Designing a Novel Multi-epitope Peptide Vaccine Against Pathogenic *Shigella* spp. Based Immunoinformatics Approaches

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Abstract

Shigella spp. causes severe diarrhea and dysenteric disease, which known as shigellosis. Until now, no licensed vaccine is available for shigellosis; therefore, development of a novel strategy to produce an effective vaccine is urgently needed. *Shigella* invasion plasmid antigens (Ipas) such as, Ipa B and Ipa D are conserved, cross-reactive and highly immunogenic antigens among *Shigella* spp. Outer membrane protein (Omp) A family of *Shigella* spp. incites effective immune; therefore, Ipa B, Ipa D and Omp A are superb candidate antigens for designing a cross-protective vaccine against *Shigella*. In this study, different immunoinformatics approaches were used to design a novel multi-epitope peptide vaccine against *Shigella* spp. CD4⁺ T cell immune response has an essential role in eradication of shigellosis; in this regard, Ipa B, Ipa D and Omp A were analyzed to identify Helper T cell lymphocytes (HTL) epitopes by various immunoinformatics servers. D0 and D1 flagellin domains of *salmonella enteric* were used as Toll like receptor 5 (TLR5) agonist which plays a role as mucosal adjuvant. All mentioned segments were fused together by proper amino acid linkers. Linear and conformational B cell epitopes were also determined in vaccine construct. Totally, we believe that the designed vaccine candidate is able to induce cellular and humoral as well as mucosal secretory IgA (sIgA) immunity against shigellosis.

Keywords Diarrhea · Shigellosis · Vaccine · Epitope · Immunoinformatics

Introduction

Shigella spp. are gram-negative, immobile and facultative anaerobic bacteria (Killackey et al. 2016). The pathogen is limited to human and primate (Mallett et al. 1993). Global reports indicate that shigellosis leads to about 169 million morbidities in developing countries (Killackey et al. 2016). According to their Lipopolysaccharide (LPS) chain, it has been divided into four pathogenic species, including: (Livio et al. 2014) *Shigella flexeneri*; the most affluent species, *Shigella sonnei*; the major agent for shigellosis in the industrial

countries, *Shigella dysenteriae* that causes pandemic dysentery, and *Shigella boydii*, which causes 7% of the shigellosis in less-developed countries (Kotloff et al. 1999). In the context of infection mechanism, *Shigella* spp. is bound and enter into M cell (an epithelial cell in the intestine) by type three secretory system (TTSS) (Phalipon and Sansonetti 2007), then propagates in the host epithelial cells and spreads to adjacent cells by Viral G (Vir G) protein (Mani et al. 2016). Another invasion mechanism which is used by *Shigella* spp. is Shiga toxin that is comprised of A and B-subunits (Gupta et al. 2011). The toxin is bound to Glycosphingolipid (GM) in the intestinal cell membrane via B-subunit, which allows penetrating of A-subunit into the host cell and subsequently leads to inhibit translation process by the ribosome inactivating (Falguieres et al. 2001). Nowadays, the appearance of antibiotics resistant strains has been caused serious concerns about shigellosis treatment, all over the world (Seidlein et al. 2006). Scientific evidence demonstrates that different vaccines have been used against *Shigella* bacteria (Mani et al. 2016). Killed and inactivated bacteria are the important types of vaccine that are used against *Shigella* infection, and they are able to stimulate immune system effectively;

