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Design of Monovalent and Chimeric Tetravalent Dengue Vaccine Using an Immunoinformatics Approach

Neeraj Kumar Dixit[1]

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Abstract
An immunoinformatics technique was used to predict a monovalent amide immunogen candidate capable of producing therapeutic antibodies as well as a potent immunogen candidate capable of acting as a universal vaccination against all dengue fever virus serotypes. The capsid protein is an attractive goal for anti-DENV due to its position in the dengue existence cycle. The widely accessible immunological data, advances in antigenic peptide prediction using reverse vaccinology, and the introduction of molecular docking in immunoinformatics have directed vaccine manufacturing. The C-proteins of DENV-1-4 serotypes were known as antigens to assist with logical design. Binding epitopes for TC cells, TH cells, and B cells is predicted from structural dengue virus capsid proteins. Each T cell epitope of C-protein integrated with a B cell as a templet was used as a vaccine and produce antibodies in contrast to serotype of the dengue virus. A chimeric tetravalent vaccine was created by combining four vaccines, each representing four dengue serotypes, to serve as a standard vaccine candidate for all four Sero groups. The LKRARNRVS, RGFRKEIGR, KNGAIKVLR, and KAINVLRGF from dengue 1, dengue 2, dengue 3, and dengue 4 epitopes may be essential immunotherapeutic representatives for controlling outbreaks.

Keywords MHC I and II · Dengue serotypes · T-cell epitopes · B-cell epitopes · c-Proteins

Introduction

Mosquitoes require a warm, humid environment in order to reproduce, and are hence categorised as tropical and subtropical animals. However, mosquitoes, which number over 3500 species, are distributed everywhere, save in Antarctica (Servadio et al. 2018; Reiter 2001). Chikungunya, dengue, and Zika viruses are mostly transmitted by *Aedes aegypti* and *Aedes albopictus*, resulting in numerous virus infections in people. The primary unresolved issue with co-infections is whether infection with two or more viruses might exacerbate illness severity in comparison to single infections (Vogels et al. 2019). In humans, co-infection occurs when a mosquito transmits two or more viruses in the same bite or when two independent mosquitoes transmit different viruses (Magalhaes et al. 2018).

About one billion individuals are infected by vector-borne diseases that lead to over a million deaths per year. Dengue fever (DF) continues to unfold across Asia, the Pacific, America, Africa, and also the Caribbean, touching a complete of 108 countries (CDC 2020). DF, also known as break bone fever, is tropical infectious diseases caused by four serotypes of the dengue virus that are (DENV 1-4) are possibly associated. When the vector comes into contact with an uninfected host, the pathogen is transferred the most common vector-borne disease is that transmitted by mosquitoes, also known as mosquito-borne sicknesses. Immunoinformatics is the use of computing methods and techniques to interpret, generate, and manipulate immunological information (Tong and Ren 2009). The fields of bioinformatics and immunoinformatics are rapidly evolving in terms of the advancement of sophisticated bioinformatics methods that aid researchers in recognizing immunodominant T-cell and B-cell epitopes, making it easier to design contenders for vaccination. The use of an immunoinformatics approach to the logical design of antigens as a B and T cell epitope based popular tetravalent vaccine has steered the current vaccine development. It has a number of advantages over traditional vaccines, including high accuracy in generating a humoral and cellular immune response and high cost efficacy. The epitope based vaccine is also easier to synthesize, purify,

✉ Neeraj Kumar Dixit
ndixitlip@gmail.com

[1] Department of Biotechnology, Saroj Institute of Technology & Management, Lucknow, Utter Pradesh, India

store, and treat than traditional vaccines (Oyarzún and Kobe 2016). The genome of the Dengue virus (DEN) contains 10,696 nucleotides. For a single open reading frame, there are 3391 amino acids (Osatomi and Sumiyoshi 1990). RNA viral genome encodes for structural Capsid protein, which aids in core formation by assembling nucleocapsid viral RNA and plays a role in budding and fusion of the virus with the membrane (Kuhn et al. 2002).

The capsid of the Dengue virus is the first viral protein that can be synthesized during translation and found at the end of the genomes 5′ terminus. The C protein is an 11 kDa protein that forms the nucleocapsid by interacting with viral genomic RNA (Sampath and Padmanabhan 2009). The capsid includes a conserved internal hydrophobic fragment that serves as a membrane anchor domain embedded in the endoplasmic reticulum membrane throughout the infection cycle, despite little sequence conservation with other DENV strains. A conserved hydrophobic domain of Protein C links it to intracellular membranes. Dengue virus-infected cells cytoplasm was used as a factory for capsid protein at the edges of lipid droplets, which are endoplasmic reticulum-derived organelles (Carvalho et al. 2012; Samsa et al. 2009). The number of lipoid droplets per cell inflated throughout infectious disease virus infection, implying a connection between viral replication and lipid-droplet metabolism (Table 1).

Because of its importance within the DF life cycle, the capsid macromolecule is a tempting choice for anti-DENV small molecules. This reasons steric quandary and systemic rigidity, stopping infectious virions from entering, collecting, or releasing. Here we'll look at how capsid can be taken into consideration into vaccine production, especially for monovalent and chimeric tetravalent dengue vaccines many mutations within the capsid macromolecule sequence end in the event of sub infective agent particles. These sub infectious agent particles are immunogenic as a result of they embody the viral surface proteins E and M, however they're not infectious because the viral ordering isn't assembled. As a result, they could be used as vaccines. Tick-borne encephalitis virus was the first flavivirus to show this (Fig. 1).

The adaptive immune system, humoral response performs a significant shielding position in DENV infection. While T cells play an important role in fighting viral infections, they have been linked to both pathological and protective effects in the sense of DENV infection (John and Rathore 2019). DENV $CD8^+$ and $CD4^+$ cells have been shown to play a significant role in DENV infection control in studies. The most important external structures of B-cells responsible for this are cells that promote memory reactions, cell activation, antigen recognition, and signal transduction (Duan et al. 2015; Wahala 2011; Sathe and Cusick 2020). B cells antigen receptor is a functional component of multi molecular protein complexes on the cell surface. CD4 T cells and CD8 T cells have been shown in animal studies to protect against DENV infection (Zellweger et al. 2014). When a T cell recognizes antigen on the surface of a B cell, the T cell becomes stimulated, and then stimulates the B cell. If a B cell is activated, it undergoes clonally expansion, and

Table 1 Predictions of allergenicity index

Sl	Protein	DENGUE 1		DENGUE 2		DENGUE 3		DENGUE 4	
		Similarity index	Result	Similarity index	Result	Similarity index	Result	Similarity index	Result
1	ANCHORED CAPSID	0.79	NON-ALLERGEN	0.79	NON-ALLERGEN	0.79	NON-ALLERGEN	0.81	NON-ALLERGEN
2	CAPSID	0.79	NON-ALLERGEN	0.8	NON-ALLERGEN	0.8	NON-ALLERGEN	0.79	NON-ALLERGEN
3	POLY PROTEIN	0.81	NON-ALLERGEN	0.82	NON-ALLERGEN	0.81	NON-ALLERGEN	0.79	NON-ALLERGEN
4	MEMBRANE GLYCOL PRECURSOR	0.77	ALLERGEN	0.78	NON-ALLERGEN	0.77	NON-ALLERGEN	0.76	NON-ALLERGEN
5	MEMBRANE			0.8	NON-ALLERGEN				
6	E			0.77	NON-ALLERGEN				

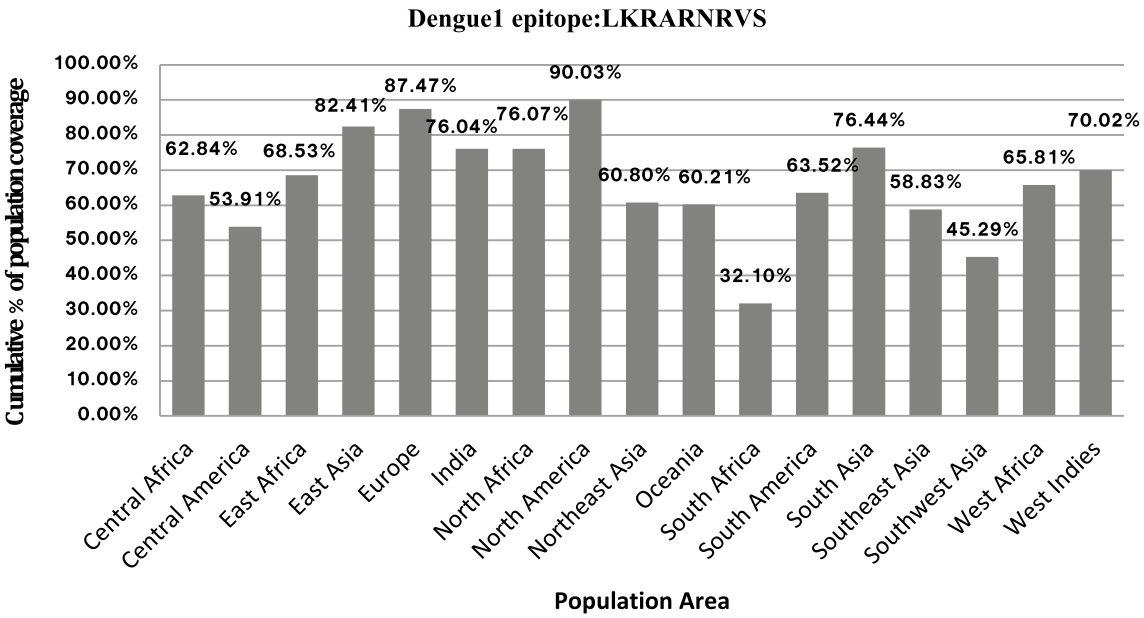

Fig. 1 Population coverage of epitope LKRARNRVS

daughter cells divide into plasma cells. T cell support consists of two components: lymphokines, which function as growth and differentiation factors for B cells. Plasma cells produce massive amounts of antibodies and function as antibody factories (Fig. 2).

