Immunoinformatics and structural vaccinology approach to design a Multi-Epitope Subunit Vaccine against Francisella tularensis through In Silico modeling

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Research Article

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Abstract

Background

Francisella tularensis is a category A pathogen with the maximum infectious and death rates, demanding the development of a vaccine. In the last eight years, growing interest in F. tularensis has resulted in the generation of data about the pathogen and its host binding method. So far, extensive information is available, but there is no authorized vaccination on the market.

Method

Using in silico techniques, present study design to develop multi-epitope vaccine. From F. tularensis, B-cell and T-cell epitopes were predicted to design a vaccine. The selection of epitope was selected on basis of lower percentile score, antigenicity, Non-toxicity, and immunogenicity scores. The 193 amino-acid long vaccine construct was obtained by joining the epitopes through EAAAK, CPGPG and AAY Linkers.

Result

Our vaccine represented overall the good stability, expression, immunostimulatory capabilities and solubility. Moreover, molecular docking and MD simulation exhibited strong interaction and stability of polypeptide vaccine construct.

Conclusion:

Design vaccine was verified as efficient in different computer-based immune response investigations and population coverage was good. But designed vaccines require scientific consensus and require in vitro experimental work also.

1 Introduction

F. tularensis causes zoonotic disease tularemia was discovered in 1911 during plaque-like disease in California. *F. tularensis* infect humans, fish, birds, and invertebrates [1]. *F. tularensis* contain 3 subspecies, type A (tularensis), type B (holarctica), and mediasiatica different from each other in terms of a life cycle, reservoirs, and geographic distribution. In humans, both type A and B cause tularemia. The disease is transmitted by eating contaminated food and drinking water from rivers, lakes, and ponds [2, 3]. Tularemia caused by *F. tularensis* highly reported in Iran such as Azerbaijan, Kazakhstan, Armenia, and Turkmenistan [4], U.S and Turkey [5]. Due to its high mortality and infectious nature, *F. tularensis* were highly studied in the respiratory tract. *F. tularensis* pathogen infect via gastrointestinal tract through ingestion and by the skin through bites of infected arthropods, insect, or handling infected animals [6]. These infection result in other 2 clinically form of tularemia such as oropharyngeal and ulceroglandular. These disease are further effect the stomach ulcers, fever, and swollen and skin ulcers [7].

F. tularensis showed resistance to different antibiotics such as Penicillin, Polymyxin B, Erythromycin, and Azithromycin. This type of resistance is mainly dependent on different strains and species, and also depends on their chromosomal genes expression. *F. tularensis* was found to be showed resistance to monobactams by the same beta-lactamase genes [8]. F. tularensis displayed its resistance to carbapenem antibodies which are proved by the *F. tularensis blaA2* gene [9]. However, the development of multidrug resistance vaccines may reduce multidrug efficacy against *F. tularensis*, therefore different approaches like immunization and vaccination are used to activate an immune response against disease [10].

Two evidence are used to support *F. tularensis* vaccine development. The first one is immunospecific protection against reinfection [11, 12] second is immunization along with a live vaccine strain that showed efficacy for wild-type humans. *F. tularensis* is a dangerous intracellular pathogen that led to finalized that cell-mediated immunity is important for protection. This assumption was born out in different research studies. Antibodies' roles are also established, which suggests a development of successful vaccine is important for both cell-mediated response and humoral. The demand for an effective *F. tularensis* vaccine is clear; in a short period, the successful vaccine will be safe for protective immunity. Therefore, in the present study immunoinformatics approaches are used to design an effective vaccine against F. *tularensis*. The step by step analysis of the vaccine design against F. *tularensis* are highlighted in the Fig. 1.

2 Materials And Methods

2.1 Retrieval of protein sequence

A web server NCBI was employed to download protein sequence of *F. tularensis* with the accession number (CAG44634.1). The Physical and Chemical properties of the protein were determined by EXPasy ProtParm [14]. To determine antigenicity of protein VaxiJen server 2.0 was employed [15]. VaxiJen performs the antigenicity analysis of a protein using the information based on physicochemical properties

2.2 T-cell epitope screening

The IEBD tool (http://www.iedb.org/) was utilized to predict T-cell epitopes, MHC-1 and MHC-2 respectively [16].

2.3 Assessment for B-cell epitope

The IEDB tool was used to anticipate B-cell epitopes. The highest rank epitopes were selected via VaxiJen tool and Allergenicity pattern were checked by AllerTop.

2.4 Designing Multi-Epitope Vaccine Construct

Carefully chosen high-affinity B-cell and T-cell epitopes were connected in a sequence-wise manner with the help of different connecting sequences known as “linkers” to construct the **Multi-Epitope Vaccine**. Epitopes were used for constructing vaccines and cluster formation. Both adjuvants and TLR-9 interaction induced immune interaction in the host. (Bhattacharya, Sharma, *et al.*, 2020) confirmed that epitopes bind with each other through linkers EAAAK, CPGPG and AAY used to combine with MHC-1 and MHC-2 respectively [17].

