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In-silico: Screening and Modeling of CTL Binding Epitopes of Crimean Congo Hemorrhagic Fever Virus

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ABSTRACT

Crimean-Congo Hemorrhagic Fever (CCHF) is a zoonotic viral disease that is asymptomatic in infected livestock but a serious threat to humans. This study is aimed at conducting the modeling of putative peptides which are suggested for vaccine development that is meant for evaluating epidemiological, clinical and laboratory characteristics of the patients diagnosed with Crimean-Congo hemorrhagic fever. In the present study, more reliable prediction of Major Histocompatibility Complex (MHC) peptide binding is based on the accurate determination of T-cell epitopes and hence the successful design of peptide and protein based vaccines. The importance of existing computational tools was used for prediction of peptide binding to Major Histocompatibility Complex (MHC) Class-I and Major Histocompatibility Complex (MHC) Class-II. With the availability of large sequence databases and computer aided design of peptide based vaccine, screening among billions of possible immune active peptides to find those likely to provoke an immune response was done. These peptides were selected by using different algorithms as Artificial Neuronal Network (ANN) and Support Vector Machine (SVM) for the T-cell epitope prediction and further characterized on the basis of binding affinity of peptide to HLA-alleles which can be finally used for the potential vaccine candidate development. A vaccine with specificity for a target population i.e., peptide based vaccine, in which small peptides derived from target proteins are used to provoke an immune reaction. Two nonameric epitopes (LRFGMLAGL) and (LLGIKCSFV) which exhibit good binding with MHC molecules and low energy minimization values providing stability to the peptide-MHC complex are reported here. These predicted peptides don't have similarity with human proteome. These peptide could be used in designing a chimeric/subunit vaccine, however, these will further be tested by wet lab studies for a targeted vaccine design against Crimean-Congo hemorrhagic fever.

Key words: Crimean-Congo hemorrhagic fever, *in-silico* vaccine design, major histocompatibility complex, human leukocyte antigen, computational vaccine, T-cell epitope

INTRODUCTION

Crimean-Congo Hemorrhagic Fever (CCHF) virus is the member of family Bunyaviridae (genus *Nairovirus*). In 1940s this virus was first described when many cases of severe hemorrhagic fever arose among agricultural workers in the Crimean peninsula but this virus was firstly reported in the former Soviet Union in 1944 (Jain *et al*., 2011). After some years, a virus with similar pathogenesis was isolated in 1956 from patient in Congo, Africa and the virus was subsequently named as Crimean-Congo Hemorrhagic Fever (CCHF) virus (Jain *et al*., 2011). Crimean-Congo

Hemorrhagic Fever (CCHF) has the most extensive geographic range of the medically significant tick-borne viruses, occurring in parts of sub-Saharan Africa, Asia, eastern Europe and the Middle East (Whitehouse, 2004). The countries widely affected in these areas include: Arabian Peninsula, Iraq, Pakistan and Xinjiang Province in northwest China (Ergonul, 2006). CCHF is a severe hemorrhagic fever in humans with a high fatality rate up to 30% (Ergonul, 2006; Vatansever *et al*., 2007). During the 21st century, recent outbreaks of CCHF virus were also reported in Gujarat, India.

The geographic distribution of CCHF virus cases corresponds most closely with the distribution of Hyalomma ticks, hosted on the migratory birds suggesting their principle vector role (Vatansever *et al*., 2007; Whitehouse, 2004). Some others species of Dermacentor and Rhipicephalus genera have also been shown to be capable of transovarial transmission. CCHFV is a member of large family of negative stranded RNA viruses denoted by *Bunyaviridae*. The family consists of more than 300 viral species and is subdivided into five genera: *Orthobunyavirus*, *Hantavirus*, *Phlebovirus*, *Tospovirus* and finally *Nairovirus* (Nichol *et al*., 2005). CCHFV has three segments of negative sense RNA viz. S, M and L which minimally encode the virus nucleocapsid, glycoproteins and polymerase proteins, respectively. M-RNA segment of CCHFV plays a major role in the immune response. In addition, members of the genera *Bunyavirus*, *Phlebovirus* and *Tospovirus* also encode a nonstructural glycoprotein referred to as NS_M (Honig *et al*., 2004; Kinsella *et al*., 2004; Meissner *et al*., 2006). But, the complete information about the M RNA fragment is not available. The M gene is responsible for immunity and pathogenicity as well as for vaccine development. The nucleotide sequences of the M RNA genome segment of CCHFV strains isolated from Xinjiang province was determined to define the molecular variability among CCHFV strains, in China. Examination of their expected amino acid sequences with the respective sequences of the orientated protein was also carried out (Meissner *et al*., 2006). Epitope based vaccine provide a new strategy for the prophylactic and therapeutic application of pathogen specific immunity (Zinkernagel and Hengartner, 2004). This strategy requires the identification and selection of promiscuous T-cell epitopes important for cytolytic and regulatory response to pathogens that helps to the vaccine development (Esser *et al*., 2003; Brusic and Agust, 2004; Pulendran and Ahmed, 2006).

