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In-silico design of an Epitope-based peptide vaccine: A Computational Biology Approach

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ABSTRACT

Lymphocytic choriomeningitis, is a rodent-borne viral infectious disease caused by *Lymphocytic choriomeningitis virus* (*LCMV*), a member of the family *Arenaviridae*, that was initially isolated in 1933. Acquired postnatal infection ranges from asymptomatic to a brief, nonspecific flu-like illness to critical self-resolving neurological disease, predominantly consisting of aseptic meningitis or meningoencephalitis. This study was undertaken to design an epitope-based peptide vaccine against *Lymphocytic choriomeningitis virus* using a computational biology approach. Twenty four sequences of *LCMV* were retrieved from UniProt database and analyzed with various in silico tools. VaxiJen was used to identify immunogenic peptides and T-cell epitopes were analysed using NetCTL server to identify T-cell epitopes. Out of 15 immunogenic peptides analysed using NetCTL server, a conservancy of 64.28% amongst all epitopes was observed. The peptide sequence VVQNLDQLY, a non-allergen, was found to be a potent T-cell epitope that interacted with 28 human leukocyte antigens (HLAs) and its interaction with HLA-A*02:06 was studied using protein-protein docking analysis. The HLA allele and the epitope VVQNLDQLY were found to effectively interact with each other and this epitope may be used as a vaccine against *LCMV*. Thus immunoinformatics based approaches can be used to predict vaccine candidates against pathogens in a timely manner and usher us into an era of T-cell based novel vaccinomics approach.

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1. INTRODUCTION

A novel approach integrating immunoinformatics and immunogenomics for the development of vaccines is known as vaccinomics [1]. The present conventional approach for vaccine development relies on antigen expression, in sufficient amount, from in vitro culture models; however, many antigens, while expressed sufficiently, may not be good candidates for vaccine. With these conventional approaches, it has not been possible to control different types of outbreaks of viral pathogens, such as recent avian, swine flu and influenza strains, due to time-consuming development process for vaccines. Hence, rapid in silico informatics-based approach has gained much popularity with the recent advancement in the sequencing of many pathogen genomes and protein sequence databases [2]. The "vaccinomics" approach has already proven to be essential for combating diseases such as multiple sclerosis [3], malaria [4], and some tumors [5]. However, these methods of vaccine development usually work through the identification of human leukocyte antigens (HLA) ligands and T-cell epitopes [6], which specify the selection of the potent vaccine candidates associated with the transporter of

antigen presentation (TAP) molecules [7-10]. The overall idea behind vaccinomics is to identify genetic and other mechanisms and pathways that determine immune responses, and thereby provide new candidate vaccine approaches [11].

Lymphocytic choriomeningitis virus (*LCMV*) is an enveloped single-stranded RNA virus of the *Arenaviridae* family. It is an important cause of neurologic disease in humans and covers a large geographic range. Wild mice are the natural host and principal reservoir of *LCMV*, but the virus can also be found in pet hamsters, golden hamsters, and guinea pigs. Humans acquire *LCMV* disease when they come into contact with the secretions of infected mice. When the virus is acquired postnatally by children or adults, the clinical manifestations are usually those of aseptic meningitis. Most people who acquire *LCMV* infection during childhood or adulthood are moderately symptomatic for several weeks, but have a full recovery. A much more severe disease ensues when the infection occurs prenatally. *LCMV* can infect the fetal brain and retina, where it leads to substantial injury and permanent dysfunction. The possibility of *LCMV* infection should be considered in all babies with evidence of congenital infection, especially those with prominent neurologic signs, such as microencephaly, periventricular calcifications, and hydrocephalus [12].

At present, vaccines are mostly based on B cell immunity. But recently, vaccine based on T-cell epitope has been encouraged as the host can generate a strong immune response by CD8+ T-cell against the infected cell. With time, due to antigenic drift, any foreign particle can escape the antibody memory response; however, the T-cell immune response often provides long-lasting immunity [13].

The aim of the present study is to predict a T-cell epitope having therapeutic potential as an effective vaccine that could be suitable to trigger a significant immune response against *Lymphocytic choriomeningitis virus*.

2. RESEARCH METHOD

2.1 Protein sequence retrieval & Evolutionary analysis

Twenty four protein sequences of *LCMV* were retrieved from the UniProtKB database [14] in the FASTA format. To construct a phylogenetic tree for the analysis of evolutionary divergence in the various protein sequences of *LCMV*, Clustal Omega [15], a multiple sequence alignment tool was used.

2.2 Immunological analysis using *In silico* tools

2.2.1 Antigenic protein identification

Antigenic protein identification was done using VaxiJen v2.0 server that classifies peptides as antigenic or non-antigenic [16].

2.2.2 T-cell epitope identification

The NetCTL 1.2 server was used for the identification of the T-cell epitope. The epitope prediction was restricted to 12 MHC-I supertypes and the method integrated peptide major histocompatibility complex class I (MHC-I) binding; proteasomal C terminal cleavage, and TAP transport efficiency [17].

