Structural and Epitope Analysis (T- and B-Cell Epitopes) of Hepatitis C Virus (HCV) Glycoproteins: An *in silico* Approach

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Background: Chronic infection with Hepatitis C Virus (HCV) poses a major risk for liver disease like cirrhosis, liver failure and hepatocellular carcinoma. In terms of percentage, the prevalence of HCV in India was found to be low to moderate (1–1.5%), but in terms of sheer numbers, India has a significant number of global HCV patients. Presently, HCV can be treated with direct acting-antibody drugs but there is no prophylactic or therapeutic vaccine available against it. In HCV infection, T- and B-cell immunity is important for clearing the virus. In the present study immunoinformatics was used to identify potent vaccine target for HCV vaccine development. **Methods:** Sequence of HCV was retrieved from NCBI and their structural analysis was done by using Protpram, PSIPRED, iTASSER and PDBsum servers. T-cell and B-cell epitopes were predicted by Immune Epitope Database and ACBPRED servers. **Results:** On epitope prediction, 25 and 55 potent MHC-I epitopes and 7 and 13 potent B-cell epitopes were predicted for E1 and E2 protein respectively. Their antigenicity score was also calculated. The most potent MHC-I epitopes were MMMNWSAV and MAWDMMMNW for HLA-A*02:01 and HLA-B*53:01 and most potent B-cell epitope was TGHMAWDMMMNWSA for E1 protein. For E2, four MHC-I epitopes having the lowest binding energy and most potent B-cell epitope was DRPYCWHYAPPCDTI. **Conclusion:** In the present study, most potent epitopes for HCV was determined on the basis of their antigenicity along with 3D modeling and docking. Identified B- and T-cell epitopes can be used for the development of potent vaccine against most prevalent HCV type in India to limit its infection. (J CLIN EXP HEPATOL 2018;8:352–361)

Infection with Hepatitis C Virus (HCV) is one of the major health problems globally. An estimated 170 million persons worldwide are infected with HCV which infects hepatocytes and different cells, including leukocytes and epithelial cells of different organs.¹ HCV has also been associated with Hepatocellular Carcinoma (HCC) and other chronic liver disease, extrahepatic abnormalities, including mixed cryoglobulinemia, malignant lymphoma and oral lichen planus.² Epidemiological evidence has indicated that persistent infection with HCV is a major risk for the development of HCC.³ HCC is the fifth most common cancer and a major cause of death in patients with chronic HCV infection, and responsible for approximately one million deaths each year. Genetic heterogeneity of HCV genotypes and varying

immunogenicity responses against these genotypes in the different human population are impending obstacle in the development of generalized vaccine design.⁴

HCVs are small, enveloped, positive, single stranded RNA viruses belonging to family *Flaviviridae* having genome size of 9.646 kb with Untranslated RNA Segments (UTRs) at both ends and a single large Open Reading Frame (ORF) encoding a polyprotein of ~3100 amino acids that is cleaved into 10 mature proteins having four structural and six non-structural (NS) proteins, including 3 structural proteins (C or core, E1 and E2), a small protein, p7, whose function has not yet been definitively defined, 6 NS proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B).⁵ The genome is surrounded by a capsid composed of the viral core protein which directly interacts with a number of cellular proteins and pathways that may be important in the viral lifecycle.⁶

Presently, HCV is treated by the pegylated Interferon (IFN) alpha, either separately or in combination with ribavirin but IFN treatment efficacy depends on various factors allied to viral genotype and status of patient's health.^{7,8} IFN treatment was shown to be non-responsive in 30–50% of HCV cases and shown serious adverse effects with treatments.⁹ Recent available Direct-Acting Antiviral (DAA) are very effective but they are not widely used due to

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Abbreviations: CHO: Chinese Hamster Ovary (CHO); DAA: Direct Acting-Antiviral; HCC: Hepatocellular Carcinoma; HCV: Hepatitis C Virus
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their higher cost.¹⁰ Furthermore, DAA treatments do not offer protection from HCV re-infection or aid as prophylaxis among high-risk individuals for incident infection. Thus, an effective vaccine to prevent HCV re-infection would still provide a significant benefit to the overall treatment of HCV infection.¹¹