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(Ashkenazi and Cohen 2013; McKenzie et al. 2006) but they have some disadvantages such as reactogenicity, instability in different storage condition and limited protection period (Nezafat et al. 2016). Until now, no approved prophylactic or therapeutic vaccine against shigellosis is available on the market (Ashkenazi and Cohen 2013). In this regard, there is an urgent need to find a new approach to struggle with these pathogenic bacteria. Multi-epitope peptide vaccines consisting of cytotoxic T lymphocytes (CTL) and helper T lymphocytes (HTL) epitopes that are designed by immunoinformatics tools are considered as an important medication against pathogenetic bacteria like *Staphylococcus aureus*, *Helicobacter pylori*, *Kelebsiella pneumonia*, *Shigella* spp, *Human papillomavirus* and *Vibrio cholerae* (Hajighahramani et al. 2017; Nezafat et al. 2016, 2017; Sette et al. 2001; Mahmoodi et al. 2017; Shahbazi et al. 2016; Farhadi et al. 2015). Some advantages such as high specificity, cost-effective production and stability in various conditions outstand these vaccines compare to others (Sette et al. 2001). Low immunogenicity and enzymatic degradation in serum are some disadvantages of epitope vaccines (Mahmoodi et al. 2016). For designing an effective multi-epitope peptide vaccine, dominant epitopes are selected from immunogenic proteins. Invasion plasmid antigens (Ipas) are important effectors in TTSS and considered as protective antigens (Martinez-Becerra et al. 2013). Ipa proteins are expressed by all virulent *Shigella* serotypes (Levine et al. 2007). Ipa D controls the formation of a tip needle for TTSS (Martinez-Becerra et al. 2013) and Ipa B interact with the host cell membrane to form a pore in it (Blocker et al. 1999). In this regard, Ipa proteins can be considered as ideal vaccine candidate antigens. Outer membrane proteins (Omps) which are immunogen and cross-reactive among *Shigella* serotypes, are another vaccine target (Pore et al. 2011; Pore and Chakrabarti 2013). Omp A is considered as protective, cross-reactive and conserved antigen among *Shigella* spp. and because of its location on the cell surface, it is highly immunogen (Pore et al. 2011). Adjuvants are one of the important components in a vaccine construct that stimulate the immune system and modulate it towards the desired immune responses (HTL or CTL) (Apostólico 2016). Due to the low immunogenicity of multi-epitope peptide vaccines, adjuvants are incorporated into vaccine construct (Smith et al. 2003). Toll-like receptors (TLRs) are conserved receptors, expressed on the immune and nonimmune cells (Kumar et al. 2009) and known as a class of the pathogen recognition receptors (PRR) which recognize specific molecules of pathogens as an agonist (Smith et al. 2003). Bacterial flagellin, as a TLR5 agonist, is able to efficiently activate both innate and adaptive immune systems via binding to TLR5; therefore it has been used as an effective mucosal vaccine adjuvant in different researches (Honko et al. 2006; Mizel et al. 2009; Mori et al. 2012). In this study, we harnessed different bioinformatics approaches

for designing a novel multi-epitope peptide vaccine against pathogenic *Shigella* spp. Our vaccine contains several T-cell, B cell and interferon gamma inducing epitopes. In order to enhance vaccine immunogenicity D0 and D1 flagellin domains were used as a strong natural adjuvant bound to TLR5.

Methods

The overall procedures used for vaccine design is demonstrated in Fig. 1.

Sequence Retrieval

Related sequences of Ipa B (Accession no P18011), Ipa D (Accession no. P18013), Omp A (Accession no. Q7X0I7) and flagellin of *salmonella enteric* subs. Enteric serovar Dublin were retrieved from the universal protein resource (uniprot) database (<http://www.uniprot.org/>).

Immunoinformatics Analyses

MHC-II Binding Epitope Prediction

MHC-II binding epitopes were selected via four different servers (IEDB, MHCpred, MHC2pred and RANKPEP). Mouse MHC-II alleles, namely H-2IAb, H-2IAd, and H-2IAs were chosen for MHC-II binding epitopes prediction. The IEDB at (<http://tools.iedb.org/mhci/>) employs four prediction methods, including consensus method, sturniolo's method, stabilized matrix method (SMM)-align and average relative binding (ARB) for MHC-II binding epitope prediction (Zhang et al. 2008). MHC-II binding epitope selection was also performed by MHCpred at <http://www.ddgpharmfa.com/mhcpred/MHCPred/>, which is based on allele specific-quantitative structure activity relationship (QSAR) models (accuracy about 90%) (Guan et al. 2003). The MHC2pred uses support vector machine (SVM) method with the accuracy of about 80% for 42 alleles in MHC-II binding epitope selection (Bordner and Mittelman 2010). RANKPEP at <http://imed.med.ucm.es/Tools/rankpep.html> predicts MHC-II binding epitopes based on the position specific scoring matrices (PSSMs); the binding threshold for the top scoring MHC-II epitopes is 4–6% (Reche et al. 2002).

