Insilico Designing of a Chimeric Vaccine Using EIS (Rv2416c) Protein Against *Mycobacterium Tuberculosis* H37Rv; An Immunoinformatics Approach

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Abstract

Mycobacterium tuberculosis (Mtb) is a respiratory pathogen that causes Tuberculosis disease (TB). There are a large number of proteins that are involved in the pathogenesis of TB. Stimulating the immune response against TB is very important to clear the pathogens from host. In the present study, an Immunoinformatics conduit is used for epitope based chimeric vaccine designing against TB. Enhanced Intracellular Survival (EIS) protein from Mtb is used for designing the chimeric vaccine. 1 B-cell epitope, 8 cytotoxic T lymphocyte (CTL) and 6 Helper T lymphocyte (HTL) epitopes were predicted based on the MHC allele binding, immunogenicity, antigenicity, allergenicity, toxicity and IFN epitopes. The selected epitopes were used for chimeric vaccine designing. Further, 3D structure elucidation, structural refinement and validation of the designed chimeric vaccine was carried out. The 3D structure was used for Protein-Protein docking studies with Toll like receptor 4 (TLR-4), followed by molecular dynamic simulation (MDS) and the interaction between the chimeric vaccine and TLR-4 complex was verified.

Introduction

Tuberculosis (TB) is a chronic infectious disease that is caused by the respiratory pathogen, *Mycobacterium tuberculosis* (Mtb). According to the WHO report, worldwide about 10 million people are known to be affected by TB every year [1]. Further, among the global causes of death, TB is ranked among the top 10. The highest rate of infection occur in countries such as Indonesia, Philippines, Afghanistan, Bolivia and Peru. New cases are also identified in densely populated nations such as China and India. Isoniazid, pyrazinamide, rifampicin and ethambutol are the first-line antituberculosis drugs[2].Control and treatment of TB is greatly hindered by development of multidrug-resistance TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). Thus, new drugs are being constantly developed to control the spread of MDR-TB and XDR-TB. Ethylsulfonyl benzodiazaborine (AN12855) isone such compound that was shown to bindto and inhibit the enoyl-ACP reductase (InhA)[3]. The efficacy of AN12855has also been established in vivo in C3HeB/FeJ mouse model that showed caseous nectrotic lung lesions, wherein, the compound exhibited remarkable potential against isoniazid-resistant strains of Mtb[4]. Treatment with a combination of drugssuch as pretomaind, linezolid and bedaquiline also showed promise in treatment of patients with XDR-TB[5].

Progression of TB is associated with the host response to Mtb infection. The innate immune cells including the macrophages, dendritic cells and the natural cells play a key role in determining the course of infection as they are the first to confront the Mtb pathogen, upon infection [6]. Once the disease progresses, the active T cells ($CD4^+$ and $CD8^+$) move to the lungs and interact with the antigen presenting cells. Cytokines including interleukins, IFN-γ, and TNF-α play important role in the immune response against TB[7, 8].In the early stage of infection, host innate immune responses like reactive nitrogen intermediates play akey role [9]. The role of B cells and humoral immunity in the early immune response against the Mtb pathogen cannot be overlooked[10]. The B cells are also known to modulate the T cells through various mechanisms, resulting in shaping of immune response against the Mtb pathogen[11, 12].Both $CD4^+$ $CD8^+$subsets of T cells play a key role in immunological response against Mtb infection

[13].In an animal model, during the early stage of infection, $CD4^+$ and $CD8^+$ T cells were found tomigrate into the lungs, thereby increasing the host immune response [14].Upon infection, the macrophages are known to identify the Mtb through the toll like receptors (TLR, most commonly TLR-2 and TLR-4)that leads to phagocytosis and control of Mtb pathogen. Further, the macrophage secret interleukin-12 (IL-12)resulting in the production of interferon-γ (IFN-γ) by the T cells[15]. Interleukinsincluding IL-2, IL-1β, IL-6, IL-10, IL-12 and chemokines are also involved in the host immune resistance to Mtb pathogen[16, 17].However, Mtb keeps evolving to successfully modulateand evade the immune responses [18]. Certain mycobacterial components expressed by the Mtb have been shown to inhibit the production of TNF, thereby evading the host immune response [19].Stimulating the immune response against TB is very important to clear the pathogens from the host.There are a large number of proteins involved in the pathogenesis of TB. One of the secretary protein from Mtb is the Enhanced Intracellular Survival (EIS) protein that plays multiple functions in the pathogenesis of TB[20]. The EIS protein from Mtb is a 42 kDa cytoplasmic secretary protein involved in progression, activation and immune response during the macrophage infection. EIS is a hexomeric form of aminoglycoside acetyltransferase enzyme that has the capacity to inhibit the activities of second line TB drugs like aminoglycoside antibiotics, through multi-acetylation mechanism[21, 22].EIS was also shown to aid in the survival of Mtb by modulating the secretion of IL-10 and TNF-α[23, 24].