Both E1 and E2, involved in the host-viral interaction, found as a potential target for the development of HCV vaccine. The antibodies directed against these proteins act to neutralize HCV.¹² The NS protein found to be prevalent in both chronic and acute patients. Immunization against these proteins showed strong and broad cellular-mediated immune responses and have most important for viral clearance. E1 and E2 glycoproteins complex expressed in Chinese Hamster Ovary (CHO) cell line and used as a vaccine candidate showed broad cross-genotype nAbs (neutralizing antibodies) in humans. A Phase 1 clinical trial in which glycosylated envelope proteins used for immunization showed potent nAbs and CD 4+ T-cell responses.^{13,14}

Now-a-days, some HCV nAbs with potent cross-genotype neutralizing activity have been identified. The epitopes in these nAbs were present in N terminal of E2 [comprising Amino Acids (aa) 412–453 and 502–535] and were mostly mapped to the “broadly neutralizing face”.^{15,16} The E2-CD81 interaction region was also thought to be within this domain. The HLA class I restricted CD8+ T lymphocytes has been isolated from liver biopsies of the chronic hepatitis C patients. These isolated epitopes were found to be localized in NS2, core protein and also in E1 and E2. The location of the epitopes in E1 (235–242) and E2 (569–578; 489–496).^{17,18}

The protection of viral infection and re-infection of the HCV can be done by providing nAbs and virus-specific T cell immunity, respectively. Therefore, the present study focused on the identification of epitopes with strong and broad B and T cell immune responses, which can be used for HCV vaccine development.

RESULTS

Sequence and Secondary Structure Analysis of E1 and E2 Protein

HCV E1 protein constitutes of 191 amino acid having molecular weight of 20,772.1 Da and extinction coefficient was 43,930 M⁻¹ cm⁻¹. On analysis of amino acid composition; Val (V) was most frequent amino acid (11.50%) whereas Lys (K) as least frequent amino acid (1%) (Supplementary Table S1). The theoretical pI was 6.71, which indicate that protein is negatively charged as its pI was less than 7. The computed Instability Index (II) was found to be 11.31 according to which it was classified as a stable protein. HCV E2 protein constitutes of 349 amino acid having molecular weight of 41,004.8 Da

and extinction coefficient was 87,985 M⁻¹ cm⁻¹. On analysis of amino acid composition; Leu (L) and Gly (G) were most frequent amino acid (8.40% and 8.40%), whereas Met (M) as least frequent amino acid (1.60%) (Supplementary Table S2). The theoretical pI was 7.85, which indicate that protein is positively charged as its pI was more than 7. The computed II was found to be 11.31 according to which it was classified as a stable protein.

PSIPRED analysis for the secondary structure prediction showed that E1 protein constitute of 45.8% helices (H), 10.9% beta sheet (E), 43.2% coils (C) (Figure 1A); where as in HCV E2 protein 15.1% helices (H), 12.4% beta sheet (E), 72.5% coils (C) (Figure 1B). Three dimensional structure prediction was done by iTASSER server showed that HCV E1 and E2 protein model 4 and model 3 was best fitting to structural parameter with the Ramachandran plot showing most of the residues ~93.9% and ~93.9%, respectively, were in favored region which is highest in comparison to other predicted structures. The predicted model with Ramachandran plot analysis for protein E1 and E2 were shown in Figure 2.

Epitope Prediction

T-Cell Epitopes: MHC class I alleles for the selected HLA allele reference set were predicted using Immune Epitope Database (IEDB) server. The potent epitopes having percentile values less than 0.5 have been considered. A total of 25 potent epitopes for E1 (Table 1) and 55 potent MHC-I binders were predicted for E2 protein (Table 2). In E1 protein, epitope ^{130–138}MMMNWSPAV and ^{126–134}MAWDMMMNW have the lowest percentile value (0.1) for HLA-A HLA-A*02:01 and HLA-B*53:01 respectively. For E2 protein, epitope ^{324–332}YLYGVGAGV, ^{74–83}SSCKPLTFFK, ^{241–250}YPCSVNFTLF, ^{339–348}WEI-VILVFL and ^{241–250}YPCSVNFTLF showed the lowest percentile value (0.15) for HLA-A*02:03, HLA-A*11:01, HLA-B*35:01, HLA-B*40:01 and HLA-B*53:01, respectively.

