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Vaccinomic Approach for Multi Epitopes Vaccine from Glycoprotein D of Virulent Strains of Avian Infectious Laryngotracheitis Virus

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Abstract Avian infectious laryngotracheitis virus (ILTV) is an alphaherpesvirus that causes an economically important respiratory chicken disease. The disease mainly controlled by vaccination. However conventional vaccinations increased the spread of the virus by latency. Therefore the aim of this study was to design multi epitopes vaccine against glycoprotein D of ILTV using immunoinformatics tools. The envelope glycoprotein D sequences were retrieved from the National Center for Biotechnology Information (NCBI) and aligned using Bioedit software for conservancy. The prediction of B and T cell epitopes were performed using Immune Epitope Database (IEDB) analysis resources. Homology modeling and docking were also performed to predict the binding affinity of the predicted epitopes to the chicken alleles. B cell prediction methods proposed nineteen linear epitopes, among them twelve epitopes were on surface and eleven antigenic epitopes using Bepipred, Emini surface accessibility and kolaskar antigenicity methods, respectively. However, only seven epitopes fulfilled the B cell prediction methods. Among these seven epitopes, two epitopes namely $_{256}$*PRPDSVPQEIPAVTKK*$_{271}$ and $_{226}$ *RHADDVY* $_{232}$ were proposed as the top B cell epitopes. For T cells, three epitopes namely $_{24}$*STAAVTYDY*$_{32,}$ $_{20}$*FASQSTAAV*$_{28}$ and $_{353}$*FAAFVACAV*$_{361}$ were proposed as cytotoxic T cells (CTL) epitopes due to their great allele's linkage to MHC class I alleles. MHC class II alleles extensively interacted with multiple epitopes. The best predicted epitopes were $_{88}$*FEASVVWFY*$_{96}$, $_{212}$*FQGEHLYPI*$_{220,}$ $_{353}$*FAAFVACAV*$_{361}$ and $_{137}$*VDYVPSTLV*$_{145}$. Moreover, molecular docking revealed high binding affinity between chicken MHCI BF alleles and MHC1 docked epitopes ($_{20}$*FASQSTAAV*$_{28}$, $_{24}$*STAAVTYDY*$_{32}$ and $_{353}$*FAAFVACAV*$_{361}$) that indicated by the lower global energy scores. The *In-silico* analysis of ILTV glycoprotein D in this study suggested eight epitopes that could be a better choice as worldwide multi epitopes vaccine. These epitopes may effectively elicit both humoral and cell-mediated immunity. Furthermore in vitro and in vivo studies are required to support the effectiveness of these epitopes as vaccine candidates.

Keywords: *ILTV, epitope vaccine, IEDB, NCBI, B cells, T cells*

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1. Introduction

Infectious laryngeotracheitis (ILT) is a contagious respiratory disease of chicken caused by an enveloped virus with a double stranded DNA genome. Infectious laryngeotracheitis virus (ILTV) belongs to the family *Herpesviridae* and subfamily *alphaherpersvirinae* [1,2]. The disease is characterized by depression, conjunctivitis, sneezing, nasal exudate and, in severe cases, gasping, dyspnea and death [3,4]. The severe epizootic form of the disease may cause morbidity up to 100% and 70% mortality [5]. ILT virus possesses at least 10 envelope glycoprotein genes, including the UL27 and US6 genes, encoding glycoprotein B (gB) and glycoprotein D (gD), respectively that are highly conserved herpesvirus structural glycoproteins [6]. Glycoprotein B is essential for infectivity and is involved in membrane fusion and virus penetration. Glycoprotein D is essential for most herpesviruses and functions as a receptor for virus binding to susceptible cells [6,7]. In addition, gB and gD elicited neutralizing antibodies and cell-mediated immune responses and has been shown to be a candidate antigen for recombinant vaccines [6,7].

Vaccination is generally considered to be the most effective method of preventing infectious diseases [8]. ILTV was the first poultry pathogen controlled by

vaccination. However, it is still a major problem in areas in which dense bird populations exist [4]. Chickens are vaccinated against ILTV using attenuated strains by multiple passages either in embryonated eggs (chicken embryo origin, CEO) or in tissue culture (tissue culture origin, TCO) [4]. Although live attenuated ILTV vaccines have been used widely, the disease remains a major concern in the poultry industry because it often occurs due to the production of latent infected birds [4,5]. Peptide-based vaccines combines immunoinformatic prediction tools with laborious experimental computational validation, making it easier to identify epitopes in protein antigens that acted as a candidate vaccine [9]. These vaccines are known to produce satisfactory results [8,9,10]. Numerous computational studies addressed the safety, accuracy, feasibility and speed of these vaccines adequately [11,12].

Recently multi epitopes vaccine using immunoinformatics tools was predicted for ILTV using glycoprotein B as an immunogenic target [13]. However no studies were conducted in glycoprotein D for vaccine design against this virus. It is important to design a vaccine that works against all immunogenic proteins of ILTV using bioinformatics. In addition, this vaccine would be safe, effective and prevent birds from being carriers of the disease. Thus this study was a continuation of the prediction of an in silico vaccine from gD as a target protein against ILTV. In this study we aimed to predict effective multi epitopes vaccine against ILTV from glycoprotein D (gD) which is essential for virus binding and attachment with high safety and accuracy.

2. Material and Method

2.1. Protein Sequence Retrieval

A total of four glycoprotein D (gD) sequences from virulent strains of ILTV were retrieved from the GeneBank of National Central Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/protein/) database in September 2017 [14]. The accession numbers, date and region of collection of the retrieved strains were listed in Table 1.

Table 1. Retrieved sequences of infectious laryngotracheitis virus (ILTV) glycoprotein D with their accession numbers, date and area of collection

Accession number	year	Country
YP182405*	2005	USA
AGN482227	2009	China
AGN 48305	2012	China
AGN48385	2012	China

*reference sequence.

2.2. Identification of Conserved Regions and Molecular Evolution

Alignment of the retrieved sequences was performed using ClustalW in the BioEdit program version 7.2.5. to obtain conserved regions using multiple sequence alignment (MSA) [15]. Moreover the retrieved strains were subjected to molecular evolution to demonstrate their common ancestors using MEGA7.0.26 (7170509) [16].

2.3. B-cell Epitope Prediction

B cell epitopes were predicted from Immune Epitope Database analysis resource IEDB (http://www.iedb.org/) [17]. BepiPred from immune epitope database was used to predict linear B-cell epitopes [18]. The surface accessibility of the predicted epitopes was investigated using Emini surface accessibility prediction tools [19]. While the antigenic epitopes were predicted through kolaskar and Tongaonkar antigenicity method [20].

2.4. MHC Class I Binding Predictions

The reference sequence of glycoprotein D of ILTV was submitted to MHCI prediction tools in IEDB (http://tools.iedb.org/mhci/) to detect and analyze the binding of peptides to MHC class I molecules [17]. To complete the analysis, the human alleles were used due to lack of chicken alleles in this tool. Artificial neural network (ANN) was used as prediction method [8,21] and all peptide lengths were set as 9amino acids. The half maximal inhibitory concentration (IC50) values of the peptides binding to MHC-I molecule was calculated. Epitopes with IC50 binding affinity equal to or less than 300 nM were suggested as promising candidate epitopes.

2.5. MHC Class II Binding Predictions

Analysis of epitopes that bound to MHC class II molecules was performed by the IEDB MHCII prediction tool at (http://tools.iedb.org/mhcii/) [17]. For MHCII binding predication, human MHC class II alleles (HLA DR, HLADP and HLADQ) were used. MHC class II groove has the ability to bind to peptides with different lengths. In this study the NN-align method was used with IC50 equal to or less than 1000 nM to predict epitopes that interacted with MHC class II alleles [22].

2.6. Homology Modeling

MUSTER server (https//zhanglab.ccmb.med.umich.edu/MUSTER/) was used for modeling the 3D structure of the glycoprotein D reference sequence (YP _182405.2) [23]. The 3D modeled of BF alleles (BF2*2101, BF2*0401) was also created using Raptor X server (http://raptorx.uchicago.edu/) [24,25,26] after retrieval of protein sequences and PDB ID of chicken alleles (BF2 *2101 & BF2*0401) from the NCBI database (PDB: 4D0C, CAK54661.1 and PDB: 4D0C CAK54660.1). Chimera software 1.8 was used to display the 3D structures of the glycoprotein D reference sequence and BF alleles [27]. The 3D structures of predicted peptides were designed using PEP FOLD3 (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/) [28,29,30]. While the 3D modeled epitopes was performed using PatchDock online autodock tools; an automatic server for molecular docking (https://bioinfo3d.cs.tau.ac.il/PatchDock/) by submitting PDB of ligands and receptors after homology

modeling by Raptor X server and PEP FOLD3 [31,32]. Firedock was used to select the best models [33]. Visualization of the result was performed using the offline UCSF-Chimera visualization tool 1.8. [27].