Constructed vaccine

EAAAKFIAGKEGTPNEIEGLEERLVSRFGFNHKDPTIEIKIQEKKVKIDNIQRIRVKDLTSNQRSRNCPGPGFIAENVRTNVDSFVVGDANKVVADFYRIR
ARPRQIAMSLEQNSNLFTVDSFVVGDANKAAYFFSTTSVEIIKIQEKKVKKTWIKPIHVRPRQIAMSLYGL
TVSVDMYRSADILLIANKIARAAAMQVSINGDANKIARAAAMQVSNKIARAAAMQVSINPHHHHHH

2.5 Examination of Allergenicity and Antigenicity

An online tool AllerTop v 2.0 was utilized to confirm the non-allergic behavior of obtained vaccine construct [18]. VaxiJen 2.0 was exploited for antigenicity behavior of vaccine constructs [15].

2.5.1 Physicochemical Aspects and Solubility analysis

Physiochemical properties of vaccine construct were identified with help of the EXPasy tool (https://web.expasy.org/protparam/), examined aliphatic index, molecular weight, grand average of the hydropathcity GRAVY, solubility, instability index of obtained vaccine constructs. secondary structure was checked via PSIPRED v3.3 server (http://bioinf.cs.ucl.ac.uk/psipred/) [19]

2.5.2 Modeling and Validating 3D structure

Scratch (http://scratch.proteomics.ics.uci.edu/)was utilized for modeling the 3D structure of vaccine construct to better understand function. The protein structure was validated via ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) server to perform Z-score and Ramachandran plot analysis respectively [20].

2.5.3 Docking

Docking was carried out with help of Cluspro2.0. (https://cluspro.bu.edu/queue.php) software to find the most probable interaction between TLR-4 and vaccine construct.

2.5.4 Codon Optimization

With help of the Java Codon Adaptation tool, codon compliance validates via optimizing the sequence of vaccine construct [21]. Three different parameters bacterial ribosomal binding sites, Rho-independent transcription termination, and restriction enzyme cleavage sites were used. For validation of increased expression, protein must have 30–70% GC content and (> 0.8) CAI values. Optimization of

the construct was designed according to the E. coli K12 strain [22]. Via the SnapGene tool, BanI and TaiI restriction enzymes are used at protein N- and C- terminals.

2.5.5 Immune simulation

C-immune tool predictor (https://kraken.iac.rm.cnr.it/C-IMMSIM/) position-specific scoring matrix that is used to understand immune response magnitude which showed the result of vaccine dosage concerning different time intervals [23].

2.5.6 Molecular dynamics simulation

MD was carried out with help of an Amber package for vaccine and TLR-4. RMSD and RMSF both were determined by using minimization, heating, equilibration, and production.

3. Results

3.1 Sequence Retrieval

In the first step, NCBI was used to retrieve protein sequences. The antigenic behavior and physical and chemical properties were checked via VaxiJen and ExPasy tools respectively. The antigenic value is greater than the threshold value of 0.4. were taken for further analysis.

3.2 Predication for B-cell and T-cell epitope

IEDB server help for B-cell and T-cell epitopes prediction. Non-allergic, antigenic, and non-toxic were identified. Most efficient B-cell epitopes recognized that can evoke B-lymphocytes. B-lymphocytes are critical for initiating pathogen-specific antibody-mediated responses. Therefore, an ideally designed vaccine construct should possess B-cell epitopes. From 20 to 100 amino acids are utilized to speed up the immune response, as result shown in Table 1. A total number of B cell epitopes with a threshold value greater than 5.0 were selected which have Non-Allergic and Non-Toxic. Then the epitopes were incorporated into the vaccine construct. Evaluation of antigenicity was performed where 0.5 was the average value, 0.5481 was the minimum value and 1.6237 was the maximum value. A value more than 0.5 is recognized as antigenic. To get the most effective T-cell epitopes, cytotoxic T-cell epitopes FIAENVRTNV, DSFVVGDANK, VVADFYRIR, ARPRQIAMSL, EQNSNLFTV, and DSFVVGDANK which show good antigenic score were selected Table 2. Here default 0.5 was set as a threshold value, where the epitope was selected on basis of the threshold value. A total number of 6 epitopes were selected with an average of 0.69 value, and 0.5053 was the minimum and 1.3369 maximum one respectively. The IEDB MHC-II tool was used to evaluate 7 HTL epitopes against seven human allele reference sets. The epitope sequences with a good antigenicity score that showed high binding affinities were finally selected for incorporation into the vaccine construct. Table 3 shows the selected epitopes and their properties.

