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Research paper

Identification of broadly reactive epitopes targeting major glycoproteins of Herpes simplex virus (HSV) 1 and 2 - An immunoinformatics analysis

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ABSTRACT

Infections due to both HSV-1 and HSV-2 constitute an enormous health burden worldwide. Development of vaccine against herpes infections is a WHO supported public health priority. The viral glycoproteins have always been the major hotspots for vaccine designing. The present study was aimed to identify the conserved T and B cell epitopes in the major glycoproteins of both HSV-1 and HSV-2 via rigorous computational approaches. Identification of promiscuous T cell epitopes is of utmost importance in vaccine designing as such epitopes are capable of binding to several allelic forms of HLA and could generate effective immune response in the host. The criteria designed for identification of T and B cell epitopes was that it should be conserved in both HSV-1 and 2, promiscuous, have high affinity towards HLA alleles, should be located on the surface of glycoproteins and not be present in the glycosylation sites. This study led to the identification of 17 HLA Class II and 26 HLA Class I T cell epitopes, 9 linear and some conformational B cell epitopes. The identified T cell epitopes were further subjected to molecular docking analysis to analyze their binding patterns. Altogether we have identified 4 most promising regions in glycoproteins (2-gB, 1-gD, 1-gH) of HSV-1 and 2 which are promiscuous to HLA Class II alleles and have overlapping HLA Class I and B cell epitopes, which could be very useful in generating both arms of immune response in the host i.e. adaptive as well as humoral immunity. Further the authors propose the cross-validation of the identified epitopes in experimental settings for confirming their immunogenicity to support the present findings.

1. Introduction

The infections caused by Herpes simplex viruses (HSV) impose an enormous health burden on the world's population commonly causing oral and genital ulcerations. Other serious but less common complications include neonatal infection, encephalitis, keratitis and meningitis. The high prevalence of HSV-2 contributes significantly to the epidemic of Human Immunodeficiency virus (HIV) (Johnston et al., 2016). The global incidence of HSV-2 infection recorded in 2012 was about 417 million in aged between 15 and 49 years with an incidence of 19 million infections per year as estimated by World Health Organization (WHO) (Looker et al., 2015).

As the infections caused by HSV are subclinical, the incidence of the disease and its prevalence data underestimate the impact of the infection. At present the treatment for HSV involves antiviral therapy using acyclovir, valacyclovir or famcyclovir (Johnston et al., 2016). Considering the fact that the current strategies for control of HSV-2 infections are partially effective, there is no permanent cure or treatment available for herpes as virus is known to persist in a latent phase and disease is well known for its recurrence (Gottlieb et al., 2017). Thus, the development of a prophylactic and therapeutic vaccine is urgently needed. The development of a prophylactic vaccine for HSV-1 and HSV-2 is a WHO-supported global public health priority (Sandgren et al., 2016). For an ideal vaccine candidate it should provide immunity against both HSV-1 and HSV-2, and contain both immunodominant T-cell epitopes as well as neutralizing antibody epitopes (Sandgren et al., 2016). Various HSV vaccines have been tried by different research groups and are in different phases of clinical trials, currently there is no commercially available vaccine (Belshe et al., 2012, 2013).

Keeping the above points under consideration, the present study aimed to identify potential epitopes in the major glycoproteins of HSV, which facilitate virus entry into the cells and could be vaccine targets.

2. Materials and methods

2.1. Retrieval of amino acid sequences, molecular and structural analysis

The primary amino acid sequences of glycoproteins B, C, D, G, H, I &

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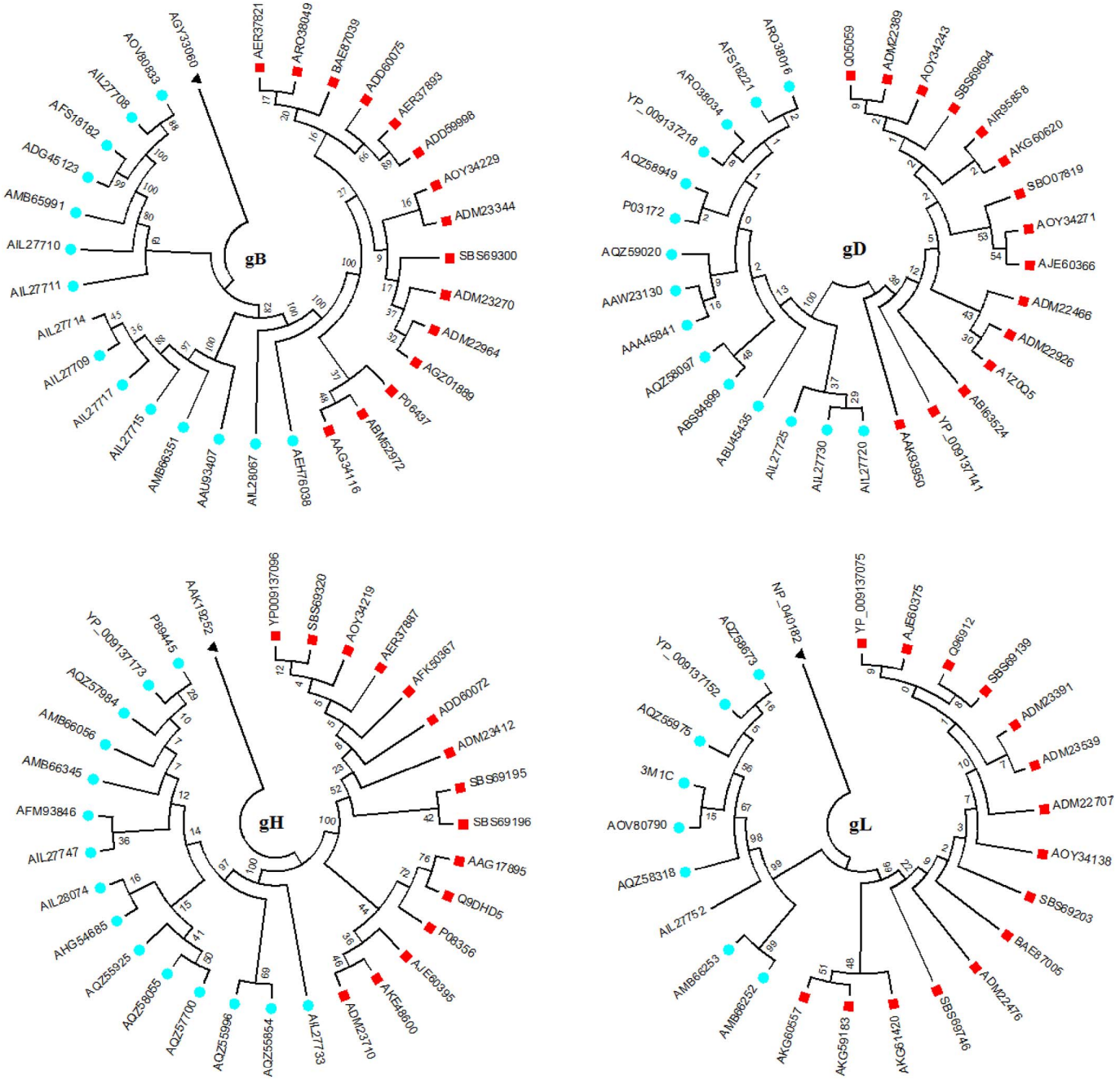

Fig. 1. Phylogenetic analysis of glycoproteins- B, D, H and L of HSV1 and 2. The accession numbers containing red boxes are HSV-1 sequences and with light blue boxes are HSV-2 sequences. The sequences denoted with black triangle are the VZV protein sequences which served as outliers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

L of HSV-1 & 2 were retrieved from NCBI database. The amino acid sequences obtained were further aligned using multiple sequence alignment tool- Clustal Omega to find out the conservancy among the above glycoproteins from HSV-1 & 2. Further, the phylogenetic analysis of the glycoproteins was carried out to analyze the evolutionary divergence between them using MEGA 7.0.14 server. The physicochemical properties of each glycoprotein were analyzed using Expasy Protparam tool (http://web.expasy.org/protparam/). The antigenicity of the target proteins were determined using VaxiJen 2.0 server (Doytchinova and Flower, 2007).

