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HHV-5 epitope: A potential vaccine candidate with high antigenicity and large coverage

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ABSTRACT

Outbreak of Human Herpes virus-5 (HHV-5) infection in emerging countries has raised worldwide health concern owing to prevalence of congenital impairments and life threatening consequences in immunocompromised individuals. Thus, there lies an impending need to develop vaccine against HHV-5. HHV-5 enters into host cells with the help of necessary components glycoprotein B (gB) and H/L. In this study, the conformational linear B-cell and T-cell epitopes for gB of HHV-5 have been predicted using conformational approaches, for their possible collective use as vaccine candidates. We examined epitope's interactions with major histocompatibility complexes using molecular docking and also investigated their stable binding with specific toll like receptor-2 (TLR2), present on host cells during HHV-5 infection. Predicted MHC-I epitope 'LVAIAVVII' with high antigenicity and large coverage of HLA alleles was found to superimpose on MHC-II epitope (Rank 1) and was also identified to be the core sequence of putative B cell epitope 'ILVAIAVVIITYLI'. Resulting epitope was found to have consistent interaction with TLR2 during long term (100 ns) MD run. We also validated this nonamer epitope for its dissimilarity with human genome and high population coverage, suggesting it to be a potential vaccine candidate with higher coverage for both the MHC alleles of Indian population.

Abbreviations: HHV-5: Human herpes virus-5; HSPGs: heparan sulphate proteoglycans; EGFR: epidermal growth factor receptor; TLR2: Toll like receptor2; CTL: cytotoxic T-lymphocytes; HTL: helper T-lymphocytes; RMSD: root mean square deviation; RMSF: root mean square fluctuation; gB: glycoprotein B; MHC: major histocompatibility complex; ns: nano seconds

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1. Introduction

Huge numbers of people are suffering from the viral disease worldwide. Although, many antiviral peptides studies have been reported and experimentally validated for blocking the viral attachment or infections to the host, but some of the antiviral peptides are also reported to associate with certain issues like target specificity, random or small sequence analysis, high cost, laborious implementations and other immunological aberration related to autoimmune disease. Overcoming the issues, scientists have employed the advanced immunoinformatics approaches with molecular dynamics simulation to design the antigenic peptides to combat the viral entry or attachment to the host for dengue virus (Abd El-Baky, Uversky, & Redwan, 2017; Anusuya & Gromiha, 2017), human immunodeficiency virus (HIV) (Vora et al., 2018), and against the HIV associated cancer (Omer & Singh, 2017). Herein, we have employed the immunoinformatics approaches along with molecular docking and molecular dynamics simulation to design the antiviral peptide against the Human Herpes virus-5 (HHV-5).

HHV-5 belongs to Herpesviridae family of viruses usually associated with asymptomatic infections causing the death of immune compromised patients (Isaacson & Compton, 2009; Ryan & Ray, 2004). HHV-5 is a complex enveloped virus with dsDNA in its core surrounded by a nucleocapsid which in turn is surrounded by a protein filled region called tegument layer and a lipoprotein envelope derived from host cells' nuclear membrane which is further studded by at least nine viral encoded glycoproteins (Phillips & Bresnahan, 2011). HHV-5 is a pathogen which can infect almost all vertebrates and also, almost every cell type or organ system can be infected in the human body (Ibanez, Schrier, Ghazal, Wiley, & Nelson, 1991; Sinzger et al., 2000). Herpes Viruses result in different illnesses in humans which include chickenpox or varicella, shingles or Herpes Zoster, cold sores, encephalitis and different cancers. These viruses are neurotropic and neuroinvasive in nature which remain latent and account for lifelong infections in human body (Ryan & Ray, 2004). As per serological surveys in India, 80%–90% women of child bearing age have pervasiveness of HHV-5 antibodies and 2.0%–2.5% of pregnant women are prone to seroconversion

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risk (Gandhoke, Aggarwal, Lal, & Khare, 2006; Lazzarotto et al., 1998). In many other reports, the prevalence of HHV-5 infection was reported for almost 40,000 infants born yearly in the United States and 0.5%–2% of infants from developing world (Demmler, 1991). HHV-5 antigenic regions have still not been studied adequately for their mechanistic interaction with HLA alleles and TLR2 which could prove beneficial for vaccine development and diagnostic purposes (Chakravarti, Kashyap, & Matlani, 2009). Hence, HHV-5 infection is still a major health concern in India and worldwide.

HHV-5 virus infects the host cells with the help of various glycoproteins, namely glycoprotein B (gB), gD, gH and gL. Among them, gB is majorly responsible for entry of HHV-5 into host cells as it is involved in the initial steps of virion-tethering followed by stable attachment as well as cell fusion (Compton, 2004; Feire, Koss, & Compton, 2004). The gB (molecular mass 170 kDa) is the most conserved glycoprotein which is abundantly present on virus envelope. The gB enhances the entry of virus into host cells by interacting with specific integrin heterodimers and epidermal growth factor receptor. Moreover, gB is also important for replication of virus. Virions devoid of gB are able to attach to the cell surface in the same manner as virions containing gB, but the entry of virus and cell-to-cell spread was defective (Isaacson & Compton, 2009). Hence, it would be a prominent target for potent vaccine development.

Binding of gB and gM to cell surface heparan sulphate proteoglycans (HSPGs) is the primary step for initiation of HHV-5 entry into host cell which is followed by interaction with more cellular receptors for a stable interaction(Compton, Nowlin, & Cooper, 1993; Wang, Huong, Chiu, Raab-Traub, & Huang, 2003). After binding with HSPGs, HHV-5 moves ahead to attain a stable binding with host cell through other cellular receptors like epidermal growth factor receptor (EGFR) which reflects stable docking and then post attachment interaction with specific integrin heterodimer further expedite viral entry/internalization into the host cell. Importantly, Toll-like receptors signalling cascade further help in amplifying the information to counteract against invading pathogen. TLRs detect pathogens by detecting pathogen-associated molecule pattern (PAMP). After recognition of membrane protein, TLRs initiate intracellular signalling mechanism which leads to expression of genes involved in inflammation and antiviral responses (Kawai & Akira, 2005). Recently, it was found that TLR2 specifically binds to gB of herpes virus-5 and acts as a host immune sensor and also plays a major role in recognising CMV infection in association with CD14 (Compton et al., 2003). TLR2 helps in activating certain transcription factors by different signalling pathways contributing to antiviral activities in the form of an inflammatory response against HHV-5. Improved understanding of viral infections and the diversity of the immune system with advancement in molecular biology provided the mechanistic insight into the host-pathogen interaction and avenues for vaccine design and development. The vaccine has already been reported to possible treatment for the viral infections and also the many types of cancer by strengthening the immune system to fight against the pathogens.

