Int J Infect. 2018 April; 5(2):e63255. doi: 10.5812/iji.63255.

Published online 2018 April 28. **Research Article**

In Silico B Cell and T Cell Epitopes Evaluation of lipL32 and OmpL1 Proteins for Designing a Recombinant Multi-Epitope Vaccine Against Leptospirosis

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Received 2017 October 25; **Revised** 2018 January 28; **Accepted** 2018 May 02.

Abstract

Leptospirosis is a widespread zoonotic disease caused by *Leptospira interrogans*. The conventional vaccines have some major problems. Therefore, recombinant vaccines such as multiple-epitope vaccine are suggested. OmpL1 and lipL32 are the most important proteins of *Leptospira interrogans* bacteria that can be used in epitope prediction process to design a multiple-epitope vaccine. Hence, in this study, the most reliable and accurate online servers were applied to predict B cell and T cell epitopes, the secondary and tertiary structures, enzyme digestion, and antigenicity score of ompL1 and lipL32. The results showed that epitopes located at 103 - 122, 210 - 232, and 272 - 291 amino acid residues are the common epitopes between T cell (MHCI) and B cell. 288 - 308 amino acid residues were introduced as common epitopes to stimulate both T cell (MHCI and MHCII) and B cell of ompL1 protein. In the case of LipL32 protein, 80 - 96 amino acid residues are recommended for T cell epitopes and 63-81 amino acid residues for stimulation of both B and T cells. All the mentioned epitopes can be considered as linear epitopes in designing a recombinant vaccine based on chimeric epitopes. It appears that these epitopes can be applied to design recombinant multiple-epitope vaccines against leptospirosis.

Keywords: Epitope, Leptospira, Vaccine, Zoonosis

1. Background

Leptospirosis is a widespread zoonotic disease that could be found in both developed and developing countries. This disease is usually seen among mammalians (in particular, human, and livestock) that are in contact with rodents or living in polluted areas. There are more reports of this disease during unforeseen events such as floods, earthquakes, etc. (1-3). Leptospirosis is a bacterial disease caused by *Leptospira interrogans*. In fact, Leptospira genus is classified into *Leptospira biflexa*, which comprises all non-pathogenic strains, and *Leptospira interrogans* that consists of all pathogenic strains (4). Leptospirosis can be transferred through direct or indirect contact with the infected cases (5). The most prevalent symptoms of this disease are meningitis, hepatitis, and nephritis and sometimes, it leads to death (6, 7). Conventional vaccines (Bacterin -type) used to prevent Leptospirosis have some side effects (fever, pain), short-term immunity and serovar- resisted protection (4); hence, it is necessary to apply new strategies to solve these problems. One of the most recent strategies for preventing leptospirosis is to use recombinant vaccines that are epitope-based or protein vaccines with a vital role in antigenicity of a pathogen (8-10). Outer membrane proteins (OMPs) are the most important proteins diagnosed by the immune system in different bacterial infections. OMPs are widely applied to design recombinant vaccines such as subunit and epitope vaccines (4). There are three classes of OMPs in Leptospira: Lip (lipoprotein) that includes lipL32, lipL42, and lipL24; transmembrane proteins that include ompL1s, and finally peripheral proteins that include lipl42 (11-15). Many studies have shown that ompL1 and lipL32 are the most conserved OMPs in most pathogenic strains of Leptospira and they can be applied to design recombinant vaccines to prevent

leptospirosis (16). Studies also showed that the application of an OMP as a subunit recombinant vaccine could not successfully prevent leptospirosis since it is not antigenic enough to stimulate the immune system. Consequently, it seems that applying multi-epitope vaccines, which use epitopes of several OMPs, is the solution to vaccine antigenicity (9, 17). Epitopes are amino acid sequences of OMPs recognized by antibodies of the immune system. In general, epitopes are divided into B cells (continuous and discontinuous) and T cells (MHCI and MHCII) (18, 19). Nowadays, by the progress in biological data, researchers are widely using intelligent methods such as machine learning for data analysis. Such methods not only are affordable, but also provide reliable data for the experiment (20). Therefore, in the present study, the most reliable and appropriate online tools and servers were applied to predict B cell and T cell epitopes of ompL1 and LipL32 (as the most important leptospirosis' OMPs) (20).

2. Methods

To predict B and T cell epitopes of ompL1 (accession number: JX532100.1) and LipL32 (accession number: JN886739.1), their amino acid sequences were collected from the National Center for Biotechnology Information (NCBI).

2.1. B Cell Epitopes Prediction of Ompl1 and Lip32

In order to predict B cell epitopes of ompL1 and LipL32 using their primary amino acid sequences, the most reliable and accurate online servers were employed. These online servers include IEDB, ABCpred, BepiPred, BCPREDS, and SVMTrip. It must be noted that all servers have been designed to predict discontinuous B cell epitopes. Required parameters of each server such as the desired length to predict epitopes were adjusted as default (18).