The progression of Congo Hemorrhagic Fever is very rapid with clinical features as flu like symptoms appears during primary 3 days of infection. After one week symptoms get resolved, 75% cases which have sign of hemorrhage, thrombosis of vessels to extremities and leading to death within 5 to 7 days. No procurement found for this viral infection. Researches for the development of effective vaccine against CCHF virus require understanding of immune response. Viral immune response is associated with MHC protein and T-lymphocytes. MHC is of two types: MHC Class I and MHC Class II (Rammensee *et al*., 1999). MHC initially recognizes the viral antigenic epitopes present on T-cells for neutralization. MHC Class I present the antigenic epitopes to $CD8^+$ T-cells and MHC Class II present to $CD4^+$ T-cells for viral antigen degradation (Adams and Koziol, 1995; Berman *et al*., 2000). CD8 T-cells also known as cytotoxic T-cells (CTL), maximum viral infections by initially recognizing and their subsequent killing infected cells and secreting cytokines. CD4 T-cells known as helper cells that play very important role in growth factor releasing and signaling for generation and maintenance of CD8 T-cells (Zielkiewicz, 2005).

T-cells recognize the antigens only when they are associated with MHC surface glycoproteins exposed on surface of all vertebrate cells. In this communication, online bioinformatics tools were used and the targeted protein of CCHFV was analyzed, to identify the putative T-cell epitopes for

the formation of peptide based vaccine. The vaccine of Congo virus is not yet available. The CCHF vaccine development is very difficult because it requires the known pathogenic human host and it is also difficult to grow the virus in culture medium. The significance of this modern approach is, it reduces the time and risk of pathogenesis; in order to overcome the problems of attenuated vaccine development. Epitope based vaccine consists of short peptide sequence which is derived from small part of virulence protein. Antigenic determinants are present in certain part of the vaccine peptide sequence. This vaccine should cover the Human Leukocyte Antigen (HLA) haplotypes of the target population, be effective against a wider spectrum of Congo virus strains and not have any self-effective epitopes and produce effective immune response (Eswar *et al.*, 2007). A No. of computational tools are now available for prediction of T-cell epitopes (Parida *et al.*, 2007; Shakyawar *et al.*, 2011), all overlapping nonamers of CCHFV to Human HLA Class I molecules have been analysed. The selected peptides have been modeled on corresponding HLA to validate the binding prediction. Some peptide identified from Bioinformatics and Molecular Analysis Section (BIMAS) and SYFPEITHI that binds to Class I HLA are also revealed. A No. of peptides were chosen for structural modeling on the bases of their binding affinity to Class I alleles by multiple analytical tools, since, the putative peptide is predicted to form stable complex with HLA allele through molecular modeling and it have not any identical peptide of Human proteome cross checked by HLApred (Berman *et al.*, 2000). The aim of the study is to suggest these peptides as potential vaccine candidate development.

MATERIAL AND METHODS

The *in silico* study was conducted at Department of Biotechnology, Mangalayatan University, Aligarh from Nov, 2010 to May, 2011.

Virus and protein: RNA-dependent RNA polymerase (ACM78472.1), nucleocapsid protein (ABB30042.1) and nucleoprotein (BAE80107.1) are the protein of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) strain isolated from Xinjiang province available in the NCBI Protein data base and hence it is used for this analysis.