A novel vaccine candidate (epitope) has been designed in this study. Epitopes are the antigenic determinants, which

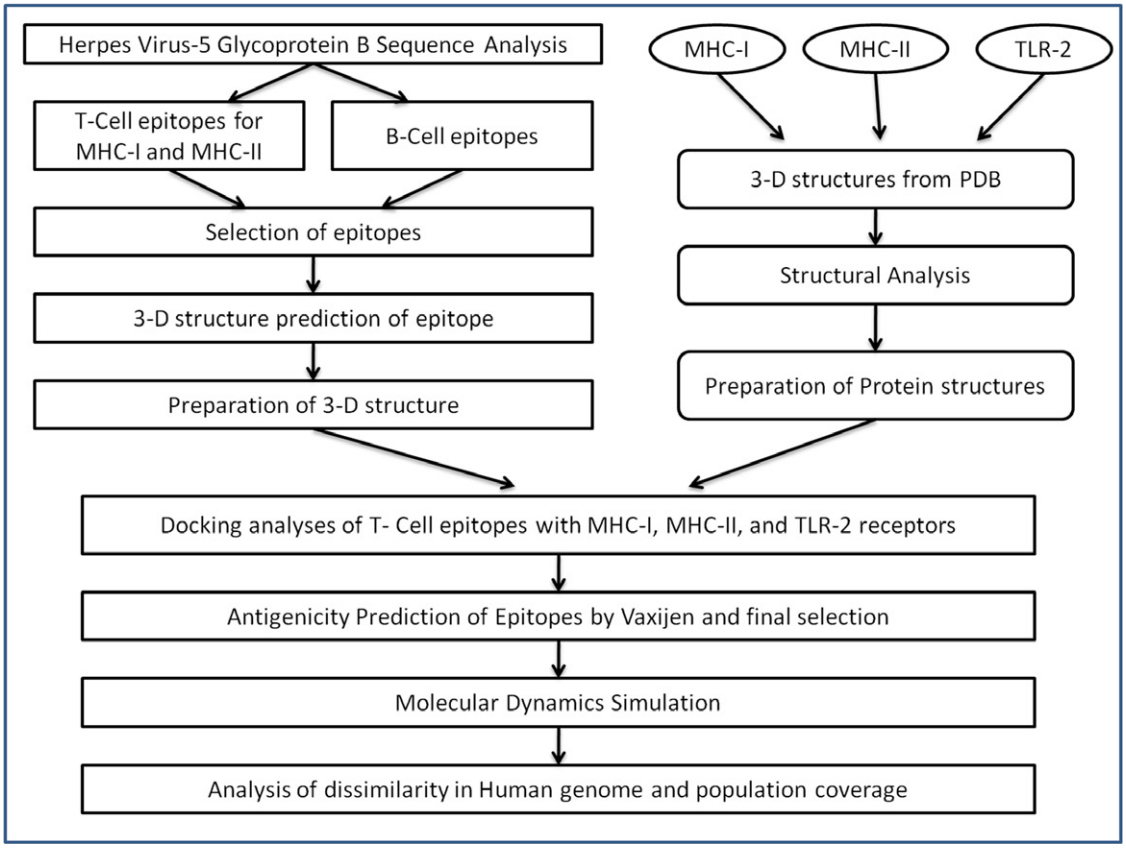

Figure 1. A brief description of methodology.

are as part of antigen, recognised by the B-cells, T-cells and other immune cells. These epitopes elicit the humoral and cellular immune response against the target antigens with high specificity to particular antigens. Immunisation with the antigens generates an appreciable immune response with the elimination of the allergenic and other reactogenic responses, chiefly they allow the immune response focus on the specific antigen and avoid the immune evasion or other side effects, which may lead to autoimmunity. Significantly, peptide antigens are reported to be easily synthesised, increased stability, low cost and relatively safer in numerous clinical studies (Bilker et al., 2007; Lin et al., s2007).

In 2009, a vaccine was analysed for prevention of HHV-5 infection, but it resulted in limited protection with only 50% protection efficacy (Pass et al., 2009). Hence, for efficient vaccine development, a specific epitopic region on gB of HHV-5 needs to be defined. Since conventional vaccine development methods are too laborious and expensive, for bypassing these, computational methods have respective advantages of structural analysis, pathogen genome and sequence screening with greater speed and lesser cost. There has been an extensive use of computational techniques which provide a high-resolution picture for conformational dynamics of proteins (Nagpal et al., 2015; Patel et al., 2015; Singh et al., 2016, 2017; Verma et al., 2016). This study focuses on the prediction of T-cell and B-cell epitopes of Glycoprotein-B of HHV-5, for the development of a potent vaccine. A brief methodology has been shown in Figure 1. Epitopes were studied for their interaction with MHC-I and MHC-II receptors and TLR2, the specific receptor of the gB present on the host. To investigate the structural and dynamics stability of the TLR2-epitope complex, we have also performed a long molecular dynamics simulation of 100 ns. Since it has been shown that epitope–receptor complex persistent for a long run results into the higher immune response against the antigen (Schreiner, Karch, Knapp, & Ilieva, 2012). Furthermore, epitope's antigenicity, dissimilarity and population coverage were also analysed.

2. Materials and methods

2.1. HHV-5 protein sequence retrieval and physicochemical properties analyses

Retrieval of the complete protein sequence of HHV-5 glycoprotein-B (GenBank ID: ADE88063) was done from National Centre for Biotechnology Information database (NCBI, 2016). HHV-5 amino acid sequence was subjected to physicochemical analysis, which included the determination of instability index, molecular weight, aliphatic index, theoretical pI, amino acid composition and grand average of hydropathicity (GRAVY) by ProtParam (Gasteiger et al., 2005). Then its phosphorylation motifs responsible for post-translational modifications with a probability of more than 80% were determined using NetPhos v2.0 (Blom, Gammeltoft, & Brunak, 1999). NetPhos defines the phosphorylation sites of Ser, Thr and Tyr in eukaryotic protein sequences using neural networks.

2.2. TLR2 structure analysis

TLR2 protein structure was retrieved from RCSB protein data bank. TLR2 structure is available as complex with TLR1, hence, TLR2 structure was extracted using Swispdbviewer tool (Guex & Peitsch, 1997). Further, an initial model of TLR2 structure was characterised and evaluated for its conformations and protein stereochemistry using Ramachandran plot and Prosa tool (Lovell et al., 2003; Wiederstein & Sippl, 2007) and also verified through protein 3D structure verification server ERRAT (Colovos & Yeates, 1993). Ramachandran plot evaluates the phi/psi bond distribution for protein structure and shows allowed and disallowed regions of the structure. Ramachandran plot was generated using Rampage server and then, 3D protein structure quality was analysed by Z-score using the Prosa tool. Z-score evaluates the quality of protein structures by comparing to a range formed by the scores of other proteins of same size in databases. Further, protein model was evaluated using ERRAT. ERRAT is protein crystal structure algorithm which identifies non-bonded interactions in different atoms by comparing the statistics with top refined structures.

2.3. Prediction of T-cell epitopes

T-cell epitopes are found on the antigen presenting cells' surface where they interact with MHCs to induce immune responses. High binding affinities of T-cell epitopes toward MHC alleles were exploited for their prediction. Cytotoxic T-lymphocytic (CTL) MHC-I restricted epitopes were predicted by using the Rankpep tool. Rankpep predicts MHC binder antigenic sequences using position-specific scoring matrices (PSSM), which represents output through the weighted frequency of peptidic sequence at every position for all amino acids (Reche, Glutting, & Reinherz, 2002).

Helper T-lymphocytes (HTL) MHC-II restricted epitopes are proteolytic cleaved pathogen antigen sequences which are presented by cells to CD4 helper cells in order to induce immune systems. This makes the MHC class-II binding antigenic sequences important to elicit the immune response against viral antigen. HTL epitopes were predicted by ProPred. It is a graphical web-based tool which uses quantitative matrices for prediction of regions which would bind to MHC-II, for any antigenic sequence to help identify the promiscuous regions which can be used as vaccine candidates (Singh & Raghava, 2001).