Peptides gift the most important organic phenomenon advanced (MHC) molecules, additionally called human white blood cell substance molecules in humans that used for identification by then lymph cell receptor as a fusion protein. Furthermore, HLA alleles linked to defense against extreme dengue disease are also linked to robust and multifunctional T cell responses, implying that T cells play a defensive role during DENV infection (Grifoni et al. 2017). T cell epitopes can play a role in a serotype-specific or cross-reactive reaction. The B-cell antigen receptor is a transmembrane receptor that crosses into the cytoplasm. These are inefficient at communicating signals and activating B cells (Tanaka and Baba 2020). The BCR (B cell receptor) is a multi-molecular protein complex that is not covalently bound to other proteins. So BCR is needed functionally (Brezski and Monroe 2008).

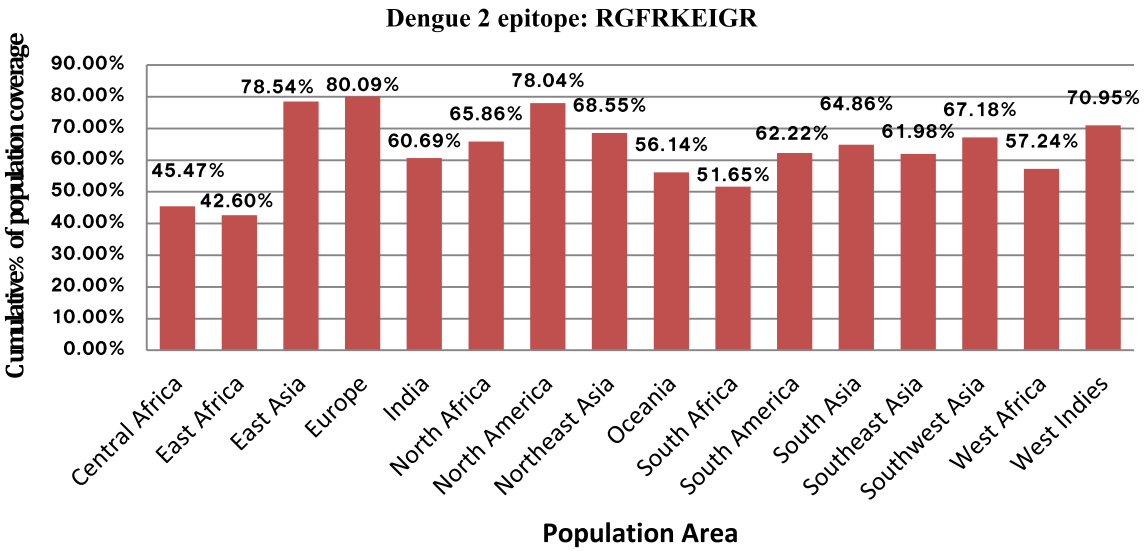

Fig. 2 Population coverage of epitope RGFRKEIGR

Dengvaxia® is a chimeric vaccine that protects against both yellow fever and DF. It had a poor overall effectiveness against DENV, with nearly 50% effectiveness against DENV 1 and 39% effectiveness against DENV 2. Latest clinical trials, however, have discovered that the CYDTDV vaccine causes a high risk of hospitalization in children under the age of nine (Hadinegoro et al. 2015). There's a chance that a better result would help for DHF/DSS (Rothman 2004). The main advantages of this epitope-based tetravalent vaccine are the reduced disease incidence and the lack of pathogen interference in the absence of any live parts, since these are specific peptide sequences that can be developed in the in the lab. Protein synthesis and purification methods could be used to obtain the pure form of proposed vaccine structures (Fig. 3).

DENV infections result in the production of neutralising antibodies (NAbs), which are associated with protection. Numerous DENV vaccine candidates are in various phases of clinical development (de Alwis et al. 2011; Guzman et al. 2007; Mathew et al. 2011). While the existence of NAbs as a correlate of protection has led the development of DENV vaccines, new research reveals that the presence of NAbs to the four serotypes following vaccination is not a reliable predictor of protection (Biswal et al. 2020; Dayan et al. 2020; Moodie et al. 2018). Dengvaxia is a live attenuated chimeric tetravalent dengue vaccine (CYD-TDV) produced by altering the live attenuated yellow fever 17D vaccine to include the envelope (E) and premembrane proteins of each DENV serotype (Thomas and Yoon 2019).

Methodology

Viral Protein Selection for Preparation of Vaccine

To predict the most successful DENV applicants for vaccination, this analysis used statistical methods. The Dengue virus amino acid sequence was obtained from the virus pathogen resource sequence database (https://www.viprbrc.org/brc/vipr-protein-serch.spg). FASTA format was used to extract viral proteins (Fig. 4).

Allergenicity of Protein Predictions

The Allergen FP algorithm, which was stated in the current research, was added (FP stands for Finger Print). Allergen FP is written in Python. It can be found online at http://ddg-pharmfac.net/Allergen FP. Proteins that aren't allergens were chosen based on their similarity index. As contrasted to known allergens, a protein is considered a possible allergen if it has > 35% sequence similarity over an 80-amino-acid window (Stadler and Stadler 2003).

Prediction of Epitopes from Shortlisted Proteins

The IEDB tool is used to identify the most promiscuous epitopes binding to the MHC class I allele. T-cell epitopes, which play a vital function in vaccine design, set off the immune response. The neural network's ability to be trained on data consisting of continuous binding affinities, improves