Linear B-Cell Epitope Prediction

Three servers (ABCpred, BCpred and BepiPred) were used for linear B-cell epitope prediction. The ABCpred at <http://www.imtech.res.in/raghava/abcpred/> predicts linear B cell epitopes by artificial neural network (ANN) which is based on applying fixed length patterns; the accuracy of ABCpred

Fig. 1 A summary of the methods that were employed for in silico design of multi-epitope peptide vaccine

is 65.93%, and the default threshold 0.51 was used (Thomas et al. 2004). The BCpred server at <http://ailab.ist.psu.edu/bcpred/predict.html> uses subsequences kernel (SSK) and a support vector machine (SVM) with accuracy and specificity, 74.57 and 75% respectively (EL-Manzalawy et al. 2008). BepiPred at <http://www.cbs.dtu.dk/services/BepiPred/> combines hidden markov model and a propensity scale method for prediction of linear B cell epitopes. The score threshold is 0.35% (Larsen et al. 2006).

Selection of the Epitope Segments

The obtained results were compared together, and the regions with the highest overlaps were determined. Selected epitopes can induce various types of immune responses, including HTL, B cell and INF-gama responses.

Prediction of Interferon-Gamma Inducing Epitopes

For designing an effective vaccine, interferon-gamma inducing epitopes from the selected MHC-II binding epitopes were predicted. IFNepitope server at <http://crdd.osdd.net/raghava/ifnepitope/> employs a dataset that composed of 3705 IFNepitope inducing and 6728 non-inducing MHC class II binders (Dhanda et al. 2013). This web server predicts interferon-gamma inducing epitopes based on three major modules, including predict, design and scan.

Vaccine Properties Evaluation

Antigenicity Evaluation

To evaluate the antigenicity of final vaccine construct two servers were used. ANTIGENpro is available at <http://scratch.proteomics.ics.uci.edu/> that predicts antigenicity based on the results obtained by protein microarray data analysis. ANTIGENpro is a sequence-based, alignment free and pathogen independent software. The accuracy is 75.51 with a prediction threshold of 0.5 (Magnan et al. 2010). Another server is Vaxijen v2.0 that predicts antigenicity based on the target organism such as bacterial, viral and tumor antigen. The server uses a new alignment-independent approach for antigenicity prediction. It's accuracy varies from 70 to 89% according to target organism (Doytchinova and Flower 2007).

Allergenicity Evaluation

To evaluate the allergenicity of the designed vaccine two servers, SDAP and PREAL, were used. Cross-referenced accesses to the sequences, structures and IgE epitopes of allergenic proteins were provided by the structural database of allergenic proteins (SDAP) at <https://fermi.utmb.edu/> (Ivanciuc et al. 2003). PREAL (Prediction of Allergenic Proteins) server at <http://gmobl.sjtu.edu.cn/PREAL/index.php> predicts allergenicity based on different biochemical and physicochemical properties of proteins, sequential features

and subcellular locations with 93.42% accuracy (Roden et al. 2000).

Physicochemical Properties Evaluation

The physicochemical features of designed vaccine, including molecular weight (Mw), theoretical pI, instability index, half-life, total number of positive and negative residues and grand average of hydropathicity (GRAVY) were calculated by protparam tool (<http://web.expasy.org/protparam>) (Gasteiger 2005).

Tertiary Structure Analysis

Homology Modeling

I-TASSER server at <http://zhanglab.ccmb.med.umich.edu/I-TASSER/> was used for homology modeling. The modeling process is performed in four steps: (1) Threading template identification (To identify structural templates based on the query protein sequence), (2) Monte Carlo simulation method and *ab initio* modeling, (3) Model selection and refinement [selection of the model by structure decoys and refinement using fragment-guided molecular dynamics simulation (FG-MD)], (4) Structure-based function annotation was performed via applying COACH approach. The obtained models are identified with a confidence score, known as C-score (the higher the value indicates the higher the confidence) (Yang et al. 2015).