2.4. Putative B-cell epitope prediction

B-cell epitope binds with lymphocytes and results in differentiation of B-lymphocytes into the plasma and memory cells which secretes antibody. Immune dominant B-cell linear epitopes have been shown to generate potent vaccine against viral disease and in autoimmunity for neutralising antibodies (Moutsopoulos, Routsias, Vlachoyiannopoulos, Tzioufas, & Moutsopoulos, 2000; Xiao, Lu, & Chen, 2001). A recurrent neural network based ABCpred tool was employed for prediction of linear B-cell epitope. ABCpred searches the

immunodominant linear epitopes in an antigenic sequence using different patterns of fix lengths method (Saha & Raghava, 2006). Resulting antigenic peptides were further analysed for their other immunological properties.

2.5. Antigenicity analysis of epitopes

To define potential lead peptides for vaccine development, quantitative structure–activity relationships based analysis of epitopes were performed to elucidate their antigenicity and pathogenicity characteristics. Antigenicity and pathogenicity of T-cell and B-cell epitopes were determined using Vaxijen v2.0, which is an alignment-free method and uses auto cross-covariance (ACC) transformation for prediction of antigenic/non-antigenic nature of epitopes (Doytchinova & Flower, 2007). Identified epitopes with a high vaxijen score would be highly probable candidates as epitopes.

2.6. Binding analysis of epitope with MHC alleles and TLR2

Predicted cytotoxic T-lymphocytes and helper T-lymphocytes epitope peptides were studied for their interaction with receptor proteins through molecular docking. Docking of 3D structure of the T-cell epitopes with their corresponding receptors MHC-I, MHC-II and TLR2 was performed. Prior to docking, 3D structure of proteins and ligands (epitope) were prepared.

2.7. Protein preparation

Retrieval of 3D structures for receptor proteins MHC-I, MHC-II and TLR2, was done from RCSB protein data bank. These proteins were prepared for molecular docking. Protein preparation includes removal of water molecules, any duplicated chain or any other unwanted heteromolecules. PDB structure of MHC-I protein (PDB: 1I1Y), MHC-II protein (PDB: 1KG0) and TLR2 protein (PDB: 2Z7X) were prepared using Swispdbviewer tool.

2.7.1. 3D structure prediction of epitopes

3D structures of epitopes were modelled using PEP-FOLD3 to carry out molecular docking and to find out the best epitopes on the basis of their binding scores with MHC-I and MHC-II (Thévenet et al., 2012). PEP-FOLD3 is a de-novo 3D structure prediction tool for peptides with size ranging from 9 to 36 amino acids. PEP-FOLD3, on the basis of Hidden Markov Model algorithm, predicts 3D structures from the sequence of protein, by performing a series of simulations and ends with giving conformations which are most representative of energy and population. Further, for molecular docking study of these ligands with their specific protein receptors, ligands were prepared using Swiss-PDB viewer molecular modelling server. Ligand preparation includes removal of water molecules, energy minimisation and optimisation.

2.8. Molecular docking analysis of epitope with MHC-I, MHC-II and TLR2

Predicted common antigenic sequence from MHC-1 restricted CTL epitope, MHC-2 restricted HTL epitope and B cell epitope was docked with MHC-1 and MHC-2 proteins. HEX 8.0 programme was used to perform molecular docking (Macindoe, Mavridis, Venkatraman, Devignes, & Ritchie, 2010). PDB files of receptors and epitope were uploaded in HEX module. A grid dimension of 0.6, ligand range of 180, step size 7.5 and receptor range of 180 with the step size of 7.5 were used to perform the rigid docking of the epitope and the receptor. Resulting epitope was also studied for its molecular interactions with TLR2, a receptor protein that binds specifically with the herpes virus glycoprotein-B antigenic sequence. Docking with TLR2 was also performed through HEX 8.0 programme, as above. Best conformation of TLR2-epitope docked complex was retrieved and used for further analysis.

2.9. MD simulation analysis of epitope with TLR2

TLR2-epitope docked complex was investigated for their stability and conformational analysis by molecular dynamics simulation for 100 ns. A stable protein-epitope complex for long run suggests stable binding for a longer time and elicits higher immune response to antigen(Kumar, Cocco, Atzori, Marrosu, & Pieroni, 2013). GROMACS 5.0 package was used to perform molecular dynamics simulation using the GROMOS96 43a1 force field of the best docking conformation of TLR2–epitope complex (Berendsen, van der Spoel, & van Drunen, 1995; Lindahl, Hess, & Van Der Spoel, 2001). For analysis of complex in a solvent, it was immersed in a cubic box filled with water molecules. In order to find a stable conformation, the complex was energy minimised using steepest descent method (100,000 steps) to liberate the conflicting contacts. Equilibration was performed in two phases (NVT and NPT) at 300 K for 100 ps and followed by production run for 100 ns. Atomic coordinates of the complex were recorded during MD simulation, at every 10 ps.

Root mean square deviation (RMSD) and root mean square fluctuation (RMSF) values of the TLR2-epitope complex were calculated to evaluate complex stability and per residue fluctuation as executed in other studies (Pandey et al., 2017; Verma et al., 2017a, 2017b). Ligplot tool was employed to map hydrogen bonds and hydrophobic interactions of pre-MD and post-MD protein complex. Radius of gyration (Rg) analysis was also performed to check the compactness and stability(Aggarwal et al., 2018; Singh et al., 2018) of the interacting complex till the 100 ns simulation run. In addition, solvent accessibility surface area (SASA) was also calculated to determine the solvent traceable area of interacting epitope-protein complex. Post MD binding energy between TLR2 and the lead epitope in the representative frame was calculated through Molecular Mechanics Poisson Boltzmann Surface Area (MMPBSA) method, using the g_mmpbsa tool of GROMACS. It is a widely used method for binding energy calculation from MD trajectory. The

methodology and values of the constants were adapted from the earlier studies (Singh et al., 2017).

2.10. Identification of dissimilarity to human genome and coverage for Indian population

During prediction of epitopes, it was also taken into consideration that predicted epitopes do not have the sequence similarity with human genome sequences. To find out the dissimilarity between lead epitope and human genome sequences, HLApred tool was used (Schirle, Weinschenk, & Stevanović, 2001). Another consideration for epitope prediction was that predicted epitope should effectively cover the human population of the country – India. Due to polymorphic nature of MHC and a large number of HLA genotypic frequencies, percentage fraction of population coverage of the lead vaccine candidate was determined. Immune epitope database (IEDB) of the various populations was accessed to determine the fraction of population coverage by epitope sequence (Bui et al., 2006).

3. Results and discussion

3.1. Physicochemical properties analysis of glycoprotein-B

Glycoprotein-B sequence of size 907 amino acids has molecular weight 101,811.7 Da. The theoretical pI is 7.05, aliphatic index 80.80 and the GRAVY score is −0.315, exhibiting the hydrophilic nature of sequences. Instability index was 37.64 which classifies the stability of glycoprotein sequence involved in binding to the receptor and consequently the infection to host. Moreover, three phosphorylation sites were predicted: Ser45, Thr14 and Tyr22. These sites are the important regulatory sites of viral protein.