Physical properties of the selected proteins: Bioinformatics tools were used for the analysis of proteome of CCHF virus. The protein sequences of were retrieved from www.ncbi.nlm.nih.gov. The expected molecular weight, highly repeated amino acids (%) of repetition, least repeated amino acid and isoelectric point (pI) values were calculated using ExPaSy (http://www.expasy.org/) (Kyte and Doolittle, 1982; Shehzadi *et al.*, 2011).

MHC-Class I binding epitope prediction: All of these targeted proteins of the CCHF virus strain found in Xinjiang namely RNA-dependent RNA polymerase, nucleocapsid protein and nucleoprotein were analyzed for the Cytotoxic T-lymphocytes (CTL) epitopes using several algorithms. BIMAS online tool was used to analyze binding of all consensus peptides with 33 human HLA allele that which helps to identify those peptides in the targeted proteins with high affinity promiscuous epitopes that binds to HLA (Parker *et al.*, 1994). The binding affinity ($T_{1/2}$) value is based on the half time (min) of dissociation of β2 microglobulin from HLA. The $T_{1/2}$ value was set at cutoff $T_{1/2} \geq 100$ for peptide selection, other several algorithms based tools viz. Propred1, SYFPEITHI and Propred are also used for prediction of putative T-cell epitopes (Singh and Raghava, 2001).

Propred1 is matrix-based method that allows prediction of MHC binders for various alleles based on the multiplication and additional matrices, proteosome cleavage site, simultaneously. This

is based on the observations made in previous studies which demonstrate that MHC binders having proteosome cleavage site at their C terminus have high potency to become T-cell epitopes. (Singh and Raghava, 2001). Endogenously synthesized peptides of 9-11 amino acids of HLA class I molecules get interacted with T-cell receptor of T-cells on the surface of infected cells. The presence of allele-specific amino acid motifs has been demonstrated by sequencing of peptides eluted from MHC molecules (Lund *et al*., 2002).

Structure-based modeling of T-cell epitope: Molecular modeling and structural analysis (Chaitra *et al*., 2005) were performed for the detection of binding peptides to their respective class I HLA alleles. Sample peptides of high affinity binders for a few alleles where structures are known (A 0201, A2, A 2402, B 1501, B 2705, B 2709, B 3501, B 4403, B 4405, B 5101, B 5301) were modeled employing their respective structural templates (1AO7, 1AKJ, 2BCK, 1XR8, 1HSA, 1UXW, 1A1M, 1SYS, 1SYV, 1A1O, 1EFX, 1IM9 and 1QQD). Two peptides viz. LLGIKCSFV and LRFGMLAGL were selected with the help of scoring based algorithms of BIMAS (Parker *et al*., 1994; Parida *et al*., 2007). These peptides have higher binding affinity. These selected peptides with highest and lowest $T_{(1/2)}$ were modeled on to their respective structural templates and the complexes were subjected to energy minimizations (Vani *et al*., 2006). The binding of the peptides was estimated by analyzing the intra-molecular hydrogen bonds, electrostatic, van der Waals and hydrophobic interactions with the protein residues in the vicinity.

The Modeller (Eswar *et al*., 2007) was used for the designing of the structures of those alleles whose structures were not available in the PDB server while the CPH model server (Nielsen *et al*., 2010) was used to design the structures of the predicted binding peptides. After designing the structures docking of selected alleles and peptides was performed with the help of Autodock. This was done to out the energy minimization (Morris *et al*., 1998; Namasivayam and Gunther, 2007; Amir *et al*., 2010) and then PMV (Python Molecular Viewer) was used for the visualization of Binding, position, H-bonding between the selected peptides and alleles.