2.2. T Cell Epitopes Prediction of Ompl1 and Lip32

In case of T cell epitopes prediction of ompL1 and LipL32, both MHCI and MHCII epitopes were evaluated by the most precise online servers: IEDB, SYFPEITH, NetCTL, NetMHC, Propred, and MHC2Pred (19).

2.3. Evaluation of the Most Important Features of the Predicted Epitopes

To investigate the antigenicity score of the predicted B and T cell epitopes, VaxiJen 2.0 server was employed with the desired threshold (0.5). Also, the most important features of the predicted epitopes such as enzymatic digestion sites, PI, and the Mass of the predicted epitopes through specialized servers were analyzed by Protein Digest server (18, 19).

2.4. Secondary and Tertiary Structure Prediction of ompL1 and Lip32

To predict secondary structures of ompL1 and LipL32 proteins, based on their primary amino acid sequences, improved self-optimized prediction method (SOPMA) server was applied and their helices, sheets, turns, and coil were evaluated. Tertiary structures of ompL1 and lipL32 proteins were designed by iterative threading assembly refinement (I-TASSER) online server which uses hierarchical approach to predict the structure and function of proteins. PDB formats of the predicted tertiary structures of the studied proteins were visualized by PyMOL V1 Viewer software (21).

3. Results

3.1. B Cell Epitopes Prediction of ompL1 and LipL32

As shown in Table 1, the initial results were theoretically obtained, based on the highest score and the most frequent epitopes among all the mentioned specialized servers. It must be mentioned that in order to predict discontinuous B cell epitopes, physic-chemical properties of amino acids such as hydrophilicity, charge, flexibility, polarity, and the exposed surface area were considered.

3.2. T Cell Epitopes Prediction of ompL1 and LipL32

To predict T cell epitopes, the most frequent Iranian alleles of MHCI (A-0101, A0201, and B-2705) and MHCII (DRB1-0101 and DRB1-0401) were used. It must be mentioned that in each server, the predicted epitopes with the highest scores were selected (data not shown). Then, T cell predicted epitopes were evaluated using the above mentioned servers and used in the following analysis in order to identify the conserved sequences in both MHCI and MHCII epitopes (Table 2).

3.3. Evaluation of the Most Important Features of Predicted Epitopes

After prediction of B cell and T ell epitopes using out put of different online servers, their antigenicity scores were evaluated, as shown in Tables 1 and 2. Epitopes with a score above 0.5 were considered as the most antigenic epitopes. The obtained results from this step were applied in further study to evaluate the enzymatic digestion. PI and Mass, reported in Table 3, were calculated by protein digestion server. According to these results, the antigenic epitopes that had the largest number of non-digestive enzymes were selected as final B cell and T cell epitopes (Table 3) (colored epitopic regions indicate the selected ones). Epitopes with the same caption are common among different categories (Table 4). In the case of ompL1 protein,

pink, green, and red regions are common between T Cell (MHCI) or B Cell epitopes, but the blue color is common among both T and B cells that are arranged between 288 and 307aa residues (Table 3). Finally, those selected sequences from lipL32 protein have the gray colored in both T cell categories (MHCI & MHCII), which are located at 80 - 93aa residues. Moreover, the blue regions indicate common epitopes between both T (MHCI & MHCII) and B cells and are located at an amino acid range of 64 - 81 (Table 3).

Table 4. Final B and T Cell Predicted Epitopes[a]

OMPL1	lip32
T Cell (MHCI)	
109 TGGAINARSTKG 120	-
210 GSNNIKGGY 218	63 VKPGQAPDGLVDGNKKA 79
272 FIELETIMSAAY 283	80 YYLYVWIPAVIAEM 93
290 SVGGATNLSPFPAY 303	238 IPGVSPLIHSNPEE 251
T Cell (MHCII)	
75 **FQNPAKPTGEGNYIGVAPR** 93	64 KPGQAPDGLVDGNKKAY 81
155 VTKADIAGY 163	88 **AVIAEMGVR** 96
290 SVGGATNLS 298	209 YRISFTTYK 217
B Cell	
34 LQLDLGQLGGTITK 47	63 VKPGQAPDGLVD 74
103 ITLDRTTGGAINARSTKGAM 122	100 PTGEIGEPGDGDL 112
213 NIKGGYDILTAAGAGAVANL 232	-
272 FIELETIMSAAYAVGKTQSV 291	-
288 TQSVGGATNLSPFPAYPIVV 307	-

[a] In first column; green, pink and red highlighted regions related to common epitopes between T cell MHCI class and B cell, and the blue ones related to the common ones among both T and B cells. In second column; the gray epitopes are common between MCHI and MHCII classes and the blue highlighted epitopes are common epitopes among both T and B cells

3.4. Secondary and Tertiary Structure Prediction of ompl1 and Lip32

As reported earlier, in order to predict the secondary structure of candidate proteins, SOPMA server was applied. OmpL1 protein included 23.79% Alpha helix, 38.26% Random coil, 27.33% Extended strand, and 10.61% Beta-turn structures (Figure 1). In addition, lipL32 protein included 38.06% Alpha helix, 19.03% Extended strand, 9.33% Beta-turn, and 33.58% Random coil structures (Figure 2). As shown in the results, these two proteins involved a high proportion of random coil structures indicating the concentration of epitopes in the mentioned areas, rather than total protein. The results of the 3DLigandSite show that all the final predicted B and T cell epitopes of ompL1 and lipL32 can be exposed on the surfaces (Figure 3A and 3B).