2.2. Identification of T cell and B cell epitopes

For the identification of 15-mer HLA class-II T cell epitopes, the servers NetMHCIIpan 3.1, IEDB-NN and IEDB-SMM were used. The alleles which are expected to cover > 95% of the worldwide population were selected for the study (Southwood et al., 1998; Moise et al., 2009). The alleles included were: HLA-DRB1*01:01, HLA-DRB1*03:01, HLA-DRB1*04:01, HLA-DRB1*07:01, HLA-DRB1*08:03, HLA-DRB1*10:01, HLA-DRB1*11:01, HLA-DRB1*12:01, HLA-DRB1*13:02 and HLA-DRB1*15:01. Based on the predicted IC_{50} values, the T cell epitopes were classified into the following groups: i) high-affinity binding epitopes (IC_{50s} < 100 nM), ii) intermediate-affinity binding epitopes (IC_{50s} < 500 nM); iii) low-affinity binding epitopes (IC_{50s} < 1000 nM); and iv) non-binding epitopes (IC_{50s} of > 1000 nM). NetMHC 4.0 Server was used for the identification of 9-mer HLA Class I epitopes (Andreatta and Nielsen, 2015). The following six HLA Class I alleles – HLA-A*01:01, HLA-A*02:01, A*03:01, HLA-A*24:02, HLA-B*07:02 and HLA-

Table 1
Predicted class II T cell epitopes along with their physicochemical properties.

Glycoprotein	Peptide sequence (code)[a]	Position[b]	Mol. wt.	Isoelectric point	Half-life in mammalian reticulocytes	Antigenicity
gB	gIAVVF**K**E**R**IAPy**K**F (A1)	145	1696.02	8.50	30 h	0.76
	IAVVF**K**E**R**IAPy**K**F**K** (A2)	146	1767.14	9.53	20 h	0.73
	IAPy**K**F**K**AtMyy**K**D**V** (A3)	154	1838.19	9.31	20 h	0.94
	A**K**gVC**R**stA**K**yV**R**nn (A4)	203	1666.92	10.31	4.4 h	0.36
	DRFKqV**D**gFyA**RD**Lt (A5)	317	1831.02	6.04	1.1 h	0.91
	RFKqV**D**gFyA**RD**Ltt (A6)	318	1817.03	8.59	1 h	0.50
	tt**R**nLLttP**K**FtVAw (A7)	340	1749.04	11.00	7.2 h	0.06
	ggsF**R**Fss**D**AIsttF (A8)	381	1579.69	5.84	30 h	0.40
	ggFLIAyqPLLsntL (A9)	446	1606.88	5.52	30 h	0.27
	sIEFA**R**LqFtynHIq (A10)	500	1867.09	6.47	1.9 h	1.34
gD	VyyAVLE**R**AC**R**sVLL (A1)	82	1755.11	8.17	100 h	0.15
	EDnLgFLMHAPAFE**t** (A2)	171	1691.88	4.13	1 h	0.34
	DnLgFLMHAPAFE**t**A (A3)	172	1633.84	4.35	1.1 h	0.63
gH	**ER**FCFVLVttAEFP**R** (A1)	87	1815.12	6.23	1 h	0.98
	DPgqLLyIP**K**tyLLg (A2)	102	1691.00	5.83	1.1 h	0.13
gL	I**D**gIFL**R**y**H**CPgL**D**t (A1)	67	1719.98	5.21	20 h	0.70
	gCVnF**D**ys**R**t**RR**CVg (A2)	148	1732.95	8.96	30 h	0.79

[a] Non polar, polar and charged residues are shown in upper case, lower case and bold respectively.
[b] The position of the epitopes indicated is of HSV-1.

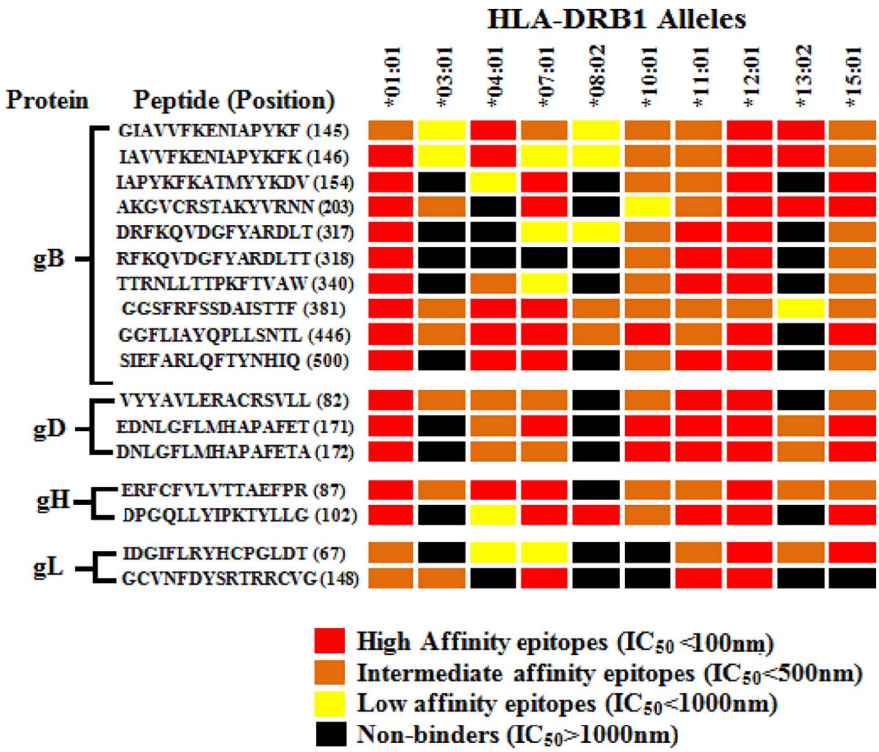

Fig. 2. Heatmap showing the high, intermediate, low and non-binding affinity of the HLA Class II T-cell epitopes towards different HLA-DRB1 alleles included in the study. Red color shows high affinity, orange- intermediate, yellow- Low and black- no affinities. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

B*44:03 alleles that are expected to cover > 90% of the worldwide population were included in the study. The peptides with rank thresholds ≤0.5 were considered as strong binders, while those between 0.5 and 2.0 were considered as weak binders. Since the majority of HLA molecules shows distinct binding specificity, the relationships between the binding patterns of the HLA Class I and Class II molecules included in the study was analyzed using MHC Cluster 2.0 server (Thomsen et al., 2013).