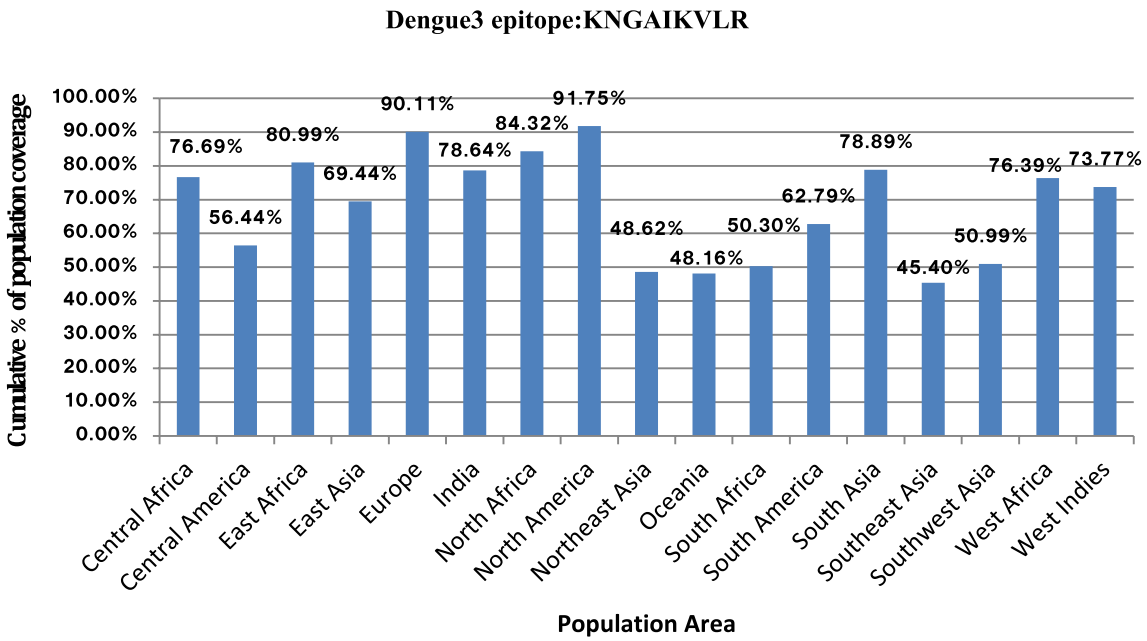

Fig. 3 Population coverage of epitope KNGAIKVLR

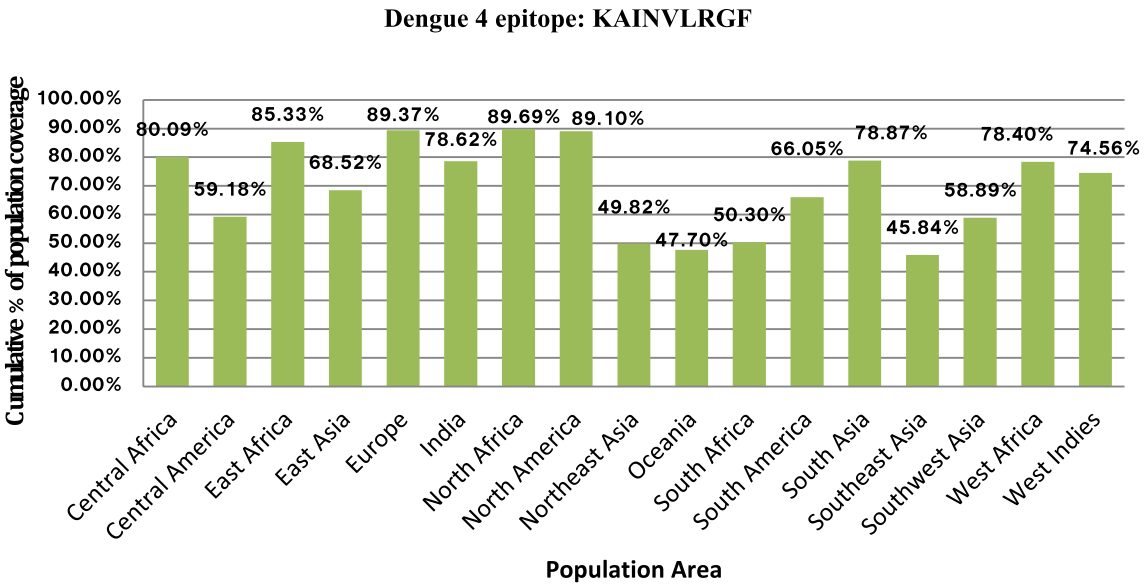

Fig. 4 Population coverage of epitope KAINVLRGF

the efficiency of the new system. T cells do this by detecting peptides bound to MHC receptors (major histocompatibility complex). Prediction methods based on alignments of insertions and deletions work considerably better than methods trained on single-length peptides.The position of deletions will help explain the peptide–MHC binding modes, As previously defined, the approach was implemented as a feed-forward artificial neural network ensemble with a single hidden layer (Nielsen and Lund 2009) (IEDB-AR, http://tools.iedb.org).

Predictions of MHC class II binding are commonly used to find epitope candidates in infectious agents. To date, the vast majority of human MHC class II prediction algorithms have focused on HLA molecules encoded in the DR locus. Immune epitopes are HLA class II peptide ligands that are recognised by T cells and trigger an immune response (Mith-Garvin et al. 2009). The immune Epitope Database's unique peptide binding specificity can be influenced by both alpha and beta chains (IEDB) (Vita et al. 2010; Peters and Sette 2007).

Instability and GRAVY of Epitope Prediction

Prot Param is software that measures physical and chemical parameters such as instability for a protein contained in Swiss-Prot or TrEMBLL, as well as for a user-entered epitope sequence (Boeckmann et al. 2003) that is a curate protein sequence database that aims to include high-quality annotations (http://www.expasy.org/sprot/).

The grand average of hydropathicity (GRAVY) comparison of the most abundant epitope in the extracellular matrix of corneal stroma between different species with the Grand Average Hydropathicity (GRAVY) values is one of the computed parameters. We've used this method to choose studies with a higher negative score (Table 2).

Toxicity, Hydrophobicity and Hydropathicity Prediction

Toxin Pred was used to assess epitope toxicity, hydrophobicity, and hydropathicity (Gupta et al. 2013). Toxin Pred is a kind in silico method for predicting peptide toxicity, hydrophobicity, and hydropathicity. It can be used to create the least toxic peptides and find toxic regions of proteins. Toxin Pred (http://crdd.osdd.net/raghava/toxinpred/) can improve peptide-based drug discovery (Table 3).

B-Cell Epitope Prediction

For all stable structural capsid proteins, the ABCpred Prediction Server was used to forecast B-cell epitope linear B-cell epitopes. The predicted epitopes were then shortlisted based on their prediction score. The aim of the ABCpred server is to use an artificial neural network to predict B cell epitope in an antigen sequence.This is the first server to use fixed length patterns and a recurrent neural network (Saha and Raghava 2006).

Consensus Epitope, Immunogenicity and Antigenic Propensity Prediction

The prediction of common epitopes between or within known serotypes may be used to produce Dengue virus monovalent and chimeric tetravalent vaccines. When the findings

Table 2 Screening of shortlisted protein epitopes binding to MHC class I allele, MHC class II allele, instability, GRAVY, toxicity, hydrophobicity and hydropathicity prediction

Serotype	Allele	Peptide	Score	Instability index	Stable	GRAVY value	Prediction toxicity	Result	Hydrophobicity	Hydropathicity
Dengue 1 MHC 1	HLA-A*03:01	KGLLSGQGPMK	0.692584	−5.48	Stable	−0.491	−0.88	Non Toxic	−0.13	−0.49
	HLA-B*27:05	ARNRVSTGSQL	0.491884	30.10	Stable	−0.809	−1.42	Non Toxic	−0.37	−0.81
	HLA-B*27:05	KRFSKGLLSGQ	0.39609	31.01	Stable	−0.709	−1.05	Non Toxic	−0.29	−0.71
	HLA-B*27:05	NRVSTGSQLAK	0.231306	18.88	Stable	−0.755	−1.24	Non Toxic	−0.31	−0.75
	HLA-A*03:01	NRVSTGSQLAK	0.203223	18.88	Stable	−0.755	−1.24	Non Toxic	−0.31	−0.75
Dengue 2 MHC 1	HLA-A*03:01	KARNTPFNMLK	0.455089	22.38	Stable	−1.027	−1.37	Non Toxic	−0.35	−1.03
	HLA-B*27:05	NRVSTVQQLTK	0.442075	26.60	Stable	−0.809	−1.49	Non Toxic	−0.28	−0.50
	HLA-A*31:01	IKKSKAINVLR	0.407682	18.88	Stable	−0.155	−1.29	Non Toxic	−0.29	−0.15
	HLA-B*27:05	KKARNTPFNML	0.346593	30.10	Stable	−1.027	−1.18	Non Toxic	−0.35	−1.03
	HLA-B*27:05	RGFRKEIGRML	0.327127	18.88	Stable	−0.791	−1.21	Non Toxic	−0.41	−0.79
	HLA-B*27:02	KKARNTPFNML	0.306116	30.10	Stable	−1.027	−1.18	Non Toxic	−0.35	−1.03
	HLA-A*02:01	GMLQGRGPLKL	0.257905	15.57	Stable	−0.127	−0.98	Non Toxic	−0.12	−0.13
	HLA-B*27:02	RGFRKEIGRML	0.232655	18.88	Stable	−0.791	−1.21	Non Toxic	−0.41	−0.79
	HLA-A*31:01	KARNTPFNMLK	0.215716	22.38	Stable	−1.027	−1.37	Non Toxic	−0.35	−1.03
	HLA-A*31:01	VLRGFRKEIGR	0.214553	36.39	Stable	−0.582	−1.09	Non Toxic	−0.39	−0.58
	HLA-B*27:02	ERNRVSTVQQL	0.208278	37.82	Stable	−1.118	−1.73	Non Toxic	−0.45	−1.12
	HLA-A*03:01	KAINVLRGFRK	0.199792	18.88	Stable	−0.327	−1.29	Non Toxic	−0.32	−0.33
	HLA-B*27:05	ERNRVSTVQQL	0.192259	37.82	Stable	−1.118	−1.73	Non Toxic	−0.45	−1.12
	HLA-B*27:05	RNRVSTVQQLT	0.185646	37.82	Stable	−0.864	−1.46	Non Toxic	−0.41	−0.86
	HLA-A*03:01	NRVSTVQQLTK	0.185015	26.60	Stable	−0.809	−1.78	Non Toxic	−0.35	−0.81
	HLA-A*33:03	IKKSKAINVLR	0.16522	18.88	Stable	−0.155	−1.29	Non Toxic	−0.29	−0.15
	HLA-A*03:01	LGMLQGRGPLK	0.161562	23.29	Stable	−0.127	−1.06	Non Toxic	−0.12	−0.13
	HLA-A*31:01	KAINVLRGFRK	0.135664	18.88	Stable	−0.327	−1.29	Non Toxic	−0.32	−0.33
	HLA-A*33:03	VLRGFRKEIGR	0.107714	36.39	Stable	−0.582	−1.09	Non Toxic	−0.39	−0.58
	HLA-B*27:02	RNRVSTVQQLT	0.10465	37.82	Stable	−0.864	−1.46	Non Toxic	−0.41	−0.86
Dengue 3 MHC 1	HLA-A*03:01	KGLLSGQGPMK	0.692584	−5.48	Stable	−0.491	−0.88	Non Toxic	−0.13	−0.49
	HLA-B*27:05	KRFSKGLLSGQ	0.39609	31.01	Stable	−0.709	−1.05	Non Toxic	−0.29	−0.71
	HLA-B*27:05	NRVSTGSQLAK	0.231306	18.88	Stable	−0.755	−1.24	Non Toxic	−0.31	−0.75
	HLA-A*03:01	NRVSTGSQLAK	0.203223	18.88	Stable	−0.755	−1.24	Non Toxic	−0.31	−0.75
Dengue 4 MHC 1	HLA-B*27:05	NRVSTVQQLTK	0.442075	26.60	Stable	−0.809	−1.78	Non Toxic	−0.35	−0.81
	HLA-A*31:01	IKKSKAINVLR	0.407682	18.88	Stable	−0.155	−1.29	Non Toxic	−0.29	−0.15
	HLA-A*02:01	GMLQGRGPLKL	0.257905	15.57	Stable	−0.127	−0.98	Non Toxic	−0.12	−0.12
	HLA-A*03:01	KAINVLRGFRK	0.199792	18.88	Stable	−0.327	−1.29	Non Toxic	−0.32	−0.33
	HLA-B*27:05	RNRVSTVQQLT	0.185646	37.82	Stable	−0.864	−1.46	Non Toxic	−0.41	−0.86
	HLA-A*03:01	NRVSTVQQLTK	0.185015	26.60	Stable	−0.809	−1.78	Non Toxic	−0.35	−0.35
	HLA-A*33:03	IKKSKAINVLR	0.16522	18.88	Stable	−0.155	−1.29	Non Toxic	−0.29	−0.15
	HLA-A*03:01	LGMLQGRGPLK	0.161562	23.29	Stable	−0.127	−1.06	Non Toxic	−0.12	−0.13

