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Modelling of Structure Based T- Cell Epitopes of Bunyaviridae viruses, as a Genus of California Encephalitis Virus [CEV].

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ABSTRACT

California Encephalitis is a severe and lethal viral disease in humans which causes inflammation of brain and is one of the main causes of viral encephalitis. In this study we tried to find out putative peptides/epitopes of Bunyaviridae virus which is a genus of California Encephalitis virus. The antigenic protein of Bunyaviridae virus is envelope Glycoprotein which is further subdivided into Gn, Nss and Gc proteins. The Nss protein is a non-essential protein and thus is eliminated from the study. The immunoinformatics tools Propred I, BIMAS, and SYFPETHI were used to predict the MHC-I epitopes of Bunyaviridae Gn and Gc protein. The molecular modeling of selected epitopes were done with the help of Swiss model finally the simulation studies for finding out the total energy was done with iGemdock. The epitope HRSILPGSM of Gn protein at position 172-180 showed maximum binding score with HLA-B2705 allele and epitope SRKSQYIGK of Gc protein at position 430-438 showed maximum binding score with HLA-B2702 allele. Other epitopes YLCYVLIP and KPYQAVSMI at positions 211-218 and 188-196 showed maximum score with HLA-A0201 and HLA-B5101 alleles respectively. These epitopes predicted by Propred I was also checked with BIMAS and SYFPETHI tools. In this study we are trying to emphasize the role of immunoinformatics in the computational vaccinology. The designing of epitope based vaccines are easy to produce, safe and cost effective.

Keywords: Epitopes, Bunyaviridae virus, alleles, Propred I, Simulation.

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INTRODUCTION

California encephalitis virus causes encephalitis in humans. Encephalitis is an acute inflammation of the brain in humans, which causes mild symptoms like headaches. It was first discovered in Kern country, California in 1943[1]. This virus is known as arbovirus (arthopode-borne virus) because the carrier of this virus is mosquitoes [1]. The incubation period can vary from a few days to a week [2]. The virus enters the body through mosquito bite and gets replicated in the skin. The primary spread of virus occurs mainly in the spleen, liver and the lymph nodes. As the virus gets replicating the secondary infection occurs in the CNS [1]. Symptoms of CEV includes Malaise, Rash, Fever, Muscle pain, Stiff neck, Headache, Light sensitivity, Lethargy, Seizures, Aphasia, Ataxia, Paralysis etc. Infants and children are mostly affected than adults who sometimes show no obvious symptoms [3].

CEV belongs to the family of RNA virus known as Bunyavirida virus of the genus Orthobunyavirus. Bunyaviridae viruses are enveloped, segmented, single strand negative sense RNA viruses. Their diameter varies from 80-120nm [4] and genome size ranging from 10.5 to 22.7 kbp [5]. The Bunyaviridae virus (BUNV) have tripartile genome consisting of three segments, the largest segment (L) codes for an RNA-dependent RNA polymerase (L protein); the medium segment (M) codes for the two virion glycoprotein's Gn and Gc and a nonstructural protein, NSm; and the smallest segment (S) encodes the nucleoprotein N and a second nonstructural protein, NSs [6]. Genomes are encapsidated by the N protein, and complementary sequences at the $3'$ and $5'$ genome termini [7]. Virus attaches to host receptors though Gn-Gc glycoprotein dimer, and is endocytosed into vesicles in the host cell [6]. The NSs proteins have been found to be not essential for virus growth and can be deleted, but contribute to viral pathogenesis [7]. BUNV lacking NSs (NSs) has a reduced growth rate as well a reduced ability to shut off mammalian host cell protein synthesis compared to wild type (wt) BUNV [7]. Thus the two envelope glycoproteins (GP), Gn and Gc are important in mediating and controlling the viral genome activities and its various functions.

Vaccination is the most successful and effective of all medical measures to save human and animal lives [8]. As compared to conventional vaccines epitope based or peptide based vaccines are less time consuming, more specific and cost effective. It is known that T-cell plays a crucial role in inducing cellular response and is important for cytolytic and regulatory response to pathogens [9]. T cells recognize foreign antigens only when they are associated with Major Histocompatibility Complex (MHC) which is exposed on the surface of cells. MHC is a heterodimer which presents a set of glycoproteins on the cell surface and induce T-cell activation therefore plays a vital role in regulating immune response [10].

Immunoinformatics is an emerging field that uses computational approaches to predict potential vaccine candidate or T-cell epitopes. One of the biggest advantages of peptide based vaccine is ability to deliver high doses of the potential immunogen at a low cost.

MATERIALS AND METHODS

Sequence retrieval

Glycoproteins Gn and Gc of Bunyaviridae viruses are the major immunogenic proteins and therefore have been used for immunogenic analysis. The amino acid sequence lengths of these are 285 and 433 respectively. The sequences were retrieved from Uniprot database. Uniprot database provides the functional information on proteins [11].

MHC-I binding epitopes prediction

The MHC-I binding epitopes were predicted using an online tool Propred-I. Propred-I is a web server for identification of MHC-I binding regions in antigens. It predicts antigenic sequences for 47 MHC-I alleles. It also allows the prediction of the standard proteasome and immunoproteasome cleavage sites in antigenic sequence [12]. The Gn and Gc protein sequence were analysed at a threshold setting of 4%. Proteasome and immunoproteasome filters were set on at a threshold of 5%. Only peptides with high score were selected for analysis.