3.2. TLR2 protein structure retrieval and structural analysis

For 3D structure of TLR2 protein, TLR2-TLR1 protein complex 3D structure was downloaded from RCSB (PDB: 2Z7X) and the TLR2 structure was extracted (Figure 2). The protein complex is made up of three chains; Chain A is TLR2 of size 549 amino acids and the chain B (TLR1) of size 520 amino acids and chain C-acylated lipopeptide of five amino acids. To obtain the TLR2 structure, we removed the TLR1 (chain-B) and tri-acylated lipopeptide (chain-C) from the protein complex structure. TLR2 structure was further validated by Ramachandran plot, Prosa and Errat analysis. Ramachandran plot of TLR2 protein structure showed that 85.7% of residues are in favourable region. Prosa tool calculated the Z-score of TLR2 protein to be −7.17, which indicates an overall good quality of the structure. ERRAT programme showed 69.907 quality factor, meaning TLR2 structure has good resolution. Ramachandran plot, Z-score and ERRAT analysis confirmed the good quality of TLR2 protein structure.

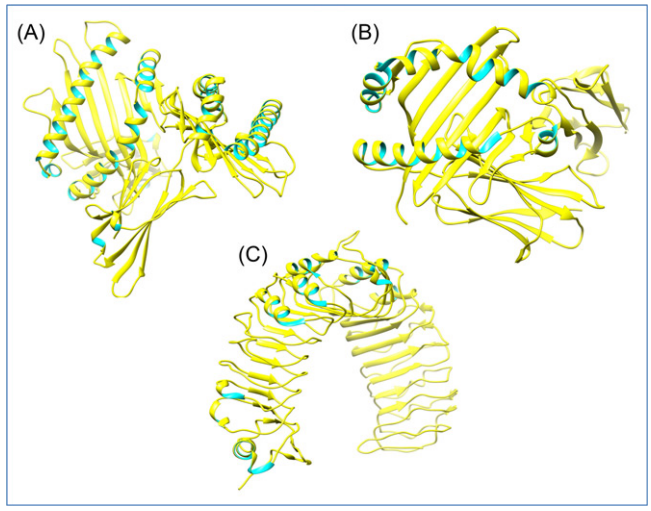

Figure 2. Protein 3D structures. (A) Three-dimensional structure of MHC-I receptor retrieved from Protein data bank (PDB), (B) 3D structure of MHC-II receptor retrieved from PDB and (C) Extracted structure of TLR2.

3.3. Identification of T cell epitopes

T-cells play a central role in the cell-mediated immune response. The key players of T-cells mediated immunity are the cytotoxicity T lymphocytes (CTLs) and helper T lymphocytes (HTLs). The CTLs express the T-cell receptor which recognises the antigens and elicit the immune response to destroy the virus's antigens or cancer antigens. Another important T cell subtypes are the HTLs, which play the arguably most potent role in adaptive immunity generation. HTLs secrete the antibodies and macrophages to eliminate the foreign antigens and also help in activation of B-cell to proliferate and generate the antibodies. HTLs also responsible for activation of a larger number of CTLs to kill the infectious pathogens and infected target cells. Both sets of T-cells are important for higher immune responses against viral disease. Both, cytotoxic and helper T-lymphocyte epitopes were predicted. Antigenic peptide sequence of CTL (MHC class-1 restricted) epitope was superimposed to HTL (MHC class-2 restricted) epitope, to find a peptide common consensus or sequence similarity, which could be more potential epitope on the glycoprotein-B sequence of HHV-5.

3.4. CTL epitope prediction and selection

Cytotoxic T-lymphocytes were predicted for glycoprotein-B. Infection of virus results in activation of T effector cells to elicit the immune response and consequently, self-destruction or killing of infected cells. After infection, epitopic sequence of virus binds to MHC molecules present on the cell surface of infected cells and this complex activates the CTL for destruction of infected cells. Computational T cell epitope prediction has been widely validated with experimental studies for the derivation of a vaccine against many infectious, cancerous and autoimmune diseases (Brusic & Petrovsky, 2005; Klemin, 2008).

To predict potential CTL epitope, Rankpep server was used, which covers almost all HLA supertypes and results in potent nanomer epitopes peptide sequences. Analysis through PPSM based Rankpep prediction tool resulted in 32

Table 1. Depiction of epitope prediction results for MHC-I, II and B cell for glycoprotein-B of the virus.

RANK MHC-I	Epitope from gB	Position	Tool score % of highest score	*Antigenicity via Vaxijen score (threshold value 0.40)
1	ILVAIAVVI	758	40.68	0.8178
2	IILVAIAVV	757	36.36	0.7615
3	AVGGAVASV	732	36.14	0.4420
4	TINQTSVKV	553	33.86	0.7379
5	LVAIAVVII	759	32.73	0.8316
MHC-II	Epitope from gB	Position	Tool score	Vaxijen score (threshold value 0.40)
1	LVAIAVVII	758	3.5800	0.8316
2	INPSAILSA	522	3.2000	0.4492
3	VLRDMTIKD	560	3.1800	0.5054
4	LKYGDVVGV	77	2.9800	1.1199
5	IFPNYTIVS	300	2.8800	0.9267
B cell	Epitope from gB	Position	Tool score	Vaxijen score (threshold value 0.40)
1	IVSDFGRPNAAQET	307	0.90	0.1265
2	GRPNAAQETHRLVA	312	0.89	0.0637
3	YPYRVCSMAQGTDL	91	0.88	0.5286
4	ILVAIAVVIITYLI	758	0.87	0.5158
5	QKSLLELERLANSS	438	0.87	0.3089

Herein different epitopes ranking with their specific position and also the antigenicity of the predicted epitopes using the Vaxijen v2.0 are shown.
*Epitopes' antigenic nature, that is, capability to bind with a product of the immune response. was predicted on the basis of their physicochemical properties using Vaxijen; Score >0.40 were considered highly antigenic.

peptide sequence with a binding threshold value of 64 for interaction with HLA alleles. Among all predicted epitopes, top scored epitope is 'ILVAIAVVI' with score of 96 for binding with HLA alleles and its molecular weight is 892.19 Da, shown in Table 1. 'ILVAIAVVI' epitope was found to interact with MHC-I alleles: HLA-A*01:01, HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:05, HLA-A*02:06, HLA-A*03:01, HLA-Cw*03:04, HLA-B*07:02, HLA-B*15:01.

3.5. HTL epitope prediction and selection

Epitopic regions in protein which bind to HTL were identified by Propred (Table 1). Propred server has coverage of 51 HLA-DR alleles, about 90% of MHC-II molecules present on antigen presenting cells. A promiscuous epitope should bind several HLA-DR protein molecule binders (Singh & Raghava, 2001). Epitopes were predicted by considering all class 2 HLA alleles. From the matrix based prediction of epitopes with amino acid position coefficient, rank one epitope is "LVAIAVVII" with a highest score of 40.68. Identified HTL epitope has high binding strengths with MHC class-2 HLA alleles, namely, HLA-DRB1*03:06, HLA-DRB1*01:01, HLA-DRB1*01:02, HLA-DRB1*03:01, HLA-DRB1*03:03, HLA-DRB1*03:04, HLA-DRB1*03:05, HLA-DRB1*03:07, HLA-DRB1*04:02, HLA-DRB1*04:06, HLA-DRB1*07:01, HLA-DRB1*07:03.