Table 2 (continued)

Serotype	Allele	Peptide	Score	Instability index	Stable	GRAVY value	Prediction toxicity	Result	Hydrophobicity	Hydropathicity
	HLA-A*31:01	KAINVLRGFRK	0.135664	18.88	Stable	− 0.327	− 1.29	Non Toxic	− 0.32	− 0.33
	HLA-B*27:02	RNRVSTVQQLT	0.10465	37.82	Stable	− 0.864	− 1.46	Non Toxic	− 0.41	− 0.86
Dengue 1 MHC II	HLA-DRB1*13:27	LKRARNRVSTG	18.85	34.51	Stable	− 1.182	− 1.09	Non Toxic	− 0.54	− 1.18
Dengue 2 MHC II	HLA-DRB1*15:01	KAINVLRGFRK	5.08	18.88	Stable	− 0.327	− 1.29	Non Toxic	− 0.32	− 0.33
	HLA-DRB1*07:01	RGFRKEIGRML	6.86	18.88	Stable	− 0.791	− 1.21	Non Toxic	− 0.41	− 0.79
	HLA-DRB1*13:01	LRGFRKEIGRM	12.57	36.39	Stable	− 0.791	− 1.06	Non Toxic	− 0.41	− 0.79
	HLA-DRB1*13:28	LRGFRKEIGRM	12.57	36.39	Stable	− 0.791	− 1.06	Non Toxic	− 0.41	− 0.79
	HLA-DRB1*13:27	NVLRGFRKEIG	12.57	36.39	Stable	− 0.491	− 1.18	Non Toxic	− 0.29	− 0.49
	HLA-DRB1*13:01	VLRGFRKEIGR	12.57	36.39	Stable	− 0.582	− 1.09	Non Toxic	− 0.39	− 0.58
	HLA-DRB1*13:28	VLRGFRKEIGR	12.57	36.39	Stable	− 0.582	− 1.09	Non Toxic	− 0.39	− 0.58
	HLA-DRB1*13:27	MNNQRKKARNT	14.85	5.74	Stable	− 2.527	− 1.11	Non Toxic	− 0.73	− 2.53
	HLA-DRB1*13:04	LRGFRKEIGRM	15.43	36.39	Stable	− 0.791	− 1.06	Non Toxic	− 0.41	− 0.79
	HLA-DRB1*13:04	VLRGFRKEIGR	15.43	36.39	Stable	− 0.582	− 1.09	Non Toxic	− 0.39	− 0.58
	HLA-DRB1*15:01	INVLRGFRKEI	20.00	36.39	Stable	− 0.045	− 1.15	Non Toxic	− 0.23	− 0.05
	HLA-DRB1*07:01	GFRKEIGRMLN	22.28	26.60	Stable	− 0.700	− 1.30	Non Toxic	− 0.31	− 0.70
	HLA-DRB1*15:06	GMLQGRGPLKL	25.71	15.57	Stable	− 0.127	− 0.98	Non Toxic	− 0.12	− 0.13
Dengue 3 MHC II	HLA-DRB1*07:01	FKKNGAIKVLR	13.71	− 23.88	Stable	− 0.273	− 0.87	Non Toxic	− 0.26	− 0.27
Dengue 4 MHC II	HLA-DRB1*07:01	GTIKKSKAINV	0.63	− 6.35	Stable	− 0.191	− 0.98	Non Toxic	− 0.18	− 0.19
	HLA-DRB1*07:01	IKKSKAINVLR	3.60	18.88	Stable	− 0.155	− 1.29	Non Toxic	− 0.29	− 0.15
	HLA-DRB1*07:03	IKKSKAINVLR	11.43	18.88	Stable	− 0.155	− 1.29	Non Toxic	− 0.29	− 0.15
	HLA-DRB1*13:27	NVLRGFRKEIG	12.57	36.39	Stable	− 0.491	− 1.18	Non Toxic	− 0.29	− 0.49
	HLA-DRB1*13:04	NVLRGFRKEIG	15.43	36.39	Stable	− 0.491	− 1.18	Non Toxic	− 0.29	− 0.49
	HLA-DRB1*15:01	INVLRGFRKEI	20.00	36.39	Stable	− 0.045	− 1.18	Non Toxic	− 0.29	− 0.49
	HLA-DRB1*15:06	GMLQGRGPLKL	25.71	15.57	Stable	− 0.127	− 0.98	Non Toxic	− 0.12	− 0.13

Table 3 Prediction of B-cell

Dengue-1				Dengue-2			
Rank	Sequence	Start position	Score	Rank	Sequence	Start position	Score
1	RWSSFKKNGAIKVLRGFKKE	55	0.86	1	KAIN**VLRGFRKEIGRML**NIL	76	0.86
2	KRFS**KGLLSGQGPMK**MVMAF	18	0.83	2	AINVL**RGFRKEIGRML**NILN	77	0.81
3	ILARWSSFKKNGAIKVLRGF	52	0.73	2	RVSTVQQLTKRFSLGMLQGR	22	0.81
4	GAIKVLRGFKKEISSMLNIM	63	0.70	3	KKS**KAINVLRGFRK**EIGRML	73	0.79
4	RFLAIPPTAGILARWSSFKK	42	0.70	3	R**ERNRVSTVQQL**TKRFSLGM	18	0.79
5	ARWSSFKKNGAIKVLRGFKK	54	0.69	4	**ERNRVSTVQQL**TKRFSLGML	19	0.77
5	SGQGPMKMVMAFIAFLRFLA	26	0.69	5	SKAINVL**RGFRKEIGRML**NI	75	0.73
6	LAIPPTAGILARWSSFKKNG	44	0.68	6	L**RGFRKEIGRML**NILNRRRR	81	0.71
7	KKNGAIKVLRGFKKEISSML	60	0.64	7	**KARNTPFNMLK**RERNRVSTV	7	0.7
7	KRAR**NRVSTGSQLAK**RFSKG	4	0.64	8	ARNTPFNMLKRERNRVSTVQ	8	0.69
7	VSTGSQLA**KRFSKGLLSGQ**G	10	0.64	9	NQRK**KARNTPFNMLK**RERNR	3	0.67
8	AIKVLRGFKKEISSMLNIMN	64	0.63	10	KRFSL**GMLQGRGPLKL**FMAL	31	0.64
9	**LKRARNRVSTG**SQLAKRFSK	3	0.62	11	KRER**NRVSTVQQLTK**RFSLG	17	0.63
9	FS**KGLLSGQGPMK**MVMAFIA	20	0.62	12	ILKRWGT**IKKSKAINVLR**GF	65	0.62
10	AFIAFLRFLAIPPTAGILAR	36	0.61	12	NTPFNMLKR**ERNRVSTVQQL**	10	0.62
11	SFKKNGAIKVLRGFKKEISS	58	0.60	13	AGILKRWGT**IKKSKAINVLR**	63	0.59
11	WSSFKKNGAIKVLRGFKKEI	56	0.60	13	LQGRGPLKLFMALVAFLRFL	38	0.59
12	LLSGQGPMKMVMAFIAFLRF	24	0.59	14	RGPLKLFMALVAFLRFLTIP	41	0.58
13	LA**KRFSKGLLSGQ**GPMKMVM	16	0.58	14	LTKRFSL**GMLQGRGPLKL**FM	29	0.58
14	KNGAIKVLRGFKKEISSMLN	61	0.54	15	NMLKR**ERNRVSTVQQLTK**RF	14	0.57
14	SQLA**KRFSKGLLSGQ**GPMKM	14	0.54	16	GRGPLKLFMALVAFLRFLTI	40	0.56
15	KVLRGFKKEISSMLNIMNRR	66	0.53	17	GT**IKKSKAINVLR**GFRKEIG	70	0.54
15	NGAIKVLRGFKKEISSMLNI	62	0.53	18	QQLTKRFSL**GMLQGRGPLKL**	27	0.53
16	TGSQLA**KRFSKGLLSGQ**GPM	12	0.52	19	PPTAGILKRWGTIKKSKAIN	60	0.52
17	MVMAFIAFLRFLAIPPTAGI	33	0.51	19	FMALVAFLRFLTIPPTAGIL	47	0.52