2.7. Molecular Docking

The interaction between 3D model of BF alleles (BF2*2101, BF2*0401) and 3D model epitopes was performed by submitting them to PatchDock online autodock tools; an automatic server for molecular docking (https://bioinfo3d.cs.tau.ac.il/PatchDock/) [33]. Firedock was used to select the top models [33]. Visualization of the result was performed using UCSF-Chimera visualization tool 1.8. [27].

3. Results and Discussion

3.1. Alignment and Molecular Evolution of the Retrieved Strains

Clustal W was used to align the retrieved sequences to obtain conserved regions between the retrieved sequences. As shown in Figure (1-a) the alignment demonstrated conservancy between the sequences despite some changes in some amino acids sequences was reported. Phylogenetic tree was constructed using MEGA7.0.26 (7170509). The evolutionary divergence among each protein was analyzed. As shown in Figure 1-b. The Chines strains AGN482227 and AGN48385 clustered together and shared common ancestor. Moreover to two strains clustered with USA strain YP182405. However the Chinese strain AGN 48305 was far related to the other strains.

3.2. Prediction of B-cell Epitopes

B-lymphocytes are differentiated into antibody-secreting plasma cell and memory cells. The B cells epitopes are characterized by being hydrophilic and accessible and flexible [34]. The reference sequence of glycoprotein D was analyzed using Bepipred Linear Epitope Prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity method in the IEDB, based on the default threshold of the prediction method. As shown in Figure 2, Bepipred Linear Epitope Prediction method; the average score of mounting glycoprotein D to B cell was 0.306. In Emini surface accessibility prediction the average surface accessibility areas of the protein was scored as 1.000. The virtual threshold of antigenicity of the protein was 1.033. All values equal to or greater than the default thresholds of B cell prediction methods were suggested to be B cell binders (linear, surface or antigenic determinants). Thus based on the binding affinity to B lymphocytes, Bepipred Linear Epitope Prediction method proposed nineteen linear epitopes with different lengths. Among them twelve epitopes were predicted as surface epitopes using Emini accessibility and eleven epitopes were suggested to be antigenic. These epitopes and their scores in the B cell prediction methods were shown in Table 2. However, only seven epitopes successfully overlapped the B cell prediction methods. Two epitopes namely $_{256}$*PRPDSVPQEIPAVTKK*$_{271}$ and $_{226}$ *RHADDVY* $_{232}$ were selected as the best B cell epitopes based on their conserved length, surface accessibility and antigenicity scores. The modeling the 3D structure of the glycoprotein D reference sequence (YP _182405.2) and the position and the 3D structure of these two epitopes in the reference sequence were shown in Figure 3 a-b.

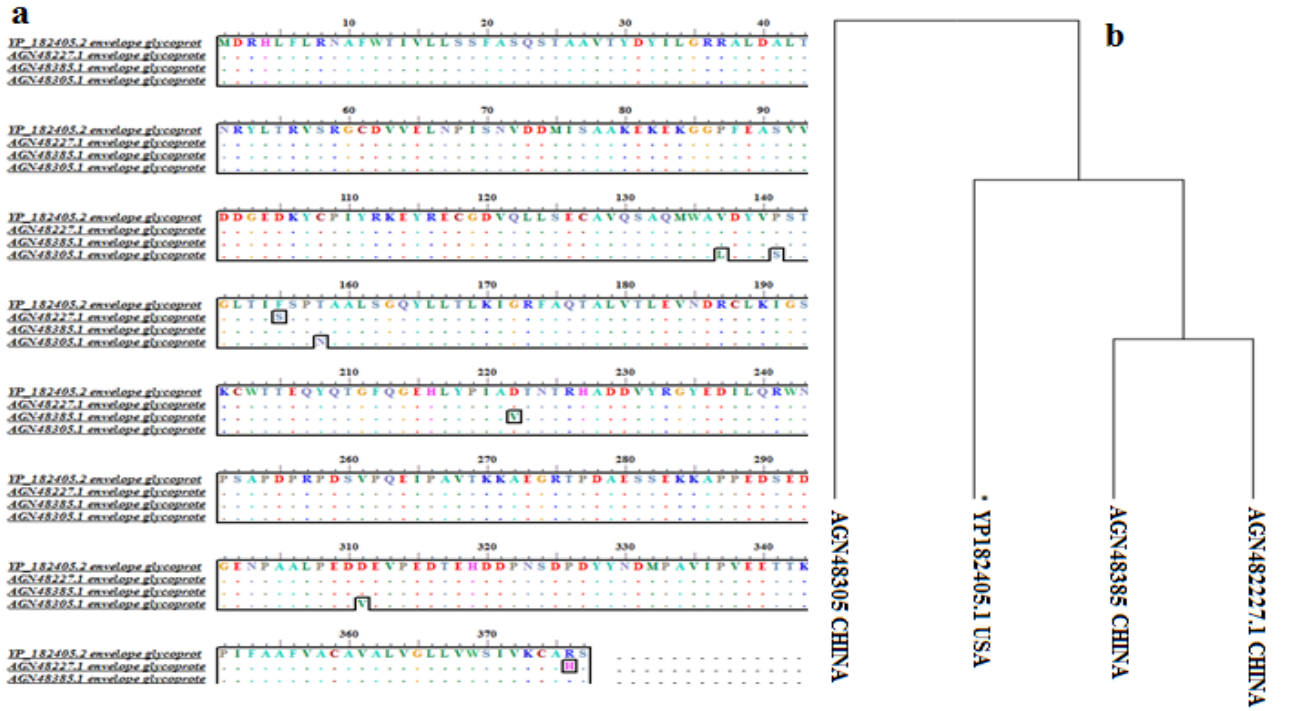

Figure 1. (**a):** Multiple sequence alignment using Bioedit software. Dots evidenced the conservancy, while letters inside square indicated the mutation or change in amino acid. (**b):** the phylogenic tree of the retrieved strains. The strains demonstrated divergence in their common ancestor