B-Cell Epitopes: B-cell epitopes play a significant role in viral immunotherapy and vaccine development. B-cell epitopes were predicted using ABCpred server at default parameters using prediction of sixteen (16) amino acid long B-Cell epitopes with 65.93% accuracy. These predicted epitopes were analyzed using VaxiJen v2.0 for obtaining their antigenicity score for the identification of potent B-cell epitope. For E1 protein, 13 B-cell epitopes were predicted and out of these predicted epitopes, seven showed good antigenicity (higher than threshold > 0.6) which means they were potent antigenic b-cell epitopes (Table 3). Most potent epitope for E1 protein was ^{162–177}GVLGLAYYSMQGNWA having binding score 0.86 and antigenicity score 0.9162. For E2 protein, 39 B-cell epitopes were predicted and out of these 13 have shown good antigenicity. The most potent epitope was

Figure 1 PSIPRED graphical result from secondary structure prediction of E6 gene ORF, (A) E1 protein; (B) E2 protein.

100–116DRPYCWHYAPRPCDTI with binding score 0.94 and antigenicity score 1.1608 (Table 4). Binding of the E1 and E2 B-cell potential epitopes with the 1JRH antibody were done by docking of the potent epitope having antigenicity score more than 0.6 to show their binding (Figures 3A and B).

DISCUSSION

According to recent epidemiological study, about 3% of world population is chronically infected with chronic HCV.¹⁹ HCV causes about 28% of liver cirrhosis and about 26% of liver cancer cases, which leads to 500,000 deaths annually.²⁰ Low-resources countries like India, East Asia, North Africa, the Middle East contributes to 80% of the global burden of HCV.²¹ India accounts major share of HCV infected people (~12–18 million) but the overall prevalence of HPV is low to moderate (1–1.5%) due to higher population size.

HCV can be treated with the use of IFN- α , telaprevir, boceprevir, and sofosbuvir like DAAs therapies, but

mostly they are unaffordable to low-resources countries like India due to poor health insurance coverage and are also expensive therapy which cannot be provided with public healthcare. Therefore, a cost effective antiviral therapies or a potent epitope based HCV vaccine is utmost needed for India and other low resources countries. The knowledge of antigenic or epitopic sites of viral protein is important for the development of effective antiviral inhibitors or vaccine. For effective vaccine, both T- and B-cells epitopes identification is very important for prevention or clearance of infection.²² Now-a-days, immunoinformatics gives an effective methods for identification of effective T-cell and B-cell epitope by predicting their binding energy and antigenicity score. Previously our group identified potent epitopes for HPV E6 and E5 proteins^{23,24} as well as we also validated our prediction results in vivo using HPV L1 as a DNA vaccine target.²⁵ Presently, two HCV vaccines, one recombinant envelope glycoproteins E1 and E2 developed by Chiron (now Novartis) and other NS proteins from recombinant viruses to induce CD8+ T cell immunity, are under clinical use.^{26,27} In present study, we

Figure 2 Structural analysis of HCV protein (A) three dimensional structure of E1; (B) Ramachandran plot analysis of E1; (C) three dimensional structure of E2; (D) Ramachandran plot analysis of E2.

performed immunoinformatics analysis for the identification of potent epitopes using E1 and E2 proteins for development of effective vaccine against HCV.

Using IEDB, MHC-I epitopes of E1 and E2 glycoprotein were predicted for improved understanding for effective immunotherapy development (T cell-based therapeutic strategies) for chronic viral hepatitis infection. Dysfunction of T cell immune response plays a very important role in persistent HCV infection and also impact disease progression.²⁸ Cellular immune (T cell) response is important for the any viral clearance (like HCV) from the patients. But in many HCV patients do not developed a robust immune response against it, which may be due to deficiency of combination of factors. In HCV cases, 8–12 weeks delayed in HCV specific T-cells immune response has been observed in the liver after initial infection, usually T cells become detectable within 4–5 days after a viral infection.²⁹ In present study, we identified 25 and 55 potent epitopes for E1 and E2 protein, respectively. Previously, a study from Pakistan showed MNWTPAVGM as a potential MHC-I epitope,³⁰ whereas in our study most potent epitope was _{130–138}MMMNWSPAV and _{126–134}MAWDDMMNW having lowest percentile score.