The B cell lymphocytes differentiate into plasma cells and memory B cells on interaction with the antigen (B cell epitopes). The hidden Markov model based BepiPred 1.0 online web server was utilized for predicting linear B cell epitopes (Larsen et al., 2007). The conformational epitopes were predicted using Ellipro server (Ponomarenko et al., 2008). In ellipro, prediction was made without altering any prediction criteria. BLASTP search was also performed against human proteome in order to find any peptides showing identity to human proteins and also the conservancy among other members of *Herpesviridae* family.

2.3. Characterization of the predicted epitopes

Different parameters like molecular weight, estimated half-life in human reticulocytes, theoretical isoelectric point (pI) of the predicted T and B cell epitopes were calculated using ExPASy ProtParam tool (http://web.expasy.org/protparam/). The antigenicity of the predicted epitopes was analyzed by VaxiJen v2.0 tool (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). The antigenicity prediction threshold was kept 0.4. The conservancy analysis of the predicted T and B cell epitopes was carried out by IEDB Conservancy Analysis tool (Bui et al., 2007).

Table 2
Predicted Class I T cell epitopes along with their physicochemical properties.

Glycoproteins	HLA Class I alleles	Epitopes (codes)[a]	Position[b]	Mol. wt.	Theoretical pI	Half-life in mammalian reticulocytes	Threshold values	Antigenicity
gB	HLA-A0201	yVyF**EE**yAy (B1)	646	1246.34	3.79	2.8 h	0.50	0.61
		FLIAyqPLL (B2)	445	1077.33	5.52	1.1 h	0.01	0.29
		TM**LED**H**E**FV (B3)	675	1120.24	4.13	7.2 h	0.17	0.45
		LLsntLA**E**L (B4)	454	973.13	4.00	5.5 h	0.40	0.41
		tMyy**KD**VtV (B5)	161	1119.30	5.50	7.2 h	0.40	1.05
		sVyPy**DE**FV (B6)	279	1118.21	3.67	1.9 h	0.50	0.005
	HLA-A2402	**R**ysqFMgIF (B7)	177	1148.34	8.75	1 h	0.02	0.05
		RygttVnCI (B8)	263	1026.18	8.22	1 h	0.06	0.56
		Cys**R**PLVsF (B9)	595	1071.26	8.22	1.2 h	0.07	−0.13
		VyPy**DE**FVL (B10)	280	1144.29	3.67	100 h	0.15	0.12
		WFgH**R**ysqF (B11)	(73	1227.35	8.75	2.8 h	0.25	1.00
		VyMsPFygy (B12)	294	1126.29	5.49	100 h	0.30	0.01
		APyK**FK**AtM (B13)	154	1056.29	9.70	4.4 h	0.05	0.85
	HLA-B4403	**EE**yAysHqL (B14)	650	1139.19	4.51	1 h	0.07	0.28
		KEnIAPy**KF** (B150	150	1109.29	8.50	1.3 h	0.08	0.50
		sqVWFgH**R**y (B16)	170	1179.30	8.49	1.9 h	0.25	0.42
		H**E**FVPL**E**Vy (B17)	680	1132.28	4.51	3.5 h	0.30	1.25
		EEVDARSVy (B18)	273	1067.12	4.14	1 h	0.30	1.19
gD	HLA-A0301	**K**IAgWHgP**K** (B1)	261	993.18	10.0	1.3 h	0.05	−0.49
gH	HLA-A0101	P**RD**PgqLLy (B1)	99	1058.20	6.26	> 20 h	0.25	−0.02
	HLA-A0201	FLAAsALgV (B2)	804	848.01	5.52	1.1 h	0.03	0.84
		ALgyqLAFV (B3)	470	981.16	5.57	4.4 h	0.25	1.37
	HLA-B0702	LP**R**gIgy**K**L (B4)	679	1016.25	9.99	5.5 h	0.03	0.24
		FP**RD**PgqLL (B5)	98	1042.20	5.84	1.1 h	0.05	0.07
		TPAg**E**VMsV (B6)	737	890.02	4.00	7.2 h	0.5	0.07
	HLA-B4403	g**E**VMsVLLV (B7)	740	946.17	4.00	30 h	0.5	0.53

[a] Non polar, polar and charged residues are shown in upper case, lower case and bold respectively.
[b] The position of the epitopes indicated is of HSV-1.

Table 3
Predicted linear B cell epitopes along with their physicochemical properties.

Glycoproteins	Linear B cell epitopes (codes)[a]	Position[b]	Mol. wt.	Theoretical pI	Half-life in mammalian reticulocytes	Antigenicity
gB	**EDR**APVPF**EE** (C1)	(187–196)	1188.26	4.00	1 h	0.90
	V**DAR**sVyPy (C2)	(276–284)	1069.18	5.81	100 h	0.93
	DAIstt (C3)	(389–394)	606.63	3.81	1.1 h	0.40
	ttnLt**E**y (C4)	(396–402)	840.89	4.00	7.2 h	0.29
	HI**K**VgqPqyyL C5)	(433–443)	1345.56	8.50	3.5 h	0.57
	EVq**RR**v (C6)	(704–709)	800.87	9.70	1 h	0.40
gD	AF**E**tAg (C1)	(182–187)	594.62	4.00	4.4 h	0.40
gH	A**EFPRD**PgqL (C1)	(97–106)	1129.24	4.37	4.4 h	−0.44
	A**R**gAAgCAA (C2)	(423–431)	746.84	8.29	4.4 h	1.06

[a] Non polar, polar and charged residues are shown in upper case, lower case and bold respectively.
[b] The position of the epitopes indicated is of HSV-1.

2.4. Modeling of epitopes, HLA alleles, target glycoproteins and molecular docking analysis

The X-ray crystallographic structures of HLA-DRB1* 01:01 and HLA-DRB1* 15:01 alleles were retrieved from Protein Data Bank (PDB) bearing PDB ID- 2g9h and 1BX2 respectively. The energy minimization for the receptors (HLA alleles) was further carried out after successfully removing the previously bound ligand. The 3D structures of the rest of the HLA alleles were modeled using Modeller 9v7 Program. The pdb files of the peptides predicted were created using PEPFOLD3 server (Lamiable et al., 2016). The pdbqt files of the receptors and ligands were prepared using AutoDock 4.2. The quality of the models of HLA alleles prepared were further checked by Ramachandran Plot analysis using Rampage server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php). Finally molecular docking analysis of the peptides with HLA alleles was carried out using AutoDock4.2 program for analyzing the binding patterns (Morris et al., 2009). Finally the interaction pattern of the peptide with HLA alleles was visualized using PYMOL version 1.7.4.4. (Schrodinger). The 3D models of all the glycoproteins B, D and L were retrieved from RCSB Protein Data Bank (PDB) with PDB ID 2GUM, 4MIV and 3MIC respectively. The 3D model of glycoprotein H was modeled using I-TASSER server (Iterative Threading ASSEmbly Refinement) homology modeling tool as the structure available in PDB contained some gaps (Yang et al., 2015).

2.5. Validation of identified epitopes and molecular docking analysis

The T cell epitopes identified by different servers were also subjected to IEDB analysis database which already contains number of well-known validated epitopes. Finally the epitopes identified by IEDB were matched with the epitopes identified by other servers and those showing exact matches were selected for further analysis. Molecular docking analysis was validated by carrying out the docking between the peptide Staphylococcal Enterotoxin I (SEI) with HLA-DRB1*01:01 (PDB id - 2g9h) using the same protocol as performed for other predicted epitopes.