Dengue-3				Dengue-4			
Rank	Sequence	Start position	Score	Rank	Sequence	Start position	Score
1	AIKVLRGF**KREISSMLNIM**N	62	0.75	1	L**RGFRKEIGRML**NILNRRRR	62	0.9
1	LARWSS**FKKNGAIKVLR**GFK	51	0.75	2	FLTIPPTAGILKRWGTIKKS	37	0.74
2	ERNRVSTGSQLAKRFSKGLL	4	0.74	3	S**KAINVLRGFRKEI**GRMLNI	56	0.73
3	ILARWSS**FKKNGAIKVL**RGF	50	0.71	4	A**INVLRGFRKEI**GRMLNILN	58	0.72
4	SGQGPMKMVMAFIAFLRFLA	24	0.7	4	GILKRWGT**IKKSKAINVLRG**	45	0.72
5	KNGAIKVLRGFKREISSMLN	59	0.67	5	**KAINVLRGFRKEI**GRMLNIL	57	0.7
6	RNRVSTGSQLAKRFSKGLLS	5	0.66	6	LFMALVAFLRFLTIPPTAGI	27	0.68
6	TGSQLAKRFSKGLLSGQGPM	10	0.66	7	AGILKRWGT**IKKSKAINVLR**	44	0.65
7	NRVSTGSQLAKRFSKGLLSG	6	0.65	8	ILKRWGT**IKKSKAINVLR**GF	46	0.62
8	A**IPPTAGILARW**SSFKKNGA	43	0.63	8	RVSTVQQLTKRFSLGMLQGR	3	0.62
8	LAIPPTAGILARWSSFKKNG	42	0.63	9	AFLRFLTIPPTAGILKRWGT	33	0.59
9	RERNRVSTGSQLAKRFSKGL	3	0.62	10	TAGILKRW**GTIKKSKAINV**L	43	0.57
9	QGPMKMVMAFIAFLRFLAIP	26	0.62	11	KKS**KAINVLRGFRK**EIGRML	54	0.56
9	GQGPMKMVMAFIAFLRFLAI	25	0.62	11	RWGT**IKKSKAINVLR**GFRKE	49	0.56
10	GAIKVLRGF**KREISSMLNIM**	61	0.61	11	TIPPTAGILKRWGTIKKSKA	39	0.56
11	VMAFIAFLRFLAIPPTAGIL	32	0.6	11	GRGPLKLFMALVAFLRFLTI	21	0.56
12	AGILARWSS**FKKNGAIKVLR**	48	0.58	11	LTKRFSL**GMLQGRGPLK**LFM	10	0.56
13	IKVLRGFKREISSMLNIMNR	63	0.57	12	GT**IKKSKAINVLR**GFRKEIG	51	0.54
14	IPPTAGILARWSSFKKNGAI	44	0.55	13	KRW**GTIKKSKAINV**LRGFRK	48	0.53
14	MAFIAFLRFLAIPPTAGILA	33	0.55	14	PPTAGILKRWGTIKKSKAIN	41	0.52

Table 3 (continued)

Dengue-3				Dengue-4			
Rank	Sequence	Start position	Score	Rank	Sequence	Start position	Score
14	GLLSGQGPMKMVMAFIAFLR	21	0.55				
15	GSQLAKRFS**KGLLSGQGPMK**	11	0.54				
16	WSSFKKNGAIKVLRGFKREI	54	0.53				
17	ARWSSFKKNGAIKVLRGFKR	52	0.52				
18	S**FKKNGAIKVLR**GFKREISS	56	0.51				

of the predicted dengue virus 1-4 serotype epitopes were compared, it was discovered that the standard peptides were consensus epitopes. The main reason for using the consensus epitope technique was to sort out potential candidates that were most likely to elicit a Dengue virus immune response (Table 4).

To be stimulated and evoke their effectors roles, T-cells must identify peptides posed on MHC molecules. Several researches suggest that some peptides are more immunogenic than others, indicating that they are more likely to be T-cell epitopes. The discovery of factors that affect immunogenicity would be a crucial next step in the study of T-cell epitopes and our knowledge of cellular immune responses (Calis et al. 2013). To be stimulated and evoke their effectors functions, T cells must recognize peptides expressed on MHC molecules in order to be activated and elicit their effectors functions (Table 5).

Kolaskar and Tongaonkar's method is used to determine antigenic peptides. The predictions are based on a table that shows the frequency of amino acid residues in segmental epitopes that have been studied experimentally (Table 6).

IC_{50} and Conservancy Analysis

Prediction server Propred (Singh and Raghava 2003) predicted the corresponding allele for each of the proposed T-cell epitopes based on IC_{50} values. In the in silico vaccinology methodology, conservancy analysis is used to determine the degree of epitope distribution in a homologous protein set. The epitope conservancy research method is for determining the conservation of epitopes (http://tools.iedb.org/conservancy/) at the IEDB was used to forecast the conservancy trend of the target epitopes. We used a method to help with the optimal degree of conservation in epitope selection and to assess the variability of epitopes within a series of protein sequences. Conservancy can be measured using given identity parameters, and minimum and maximal conservancy amounts can be determined.

Population Coverage Analysis

For successful vaccination, a vaccine molecule must have broad-spectrum defense against the disease in various world populations (Robinson et al. 2016). However, due to the strong polymorphism of MHC molecules, individuals of different ethnicities/countries have different MHC related pools/frequencies. This issue was resolved for analysis using the IEDB population coverage method (http:/tools.iedb.org/population/).

The fraction of people who are most likely to react to a given epitope range, vaccine, was calculated using an algorithm based on HLA genotypic frequencies. We used a web-based method to forecast population coverage of T-cell epitope-based diagnostics and vaccines. As a result, epitope-based vaccinations or diagnostics may be optimized to optimize population distribution while minimizing uncertainty and heterogeneity in coverage achieved or predicted across ethnic groups.

Table 4 Linear B-cell epitopes, immunogenicity and antigenic propensity

Serotype	Sequence	Start position	Score	Immunogenicity	Antigenic propensity
Dengue-1	**LKRARNRVS**TGSQLAKRFSK	3	0.62	0.07259	0.9834
Dengue-2	KAINVL**RGFRKEIGR**MLNIL	76	0.86	0.08016	0.9515
Dengue-2	AIN**VLRGFRKEI**GRMLNILN	77	0.81	0.23167	1.0022
Dengue-3	LARWSSFK**KNGAIKVLR**GFK	51	0.75	0.00533	1.0258
Dengue-4	S**KAINVLRGF**RKEIGRMLNI	56	0.73	0.12993	1.178
Dengue-4	A**INVLRGFRK**EIGRMLNILN	58	0.72	0.21358	1.0186

