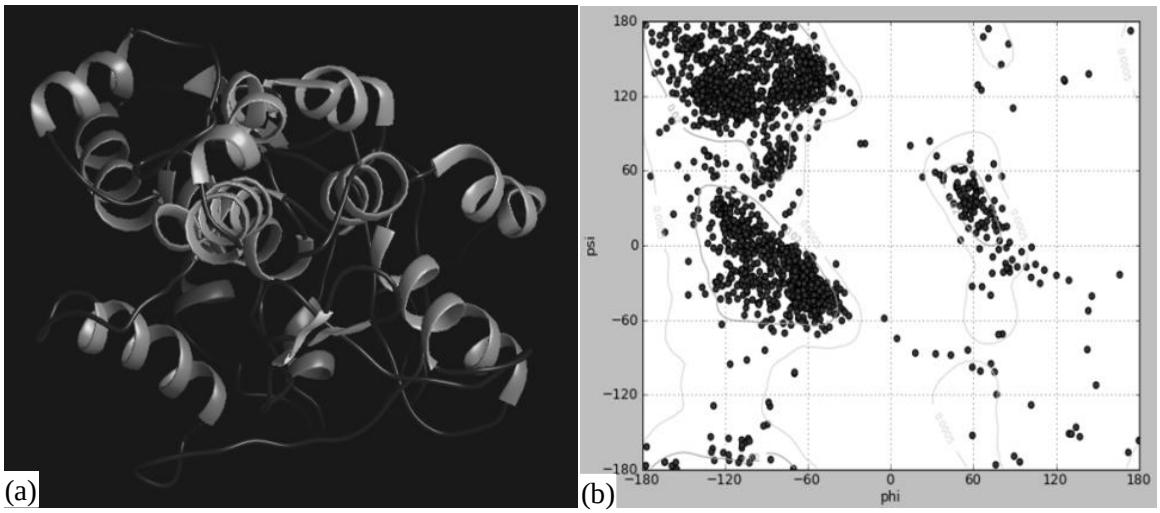

Figure 1. (a) Tertiary structure prediction for the multi-epitope subunit vaccine construct using I-Tasser server. (b) Ramachandran plot indicating 92.5% amino acids in Rama favoured regions.

Protein-Protein Docking

Protein-protein molecular docking of the multi-epitope vaccine construct and Toll-like receptor-4 (PDB ID: 4G8A) was performed using ClusPro server and the docked structure having the most favourable binding energy of –951.4 kJ/mol showed proper orientation of vaccine construct in the TLR4-receptor indicating effective and favourable binding (Figure 2(a and b)) [26]. The multi-epitope subunit vaccine designed in the present study using an immune-informatics approach may be effective in controlling the spread of various pathogens by members of *Aedes* genus, though the vaccine requires experimental validation.

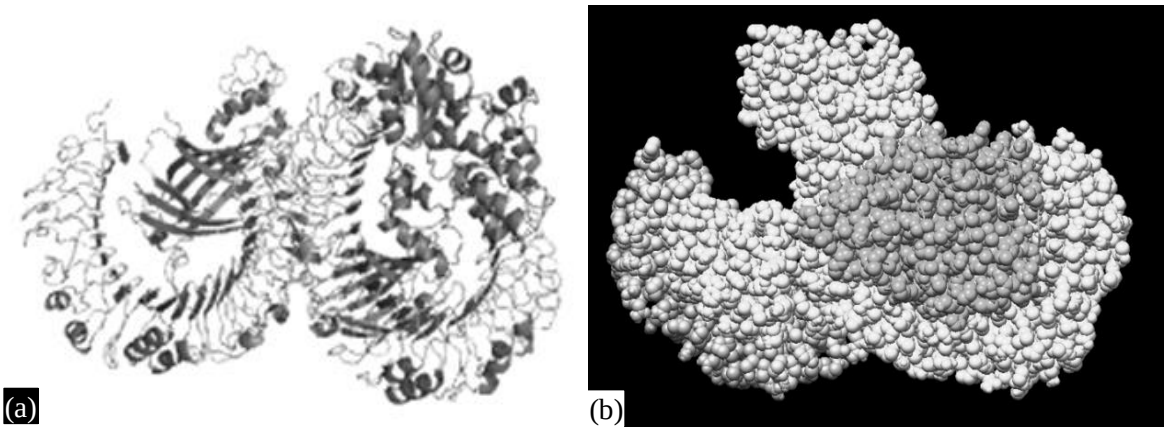

Figure 2. The docked complex of the multi-epitope subunit vaccine construct and TLR-4 (PDB ID: 4G84) ligand having a binding energy of –951.4 kJ/mol (a) Ribbon view (b) Surface view.

CONCLUSION

Vector-borne diseases (VBD) are responsible for significant global morbidity and mortality and there is an imminent need to combat them. Mosquitoes belonging to *Aedes* genus are vectors for dengue, Zika, and West Nile. Over the years, several chemical and biological methods for vector eradication have been implemented none of which have been completely effective. Using conventional approaches, successful vaccine development against a disease can take decades due to the cumbersome process of locating a region that can initiate a significant immune response. Therefore, in the present study, a multi-epitope subunit vaccine was constructed against the critical proteins of *Aedes* genus that have been reported to be involved in various vector specific pathways and mechanism such as host-seeking behaviour, odorant receptors, pigmentation, oocyte formation, and neuropeptide activity using an

immuno-informatics approach. Vaccinomics based approach used in the present study can help achieve cost-efficient and timely development of vaccines by narrowing down the probable effective epitopes that can be validated by the wet lab researcher.

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