3. Results

3.1. Protein targets

The accession numbers of all the sequences of the target proteins

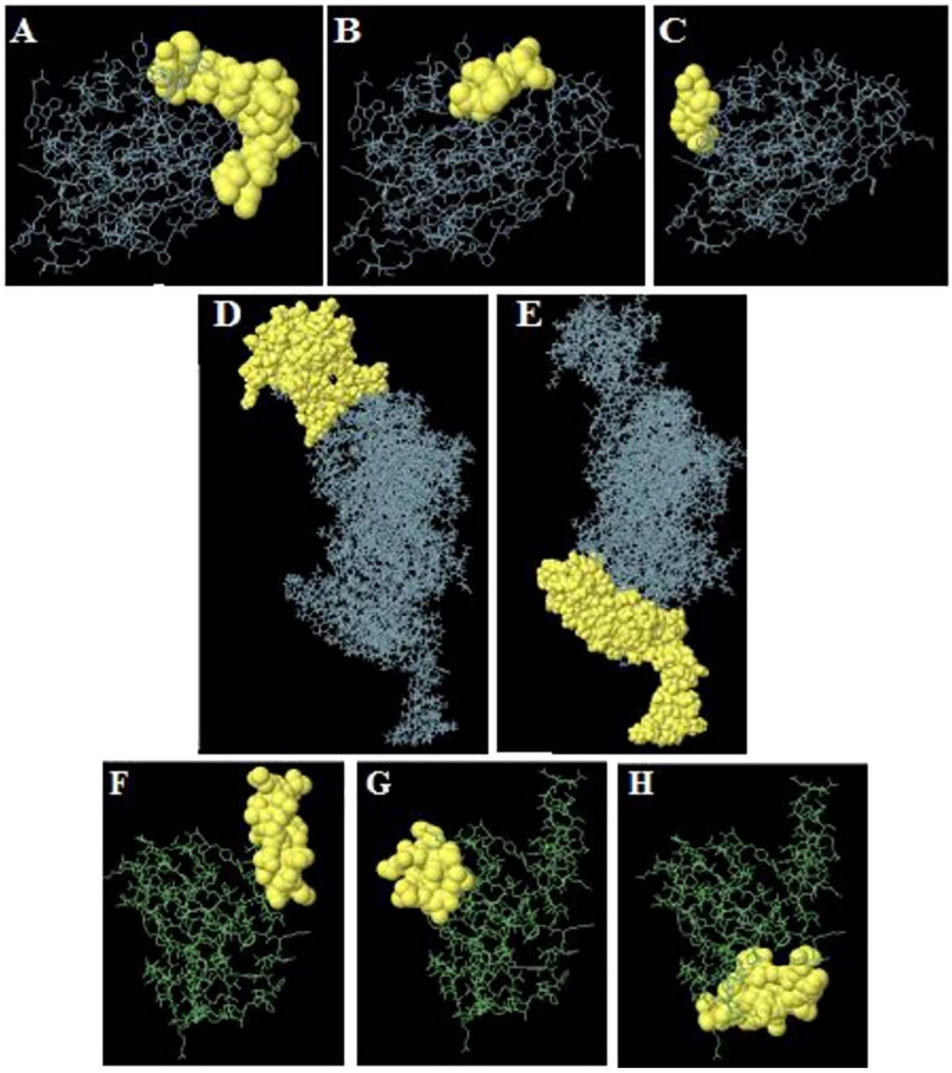

Fig. 3. Conformational B cell epitopes predicted by Ellipro server. In ball-and-stick model, the predicted epitope residues are shown as yellow balls (abbreviated amino acids) and non-epitope and core residues are shown in sticks (superscript numerals): A–C are the conformational epitopes predicted in gD, D–E in gH and F–H in gL. A- YAVLERACRS$^{59–68}$, DSF$^{139–141}$, HAPAFE$^{154–159}$; B- LRIPP$^{195–199}$; C- VYHI$^{37–40}$; D: EQ$^{25–26}$, DPW$^{28–30}$, YWRDTNTGRLWLPNTPD$^{41–57}$, ELNLTTAS$^{71–78}$, ERFCFVLVTTAE$^{87–98}$ RDPGQLLYIPKTYLLGRP$^{101–118}$; GLL HN$^{144–148}$, AS$^{151–152}$, LR$^{155–156}$, E- VPEASHRCGGQSANV$^{645–659}$, ITHNASYVVTH$^{667–677}$, PLPRGIGYKLTGVDVRRPLF$^{679–698}$, TYLTATCEG$^{700–707}$, VDTD7$^{49–752}$, TQQQ$^{754–757}$, FSSDVP770–775, FPNGTVIHLLAFDT$^{782–795}$, IAPGFLAASALGVVMITAALAGIL$^{801–824}$, VPFFWRRE$^{831–638}$; F: SH$^{108–109}$, FPA$^{105–112}$; TQETTTR$^{116–122}$, ALYKEIR$^{124–130}$, G: EVGDILR$^{35–41}$, HAQ$^{87–89}$, H: AGCVNF$^{147–152}$, TRRCVG$^{157–162}$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

included in the present study are shown in Table S1. The multiple sequence alignment results revealed a high degree of similarity in the amino acid sequences of the glycoproteins- gB (~88%) and gD (~82%) followed by gH (~79%), gC (~74%), gL (~70%), gI (~68%) and gG (~49%) among HSV-1 and HSV-2 respectively. As no conservancy was seen in any T cell or B cell epitopes of HSV1 and 2 in glycoproteins C, I and G, they were not carried out for further analysis. The phylogenetic analysis of the glycoproteins B, D, H and L was carried out using UPGMA statistical method for testing phylogeny with 1000 bootstrap replications using Poisson model (Fig. 1).

3.2. Identified T and B cell epitopes

The criteria selected for predicting HLA Class II T cell epitopes was that it should be conserved in both HSV 1 and 2, promiscuous, high affinity towards HLA Class II alleles (least IC_{50} value) included in the study, should not be present in the glycosylation sites and should be located on the surface of glycoproteins. Based on all these criteria, 17 HLA Class II T cell epitopes (gB-10, gD-3, gH-2 and gL-2) were predicted (Table 1) (Fig. 2). The same criterion was set for identification of HLA Class I epitopes except for promiscuousity. On the basis of these criteria 26 HLA Class I T cell epitopes (gB-18, gD-1, gH-7) were predicted (Table 2). Similarly only those linear and conformational B cell epitopes were selected which were conserved in both HSV 1 and 2 and strictly lying within the threshold values of the respective servers used for their identification. Based on these parameters, 9 linear (gB-6, gD-1, gH- 2) (Table 3) and 7 set of conformational B cell epitopes were identified (Fig. 3). The predicted epitopes showed almost 100% conservancy as revealed by IEDB Conservancy Analysis tool. The predicted T and B cell epitopes were further localized on the 3 D models of the respective glycoproteins to locate their surface accessibility (Fig. 4). Because the epitopes located at the surface of the protein are only accessible to immune cells, this is an important factor to be considered while screening the T cell and B cell epitopes. The overall analysis predicted four most promising regions (gB-2, gD-1, gH-1) in the glycoproteins of HSV-1 and 2 containing overlapping HLA Class I and HLA Class II T cell epitopes as well as B cell epitopes (Fig. 5).