4. Discussion

Vaccines prevent infectious diseases. Although conventional vaccines (attenuated and killed vaccines) are able to save millions of lives, there are some drawbacks such as long production process (around 15 years), the diverse effects on different cases, and their side effects (even death) (22-24). Therefore, it seems that the new generation of vaccines must be substituted with the conventional ones. Today, bioinformatics is widely being used. In fact, bioinformatics uses both computer and biology to accelerate data analysis and decrease the expenditure of the experiments. One of the most important applications of bioinformatics is vaccine production. In fact, advancements in bioinformatics tools along with the advances in recombinant DNA technology and genetics can decrease the time (around 2 years) and expenses of vaccine production (20, 25). As noted before, since Leptospirosis is a zoonotic and widespread disease in most developing countries including Iran, certain considerations should be taken into account to fight this disease. Many studies have reported that subunit recombinant vaccines, which have been designed based on LipL32 and ompL1 proteins (as the most important OMPs to design subunit vaccine), cannot be successfully applied to treat Leptospirosis, because these recombinant vaccines are not strong enough to stimulate the immune system against the disease (16, 26). However more recently, it has been reported that the use of chimeric epitope vaccines designed based on epitopes of OmpL1 and Lep32 is a promising method of fighting leptospirosis (16). Therefore, it seems in order to produce chimeric epitope vaccine, the epitope prediction of ompL1 and LipL32 for Leptospirosis is of great importance. In this study, the most accurate and reliable bioinformatics tools were applied to predict B cell and T cell epitopes of ompL1 and LipL32 (18-20). As our result showed, 20 to 25 amino acids, at the beginning of both proteins, cannot be epitopes because these amino acid sequences are usually considered as a signal peptide to translocate proteins to the endoplasmic reticulum and they are then cleaved by signal peptidase (27, 28). Therefore, these amino acid sequences cannot be exposed as epitopes on the surface of the bacteria to stimulate the immune system.

The results of the current study obviously showed that the epitopic region of ompL1 protein including 103 - 122, 210 - 232, and 272 - 291 aa residues are the most common epitopes between T cell (MHCI) and B cell. In addition, 288-308 aa residues could be considered as a unique epitopic region to stimulate both T cell (MHCI&MHCII) and B cell. Moreover, the amino acids that have been arranged in 80 - 96aa residues are recommended for T cell epitope and 63 - 8196aa residues are suggested for both B and T cells in

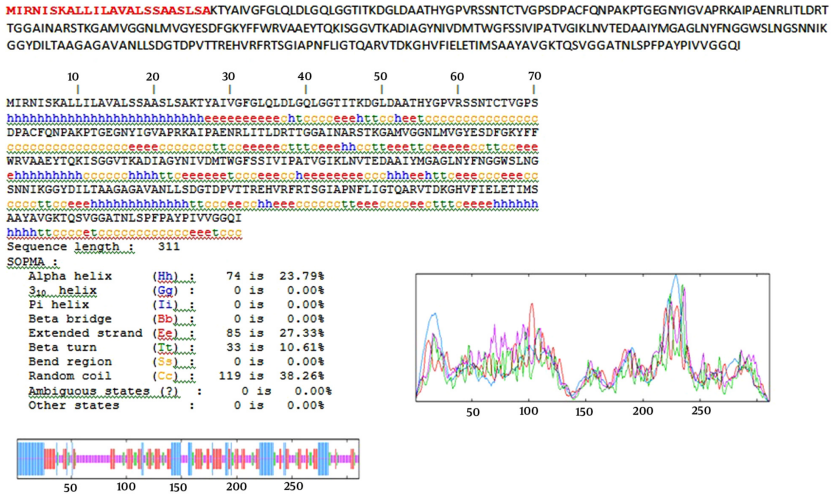

Figure 1. Secondary structure prediction results of OMPL protein. Amino acids with different colors represent different secondary structures. Blue: α helix, green: β turn, red: extended strand, and yellow: random coil.