3.6. Prediction and selection of putative B-cell epitope

On the basis of physicochemical properties (hydrophilicity, flexibility, turns, solvent accessibility propensity, exposed surface and accessibility) of experimentally derived epitopes, linear B-cell epitopes were predicted using ABCpred through recurrent neural network-based method. This method has been known to have more than 70% experimental validation accuracy (Saha & Raghava, 2006). ABCPred resulted in epitopes ranked form 1 to 36 for gB, among them top 5 epitopes have been listed in Table 1. From all predicted linear B cell epitopes, 'IVSDFGRPNAAQET' at 307th position is first rank epitope with ABCpred score of 0.90. At similar position to MHC class-I and II epitope at 758th position of gB, 'ILVAIAVVIITYLI' B-cell epitope has been found with server score of 0.87 which comes at fourth rank among all predicted B-cell epitopes. Predicted linear B-cell epitope has immunodominance which could elicit higher immune response and could be used as a potent anti viral peptide. It should be noted here that B-cell linear epitope starts at leucine 758th position of glycoprotein-B and has a common consensus with cytotoxic T-lymphocytes epitope and helper T-lymphocytes epitope which significantly increase the possibility of this region to be used a potent vaccine candidate.

3.7. Antigenicity analysis and selection of epitope

Top scored CTL, HTL and B cell epitopes were also evaluated to determine their antigenicity, efficacy, and pathogenicity to serve as potential lead molecules for vaccine development. A vaccine candidate should possess the high antigenicity and pathogenicity characteristics. The immune system considers the epitope as a foreign agent so epitope with high values of antigenicity will generate the strong immune response. Elicitation of strong immune response will produce antigen-specific antibodies to destroy the pathogens and also memorise the immune system so that in future when the body will encounter the microbial infection, memory immune cells will prime to elicit the strong immune response against the pathogenic antigens and prevent the microorganism infection and growth. Antigenicity of all epitopes was predicted by using Vaxijen v2.0, mentioned in Table 1. High vaxijen score (threshold value 0.4) suggests more antigenicity of predicted epitope and potential candidate for vaccine development. For MHC-I epitopes, 'LVAIAVVII' epitope resulted in a highest vaxijen score of 0.8316 and first rank epitope 'ILVAIAVVI' resulted in vaxijen score of 0.8178. So, with regard to high antigenicity, 'LVAIAVVII' was considered as a potential CTL epitope. Prediction accuracy of vaxijen has

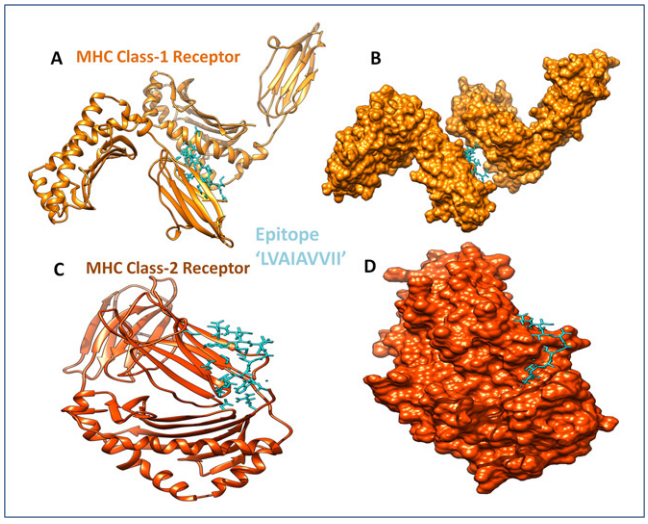

Figure 3. Epitope-MHC-I and II interactions: Figure depicts (A) Docked protein complex of MHC-I receptor (Orange colour) and epitope 'LVAIAVVII' (Cyan colour), a ribbon view of the protein complex and, (B) Surface view of MHC-I/epitope protein complex, (C) Docked protein complex (Ribbon view) of MHC-II receptor (Orange red colour) and epitope 'LVAIAVVII' (Cyan colour) and (D) Surface view of MHC-II/epitope protein complex.

been reported to have higher validation accuracy from 70 to 80% (Doytchinova & Flower, 2007).

Among HTL epitopes, first rank epitope 'LVAIAVVII' showed vaxijen score 0.8316 and ranked four epitope showed vaxijen score of 1.1199. However, rank one epitope 'LVAIAVVII' showed similarity with the CTL epitope and also had a good vaxijen score. Hence, it could be considered as more efficient epitope than rank four epitope. For B cell epitopes, 'YPYRVCSMAQGTDL' and 'ILVAIAVVIITYLI' epitopes have a high vaxijen score of 0.5286 and 0.5158, respectively, among all top five rank B cell epitopes. CTL and HTL selective epitope 'LVAIAVVII' is having sequence similarity with the core sequence of B cell epitope 'ILVAIAVVIITYLI'. Hence 'LVAIAVVII' was considered as a potent epitope for both classes of MHC as well as B cells.

3.8. Binding affinity analysis of epitopes for MHC-I, MHC-II and TLR2

Binding affinity of MHC-I, MHC-II and B cell epitopes were determined through molecular docking. Since we found 'LVAIAVVII' common epitope candidate for both MHCs and B cells, we docked it with specific protein receptors: MHC

Table 2. Molecular interaction analysis of Lead antigenic peptide sequence (LVAIAVVII) with MHC-1 protein receptor, MHC-2 protein receptor and TLR2 receptor present on the host.

Protein complex	Binding energy (kJ/mol)	Hydrogen bond	Interacting hydrophobic residues
Epitope-MHC class-1 protein	−375.79	Thr 187 (N)–Val 6 (O) 3.77 Å	Ala 158, Glu 161, Gly 162, Leu 266, Pro 185, Leu 270, Lys 268, Prol 267, Ala 184, Trp 274, Lys 186, Glutamate 275, Met 189, Arg 273, Leu 272
Epitope-MHC class-2 protein	−329.36	Trp 168 (NE1)–Ile 4 (O) 3.64 Å	Leucine 175, Glycine 125, Leucine 122, Glu 172, Asp 171, Trp 168, Glu 166, Val 117, Asn 118, Val 116 , Thr 3
Epitope-TLR2 protein (PreMD)	−352.83	Thr 391 (OG1)–Ala 5 (N) 2.72 Å, Arg 340 (NE)–Valine 6 (O) 2.85 Å, Gln 310 (OE1)–Ile 9 (N) 3.34 Å	Asp 419, Tyr 364, Gln 390, Ile 393, Arg 395, Asp 366, Glu 363, Thr 311, Glu 281, Asp 366, Tyr 440, Asp 417, Lys 339
Epitope-TLR2 protein (PostMD)	−403.928	Asp 419 (OD2)–Val 2 (N) 2.79 Å, Tyr 440 (OH)–Leu 1 (N) 2.92 Å, Asn 442(OD1)–Leu 1 (N) 2.92 Å, Tyr 440 (OH)–Val 2 (N) 3.63 Å, Asp 419–Ala 3 (N) 3.43 Å	Thr 391, Arg 395, Ser 421, Asp 463

The depiction of binding energy scores of the best-ranked peptide-receptor complex derived through the HEX 8.0 and molecular interactions in the form of hydrogen bonds and hydrophobic interactions. Also showing the PreMD and PostMD molecular dynamics simulation analysis of TLR2-Lead epitope complex using GROMACS.

