



Reverse vaccinology approach for multi-epitope centered vaccine design against delta variant of the SARS-CoV-2

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Abstract

The ongoing COVID-19 outbreak, initially identified in Wuhan, China, has impacted people all over the globe and new variants of concern continue to threaten hundreds of thousands of people. The delta variant (first reported in India) is currently classified as one of the most contagious variants of SARS-CoV-2. It is estimated that the transmission rate of delta variant is 225% times faster than the alpha variant, and it is causing havoc worldwide (especially in the USA, UK, and South Asia). The mutations found in the spike protein of delta variant make it more infective than other variants in addition to ruining the global efficacy of available vaccines. In the current study, an *in silico* reverse vaccinology approach was applied for multi-epitope vaccine construction against the spike protein of delta variant, which could induce an immune response against COVID-19 infection. Non-toxic, highly conserved, non-allergenic and highly antigenic B-cell, HTL, and CTL epitopes were identified to minimize adverse effects and maximize the efficacy of chimeric vaccines that could be developed from these epitopes. Finally, V1 vaccine construct model was shortlisted and 3D modeling was performed by refinement, docking against HLA and TLR4 protein, simulation and *in silico* expression. *In silico* evaluation showed that the designed chimeric vaccine could elicit an immune response (i.e., cell-mediated and humoral) identified through immune simulation. This study could add to the efforts of overcoming global burden of COVID-19 particularly the variants of concern.

Keywords Reverse vaccinology · Delta variant · Chimeric vaccine · Spike protein · SARS-CoV-2

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Introduction

Coronavirus disease 2019 (COVID-19) has appeared as one of the most life-threatening infectious diseases caused by the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). It was initially diagnosed in Wuhan, China in December 2019 (Ito et al. 2021). The COVID-19 cases spread with reproduction number (R0) (<https://www.gov.uk/guidance/the-r-value-and-growth-rate>) reaching 5.82 million deaths globally and leaving severe impact on infected survivors (Kar et al. 2020; Z. Yang et al. 2021). The WHO has confirmed over 410 million cases with 5.8 million reported deaths (as of 16 February 2022 <https://www.who.int/emergencies/diseases/novel-coronavirus-2022/situation-reports>). Currently, due to the mutations in SARS-CoV-2 genome, a number of variants such as alpha, beta, gamma, delta, kappa, and eta (Lazarevic et al. 2021) have been observed. These variants have higher transmissibility rates and immune escape mechanisms (Brown et al. 2021; Uddin et al. 2022)

One of the recent and hazardous variants of concern (VOC) of SARS-CoV-2 is the delta variant (also called lineage B.1.617.2) derived from the alpha lineage B.1.617 (Ito et al. 2021). It was initially discovered in the Indian state of Maharashtra in the late 2020s (Salvatore et al. 2021), introduced by a traveler from the UK on 31st May 2021. It was designated the delta variant by the World Health Organization (WHO), as India witnessed a devastating second wave of it and it peaked during May 2021 (W. Yang and Shaman 2021). Lately, it spread to more than 93 countries (<https://www.gisaid.org/hcov19-variants/>) and became globally dominant variant, displacing the B.1.1.7 (alpha) variant and other pre-existing lineages (Campbell et al. 2021; Shu and McCauley 2017). It sustained an increase in the reproduction number (R_0) to 1.2–1.4 (in the UK) (Riley et al. 2021). The B.1.617.2 carried the following mutations in the spike protein: *G142D*, *T19R*, *R158G*, *L452R*, *T478K*, *D614G*, *P681R*, *D950N*, *T95I*, *E156del*, and *F157del* (Kumar, Dwivedi, et al., 2021) at *N*-terminal domain. The *N*-terminal domain (NTD) mutations reduce the flexibility and revealed rigidity in spike protein of B.1.617.2 (Behl et al. 2022; Brown et al. 2021). These multiple mutations appear to give an advantage to Delta variant over other variants. The notable effect caused by these mutations is the increased transmission rate (within 10 s, 60% more transmissible than alpha variant) rendering it as most dangerous and dominant variant globally (<https://nextstrain.org/ncov/gisaid/global?l=clock>). In England, ~38,805 genotyped SARS-CoV-2 cases are linked with the delta variant with 2.61 times higher risk of hospitalization (<https://nymag.com/intelligencer/2021/06/covid-b-1-617-2-delta-variant-what-we-know.html>) and 1.67 higher risk of A&E care within 14 days of specimen as compared to the alpha variant (Angelo et al. 2017; Riley et al. 2021). Since the beginning of the COVID-19 pandemic, various variants (such as alpha, beta, gamma, delta, kappa, and currently omicron) have emerged as a consequence of mutations in the SARS-CoV-2 genome. These alterations have a greater influence on the rate of transmission and the immune system's ability to escape (Negrut et al. 2021; Tao et al. 2021; Thakur et al. 2021). Lately, the WHO reported on November 24, 2021 that a novel mutant variant of SARS-CoV-2 was identified in Botswana, South Africa. On November 26, 2021, WHO designated the novel variant omicron as a variant of concern (VoC) (Poudel et al. 2022). The mutational profile of omicron is crucial for determining whether it shares or varies in clinical symptoms from other SARS-CoV-2 variants. As a result of the substantially modified form's development, several nations have taken rigorous measures to reduce the transmission of the variants of SARS-CoV-2.