RESULT

In this present study, three putative proteins of CCHF virus were used for the physicochemical analysis (Stevenson *et al*., 2007) such as molecular weight, isoelectric point (pI value) and antigenic nature. The RNA-Dependent RNA Polymerase protein has the highest molecular weight of about 447836.7 KDa which consists of Leucine (L) a neutral nonpolar amino acid residue has the highest percentage of repetition (12.4%). The least repeated residue of L-segmented protein of CCHFV is a nonpolar Tryptophan (W) (0.9%). The M-segment viral peptide encoded by nucleocapsid protein is NC_M (53955.5 KDa molecular weight) comprising 482 amino acid residues. Lysine (K) has highest percentage and Cysteine (C) is the least repetitive amino acid residues(1.2%) of this protein; another targeted protein has the lowest molecular weight of about 8223.4 KDa (i.e., S-segment encoded the nucleoprotein), Lysine(K) has the highest percentage of repetition (13.7%), Histidine (H) and Proline (P) are the least repeated amino acid residues (1.4%) of nucleoprotein. The physicochemical properties of putative proteins were given in Table 1. The pI value of any protein indicates the stability of protein in that particular isoelectric point. Isoelectric points of these proteins were ranged between 7.14 to 9.48.

Binding specificity of promiscuous T-cell epitopes to HLA class-I molecules: The prediction of epitopes with their position and corresponding promiscuous HLA alleles by using different tools has been summarized in Table 2. The promiscuity of binding of a peptide to HLA

Table 1: It comprises the data of CCHFV proteins, molecular weight and percentage of highly repeated and least repeated amino acid residues in individual protein. The percentage of amino acid residues gives an outlook for their pI value and their probability of incidence in the antigenic epitopes

Proteins	Amino acid No.	Mol. Wt	Highly repeated amino acid	Percentage of repetition	Least repeated amino acid	Percentage of repetition	pI
RNA-dependent RNA polymerase	3945	447836.7	L*	12.4	W#	0.9	7.14
Nucleocapsid protein	482	53955.5	K**	9.5	C	1.2	8.60
Nucleoprotein	73	8223.4	K	13.7	H/P***	1.4	9.48

L*(Lucine) and W# (Tryptophan) are non-polar anchor residue for HLA predicted epitopes. K**(Lysine) is an anchor residue and also for HLA predicted epitopes. H/P***(Histidine/Proline) are least repeated amino acids

Table 2: The predicted peptides from target protein binds to different HLA class I alleles (BIMAS $T_{(1/2)} \geq 100$ and SYFPEITHI value≥15)

Protein	Start position	Peptide	$T_{(1/2)}$ (min)	Alleles	No. of peptide	SYFPHETHI
Nucleocapsid protein	66	ALVEATKFC	112.028	A_0201	1	15
	430	IVKLFEIQK	180	A68.1	2	20
		WVSSTGIVK	120	A68.1		19
	115	VEVPKIEQL	160	B40	1	24
	115	VEVPKIEQL	352	B60	3	24
	451	SEHLLHQSL	176	B60		23
	18	EEFKKGNGL	160	B60		24
	351	TPMKWGKKL	240	B7	1	
	201	CREFVKGKY	200	B_2702	1	
	175	RRRNLILNR	3000	B_2705	19	31
	182	NRGGDENPR	1000	B_2705		23
	328	SQFLFELGK	1000	B_2705		15
	201	CREFVKGKY	1000	B_2705		22
	273	ADNMITNLL	300	B_3701	1	22
	282	KHIAKAQEL	180	B_3901	2	21
	408	GHTKSILNL	135	B_3901		26
	55	DDAQKDSIY	135	B_4403	2	
	80	CAWVSSTGI	484	B_5101	10	23
	69	EATKFCAPI	440	B_5101		21
	238	LAETEGKGV	143	B_5101		22
	129	AALKWRKDI	120	B_5103	4	
	115	VEVPKIEQL	100	Cw_0301	1	
	35	SFCESVPNL	480	Cw_0401	5	
Nucleoprotein	30	VEVPKIEQL	160	B40	1	24
	30	VEVPKIEQL	352	B40	1	24
	42	QQAALKWRK	200	B_2705	3	15
	44	AALKWRKDI	242	B_5101	1	24
	44	AALKWRKDI	726	B_5102	1	
	44	AALKWRKDI	120	B_5103	1	
	30	VEVPKIEQL	100	Cw_0301	1	
RNA Polymerase	1841	GTENKKIVK	225	A1	3	15
	1918	LTEDGNLIF	112.5	A1		19
	64	LTELAARKY	112.5	A1		31
	1280	KLMKNKQPV	900.698	A_0201	42	22

