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In Silico Study on the Binding Pattern of cTap Binding Epitopes of S-27 Strain with the Common HLA Alleles for the Chikungunya Vaccine Development

Sanjay Mishra[1*], Prashant Saxena[1,2] and Madhvi Saxena[3]

[*1]*School of Biotechnology, IFTM University, Moradabad- 244102, UP (W), India*

[2]*Department of Biotechnology, KS Vira College of Engineering & Management, Bijnor UP (W), India*

[3]*Department of CSE, University College of Engineering & Technology, Vinoba Bhave University, Hazaribagh, Jharkhand, India.*

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ABSTRACT

Chikungunya is a reemerging disease caused by the CHIK virus of Togaviridae family. *Aedes aegypti* and *Aedes albopictous* are the carrier mosquitoes of this disease. The disease is spreading globally with the increasing number of cases every day, the first outbreak of which was noticed in the year 1952 in Tanzania, while the latest outbreak found in 2016 in India. The symptoms may be chronic if disease persists more than 2-3 weeks and cause severe joint pain, probably leading to arthritis. There is no medicine or vaccine available for the treatment of this disease. In this study, authors collected the African genotype of CHIK virus and predicted T cell epitopes. The predicted epitopes were tested for the sharing with B cell, TAP binding activity. The selected epitopes tertiary structure was modeled and then docked with the cTAP1 (1JJ7) protein. Those epitopes showed the interaction with the cTAP1, further docked with the respective HLA allele. The epitope-HLA complex was tested for stability using NAMD-VMD molecular dynamics simulation method. ^{128}**FLARNYPTV**^{136} were found as the promiscuous epitopes that function as the vaccine candidate for the designing of vaccine of chikungunya. Data obtained from the present study provide new insights into development of novel process of vaccine research of chikungunya and ultimately shedding off the burden from the human society.

Keywords: Chikungunya, Peptide vaccine, cTAP, HLA, Vaccine designing, Immunoinformatics

BACKGROUND

Chikungunya is a viral disease caused by the CHIK virus, belongs to the genus alphavirus and family Togaviridae. The disease is transmitted via the bite of female mosquito, i.e., *Aedes aegypti* & *Aedes albopictus* of genus Aedes and family culicidae. The threat of the disease is increasing with the increased number of cases around the globe. The common symptoms of chikungunya are high grade fever, nausea, headache and joint pain. The disease has both acute and chronic symptoms. The chronic symptoms are characterized by the severe joints pain with arthralgia [1]. Till date there is no direct treatment available for this disease. Medical practitioners adopt the method for the lowering down the fever and pain-relieving therapy. Due to this the disease is progressively expanding the epidemic area.

Name Chikungunya derived from the place where this disease first appeared that is Makonde Plateau. The meaning of Makonde is the "Bends Up", such symptoms appears in the disease [2]. The disease first appeared in 1952 in Africa and first outbreak was found in the Tanzania (Previously known as Tanganyika) in 1953. Till then the virus has been kept circulating in the area and spreading globally and increasing the number of registered cases. The disease shed off from most of the regions from long time but re-emerged as a drastic one. In India disease got disappear in 1973 but after the 32 years disease again reemerged in 2005 and affected nearly 1.4 million people in the country. The recent outbreak occurred in 2016in Capital region of India [3].

CHIK virus contains a single stranded RNA as a genome with the size of 11.8 Kb and encoding two polyproteins, Structural Polyprotein and Non-Structural Polyprotein namely. The Structural Non-Structural polyprotein is further subdivided into six and four proteins, respectively. Structural polyproteins contain envelope protein (E1, E2 & E3), Nuclear capsid protein and a 6K protein domain, while

Corresponding author: Sanjay Mishra, School of Biotechnology, IFTM University, Delhi Road (NH 24), Moradabad 244102, Uttar Pradesh, India, Tel: +91-9837006059; E-mail: sanjaymishra@iftmuniversity.ac.in

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nonstructural polyprotein contains P1234 unit, which further converts into nSP1, nSP2, nSP3 and nSP4 (Non-Structural Protein) [4]. The CHIKV have three main genotypes that had evolved according to the climatic condition of surroundings, namely, African genotype, Asian genotype and ECSA (East central South Africa) genotype.