Table 1
Final selected CTL epitopes for incorporation into the vaccine construct with their antigenicity and Toxicity

Epitopes	Position	Antigenicity Score	Allergenicity	Toxicity
FIAGKEGT	278–285	0.5481	Non-Allergic	None-Toxic
PNEIEGLEERLVSRFG	311–326	0.7178	Non-Allergic	None-Toxic
FNHKDPTIE	388–396	0.8271	Non-Allergic	None-Toxic
IKIQEKKVKIDNIQ	406–419	1.2267	Non-Allergic	None-Toxic
RIRVKDLTSNQRSRN	427–441	1.3676	Non-Allergic	None-Toxic
NAFGRD	466–472	1.6237	Non-Allergic	None-Toxic
TKLRQSNTSISDDYE	484–498	0,7693	Non-Allergic	None-Toxic

Table 2
Helper T lymphocyte epitopes along with their percentile rank for the final vaccine construct

Epitopes	Position	Antigenicity Score	IC_{50} Value (nM)	Percentile rank
FIAENVRTNV	13–22	1.3369	46.56	0.42
DSFVVGDANK	38–47	0.5053	22.84	0.2
VVADFYRIR	57–65	0.688	39.16	0.34
ARPRQIAMSL	23–32	0.588	8.74	0.03
EQNSNLFTV	45–53	0.578	29.81	0.32
DSFVVGDANK	38–47	0.5053	22.84	0.2

Table 3
Linear B-cell epitopes ranging in antigenicity from 0.5481 to 1.6237

Epitopes	Position	Residues number	Antigenicity	Allergenicity
FFIMTTWDK	11–19	0.5481	0.9357	Non-Allergic
FFSTTSVEI	34–43	0.7178	0.5819	Non-Allergic
IKIQEKKVK	51–60	0.8271	1.6229	Non-Allergic
KTWIKPIHV	30–38	1.2267	0.5995	Non-Allergic
RPRQIAMSL	22–31	1.3676	0.5156	Non-Allergic
YGLTVSVDM	41–50	1.6237	1.726	Non-Allergic
YRSADILLI	52–59	0.7693	0.5476	Non-Allergic

3.3 MHC-1 and MHC-2 predication

SMM process was used for estimation of MHC HLA alleles on basis of IC50 value. The lower IC50 value represents the highest binding affinity which may bind with MHC-1 molecules. Lower the value shows maximum interaction. A value less than 0.4 considered toxic and allergic, was removed. Epitopes that have antigenic, Non-toxic, and Non-allergic were selected for further screening. The MH Class-1 epitopes were finalized and HLA-B*07:02, HLA-A*68:01, HLA-A*02:06, HLA-A*02:01, and HLA-A*68:01 alleles were recognized as dominant. The epitopes FIAENVRTNV determined the highest antigenic score 1.3369. MHC-2 epitopes were finalized on basis of IC50 value. Out of 100 epitopes for MHC-II, 7 were selected on basis of IC50 values. Epitopes were finalized on basis of toxicity, Allergenicity, and antigenicity for further screening. The epitopes YGLTVSVDM, IKIQEKKVK, and FFIMTTWDK are predicated as a higher binder that interacts with alleles (HLA-DRB5*01:01, HLA-DRB5*01:01, HLA-DRB4*01:01, and HLA-DRB1*07:01).

3.4 Constructed vaccine

B cell epitopes, MHC-1, and MHC-2 epitopes are used for generating vaccine ensembles. For developing vaccine construct, 50S ribosomal protein is considered an adjuvant. Specific immune response produced, when adjuvant and B-cell epitope coupled through EAAAk linker. MHC-1 and B-cell linkage was achieved using CPGPG linkers. For reduction of vaccine size, the B cell epitopes, CTL, and HTL epitopes are combined through the AAY linker Fig. 2. For protein determination and purification, a 6x His tag was inserted in the vaccination sequence (at C-terminus).

3.5 Physical and chemical properties

The characterization of vaccine construct was performed on physical and chemical properties and physiochemical properties of *Francisella tularensis* demonstrated via ProtParm which recognizes 193 amino acids. A constructed sequence such as non-toxic, antigenic, and non-reactogenic behavior was checked. The molecular formula is C982H1572N286O281S4 and the molecular weight is 22009.27 KDa and the PI value is 9.65. Instability index 36.82 and Aliphatic index 89.38 was. The GRAVY score was − 0.390.

3.5.1 Structural Aspects of the Vaccine

Secondary structure showing 45% alpha-helix 20% beta-sheets and 35% coil structure

Figure. The PROSA 3D server was used to predict the 3D structure of the vaccine sequence, resulting in ten predicted structures for a given query sequence. The fifth model was taken for further investigation Fig. 3. The ERRAT, ProSA-web, and PROCHECK services were used to validate the structure, identifying and correcting any potential mistakes in the projected tertiary structure. The ERRAT server projected the overall quality of the vaccine 3D structure, and the estimated quality score was 90.0%S. The Z-score was calculated to see if the input structure was within the range of similar-sized natural proteins. Figure 3b shows that the computed Z-score for the input structure was − 9.77, indicating that it was outside the normal range for natural proteins of the same size. For Ramachandran analysis, the PROCHECK server computed 92.2% of the residues in the most favored areas, 7.3% in additional allowed regions, 0.5% in generously allowed regions, and 0.0% in residues in disallowed regions.