Table 2: Continued

Protein	Start position	Peptide	$T_{(1/2)}$ (min)	Alleles	No. of peptide	SYFPHETHI
	852	LLGIKCSFV	650.311	A_0201		23
	3317	GLTDLLDYL	542.901	A_0201		26
	3542	LLLNSLTLL	309.050	A_0201		28
	304	KLYVTKDLL	117.237	A_0201		22
	383	KVYKVLGNL	252	A_0205	2	
	3699	LYEEVLMNL	504	A24	20	
	410	ALFGKQINK	300	A3	8	26
	3443	RLLKFVPLK	270	A3		26
	1299	EVAAECMKR	1200	A68.1	35	20
	2049	EVLIKRLEK	720	A68.1		20
	1525	LRFGMLAGL	300	B14	5	26
	277	GEVMSLRQL	160	B40	1	22
	277	GEVMSLRQL	352	B60	39	22
	570	IEIKRLYAL	640	B60		22
	3726	LELESLLTL	640	B60		26
	2578	SEFMMGYRV	180	B61	1	
	3932	ALKTGNLGF	288	B62	8	
	1255	FVRNNDKLL	200	B7	7	
	1850	MLRGKLKKL	160	B8	2	33
	3745	KRDGPRCSF	600	B_2702	18	
	1525	LRFGMLAGL	300	B_2702	18	
	1525	LRFGMLAGL	10000	B_2705	203	27
	3399	KRRTEVITK	6000	B_2705		26
	2433	LRKLLVDNL	2000	B_2705		25
	1272	KCFDVQSFK	150	B_2705		19
	1256	VRNNDKLLI	600	B_2705		18
	20	NPRFNISDY	120	B_2705	1	
	2114	LDLSVSKLL	300	B_2705	3	24
	150	THFDALRIL	540	B_3901	4	23
	3329	TELLKKKPY	540	B_4403	15	
	3016	EPSLFNPNI	880	B_5101	54	21
	3209	VAELVSYGI	286	B_5101		23
	1657	DARTARLLL	110	B_5101		22
	741	TPLNEVHSI	1320	B_5102	58	25
	2679	VALEDAEVI	726	B_5102		
	508	VANAEEFII	200	B_5102		
	2679	VALEDAEVI	145.2	B_5103	15	
	508	VANAEEFII	110	B_5103		
	3774	RANNELGDV	110	B_5103		
	1424	QQYRCLEVI	605	B_5201	9	
	1288	VPFQVDCIL	150	B_5201		
	3934	KTGNLGFNW	396	B_5801	13	
	2666	LFVPTYSGL	600	Cw_0301	10	
	2944	LSLPIYTIF	125	Cw_0301		

alleles is important since inclusion of such peptides in the vaccine construct provides a greater population coverage which helps to short out the promiscuous peptide that needs to be in vaccine developments (Herrera *et al*., 2010) . BIMAS is the immunoinformatics tool freely available to be

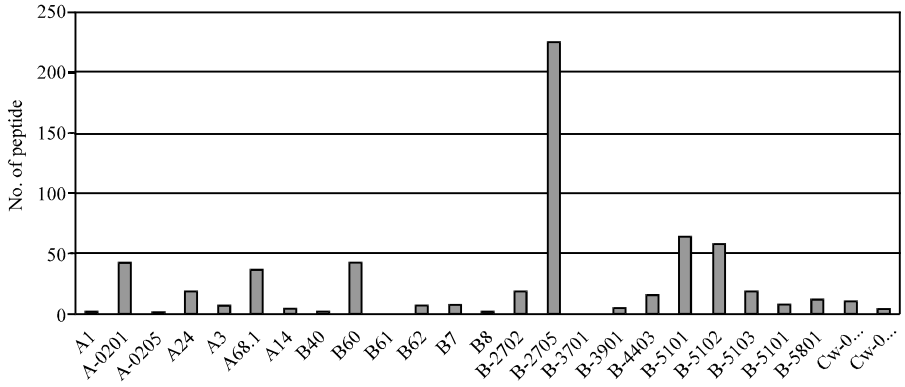

Fig. 1: This conservation plot represents the number of peptides of all three protein of CCHFV that binds to 33 Class I HLA alleles at cutoff $T_{(1/2)} \geq 100$. B_2705, B_5101, B_5102, A_0201, B_60, A_68.1 are some strong binding alleles which bind to most of the peptides and also shown by the tallest bar. HLA alleles A3, A24, B14, B62, B7, B_2702, B_4403, B_5103, B_5201, B_5801 and Cw_0301, Cw_0401 bind to the less No. of predicted epitopes

used for prediction of the antigenic epitopes in the complete protein with more effective and accurate prediction of MHC binding affinity (i.e., $T_{(1/2)}$ value). The binding analysis of all conserved nonamers of all three consensus CCHFV proteins to 33 HLA class I alleles at different binding affinities.