Our results also showed that the region 130–150 amino acid stretch showed maximum of the potent predicted MHC-I epitopes (Table 1). These results indicate the importance of this region for the development of effective E1 based vaccine. According to recent study, this region in E1 protein of HCV 3 genotypes has lowest possibility for the positive selective pressure for individual HCV genotypes.³¹ For E2 protein, _{324–332}YLYGVGAGV was predicted as a good binder with percentile rank 0.15, whereas a study from Pakistan showed two potent epitope FFNQGWGPL and TPSPVVVGT having good antigenicity score. Both these predicted epitopes were not observed in the present study. This may be due to the different HCV genotype prevalent in India and Pakistan.³⁰

The multi-epitope based vaccine is essential for the development of the good immune response. Recently, Czarnota et al., used the HCV E2 region (amino acid 412–425) inserted into the hydrophilic loop of sHBsAg for a proposed bivalent vaccine candidate on novel chimeric particle.³²

To our knowledge, in India no study has been done so far for the identification of the potent epitope for HCV. Zhang et al., shows the two important B cell epitope in

Table 1 Predicted Epitope for MHC-I Alleles of HCV E1 Protein.

Allele	Start	End	Length	Peptide	Percentile_rank
HLA-A*02:01	130	138	9	MMMNWSPAV	0.1
HLA-B*53:01	126	134	9	MAWDMMMNW	0.1
HLA-A*02:06	130	138	9	MMMNWSPAV	0.15
HLA-A*02:03	130	138	9	MMMNWSPAV	0.2
HLA-B*51:01	148	156	9	LPQTLFDII	0.2
HLA-B*57:01	126	134	9	MAWDMMMNW	0.2
HLA-B*58:01	126	134	9	MAWDMMMNW	0.2
HLA-B*58:01	125	134	10	RMAWDMMMNW	0.2
HLA-A*24:02	57	66	10	RYVGATTASI	0.25
HLA-A*68:01	97	105	9	QAFTIRPRR	0.3
HLA-A*68:02	62	70	9	TTASIRKHV	0.3
HLA-B*35:01	117	126	10	YPGHLTGHRM	0.3
HLA-B*08:01	63	72	10	TASIRKHVDL	0.3
HLA-B*35:01	84	93	10	YVGDMCGAVF	0.3
HLA-A*02:03	151	159	9	TLFDIIAGA	0.35
HLA-A*02:01	93	101	9	FLVGQAFTI	0.4
HLA-A*32:01	130	138	9	MMMNWSPAV	0.4
HLA-B*07:02	117	126	10	YPGHLTGHRM	0.4
HLA-B*58:01	167	176	10	LAYYSMQGNW	0.45
HLA-A*23:01	57	66	10	RYVGATTASI	0.45
HLA-B*53:01	117	126	10	YPGHLTGHRM	0.45
HLA-B*53:01	167	176	10	LAYYSMQGNW	0.45
HLA-A*02:01	151	159	9	TLFDIIAGA	0.5
HLA-A*02:06	180	189	10	VIIMVMFSGV	0.5
HLA-A*30:02	76	84	9	AATMCSALY	0.5

HCV E2 protein located at region 412–426 and 434–446 but in the present study these epitopes were not predicted in our study, which may be due to the larger amino acid stretch has been used in our study, i.e. 16 amino acid.³³ In E1 protein, the most potent epitope was AFTIRPRRHQTVQTCN having the highest score (0.87) and good antigenic score (0.9016). On the basis of our docking studies, epitope TGHMAWDMMMNWSPA has the highest global binding energy than other predicted epitopes also showed good binding energy (Figure 3A). In E2 protein, DRPYCWHYAPRPCDTI B-cell epitope has the highest score and the good antigenicity score which can be used for effective epitope based vaccine (Figure 3B). A mapping of the HCV epitope of HCV glycoprotein was done by a previous study,³⁴ but study would also help in the identification of cost effective epitope target for neutralizing antibodies. Identified B-cell epitope can be utilized in the development of the effective HCV vaccine because it is a vital step for development of epitope-based vaccines and diagnostic tools.