Despite the availability of several vaccines, it is an immense logistical challenge to achieve universal coverage especially in populous nations (Mlcochova et al. 2021). The

vaccination process has effectively controlled the hospitalization and deaths related to COVID-19 infection. However, the new viral variants with novel mutations and antigenic profiles are posing serious threats and diminishing the efficacy of the available vaccines (Angelo et al. 2017). It has been observed through sensitivity sera test performed by Davis et al. (2021) against SARS-CoV-2 variants that B.1.617.2 conferred reductions in neutralization of sera (for Pfizer and Oxford vaccine) 5.11-fold while ~eightfold reduction in sensitivity observed through in vitro analysis in contrast to the wild type SARS-CoV-2 from Wuhan-1 (Krause et al. 2021). More recently, a study performed by the UK government found that only 33% protection was provided by single dose of COVID-19 vaccine against B.1.617.2 (compared with 51% protection against B.1.1.7) (Callaway 2021; W. Yang and Shaman 2021). Nevertheless, Israel on June 26, 2021 reported COVID-19 cases among which, about 90% of infections were likely caused by the delta variant, infecting ~50% of fully vaccinated adults (Papenfuss 2021). Therefore, new vaccine constructs are of dire necessity for efficacious operative long-term management of delta variant infections in current situation of pandemics (Kabir et al. 2020; Mlcochova et al. 2021).

Traditionally, vaccine designing requires a pathogen growth (i.e., inactivation, isolation, and injection of the disease-causing virus) and assays (both in vivo and in vitro) that are time and money consuming approaches (Rappuoli et al. 2016). Traditional process requires more than a year for the production and availability of an efficacious vaccine, consequently contributing very little to control the current spread of infection (Ojha et al. 2020; Tagde et al. 2021). On the contrary, the development in computational biology and bioinformatics approaches has led the swift design of useful constructs, reducing the conventional laboratory-based experimentations (Sharma et al. 2021). One of the widely used computational approaches for the development of vaccine model is "Reverse Vaccinology" (D'Mello et al. 2019). It enables vaccine construction and designing based on the organism genome sequence information without the need to grow pathogens. It works on the construction of multiple fragments (i.e., epitopes) from viral proteins so that it can elicit cellular and humoral immune responses and reduces the adverse effects (Gheorghie et al. 2021; Shey et al. 2019). Reverse vaccinology approach has been successfully employed in prioritizing and designing vaccine targets against multiple pathogens (Jalal et al. 2022; Khan et al. 2022; Nosrati et al. 2019; Srivastava et al. 2019; Tosta et al. 2021).

Previous studies on SARS-CoV-2's spike protein revealed that this protein is the key component that is enabling the entry of virus in human cells and playing a decisive role in infection (Kar et al. 2020). The emergence of multiple variants leads to the emergence of several waves of devastating

pandemics around the world (Kumar et al. 2021a, b; Li et al. 2021). As a result, vaccines required a booster dose after 6 months due to high transmission of these SARS-CoV-2 variants. Therefore, current study applied the reverse vaccinology approach against the spike protein of delta variant to design multi-epitope-based vaccine model that can induce cellular (i.e., the activation of T helper cells, T cytotoxic cells), interferon- γ (i.e., IFN- γ), and humoral (i.e., B-cells activations and antibodies production) responses. Furthermore, the shortlisted vaccine molecules can be effectively expressed in *E. coli* vector model. We strongly believe that our findings may provide prolific information and better guidance for further vaccine development against the delta variant.