3.3. Modeling of target proteins, HLA alleles and molecular docking analysis

The 3D models of glycoproteins B, D and L were retrieved from protein data bank while the glycoproteins H was successfully modeled using I TASSER server. The server predicted crystal structure of conserved fusion regulator complex gH-gL of *Herpesvirus* (PDB ID-3M1C) as template. The modeled gH showed the C-score of −0.57, which is a

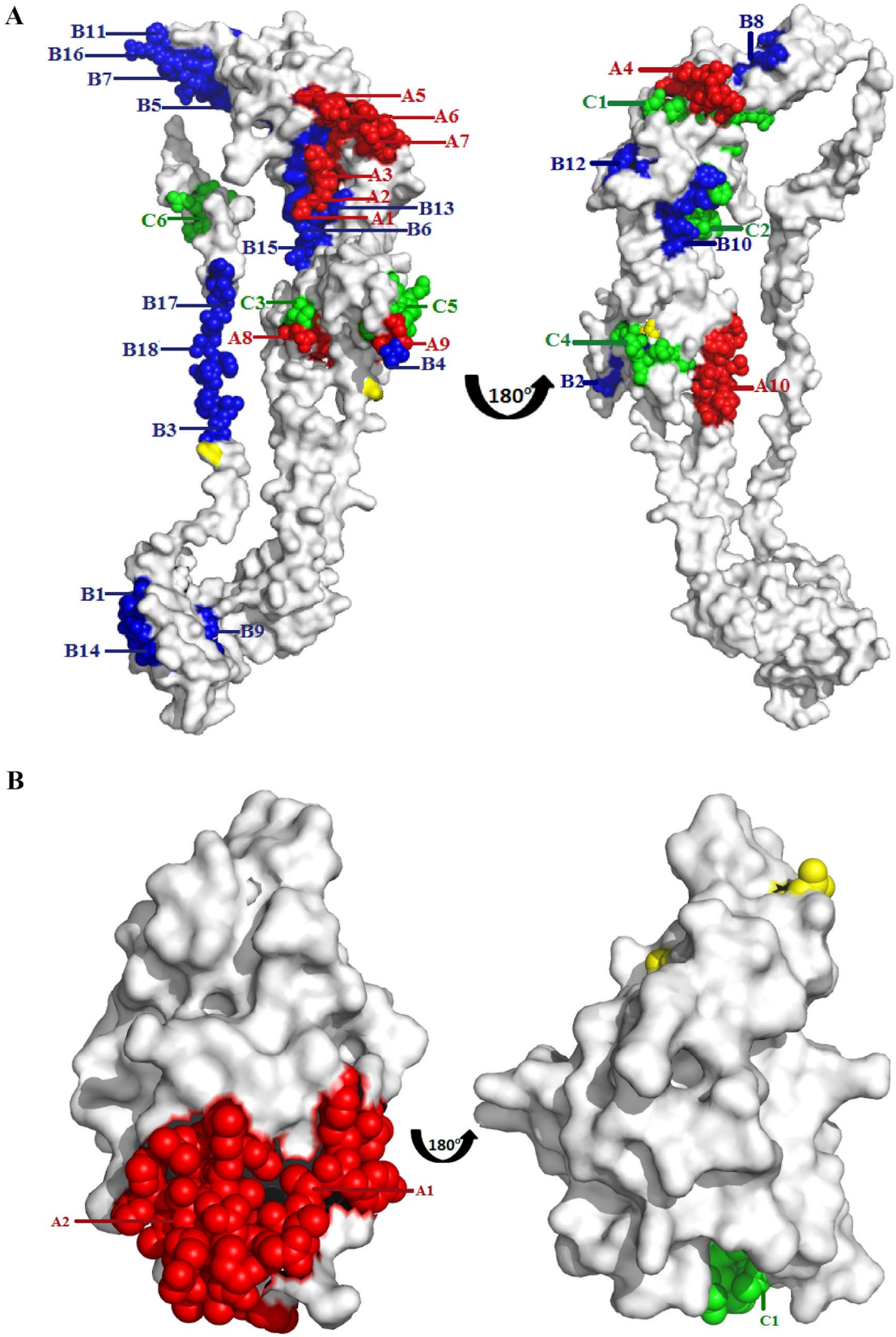

Fig. 4. 3-D models of the glycoproteins in surface view with superimposed T and B cell epitopes. A: 3D model of glycoprotein-B of HSV-1 (PDB ID 2GUM); B: 3D model of glycoprotein-D of HSV-2 (PDB ID 4MYV); C: 3-D model of glycoprotein-H of HSV-2; and D: 3-D model of glycoprotein-L of HSV-2 (PDB ID 3M1C), showing the surface location of HLA Class I (blue color), HLA Class 2 (red color) and B cell epitopes (green color). In yellow color are the glycosylation sites. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

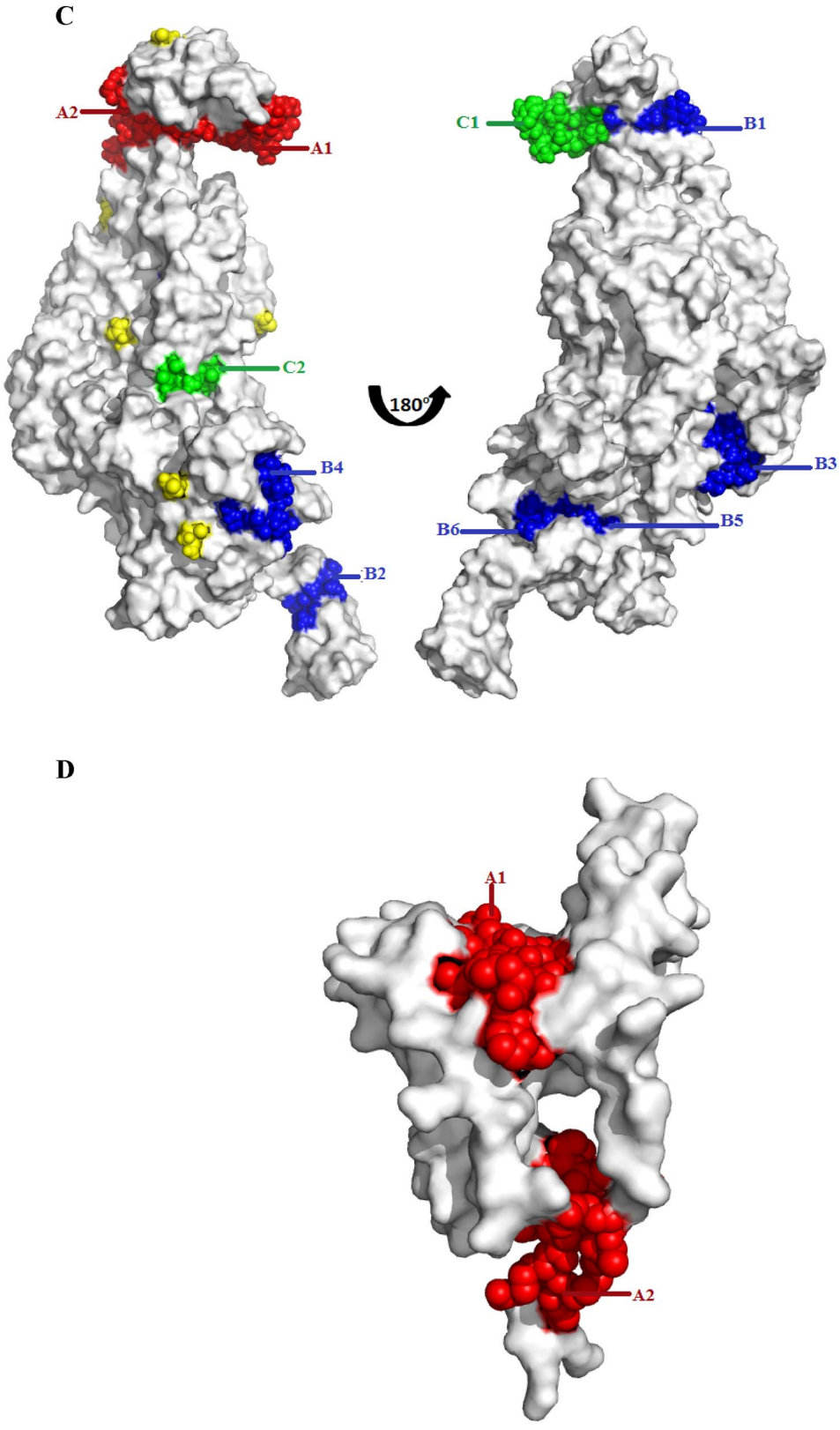

Fig. 4. (*continued*)