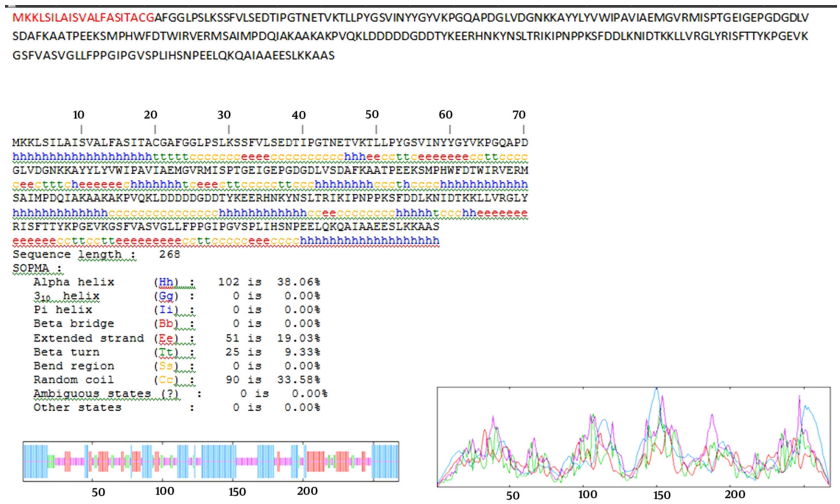

Figure 2. Secondary structure prediction results of Lip32 protein. Amino acids with different colors represent different secondary structures. Blue: α helix, green: β turn, red: extended strand, and yellow: random coil.

LipL32 protein. All of them can be considered to design a chimerical epitopic vaccine.

According to our prediction, it appears these epitopes could not only evade from protease system but also provide enough immune response against Leptospirosis. In fact, the evasion of protease system can increase epitopes half-life and can lead to the stimulation of immune system. It should be noted that the final epitopes, which have been predicted in this study, could simultaneously stimulate B and T cells. Therefore, the use of these epitopes with the appropriate arrangement could lead to a proper immune response.

4.1. Conclusions

Nowadays, bioinformatics is widely being used to analyze biological data. This area of science can accelerate data analysis and decrease the expenses at the same time. In this study, a wide variety of the most reliable and precise online tools and servers were applied to predict B cell, T cell, and common B and T cell epitopes of ompL1 and lipL32. In addition, 288 - 308aa residues of ompL1 protein can be considered as common epitopes to stimulate both T cell (MHCI

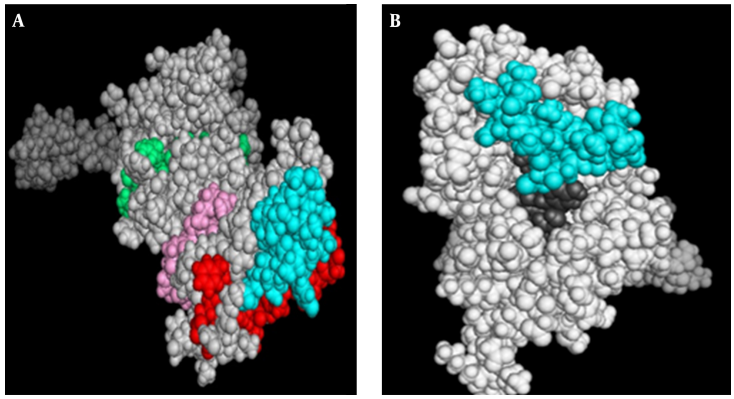

Figure 3. Tertiary structure prediction results for the OMPL1 (A) and Lip32 (B) proteins. A: green, pink, and red highlighted regions related to common epitopes between T cell MHCI class and B cell, and the blue ones related to the common ones among T and B cells. B: the gray regions are common between T cell MCHI and MHCII classes and the blue highlighted regions are common epitopes among both T and B cells.

and MHCII) and B cell. For LipL32 protein, 63-81aa residues are suggested for the epitopic region of both B and T cells. However, the results of this study need to be confirmed by further experimental studies.

Footnote

Conflict of Interest: The authors declare that there is no conflict of interest.

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Table 1. The List of High Scored Predicted T Cell Epitopes Using Online Software and Their VaxiJen Score[a]