Material and methods

The current study's pipeline is based on reverse vaccinology for the identification of novel vaccine constructs against the delta variant spike protein.

Data retrieval

The delta variant spike glycoprotein sequence was retrieved from Zhanglab's database (Huang et al. 2020) and mutated with reported amino acid changes, i.e., *T19R*, *G142D*, *E156del*, *F157del*, *R158G*, *T478K*, *L452R*, *D614G*, *P681R*, *D950N*, and *T95I* (Quinonez et al. 2021) using PyMol software. The FASTA format was used for saving the variant spike protein for further analysis.

Reverse vaccinology

The recent advancements in vaccinomics, immunology, biochemistry, molecular biology, genomics, proteomics, and the conventional vaccinology have transformed into reverse vaccinology (RV). The RV is one of the novel and emerging computational approaches that have been tremendously used to optimize the vaccine target and vaccine model prediction, particularly those microbes which are difficult to cultivate in the laboratory (Rappuoli et al. 2016). It combines the immunology, molecular biology, biochemistry, genomics, and proteomics based in silico approaches to screen whole proteome of pathogens in order to determine novel vaccine candidates and assess its ability to induce host immune response.

Antigenicity identification

The antigenic analysis of delta spike protein was performed through VaxiJen v 2.0 (Doytchinova and Flower 2007) server using a threshold value of 0.4, in order to be recognized by the immune system.

MHC-I T-cell epitope prediction

Different T cell epitopes that can potentially activate human immune system and produce memory cells (immunomodulatory effects) were analyzed for delta spike protein through NetCTL server (Larsen et al. 2007). The predicted epitopes were chosen on the parameters of overall intrinsic peptide potential scores integrated with transporter-associated efficiency prediction, protease cleavage, prediction score for MHC I epitope affinity, along with combined score of predicted parameters with threshold value of 0.75.

Additionally, the binding analysis of identified T-cell epitopes was studied by employing Immune Epitope Database and Analysis Resource (IEDB AR) server (Kim et al. 2012) through which T-cell recognized antigen represented by MHC-I. Default parameters of consensus method, i.e., ANN (Nielsen et al. 2003), SMM (Chen et al. 2009), CombiLib (Sidney et al. 2008), and NetMHCpan (Lundegaard et al. 2008) and all HLA alleles were used for MHC-I prediction. The HLA alleles selected for the MHC-I analysis were HLA-A 0205, HLA_0201, HLA-A2, HLA-A 2.1, HLA-A3, HLA-B 5401, and HLA-B 5102. The threshold parameters based on IC50 < 100 μ M and percentile rank (< 0.2) were set as cut-off values for the shortlisting of MHC-I epitopes (Solanki and Tiwari 2018).

MHC-I immunogenicity prediction

The predicted MHC-I epitopes should be immunogenic enough so that they can stimulate either CD4 or CD8 T-cells. Therefore, IEBD AR tool (Dhanda et al. 2019) was used for the prediction of MHC-I immunogenicity analysis. The score having positive value for MHC-I epitopes was selected for further study.

Antigenicity, toxicity and conservancy assessments for MHC-I predicted epitope

The shortlisted MHC-I epitopes having promiscuous immunogenic scores were further analyzed to predict their toxicity, conservancy, and antigenic properties. The conservancy analysis was performed through IEBD server (Angelo et al. 2017). The conservancy analysis is important to develop a broad-spectrum peptide-based vaccine against specific virus. Furthermore, antigenicity of conserve epitopes was predicted through VaxiJen server (Doytchinova and Flower 2007) with an accuracy of 70–80% and 0.5 probability threshold score. Finally, ToxinPred server was utilized for the prediction of relative toxicity levels for shortlisted antigenic MHC-I epitopes with cut-off value set as 0.5.