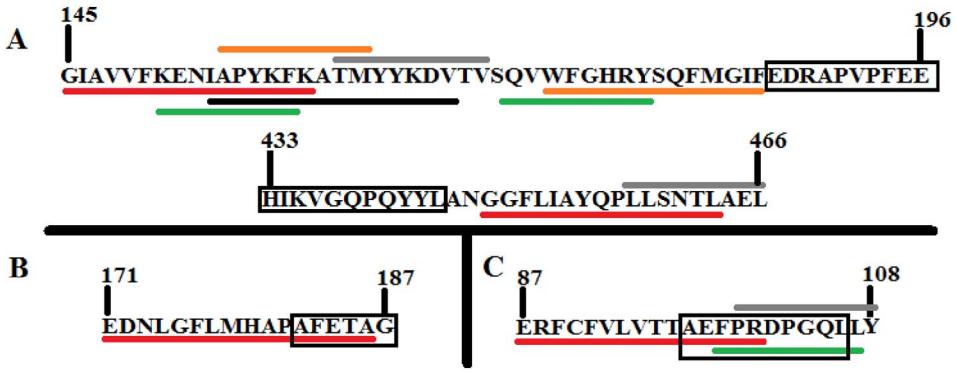

Fig. 5. Most promising regions in glycoprotein B, D and H. These regions are conserved in HSV1 and HSV2 having affinity towards number of HLA Class I and Class II alleles, thus are highly promiscuous. In addition they also possess linear B cell epitopes (shown in black box). (A) Glycoprotein B region from 145 to 196 amino acid. Red (affinity with HLA-DRB1 01:01, 04:01, 12:01, 13:02); black (01:01, 07:01, 12:01, 15:01); grey (HLA-A02:01); orange (HLA-A24:02); green (HLA-A44:03). Box (B cell epitope) and from 145–196 amino acid 433–466- red (affinity with HLA-DRB1 01:01, 04:01, 07:011, 10:01, 12:01, 15:01), grey (HLA-A02:01); box (B cell epitope). (B) Glycoprotein D region from 171 to 187 amino acid - red (affinity with HLA-DRB1 01:01, 07:011, 10:01, 11:01, 12:01, 15:01); box (B cell epitope). (C) Glycoprotein H region from 87 to 107 amino acid - red (affinity with HLA-DRB1 01:01, 04:01, 07:01, 12:01); grey (HLA-A02:01), green (HLA-B0702); box (B cell epitope). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

confidence score predicted by I-TASSER server for estimating the quality of models prepared. According to the server, the C score should be typically between −5 and 2, higher the value, higher is the confidence of the model prepared and vice versa. In addition, the TM-score of the modeled gH was 0.64 ± 0.13, which is used for measuring the structural similarity between two structures. According to the server the model of correct topology should have a TM-score > 0.5 and if it is below < 0.17 it means a random similarity between two structures exists. These results indicate that the quality of the model prepared was quite satisfactory.

The Ramachandran Plot analysis of the 3D structures of HLA alleles prepared through homology modeling (HLA-DRB1*07:01, *11:01 and *13:02) revealed satisfactory results with > 98% residues lying in the favoured and allowed region (Fig. S1, Table S2). Some of the predicted HLA Class II T cell epitopes were further subjected to molecular docking analysis in order to analyze their binding patterns and affinity towards different HLA alleles. The relationships between the binding patterns of the HLA Class I and Class II molecules included in the study as well as the docking energies obtained after docking analysis is shown in Fig. 6 in the form of heat maps. The docking patterns of some of the peptides with HLA-DRB1*01:01 is shown in Fig. 7.

For validation of docking, the Staphylococcal Enterotoxin I (SEI) peptide was docked with HLA DRB1*0101 allele using the same strategy as followed for other predicted epitopes. The docked figure is shown in Fig. S2. The overall strategy involved in epitopes prediction in the present study is summarized in Fig. 8.

4. Discussion

Though many candidate HSV vaccines are in pipeline and are undergoing various phases of clinical trials, there is still no commercial vaccine available. Most of the vaccines developed so far are based on the conventional vaccinology approaches; there is scarcity of literature available for identifying immunodominant epitopes using immunoinformatics approach. Since the peptide based vaccines use only a minute amount of antigenic component of the microbes which could elicit protective immune response in the host, such a technology has a great potential in future (Skwarczynski and Toth, 2016). However the major challenge in the post genomic era of vaccine development, is to locate or identify the most immunogenic regions in the target protein that can stimulate different arms of the immune responses (Dhanda et al., 2016). In order to screen out potential epitopes from a peptide sequence, a recent advances in the field of immunology combined with computational methods has led to the development of immunoinformatics methods (Dhanda et al., 2016). This approach of utilizing peptide based vaccines has provided promising results against influenza virus (Tan et al., 2011), human papilloma virus (Kim et al., 2014), and also in developing vaccines against common allergens (Kwon et al., 2005).

The aim of the study was to identify broadly reactive T cell and B cell epitopes targeting major glycoproteins of HSV-1 and HSV-2. Vaccines targeting T-cell epitopes are considered to be more promising as they are capable of evoking a long-lasting immune response. In addition, the antigen could escape the memory response generated by antibody due to antigenic drift. It is a well-known fact that the identification of HLA Class II binding epitopes is necessary (although not sufficient) for developing protective immunity, as in response to exogenously administered proteins, the CD4 cell responses are mediated by HLA Class II molecules. Recently Dhanda et al. (2017), have discussed the importance of the promiscuous epitopes as they have the ability to interact with multiple allelic forms of HLAs. As the HLA alleles are highly polymorphic in human population, the identification of epitopes which may have the affinity towards multiple HLA alleles is of utmost importance. Brusic and colleagues evaluated the prediction accuracy of the HLA Class II peptide identification servers in terms of area under the receiver operating characteristic curve (AROC) and identified NETMHCIIPAN server (AROC > 0.9) as the best predictor followed by PROPRED, IEDB, and MULTIPRED servers (Lin et al., 2008; Nielsen et al., 2008; Singh and Raghava, 2001; Peters et al., 2005; Zhang et al., 2005). In the present study, HLA Class II T cell epitopes were predicted using NetMHCIIpan3.1 and IEDB tools thus supporting our study. The criteria for selecting the HLA Class II T cell epitopes in the present study was that it should have least IC_{50} value, should be conserved, promiscuous, surface accessibility and should not be present in the glycosylation sites. In addition, the HLA alleles targeted in the present study for epitope identification covers > 90% of the worldwide population, thus supporting the promising nature of the identified epitopes. Several epitopes with strong binding affinity with MHC molecules have been proposed from different proteins (Adorini et al., 1988; Moudgil et al., 1996). The abundance of the peptide to be presented by antigen presenting cell (APC) to T cells can be determined directly by evaluating the binding affinity of the peptide-MHC complex, the higher the affinity, more likelihood of immunodominant nature of specific peptide (Adorini et al., 1988; Nelson et al., 1992; Ma et al., 1999). Therefore in the present study, only those epitopes were selected which showed strong affinity (IC ≤ 100 nM) towards HLA molecules. On narrowing down the prediction method based on all these criteria, a total of 17 HLA Class II T cell epitopes were predicted. The gB region was predicted to have maximum number of T cell and B cell epitopes followed by gH, gD and gL. In the present study, we have also identified some