Sequence	Server	SCORE	Vaxijen Score	Sequence	Server	SCORE	Vaxijen Score
MHCI							
LipL32				**OmpL1**			
$_{63}$VKPGQAPDGLVDGNKKA$_{79}$	IEDB	98	1.0042 (Probable ANTIGEN)	$_{72}$PACFQNPAKPTG$_{83}$	IEDB	99	NON-ANTIGEN
$_{148}$IAKAAKAKPVQKLDDDDDGDDTYKEERHNK$_{177}$		99.5	1.2556 (Probable ANTIGEN)	$_{74}$CFQNPAKPTGEG$_{85}$		100	NON-ANTIGEN
$_{179}$NSLTRIKIPNPPKS$_{192}$		98	-0.1699 (Probable NON-ANTIGEN)	$_{202}$FNGGWSLNGSNN$_{213}$		100	0.6696 (Probable ANTIGEN)
$_{185}$KIPNPPKSFDDLKN$_{198}$		97	-0.3322 (Probable NON-ANTIGEN)	$_{109}$TGGAINARSTKG$_{120}$		99	2.2206 (Probable ANTIGEN)
$_{98}$ISPTGEIGEPGDGDLVSDAFKAATPEEKSMPHWFD$_{132}$		95.87	0.4244 (Probable NON-ANTIGEN)	$_{258}$LIGTQARVTDKGH$_{270}$		95	0.9339 (Probable ANTIGEN)
$_{152}$AKAKPVQKLDDDDDGDDTYKEERHNKYNS$_{170}$		100	1.1642 (Probable ANTIGEN)	$_{67}$VGPSDPACFQNP$_{78}$		100	NON-ANTIGEN
$_{31}$SSFVLSEDTIPGTNETVKT$_{49}$		96.25	0.5263 (ProbableANTIGEN)	$_{81}$PTGEGNYIGVAP$_{92}$		99	1.4730 (Probable ANTIGEN)
$_{97}$MISPTGEIGEPGDGDLVSDA$_{117}$		99	0.4157 (ProbableNON-ANTIGEN)	$_{178}$PATVGIKLNVTE$_{189}$		99	1.6638 (Probable ANTIGEN)
$_{153}$KAKPVQKLDDDDDGDDTYKEERHNK$_{177}$		99.88	1.2193 (ProbableANTIGEN)	$_{299}$PFPAYPIVVGGQ$_{310}$		100	0.8669 (Probable ANTIGEN)
$_{187}$PNPPKSFDDLKNIDT$_{201}$		99	0.0200 (Probable NON-ANTIGEN)	$_{272}$FIELETIMSAAY$_{283}$	NetMHC	0.432	0.5005 (Probable ANTIGEN)
$_{235}$PPGIPGVSPLIHSNPEELQKQAIAAEES$_{262}$		95.62	0.4033 (Probable NON-ANTIGEN)	$_{290}$SVGGATNLSPFPAY$_{303}$		0.353	0.7905 (Probable ANTIGEN)
$_{238}$IPGVSPLIHSNPEE$_{251}$		100	0.5522 (Probable ANTIGEN)	$_{120}$GAMVGGNLMV$_{129}$		0.513	0.8084 (Probable ANTIGEN)
$_{34}$VLSEDTIPGTNETV$_{47}$	NetMHC	0.537	-0.3071 (Probable NON-ANTIGEN)	$_{231}$NLLSDGTDPV$_{240}$		0.649	NON-ANTIGEN
$_{76}$NKKAYYLYVWIPAV$_{89}$		0.751	-0.3071 (Probable NON-ANTIGEN)	$_{256}$NFLIGTQARV$_{265}$		0.594	NON-ANTIGEN
$_{80}$YYLYVWIPAVIAEM$_{93}$		0.600	-0.3071 (Probable NON-ANTIGEN)	$_{272}$FIELETIMSA$_{281}$		0.668	0.5322 (Probable ANTIGEN)
$_{207}$RGLYRISFTTYKPG$_{220}$		0.425	0.6082 (ProbableANTIGEN)	$_{296}$NLSPFPAYPI$_{305}$		0.582	1.2997 (Probable ANTIGEN)
$_{45}$ETVKTLLPY$_{53}$	NetCTL	1.42	0.0981 (Probable NON-ANTIGEN)	$_{210}$GSNNIKGGY$_{218}$	NetCTL	1.67	1.8330 (Probable ANTIGEN)
$_{54}$GSVINYYGY$_{62}$		1.24	-0.1594 (Probable NON-ANTIGEN)	$_{265}$VTDKGHVFI$_{273}$		1.27	NON-ANTIGEN
$_{70}$DGLVDGNKKAYYLY$_{83}$	Syfpeithi	17	0.3399 (Probable NON-ANTIGEN)	$_{274}$ELETIMSAAY$_{283}$	Syfpeithi	24	NON-ANTIGEN
$_{200}$DTKKLLVRGLY$_{210}$		24	-0.3071 (Probable NON-ANTIGEN)	$_{294}$ATNLSPFPAY$_{303}$		23	NON-ANTIGEN
$_{142}$AIMPDQIAKA$_{151}$		25	0.4060 (Probable NON-ANTIGEN)	$_{176}$VIPATVGIKLNVTEDAAI$_{193}$		22	1.1858 (Probable ANTIGEN)
$_{203}$KLLVRGLYRI$_{212}$		26	-0.5296 (Probable NON-ANTIGEN)	$_{92}$PRKAIPAENR$_{101}$		23	NON-ANTIGEN
$_{232}$LLFPPGIPGV$_{241}$		31	0.7159 (Probable ANTIGEN)	$_{141}$WRVAAEYTQK$_{150}$		24	0.9085 (Probable ANTIGEN)
				$_{248}$FRTSGIAPNF$_{257}$	Syfpeithi	24	1.1327 (Probable ANTIGEN)
MHCII							