T-cell MHC-II prediction

Additionally, the MHC-II epitopes were also identified using IEBD server based on consensus method w. The cut-off value for MHC-II epitopes shortlisting was set as < 0.2 peptide rank and $IC_{50} < 100$ nM for top binders against the 95% HLA variability found in worldwide human population, i.e., DRB1*1301, DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB3*01:01, DRB1*0801, DRB1*1101, HLA- HLA-DRB3*02:02, HLA-DRB5*01:01, HLA-DRB4*01:01, and DRB1*1501 (Solanki and Tiwari 2018). In the current analysis, multiple epitopes with 9–14 residues length were shortlisted for further study.

MHC restricted alleles cluster analysis

The MHCcluster server (Thomsen et al. 2013) was used to cluster MHC restricted alleles with appropriate MHC epitopes for further validation of identified MHC-I/II epitopes. The tool results in clustering of MHC-I and II epitopes generating heat map and phylogenetic tree highlighting the functional relationship between HLAs and epitopes.

B-cell epitope identification

An ideal peptide vaccine must have the property of inducing long-lasting humoral immunity similar to the natural immune response generated by certain infections. B-cell epitopes are responsible for the stimulation of humoral immunity having the ability to eliminate the virus by producing antibodies against antigen exposed in human body through B lymphocytes. ABCpred, FBCpred, and BCpred (Saha and Raghava 2007) using sequence-based methodology with a cut-off score of > 0.51 and 75% specificity were employed for the identification of B-cell epitopes, respectively. Moreover, ElliPro server (Ponomarenko et al. 2008) was also used to characterize B-cell epitopes based on their hydrophobicity content (El-Manzalawy et al. 2008), flexibility (Karplus and Schulz 1985), antigenicity (Emini et al. 1985), accessibility, beta-turn prediction through Chou and Fasman tool (Chou and Fasman 1978), Karplus and Schulz flexibility scale, and Parker hydrophilicity scale (Parker et al. 1986), respectively.

Identified epitope mapping

Consequently, the epitopes having ability to induce immune cells (B and T-cells) response are significant for the designing of epitope-based vaccine (Solanki and Tiwari 2018). Therefore, the shortlisted MHC-I-II and B-cell epitopes of delta spike protein were mapped for the identification of binding affinity and similarity among

them. The manual comparison was performed and overlapping epitopes characterized as probable peptides sequences were assembled and considered a final predicted epitopes for vaccine modeling.

Vaccines construction and structure modeling

In order to construct vaccine with reduced toxicity and allergenicity and increased immunogenicity, the sequential conjugations of shortlisted epitopes were performed with relevant adjuvant (beta-defensin, L7/L12 ribosomal protein, HBHA protein, and HBHA conserved sequence), PADRE (Pan HLA-DR reactive epitope) and linker (GGGS, HEYGAEALERAG and EAAAK) (Solanki and Tiwari 2018). The use of linkers enhances immunogenicity while the induction of $CD4^+$ T-cells by PADRE peptide makes the vaccine efficacious and potent. HEYGAEALERAG and GGGS linkers were utilized to conjugate HTL, CTL, and B-cell epitopes, whereas EAAAK linkers were used to join adjuvant sequences at both N and C-terminus (Solanki and Tiwari 2018).

Antigenicity, allergenicity, and solubility assessment for vaccines constructs

Adverse allergic reactions may be associated with vaccine outcomes. In order to overcome the allergic features of vaccine model, AlgPred tool was used to examine the allergenicity of model vaccine sequences with cut-off score of -0.4 and 85% accuracy (N. Sharma et al. 2020). Predicted scores less than the threshold value were considered non-allergenic vaccines. To predict the antigenic nature of vaccine models, the VaxiJen and ANTIGENpro server (Magnan et al. 2010) were used with the threshold value > 0.5 . Furthermore, to identify the solubility property of vaccine model upon over-expression in *E. coli*, SOLpro program was used having default parameters of 74% accuracy and corresponding probability (≥ 0.5) (Magnan et al. 2009).

Physiochemical analysis of constructed vaccines

The widely used ExPASy ProtParam tool (Gasteiger et al. 2005) was utilized for the physiochemical analysis and functional characterization of vaccines based on pK values of different amino acids, GRAVY values, instability index, molecular weight, aliphatic index, approximate half-life, hydrophobicity, and isoelectric pH parameters (Gasteiger et al. 2005). It is essential to evaluate physiochemical properties to determine the safety and efficacy of vaccine candidates.