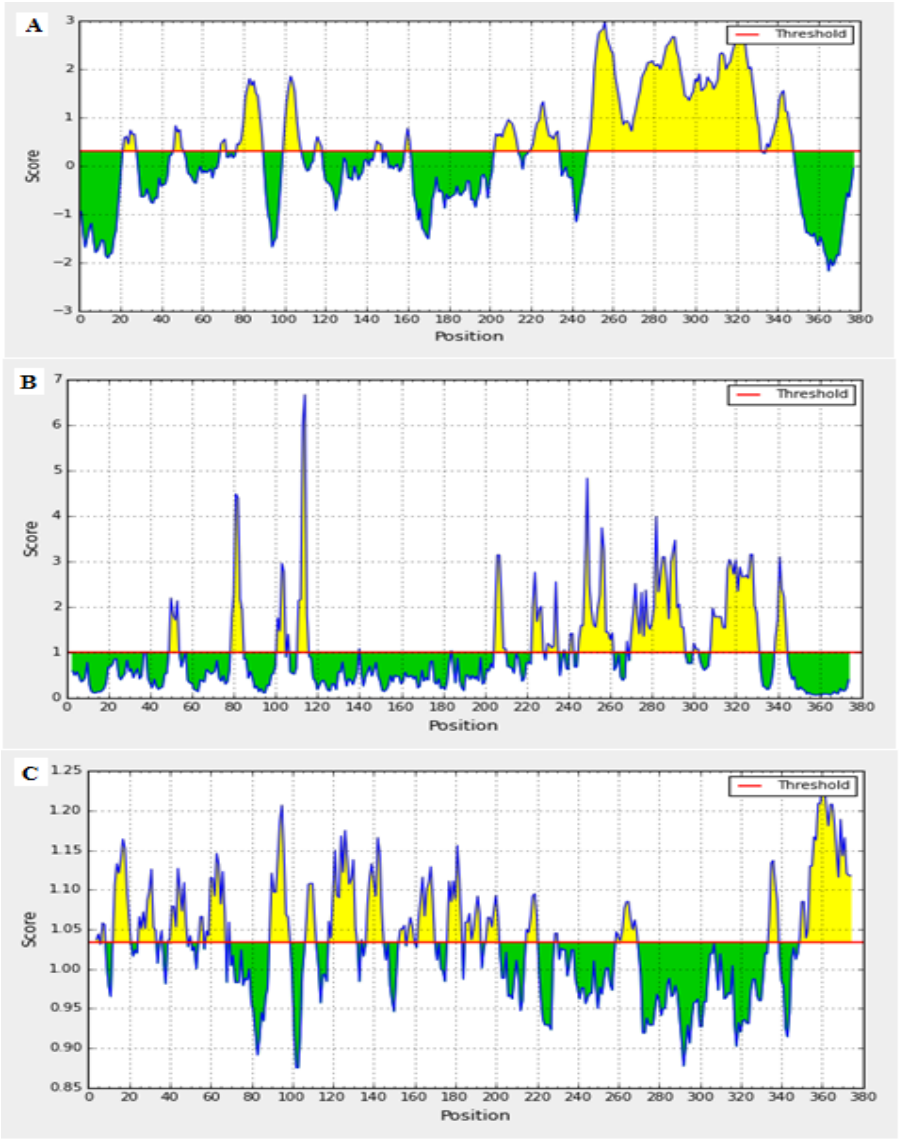

Figure 2. B-cell prediction epitopes using (a) Bepipred linear epitope method (b) Emini surface accessibility method (c) kolaskar or Tongaonkar Antigenicity method. The yellow regions above threshold (red line) are proposed to be part of B cell epitope, while green regions are not

Table 2. B cell epitopes prediction by different scales of B cell epitopes prediction methods. The start and end of peptides and their length were according to their position in the envelop protein

No	Peptide	Start	End	Length	Emni[1]	Antigenicity[2]
1	AALS	159	162	4	0.375	1.098
2	LYPIA	217	221	5	0.35	1.138
3	KEYREC	13	118	6	1.827	1.013
4	ASQSTAAV	21	28	8	0.349	1.065
5	IPAVGPYNR.	44	52	9	0.455	1.046
6	STLVSRNGA	142	150	9	0.371	1.017
7	KGDDGEDKYCP	99	109	11	1.59	0.972
8	EDKYCP*	104	109	6	1.391	1.047
9	DKYCP *	105	109	5	1.074	1.087
10	TNTRHADDVYRG	223	234	12	1.574	0.972
11	RHADDVY*	226	232	7	1.206	1.045
12	ADDVYR*	228	233	6	1.188	1.036
13	CWTTEQYQTGFQGE	202	215	14	0.505	0.984
14	PISNVDDMISAAKEKEKGGPFEA	68	90	23	0.186	0.98
15	EVPEDTEHDDPNSDPDYYNDMPAVIPVEETTKSSNAV	312	348	37	1.037	0.996
16	EVPE*	312	315	4	1.144	1.037
17	RKKNPSAPDPRPDSVPQEIPAVTKKAEGRTPDAESSEKKAPPEDSEDDMQAEASGENP AALPED	247	310	64	6.185	0.975
18	PRPDSVPQEIPAVTKK*	256	271	16	1.238	1.039
19	PRPDSVPQ*	256	263	8	2.119	1.043

[1]hypothetical threshold 1.00, [2]hypothetical threshold 1.033, [*]Peptide after being shortened.

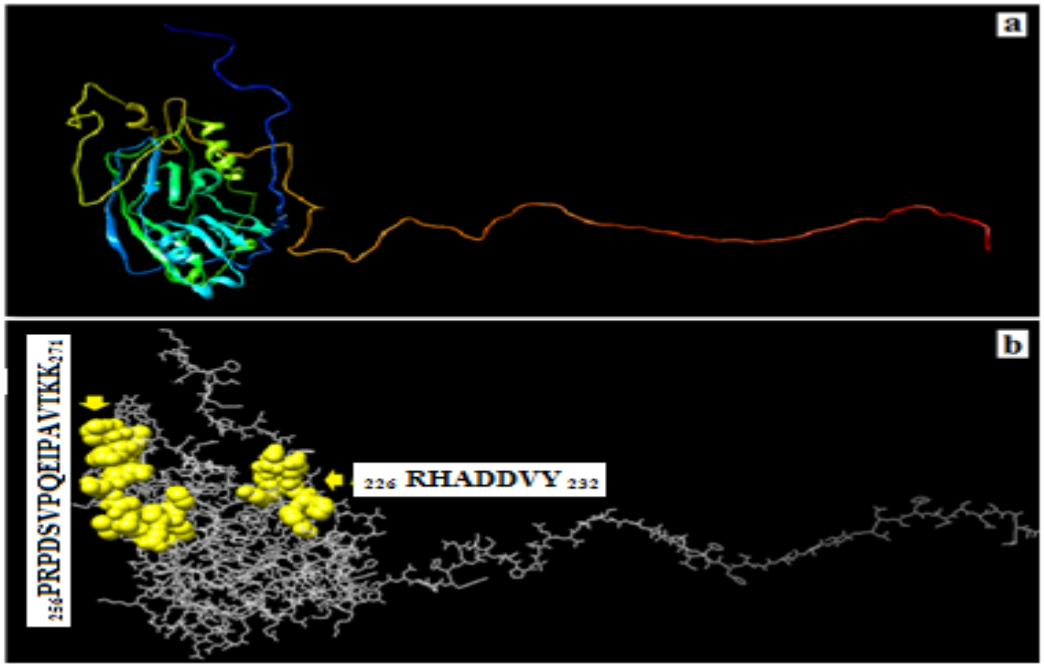

Figure 3. (a): The 3 D structure of glycoprotein D of ILTV using chimera visualization tool. **(b):** the position of proposed B-cell epitopes in the 3D structure of reference glycoprotein D of ILTV

3.3. Predicted T-cell Epitopes and Interaction with MHC1

Although MHC-I analysis tools supported epitopes prediction of many organisms, it lacked certain organisms such as chickens. However, several studies suggest some similarities between human HLA alleles and chicken MHC alleles [35]. In the present study, the reference glycoprotein D was analyzed using MHC-1 binding prediction tool in IEDB to predict T cell epitopes that interacting with different HLA MHC1alleles. The result predicted 91 CTL epitopes that interacted with MHC-I alleles. These epitopes, their positions in glycoprotein D and their interacted alleles were shown in Table 3. Three epitopes namely $_{24}STAAVTYDY_{32,}$ $_{20}FASQSTAAV_{28}$ and $_{353}FAAFVACAV_{361}$ demonstrated strong interaction with MHC-I alleles. Thus they were proposed as CTL cells epitopes. The positions of these epitopes at the 3D structural level of glycoprotein D were shown in Figure 4.

Table 3. List of epitopes and their positions that interacted with different human MHC class I alleles. IC50: Inhibitory concentration needed for binding MHC depending on the IEDB ANN method, the lower is better (# The proposed epitopes as a vaccine candidate)

Peptide	Start	End	Allele	IC50
RHLFLRNAF	3	11	HLA-A*32:01	34.98
HLFLRNAFW	4	12	HLA-B*57:01	201.25
FLRNAFWTI	6	14	HLA-A*02:01	19.04
			HLA-A*02:06	9.22
			HLA-A*32:01	290.83
			HLA-B*08:01	17.3
LRNAFWTIV	7	15	HLA-B*27:05	292.55
RNAFWTIVL	8	16	HLA-A*32:01	135.4
NAFWTIVLL	9	17	HLA-A*68:02	49.91
			HLA-B*39:01	209.97
			HLA-C*12:03	60.08
WTIVLLSSF	12	20	HLA-A*25:01	108.38
			HLA-A*26:01	175.09
			HLA-B*15:01	51.4
			HLA-B*58:01	296.56
TIVLLSSFA	13	21	HLA-A*68:02	141.26
SSFASQSTA	18	26	HLA-A*68:02	256.91
FASQSTAAV#	20	28	HLA-A*02:06	21.09
			HLA-A*68:02	9.32
			HLA-B*35:01	24.07
			HLA-B*39:01	140.61
			HLA-B*46:01	164.5
			HLA-C*03:03	4.1
			HLA-C*05:01	57.09
			HLA-C*12:03	10.07
			HLA-C*14:02	159.29