Immunoinformatics is an interface between computational biology and experimental immunology. It involves analysis of biological information using statistical techniques and computer. It acts as the platform to understand the immunological information through the computational methods and resources[25].Immunoinformatics is a new subclass of bioinformatics with variety of different approaches and tools to analysis the immunological data. It involves using different methods and tools to understand the immune response and immune system against pathogens, thereby aiding in accelerating the progression of immunological research[26].In the present study, insilico approach has been applied to design a chimeric vaccine against TB, incorporating the EIS gene from Mtb. Initially, the B cell, cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) epitopes were predicted. The antigenicity, allergenicity, toxicity and the IFN-inducing ability of the predicted epitopes were evaluated. Subsequently, highly antigenic, non-allergenic and non-toxic B-cell, CTL and HTL epitopes were selected for construction of the chimeric vaccine. To increase the immune response, 50S ribosomal protein Rv0652 from Mtb H37Rv was used as an adjuvant. The physiochemical properties such as molecular-weight, theoretical isoelectric point (pI),instability index, aliphatic index, grand average hydropathicity (GRAVY), hydrophobicity and hydrophilicity were determined using the ProtScale and ProtParamtools from ExPASy. After the tertiary structure of the chimeric vaccine was predicted, refined and validated, the interaction between the constructed chimeric vaccine with TLR-4 was evaluated by molecular docking studies. During Mtb infection, increased expression of TLR-4 in host immune cells are observed. Thus, to corroborate the host immune response that develops as a result of interaction between the immune cells and Mtb, TLR-4 was chosen as the immune receptor. Finally, molecular dynamic simulation of the chimeric vaccine (EISvac) with immune receptor was performed to determine if the interaction between the ligand and the receptor are stable.

Materials And Methods

Sequence retrieval

FASTA format of the EIS protein amino acid sequence from H37Rv Mtb strain was extracted from Mycobrowser (https://mycobrowser.epfl.ch/genes/Rv2416c). The structures of EIS (4JD6) and TLR-4 (4G8A) were downloaded from the Protein Data Bank (PDB).

Prediction of B-cell epitopes

B-cell epitope prediction was done using the IEDB (Immune Epitope DataBase and analysis resource) server. The linear B-cell epitopes were predicted based on the accessibility, flexibility, hydrophilicity and antigenicity of the B-cells[27].Emini's method was used for surface accessibility prediction and Karplus and Schulz's algorithm was used for flexibility determination [28, 29]. The method of kolaskar and tongaonkar was used for antigenicity prediction [30]. Further, to predict the hydrophilicity, themethod proposed by parker et al., was used[31].Conformational B-cell epitopes were predicted using the Epitope prediction based upon structural protrusion(ElliPro) tool from IEDB (http://tools.iedb.org/ellipro/)[32].

Cytotoxic T-lymphocyte (CTL) epitopes andimmunogenicityprediction

CTL epitopes were predicted using the IEDB MHC-I tool (http://tools.iedb.org/mhci/). The FASTA sequence of EIS protein was used for CTL epitope prediction. This server predicts $CD8^+$ T cell epitopes based on the proteasomal C terminal cleavage, MHC-I binding and TAP transport efficiency. IEDB MHC-I tool predicts the proteasomal C terminal cleavage, MHC-I binding and TAP transport efficiency using weight matrix, artificial neural networks and IC50 value. The epitopes were predicted for the human alleles (HLA-A*01:01, HLA-A*01:01, HLA-A*02:01, HLA-A*02:01, HLA-A*02:03, HLA-A*02:03, HLA-A*02:06, HLA-A*02:06, HLA-A*03:01, HLA-A*03:01, HLA-A*11:01, HLA-A*11:01, HLA-A*23:01, HLA-A*23:01, HLA-A*24:02, HLA-A*24:02, HLA-A*26:01, HLA-A*26:01, HLA-A*30:01, HLA-A*30:01, HLA-A*30:02, HLA-A*30:02, HLA-A*31:01, HLA-A*31:01, HLA-A*32:01, HLA-A*32:01, HLA-A*33:01, HLA-A*33:01, HLA-A*68:01, HLA-A*68:01, HLA-A*68:02, HLA-A*68:02, HLA-B*07:02, HLA-B*07:02, HLA-B*08:01, HLA-B*08:01, HLA-B*15:01, HLA-B*15:01, HLA-B*35:01, HLA-B*35:01, HLA-B*40:01, HLA-B*40:01, HLA-B*44:02, HLA-B*44:02, HLA-B*44:03, HLA-B*44:03, HLA-B*51:01, HLA-B*51:01, HLA-B*53:01, HLA-B*53:01, HLA-B*57:01, HLA-B*57:01, HLA-B*58:01, HLA-B*58:01). The epitopes with IC50 value less than 50 were selected for further studies[33, 34].Immunogenicity of the predicted CTL epitopes were analyzed using the IEDB class I Immunogenicity prediction tool (http://tools.iedb.org/immunogenicity/) and highly immunogenic CTL epitopes were used for further studies[35].

Helper T lymphocyte (HTL) epitopesprediction

HTL epitopes were predicted using MHC2Pred tool from Raghava online bioinformatic tools (http://crdd.osdd.net/raghava/mhc2pred/index.html). In this tool HTL epitopes were predicted based on

the MHC-II allele binding, using SVM support Vector Machine algorithms. FASTA format of the protein sequence was submitted to the server and default parameters were applied[36, 37].

Antigenicity and allergenicity prediction

For Antigenicity prediction, conformational B-cell epitope, CTL epitopes and HTCepitopeamino acid sequenceswere submitted to the VaxiJen v2.0 server tool from pharmfac server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) and highly antigenic epitopes were selected for further analysis[38].Allergenicity of the B-cell, CTLand HTL epitopes were predicted using freely available allergenicity prediction tool (http://www.ddg-pharmfac.net/AllerTOP/index.html) AllerTOPv. 2.0. AllerTOPv. 2.0 predicts the allergens based on the machine learning methods like auto and cross covariance transformation, k nearest neighbors and amino acid E-descriptors[39].