Refinement of the 3D Modeled Structure

Two servers: GalaxyRefine and 3Drefined were used to refine the best 3D model resulted from I-TASSER. GalaxyRefine server at <http://galaxy.seoklab.org/cgi-bin/submit.cgi>, applies mild and aggressive relaxation methods to refine the 3D model. Mild relaxation method refines side chains while secondary structure segments and loops are refined by the aggressive method (Shin et al. 2014). The 3Drefined at <http://sysbio.rnet.missouri.edu/3Drefine/> is a server for efficient protein structure refinement. This server optimizes hydrogen binding network and performs atomic-level energy minimization (Bhattacharya et al. 2016).

Validation of the 3D Structure

3D refined vaccine structures were validated using Verify3D and ERRAT servers. The ERRAT server (<http://services.mbi.ucla.edu/ERRAT/>) performs differentiating between correctly and incorrectly regions of protein structures based on non-bonded atomic interactions (Colovos and Yeates 1993). Verify 3D (<http://services.mbi.ucla.edu/Verify3D/>) was used to validate the 3D structure of vaccine through

measuring the compatibility of the 3D protein model with its sequence (Lüthy et al. 1992).

Prediction of Conformational B-Cell Epitopes

Conformational B-cell epitopes were predicted by DiscoTope server from the 3D model. DiscoTope method is based on the protein structure and epitope propensity scores. Its sensitivity and specificity are 0.47 and 0.75 respectively, the default threshold (− 3.7) (Kringelum et al. 2012).

In Silico Cloning

To perform cloning and expression of protein vaccine in a vector, the reverse translation and codon optimization of vaccine protein sequence were performed by Codon Usage Wrangler server (<http://www.mrc-lmb.cam.ac.uk/ms/methods/codon.html>), also using the GenScript Rare Codon Analysis Tool (<https://www.genscript.com/tools/rare-codon-analysis>), different features of sequences, including Codon Adaptation Index (CAI), GC content, and Codon Frequency Distribution (CFD) were evaluated.

Results

Immunoinformatics Assays

MHC Class II Binding Epitopes

Ipa B, Ipa D and Omp A were used for MHC class II binding epitopes prediction by four different servers (IEDB, MHCpred, MHC2pred and RANKPEP) (Tables 1, 2, 3). Also, the high-scored and overlapped regions between selected epitopes were determined (Table 4).

Interferon-Gamma Inducing Epitopes and Linear B Cell Epitopes Prediction

The predicted peptide segments were submitted to the IFNepitope server to project interferon-gamma inducing epitopes (Table 5). Linear B cell epitopes were selected among the predicted HTL epitopes by ABCpred, BCPred and BepiPred; the results are demonstrated in Table 6.

Evaluation of Physicochemical and Immunological Properties of the Designed Vaccine

The physicochemical properties of the designed vaccine such as molecular weight, amino acid composition, instability index, half-life, aliphatic index, theoretical pI and GRAVY, as well as the immunological properties, including antigenicity and allergenicity are shown in Table 7.

Table 1 Prediction of HTL epitopes from Ipa B by four different servers

Server	Start–end position	Best ranked epitope in the region
IEDB		Percentile rank ^a
	322–354	2.99
MHC2pred		Score ^b
	395–421	0.85
	90–104	0.71
	436–448	0.12
MHCpred		–Log IC 50 ^c
	546–562	8.4
	3–117	8.15
	158–183	7.6
	238–280	7.25
	571–580	7.07
	288–541	6.99
	121–133	6.6
	190–206	6.16
		Score ^b
RANKPEP	482–521	19.23
	135–145	15.06
	67–76	10.96
	292–301	10.78
	334–357	10.43
	412–433	9.56
	533–545	9.06

^aLow percentile rank = good binder^bHigh score = good binder^cHigh –Log IC₅₀ = good binder

Homology Modeling

The 3D structure model of the designed multi-epitope peptide vaccine was obtained from the I-TASSER server. Five models with the C-score value in the range of [–5, 2] were suggested by the server. The model with the higher C-score is the best model; therefore, a model with C-score value 0.11 was selected for more evaluations.