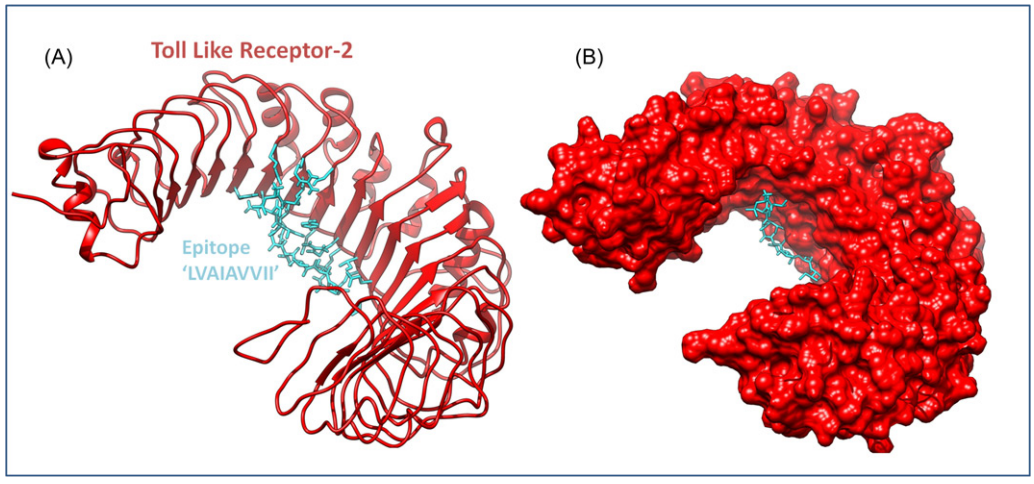

Figure 4. TLR2-epitope binding interactions. (A) It depicts the epitope 'LVAIAVVII' (Cyan colour) interaction with TLR2 protein (Red colour) to its binding groove and (B) Surface view of their interactions.

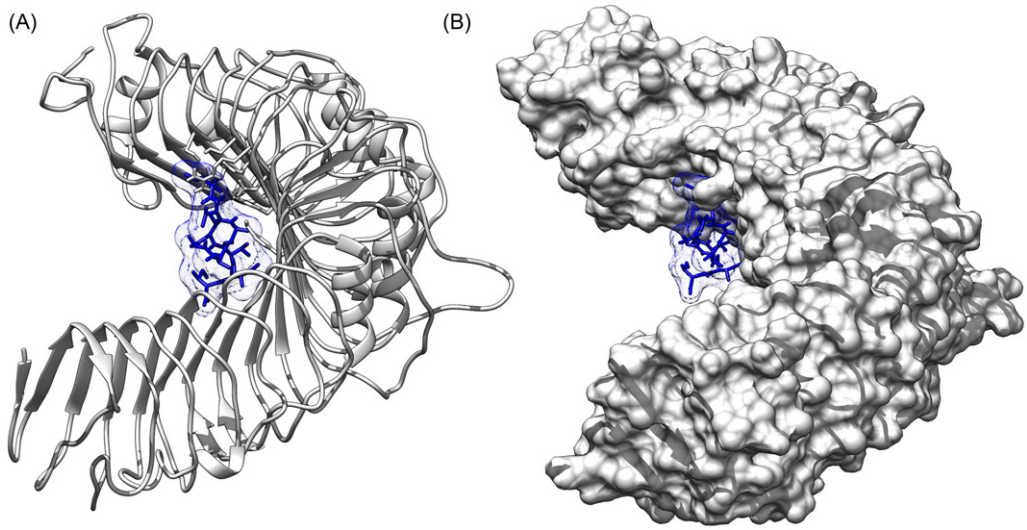

Figure 5. Binding pocket interaction. (A) Post MD diagram of epitope 'LVAIAVVII' (Blue colour) interaction with TLR2 protein (Silver colour) in its binding groove and (B) Surface view of their interactions.

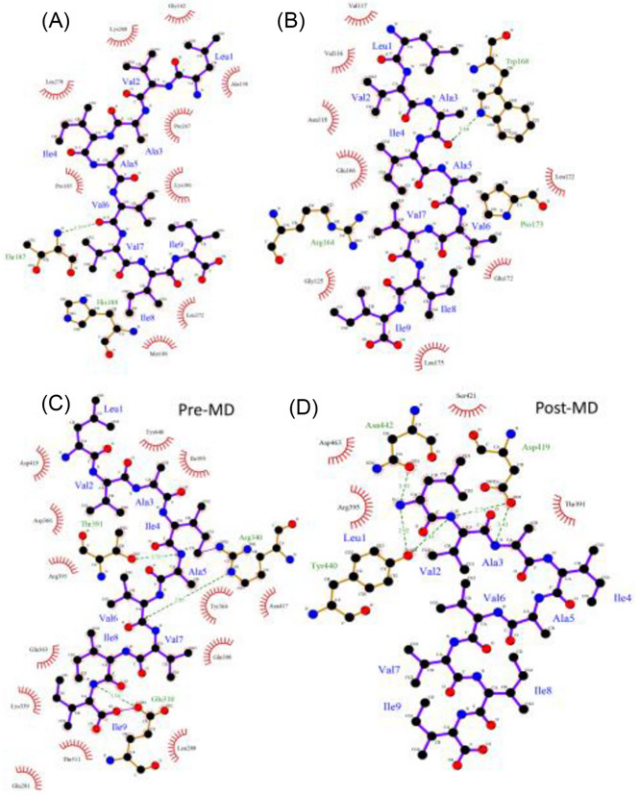

Figure 6. Pre-MD and post MD interactions. (A) Ligplot analysis of docked complex of MHC-I receptor and lead epitope (LVAIAVVII), showing the hydrophobic interactions involved (red colour comb arcs) and two external bond between Ile 8 and His 188 of TLR2. (B) Interaction analysis of MHC-II receptor and lead epitope docked protein complex showing the hydrophobic interactions involved (red colour comb arcs) and three external bonds between Val 6-Pro 173, Val 7-Arg 164 and Ile 4-Pro 173, (C) Pre-MD and (D) Post-MD TLR2-epitope complex analysis, three H- bonds (green colour) were found between Ala 5-Thr 391, Val 6-Arg 340 and Ile 9-Glu 310 before MD simulation and also three H-bonds were observed between Val 2-Asp 419, Leu 1-Tyr 440 and Leu 1-Asn 442 after the MD simulation.

class-1 receptor, MHC class-2 receptor and membrane protein receptor TLR2.

3.9. Molecular docking analysis of epitopes for MHC-I, MHC-II receptors

Protein structures of MHC-I and MHC-II were extracted from protein complexes with PDB ID: 1I1Y and 1KG0, respectively, which were then prepared for molecular docking study, accomplished using HEX 8.0. After docking 'LVAIAVVII' with MHC-I protein, resulting protein complex (Figure 3) exhibited an E_{total} (energy total) binding score of −375.79 kJ/mol. Epitope formed a hydrogen bond with Thr 187 and had strong hydrophobic interactions with Gly 162, Glu 161, Ala 158, Leu 266, Pro 185, Leu 270, Lys 268, Pro 267, Ala 184, Trp 274, Lys 186, Glu 275, Met 189, Arg 273 and Leu 272. Next, we docked 'LVAIAVVII' with MHC-II receptor and found binding E_{total} score −329.36 kJ/mol. Epitope formed a hydrogen bond with Trp 168 and had hydrophobic interactions with Leu 175, Gly 125, Leu 122, Glu 172, Asp 171, Trp 168, Glu 166, Val 117, Asn 118, Val 116 and Thr 3 residues (Table 2).