Toxicity and IFN-γ inducing epitope prediction

Toxicity of the B-cell, CTL and HTL epitopes were predicted using the freely available toolToxinPred (http://crdd.osdd.net/raghava/toxinpred/). This tool utilizes the quantitative matrix properties of the peptide and machine learning methods to predict the toxicity of the peptides. The advantage of this tool is that it also predicts the physiochemical properties of the epitopes[40].The IFN-γ inducing epitopes from B-cell, CTL and HTL epitopes were predictedusing the IFN-γ inducing epitopes predicting and designing tool(https://webs.iiitd.edu.in/raghava/ifnepitope/predict.php). This tool works based on motif, support vector machine and hybrid approach to predict the IFN-γ inducing epitopes[41, 42].

Designing of the chimericvaccine (EISvac)

To design the chimeric vaccine, highly antigenic, non-allergenic and non-toxic B-cellepitopes, CTL epitopes and HTL epitopes wereselected. For increasing the immune response, 50S ribosomal protein Rv0652 from Mtb H37Rv was used as adjuvant. EAAAK, AAY and GPGPG were the peptide linkers used for linking different epitopes[43, 44].

Tertiary structure prediction and Refinement ofthe chimeric vaccine (EISvac)

The 3D structure of the chimeric vaccine was predicted using the RaptorX server tool. FASTA format of the chimeric vaccine construct was submitted to the server (http://raptorx.uchicago.edu/)[45, 46]. After the tertiary structure of the vaccine was predicted, the structure was refinedusing the 3Drefine server tool, The highly ranked model was submitted(http://sysbio.rnet.missouri.edu/3Drefine/index.html)[47].

Tertiary Structural validation of chimeric vaccine (EISvac)

Tertiary structural validation is a process to detect the errors in the predicted 3D model of the vaccine constructs.3D structure validation was done using different, freely available structure validation tools like PROCHECK from SAVEv 5.0, ERRAT server and ProSA-web[48]. ERRAT server was used to identify the overall quality factor of the 3D model[49]. ProSA-web is a web server to detect the overall quality and

local model quality of 3D the model using Z-score value[50].Hydropathicity of the chimeric vaccine (EISvac) was predicted using the ProtScale server Tool from ExPASy[51].

Evaluation of Physiochemical properties of chimeric vaccine (EISvac)

Physiochemical properties like molecular weight, number of amino acids, number of nucleobases, theoretical pI, instability index, aliphatic index, grand average hydropathicity(GRAVY), hydrophobicity and hydrophilicity of the amino acidswereevaluated using freely available bioinformatic servers like ProtScale and ProtParam from ExPASy [51–53].

Molecular Docking of the chimeric vaccine (EISvac) with the immune receptor

It is important to know the predicted chimeric vaccine's interaction with the immune receptor and whether the construct will generate the immune response. Thus, molecular docking between the EISvac (ligand) and TLR-4 (receptor) was performed using ZDOCK model from Discovery Studio client 2017. Default parameters were set and angular step size was set as 15[54–56].

Molecular Dynamic Simulation (MDS) of the chimeric vaccine (EISvac) with Immune receptor

MDS was performed to know the physical basis of the protein structure and function of the biological macromolecules. MDS for the chimeric vaccine (EISvac), TLR-4 and the complex (TLR-4 with EISvac) was performed using Discovery Studio client 2017, CHARMm force-field was applied to determine the intramolecular interaction and stability. To remove the high energy configuration of the macromolecules, energy minimization step was performed. The MDS was performed at a temperature of 300K for 0.4ns. To determine the standard deviation and protein backbone fluctuation, the trajectory analysis was run and root mean square deviation (RMSD) and root mean square fluctuation (RMSF)were obtained. Overall, five step MDS run was conducted. The steps were followed by minimization one and two, heating, equilibration and production[57, 58].

Results And Discussion

Primary analysis of the sequenceretrieved from Mycobrowser

The chimeric vaccine was constructed using the EIS protein sequence from Mtb H37Rv strain. The FASTA sequence and structure of the proteinwere retrieved from Mycobrowser and Protein Data Bank, respectively. Immunoinformatics analysis were used to predict the antigenicity and allergenicity of the protein. The EIS protein was predicted as a probable antigen (0.5344) and non-allergen. Further, B cell, CTL and HTL epitopes were predicted using FASTA sequence of the EIS protein.

B-cell epitope prediction

B-cell epitopes were predicted using the ElliPro tool from IEDB server. The epitopes were predicted based on the properties of B cell epitopes like surface accessibility, surface antigenicity, surface flexibilityand surface hydrophilicity(Fig. 1a-d). In the ElliPro server, epitopes were predicted based on the tertiary structure of the protein. The sequences of the 42 B cell epitope epitopes that were predicted are mentioned in Table 1.

Prediction of CTL epitopes andimmunogenicity

From the EIS protein sequence, CTL epitopes were predicted using the MHC-I tool from IEDB server. This server predicts the epitopes based on the MHC-I allele binding, proteasomal C terminal cleavage and TAP transport efficiency. From the IEDB server, reference allele set was selected for epitope prediction. Around 87 epitopes were predicted based on the MHC-I allele binding and IC50 value less than 50(Supplementary material Table S1). Immunogenicity for all 87 epitopes were also predicted using class I immunogenicity prediction tool from IEDB server.

Helper T lymphocyte (HTL) epitopesprediction

HTL epitopes were predicted from EIS protein using MHC2Pred tool from Raghava server tool. Epitopes were predicted based on the MHC-II binding and top rank score epitope from each MHC-II allele was short listed. In all, 41 HTL epitopes were predicted based on the top score and MHC-II allele binding(Table S2).