In conclusion, so far no vaccine against HCV has been developed and it is now utmost needed for the low-

resources countries like India. The present study identifies the good predicted MHC-I binders as well as good B-cell epitopes. These predicted epitopes can be used as potential targets for the development of the effective vaccine against HCV serotype 3a and these strategies can also be used for other HCV types.

METHODS

Amino Acid Sequence: HCV 3 serotypes genome id AFH74070.1 were retrieved from NCBI databank for E1 and E2 protein sequence.

Sequence Analysis and Secondary Structure Prediction: The various physical and chemical parameters of protein sequences was analyzed by Protparam (<http://web.expasy.org/protparam/>) to compute amino acid composition, molecular weights and percentage of strongly basic, acidic, hydrophobic and polar amino acids. The secondary structure was predicted by PSIPRED, online server (<http://bioinf.cs.ucl.ac.uk/psipred/>).³⁵

T-Cell Epitopes Prediction: MHC-I and MHC-II epitopes for retrieved protein sequence were predicted by using

Table 2 Predicted Epitope for MHC-I Alleles of HCV E2 Protein.

Allele	Start	End	Length	Peptide	Percentile_rank
HLA-A*02:03	324	332	9	YLYGVGAGV	0.15
HLA-A*11:01	74	83	10	SSCKPLTFFK	0.15
HLA-B*35:01	241	250	10	YPCSVNFTLF	0.15
HLA-B*40:01	339	348	10	WEIVLVFLL	0.15
HLA-B*53:01	241	250	10	YPCSVNFTLF	0.15
HLA-A*02:03	324	333	10	YLYGVGAGVV	0.2
HLA-A*11:01	243	251	9	CSVNFTLFK	0.2
HLA-A*30:01	251	259	9	KVRMFVAGV	0.2
HLA-A*31:01	262	271	10	RFHAACNWTR	0.2
HLA-A*33:01	80	88	9	TFFKQGWGR	0.2
HLA-A*33:01	153	161	9	DVFLLESRL	0.2
HLA-A*68:01	153	161	9	DVFLLESRL	0.2
HLA-A*68:01	54	63	10	FIAGLFYYHK	0.2
HLA-A*68:01	79	88	10	LTFFKQGWGR	0.2
HLA-B*35:01	227	236	10	TPRCMVDYPY	0.2
HLA-B*40:01	148	156	9	GENETDVFL	0.2
HLA-B*40:01	339	347	9	WEIVLVFL	0.2
HLA-B*51:01	305	313	9	MPALSTGLI	0.2
HLA-A*24:02	240	249	10	HYPCSVNFTL	0.25
HLA-A*30:01	63	72	10	KFNSTGCPQK	0.25
HLA-A*30:02	117	125	9	ALNVCGPVY	0.25
HLA-A*33:01	201	210	10	DLFCPTDCFR	0.25
HLA-B*40:01	148	157	10	GENETDVFL	0.25
HLA-B*53:01	96	105	10	GPSEDRPYCW	0.25
HLA-A*01:01	136	145	10	TTDRKGAPTY	0.3
HLA-A*68:01	3	11	9	YTTGGAAR	0.3
HLA-B*07:02	305	313	9	MPALSTGLI	0.3
HLA-B*44:02	350	359	10	ADARVCVAFW	0.3
HLA-B*44:03	293	302	10	TELAILPCSF	0.3
HLA-B*51:01	356	364	9	VAFWLMLMI	0.3
HLA-B*53:01	230	239	10	CMVDYPYRLW	0.3
HLA-A*01:01	51	60	10	NTGFIAGLFY	0.35
HLA-A*03:01	243	251	9	CSVNFTLFK	0.35
HLA-A*11:01	54	63	10	FIAGLFYYHK	0.35
HLA-A*23:01	238	247	10	LWHYPCSVNF	0.35
HLA-A*68:01	201	210	10	DLFCPTDCFR	0.35
HLA-B*07:02	303	312	10	VPMPALSTGL	0.35
HLA-B*35:01	305	314	10	MPALSTGLIH	0.35
HLA-B*44:02	195	203	9	VENNESDLF	0.35
HLA-B*44:02	98	107	10	SEDRPYCWHY	0.35
HLA-B*44:03	98	107	10	SEDRPYCWHY	0.35
HLA-B*58:01	326	335	10	YGVGAGVVGW	0.35
HLA-A*02:01	230	238	9	CMVDYPYRL	0.4
HLA-A*02:01	237	245	9	RLWHYPCSV	0.4
HLA-A*02:01	324	332	9	YLYGVGAGV	0.4