Peptide	Start	End	Allele	IC50
SQSTAAVTY	22	30	HLA-A*30:02	124.01
			HLA-B*15:01	35.17
STAAVTYDY#	24	32	HLA-A*01:01	27.91
			HLA-A*11:01	30.39
			HLA-A*26:01	234.14
			HLA-A*29:02	8.52
			HLA-A*30:02	13.71
			HLA-A*32:01	210.42
			HLA-A*68:01	85.58
			HLA-A*68:02	292.62
			HLA-B*35:01	96.17
			HLA-B*58:01	189.96
TAAVTYDYI	25	33	HLA-A*68:02	27.13
AAVTYDYIL	26	34	HLA-C*03:03	43.08
VTYDYILGR	28	36	HLA-A*03:01	97.5
			HLA-A*11:01	12.35
			HLA-A*31:01	30.04
			HLA-A*68:01	15.67
DYILGRRAL	31	39	HLA-C*14:02	50.92
RRALDALTI	36	44	HLA-B*27:05	26.06
ALDALTIPA	38	46	HLA-A*02:01	16.07
			HLA-A*02:06	32.98
LTIPAVGPY	42	50	HLA-A*26:01	15.88
			HLA-A*29:02	27.48
			HLA-A*30:02	76.55
			HLA-B*15:01	7.45
			HLA-B*35:01	30.65
			HLA-B*58:01	126.12
			HLA-C*12:03	191.93
AVGPYNRYL	46	54	HLA-C*07:01	124.02
NRYLTRVSR	51	59	HLA-B*27:05	103.1
VELNPISNV	64	72	HLA-B*40:02	265.05
NPISNVDDM	67	75	HLA-B*35:01	32.62
NVDDMISAA	71	79	HLA-A*02:06	51.86
KEKEKGGPF	80	88	HLA-B*40:02	293.04
KEKGGPFEA	82	90	HLA-B*40:02	68.61
GPFEASVVW	86	94	HLA-B*53:01	63.95
FEASVVWFY	88	96	HLA-A*29:02	123.64
			HLA-A*30:02	126.76
			HLA-A*68:01	133.58
			HLA-B*18:01	3.19
			HLA-B*35:01	144.62
			HLA-B*44:02	15.2
			HLA-B*44:03	34.6
EASVVWFYV	89	97	HLA-A*02:06	247.14
			HLA-A*68:02	3.18
ASVVWFYVI	90	98	HLA-B*58:01	222.25
			HLA-C*15:02	164.07
SVVWFYVIK	91	99	HLA-A*03:01	258.56
			HLA-A*11:01	12.29
			HLA-A*30:01	108.2
			HLA-A*31:01	146.89
			HLA-A*68:01	13.23
YCPIYRKEY	107	115	HLA-C*14:02	65.33
CPIYRKEYR	108	116	HLA-A*31:01	254.1
			HLA-A*68:01	221.58
KEYRECGDV	113	121	HLA-B*40:02	157.75
YRECGDVQL	115	123	HLA-B*39:01	30.47
			HLA-C*07:02	133.78
RECGDVQLL	116	124	HLA-B*40:01	32.99
			HLA-B*40:02	197.98
VQLLSECAV	121	129	HLA-A*02:06	24.67
CAVQSAQMW	127	135	HLA-B*53:01	62.32
			HLA-B*57:01	68.86
			HLA-B*58:01	6.82

Peptide	Start	End	Allele	IC50
VQSAQMWAV	129	137	HLA-A*02:01	31.72
VQSAQMWAV	129	137	HLA-A*02:06	3.62
SAQMWAVDY	131	139	HLA-A*29:02	134.97
			HLA-A*30:02	100.48
			HLA-B*35:01	96.18
AQMWAVDYV	132	140	HLA-A*02:01	12.18
			HLA-A*02:06	3.2
WAVDYVPST	135	143	HLA-A*02:06	263.88
AVDYVPSTL	136	144	HLA-C*03:03	238.28
			HLA-C*05:01	70.92
YVPSTLVSR	139	147	HLA-A*68:01	22.02
STLVSRNGA	142	150	HLA-A*30:01	189.18
SRNGAGLTI	146	154	HLA-B*39:01	82.29
GLTIFSPTA	151	159	HLA-A*02:01	208.71
LTIFSPTAA	152	160	HLA-A*68:02	113.93
TIFSPTAAL	153	161	HLA-A*02:06	266.47
			HLA-A*68:02	18.27
			HLA-B*39:01	131.2
			HLA-C*03:03	17.64
			HLA-C*12:03	285.65
			HLA-C*14:02	38.1
PTAALSGQY	157	165	HLA-A*01:01	106.45
			HLA-A*29:02	66.62
			HLA-A*30:02	119.53
TAALSGQYL	158	166	HLA-C*03:03	15.69
AALSGQYLL	159	167	HLA-C*03:03	32.69
LSGQYLLTL	161	169	HLA-B*58:01	280.59
SGQYLLTLK	162	170	HLA-A*11:01	124.17
GQYLLTLKI	163	171	HLA-A*02:06	201.64
			HLA-B*27:05	177.77
YLLTLKIGR	165	173	HLA-A*31:01	115.14
GRFAQTALV	172	180	HLA-B*27:05	139.37
			HLA-C*06:02	128.34
			HLA-C*07:01	87.99
FAQTALVTL	174	182	HLA-A*02:06	45.3
			HLA-B*35:01	152.3
			HLA-B*39:01	126.47
			HLA-C*03:03	9.33
			HLA-C*12:03	27.22
QTALVTLEV	176	184	HLA-A*02:06	119.29
			HLA-A*68:02	4.95
QTALVTLEV	176	184	HLA-C*15:02	137.88
EVNDRCLKI	183	191	HLA-C*15:02	282.99
FLPSKCWTT	197	205	HLA-A*02:01	23.34
			HLA-A*02:06	71.2
TGFQGEHLY	210	218	HLA-A*29:02	20.79
			HLA-A*30:02	83.47
FQGEHLYPI	212	220	HLA-A*02:06	2.12
			HLA-B*39:01	48.83
			HLA-C*12:03	93
HADDVYRGY	227	235	HLA-B*35:01	60.02
			HLA-C*05:01	26.86
			HLA-C*12:03	208.26
VYRGYEDIL	231	239	HLA-C*14:02	165.14
GYEDILQRW	234	242	HLA-A*23:01	156.43
ILQRWNNLL	238	246	HLA-A*02:01	127.17
LQRWNNLLR	239	247	HLA-A*31:01	81.34
QRWNNLLRK	240	248	HLA-B*27:05	19.45
RWNNLLRKK	241	249	HLA-A*30:01	203.86
			HLA-A*31:01	142.84
LLRKKNPSA	245	253	HLA-A*30:01	103.26
			HLA-B*08:01	14.45
RPDSVPQEI	257	265	HLA-B*07:02	221.09
SVPQEIPAV	260	268	HLA-A*02:06	26.96
			HLA-C*14:02	139.72

Peptide	Start	End	Allele	IC50
AEASGENPA	297	305	HLA-B*40:02	248.39
ASGENPAAL	299	307	HLA-C*03:03	34.97
GENPAALPE	301	309	HLA-B*40:01	143.99
DPNSDPDYY	321	329	HLA-B*35:01	37.12
			HLA-B*53:01	140.43
DYYNDMPAV	327	335	HLA-C*14:02	14.02
YYNDMPAVI	328	336	HLA-A*23:01	89.52
			HLA-A*24:02	34.31
			HLA-C*03:03	186.09
			HLA-C*07:02	85.04
			HLA-C*12:03	79.7
			HLA-C*14:02	42.73
NDMPAVIPV	330	338	HLA-A*02:06	121.18
			HLA-A*68:02	55.06
MPAVIPVEE	332	340	HLA-B*35:01	75.47
ETTKSSNAV	340	348	HLA-A*68:02	6.91
FAAFVACAV#	353	361	HLA-A*02:01	264.35
			HLA-A*02:06	12.45
			HLA-A*68:02	5.7
			HLA-B*35:01	64.72
			HLA-B*39:01	177.44
			HLA-B*51:01	297.92
			HLA-C*03:03	6.1
			HLA-C*12:03	7.44
			HLA-C*14:02	262.17
AFVACAVAL	355	363	HLA-C*14:02	101.38
FVACAVALV	356	364	HLA-A*02:06	3.94
			HLA-A*68:02	2.26
			HLA-C*12:03	69.44
AVALVGLLV	360	368	HLA-A*02:06	126.6
VALVGLLVW	361	369	HLA-B*53:01	141.86
			HLA-B*57:01	27.41
			HLA-B*58:01	3.03
LVGLLVWSI	363	371	HLA-A*32:01	199.21
GLLVWSIVK	365	373	HLA-A*03:01	59.95
			HLA-A*11:01	68.1