Antigenicity and allergenicity prediction

The amino acid sequences of the predicted 42 B-cell epitopes, 87 CTL epitopes and 41 HTL epitopes were submitted in the VaxiJen v2.0 server tool to predict the antigenicity. The B-cell epitope with the highest antigenic naturewas selected for further studies (Table 1). The CTL epitopes and the HTL epitopes that showed antigenicity score of more than 0.6 were selected for further studies. From the 87 CTL epitopes, 13 epitopes(Table 2) and out of the 41 T Helper cell epitopes, 14 epitopes(Table 3) were thus selected. The short listed epitope sequences of B-cell, CTL and HTLwere submitted to AllerTOPv. 2.0 server tool to predict the allergenicity of the epitopes.

Toxicity and IFN-γ inducing epitope prediction

IFN- γ is a key cytokine that plays a pivotal role in host defense against bacterial pathogens including Mtb, through various mechanisms [59, 60]. Thus, after predicting the antigenicity and allergenicity, the final shortlisted B-cell, CTL and HTL epitope sequences were submitted to tools that predict the non-toxicand IFN-γ inducing epitopes. For toxicity prediction, ToxinPred tool was used. Those sequences that are non-toxic are shown in Table 4. Only 5 positive epitopes that can induce IFN- γ production were identified by the IFN-γ inducing epitope prediction tool (Table 4).

Designing of chimericVaccine (EISvac)

For the chimeric vaccine (EISvac) construction, epitopes that were highly antigenic, non-allergenic and non-toxic,and some that were capable of inducing IFN-γ production were selected. Accordingly, one B-cell epitope, 8 CTL epitopes and 6 HTL epitopes were selected (Table 4). The selected epitopes were linked with amino acid linkers like EAAAK, GPGPG and AAY. Further, to increase the immune response, 50S ribosomal protein Rv0652 was added as an adjuvant to the N-terminal end of the vaccine. The amino acid sequence of the constructed chimeric vaccine is mentionedin Table 5.

Tertiary structure prediction and refinement of chimeric vaccine (EISvac)

The tertiary structure of the chimeric vaccine was predicted using the RaptorX protein structure prediction tool. RaptorX is a web based server that does not require any template to predict the secondary and tertiary structure of proteins [61]. Five best models were generated after the amino acids sequence of the designed EISvac complex was submitted to RaptorX. The top ranked model from RaptorX was subjected to further refinement using the 3Drefine tool. Refinement of the initial, best modelsby the 3Drefine server generated five best models. Based on the scores of all the refined models, one model was used for structure validation (Fig. 2)

Tertiary structural validation of chimeric vaccine (EISvac)

Further analysis of the selected model by Ramachandran plot analysisrevealed that 89.2% residues were in the most favoured regions, 9.0% residues were in additional allowed regions, 1.4% residues were in generously allowed regions and 0.4% residues were in disallowed regions (Fig. 3a). The overall quality of the model was substantiated byPROCHECK and ERRAT from SAVEv 5.0. The overall quality factor of the model after refinement with ERRAT was 98.476(Fig. 3b) and the Z-score of this model as predicted byProSA-web was -7.0 (Fig. 3c).

Evaluation of Physiochemical properties of chimeric vaccine (EISvac)

Based on the 3D refined models, favorable regions from Ramachandran plot and ERRAT results, the first model was selected for molecular docking and dynamic simulation.Physiochemical properties of chimeric vaccine (EISvac) were predicted using ProtScale and ProtParam from ExPASy(Table 6). The molecular weight of the chimeric vaccine was 34.8 kDa andthe theoretical pI of the construct wasfound to be 6.52. The total number of the amino acids were 336 and the number of nucleobase was 1008. The instability index of the chimeric vaccine was evaluated to be 21.81, which denotes that the constructed vaccine is stable in nature. The aliphatic index of the vaccine was found to be 82.50 and indicates that the construct (EISvac) is thermostable.Hydropathicity of the vaccine (EISvac) was predicted by the ProtScale server Tool form ExPASy. The positive value denotes that those amino acid residues are hydrophobic and will not interact with water molecules while negative value denotes that the residues are hydrophobic (Fig. 3d). The grand average hydropathicity (GRAVY) of the chimeric vaccine was 0.001. The positive value denotes that the molecule is hydrophobic and will not interact with water molecules, thereby, it can be delivered using liposome modules.

Molecular Docking of the chimeric vaccine (EISvac) with the immune receptor

After validation of the 3D refined model of final chimeric vaccine construct (EISvac), it was deemed important to know the interaction of vaccine construct with TLR-4. Molecular docking studies between the TLR-4 and chimeric vaccine was performed using Discovery Studio client 2017. From the best docked2000 poses obtained from the docking server, the top ranked and lowest ZRank score pose was selected for interaction and simulation studies. Accordingly, pose 1 for which docking rank was 1 and ZRank score was -124.008 was used for further studies. The structure of pose 1 obtained from PDB was visualized in surface mode using Discovery Studio client 2017 (Fig. 4). Around 33 interactions were identified between the construct (EISvac) and the TLR-4. Among the interactions, 8 carbon hydrogen Bonds, 9 conventional hydrogen bonds, 6 hydrophobic interactions (4 alkyl & 2 Pi-Alkyl), 7 electrostatic interaction and 3 salt bridge were identified. All the interactions and bond distances are mentioned in the Table 7.