$_{64}$KPGQAPDGLVDGNKKAYY$_{81}$	IEDB	89.97	0.7241 (Probable ANTIGEN)	$_{75}$FQNPAKPTGEGNYIGVAPR$_{93}$	IEDB	91.61	0.7 (Probable ANTIGEN)
$_{118}$KAATPEEKSMPHWFDTW$_{134}$	IEDB	92.65	0.2905 (Probable NON-ANTIGEN)	$_{233}$LSDGTDPVTTREHVRFRTS$_{251}$	IEDB	85.64	1.1127 (Probable ANTIGEN)
$_{186}$IPNPPKSFDDLKNID$_{200}$			-0.0292 (Probable NON-ANTIGEN)	$_{59}$VRSSNTCTV$_{67}$	Propred	3.4	1.4325 (Probable ANTIGEN)
$_{99}$SPTGEIGEPGDGDLVSD$_{115}$		83.37	0.4243 (Probable NON-ANTIGEN)	$_{183}$IKLNVTEDA$_{191}$		4.1	0.9238 (Probable ANTIGEN)
$_{150}$KAAKAKPVQKLDDDDDGDDTYKEER$_{174}$		83.58	1.1462 (Probable ANTIGEN)	$_{218}$YDILTAAGA$_{226}$		1.1	NON-ANTIGEN
$_{82}$YVWIPAVIA$_{90}$	Propred	55	1.1663 (Probable ANTIGEN)	$_{272}$FIELETIMS$_{280}$		3.38	0.5775 (Probable ANTIGEN)
$_{177}$YNSLTRIKI$_{185}$		23.33	0.1128 (Probable NON-ANTIGEN)	$_{142}$RVAAEYTQK$_{150}$	MHC2Pred	1.528	0.8696 (Probable ANTIGEN)
$_{229}$VGLLFPPGI$_{237}$		32.83	0.2905 (Probable NON-ANTIGEN)	$_{155}$VTKADIAGY$_{163}$		1.009	0.6261 (Probable ANTIGEN)
$_{22}$FGGLPSLKS$_{30}$		45.35	0.4137 (Probable NON-ANTIGEN	$_{172}$SIVIPATVG$_{182}$		1.793	NON-ANTIGEN
$_{33}$VLSEDTIPG$_{41}$		31.16	0.0022 (Probable NON-ANTIGEN)	$_{227}$GAVANLLSD$_{235}$		1.431	NON-ANTIGEN
$_{82}$YVWIPAVIA$_{90}$		34.65	1.1663 (Probable ANTIGEN)	$_{257}$FLIGTQARV$_{265}$		1.507	0.5925 (Probable ANTIGEN)
$_{209}$YRISFITTYK$_{217}$		69.77	0.7222 (Probable ANTIGEN)	$_{290}$SVGGATNLS$_{298}$		1.464	0.9973 (Probable ANTIGEN)
$_{83}$YVWIPAVIA$_{41}$	MHC2Pred	1.607	1.1663 (Probable ANTIGEN)	$_{103}$ITLDRTTGG$_{111}$		1.105	1.5169(Probable ANTIGEN)
$_{88}$AVIAEMGVR$_{96}$		1.52	0.8893 (Probable ANTIGEN)				
$_{136}$RVERMSAIM$_{144}$		1.65	-0.4057 (Probable NON-ANTIGEN)				
$_{115}$DAFKAATPE$_{143}$		1.406	0.8056 (Probable ANTIGEN)				
$_{130}$WFDTWIRVE$_{138}$			0.0030 (Probable NON-ANTIGEN)				
$_{138}$ERMSAIMPD$_{146}$			0.2385 (Probable NON-ANTIGEN)				

[a]Highlight parts related to selected epitopes with more than 0.5 score of VaxiJen for digestion analysis.

Table 2. The List of High Scored Predicted B Cell Epitopes Using Online Software and Their VaxiJen Score[a]