Comparative structure modeling

Modeler Phyre2 tool, whereas PSIPRED (Buchan and Jones 2019), ProSA-web (Wiederstein and Sippl 2007), and PRO-CHECK (R. Laskowski et al. 1993) were applied for the model structure evaluation based on secondary structure analysis, error in 3D modeled identification, and tertiary structure stereochemistry analysis, respectively. The best-modeled vaccine construct was selected for further structure-based analysis.

Molecular docking studies

The interaction of vaccine construct was modeled with its receptors to generate the stable immune response of vaccine model to target cells. The molecular docking approach is an ideal method to study such interaction studies in terms of binding energies between epitopes and HLA proteins (Solanki and Tiwari 2018). The final potential vaccine constructs fulfilling all filters of the framework were docked into the binding cavity of six most common human alleles in human population HLA alleles, i.e., 3C5J (HLA-DRB3*02:02), 2Q6W (HLA-DRB3*01:01), 1H15 (HLA-DR B5*01:01), 2FSE (HLA-DRB1*01:01), 1A6A (HLA-DR B1*03:01), and 2SEB (HLA-DRB1*04:01) retrieved from Protein Data Bank. The PatchDock server (Schneidman-Duhovny et al. 2005) was used to estimate the HLA and vaccine interactions while the FireDock (fast interaction refinement in molecular docking) server was applied to further refine and re-score the docked complex obtained through PatchDock (Mashiach et al. 2008). Moreover, the docking step was validated by GRAMMX tool for vaccine and TLR4/MD complex. The TLR4 is implicated in viral protein recognition, leading to the production of inflammatory cytokines. According to several studies, TLR4 is critical for generating an efficient immune response against SARS-CoV-2 (Hu et al. 2012). Therefore, the molecular docking studies were performed for the vaccine candidate with TLR4. The UCSF Chimera (Pettersen et al. 2004) and PDBsum (Laskowski et al. 2018) tools were used for the binding interpretation based on hydrogen bond pattern and docked scores.

Molecular dynamic simulation studies

The molecular dynamic simulation (MDS) and energy minimization were performed through GROMACS (GROningen MAchine for Chemical Simulations) to determine the stability and flexibility of the vaccine construct. The MDS was performed to examine how vaccine model acts in biological system (Abraham et al. 2015). The topology files needed for energy minimization and equilibrium were generated using GROMACS MDS's published approach.

The solvation was executed with SPC216 water model with energy minimization using steepest algorithm and NVT and NPT ensembles for 50,000 steps (100 ps). Furthermore, the neutralization of vaccine construct was performed by adding charged ions to MDS system. Finally, the vaccine molecular dynamics simulation was carried out for 10 ns to find root mean square deviation (RMSD). The graphs obtained from MDS were visualized employing the Xmgrace plotting tool (Cowan and Grosdidier 2000). Furthermore, molecular dynamic simulation of docked complex (vaccine with TLR4) was performed using iMODS web-server (López-Blanco et al. 2014). It explains the collective movements of protein complex with vaccine construct through normal mode analysis (NMA). The intrinsic complex motion extent and direction were evaluated in terms of covariance, deformability, eigenvalue, and B-factors.

Immune simulation of final vaccine construct

The immunogenicity and immune response profile of a chimeric peptide vaccine were characterized using the C-ImmSim service. The C-ImmSim server is an in silico-based immune simulation method (Rapin et al. 2010). At three different intervals for 4 weeks, three injections of molded prophylactic delta variant vaccine at 1, 82, and 126 h time periods and 12,345 random seed were administered keeping all simulation parameters at default containing no LPS, volume and the stages of the simulation at 10, and 1000, respectively, with homozygous host haplotypes HLA-A*0101, HLA-A*0201, HLA-B*0702, HLA-DRB1*0101, and HLA-DRB1*0401 (Rahman et al. 2020).

Codon optimization and in-silico cloning

The Java Codon Adaptation Tool (JCAT), based on codon adaptation approach, was used to reverse translate vaccine amino acid sequence in cDNA for the effective expression of vaccine construct in *E. coli* vector (Grote et al. 2005). Computed Codon Adaptation Index (CAI) values and GC content were used as a parameters for vaccine adaptation while avoiding the ribosome-binding prokaryote site, termination of Rho's independent transcription, and the cleavage of restriction enzymes (Bibi et al. 2021). Finally, the insertion of adapted codon sequence was performed through SnapGene cloning module into the pET-28a (+) vector.