Molecular dynamic simulation (MDS) of the chimeric vaccine (EISvac) with the immune receptor, TLR-4

Five step MDS of EISvacvaccine, TLR-4 andTLR-4-EISvac complex was done using Discovery studio client 2017. CHARMm Forcefield was applied for energy minimization and other steps. The overall energy minimization for the chimeric vaccine construct, TLR-4 and the complex (TLR-4+EISvac) are mentioned in the Supplementary material Tables S3, S4and S5, respectively. In dynamic Simulation, during production step the potential energy was found to be-17495.972 kcal/mol for EISvac (Table S3), -90849.87 kcal/mol for TLR-4 (Table S4) and the potential energy of the complex was -109207.19kcal/mol (Table S5). The Total energy of the ligand EISvac during the start of equilibration step was -13654.464kcal/mol and decreased to -13845.511kcal/mol at the end(Fig. 5a). Likewise, the total energy of TLR-4 decreased from -71400.23kcal/mol to -73143.877 kcal/mol (Fig. 5b) and the total energy of the complex(TLR-4+EISvac) decreased from -85716.75kcal/mol to -87927.802kcal/mol (Fig. 5c).The initial and the final RMS gradient and other details of both the proteins and the complex are mentioned in the energy minimization tables (Supplementary Table S3-S5). From the time vs temperature plot, it is clear that the EISvac chimeric vaccine stabled at 301.171K (Fig. 6a), the TLR-4 protein stabled at 300.407K(Fig. 6b) and the complex(TLR-4+EISvac) stabled at 299.427K(Fig. 6c).

After MDS, trajectory analysis to know the changes in the RMSD values and RMSF wereanalyzed using Discovery Studio client 2017. The RMSDof the chimeric vaccine (EISvac) for 100 conformations was around 1.3(Fig.7a) A slight change in the RMSD was observed between the TLR-4 and the complex(Fig. 7b). This indicates that binding of chimeric vaccine with the TLR-4 results in changes to the RMSD of the TLR-4 amino acids. The analysis of RMSF results of the chimeric vaccine and revealed that after binding, there are fluctuation. Fluctuations in the root mean square in the amino acid residues of the ligand molecule (EISvac) before and after docking with TLR-4 receptor (Fig. 8a) and TLR-4 receptor amino acids were also observed (Fig. 8b). These fluctuations are responsible for the conformational changes of the binding proteins. The final results of MDS concludes that the binding of EISvac chimeric vaccine with

TLR-4 induces the immune response and the final conformational changes may attribute to immunomodulation.

Conclusion

TB remains among the top 10 causes of death worldwide. Mtb, the pathogen that causes TB keeps evolving, thereby evading the host immune response. The development of MDR-TB and XDR-TB has further weakened the battle against eradication of TB. Thus, it was deemed significant to develop a multi-epitope vaccine candidate, capable of eliciting a strong immune response. In the present study, Immunoinformatics and immunological approaches were used to design a chimeric vaccine construct against TB. Highly antigenic epitopes of B-cell, CTL and HTL epitopes were predicted and used to construct the vaccineso that it is capable of inducing innate, cell mediated and humoral immunity. The molecular docking studies revealed that around 33 different interactionare involved in binding of the chimeric vaccine with TLR-4. Among the interaction, 17 hydrogen bonds and 3 salt bridges were identified. Among the non-covalent interactions, hydrogen bond and salt bridge are very strong interactions. Thus, it can be concluded that the interaction between the chimericvaccine and TLR-4 was stable. [62–64]. The stability of the chimeric vaccine was confirmed by MDS and the interaction of the chimeric vaccine with the immune receptor, TLR 4 was found to be stable. Thus, this predicted chimeric vaccine candidate can be further validated by in vitro and in vivo methods.

Declarations

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Code availability: Not applicable

Authors' contributions

NA and SJ are conceived and designed the methodology and research. LR conducted the experiments and analyzed the data. LR and LV wrote the manuscript and analyzed the final results. All the authors read and approved the manuscript.

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Tables

Due to technical limitations, table 1-7 is only available as a download in the Supplemental Files section.