3.10. Molecular docking analysis of epitope with TLR2

Shortlisted top scoring epitope was further studied for its interaction with HHV-5 specific receptor TLR2, which is present on host cells during the process of infection. Initially for docking, binding cavity of TLR2 was predicted using META pocket 2.0 and binding cavity-1 was at Pro 306, Gly 307, Ile 341, Val 351, Leu 359, Leu 365, Trp 386, Ser 333, Leu 317, Ile 276, Ser 277, Gly 278, Ser 333, Leu 317, Ile 276, Ser 277, Gly 278.

After selection of binding cavity 1 from TLR2 protein, it was docked with the lead epitope which resulted in an E_{total} score of −352.83kJ/mol (Figure 4). The epitope-TLR2 protein

complex showed three H-bonds during Pre-MD analysis. First H-bond formed between amino acid 'Ala 5' of epitope with 'Thr 391' of TLR2 of bond length 2.72Å and second H-bond between 'Val 6' of epitope with 'Arg 340' of TLR2 of 2.85Å bond length and third H-bond formed between 'Ile 9' of epitope with 'Glu 310' of TLR 2 protein having 3.34 Å bond length (Table 2). TLR2 protein residues, namely Asp 419, Tyr 364, Gln 390, Ile 393, Arg 395, Asp 366, Glu 363, Thr 311, Glu 281, Asp366, Tyr 440, Asn 417 and Lys 339 were found to be involved in the hydrophobic interactions with 'LVAIAVVII.'

3.11. MD simulations analysis of TLR2-eptiope

TLR2–epitope complex was subjected to MD simulation using water solvent model through GROMACS. A stable trajectory was observed throughout the course of 100 ns MD simulation and a representative frame was extracted (Figure 5). Post-MD analysis of TLR2–epitope complex showed an increase in the number of hydrogen bonds from three to five while a visible decrease in the number of hydrophobic interactions (Figure 6). Asp419, Tyr440 and Asn 442 residues of TLR2 were observed to form hydrogen bonds of 2.79, 2.92 and 2.92 Å bond lengths, respectively, with the epitope. At the same time, Asp 419 and Tyr 440 also formed two longer hydrogen bonds of 3.43 and 3.63 Å lengths each. Residues Thr391, Arg395, Ser421 and Asp463 were found to be involved in hydrophobic interaction.

Post MD binding energy calculation was done using MMPBSA method and the total MMPBSA energy was estimated to be −403.928 kJ/mol (Table 2) in which the polar solvation energy contributed highest, that is, −396.899 kJ/mol. Higher binding energy of the post MD TLR2-epitope complex demonstrated a stable complex.

Molecular stability and conformational changes of the complex were studied through the RMSD and RMSF analysis. Stability and flexibility properties are acquired for the biochemical functions, activity and regulation. RMSD plot shows low deviation within a range of approximately 0.8 to approximately 1.2 nm for the epitope bound to TLR2 during the simulation of 100 ns time length (Figure 7(A)), suggesting the stability of the epitope-TLR2 protein complex. During the time period of 24–60 ns, RMSD had an approximate value around 1 nm but in later phase, from 80 to 100 ns, it centred on approximately 1.2nm. To observe the fluctuation and movement of residues in the complex we calculated residue wise RMSF values (Figure 7(B)). RMSF measured the

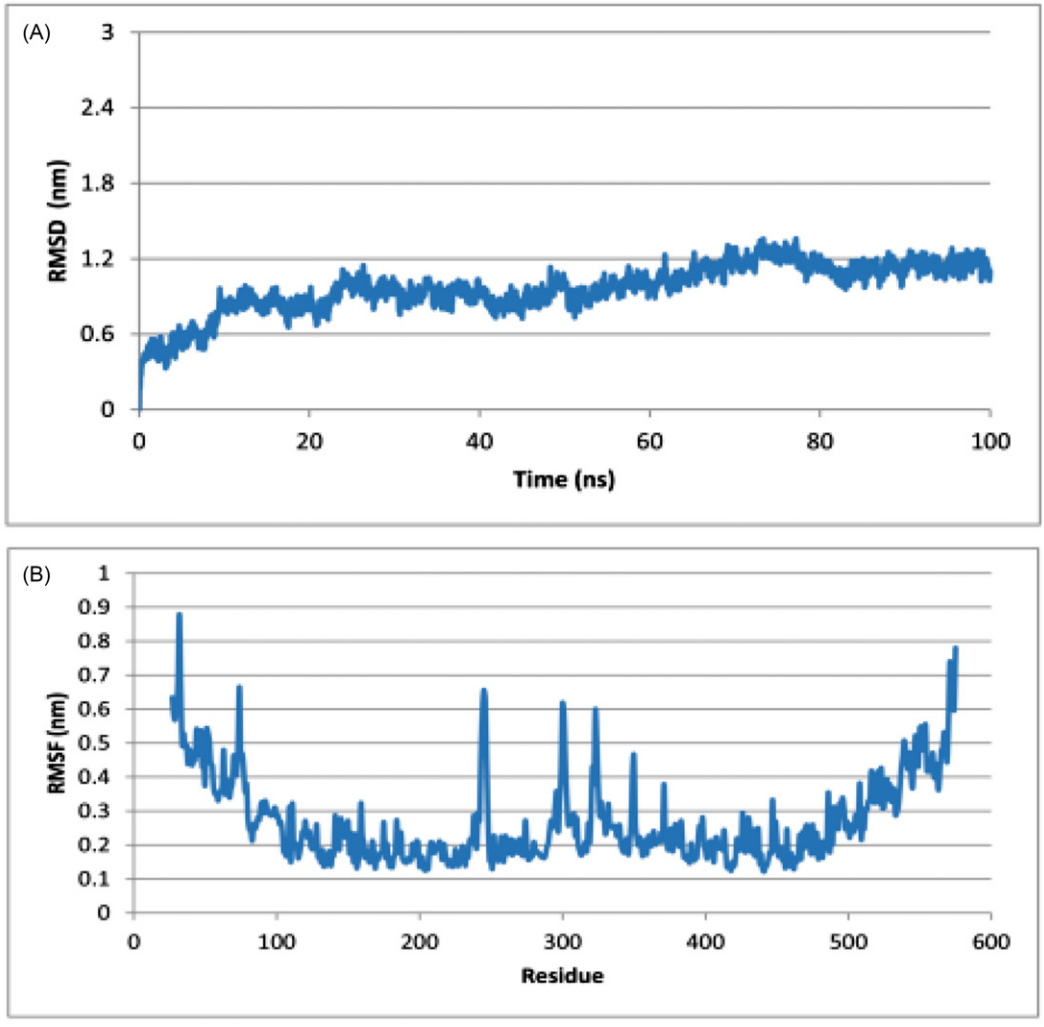

Figure 7. RMSD and RMSF plots (A) TLR2-Epitope (LVAIAVVII) protein complex RMSD analysis showing very less deviation of the protein complex in 100 ns simulation run and (B) RMSF profile of TLR2-epitope from the initial run till the end of the simulation.