The expansion of disease keeps increasing around the world and there is no vaccine and medication available for the treatment of this disease. Peptide based vaccine could be the fruitful solution to cope up with this emerging disease. The peptide-based vaccine uses the small fragment of microbial component, which are capable of inducing long lasting effects against the microbial invader. Due to this powerful feature the upcoming era is for peptide based synthetic vaccines. For synthesizing the peptide-based vaccine, selection of epitopes are the crucial steps as this identification of appropriate microbial protein content is needed. Designing a peptide-based vaccine [5] is not an easy process, it is a challenge and the major task is the identification of the short peptides as a promiscuous T cell epitope. The small size of epitope is needed to get identified by the T cell, if the size is larger the T cell would not recognize them. The identified epitopes should be recognized by the MHC class I molecule before analyzed by the T-cell.

The MHC molecules present on the surface of T cell, i.e., T_c (CD8-Class-I MHC) and T_H (CD4-Class-II MHC). These CD8 & CD4 cells help in the processing of the epitopes bound with T cell and produce the effective immunological response to degenerate the microbial invader and provide the immunity to the host cell.The small antigenic peptide should reach to the RER lumen where they will bind with the class I MHC molecule and then the complex is processed by the T cell [6]. To achieve this process the TAP (Transporter Associated Protein) provides the cavity through the TAP1 and TAP2. This process required energy in form of ATP, ATP binds on the site of TAP protein to continue the process [7].

Peptide based vaccine designing is still a challenge in recent era due to the less available methods for identification of small peptides, which could function as a promiscuous T cell epitope. Therefore, the present research work was undertaken using the concept of immunoinformatic techniques to identify and analyze the small peptides, which may function as a vaccine candidate and become a valuable asset in designing the vaccine for the treatment of chikungunya.

METHODOLOGY

Chikungunya African strain S-27 (Accession No: AF369024) were retrieved from the NCBI (National Centre for Biotechnology Information) database (https://www.ncbi.nlm.nih.gov/) [8]. The complete genome of African genotype contains the structural (Accession No: AAN05102.2) and non-structural (Accession No: AAN05101.1) proteins.

PREDICTION OF T CELL & B CELL EPITOPES

All the selected structural and non-structural proteins were analyzed by using IEDB (Immune Epitope Database and Analysis Resource) database (http://tools.iedb.org/mhci/) against the common HLA alleles frequent in the human population. The IEDB is a machine learning based tool which used the concept of ANN and SMM method for the prediction of MHC class I epitopes. The cleft of the MHC class I molecule is suitable for the small size peptides, so in this study, authors used the nanomeric peptides having the IC_{50} value less than 50 nm. Predicted peptides have IC_{50} value less then 50 considered as good binders. The selected epitopes were tested for the conservancy analysis using the IEDB conservancy tool and only peptides having conservancy in the range of 88-100 % were only selected in the study [9].

The retrieved structural and non-structural sequences were tested again by the BCPred tool for the identification of B cell epitopes. BCPred (http://ailab-projects1.ist.psu.edu:8080/bcpred/predict.html) is a machine learning algorithm uses the concept of SVM and AAP method and predicts the linear B cell epitopes of the length of 20 amino acids. The antibodies approach to the T cell and B cell epitopes and the epitopes become more achievable for the antibody if the T cell epitopes shares part with the B cell epitopes. In this study only those T cell epitopes were selected who shares the part of B cell epitopes [10].