3.5.2 Molecular Docking

The designed protein was docked with TLR9 (PDB id 3WPF) with help of Cluspro to find interaction. Docking provides multiple complexes and is further analyzed by the Pymol server. PDBePISA and PDBsum have used a representation of different interactions. The H-bond result revealed the interaction formed between the residues are ASP259-SER122, ARG346-ASP127, ARG377-ASP127, PHE402-LYS130, HIS260-SER136, ALA261-LYS152, PRO262-LYS152, ARG482-TYR179, ASP424-TYR179, ARG482-ASP183, ARG482-ASP183, ARG470-ASN189, ARG470-LYS190, GLN399-ARG193, THR395-ARG193, ASN473-ASP204, LYS472-ASP204 and ASN466-LYS219 showing the distance of 2.83, 2.87, 3.15, 2.51, 2.66, 2.69, 2.61, 2.80, 2.90, 2.74, 2.88, 2.77, 2.65, 2.55, 2.67, 2.61, 2.48 and 2.48 respectively Fig. 4.

3.5.3 Immune Simulation

We evaluated the vaccine's ability to trigger an effective immune response in practical use using the C-ImmSim webserver. After the primary reaction, the secondary and tertiary immune responses rose steadily. Antibodies (IgM, IgM + IgG, and IgG1 + IgG2) levels have risen dramatically. Fig represents immune response showed a similar response to other immune responses in the human body. The antibodies IgM and IgG were detected along with memory cell formation Fig. 5a. Similarly, there was a large rise in B-cell counts, with IgG1 and IgM biotypes observed as well as significant memory cell development Fig. 5b. Active T-cells increased dramatically after getting a secondary and tertiary injection dose, although they gradually declined at later stages Fig. 5c, d. an increasing amount of IFN-γ and TH cell population is also noted in Fig. 5e, f.

3.5.4 Optimization of Codons

In *E. Coli*, codon optimization and reverse translation were determined, which find high expression in vaccines via the Jcat tool. Vaccine construct codons were tailored to E. coli expression system codons using the Jcat web tool. The cDNA sequence generated was 720 nucleotides long. In the vaccine, the GC value was 64.42 and CAI was 0.95, these expressions showing expression was high. The designed construct's 5 and 3 ends were restricted using BanI and TaiI restriction enzymes. The restricted sequence was successfully ligated into the pET28a (+) vector, yielding a 582 bp clone. The cloned map was created with SnapGene Fig. 6.

3.5.5 Analysis of MD Simulation

MD simulation is a popular method for efficiently analyzing protein structural reliability in a simulated environment. To get information about the stability and flexibility of the protein MD simulation was performed via Amber under 100ns to identify RMSD and RMSF analysis. Till 100ns, both RMSD and RMSF were stable. The construct was first energy minimized by using Pymol. Then topology file for both vaccine construct and TLR-9 was generated. To neutralize the system 7 Na^+ ions were added to the system. The generated trajectories were evaluated to learn more about the construct's RMSD and RMSF. The calculated RMSD of the backbone atoms reached 8(Å) and oscillated between 4 and 8(Å) throughout the simulation, indicating strong protein stability. The estimated RMSF values also identified flexible regions with peak-like patterns Fig. 7.

Discussion

For *F. tularensis, the v*accine is not available yet. Vaccines are seen to be most protective against different pathogens and diseases [24]. Multiple epitopes are highly recommended because of their cost-effectiveness, and higher efficiency [25]. Different multi-epitope

vaccines are developed against bacteria such as *Klebsiella pneumonia* [26], *Aeromonas hydrophilla* [27], *Helicobacter pylori* [28], and so on. Due to the efficiency of these vaccines, the multi-epitope-based vaccine was designed against *F. tularensis.*

The study aims to design a vaccine against *F. tularensis*, by using the immunoinformatics technique. The first protein sequence was obtained from NCBI, after which the antigenicity, Non-Allergenicity, and non-toxic properties of the protein were investigated. Mapping of epitopes was performed for further analysis. MHC-1 and MHC-2 were predicated. The selected epitopes are combined by the linker (EAAAK, CPGPG, and AAY). The stability and efficiency of the vaccine constructs was checked via an online server. The antigenic score 0.7218 was and the protein was non-allergic, which revealed our vaccine is non-allergic and antigenic. The molecular weight of protein was 22.27 KDa, the normal range was 30–60 KDa, and the instability index was 36.82, which is 4 or less than 40, indicating that our vaccine is optimal. The GRAVY value of -0.390 indicates that the vaccination is hydrophobic. The aliphatic index was 89.38, indicating that the vaccine was stable. Secondary and 3D structure is constructed using PSIPRED V3.3 and Scratch. The secondary structure represents the alpha-helix (45%), beta-strand (20%), and coil (35%). Different servers PROCHECK, ERRAT and were used for validations. Docking analyses were carried out by the Cluspro server and the PDB sum was used to generate a full set of PDBsum structural analyses. MD simulations were performed via Amber20. To gain high protein expression in *the E. Coli* K12 strain, Jcat software was utilized for optimization of codon to increase translation and transcription efficacy. Protein expression linked with GC and CAI of reverse translated optimized sequence. Via SnapGene software, constructed vaccine cloned in *E. coli* K12 strain plasmid pET28a (+). As a result, a reliable immunoinformatics technique was applied to build a stable and safe vaccination in this work. Vaccines, on the other hand, require scientific validation and consensus.