(Continued on next page)

Table 2 (Continued)

Allele	Start	End	Length	Peptide	Percentile_rank
HLA-A*02:01	346	354	9	FLLLADARV	0.4
HLA-A*24:02	238	247	10	LWHYPCSVNF	0.4
HLA-A*33:01	2	11	10	TYTTGGAAAR	0.4
HLA-B*44:02	293	302	10	TELAILPCSF	0.4
HLA-A*31:01	253	262	10	RMFVAGVEHR	0.45
HLA-B*58:01	330	339	10	AGVVGWALKW	0.45
HLA-A*02:03	359	367	9	WLMLMISQA	0.45
HLA-A*23:01	338	346	9	KWEIVILVF	0.45
HLA-A*33:01	102	110	9	PYCWHYAPR	0.45
HLA-A*33:01	202	210	9	LFCPTDCFR	0.45

Table 3 B-Cell Epitopes with Their Antigenicity Score for HCV E1 Protein.

Rank	Sequence	Start position	Score	Antigenicity score
1	VYEADDVILHTPGCIP	21	0.89	-0.1321
2	AFTIRPRRHQTVQTCN	98	0.87	0.9016
2	CSLYPGHLTGHRMAWD	114	0.87	0.4645
3	GVLAGLAYYSMQGNWA	162	0.86	0.9162
3	VGMIVAHVLRPLQTLF	138	0.86	-0.2034
4	CWTPVTPTVAVRYVGA	46	0.84	0.9042
5	TGHRMAWDMMMNWSA	122	0.83	0.7591
5	GHLTGHRMAWDMMMNW	119	0.83	0.3814
6	TASIRKHDLLVGAAT	63	0.82	0.4985
6	AGLAYYSMQGNWAKVV	165	0.82	0.5682
6	HWGVLGAYYSMQGN	160	0.82	0.7804
6	LFDIAGAHWGVLAGL	152	0.82	0.9319
7	NGSESTCWTPVTPTVA	40	0.81	0.9424

Epitopes in bold are potent B-cell epitopes.

IEDB: analysis resource (<http://www.iedb.org/>). IEDB was supported by National Institute of Allergy and Infectious Diseases, a component of the National Institutes of Health in the Department of Health and Human Services. The epitopes for MHC class I alleles were predicted for ORF of E1 and E2 proteins, using default server parameters on the basis of the percentile rank less than 0.5 in IEDB (lower the percentile rank higher the binding affinity of the predicted epitope). The immunogenicity of the predicted epitope was calculated by IEDB T cell class I pMHC immunogenicity predictor (<http://tools.immuneepitope.org/immunogenicity/>).

B-Cell Epitopes: B-cell epitopes for HCV E1 and E2 protein was predicted by ABCpred server (<http://www.imtech.res.in/raghava/abcpred/ABCsubmission.html>), which uses artificial neural network. This server is able to predict epitopes with 65.93% accuracy using recurrent neural network.³⁶

Structure Prediction: Three dimensional structure of the HCV E1 and E2 protein were predicted by iTASSER servers, which used de novo modeling approaches as in PDB database. Structure of E1 and E2 protein were not available for homology modeling. iTASSER server predicted five models out of which the best model was selected and further structure analysis and stereo chemical properties using PROCHECK tool was done. The visualization of the model was done by using UCSF Chimera, a visualizing tool developed by the resource for bio-computing, visualization and informatics.³⁷

Molecular Docking of Epitopes: Predicted B-cell epitopes were docked with 1JRH PDB, which showed a complex of neutralizing epitopes on the extracellular IFN gamma receptor. Files of the predicted HLA alleles were retrieved from RCSB Protein Data Bank (RCSB PDB). The structure of the predicted epitopes were generated by using 3D structure modeled by using iTASSER server and epitope

Table 4 B-Cell Epitopes with Their Antigenicity Score for HCV E2 Protein.