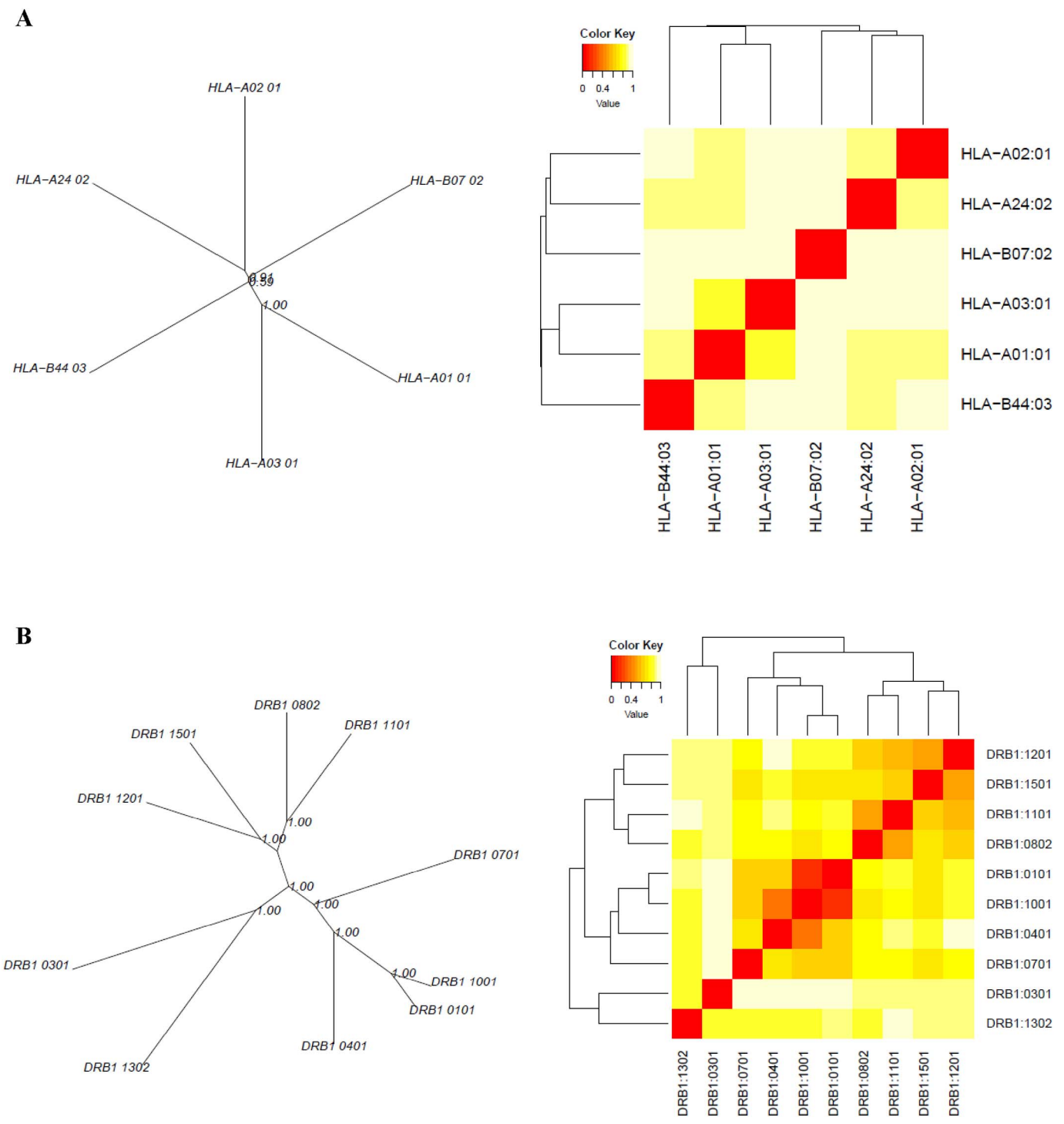

Fig. 6. Heat map of MHC cluster and molecular docking analysis. (A) MHC Cluster analysis of HLA Class I alleles included in the study. (B) MHC Cluster analysis of HLA Class II alleles included in the study. (C) Docking energies (in kcal/mol) of the predicted T cell epitopes with different HLA Class II alleles.

linear and discontinuous B cell epitopes which could be utilized in vaccine development and could elicit a humoral immune response in host.

Recently, Sheikh et al. (2016), predicted the CD8+ and CD4+ T cell epitopes against influenza virus using an *in-silico* approach. Alam et al., 2016, have also predicted some promising T cell epitopes against Zika virus using an *in-silico* strategy. These researches indicate the potential of this technology in the field of vaccinology. Khan et al. (2014) have experimentally validated their *in-silico* findings of predicted HLA Class I and Class II T cell epitopes targeting secretory protein (Ag85B) of *Mycobacterium tuberculosis*. These authors have selected the epitopes with affinity (IC_{50} < 500 nm), however, in the present study, only

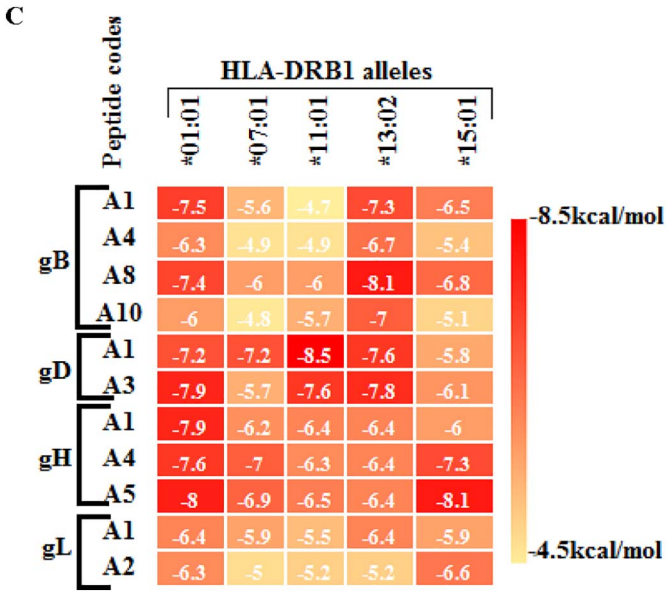

Fig. 6. (*continued*)

those epitopes showing affinity ≤100 nm have been selected, thus making our results more stringent.