Abbreviations

NCBI
National Center for Biotechnology Information
IEBD
Immune Epitope Database and Analysis Resource
MHC
Major histocompatibility complex
RMSD
Root-mean-square deviation
RMSF
Root-mean-square fluctuation

Declarations

The submitting research article "**Immunoinformatics and structural vaccinology approach to design a Multi-Epitope Subunit Vaccine against *Francisella tularensis* through *In Silico* modeling**" for publication in your journal of repute, is a unique article and nobody did it earlier.

Ethics approval and consent to participate

This study does not include any experiments with humans or other living animals, hence ethical approval and consent to participate is not applicable.

Consent to publication

Not applicable.

Data availability statement

NCBI database in FASTA format with ID (CAG44634.1).

Conflict of interest

The authors declare that they have no conflicts of interest.

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Not applicable

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Author's Contributions:

C.H: methodology and writing. C.L, X.D: Investigation. M.S, H.S: software. G.Z: supervision, review, and editing

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Figures

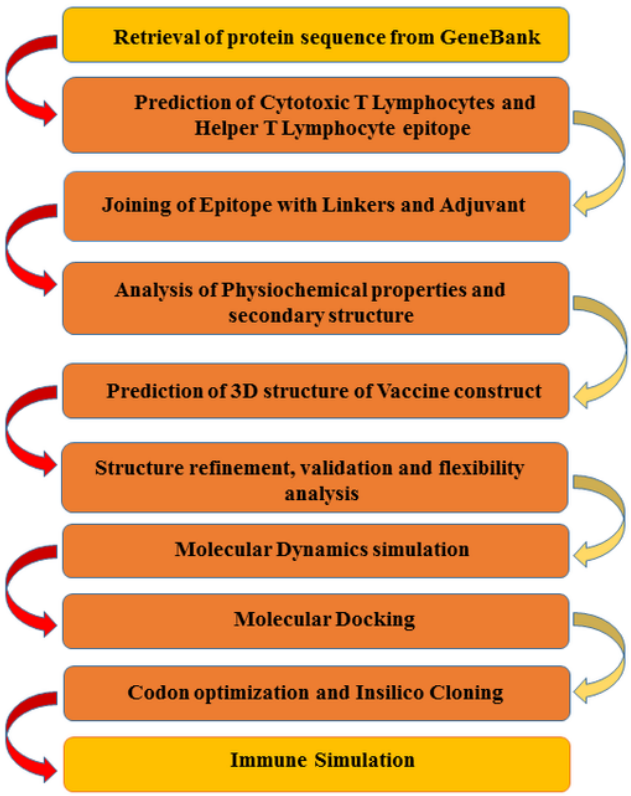

Figure 1

Steps taken to develop a multiepitope vaccine against F. *tularensis*[13].