THREE-DIMENSIONAL MODELLING OF PREDICTED EPITOPES AND HLA ALLELES

The selected epitopes who satisfy all the criteria of selection i.e. IC_{50}<50, conservancy score in range 88-100% and shares the part with B cell linear epitopes, were modeled using the PepStrMOD tool (https://webs.iiitd.edu.in/raghava/pepstrmod/) [11]. The tool is able to model the peptide of length 7 to 25 amino acids. The modelled tertiary structure of epitopes was tested for the stability using the molecular dynamics simulation using the Amber 6.0. The HLA allele structure of HLA-A-02:02 was modelled using the MODELLER 9.21 [12] (https://salilab.org/modeller/) by using the 6APN as the template retrieved from the PDB database. The modelled HLA allele were tested for various tools to check the stability of the modelled structure.

DOCKING STUDY OF SELECTED EPITOPES WITH cTAP1 AND HLA ALLELE

The modelled epitopes were docked against the cTAP1 (PDB ID: 1JJ7) protein using the AutoDock 4.2 [13] (http://autodock.scripps.edu/) and Cygwin terminal. Those epitopes who shows the binding with the cTAP1 protein exposes for the binding with the favorable HLA allele of

epitopes. The binding poses of docking study were analysed using the Discovery Studio Visualizer [14]. The quality of binding was analysed by the various parameters such as, Binding energy, H-Bonds, H-Bonds Distance, Ki value and RMSD value.

MOLECULAR DYNAMICS & SIMULATION STUDIES OF EPITOPE-HLA ALLELE COMPLEX

Epitope-HLA allele complex were tested for the stability by using the molecular dynamics & simulation analysis methods. NAMD-VMD [15,16] tool is used for preparation of ATOM file and PSF files and for running the molecular dynamics using the CHARMM force field at 310°K with 100000 runs. The RMSD value was calculated using the command line of VMD and then rmsd.dat file is generated and using the Origin tool interactive RMSD plot was generated.

RESULTS & DISCUSSION

Identification of Epitopes

Chikungunya S-27 African strain was retrieved from the NCBI database and the T cell and B cell epitopes were predicted using the IEDB analysis resource and BCPred tool respectively. Twenty different HLA alleles were used for the prediction of epitopes. The tool predicted 134 structural and 275 non-structural T cell epitopes. These predicted epitopes were tested for the conservancy analysis and all epitopes were fall in the range of 88-100 % conservancy. The identified epitopes were tested for the B cell epitopes and only 26 structural and 50 non-structural epitopes were identified that shares part with the B cell epitopes. These identified epitopes were tested by TAPPred tool [17] to predict the binding affinity of the predicted epitopes. Among these 76 epitopes 13 were found with the high binding affinity towards the cTAP protein. So, these 13 epitopes **(Table 1)** were selected for the study and further analysis.

Table 1. Predicted structural and non-structural epitopes along with the prediction score of BCPred and TAPPred.

S.No	HLA Allele HLA-	Epitope with Start and End Position	Protein Name	IEDB IC_{50} Value	BCPred Score	TAPPred Score
1	A-68:01	^{201}FTIPTGAGK^{209}	C	8.79	1	1.280
2	A-02:02	^{482}SLVPILETA^{490}	nSP2	26.09	1	3.244
3	A-02:03	^{482}SLVPILETA^{490}		15.21		
4	A-02:06	^{482}SLVPILETA^{490}		19.34		
5	A-02:01	^{284}IIVCSSFPL^{292}	nSP3	33.26	1	3.249
6	A-02:02	^{284}IIVCSSFPL^{292}		15.21		
7	A-02:06	^{284}IIVCSSFPL^{292}		19.34		
8	A-02:01	^{128}FLARNYPTV^{136}	nSP4	6.01	1	3.277
9	A-02:02	^{128}FLARNYPTV^{136}		4.41		
10	A-02:03	^{128}FLARNYPTV^{136}		2.63		
11	A-02:06	^{128}FLARNYPTV^{136}		6.14		
12	A-02:02	^{121}AVAACNEFL^{129}		6.43		3.037
13	B-15:01	^{455}LTKSACAAF^{463}		10.55		3.801