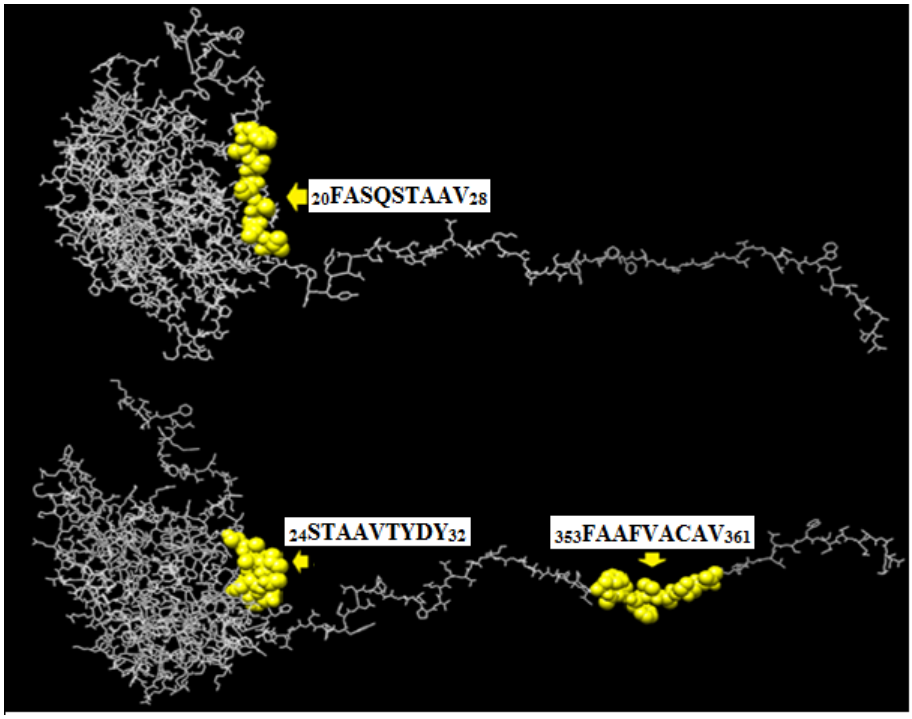

Figure 4. The position of proposed cytotoxic T cell epitopes ($_{24}$STAAVTYDY$_{32,}$ $_{20}$FASQSTAAV$_{28}$ and $_{353}$FAAFVACAV$_{361}$) of ILTV glycoprotein D suggested to be interact with MHC I alleles illustrated by UCSF-Chimera visualization tool

3.4. Predicted T-cell Epitopes and Interaction with MHC II

The activation of the T-helper subtypes and their corresponding cytokines secretion is one of the important characteristics of immune responses as they are required for almost all adaptive immune responses [36]. Peptides binding to MHC class II molecules were evaluated using human alleles. Several methods were used for analysis of MHC-II epitopes binding grooves. However the NN- align is important for instantaneous identification of the MHC class II binding core epitopes [37,38]. Several core peptides were predicted to interact with considerable number of MHCII alleles. Four epitopes namely $_{88}FEASVVWFY_{96}$, $_{212}FQGEHLYPI_{220}$, $_{353}$FAAFVACAV$_{361}$ and $_{137}VDYVPSTLV_{145}$ exhibited great binding interaction to 82, 71, 62 and 69 alleles to MHC-II, respectively. These epitopes, their positions in glycoprotein D and their interacted alleles were shown in Table 4. Also the positions of these epitopes at the 3D structural level of glycoprotein D were shown in Figure 4 and Figure 5.

Table 4. List of top four epitopes that bind with high affinity with the human MHC class II alleles. IC50: Inhibitory concentration needed for binding MHC depending on the IEDB NN-aligned method, the lower is better

Core Sequence	Peptide Sequence	Start	End	Allele	IC50
FEASVVWFY	KEKGGPFEASVVWFY	82	96	HLA-DPA1*01:03/DPB1*02:01	520.6
				HLA-DQA1*03:01/DQB1*03:02	591.8
				HLA-DQA1*05:01/DQB1*02:01	238.6
				HLA-DRB1*07:01	26.3
				HLA-DRB1*09:01	175.5
				HLA-DRB1*15:01	456.1
				HLA-DRB3*01:01	272.8
	EKGGPFEASVVWFYV	83	97	HLA-DPA1*01/DPB1*04:01	693.5
				HLA-DPA1*01:03/DPB1*02:01	230.5
				HLA-DPA1*02:01/DPB1*01:01	925.2
				HLA-DQA1*03:01/DQB1*03:02	339.3
				HLA-DQA1*05:01/DQB1*02:01	233.8
				HLA-DRB1*01:01	667.6
				HLA-DRB1*04:05	812.5
				HLA-DRB1*07:01	27
				HLA-DRB1*09:01	166.5
				HLA-DRB1*15:01	348.5
				HLA-DRB3*01:01	250.5
	KGGPFEASVVWFYVI	84	98	HLA-DPA1*01:03/DPB1*02:01	161.1
				HLA-DPA1*02:01/DPB1*01:01	375.1
				HLA-DQA1*03:01/DQB1*03:02	301.8
				HLA-DQA1*05:01/DQB1*02:01	283.7
				HLA-DQA1*05:01/DQB1*03:01	52.4
				HLA-DRB1*01:01	444.9
				HLA-DRB1*04:05	711.9
				HLA-DRB1*07:01	28.3
				HLA-DRB1*09:01	156.2
				HLA-DRB1*15:01	285.9
				HLA-DRB3*01:01	243.3
	GGPFEASVVWFYVIK	85	99	HLA-DPA1*01/DPB1*04:01	172.3
				HLA-DPA1*01:03/DPB1*02:01	172.9
				HLA-DPA1*02:01/DPB1*01:01	300.3
				HLA-DPA1*02:01/DPB1*05:01	693.4
				HLA-DQA1*03:01/DQB1*03:02	371.8
				HLA-DQA1*05:01/DQB1*02:01	380.2
				HLA-DQA1*05:01/DQB1*03:01	51.5
				HLA-DRB1*01:01	302.5
				HLA-DRB1*04:05	728.2
				HLA-DRB1*07:01	42
				HLA-DRB1*09:01	153.7
				HLA-DRB1*15:01	250.3
				HLA-DRB3*01:01	268.6

	GPFEASVVWFYVIKG	86	100	HLA-DPA1*01/DPB1*04:01	188.2
				HLA-DPA1*01:03/DPB1*02:01	183.3
				HLA-DPA1*02:01/DPB1*01:01	328.4
				HLA-DPA1*02:01/DPB1*05:01	726.3
				HLA-DQA1*03:01/DQB1*03:02	441.2
				HLA-DQA1*05:01/DQB1*02:01	406.8
				HLA-DQA1*05:01/DQB1*03:01	248.1
				HLA-DRB1*01:01	350.4
				HLA-DRB1*04:05	603.1
				HLA-DRB1*07:01	43.7
				HLA-DRB1*09:01	213.6
				HLA-DRB3*01:01	367.3
	PFEASVVWFYVIKGD	87	101	HLA-DPA1*01:03/DPB1*02:01	205.8
				HLA-DPA1*02:01/DPB1*01:01	383.2
				HLA-DPA1*02:01/DPB1*05:01	883.4
				HLA-DQA1*03:01/DQB1*03:02	535.4
				HLA-DQA1*05:01/DQB1*02:01	481.3
				HLA-DQA1*05:01/DQB1*03:01	324.4
				HLA-DRB1*04:05	596.2
				HLA-DRB1*07:01	53
				HLA-DRB1*09:01	310.6
				HLA-DRB3*01:01	506.8
	FEASVVWFYVIKGDD	88	102	HLA-DPA1*01:03/DPB1*02:01	221.5
				HLA-DQA1*03:01/DQB1*03:02	545.7
				HLA-DQA1*05:01/DQB1*02:01	937.5
				HLA-DQA1*05:01/DQB1*03:01	322.4
				HLA-DRB1*07:01	67.9
				HLA-DRB1*09:01	505.3
				HLA-DRB3*01:01	704
FQGEHLYPI	EQYQTGFQGEHLYPI	206	220	HLA-DPA1*01/DPB1*04:01	164.1
				HLA-DPA1*01:03/DPB1*02:01	58.6
				HLA-DPA1*02:01/DPB1*01:01	251.8
				HLA-DPA1*03:01/DPB1*04:02	216.3
				HLA-DQA1*05:01/DQB1*02:01	430.9
				HLA-DQA1*05:01/DQB1*03:01	675.6
				HLA-DRB1*01:01	306.6
				HLA-DRB1*04:01	391.7
				HLA-DRB1*04:05	736.6
				HLA-DRB1*07:01	80.3
				HLA-DRB3*01:01	26.5
				HLA-DRB5*01:01	152.6
	QYQTGFQGEHLYPIA	207	221	HLA-DPA1*01/DPB1*04:01	132.8
				HLA-DPA1*01:03/DPB1*02:01	52.2
				HLA-DPA1*02:01/DPB1*01:01	241.8
				HLA-DPA1*03:01/DPB1*04:02	144.5
				HLA-DQA1*05:01/DQB1*02:01	516.2
				HLA-DQA1*05:01/DQB1*03:01	629.8
				HLA-DRB1*01:01	143.2
				HLA-DRB1*04:01	295.9
				HLA-DRB1*04:05	680
				HLA-DRB1*07:01	95.5
				HLA-DRB3*01:01	24.2
				HLA-DRB5*01:01	124.1