3D Structure Validation The potential errors and quality of the initial and refined 3D model were evaluated by ERRAT and Verify 3D servers. The ERRAT results demonstrated that the overall quality factor of the initial 3D model is 88.512 (Fig. 2a) and based on Verify 3D score, 57.43% of residues had an average 3D-1D score greater than 0.2 (Fig. 3a). Among the refined models, which were refined by GalaxyRefine, model no. 3 was selected as the best 3D model; the ERRAT factor and Verify 3D score residues of the final model were calculated 94 and 65%, respectively (Figs. 2b, 3b); therefore, this 3D model was used for the fol-

Table 2 Prediction of HTL epitopes from Ipa D by four different servers

Server	Start–end position	Best ranked epitope in the region
IEDB		Percentile rank ^a
	69–76	1.98
MHC2pred		Score ^b
	127–145	3.31
	293–311	6.71
		Score ^b
MHCpred		–Log IC 50 ^c
	68–81	0.85
	189–213	0.82
	222–235	0.53
		–Log IC 50 ^c
	214–331	7.95
	159–198	7.32
	1–83	6.45
	87–125	6.25
		Score ^b
RANKPEP	112–120	19.88
	160–176	14
	63–76	12.71
	242–258	12.61

^aLow percentile rank = good binder^bHigh score = good binder^cHigh –Log IC₅₀ = good binder**Table 3** Prediction of HTL epitopes from Omp A by four different servers

Servers	Start–end position	Best ranked epitope in the region
IEDB		Percentile rank ^a
	252–271	2.23
MHC2pred		Score ^b
	113–127	0.78
	316–327	0.63
	177–207	0.46
MHCpred		–Log IC 50 ^c
	7–327	7.29
RANKPEP		Score ^b
	70–83	14.15
	258–271	12.39
	143–155	10.66
	168–200	10.46
	99–111	10.45

^aLow percentile rank = good binder^bHigh score = good binder^cHigh –Log IC₅₀ = good binder

Table 4 Final selected HTL epitopes from Omp A, Ipa D, and Ipa B antigens

Protein	Start–end position	HTL epitopes
Omp A	113–127	GASFKDHDGTGVSPVF
	143–155	LEYQWTNNIGDAN
	177–207	GEAAPVVAPAPAEVQTKHFTLKSDVLFNFN
Ipa D	68–81	KTLTKTSLEEIALH
	112–120	ELLHSAPKE
	160–176	YTQMYQDFSAVLSSLAG
	222–235	QANKWLTELGGTIG
	242–258	GYVVSINMTPIDNMLK
Ipa B	293–311	NLQTLVQKYSNANSIFDNL
	67–76	LKAPKSLNAS
	90–104	LGEKSLTALTNKITA
	292–301	EYAAEVRKAE
	334–357	LALAAVGLALMVTDIAVQAATGNS
	395–433	KAKMIGSILGAIAGALVLVAAVVLVATVG-KQAAAKLAEN
	436–448	KIIGKTLTDLIPK
	533–545	EQLSKYISEAIEK

Table 5 Analysis of selected HTL epitopes potentiality are able to inducing IFN gamma secretion, by IFNepitope server

Protein	Start–end position	IFN gamma score ^a	Sequence
Omp A	177–206	+1	GEAAPVVAPAPAEVQTKHFTLKSDVLFNF
Ipa D	159–176	+2	SYTQMYQDFSAVLSSLAG
	242–258	+0.37	GGYVVSINMTPIDNMLK
	293–311	+0.26	NLQTLVQKYSNANSIFDNL
Ipa B	292–301	+0.43	EYAAEVRKAE

^aPositive score = the epitope potentiality to inducing IFN gamma secretion

lowing studies (Fig. 4). According to the above-mentioned results, the quality of the 3D structure was improved after refinement. The initial and refined 3D models are compared in Fig. 5.

Prediction of Conformational B-cell Epitopes

Conformational B cell epitopes were determined by Discotope server. Out of 404 total residues, 76 conformational B-cell epitope residues were identified in the final vaccine construct (Table 8).