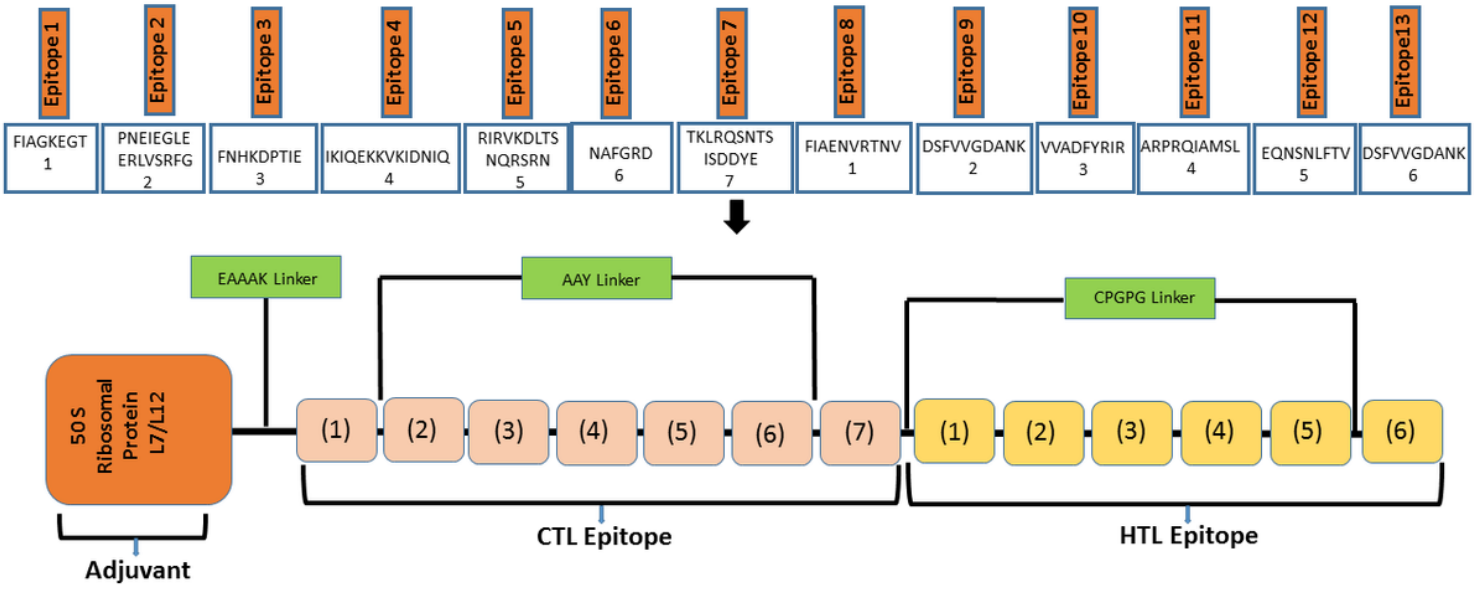

Figure 2

Vaccine design illustration. Sequences of selected CTL and HTL epitopes, with bracketed numbers indicating vaccine location. Vaccine structure containing adjuvants, linkers, and epitopes.

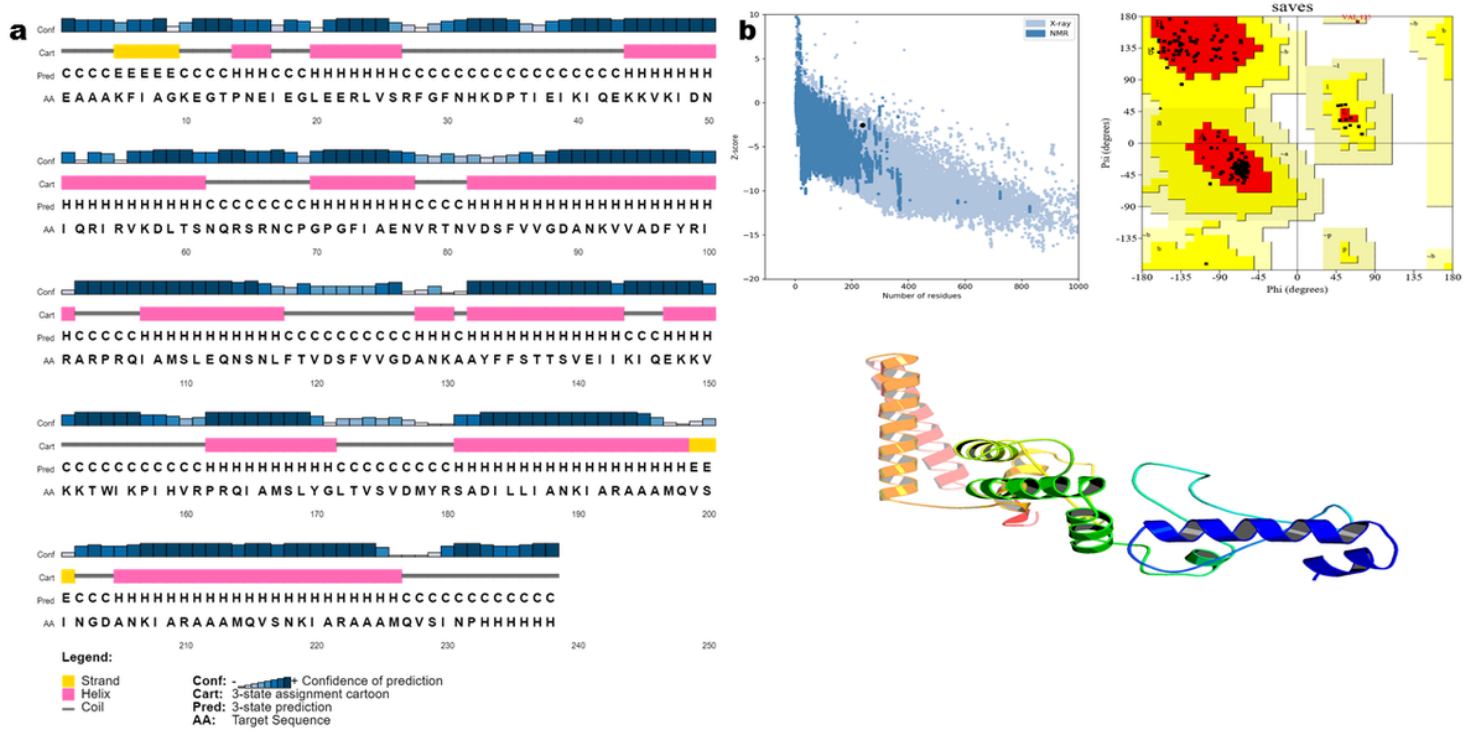

Figure 3

Secondary structure prediction by PSIPREDV 3.3 showing 45% alpha-helix, 20 % beta-strand, and 35% coil. ERRAT server was used to predict 3D structure.