YQTGFQGEHLYPIAD	208	222	HLA-DPA1*01/DPB1*04:01	130.4
			HLA-DPA1*01:03/DPB1*02:01	58.5
			HLA-DPA1*02:01/DPB1*01:01	269.4
			HLA-DPA1*03:01/DPB1*04:02	128.9
			HLA-DQA1*05:01/DQB1*02:01	646
			HLA-DQA1*05:01/DQB1*03:01	624.5
			HLA-DRB1*01:01	152.1
			HLA-DRB1*04:01	296.2
			HLA-DRB1*04:05	540.9
			HLA-DRB1*07:01	147.2
			HLA-DRB1*09:01	519.9
			HLA-DRB3*01:01	21.1
			HLA-DRB5*01:01	135.5
QTGFQGEHLYPIADT	209	223	HLA-DPA1*01/DPB1*04:01	136.1
			HLA-DPA1*01:03/DPB1*02:01	68.5
			HLA-DPA1*02:01/DPB1*01:01	321.3
			HLA-DPA1*03:01/DPB1*04:02	172.6
			HLA-DQA1*05:01/DQB1*02:01	862.1
			HLA-DQA1*05:01/DQB1*03:01	610.8
			HLA-DRB1*01:01	71.7
			HLA-DRB1*04:01	263.1
			HLA-DRB1*04:05	447.4
			HLA-DRB1*07:01	199
			HLA-DRB1*09:01	864.4
			HLA-DRB3*01:01	22.4
			HLA-DRB5*01:01	136.7
TGFQGEHLYPIADTN	210	224	HLA-DPA1*01/DPB1*04:01	181.9
			HLA-DPA1*01:03/DPB1*02:01	92.6
			HLA-DPA1*02:01/DPB1*01:01	441.3
			HLA-DPA1*03:01/DPB1*04:02	231
			HLA-DQA1*05:01/DQB1*03:01	450.9
			HLA-DRB1*01:01	150.5
			HLA-DRB1*04:01	261.1
			HLA-DRB1*04:04	844
			HLA-DRB1*04:05	563.7
			HLA-DRB1*07:01	305.6
			HLA-DRB3*01:01	49.1
			HLA-DRB5*01:01	227.1
GFQGEHLYPIADTNT	211	225	HLA-DPA1*01/DPB1*04:01	649.3
			HLA-DPA1*01:03/DPB1*02:01	274.4
			HLA-DPA1*03:01/DPB1*04:02	373.7
			HLA-DQA1*05:01/DQB1*03:01	560.7
			HLA-DRB1*01:01	319.3
			HLA-DRB1*04:01	222.6
			HLA-DRB1*04:04	640.1
			HLA-DRB1*04:05	520.5
			HLA-DRB1*07:01	385.2
			HLA-DRB3*01:01	119.4
			HLA-DRB5*01:01	374.9
FQGEHLYPIADTNTR	212	226	HLA-DPA1*01:03/DPB1*02:01	505.9
			HLA-DPA1*03:01/DPB1*04:02	655
			HLA-DQA1*05:01/DQB1*03:01	601.9
			HLA-DRB1*01:01	507.1
			HLA-DRB1*04:01	158
			HLA-DRB1*04:05	593.5
			HLA-DRB1*07:01	468.1
			HLA-DRB3*01:01	305.4
			HLA-DRB5*01:01	367.2

FAAFVACAV	NAVSMPIFAAFVACA	346	360	HLA-DPA1*02:01/DPB1*01:01	999.6
				HLA-DQA1*01:02/DQB1*06:02	86.8
				HLA-DQA1*03:01/DQB1*03:02	522.5
				HLA-DQA1*05:01/DQB1*02:01	516.1
				HLA-DQA1*05:01/DQB1*03:01	33.5
				HLA-DRB1*04:01	816.2
				HLA-DRB1*08:02	835.5
				HLA-DRB1*09:01	346.5
	AVSMPIFAAFVACAV	347	361	HLA-DPA1*02:01/DPB1*01:01	727.7
				HLA-DQA1*01:01/DQB1*05:01	704.7
				HLA-DQA1*01:02/DQB1*06:02	38.5
				HLA-DQA1*03:01/DQB1*03:02	377.3
				HLA-DQA1*04:01/DQB1*04:02	231.6
				HLA-DQA1*05:01/DQB1*03:01	18.8
				HLA-DRB1*04:01	754.5
				HLA-DRB1*04:05	870
				HLA-DRB1*08:02	891.8
				HLA-DRB1*15:01	330
	VSMPIFAAFVACAVA	348	362	HLA-DPA1*02:01/DPB1*01:01	730
				HLA-DQA1*01:01/DQB1*05:01	559.8
				HLA-DQA1*01:02/DQB1*06:02	32.1
				HLA-DQA1*03:01/DQB1*03:02	337.7
				HLA-DQA1*05:01/DQB1*03:01	14.7
				HLA-DRB1*04:01	711.3
				HLA-DRB1*08:02	867.9
				HLA-DRB1*15:01	287.6
	SMPIFAAFVACAVAL	349	363	HLA-DPA1*02:01/DPB1*01:01	606.3
				HLA-DQA1*01:01/DQB1*05:01	490.9
				HLA-DQA1*01:02/DQB1*06:02	30.1
				HLA-DQA1*03:01/DQB1*03:02	306.5
				HLA-DQA1*04:01/DQB1*04:02	228.7
				HLA-DQA1*05:01/DQB1*03:01	11.1
				HLA-DRB1*04:01	525.4
				HLA-DRB1*08:02	702.5
				HLA-DRB1*15:01	225.9
	MPIFAAFVACAVALV	350	364	HLA-DPA1*02:01/DPB1*01:01	594.3
				HLA-DQA1*01:01/DQB1*05:01	547.4
				HLA-DQA1*01:02/DQB1*06:02	33.1
				HLA-DQA1*03:01/DQB1*03:02	349.1
				HLA-DQA1*04:01/DQB1*04:02	263.3
				HLA-DQA1*05:01/DQB1*03:01	9.5
				HLA-DRB1*04:01	553
				HLA-DRB1*08:02	595.7
				HLA-DRB1*15:01	333.5
	PIFAAFVACAVALVG	351	365	HLA-DPA1*02:01/DPB1*01:01	875
				HLA-DQA1*01:01/DQB1*05:01	684.4
				HLA-DQA1*01:02/DQB1*06:02	37
				HLA-DQA1*03:01/DQB1*03:02	434.8
				HLA-DQA1*04:01/DQB1*04:02	470.1
				HLA-DQA1*05:01/DQB1*03:01	9.1
				HLA-DRB1*04:01	604.1
				HLA-DRB1*08:02	750
				HLA-DRB1*15:01	372.9