Table 5 IC_{50} value and the potential T cell epitopes conservancy

Types of dengue	Epitope sequence	IC_{50} value	Percent of protein sequence matches at identity < = 100%	Minimum identity (%)	Maximum identity (%)
Dengue 1	LKRARNRVS	HLA-DRB1*01:01(6.937), HLA-DRB1*03:01(44.443), HLA-DRB1*04:01(6.447), HLA-DRB1*04:04(4.599), HLA-DRB1*04:05(6.679), HLA-DRB1*07:01(6.166), HLA-DRB1*08:02(6.005), HLA-DRB1*09:01(6.007), HLA-DRB1*11:01(6.177), HLA-DRB1*12:01(4.444), HLA-DRB1*13:02(6.059), HLA-DRB1*15:01(4.724)	60.00% (60/100)	22.22	100.00
Dengue 2	RGFRKEIGR	HLA-A*02:01(0.35), HLA-A*02:05(0.005), HLA-A*11:01(0.006), HLA- A*31:01(0.12), A*33:02(0.1), HLA- A*68:01(.01), HLA-B*27:02(0.15), HLA-B*35:01(0.6), HLA-B*37:01(0.2), HLA-B*38:01(0.0078), HLA-B*39:01(0.45), HLA-B*39:02(0.3), HLA-B*27:05(35.5), HLA-B*44:03(0.01), HLA-B*51:01(0.34), HLA-B*51:02(.363), HLA-B*51:03(0.22), HLA-B*52:01(0.25)	98.94% (93/94)	88.89	100.00
Dengue 2	VLRGFRKEI	HLA-DRB1*01:01(8.445), HLA-DRB1*03:01(5.625), HLA-DRB1*04:01(6.819), HLA-DRB1*04:04(5.63), HLA-DRB1*04:05(7.021), HLA-DRB1*07:01(7.747), HLA-DRB1*08:02(5.943), HLA-DRB1*09:01(6.716), HLA-DRB1*11:01(6.313), HLA-DRB1*12:01(4.806), HLA-DRB1*13:02(5.531),	100.00% (94/94)	100.00	100.00
Dengue 3	KNGAIKVLR	HLA-DRB1*01:01(0.02), HLA-DRB1*01:02(0.4), HLA- DRB1*03:01(2.6), DRB1*03:05(1.4), HLA-DRB1*04:01(1.1), HLA-DRB1*04:04(1.3), HLA-DRB1*04:05(1.65), HLA-DRB1*07:01(3.62), HLA-DRB1*08:02(0.6), HLA-DRB1*08:17(2.4), HLA-DRB1*11:01(0.7), HLA-DRB1*11:02(1.5), HLA-DRB1*13:01(2.26), HLA-DRB1*13:02(1.8), HLA-DRB1*15:01(2.9)	97.00% (97/100)	88.89	100.00
Dengue 4	KAINVLRGF	HLA-DRB1*01:01(0.2), HLA-DRB1*01:02(0.4), HLA- DRB1*03:01(2.6), DRB1*03:05(1.4), DRB1*03:06(1.7), DRB1*03:07(1.7), DRB1*03:09(2.1), HLA-DRB1*04:02(1.35), HLA-DRB1*04:04, (1.35), HLA-DRB1*04:05(1.65), HLA-DRB1*07:01(3.62), HLA-DRB1*08:02(0.6), HLA-DRB1*08:04(1.2), HLA-DRB1*08:13(1.5), HLA-DRB1*11:01(0.7), HLA-DRB1*11:02(1.5), HLA-DRB1*11:04(1.6), HLA-DRB1*13:01(2.26), HLA-DRB1*13:02(1.8), HLA-DRB1*15:01(2.9)	100.00% (97/97)	100.00	100.00

Table 5 (continued)

Types of dengue	Epitope sequence	IC_{50} value	Percent of protein sequence matches at identity < = 100%	Minimum identity (%)	Maximum identity (%)
Dengue 4	INVLRGFRK	HLA-DRB1*01:01(7.1037), HLA-DRB1*03:01(4.288), HLA-DRB1*04:01(6.638), HLA-DRB1*04:04(4.719), HLA-DRB1*04:05(6.572), HLA-DRB1*07:01(6.442), HLA-DRB1*08:02(6.337), HLA-DRB1*09:01(6.941), HLA-DRB1*11:01(6.263), HLA-DRB1*12:01(4.014), HLA-DRB1*13:02(6.09), HLA-DRB1*15:01(3.748)	100.00% (97/97)	100.00	100.00

Table 6 Epitope, template structures modeling of alleles and docking

Serotype	Peptide sequence	Allele	Template (PDB ID)	Crystal structure/model	Docking score
DENGUE-1	LKRARNRVS	DRB1_1327	2WBJ	Model	−213.922
DENGUE-2	RGFRKEIGR	HLA-B*2705	2BSR	Crystal structure	−222.148
DENGUE-3	KNGAIKVLR	DRB1_0701	3PDO	Model	−206.13
DENGUE-4	KAINVLRGF	DRB1_1501	1BX2	Crystal structure	−178.031

Modeling, Refining, and Validation of Tertiary Structures

The PEPstr server predicts the tertiary structure of small peptides with sequence lengths ranging from Residues range from 7 to 25. This is a server that models small peptide structures in three dimensions (http://www.imtech.res.in/raghava/pepstr/).

Structure-Based Modeling and Evaluation of MHC Alleles

Allele structures are prepared using the IMGT/HLA Database (http://www.ebi.ac.uk/ipd/imgt/hla/intro.html). The Anthony Nolan Research Institute's HLA Informatics department has launched the IMGT/HLA Database. The member libraries of the International Nucleotide Sequence Database Collaboration provide access to all of the submissions submitted by the IPD-IMGT/HLA database (Kodama et al. 2019; Sayers et al. 2019).

Computational approaches were used to model some of the allele configurations that were not present in the IMGT/HLA database. Homology simulation modeling technique was used to construct the allele structures. Procheck (http://www.biochem.ucl.ac.uk/) is software that evaluates the stereochemical content of a protein structure by comparing it to well-refined structures of the same resolution and indicating its local, residue-by-residue consistency.

Docking

Signal transduction, immune responses, and cellular modulation are all examples of peptide–protein interactions play an important role (Petsalaki and Russell 2008; London et al. 2013). The composition of protein–peptide complexes must be determined in order to understand the molecular dynamics of related biological processes and to manufacture peptide vaccines. Protein–peptide docking predicts the complex structure from the arrangement of proteins and a sequence of peptides by sampling possible peptide binding conformations and ranking the putative protein–peptide complexes with an energy scoring feature (htt:huanglab.phys.hust.edu.cn/hpepdock/).

HPEPDOCK is a web based server that uses a hierarchical algorithm to perform blind protein–peptide docking. HPEPDOCK considers peptide versatility by an ensemble of peptide conformations created by our MODPEP software, rather than running lengthy simulations to refine peptide conformations (Fosgerau and Hoffmann 2015).

Results and Discussion

Selection of Viral Proteins for Vaccine Preparation

The virus pathogen resource sequence database was used to produce the tetravalent vaccine (https://www.viprbrc.org/brc/vipr-protein-serch.spg). Dengue virus 1-4 was used to

determine the amino acid sequence of the structural Capsid protein. The structural protein assists the virus in invading the host and assembling viral particles. The C-proteins of DENV 1-4 Flavivirus serotypes had been selected for this analysis and an epitope was designed in such a way that it generated both B and T cell immunity.

Protein Predictions for Allergenicity

The algorithm described in this study was released on a specially designed website known as Allergen F P. Following the allergenicity test, four non-allergenic capsid proteins were selected for further research based on the index of similarity.

Epitope Estimation for Cytotoxic T Lymphocytes (CTL)

The most promiscuous epitopes absolute to the MHC category I allelomorph for capsid super molecule were classified victimization CTL epitopes projected from the IEDB tool. The immune response is triggered by T cell epitopes. The IEDB server assigns a score to each epitope. A high ranking indicates high precision. A high score indicates that the binder is of high quality.

All epitopes non allergenic structural capsid proteins together with longer chain of peptides, the higher the number of epitopes, based on a higher score of more than 0.1. As a result, for dengue 1, dengue 2, dengue 3, and dengue 4, a total of 29, 69, 40, and 53 $CD8^+$ T cell epitopes were selected in my study.

Helper T Lymphocyte (HTL) Epitope Prediction

For structural capsid protein, the IEDB server for MHC II predicted HTL epitopes. IEDB recommended 2.22 as a prediction process, with Low adjusted rank binders being strong binders. Actual Score 30 is the cutoff value. The epitopes with a score of more than 30 were short out from my data. For dengue 1, dengue 2, dengue 3, and dengue 4, a total of 34, 55, 29, and 47 $CD4^+$ T cell epitopes were eliminated based on a lower score of less than 30 for next study.

Instability and GRAVY of the Epitope Predictions

Prot Param is a way of calculating an index of parameter instability. The epitope is stable if the value is less than 40. Calculate the GRAVY value for hydropathy in protein sequences. The GRAVY value is calculated by multiplying the hydropathy values of all amino acids by the epitope length. Analysis chooses a higher negative score for the next study. On the basis of stability index and negative GRAVY value, 5 epitopes for dengue 1 were shorted out, 20 epitopes for dengue 2, 4 epitopes for dengue 3, and 10 epitopes for dengue 4 were shorted out as CTL epitopes. On the other hand, HCL epitopes are stable and had a negative GRAVY value. 1 epitope, 13 epitopes, 1 epitope and 7 epitopes short out from Dengue 1, Dengue 2, Dengue 3 and Dengue 4.

Toxicity, Hydrophobicity, Hydropathicity prediction

The toxicity of epitopes was determined using the Toxin Pred program, which classified them as toxic or non toxic. Only the negative SVM score indicates that chosen epitopes were non toxic, hydrophobic, and hydropathic, indicating that they should be investigated further study.