Figures

Fig. 1

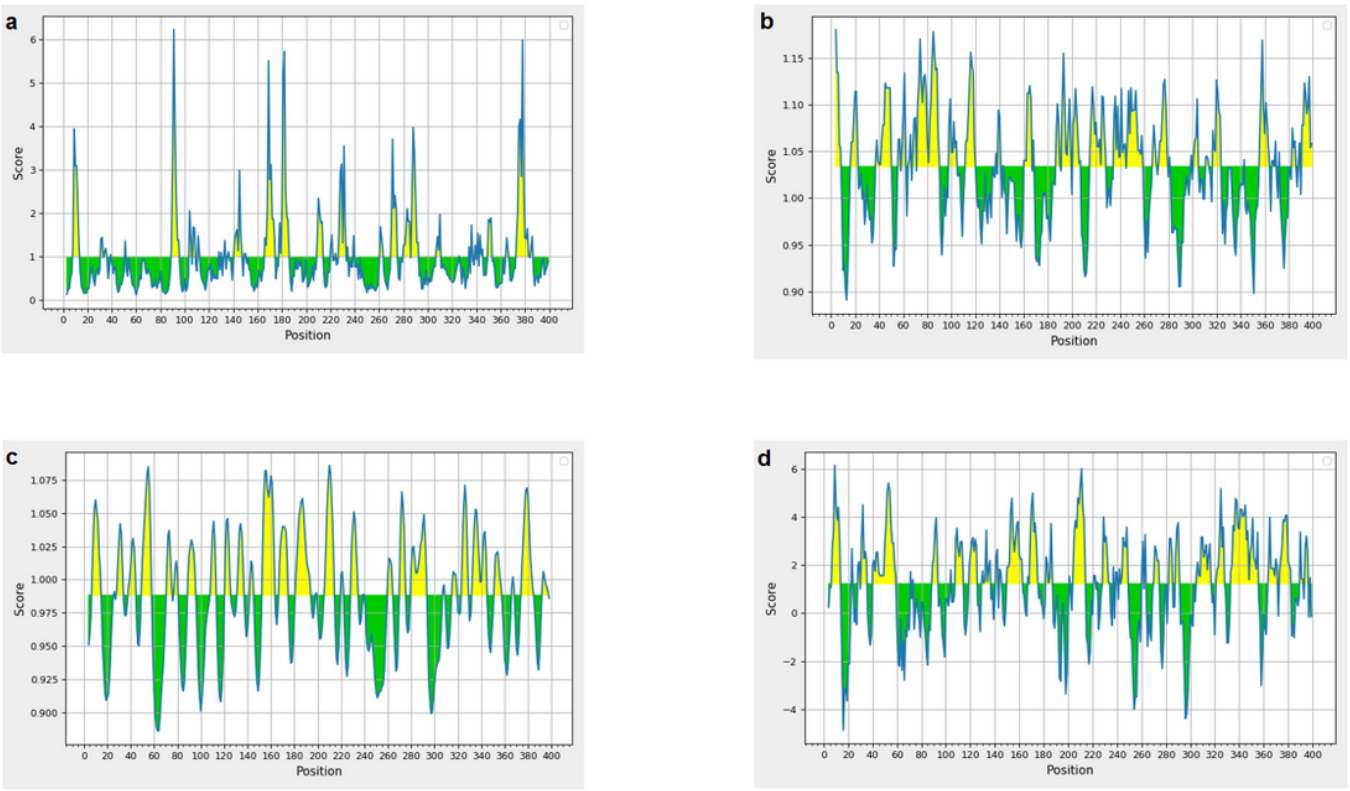

Figure 1

a-dB cell epitopes predictedbased on the accessibility, antigenicity flexibility and hydrophilicity, using IEDB server Tools.

Fig. 2

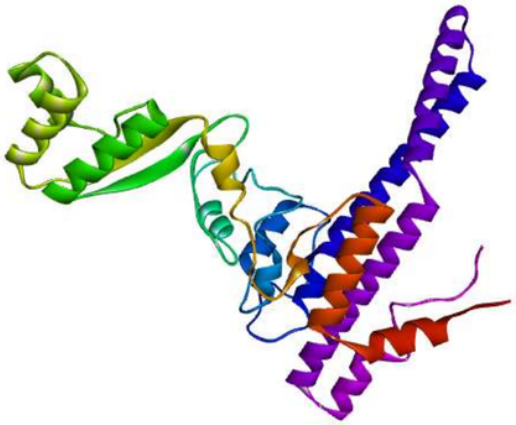

Figure 2

Refined 3D structure of the predicted vaccine model (EISvac). Tertiary Structure was predicted using RaptorX server, 3D refinement was done by 3Drefine server and the structure was visualized in Discovery studio 2017.