Sequence	Server	SCORE	VaxiJen Score	Sequence	Server	SCORE	VaxiJen Score
LipI32				OmpI1			
$_{119}$AATPEEKS$_{126}$	IEDB	1.368	0.9680 (ProbableANTIGEN)	$_{45}$ITKDGLDAATHYGPVRSS$_{62}$	IEDB	0.816	NON-ANTIGEN
$_{158}$QKLDDDDDGDDTYKEE$_{173}$		1.762	1.5742 (Probable ANTIGEN)	$_{66}$TVGPSDPACFQNPAKPTGEGN$_{86}$		1.46	NON-ANTIGEN
$_{137}$VEERMSAIMPDQIAKA$_{152}$	ABCpred	0.9	0.2977 (Probable NON-ANTIGEN)	$_{141}$WRVAAEYTQKISGG$_{154}$	ABCpred	0.88	1.0726 (Probable ANTIGEN)
$_{127}$MPHWFDTWIRVEERMS$_{142}$		0.77	0.3736 (Probable NON-ANTIGEN)	$_{213}$NIKGGYDILTAAGAGAVANL$_{232}$		0.84	0.8346 (Probable ANTIGEN)
$_{144}$MPDQIAKAAKAKPV$_{157}$		0.91	0.3754 (Probable NON-ANTIGEN)	$_{89}$GVAPRKAIPAENRLITLDRTTG$_{110}$		0.78	-0.1147 (Probable NON-ANTIGEN)
$_{63}$VKPGQAPDGLVD$_{74}$	BepiPred	1.39	1.1553 (Probable ANTIGEN)	$_{34}$LQLDLGQLGGTITK$_{47}$	BCPREDS (BCPred)	0.977	0.5749 (Probable ANTIGEN)
$_{102}$GEIGEPGDGDL$_{112}$		1.71	0.8654 (Probable ANTIGEN)	$_{53}$ATHYGPVRSSNTCTVGPSDP$_{72}$	BCPREDS (AAP)	1	0.8607 (Probable ANTIGEN)
$_{117}$FKAATPEEKSMP$_{128}$		1.11	0.2487 (Probable NON-ANTIGEN)	$_{103}$ITLDRTTGGAINARSTKGAM$_{122}$		1	1.4181 (Probable ANTIGEN)
$_{230}$VGLLFPPGIPGVSPLIHSNP$_{249}$	BCPREDS (BCPred)	1	0.3560 (Probable NON-ANTIGEN)	$_{130}$GYESDFGKYFFWRVAAEYTQ$_{149}$		0.973	NON-ANTIGEN
$_{39}$TIPGTNETVKTLLPYGSVIN$_{58}$	BCPREDS (AAP)	1	0.0138 (Probable NON-ANTIGEN)	$_{165}$IVDMTWGFSSIVIPATVGIK$_{164}$		1	0.7011 (Probable ANTIGEN)
$_{107}$PGDGDLVSDAFKAATPEEKS$_{126}$		1	0.8044 (Probable ANTIGEN)	$_{202}$FNGGWSLNGSNNIKGGYDIL$_{221}$		0.979	0.9594 (Probable ANTIGEN)
$_{184}$IKIPNPPKSFDDLKNIDTKK$_{203}$		1	0.2565 (Probable NON-ANTIGEN)	$_{223}$AAGAGAVANLLSDGTDPVTT$_{242}$		1	NON-ANTIGEN
$_{58}$NYYGYVKPGQAPDG$_{71}$	BCPREDS (FBCPred)	0.999	0.5762 (Probable ANTIGEN)	$_{288}$TQSVGGATNLSPFPAYPIVV$_{307}$		1	1.1034 (Probable ANTIGEN)
$_{100}$PTGEIGEPGDGDLV$_{112}$		1	0.6637 (Probable ANTIGEN)	$_{227}$FIELETIMSAAYAVGKTQSV$_{291}$	SVMTrip	1	0.5332 (Probable ANTIGEN)
$_{47}$VKTLLPYGSVINYYGYVKPG$_{66}$	SVMTrip	1.000	-0.3640 (Probable NON-ANTIGEN)				

[a]Highlight areas related to selected epitopes with more than 0.5 score of VaxiJen for digestion analysis.

Table 3. Protein Digestion Analysis of Final B- and T-Cell Epitopes[a]

Sequence	PI	Mass	Non-Digestive Enzyme	Sequence	PI	Mass	Non-Digestive Enzyme
T Cell, MHCI							
OmpL1				**LipL32**			
210 GSNNIKGGN 218	8.59	908.97	Chymotrypsin, Clostripain, Cyanogen_Bromide, Proline_Endopept, Staph_Protease, IodosoBenzoate, Trypsin_R, AspN , Chymotrypsin (modified)	31 SSFVLSEDTIPGTNETVK 48	4.14	1924.09	Trypsin,Clostripain, Cyanogen_Bromide, IodosoBenzoate, Trypsin_K,Trypsin_R,
272 FIELETIMSAAY 283	3.79	1387.61	Trypsin, Chymotrypsin, Clostripain, IodosoBenzoate, Proline_Endopept, Trypsin_K, Trypsin_R, AspN,	63 VKPGQAPDGLVDGNKKA 79	8.47	1693.92	Chymotrypsin,Clostripain, Cyanogen_Bromide,IodosoBenzoate, Staph_Protease,Trypsin_R,
290 SVGGATNISPTPAY 303	5.24	1380.52	Trypsin, Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Staph_Protease,Trypsin_K, Trypsin_R, AspN,	76 NKKAYYLYVWIPAV 89	9.40	1728.07	Clostripain, Cyanogen_Bromide, Staph_Protease, Trypsin_R,AspN,
109 TGGAINARSTKG 120	11.00	1132.24	Chymotrypsin, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease, AspN, Chymotrypsin (modified),	80 YYLYVWIPAVIAEM 93	4.00	1731.08	Trypsin, Clostripain,Cyanogen_Bromide, Trypsin_K,Trypsin_R, AspN
81 PTGEGNYIGVAP 92	4.00	1077.16	Trypsin, Cyanogen_Bromide, Clostripain, IodosoBenzoate, Trypsin_K, Trypsin_R,AspN,	153 KAKPVQKLDDDDDGDDTYKEERHNK 177	4.70	2960.12	Cyanogen_Bromide, IodosoBenzoate
178 PATVGIKLNVTE 189	6.64	1241.45	Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Staph_Protease, Trypsin_R, AspN	207 RGLYRISFTIYKPG 220	10.28	1658.92	Cyanogen_Bromide, IodosoBenzoate
248 FRTSGIA PNF 257	9.75	1109.25	Cyanogen_Bromide, Trypsin_K, AspN, IodosoBenzoate, Staph_Protease,	232 LLFPPGIPGV 241	5.52	1009.26	Trypsin ,Chymotrypsin, Clostripain,Cyanogen_Bromide,IodosoBenzoate, Staph_Protease,Trypsin_K, Trypsin_R, AspN
				238 IPGVSPLIHSNPEE 251	4.81	1488.66	Trypsin, Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate,Trypsin_K, Trypsin_R, AspN,
				31 SSFVLSEDTIPGTNETVK 48	4.14	1924.09	Trypsin,Clostripain, Cyanogen_Bromide, IodosoBenzoate, Trypsin_K,Trypsin_R,
T Cell, MHCII							
OmpL1				**LipL32**			
103 ITLDRTTGG 111	5.84	933.03	Chymotrypsin, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease,Trypsin_K,	64 KPGQAPDGLVDGNKKAY 81	8.43	1757.96	Clostripain, Cyanogen_Bromide, IodosoBenzoate, Staph_Protease, Trypsin_R,
142 RVAAEYTQK 150	8.59	1065.19	Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Trypsin_K, AspN	83 YVWIPAVIA 91	5.52	1031.26	Trypsin, Clostripain, Cyanogen_Bromide, Staph_Protease, Trypsin_K, Trypsin_R, AspN,
75 IQNPAKPTGEGNYIGVAPR 93	8.59	2016.24	Trypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate,Trypsin_K, Trypsin_R, AspN	88 AVIAEMGVR 96	6.05	945.14	Trypsin, Chymotrypsin, Clostripain, IodosoBenzoate, Proline_Endopept, Trypsin_K, Trypsin_R, AspN, Chymotrypsin(modified),
155 VTKADIAGY 163	5.81	937.06	Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease, Trypsin_R, Chymotrypsin (modified)	115 DAFKAATPE	4.75	949.03	Cyanogen_Bromide, IodosoBenzoate, Staph_Protease, Trypsin_R, AspN,
183 IKLNVTEDA 193	4.37	1002.13	Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Trypsin_R	150 KAAKAKPVQKLDDDDDGDDTYKE 172	4.49	2565.73	Clostripain, Cyanogen_Bromide, IodosoBenzoate, Staph_Protease,Trypsin_R,
233 LSDGTDPVTTREHVRFRTS 251	6.76	2174.36	Cyanogen_Bromide, IodosoBenzoate, Trypsin_K	209 YRISFTIYK 217	9.70	1178.35	Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease, Trypsin_K, AspN,
290 SVGGATNIS 298	5.24	804.86	Trypsin, Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease, Trypsin_K, Trypsin_R, AspN				
B Cell							
OmpL1				**LipL32**			