	IFAAFVACAVALVGL	352	366	HLA-DPA1*02:01/DPB1*01:01	886
				HLA-DQA1*01:01/DQB1*05:01	761
				HLA-DQA1*01:02/DQB1*06:02	57.8
				HLA-DQA1*03:01/DQB1*03:02	493.5
				HLA-DQA1*04:01/DQB1*04:02	520
				HLA-DQA1*05:01/DQB1*03:01	8.7
				HLA-DRB1*04:01	663.8
				HLA-DRB1*15:01	410.4
VDYVPSTLV	SAQMWAVDYVPSTLV	131	145	HLA-DPA1*02:01/DPB1*01:01	254.3
				HLA-DPA1*03:01/DPB1*04:02	539
				HLA-DRB1*01:01	32.7
				HLA-DRB1*04:05	412.2
				HLA-DRB1*07:01	3.9
				HLA-DRB1*09:01	363.5
				HLA-DRB1*15:01	716.3
				HLA-DRB5*01:01	230.3
	AQMWAVDYVPSTLVS	132	146	HLA-DPA1*01:03/DPB1*02:01	867.1
				HLA-DPA1*02:01/DPB1*01:01	255
				HLA-DPA1*03:01/DPB1*04:02	410.9
				HLA-DRB1*01:01	23.3
				HLA-DRB1*03:01	658
				HLA-DRB1*04:05	406.3
				HLA-DRB1*07:01	4.3
				HLA-DRB1*09:01	290.9
				HLA-DRB1*15:01	589
				HLA-DRB5*01:01	149.4
	QMWAVDYVPSTLVSR	133	147	HLA-DPA1*01:03/DPB1*02:01	633.8
				HLA-DPA1*02:01/DPB1*01:01	152.9
				HLA-DPA1*03:01/DPB1*04:02	255.4
				HLA-DRB1*01:01	15.7
				HLA-DRB1*03:01	471.2
				HLA-DRB1*04:05	439
				HLA-DRB1*07:01	5
				HLA-DRB1*09:01	262
				HLA-DRB1*13:02	738.7
				HLA-DRB1*15:01	471.2
				HLA-DRB4*01:01	873.3
				HLA-DRB5*01:01	52.5
	MWAVDYVPSTLVSRN	134	148	HLA-DPA1*01:03/DPB1*02:01	720.2
				HLA-DPA1*02:01/DPB1*01:01	136
				HLA-DPA1*03:01/DPB1*04:02	193.6
				HLA-DRB1*01:01	13.5
				HLA-DRB1*03:01	426.3
				HLA-DRB1*04:05	468.5
				HLA-DRB1*07:01	6.7
				HLA-DRB1*08:02	78.2
				HLA-DRB1*09:01	296.9
				HLA-DRB1*13:02	691.3
				HLA-DRB1*15:01	448.3
				HLA-DRB5*01:01	40.9

WAVDYVPSTLVSRNG	135	149	HLA-DPA1*01:03/DPB1*02:01	921.6
			HLA-DPA1*02:01/DPB1*01:01	161.6
			HLA-DPA1*03:01/DPB1*04:02	216.1
			HLA-DRB1*01:01	23.3
			HLA-DRB1*03:01	795.1
			HLA-DRB1*04:05	662.6
			HLA-DRB1*07:01	8.9
			HLA-DRB1*08:02	93
			HLA-DRB1*09:01	359.6
			HLA-DRB1*13:02	880.5
			HLA-DRB1*15:01	532.5
			HLA-DRB5*01:01	63.2
AVDYVPSTLVSRNGA	136	150	HLA-DPA1*02:01/DPB1*01:01	186.1
			HLA-DPA1*03:01/DPB1*04:02	286.1
			HLA-DQA1*05:01/DQB1*03:01	666.3
			HLA-DRB1*01:01	39.1
			HLA-DRB1*04:04	138.6
			HLA-DRB1*07:01	13.1
			HLA-DRB1*09:01	464.4
			HLA-DRB1*15:01	757.4
			HLA-DRB5*01:01	91.1
VDYVPSTLVSRNGAG	137	151	HLA-DPA1*02:01/DPB1*01:01	253.8
			HLA-DPA1*03:01/DPB1*04:02	398.5
			HLA-DRB1*01:01	69.4
			HLA-DRB1*07:01	18.4
			HLA-DRB1*09:01	829.5
			HLA-DRB5*01:01	197.8

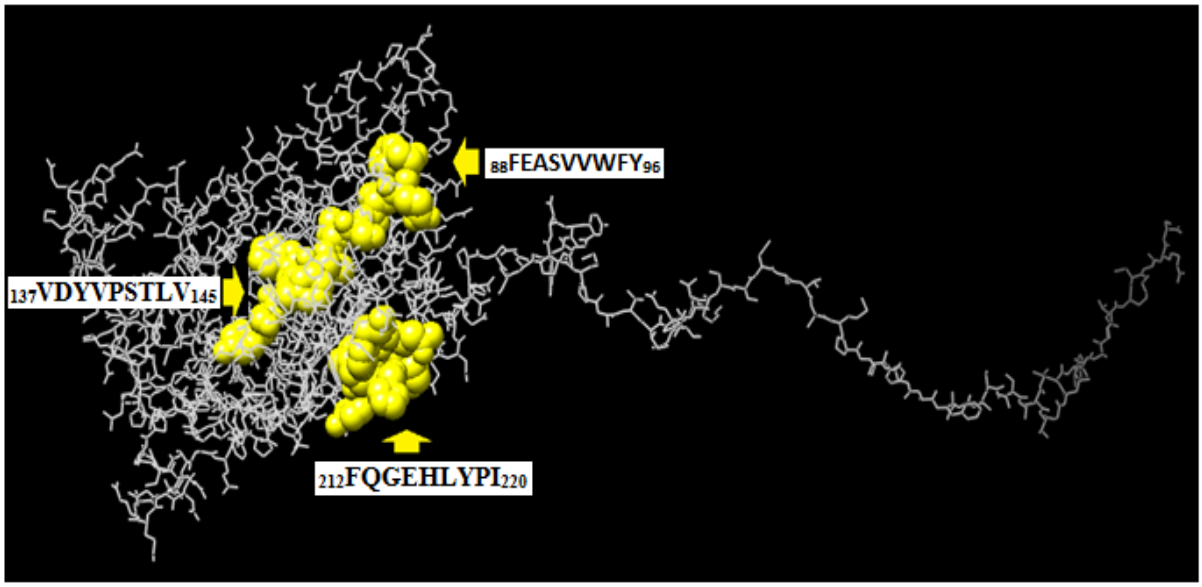

Figure 5. The position of proposed T helper cells epitopes of ILTV glycoprotein D suggested to be interact with MHC II alleles illustrated by UCSF-Chimera visualization tool. The epitope FAAFVACAV was shown in Figure 4

3.5. Overlapping Epitopes

In this study several epitopes were predicted to interact with both MHC I and MHC II alleles. As shown in table (5) the three proposed epitopes that strongly interacted with MHC class I alleles were also strongly interacted with MHC II alleles. For instance $_{20}$FASQSTAAV$_{28}$ interacted with 9 and 52 alleles in MHC I and MHC II respectively. The same epitope ($_{20}$FASQSTAAV$_{28}$) overlapped with epitope $_{24}$STAAVTYDY$_{32}$ that interacted with 10 and 1 alleles in MHC I and MHC II respectively. Moreover the epitope $_{353}$FAAFVACAV$_{361}$ was interacting with 9 and 37 alleles of MHC I and MHC II respectively. Concerning epitopes proposed for MHC II the best epitope was $_{88}$*FEASVVWFY*$_{96}$ since it interacted with 7 and 71 alleles in MHCI and MHC II, respectively. Moreover the epitope $_{212}$*FQGEHLYPI*$_{220}$ that associated with 82 alleles for MHC II was linked to 3 alleles only from MHC I. However the epitope $_{137}$*VDYVPSTLV*$_{145}$ was found only interacting with MHC II alleles.

Table 5. Comparison between the numbers of alleles linked with top proposed epitopes in MHCI and MHCII

No	Peptide	Start	End	MHCI alleles	MHCII alleles
1	FAAFVACAV	353	361	9*	62#
2	FASQSTAAV	20	28	9*	52
3	STAAVTYDY	24	32	10*	1
4	FEASVVWFY	88	96	7	71#
5	FQGEHLYPI	212	220	3	82#
6	VDYVPSTLV	137	145	0	69#

* Top MHCI epitopes; # top proposed MHCII epitopes.