In Silico Cloning

Reverse translation and codon optimization of the designed vaccine construct were performed by the Wrangler server. The important properties of the gene sequence in order to achieve a high-level protein expression in the *E. coli* host, including Codon Adaptation Index (CAI), GC content, and Codon Frequency Distribution (CFD), were determined by the GenScript Tool. CAI of optimized nucleotide sequence was 1, a CAI of >0.80 is considered proper for expression in the selected host (Fig. 6a). The average GC content of

Table 6 Prediction of linear B cell epitopes by three different servers (BCpred, ABCpred, BepiPred)

Proteins	Start–end position	Best ranked epitope in the region
Omp A		Score ^a
	145–203	0.98
	278–325	0.96
	14–76	0.91
	240–259	0.77
	102–126	0.69
Ipa D		Score ^a
	200–219	0.98
	231–259	0.93
	126–141	0.93
	174–194	0.84
	268–310	0.81
	5–49	0.75
Ipa B	57–119	0.57
		Score ^a
	1–80	0.98
	512–554	0.86
	281–304	0.8
	476–492	0.76
	99–272	0.72

^aHigh score = good binder

Table 7 Physicochemical and immunological properties of designed vaccine construct

Physicochemical properties	Result
Instability Index	32.06
GRAVY	−0.34
Theoretical pI	5.01
Aliphatic Index	87.50
Molecular weight	42562.27
No. of amino acids	404
Instability Index	32.06
Total number of negatively charged residues	40
Total number of positively charged residues	33
Immunological properties	
Antigenicity	
ANTIGENpro	0.95
VaxiJen	0.64/probable antigen
Allergenicity	
SDAP	No-allergen
PREAL _W	Non-allergen

our vaccine sequence is 58.55% (Fig. 6b), the percentage GC content between 30–70% is optimal. Codons with a frequency distribution (CFD) < 30% are suitable for transcription and translation with high efficacy. The results of 100% CFD value was obtained for the sequence (Fig. 6c).

Discussion

Different species of *Shigella* bacteria lead to dysenteric and diarrheal disease by invading to intestinal epithelium cells (Kweon 2008). The traditional approach for treating *Shigella* infection is using of different antibiotics such as Nalidixic acid, Ampicillin and Ciprofloxacin (Kosek et al. 2010); however, nowadays increasing of antibiotic resistance *Shigella* spp. has been known as a major challenge in the worldwide (Ashkenazi and Cohen 2013). Hence an effective therapeutic approach should be considered for *Shigella* infection. Until now, several types of *Shigella* vaccines including live attenuated, polysaccharide conjugated, killed bacteria and recombinant vaccines have been presented against *Shigella* (Martinez-Becerra et al. 2012). In spite of extensive researches to develop an effective *Shigella* vaccine, up to date, no licensed vaccine is available on the market, therefore; there is an essential need to search for new generation of vaccines against *Shigella* (Ashkenazi and Cohen 2013). Multi-epitope peptide vaccines that are designed with the help of bioinformatics tools have the high degree of accuracy (Davies and Flower 2007). Moreover, harnessing bioinformatics approaches for designing multi-epitope vaccines are less expensive and time-saving (Mahendran et al. 2016). These vaccines are used as a novel modality to fight different bacterial pathogens such as *Helicobacter pylori*, *Staphylococcus aureus*, *Vibrio cholerae*,

Fig. 2 The overall quality factor plot (ERRAT) of **a** the initial model is 88.512% and **b** the final model after refinement is 94%; regions of the 3D model that can be rejected at the 99% level are shown with

gray lines in ERRAT plot, and regions that can be rejected at the 95% confidence are demonstrated with black lines. An overall quality factor value for good high resolution structures is about 95% or higher

Fig. 3 Verify 3D score of predicted model. The Verify 3D of **a** the initial model is 57.43 and **b** the model after refinement processes is 65. Reliable residues have an average 3D-1D score greater than zero

Fig. 4 The refined 3D model of the multi- epitope peptide vaccine. The 3D structure of the designed vaccine was determined by homology modeling using I-TASSER server, and was refined by GalaxyRefine and 3Drefine servers

Fig. 5 Superimposition of 3D protein vaccine structure, the initial model and the refined model are colored in magenta and green, respectively. After overall refinement processes, the quality of refined 3D model was improved