Modelling of Selected Epitopes and HLA Alleles

The selected epitopes were modelled using the PepStrMOD tool, a small peptide modelling tool uses the NCAA and PTM forcefield libraries to perform the molecular dynamics analysis to check the stability of modelled epitope by using AMBER 6.0 [18]. HLA allele structure was modeled using the Modeller 9v21. Tertiary structure of HLA alleles was retrieved from the PDB (Protein Data Bank) database. The tertiary structure of HLA-A-68:01 (6PBH), HLA-A-02:03 (3OX8), HLA-A-02:06 (3OXR), and HLA-A-02:01 (4U6X) was available in the RSCB-PDB database [19] and these structures were retrieved. Structure of HLA-A-02:02 were not available in the PDB database, so tertiary structure was modelled using the method of homology modelling by modeler tool using the 6APN as a template. The modelled structure was analyzed by several tool to check its quality factor **(Table 2)** and it was found that modelled structure possess good quality and could be used further.

Table 2. Comparative Stability Analysis of the Modelled Structure using the various computational Tools.

HLA-A-	[1]Verify 3D	[2]ERRAT	[3]PROVE	[4]PROCHECK	[5]PROSA	[6]PROQ	[7]RAMPAGE
02:02	Pass 98.18	94.8207	3.8% Warning	Error:0 Warning:6 Pass:3	-9.04	2.920	F~98.5 A~1.5% DA~0.0%

1. At least 80% amino acid residue must have 3D score >= 0.2.
2. It defines the quality factor of the modelled protein structure.
3. On the basis of Voronai Radical planes predicts the quality of the structure
4. Defines the available **E**rror **W**arning and **P**ass of the modelled structure.
5. The quality of the modelled structure is predicted in terms of Z Score.
6. The quality of the modelled score is predicted in terms of LG score.
7. The data represents the number of amino acids available in the favored region in the Ramachandran Plot. F: Favored Region, A: Allowed Region, DA: Disallowed Region.

Molecular Docking Analysis

The molecular docking study was performed between the modelled epitopes and cTAP1 (1JJ7) and the process was repeated again between epitopes who was found interacting with cTAP1 and respective HLA allele using the AutoDock 4.2. The study reveals that an epitope shows the interaction with the cTAP1 and they also show the good binding energy with the HLA allele. Epitope [128]**FLARNYPTV**[136] shows the binding energy of -2.38Kcal/mol with the 6 H-bond when docked with the cTAP1 protein. Further same epitope was docked with the four different alleles i.e. HLA-A (0201), (0202), (0203), and (0206) and it shows the best binding energy -4.25 Kcal/mol & -3.53 Kcal/mol with HLA-A-02:02 and HLA-A-02:01 allele **(Table 3)**.

Table 3. Docking Analysis Table, shows the binding analysis with the cTAP1 protein and the respective HLA allele.

Epitope	BE (Kcal/Mol)		Ki mM/µM		RMSD (Å)		H-Bond		H-Bond Distance (Å)	
FLARNYPTV	**cTAP**	**HLA**	**cTAP**	**HLA**	**cTAP**	**HLA**	**cTAP**	**HLA**	**cTAP**	**HLA**
HLA-A-02:01	-2.38	-3.5	17.95	2.57	140.5	61.25	ALN583-PRO7 GLY585-VAL9 TYR6-GLN552 TYR6-HIS574	GLN96-THR8 ARG4-TYR27 PHE1-ASP30 VAL9-GLN115	3.20 2.81 3.21 3.16	2.85 3.05 2.53 3.62
HLA-A-02:02		-4.2		765.8		16.61	ASN5-GLU587 ARG4-SER545	ARG4-ASP54 GLN120-PRO7 GLN120-VAL9	2.36 2.35	3.12 2.99 3.14