Total 71 epitopes were predicted against 33 alleles of MHC Class I by using the tool BIMAS. The maximum number of epitopes were represented by RNA dependent-RNA polymerase protein comprising 65% of all MHC Class I predicted epitopes, 29.5% of MHC Class I predicted epitopes from nucleocapsid protein and minimum number of epitopes from nucleoprotein 5.6% (Table 2). LLGIKCSFV, LRFGMLAGL, EPSLFNPNI and SQFLFELGK are the promiscuous binders of MHC Class I alleles. In case of nucleocapsid protein RRRNLLLNR and CAWVSSTGI are the best binders in terms of quantitative scores of HLA alleles (MHC Class I) coverage. For the nucleoprotein not also have the epitope of good quantity covering HLA alleles available in BIMAS. Out of these 33 HLA alleles B_2705 is capable for binding to the highest number of predicted promiscuous epitopes all proteins show the tallest bar in Fig. 1. Other HLA alleles binding to the less number of promiscuous epitopes are also shown in same figure.

Conserved epitope of CCHF virus protein: It is important to identify those peptides which are conserved across the various strains of CCHF virus and in this study that has been shown for the conserved peptides present in the constituent proteins of CCHF virus.

The analysis reveals that there are number of suitable peptides from RNA dependent-RNA polymerase which may be included in the construction of poly epitopes T-cell vaccine (Parida *et al.*, 2007). Some of the conserved peptides (LLGIKCSFV, EPSLFNPNI, LRFGMLAGL and SQFLFELGK) with class I presentation potential along with their interaction energies are, -29.31, -26.85, -35.05 and -23.97 (in kcal mol^{-1}), respectively given in Table 3. High $T_{(1/2)}$ and interaction energy indicate high HLA binding affinity. These peptides are promiscuous HLA binders. It will be useful to include these peptides in a chimeric constructs containing both cytotoxic and helper epitopes. It is expected that though this T-cell vaccine would not prevent CCHF virus infection, it would aid in quick clearance of the virus and prevent the severe infection (Parida *et al.*, 2007).

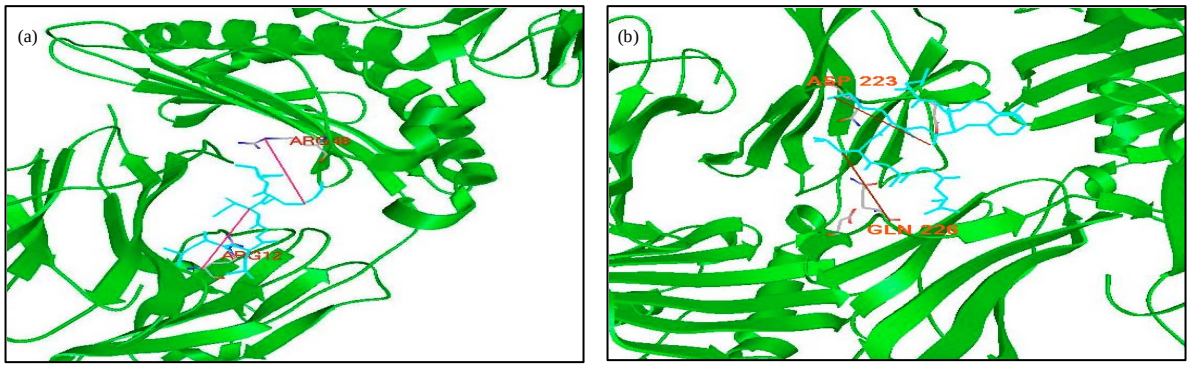

Fig. 2: The peptide binding to the HLA class I molecule. The peptides (shown in green colour) predicted to a have very high affinity for the allele A_0201 and B_2705 modeled on to the crystal structure (1AO7 and 1 HAS) based on the position of the peptide. Potential hydrogen bonds are shown. The high binder peptide (a) LLGIKCSFV and (b) LRFGMLAGL are derived from the CCHF Virus