Shigella spp. and many others (Nezafat et al. 2016, 2017; Shahbazi et al. 2016; Sharma et al. 2016). As mentioned

in introduction, multi-epitope peptide vaccines compare to other types vaccines have some advantages but one of the most important drawbacks related to such vaccine is their low immunogenicity (Groot 2009). To conquer this drawback, adjuvants are added to multi-epitope peptide vaccines construct that play a significant role in stimulation of immune system as well as vaccine delivery (Farhadi et al. 2016; Liu et al. 2008). Toll-like receptors (TLRs) are bound to pathogen-associated molecular patterns (PAMPs) are caused activation of innate immune responses (Duthie et al. 2011). Based on the scientific evidence, bacterial flagellin via binding to TLR5 triggers antigen-presenting cells (APC) to induce innate immune system and acts as a mucosal adjuvant (Lycke 2012). Therefore, vaccines based flagellin adjuvant can be administrated via the nasal and vaginal routes (Moyle 2017). Most experimental evidence suggests that bacterial flagellins consists of four different domains namely D0, D1, D2 and D3, D2 and D3 are considered as the variable and central domains. The D0 and D1 domains of flagellin are located in the N and C terminal parts of the flagellin and contribute to TLR5 activation (Smith et al. 2003; Song 2017). Therefore, in this study, D0 and D1 domains of *Salmonella enterica* subsp. *enterica* serovar Dublin which have high affinity for binding to TLR5 were used as an adjuvant in vaccine construct. An appropriate vaccine against pathogenic bacteria should provide broad protection against different spp (Martinez-Becerra et al. 2012). Ipa proteins play a critical role in *Shigella* pathogenesis and are highly conserved among *Shigella* spp

Table 8 Identification of conformational B-cell epitopes from 3D protein vaccine structure using DiscoTope

Residues number	Amino acid	Contact number	DisCoTope score
38–40	I–N–S	0–11–2	–2.629, –3.275, –2.770
43–47	N–N–A–A–G	2–6–4–0–7	–2.350, –2.061, –2.645 2.767, –3.329
49	A	3	–3.500
59	K	6	–3.302
62–63	T–Q	9–10	–2.982, –3.272
66	R	9	–2.722
101–105	N–G–T–N–S	16–0–2–21–3	–3.349, –1.120, –0.938, –3.291, –0.248
107–108	S–N	1–10	–1.772, –2.324
111	S	11	–2.656
131	Q	5	–3.137
133–134	N–G	12–0	–1.952, –1.378
143	Q	7	–3.691
145	K	7	–3.341
151–153	N–G	6–0	–2.882, –0.729, –1.995
187–197	V–R–K–A–E–G–P–G–P–P	2–21–7–13–13–15–8–10–0–5–2	–1.138, –3.550, –1.945, –2.530, –2.114, –1.869, –1.096, –0.395, 0.634, –1.316, –2.193
199	A	5	–3.390
208–214	A–P–E–V–Q–T–K	5–3–9–10–0–0–5	–3.662, –2.362, –3.444, –3.576, –1.741, –1.852, –3.202
219	K	0	–3.570
224–236	F–N–F–G–P–G–P–G–S–Y–T–Q–M–Q	6–8–0–7–6–16–4–3–2–22–13–12–10	–2.827, –1.634, –0.189, –1.628, –1.850, –2.002, 0.618, 0.324, 0.336, 0.618, 0.324, 0.336, –2.799, –1.248, –1.701, –1.170
238	P	11	–3.563
248–251	A–G–G–P	0–6–15–10	–2.269, –2.328, –3.007
253	P	9	–3.015
263–265	Q–N–P	4–1–0	–2.858, –2.780, –2.920
287	T	2	–3.343
301	N	9	–3.528
351	T	6	–3.471
404	R	3	–3.309