2.2.3 Identification of MHC-I allele – T-cell epitope interaction & Epitope conservancy analysis

Tools from the Immune Epitope Database (IEDB) [18] that is based on stabilized matrix base method (SMM) [19] was used to calculate the half-maximal inhibitory concentration (IC_{50}) values of peptide binding to MHC-I molecules. The analysis of the epitope conservancy was done using web-based tool from IEDB analysis resource [20].

2.2.4 Allergenicity assessment

Allergenecity *i.e* whether the epitope is allergen or non-allergen was predicted by AllerTOP 1.0 and AlgPred. AllerTOP is the first alignment-free server for *in silico* prediction of allergens based on the main physicochemical properties of proteins [21]. Allergenecity was also predicted using another tool *i.e.* AlgPred that allows prediction of allergens based on similarity of known epitope with any region of protein [22].

2.3 Prediction and validation

The structure of the epitope was predicted using I-TASSER server [23]. This server modelled five 3D structures of the epitope, and the structure with highest C-mean score was selected for further studies. The 3D structure of the protein was predicted using I-TASSER was validated by Verify3D [24] that determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc) and comparing the results to good structures.

2.4 Docking Studies

To analyse the interaction between HLA molecule and the epitope, a protein-protein docking study was performed using PatchDock [25] and ZDOCK [26] servers. The structure of HLA was obtained from PDB. The docked models obtained from PatchDock and ZDOCK were superimposed using online tool, SuperPose [27] to compare the docking and interactions between T-cell epitope and HLA.

3. RESULTS AND ANALYSIS

Twenty four protein sequences of *LCMV* were retrieved from UniProtKb and multiple sequence alignment was performed amongst various strains followed by construction of phylogenetic tree using Clustal Omega (Figure 1).

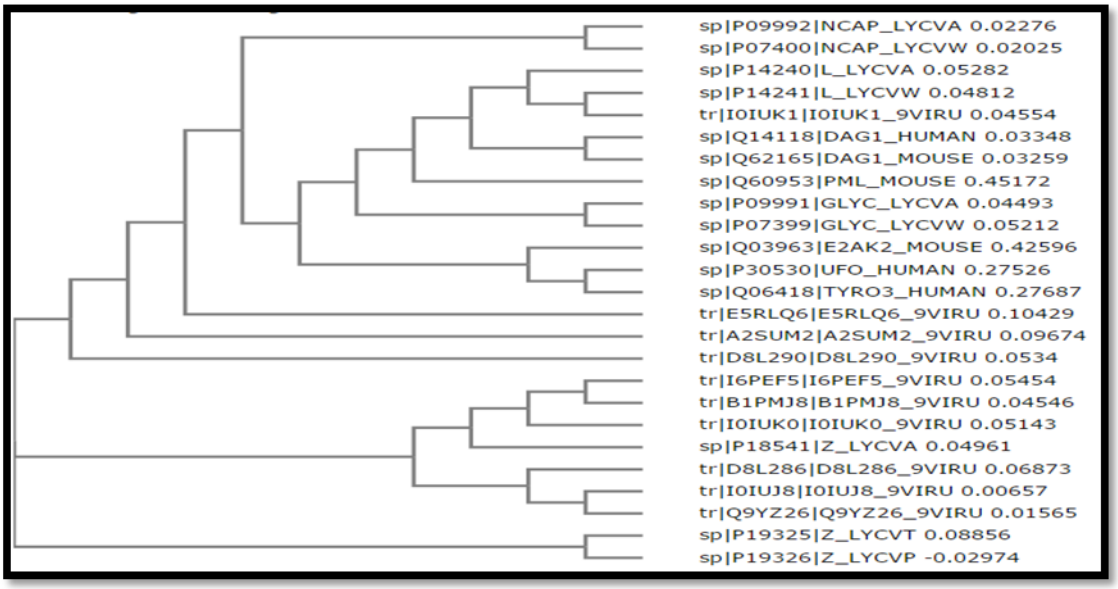

Figure 1: Phylogenetic Tree of various strains of *LCMV* obtained using Clustal Omega.

All 24 peptide sequences were then investigated using VaxiJen server out of which 15 peptides were found to be antigenic. These 15 sequences were further investigated for the prediction of T-cell epitopes using NetCTL server.

Top five best epitopes having highest combinatorial score were selected (Table 1) and further investigated for HLA-allele interactions and epitope conservancy using IEDB conservancy analysis tool. Amongst these T-cell epitopes, a 9mer epitope, VVQNLDQLY was found to interact with most number of HLA alleles i.e. 28 with two other 9-mer epitopes QMHGVAITY and HRHDGIMLY having similar epitope conservancy of 66.28%. Therefore based on both conservancy and maximum number of interactions of epitope with MHC-I alleles, the epitope VVQNLDQLY was selected.

Table 1: Epitopes selected on the basis of overall score predicted using NetCTL sever.