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BIMAS and SYFPETHI

An online tool BIMAS was used to analyze all peptides with HLA alleles that indentifies those epitopes/peptides which will bind to HLA with high affinity [10, 13]. The binding affinity ($T_{1/2}$) value on BIMAS is based on the half time (min) of dissociation of beta 2 microglobulin from HLA. SYFPETHI is a database of MHC ligands and peptide motifs. It also predicts the binders for MHC class 1 molecule [14].

Modelling of T-cell epitope

Molecular modeling and structure analysis was done for the detection of peptides binding to their respective class I MHC alleles. The protein sequences of alleles were retrived from uniprot. The Swiss model was used for designing of those alleles whose structure is not available at PDB server. The predicted binding peptides were converted into pdb format by using open babel software. After designing the structure of alleles and binding epitopes the docking was done with *i*Gemdock for finding out the total energy between predicted epitopes and alleles [15].

RESULTS

Analysis of class I MHC molecules by Propred, BIMAS, and SYFPETHI

Propred was used to predict the highest scoring epitopes of Gn and Gc proteins which binds to maximum number of MHC-I alleles. After identification of epitopes by Propred I the results were evaluated by BIMAS and SYFPETHI for confirmation of predicted binders. The cut off value for BIMAS ($T_{1/2}\geq100$) was set at a threshold of 100 and for SYFPETHI the cut off value was set at ≥ 15 for peptide selection, indicated in table 1. Finally eight peptides are selected i.e; VRFLHRSIL, MRMHRESGL, YLCYVLIP, HRSILPGSM, KPYQAVSMI, TYLSEASLL, SRKSQYIGK, MHLLEAVFL with the help of scoring based algorithms of Propred I, BIMAS and SYFPEITHI. These peptides have higher binding affinity which was estimated by analyzing all interactions like hydrogen, Vander Waals and electrostatic force.

Table 1: Predicted peptides from target proteins bind to different HLA class I alleles

Protein	Position	Peptide	Allele	Propred I	BIMAS	SYFPEITHI
Gn	168-176	VRFLHRSIL	HLA-B14	1500	1500	27
	267-275	MRMHRESGL	HLA-B14	600	600	22
	211-218	YLCYVLIP	HLA-A0201	101.18	101.18	23
	172-180	HRSILPGSM	HLA-B2705	6000	6000	20
Gc	188-196	KPYQAVSMI	HLA-B5101	572	572	26
	31-39	TYLSEASLL	HLA-A24	300	300	25
	430-438	SRKSQYIGK	HLA-B2702	2000	2000	21
	76-84	MHLLEAVFL	HLA-B3901	180	180	25

Simulation Studies

Simulation studies of epitope HRSILPGSM of Gn protein and SRKSQYIGK of Gc protein formed stable HLA-peptide complexes with the energy minimization of -106.55 kcal/mol and -96.03 kcal/mol respectively. The other peptides YLCYVLIP and KPYQAVSMI identified were also found antigenically variable with total energy of -60.733 kcal/mol and -64.86 kcal/mol. This can possibly be targeted for designing of vaccine against Bunyavirida virus. The energies of predicted epitopes with their binding alleles are shown in table 2.

Table 2: Docking results of peptides and alleles using *i*Gemdock

S.No.	Peptide	Allele	Total energy kcal/mol	VDW	HBond	Elec	Aver.Con Pair
1.	VRFLHRSIL	HLA-B14	-32.19	-10.163	-22.027	0	9.73404
2.	MRMHRESGL	HLA-B14	-81.181	-64.664	-15.278	-1.2381	8.70513
3.	YLCYVLIP	HLA-A0201	-60.733	-48.366	-12.366	0	7.6852
4.	HRSILPGSM	HLA-B2705	-106.55	-85.38	-20	-1.17	9.4078
5.	KPYQAVSMI	HLA-B5101	-64.86	-65.01	-0.15	0.3	8.36782
6.	TYLSEASLL	HLA-A24	-11.19	23.23	-34.42	0	10.7467
7.	SRKSQYIGK	HLA-B2702	-96.03	-95.5	-0.44	0.98	9.72619
8.	MHLLEAVFL	HLA-B3901	-3.02	8.67	-11.43	0.27	8.3253

DISCUSSION

With the emergence in the field of computational biology it is now possible to reduce the time for identification of putative antigenic peptides. In this study we use immunoinformatics in computational vaccinology to find probable epitopes against the virus. These approaches were used for determination of antigenic peptides in the protein sequence of Gn and Gc of Bunyaviridae virus without using wet lab procedures. The prediction of epitopes of these Bunyaviridae proteins is recognized against MHC class I molecules. Propred I, BIMAS and SYFPETHI tools are used to find out the binding peptides and alleles. The predicted epitopes can be used for evaluating T-cell responses in the context of natural infections and also might be helpful in designing novel vaccine candidates against particular virus.

CONCLUSION

After screening of epitopes it is clear that protein Gn and Gc of Bunyaviridae virus can be used for the preparation of immunological constructs. Simulation and docking studies suggests that epitopes HRSILPGSM and SRKSQYIGK have lowest binding affinity with MHC class I molecules and thus can be used as a potential vaccine candidate against Bunyaviridae virus. Therefore by using similar approach the epitopes of other proteins can also be targeted for vaccine design that would reduce time and money.

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