In addition, 9 linear B cell epitopes were also predicted and further the antigenicity of each peptide was also predicted, as such peptides are sometimes considered to be weak immunogens. Most of the peptides showed an antigenicity score above the threshold value 0.4 which is used as a cut off value for antigenicity prediction by VaxiJen v2.0 server. This server has already been utilized by several researchers for antigenicity determination of their predicted epitopes against number of pathogens like Zika Virus (Alam et al., 2016), Bronchitis Virus (Bande et al., 2016), influenza virus (Sheikh et al., 2016). Recent advances in molecular modeling tools, the post-docking interactions between peptide-MHC complexes can be analyzed which may further help in selection of potential candidates for peptide based vaccines (Mirza et al., 2016). In the present study the binding pattern of the predicted epitopes with HLA alleles was investigated by molecular docking analysis using Autodock 4.0 tool. Almost all the peptides showed strong binding affinities to the HLA alleles with relevant hydrogen bonding.

5. Conclusion

As the burden of HSV related infections is very high throughout the world, a vaccine is need of the hour. To the best of our literature search, this is the first study to carry out identification of immunodominant peptides which are conserved in both HSV-1 and 2 using an immunoinformatics approach. Overall our analysis proposed four most promising regions which may be useful in developing a peptide pool based vaccine as the authors believe that these peptides can be potential vaccine candidates owing to their promiscuousity towards different HLAs (both HLA Class I and Class II), B cell epitope region and are also highly conserved in both HSV 1 and HSV 2. The authors further propose that in support of the present *in-silico* findings, the peptides should further be tested for their immunogenicity in laboratory setup.

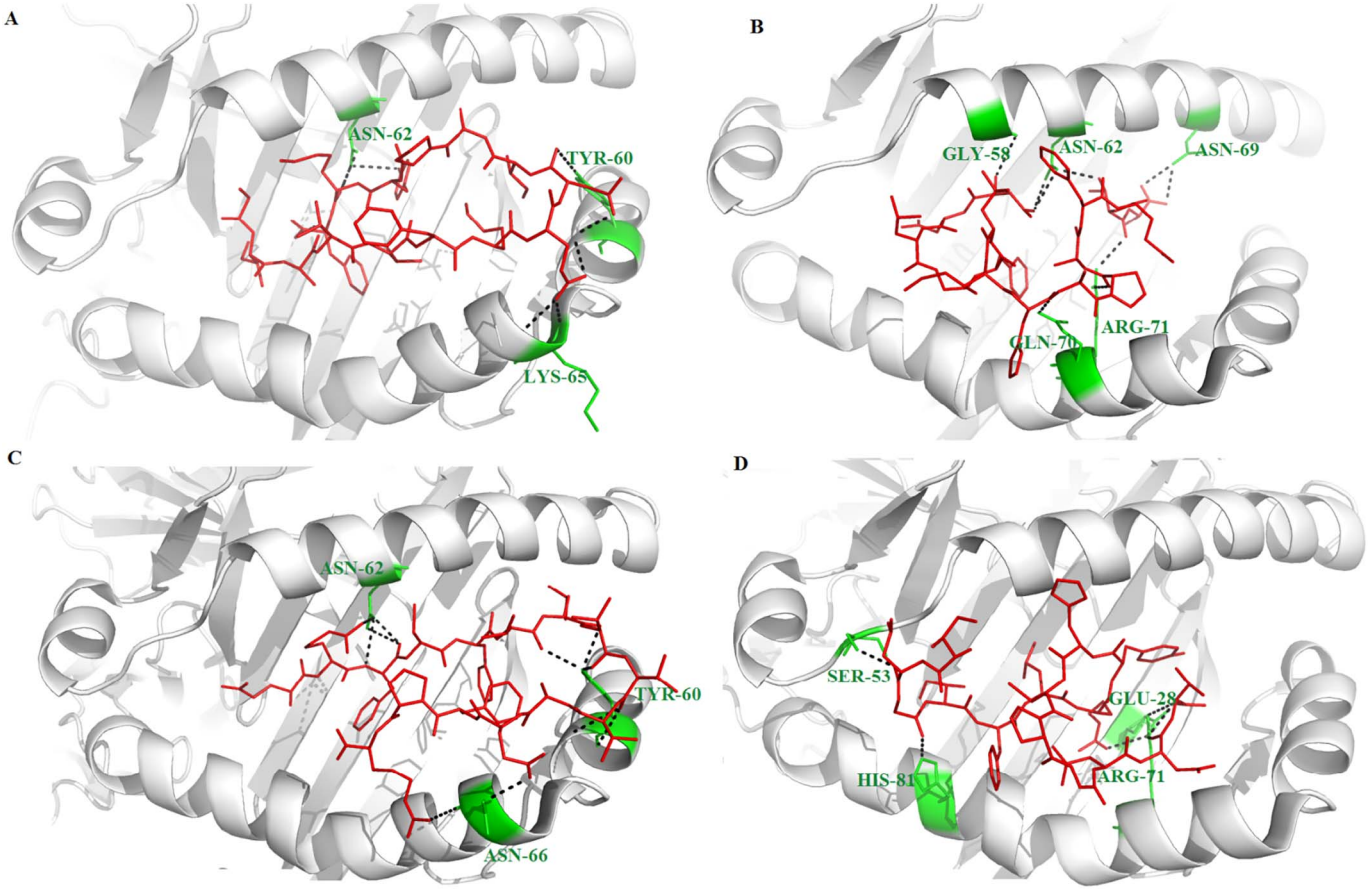

Fig. 7. Binding patterns of epitopes with HLA DRB1*0101 allele. A- glycoprotein B peptide A1. B- glycoprotein D peptide A3. C- glycoprotein H peptide A5. D- glycoprotein L peptide A1.

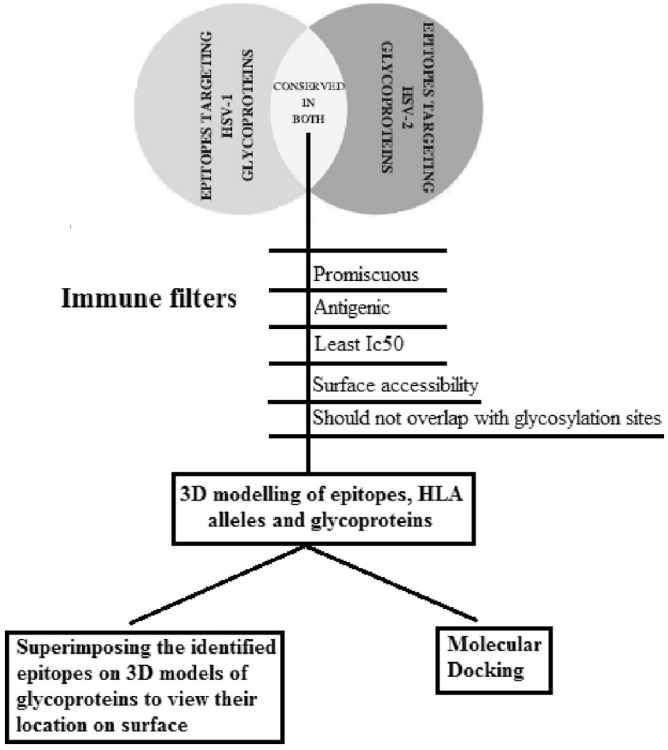

Fig. 8. Overall pipeline of the present study.

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Varun Chauhan carried out the immunoinformatics analysis and wrote the article.

Kapil Goyal provided the computer facility and various softwares required for carrying out the analysis.

Mini P Singh designed the present study and helped in writing and reviewing the article.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2018.03.004.

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