Table 3: The conformational properties of the peptides with efficient binding energy and present on the variable regions of predicted peptide as investigated by molecular dynamics simulation using Autodock tool v3.0

Peptide	Energy (kcal mol^{-1})	Hydrogen bond	Position	$T_{1/2}$	Alleles
LLGIKCSFV	-29.31	2	852	650.311	A_0201
EPSLFNPNI	-26.85	2	3016	880	B_5101
LRFGMLAGL	-35.05	2	1525	10000	B_2705
SQFLFELGK	-23.97	2	328	1000	B_2705

DISCUSSION

It is reported that, the primary function of T-Cell vaccine is to generate CTLs to degrade the virus infected cells. The viral antigens released when get lysed are capable of stimulating antibody response against these antigens get leading to neutralization of reinfecting and residual viruses in the system. Since these events take place in during the incubation phase of the virus infection, if any. It is likely to be very mild. Beside the idiotype and anti-idiotypic antibody cascades generated by T-Cell epitopes would reinforce T-Cell memory (Lal *et al*., 2006; Nayak *et al*., 2001, 2005; Mohabatkar and Mohammadzadegan, 2007).

The characterization of putative peptides on the basis of antigenic variability depends on the surface exposed regions of target CCHFV protein revealed that 6 of the total 71 predicted epitopes were present. Out of six short listed peptides, four peptides were chosen here for their further characterization on the basis of their energy minimization value. Moreover with the SYFPEITHI, it scored high with a value of 27 and 23 for the binding to HLA alleles like B_2705 and A_0201 corresponding to their short listed peptides LRFGMLAGL and LLGIKCSFV, respectively. Figure 2a and b illustrates the interaction of these two peptides with their respective alleles (Kavita *et al*., 2010). The resulting peptides LRFGMLAGL, of RNA polymerase protein binds to HLA B_2705. It is seen to make two hydrogen bonds from its arginine in the 2nd position to asparagine at position 223 of the B_2705 and leucine in the 1st position to a glutamine at position 226 of this allele. Similarly the peptide LLGIKCSFV, of same protein binds to HLA A_0201. It also

makes two hydrogen bonds from its lysine in the 5th position to a arginine at position 48 of the allele A_0201 and glycine in the 3rd position to a arginine at position 12th of this allele with its high affinity (Parida *et al*., 2007; Tambunan and Parikesit, 2010).

It must be noted that MHC class I a peptides have preference for hydrophobic or positively charged amino acid residues at carboxyl end for proper biding in pockets (Brusic *et al*., 2002). The screening in this work also listed two more peptides TPLNEVHSI and GEVMSLRQL that were presented on class I allele of RNA dependent-RNA polymerase and scored 1320 and 352 with BIMAS. These epitopes were, however, not included for simulation analysis. Simulation studies of the epitope LRFGMLAGL and LLGIKCSFV formed stable MHC-peptide complexes with the energy minimization of -35.05 and -29.31 (kcal mol^{-1}), respectively. The other two peptides EPSLFNPNI and SQFLFELGK identified in the present study were found to be antigenically variable with energy minimization value of -26.85 and -23.97 (kcal mol^{-1}), respectively (Kavita *et al*., 2010). This can possibly be targeted for designing of vaccine against CCHF virus strains of Xinjiang province.

CONCLUSION

The screening of putative epitopes using bioinformatics tools thus suggests that protein RNA dependent-RNA polymerase of CCHFV could be used for preparation of immunological constructs. Molecular simulation and binding tests also suggest that the two nonameric epitopes LRFGMLAGL and LLGIKCSFV predicted and reported for the first time have considerable binding with MHC molecules and low energy minimization values providing stability to the peptide-MHC complex. These peptide construct will further undergo wet lab studies, for the development of targeted vaccine against CCHF virus strains. Using a similar approach the short listed candidate epitopes for vaccine design using other proteins can also be targeted that would reduce time and experimental expense.

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