Epitopes	Overall score	Epitopes	Overall score	Epitopes	Overall score
RTWENHCRY	3.4600	AALKNLCFY	3.1390	LIKEFSELY	3.0350
RTWENHCTY	3.4200	ALVKRMKLY	3.1360	WTPGLSGIY	3.0156
VVQNLDQLY	3.2470	FYMDKLNKY	3.1290	QTFVSQPGY	3.0050
HRHDGIMLY	3.2240	SSVFSIEVY	3.1060	ALTDLGLLY	3.0030
QMHGVAITY	3.2210	MDSDTPGFY	3.0180	SLVRCHDHY	2.9360

Allergenecity was predicted by AllerTOP and AlgPred server and the epitope, VVQNLDQLY was found to be a non-allergen. The tertiary structure of the epitope, VVQNLDQLY was predicted using I-

TASSER. This was validated using Verify3D and the residue numbers 20-400, 500-780 and 820-880 were found to be well predicted as they had a score close to +1 while only few residues (400-500 and 830-870) were seen to have not been predicted reliably. Therefore it may be concluded that the overall quality of the structure of the epitope, VVQNLDQLY predicted using I-TASSER was good.

The HLA-A*02:06 which interacted with our proposed epitope was selected for docking as its structure was available in PDB (PDB ID: 3OXR). This structure of HLA A*02:06 had a bound HBV Core 18-27 which was removed before performing docking studies. Protein-protein docking analysis of allele HLA-A*02:06 and the epitope VVQNLDQLY was performed using PatchDock and ZDOCK (Figure 2).

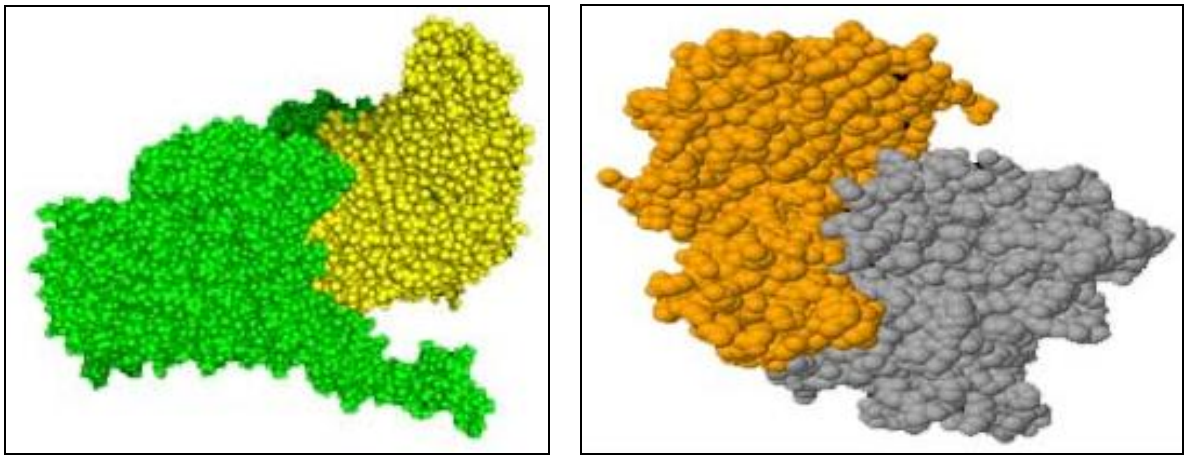

Figure 2: Docking of epitope, VVQNLDQLY and HLA-A*02:06 obtained from (A) PatchDock and visualised using Pymol (Yellow color represents the interacting allele and Green color represents the epitope, VVQNLDQLY) (B) zDOCK (Grey color represents the epitope, VVQNLDQLY and yelow color represents the interacting allele)

On superimposing the docked structures of HLA allele and epitope using SuperPose tool, the RMSD was zero thereby validating the interaction and results obtained from PatchDock & zDock. Therefore it can be concluded that the allele HLA-A*02:06 and epitope VVQNLDQLY effectively interact with each other and the epitope may be used as a vaccine against *LCMV*.

Vaccinomics helps in the development of a new vaccine in a timely fashion which is very crucial in recent times due to ever rising global burden of disease. The advancement of *in-silico* tools and sequence-based technology has resulted in availability of genomic and proteomic data of different viruses which can be used to design peptide vaccines based on neutralizing epitopes. Knowledge about the peptide's epitopes has a key role for manufacturing epitope-based vaccines, which when injected into the recipient, can induce an immune response.

Therefore in the present study we have been able to design a T-cell epitope based vaccine for *Lymphocytic choriomeningitis virus* using immunoinformatics based approach. This approach may be used to predict vaccine candidates for other pathogens in a timely manner.

4. CONCLUSION

The immunoinformatics based approach to design vaccines can help achieve effective, cost- efficient development of vaccines or vaccine components against pathogens such as *LCMV* with the validation of the prediction expected to be done by the wet lab researcher.

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