Rank	Sequence	Start position	Score	Antigenicity score
1	DRPYCWHYAPRPCDTI	100	0.94	1.1608
2	PEATYSRCGAGPWLTP	213	0.93	0.1627
2	CDTIPALNVCGPVYCF	112	0.93	-0.8067
3	AGPWLTPRCMVDYPYR	222	0.92	0.2249
3	TDRKGAPTYTWGENE	136	0.92	0.1774
4	DANITGPSEDRPYCWH	91	0.9	0.9863
4	VKTCGAPPCNIYGGNN	179	0.9	-0.0924
5	CGAPPCNIYGGNNVEN	182	0.88	0.398
5	GCVWMNSTGFVKTCGA	169	0.88	-0.327
5	APRPCDTIPALNVCGP	108	0.88	-0.4622
6	YRLWHYPCSVNFTLFK	236	0.86	0.2442
6	TPRCMVDYPYRLWHYP	227	0.86	-0.1395
6	FRKHPEATYSRCGAGP	209	0.86	0.3482
7	PSEDRPYCWHYAPRPC	97	0.85	1.2517
7	FKQGWGRLTDANITGP	82	0.85	0.1694
7	AACNWTRGERCNIEDR	265	0.85	0.9304
7	RCMVDYPYRLWHYPCS	229	0.85	0.2636
7	SGRWFGCVWMNSTGFV	164	0.85	-0.4662
8	TGPSEDRPYCWHYAPR	95	0.84	1.2589
8	KLSSCKPLTFFKQGWG	72	0.84	0.061
8	NVENNESDLFCPTDCF	194	0.84	0.4652
8	APPCNIYGGNNVENNE	184	0.84	0.4072
9	GRLTDANITGPSEDRP	87	0.83	0.5849
9	GERCNIEDRDRSEQQP	272	0.83	1.4506
9	RFHAACNWTRGERCNI	262	0.83	0.4693
9	HRFHAACNWTRGERCN	261	0.83	0.7372
9	RCGAGPWLTPRCMVDY	219	0.83	0.061
9	VWMNSTGFVKTCGAPP	171	0.83	-0.1337
9	PSPVVVGTTDRKGAPT	129	0.83	1.537
10	PVVVGTTDRKGAPTYT	131	0.82	1.3491
11	TGFIAGLFYYHKFNST	52	0.81	0.2581
11	TELAILPCSFVMPAL	293	0.81	0.6062
11	CGAGPWLTPRCMVDYP	220	0.81	-0.0223
11	DRKGAPTYTWGENETD	138	0.81	0.2368
12	SWHINSTALNCNESIN	36	0.8	0.789
12	RGERCNIEDRDRSEQQ	271	0.8	1.303
12	RKGAPTYTWGENETDV	139	0.8	0.1448
12	VGTTDRKGAPTYTWGE	134	0.8	0.5251
12	GPVYCFTSPVVVGTT	122	0.8	1.1613
12	PALNVCGPVYCFTSP	116	0.8	-0.6584

Epitopes in bold are potent B-cell epitopes.

Figure 3 Docking of 1JRH antibody with (A) E1 potential B cell epitope; (B) E2 potential B cell epitope [only epitope having higher antigenicity score than 0.6].

structures were docked using rigid-body docking methods, PatchDock algorithm (<http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html>), and further refinement was done by using Firedock^{38,39} with 1JRH antibody.

AUTHORS' CONTRIBUTION

A.K., R.P. and I.S.Y. performed the software analysis, analyzed data and manuscript writing. M.B. analyzed experiments and data, final approval of the manuscript.

CONFLICTS OF INTEREST

The authors have none to declare.

APPENDIX A SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.jceh.2017.12.010](https://doi.org/10.1016/j.jceh.2017.12.010).

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