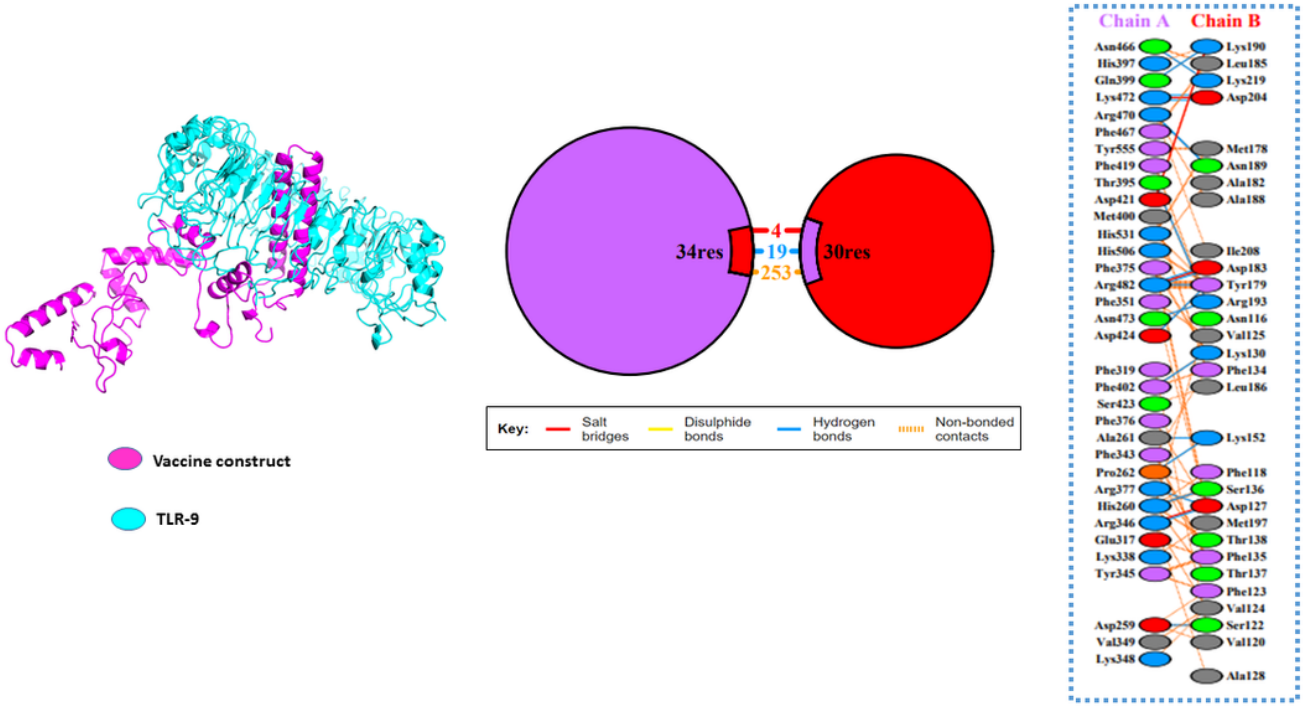

Figure 4

The docked complex of TLR-9 and vaccine. (A) The vaccine construct was in yellow and TLR-9 is in purple. (B) Hydrogen bond interaction between vaccine construct and TLR-9.