34 LQLDTGQLGGTITK 47	5.84	1456.70	Trypsin, Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease, Trypsin_K,Trypsin_R	58 NYYGYVKPGQAPDG 71	5.83	1528.64	Trypsin, Clostripain, Cyanogen_Bromide,IodosoBenzoate,Staph_Protease, Trypsin_K, Trypsin_R,
29 ATHYGPVRSSNTCTVGPSDP 48	6.79	2046.20	Cyanogen_Bromide, IodosoBenzoate, Staph_Protease, Trypsin_K	63 VKPGQAPDGLVD 74	4.54	1195.34	Trypsin, Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Staph_Protease, Trypsin_K, Trypsin_R,
103 VTLDRTTGGAINARSTKGAM 122	10.84	1987.22	Chymotrypsin, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease	100 PTGEIGEPGDGDL 112	3.43	1256.29	Trypsin, Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Trypsin_K,Trypsin_R, Chymotrypsin(modified)
165 IVDMTWGFSSIVIPATVGIK 184	5.84	2134.56	Trypsin, Clostripain, Staph_Protease, Trypsin_K, Trypsin_R	119 AATPEEKS 126	4.53	831.88	Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Trypsin_R, AspN, Chymotrypsin(modified),
202 FNGGWSLNGSNNIKGGYDIL 221	5.83	2126.31	Clostripain, Cyanogen_Bromide, Proline_Endopept, Staph_Protease, Trypsin_R	158 QKLDDDDGDDTYKE 172	3.85	1771.72	Clostripain, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease, Trypsin_R,
213 NIKGGYDILTAAGAGAVANL 232	5.83	1889.14	Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease, Trypsin_K,Trypsin_R,				
272 FIELETIMSAAYAVGKTQSV 291	4.53	2158.49	Clostripain, IodosoBenzoate, Proline_Endopept, Trypsin_R, AspN,				
288 TQSVGGATNLSPFPAYPIVV 307	5.18	2018.30	Trypsin, Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Staph_Protease, Trypsin_K, Trypsin_R, AspN				

[a] All colored epitopes related to final selected epitopes with maximum of Non-digestive enzymes; ompL1 protein: pink, green and red region are common epitopes between T cell MHCI class and B cell epitopes but the blue one is the common epitopes among both T and B cells which arranged between 288-307 amino acid residuals. LipL32 protein; gray colored epitopes are common ones among the T cell epitopes and located in 80-93 amino acid residuals. And the blue ones indicate the common ones between both T and B cell epitopes and are in amino acid 64-81 residuals.