Fig. 3

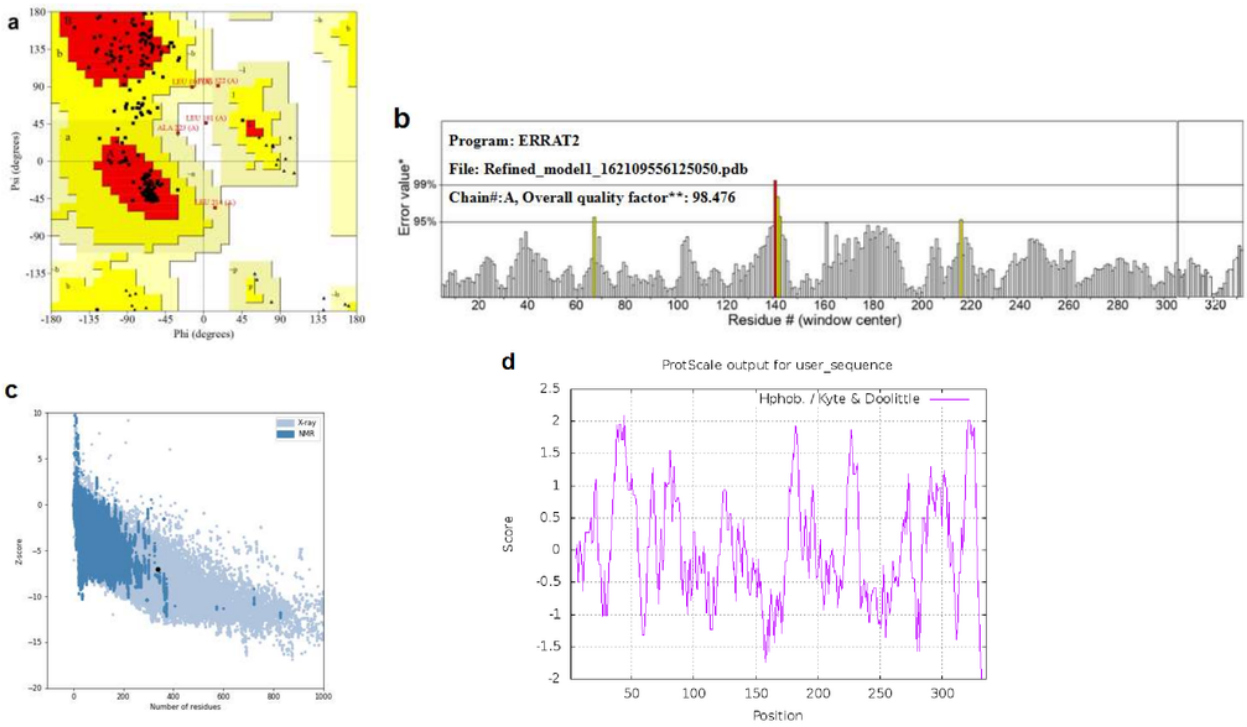

Figure 3

Validation of refined 3D structure of the predicted vaccine model (ElSvac). (a) Ramachandran plot analysis reveals that 89.2% of the amino acid residues are in the most favoured region. (b) Z-score of the refined model was found to be -7,as predicted by ProSA-web tool. (c) Overall quality of the modelled protein structure was 98.476, as predicted from the ERRAT tool. (d) Predicted hydrophobic regions of the vaccine model (ElSvac) by ProtScale Tool. In the vertical axis positive value indicates that the amino acids residues are hydrophobic and negative values indicate that those residues are hydrophilic in nature.

Fig. 4

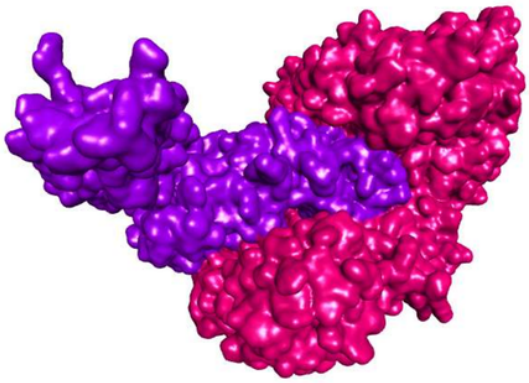

Figure 4

Visualization of Protein-Protein docking of TLR-4 with the predicted ElSvac vaccine model in Discovery studio client 2017. In the surface mode, TLR-4 is represented in pink and the predicted vaccine model (ElSvac) is represented in violet.

Fig. 5

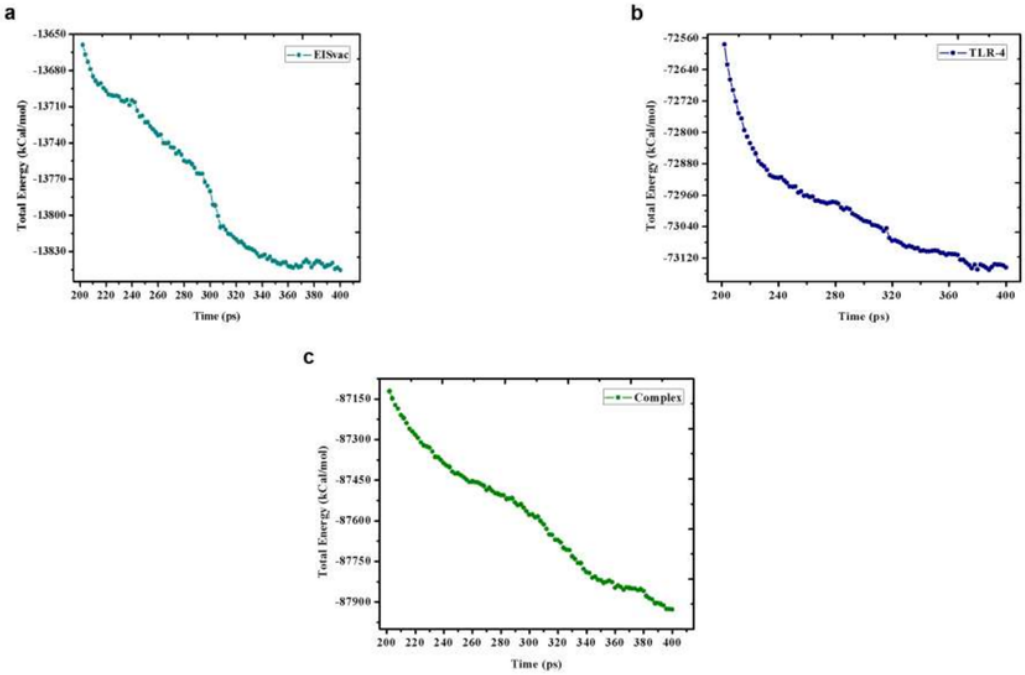

Figure 5

Molecular dynamic simulation. Time (ps) vs Total energy (kCal/mol) plot of (a) the predicted vaccine model (EISvac), (b) TLR-4 and (c) the complex (TLR-4+EISvac).