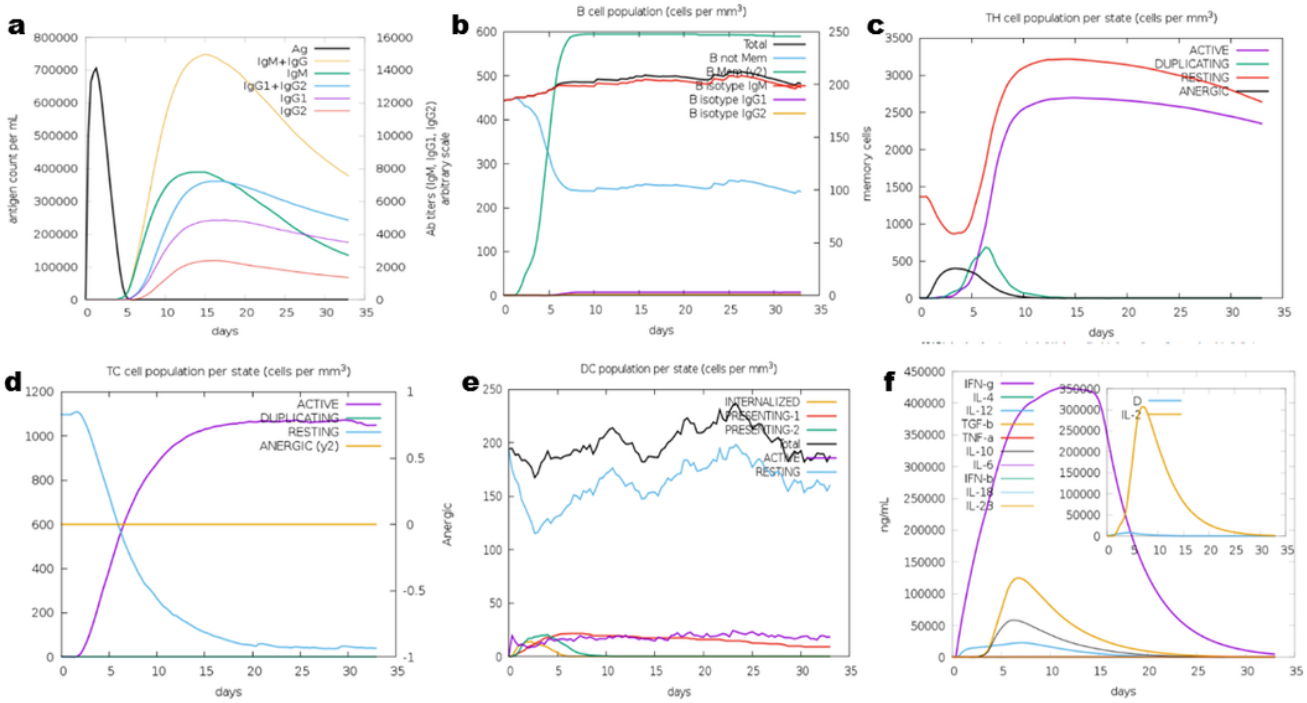

Figure 5

vaccine immune simulation through C- ImmSim server

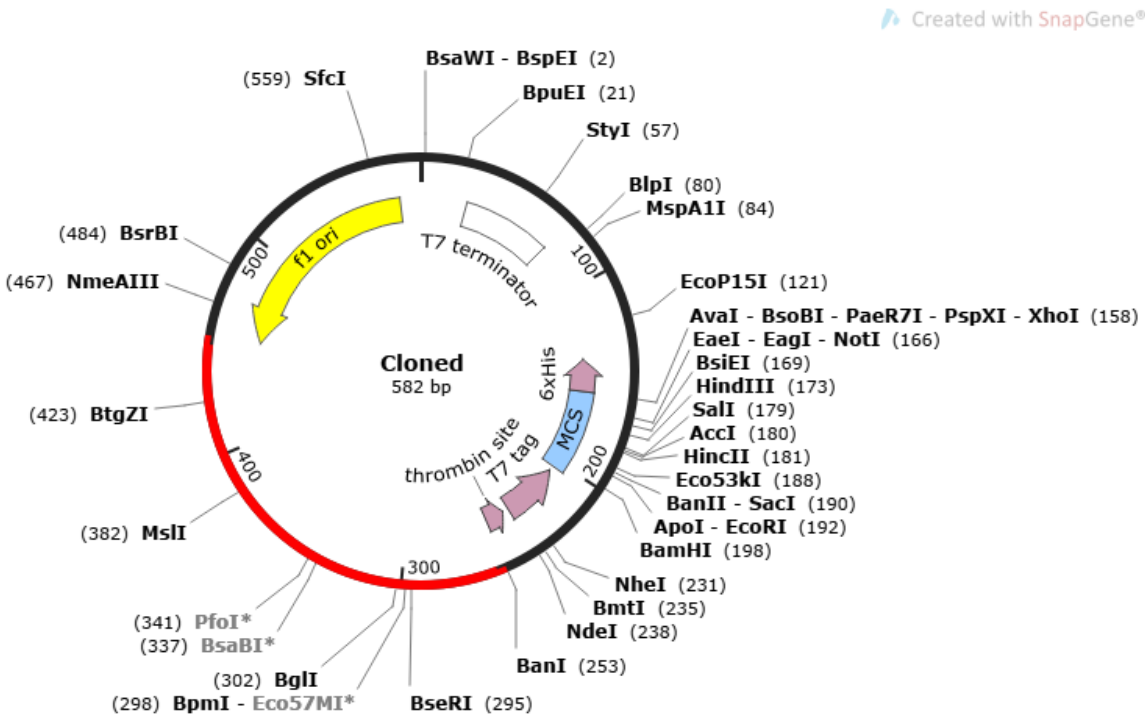

Figure 6

represents the cloning of the final vaccine where black shows the vector and Red shows the vaccine insert.

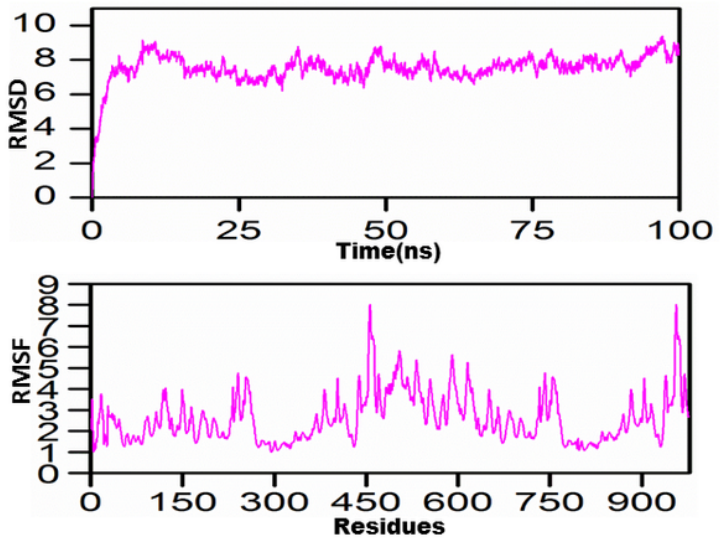

Figure 7

Represent RMSD and RMSF of the complex.