Prediction of B-Cell Epitope Vaccine Sequence Construction

All epitopes expected via way of means of ABCpred with a score more than 0.50 have been selected for every capsid protein. From all capsid proteins, a total of 25, 25, 25, and 20 B cell epitopes are predicted for dengue 1, dengue 2, dengue 3, and dengue 4, respectively. Predicted B-cell epitopes were used as a basis for CTL and HTL epitopes in the development of the final vaccine, and those epitopes whose sequences in B cell epitopes overlapped were shortlisted and chosen for inclusion in the vaccine's final build.

Prediction of Consensus Epitope, Immunogenicity, and Antigenic Propensity

A total of 47 consensus epitopes were predicted in my study. For break bone fever dengue 1, dengue 2, dengue 3, and dengue 4, consensus epitopes of 8, 18, 8, and 13 are expected. The immunogenicity and average antigenic propensity of these epitopes were considered for further investigation. Highly antigenic Consensus B cell epitopes were chosen from among B cell epitopes to predict T cell epitopes.

We constructed peptide datasets with equivalent MHC binding affinity and distinguished those that were recognized by T cells from those that were not. T cells with a high Immunogenicity score are more likely to be identified, whereas those with a negative score are less likely to be accepted. For dengue 1, LKRARNRVS had a higher score of 0.07259, RGFRKEIGR and VLRGFRKEI had a higher score of 0.08016 and 0.23167, respectively, for dengue 2, and KNGAIKVLR had a higher score of 0.00533 for dengue 3. For dengue 4, the epitopes KAINVLRGF and INVLRGFRK have higher scores of 0.12993 and 0.21358, respectively. Epitopes that failed to generate a positive value were discarded for further investigation.

The vaccine construct's antigenicity probability was determined to be 0.4 by ANTIGENpro, indicating that it is antigenic. LKRARNRVS has a higher dengue 1 score of 0.9834, RGFRKEIGR and VLRGFRKEI have a higher

dengue 2 score of 0.9515 and 1.0022, respectively, and KNGAIKVLR has a higher dengue 3 score of 1.0258. For dengue 4, KAINVLRGF and INVLRGFRK have better scores of 1.178 and 1.0186 respectively. Both results suggest that the final vaccine construct is a powerful antigen as a result of this process.

IC_{50} Values and Conservancy Prediction Through Consensus Sequences

Peptides with IC50 values of 50 nM are thought to have a high affinity, 500 nM to be intermediate and 5000 nM to be weak in the Informatics in Medicine Unlocked 20 (2020) 1004306 IEDB. Alleles with IC_{50} values were chosen as the best binders for further investigation.

Three out of six epitopes demonstrated 100% conservation in all consensus sequences of all DENV at the 60% sequence identity threshold. In my research, we discovered that the epitope LKRARNRVS of DENV-1 is 60% conserved, while the epitopes RGFRKEIGR and VLRGFRKEI of DENV-2 are 98.94% and 100.00% conserved, DENV-3 epitope KNGAIKVLR is 97.00% conserved, while DENV-4 epitopes KAINVLRGF and INVLRGFRK are also 100.00% conserved respectively.

Population Coverage Study

Selecting a group of epitopes with many HLA binding capacities will help to expand global coverage. Exploitation HLA constitution frequencies; we have a tendency to be ready to establish the response of every human fraction to a given epitope. In my research, we discovered that the DENV-1 epitope LKRARNRVS covers 76.04% of the population in India, while the average population coverage is 66.49%, and the DENV-2 epitopes RGFRKEIGR and VLRGFRKEI cover 60.69% and 39.65% of the population in India, respectively, while the average population coverage is 98.64% and 47.95%. DENV-3 epitope KNGAIKVLR covered 78.64% of the population in India, while the average population coverage was 68.45%, and DENV-4 epitopes KAINVLRGF and INVLRGFRK covered 78.62% and 76.04% of the population in India, respectively, while the average population coverage was 70.02% and 66.49%. The DENV-1 epitope LKRARNRVS, DENV-2 epitope RGFRKEIGR, DENV-3 epitope KNGAIKVLR, and DENV-4 epitope KAINVLRGF were chosen for further study due to their higher population cover.

Tertiary Structure Modeling, Refinement, and Evaluation

The method makes use of both PSIPRED's projected standard secondary structure information and Beta Turns' predicted information. A typical backbone-dependent rotamer library is used to position the side-chain angles.

Allele Structure

The IPDIMGT/HL allele Structure Prediction server was used to build the 3D structure of the chosen allele HLA B*2705 (2BSR).Using the IPD IMGT/HLA allele Structure Prediction server, the 3D structure of the selected allele DRB1 1501 (1BX2) was created. MODELLER 9.10 is homology simulation software chosen Sample Template (PDB ID) 3PDO by the model with allele DRB1 0701. The model with allelomorph DRB1 1327 selected model (PDB ID) 2WBJ by MODELLER 9.10. PROCHECK was used to check the allele's stereo chemical properties.

Docking

The Hpepdock online server, which was chosen for protein docking, was used for blind global peptide docking to measure the relationship between the refined model and the immune receptor. The success of Hpepdock docking generates a large number of outcomes, from which the top ten had been selected for analysis. After analyzing all 10 docked conformations, result number one was the best-docked model, indicating the best interactions between the receptor and ligand (Fig. 5). Peptide LKRARNRVS, RGFRKEIGR, KNGAIKVLR and KAINVLRGF for DENGUE-1, DENGUE-2, DENGUE-3 and DENGUE-4 bind with Allele DRB1_1327, HLA-B*2705, DRB1_0701 and DRB1_1501 Docking score – 213.922, – 222.148, – 206.13, – 178.031 were found to be geometric form complexity docking score.

Discussion

DF has emerged as a life-threatening disease that has spread exponentially across the world in recent years. Many efforts have been made to develop the dengue virus vaccine, but none have been effective. Dengvaxia was the first vaccine approved, but it has a number of flaws that make it useless against all DEN serotypes. According to the WHO, Dengvaxia can only be used in patients aged 9 to 45 years old and in countries where DF is widespread. Dengvaxia's ineffectiveness and shortcomings necessitate the creation of a more effective dengue vaccine right away. Using an immunoinformatics approach, an epitope-based tetravalent DENV vaccine was created to provide increased protection over previously manufactured vaccines.

To anticipate capsid protein epitopes, the entire DENV viral proteome was retrieved from the VPRS database. Capsid's role is to bundle the viral genome so that it can be efficiently transferred from one host cell to another. Capsid