(Turbyfill et al. 2008). Ipa B and D have been demonstrated are able to stimulate immune responses in animal models of *Shigella* infection (Heine et al. 2013). In addition, antibody against Ipa proteins after human *Shigella* infection was also detected (Barry et al. 2013), therefore, Ipa proteins can be considered as superb candidate antigens in vaccine development. Another *Shigella* protective antigen is Omps; Omp A in *Shigella flexneri* 2a causes to elicit Th1 immune response against shigellosis in animal models (Mukhopadhyaya et al. 2006). In this regard, we used Ipa B, Ipa D and Omp A as candidate antigens for epitope selection. Although, antigen-specific T CD8⁺ possesses a primary role in stimulation of adaptive immune system against cytosolic bacterial infection, T CD8⁺ response does not involve in adaptive immune response against *Shigella* infection (Jehl et al. 2011). Accumulating evidence indicate that, T CD4⁺ response has a critical role in eradicating of *Shigella* infection (Mukhopadhyaya

et al. 2006); therefore immunodominant HTL epitopes were selected from the mentioned antigens (Tables 1, 2, 3, 4). In addition to cellular immune responses, induction of humoral immunity against *Shigella* infection has an important role in eradication of it, so to reach this goal, linear and conformational B cell epitopes were determined from the antigenic protein sequence and 3D structure of the peptide vaccine respectively (Tables 6, 8). HTL epitopes activate T-helper cells, including Th1, Th2 and Th17 (Rincón and Flavell 1997; Agnello et al. 2003). Specific type of cytokine is secreted by different type of helper cells, as an example, IFN-gamma that is essential for eradication of intracellular pathogens is released by Th1 (Dhanda et al. 2013); therefore, the presence of IFN-gamma inducing epitopes in selected HTL epitopes were evaluated (Table 5). Linkers are the essential components of the multi-epitope peptide vaccines (Le-Barillec et al. 2005) that have functional

Fig. 6 Investigation of important parameters of the codon-optimized gene for high-level protein expression in *E. coli* host. **a** CAI of gene sequence is 1.00. A CAI of >0.8 is considered as good for expression in selected host. **b** The average GC content of the sequence is 58.55%. **c** Codon frequency distribution (CFD) value of gene sequence is 100%

and structural roles in vaccine construct (Bai et al. 2005; Aurora et al. 1997); as an example, EAAAK motif that has α -helical structure and is known as a rigid linker, makes distance between domains (Takamatsu et al. 1990). Accordingly, EAAAK was used to join D0 and D1 domains of flagellin to selected HTL epitopes in our designed vaccine construct (Fig. 6). Also, GPGPG motif that is known as a flexible linker and leading enhancement of antigen presentation to MHC II molecules (Livingston et al. 2002) was employed in designed vaccine to link HTL epitopes together (Fig. 6). The important properties of the designed vaccine were evaluated by bioinformatics tool (Table 7). The 3D structure of designed vaccine was determined and evaluated

by different servers. Based on the obtained results, the initial 3D model was refined, and then the best refined model was selected (Fig. 4), for identification of conformational B cell epitopes (Table 8). In this study the high immunogenic antigens from *Shigella* spp. including Ipa B, Ipa D and Omp A were analyzed bioinformatically to select HTL and B cell epitopes; furthermore, Flagellin domains were applied as a natural adjuvant. The immunological properties of designed vaccine showed that the designed vaccine is potent immunogen and non-allergen. We expect that our designed multi-epitope peptide candidate vaccine could be able to eradicate shigellosis via triggering cellular and especially humoral immune response.

Fig. 7 Schematic diagram of vaccine construct consists of adjuvant (D0 and D1 domains of flagellin in N and C terminal), HTL epitopes, which were selected from Ipa B, Omp A and Ipa D antigens and fused together by proper linkers

Conclusion

Developing multi-epitope peptide vaccines against pathogenic bacteria have attracted more attentions in recent years. In this study, we used in silico techniques to design a novel multi-epitope peptide vaccine against *Shigella* spp. In order to trigger T CD4⁺ immune response, that is critical in fighting *Shigella*, HTL epitopes were selected from conserved antigens (Ipa B, D) and Omp A in *Shigella* spp. But in vitro and in vivo immunological tests are needed to evaluate the efficiency of designed multi-epitope peptide vaccine (Fig. 7).

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Compliance with Ethical Standards

Conflict of interest The author(s) confirms that this article content has no conflict of interest.

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