Molecular Dynamics & Simulation

Molecular dynamics aids to the understanding the molecular behavior in the defined parameters [20]. The molecular dynamics and simulation study of 2 predicted complex (Epitope-HLA allele) was performed using the NAMD-VMD tool. The MD simulation was run for 100000-time step and the energy minimization was performed for the 10000 steps at 310°K and default parameters and the simulation was run for the 1 FS (femtosecond). The result analysis of simulation study confirms that **FLARNYPTV-HLA-A-02:02** complex **(Figure 1)** is more stable in comparison to the other complexes. The graph was plotted between the RMSD and Time (PS), for building the graph VMD tool was used. The protein's file was loaded first and then the protein_md.dcd file was uploaded for further analysis. The RMSD trajectory tool was used to plot the RMSD vs Time graph. In the RMSD vs Time graph protein_wb.psf and protein_wb_md.dcd files were used. The RMSD trajectory tool first performs the alignment and then plot the RMSD vs Time graph using the time slot 0.0 to 1.0. Among the 2 identified epitopes HLA complexes **^{128}FLARNYPTV^{136}** epitopefound stable in the MD simulation analysis.

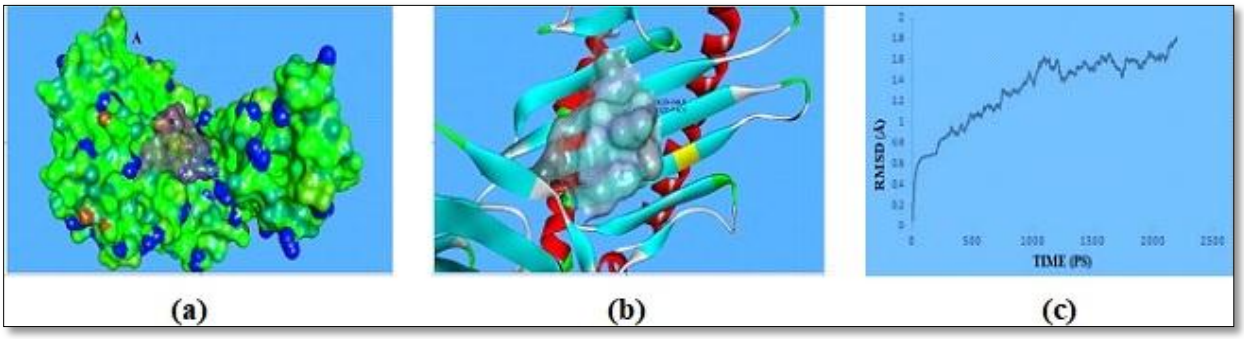

Figure 1. (a) Binding pocket of HLA alleles shows the settlement of epitope in the pocket. The epitope shows the groove binding, refer to the stable binding. **(b)** HLA allele and epitope show the binding pattern. 3 H-bonds are visible showing the interaction between the **^{128}FLARNYPTV^{136}** and **HLA-A-02:02**. **(c)** The molecular dynamics and simulation study show the RMSD vs Time (PS) graph. The graph depicts that complex molecule reaches to the stability state after the completion of MD.

CONCLUSION

The immunoinformatics approaches and the advancement in the computational methods have shown the new path in the field of vaccine technology [21]. The designing of short peptide vaccine was a time taking and tedious process and the chances of success was very less but these methods made possible to cut short the time with the high success rate [22]. In this study, authors reported a T cell epitope which is interacting with the HLA class I allele. The nanomeric epitope **^{128}FLARNYPTV^{136}** was found matching with all the criterion and conditions to become as a vaccine candidate. Hence, authors suggest that this epitope is most promiscuous and can function as a vaccine candidate for the treatment of chikungunya. Authors are looking forward to use this result in the process of vaccine designing for the chikungunya.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

ETHICAL APPROVAL

The authors declare that there were no animal or human objects involved in this present study.

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