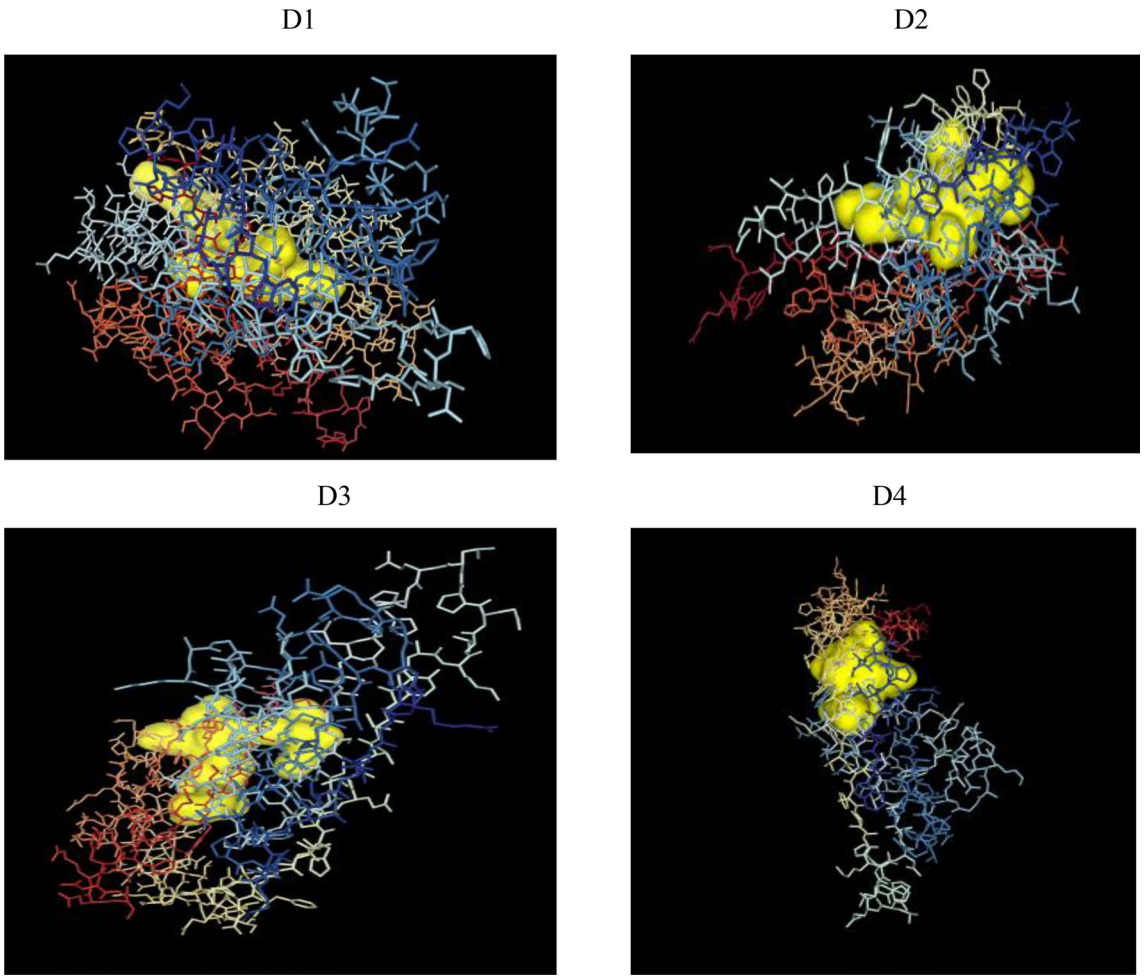

Fig. 5 Graphical representation of the **D1** T-cell epitope (LKRARNRVS) docked with DRB1_1327, **D2** T-cell epitope (RGFRKEIGR) docked with HLA-B*2705, **D3** T-cell epitope (KNGAIKVLR) docked with DRB1_0701 and **D4** T-cell epitope (KAINVLRGF) docked with DRB1_1501

will form the nucleocapsid before the processing of assembly or packaging faulty viruses, which has become a significant focus of vaccine development strategy (Patkar et al. 2007). It is contained in lipid droplets that form as a result of interactions with host proteins and other factors (Martins et al. 2012; Byk and Gamarnik 2016; Iglesias et al. 2015; Samsa et al. 2009). The dengue C protein is important in virus assembly to ensure precise encapsidation of the viral genome. The presence of sub viral particles emitted from infected cells shows Capsid's essential function currently, information from the capsid structures is used to explain the role of flavivirus capsid in the virus life cycle (Alvarez et al. 2005; Doklandet al. 2004). Encapsidation's function is unclear, although it is believed to include non structural viral proteins as well as C protein (Villar et al. 2015). In order to find possible drug targets (Oliveira, et al. 2017; Byrd et al. 2013; Soto-Acosta et al. 2014). As the viral polyprotein is cleaved by signal peptidase, the capsid may interact with vesicular transport proteins and pass into lipid droplets for storage or the nucleus (Hasan et al. 2018). Now that established, capsid proteins play a variety of other roles and interact in the pathogenesis of these viruses (Slomnicki et al. 2017; Faizan et al. 2016). Capsid contact with lipid droplet membranes aids in both viral packing and storage capsid prior to packaging (Martins et al. 2012; Samsa et al. 2009; Shang et al. 2018). Capsid of flaviviruses appears to bind nucleic acids (Byk et al. 2016; Samuel et al. 2016; Varjak et al. 2017) in a non-specific manner. Capsid isn't the only soluble viral protein that can bind double-stranded nucleic acids (Cortese et al. 2017; Paul and Bartenschlager 2015). Despite the fact that different types of host proteins have been shown to interact with the capsid protein and despite the fact that certain medications have been engineered to target these interactions. However, it's possible that during the compacting of the genome, the capsid binds both ss- and dsRNA. As a result, a comparison of the nucleic acids

that the capsid binds in vivo is needed (Garcia-Blanco et al. 2016).

Antibodies, B cells, and T cells accept the epitope as a component of an antigen that is recognised by the immune system. MHC binding estimates are now highly precise, covering the majority of established HLA specificities. These structures are excellent for epitope exploration (Hope and McLauchlan 2000).

Experimentally determining B cell epitopes is costly in terms of resources and time (Thiele and Spandl 2008). In my research, the ABCpred method was used to estimate B cell epitopes. B cell epitopes, which can only cause B cell mediated immunity, are expected at first. Highly antigenic Consensus B cell epitopes were chosen from among B cell epitopes to predict T cell epitopes.

Many researchers have recently looked at B cell epitope sequences in order to predict T cell epitopes that could interfere with both MHC groups with the most alleles, resulting in a stronger antigenic response (Boulant et al. 2007). Docking the receptor ligand molecule is an effective technique for determining the receptor's relative binding affinity for the ligand. Molecular docking of the expected immunogenic epitope LKRARNRVS (DRB1 1327) of DENV-1, epitope RGFRKEIGR (HLAB*2705) of DENV-2, epitope KNGAIKVLR (DRB1 0701) of DENV-3, and epitope KAINVLRGF (DRB1 1501) of DENV-4 Docking score − 213.922, − 222.148, − 206.13, − 178.031 was performed to suggest structural insight into the epitope. The constructs were found to be effective after testing antigenicity, solubility, and allergenicity. These T cell epitopes may be used as a candidate for a monovalent vaccine that would be safe and immunogenic against DENV serotypes.

A tetravalent dengue DNA vaccine was created by combining four monovalent vaccines in a non-lipid adjuvant and testing it in rhesus monkeys. Antibodies of all four dengue serotypes may be neutralized by the vaccine (Zhu et al. 2007). A dengue vaccine would elicit defensive immune responses to all four serotypes of the disease. In the current study, a monovalent 9-mer epitope induces immunity against a single DENV serotype. This tetravalent vaccine should protect against all four dengue serotypes.

Similar studies were performed by different researchers such as Ali et al. (2017) employed immunoinformatics to develop a multi-epitope-based dengue subunit vaccine capable of eliciting a variety of immune responses within the host. For dengue virus structural and non-structural proteins, distinct B-cell, TC cell, and TH cell binding epitopes were predicted. Final vaccine constructs include epitopes from TC and TH cells and an adjuvant (β-defensin) at the N-terminus. Subramaniyan et al. (2017) investigated E-proteins from DENV-1-4 serotypes as antigens and hypothesised that T cell and B cell epitopes could act as peptide vaccine candidates. The chimeric tetravalent vaccine was developed by conjugating four vaccines, one from each of the four dengue serotypes, to produce a vaccine candidate that is effective against all four dengue serotypes. They determined that the monovalent 9-mer T cell epitopes for each DENV serotype can be exploited to generate unique antibodies against dengue virus, as well as a chimeric common tetravalent vaccine candidate capable of covering any of the four dengue virus serotypes. Verma et al. (2019) conducted a comparative genomes study of Dengue virus (DENV) to identify novel vaccine targets. They discovered 100% conserved epitopes in the envelope protein (RCPTQGE), the NS3 (SAAQRRGR, PGTSGSPI), the NS4A (QRTPQDNQL), the NS4B (LQAKATREAQKRA), and the NS5 (QRGSGQV) proteins in all DENV serotypes. Additionally, conserved serotype-specific motifs were discovered in NS1, NS5, Capsid, PrM, and Envelope proteins. Chan et al. (2020) developed four multi-epitope peptides (P1–P4) against all four DENV serotypes by connecting a universal T-helper epitope (PADRE or TpD) to a highly conserved CD8 T cell epitope and a B cell epitope (B1 or B2). Four nanovaccines (NP1–NP4) were developed using the multi-epitope peptides conjugated to polystyrene nanoparticles (PSNPs). They concluded that NP3, which contained the TpD T-helper epitope coupled to the highly conserved B1 epitope derived from the E protein, was capable of eliciting large quantities of IFN-γ and NAbs against all four dengue serotypes.

Similar studies were performed on different viruses by different researchers such as Pandey et al. (2018) developed a multiepitope subunit vaccine employing Zika virus structural and nonstructural proteins using a combinatorial immunoinformatics method. The subunit vaccine is composed of cytotoxic T-lymphocyte and helper T-lymphocyte epitopes, as well as an adjuvant and linkers. Shahid et al. (2020) developed a MEBP vaccine using a combination of immunoinformatics and molecular docking. Prediction of B-cell, T-cell and IFN-epitopes was performed using the ZIKV proteome. Tahir ul Qamar et al. (2018) conducted a study to identify conserved B and T cell epitopes on CHIKV structural proteins using immunoinformatics and computational techniques. These epitopes may play a critical role in eliciting immunological responses against CHIKV. Numerous conserved CTL epitopes, linear and conformational B cell epitopes, and their antigenicity were predicted for the CHIKV structural polyprotein.

Conclusion

DENV-1 epitope LKRARNRVS, DENV-2 epitope RGFRKEIGR, DENV-3 epitope KNGAIKVLR, and DENV-4 epitope KAINVLRGF were selected as the top four T cell epitopes. Chosen as monovalent vaccines were conjugated to create a compound tetravalent vaccine in

this research. Further wet-lab replication using cell-based approaches and animal-based models will boost the credibility of this study.

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