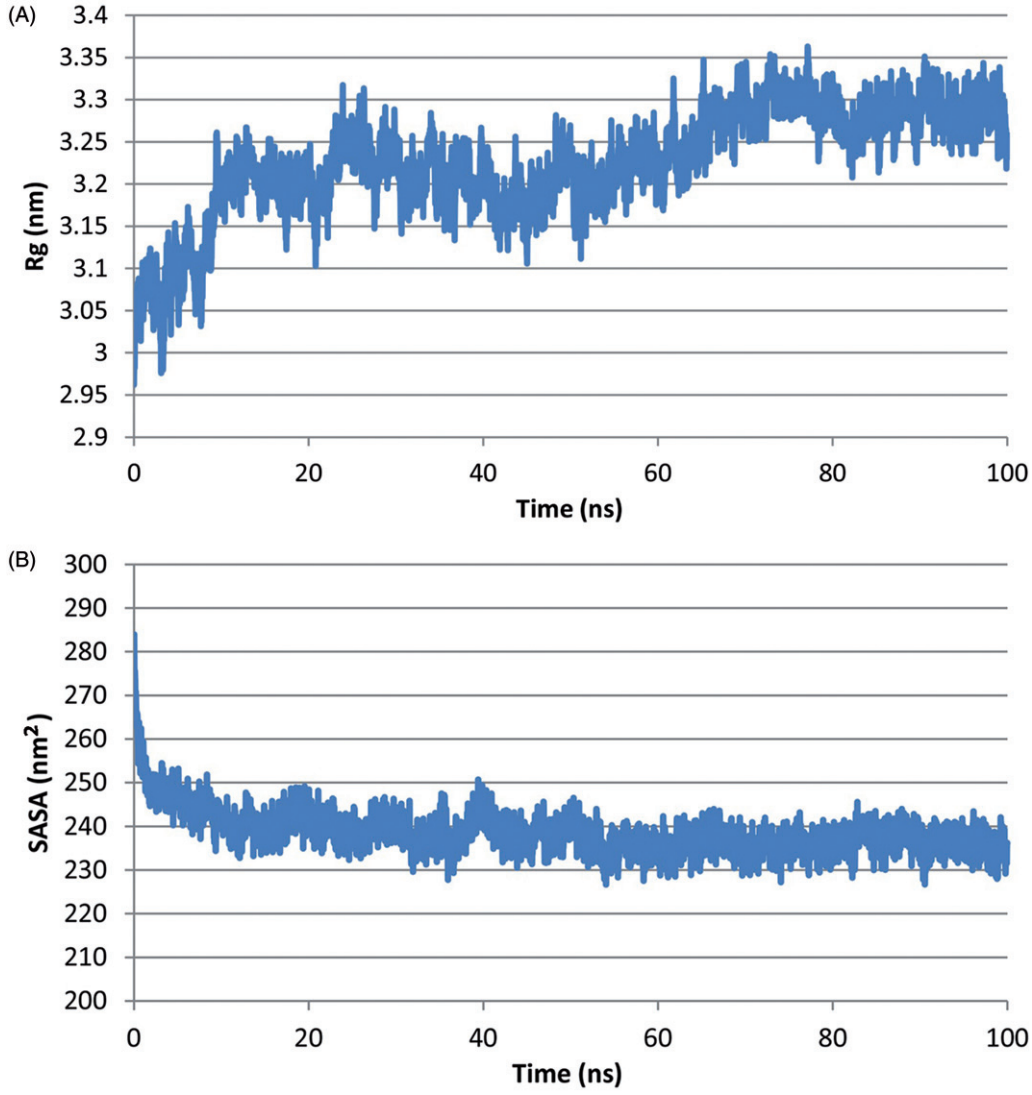

Figure 8. Rg and SASA plots (A) TLR2-epitope radius of gyration plot, showing a small deviation at the beginning and then very similar Rg score from initial run till the end of the simulation (B) SASA profile of TLR2-epitope protein complex.

mobility of C-alpha atoms of TLR2 during MD simulation run. We observed comparatively less variation in RMSF values of residues from 390 to 480 corresponding to the binding region of the epitope to TLR2 in post MD complex. In pre-MD complex, epitope was also interacting along the residue region from 250 to 380 which is having visibly very high RMSF values throughout MD run and may be a significant reason behind losing the interactions during molecular dynamics. In this region (250–380), RMSF profile fluctuates between 0.15 and 0.65 nm while for the binding region fluctuation can be noted between 0.12 and 0.3 nm. This indicates that lesser fluctuation per residues in this protein region is suitable for epitope binding and provides stability to the TLR2-epitope complex.

Change in compactness of protein during molecular dynamics run was calculated by Rg analysis. Rg plot shows that at the beginning TLR2 had a slightly fluctuating Rg and then it gets stabilised with Rg score of 3.093 Å and remained approximately same till the end of 100 ns run with Rg score of 3.251 Å (Figure 8(A)). Thus protein gets a little less compact that improves its interaction with the epitope. Same was analysed with solvent accessible surface area (SASA) analysis which indicates the solvent exposed surface of protein and its folding by considering the change in buried and exposed surface area. TLR2 exhibited SASA value of approximately 230 to approximately 250 nm^2 for 100 ns simulation run (Figure 8(B)). This solvation profile plays an important role in stabilisation and folding of protein complex. A considerable increase in SASA also supports the hypothesis that protein became slightly unfolded and provided more surface to interact with the epitope.

3.12. Identification of lead epitope's dissimilarity to human genome and population coverage for India

The immune response is generation of antibodies by the immune system and manifests itself in the ability for host organism to tolerate the self-proteins or endogenous substances and eliminate the exogenous antigens (foreign materials). This proteome discrimination is a requisite property for proper functioning of immune system. Else it would result in the generation of antibodies for self-protein and may lead to

autoimmune disease or immune hypersensitivity. Hence it was taken into the consideration that vaccine candidate has the dissimilarity with the human genome for avoiding immune problems. HLApred tool was used to analyse the lead epitopes of HLA class-1 and class-2 for their dissimilarity with human genome. We analysed the lead epitope 'LVAIAVVII' and B cell epitope 'ILVAIAVVIITYLI'. There were no similarities found for 'LVAIAVVII' and 'ILVAIAVVIITYLI' with human genome, which clearly indicates that, predicted epitopes are potential vaccine candidates. HLApred server works by identifying the matching amino acid sequences of the epitope with eukaryotic organisms. Predicted epitopes were further evaluated for population coverage in India. For population coverage analysis both MHC alleles were considered. Predicted nonamer epitope 'LVAIAVVII' showed the population coverage of 73.03% with Indian population. Hence, nonameric epitope of HHV-5 gB can be used as a potent vaccine candidate against HHV-5 infection.

4. Conclusions

We have predicted and validated a lead epitope against TLR2, the major receptor responsible for HHV-5 infection. Our approach was based on structural analysis integrated with molecular docking and MD simulations study. Epitope possesses the high antigenicity and pathogenicity characteristics which will induce the strong immune response in the host and strengthen the immunity to fight and prevention against viral infections and progression. Importantly, TLR2-epitope interacting complex was found to be stable with strong molecular interactions through its persistent long run (100 ns) RMSD and similar Rg values. Higher binding energy even after the molecular dynamics of 100ns also confirmed the stability and specificity of the TLR2-epitope interaction. Epitope was also found dissimilar with the human genome, which significantly avoid any immunological aberration and moreover, epitope also showed the high population coverage for Indian population. In conclusion, this epitope appears to be a promising candidate for designing a vaccine against HHV-5.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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