Fig. 6

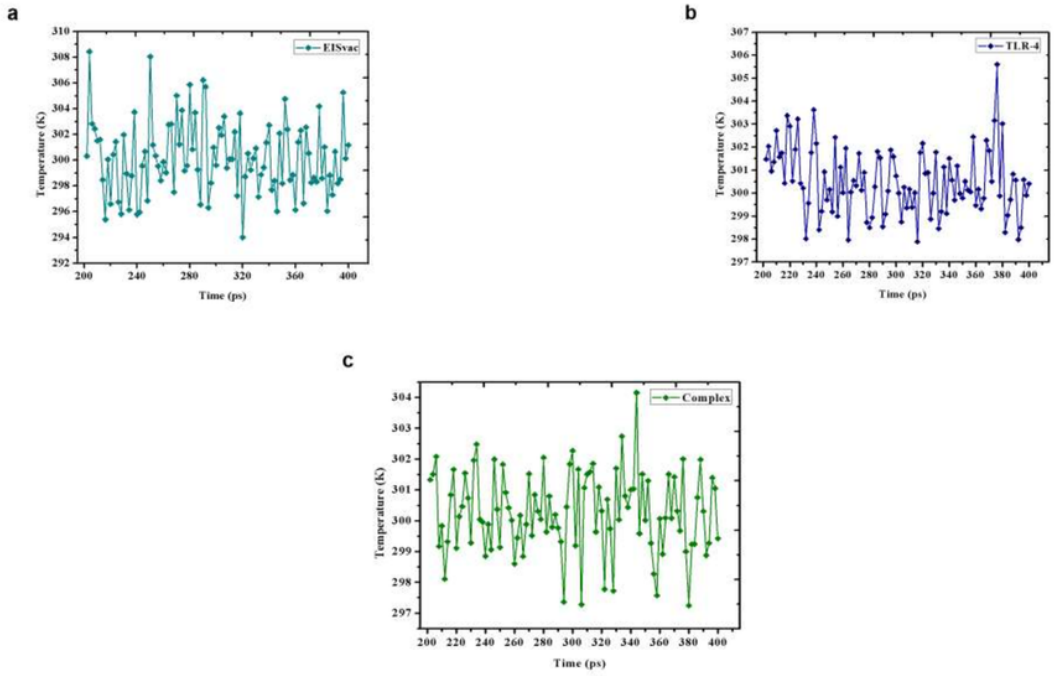

Figure 6

Molecular dynamic simulation. Time (ps) vs Temperature (K) plot in Dynamic Simulation. (a) the predicted vaccine model (EISvac), (b) TLR-4 and (c) the complex (TLR-4+EISvac).

Fig. 7

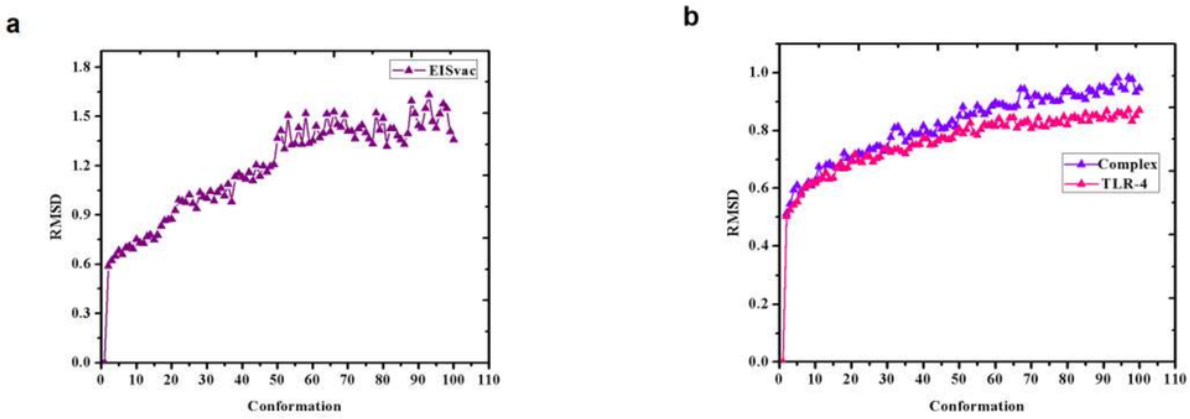

Figure 7

Trajectory analysis of the predicted vaccine model (EISvac), TLR-4 and Complex (TLR-4+EISvac). (a) RMSD value of 100 conformation of predicted vaccine model (EISvac). (b) RMSD value of 100 conformation of TLR-4 and Complex (TLR-4+EISvac).

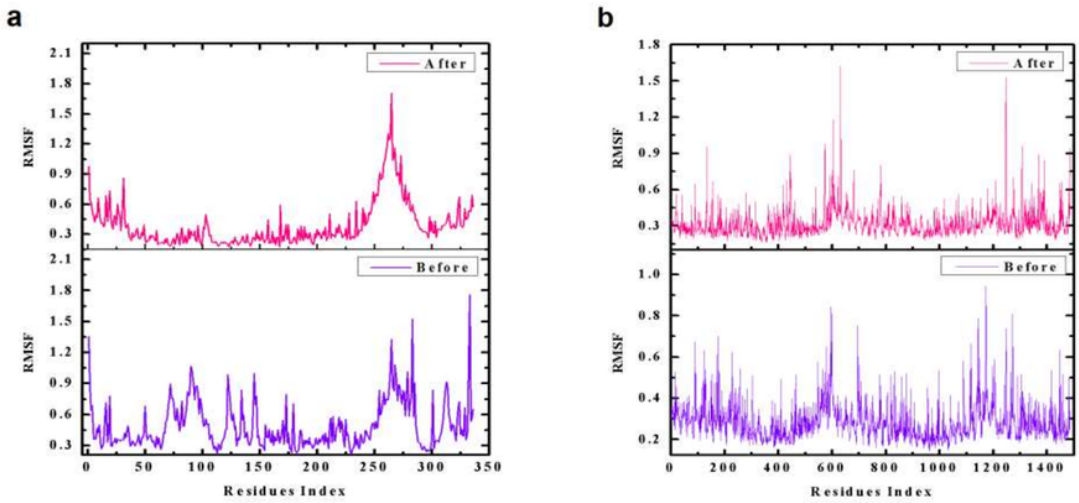

Figure 8

Trajectory analysis of both the receptor (TLR-4) and ligand (EISvac) before and after molecular docking. (A) RMSF of amino acid residues of the predicted vaccine model (EISvac) before and after protein-protein docking with TLR-4. (B) RMSF of amino acid residues of the receptor TLR-4 before and after Protein-Protein docking with the predicted vaccine model (EISvac).

Supplementary Files

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- Supplementarymaterial.docx
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