3.6. Molecular Docking

Docking is commonly known for its wide application in computer-aided drug design. However, it is also used for designing novel peptides exhibiting binding affinity towards MHC molecules. Originally, the docking studies were mainly used for investigation of peptides that bind MHC class I molecules [8]. Molecular docking was performed using peptide-binding groove affinity. This help in the prediction and symbolization of the real image of CTL epitopes interaction (ligands) with chicken alleles (receptors). For this purpose, as shown in Figure 6, two types of chicken BF alleles (BF2*2101 & BF2*0401) were used for docking with the epitopes $_{24}STAAVTYDY_{32}$, $_{20}FASQSTAAV_{28}$ and $_{353}FAAFVACAV_{361}$ that proposed for CTL. The lowest binding energy (kcal/mol) was selected to predict probable CTL epitopes based on the score of global energy and attractive VDW in kcal/mol unit of docked molecules. As shown in Table 6, docking of $_{353}FAAFVACAV_{361}$ epitope with BF_2 2101 and BF2*0401alleles produced -67.87 and -50.57 global energy respectively. This indicated the strong binding affinity between the ligand and both receptors compared to other epitopes. Moreover the epitopes $_{24}STAAVTYDY$ and $_{20}FASQSTAAV_{28}$ both demonstrated favorable binding affinity with BF2 alleles. The BF2*2101 chicken alleles displayed strong binding affinity with CTL proposed peptides compared to BF2*0401. Moreover, the docked epitopes demonstrated deep binding grooves in both BF alleles. Figure 7 illustrated the deep binding between the BF2 alleles and the docked molecules using Patch Dock server for molecular docking.

Compared to the recent in silico study on glycoprotein B of ILTV similarity and difference were observed in the docked epitopes form glycoprotein D to the chicken alleles [13]. For instance epitopes predicted from the glycoprotein B and D of ILTV demonstrated similarity in their high binding affinity of the ligands to the receptor BF2*2101 alleles compared with BF_2 0401 alleles. However, the docked molecules from glycoprotein B and D demonstrated different binding sites for BF2*2101 allele and similar binding sites for BF2*0401 allele (Figure 6 and Figure 7). Moreover, the overall binding affinity of glycoprotein D to BF alleles was less compared to that obtained by glycoprptein B [13].

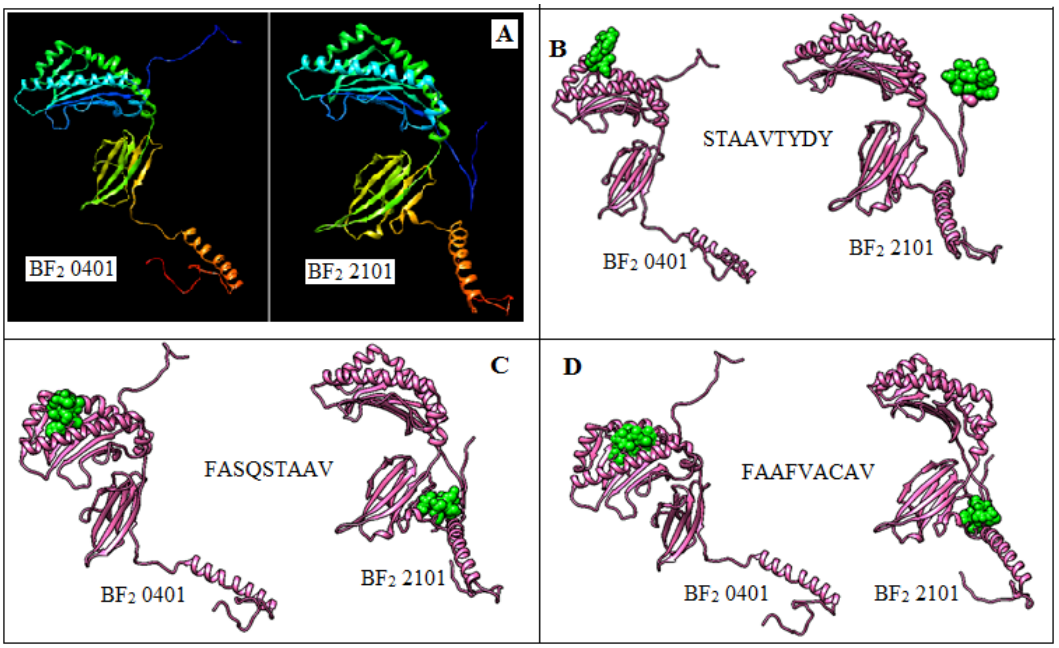

Figure 6. A: The 3D structure of BF_2 alleles of chicken using chimera visualization tool. **B, C and D**: The epitopes $_{24}STAAVTYDY_{32}$, $_{20}FASQSTAAV_{28}$ and $_{353}FAAFVACAV_{361}$ that proposed for CTL for docking with chicken BF allele, respectively. Each epitope was docked with two types of chicken BF alleles (BF2*2101 & BF2*0401).

Table 6. The binding energy and Attractive VDW scores for the proposed epitopes with chicken BF2 alleles using PatchDock server

Peptide	Receptor	Energy	Attractive vdw
STAAVTYDY	BF_2 2101	- 64.94	-28.46
	BF_2 0401	- 44.19	-21.63
FASQSTAAV	BF_2 2101	- 60.18	-25.98
	BF_2 0401	- 34.05	-22.42
FAAFVACAV	BF_2 2101	- 67.87	- 27.16
	BF_2 0401	- 50.57	- 25.03

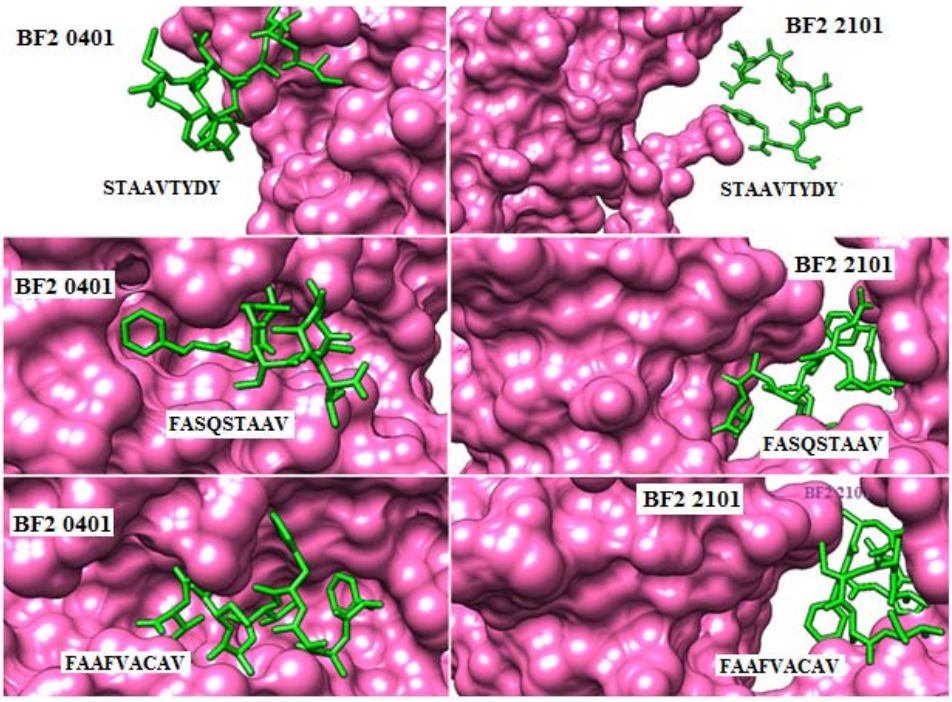

Figure 7. Visualization of PatchDock Molecular docking of MHCI proposed epitopes and chicken BF_2 alleles receptors using UCSF-Chimera visualization tool. Receptors (BF alleles) represented by rounded ribbon structure pink colour while CTL Epitopes represent by cyan one

4. Conclusion

Vaccines evoke profound changes in the cellular components of adaptive immunity, comprising T- and B-cells. Peptide vaccine can induce specific immune responses because it contains immunodominant peptides. These peptides are constructed on the basis of a chemical approach to synthesize the identified B cells and T cells epitopes. B-cell epitope of a target molecule can be linked with a T-cell epitope to make it immunogenic.

The immunoinformatics tools are used in diversity of applications from basic immunological data to computational techniques and assays. For example conduction of potent biomedical research for prediction of new epitopes, vaccines design and design of immune-based. The traditional peptide vaccine is costive and takes long time to produce.

In vitro and *in vivo* tests are needed to achieve and exemplify the effectiveness of the proposed epitopes to induce an immune response. Peptide vaccine against glycoprotein is strongly supersedes the conventional vaccines, this new universal predicted vaccine for chicken.

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Conflict of Interests

The authors declared that they have no conflict of interests regarding the publication of this paper.

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