Results

Antigenicity prediction for delta spike protein

The antigenicity analysis for delta variant spike protein predicted through VaxiJen v2.0 server was found to be 0.4703

using a cut-off value of 0.4 characterizing it as antigenic protein that can induce host-immune system response.

Identification of MHC-I T-cell epitope

The NetCTL server resulted in the prediction of 1262 T-cell epitopes using a threshold value of 0.75. MHC-I binding study was performed on these epitopes using IEBD tools. It identified ~34,075 epitopes for MHC-I. However, 284 epitopes were shortlisted that elicited high binding affinity using a cut-off parameter, i.e., $IC_{50} < 100$ and percentile rank ≤ 0.2 based on MHC-I and T-cell interaction. All these shortlisted epitopes were identified as optimal binders to T-cells and therefore assessed for further analysis.

Immunogenicity prediction for shortlisted epitopes

The efficacy of epitopes to induce T-cells response is based on their immunogenicity level. The shortlisted epitopes were analyzed for immunogenicity prediction. The greater the immunogenicity score, the higher will be the ability of epitopes to simulate naive T-cells and cellular immunity. From above 284 shortlisted MHC-I epitopes, 150 immunogenic epitopes with a cut-off value of the positive score were identified as having significant immunogenic values predicted through IEBD server. These immunogenic shortlisted epitopes were used for further study in vaccine designing.

Antigenicity, conservancy, and toxicity analysis

Furthermore, toxicity, antigenicity, and conservancy analysis were performed for the shortlisted 150 immunogenic epitopes. The ToxinPred and IEBD conservancy results revealed that all 150 sequences were non-toxic and 100% conserved within the spike protein of delta SARS-CoV-2. However, antigenicity analysis performed through VaxiJen server identified a total of 37 epitopes (Table 1) that are characterized as antigenic, i.e., scoring from 0.5 to 1.0 and selected for further evaluation while the remaining less/non-antigenic immunogenic epitopes were discarded.

MHC-II epitopes identification and antigenicity, toxicity, and conservancy analysis

Beside the MHC-I epitopes prediction, MHC-II epitopes were also identified using IEBD server. The epitopes having low percentile rank (> 0.2) and high binding affinity ($IC_{50} < 100$ nM) were analyzed. The server resulted in the prediction of 14 MHC-II epitopes. The ToxinPred and IEBD conservancy tools demonstrated that all 150 sequences inside the spike protein of delta SARS-CoV-2 were non-toxic and 100% conserved. However, using the VaxiJen server, antigenicity analysis revealed a total of 14 epitopes

Table 1 Predicted antigenicity of MHC-I epitopes (all epitopes are non-toxic, and conservancy is 100% for each)

S. no	Epitopes	Antigenicity
1	YIKWPWYIW	0.963
2	VTWFHAIHV	0.5426
3	QLTPTWRVY	1.2119
4	TLADAGFIK	0.5781
5	QYIKWPWYI	1.4177
6	IAIPTNFTI	0.7052
7	IPNFTISV	0.882
8	SVYAWNRRK	0.765
9	KEIDRLNEV	0.53
10	WTAGAAAYY	0.6306
11	DIADTTDAV	1.0904
12	FNATRFASV	0.5609
13	YQPYRVVVL	0.5964
14	VVFLHVTYV	1.5122
15	LPFNDGVYF	0.5593
16	GVYFASTEK	0.7112
17	ASANLAATK	0.7014
18	NYNRYRLF	0.9855
19	NATNVVIKV	0.6726
20	STQDLFLPF	0.6619
21	NASVVNIQK	0.8372
22	PFFSNVTWF	0.6638
23	YEQYIKWPW	0.869
24	IGAGICASY	0.6368
25	NLNEIDL	0.6827
26	HWFVTQRNF	0.746
27	LPFFSNVTW	1.0808
28	FTISVTTEI	0.8535
29	PYRVVLSF	1.0281
30	KVGGNYNYR	1.5212
31	VTYVPAQEK	0.8132
32	GYLQPRTFL	0.6082
33	GLTVLPPLL	0.6621
34	RLDKVEAEV	0.0765
35	AEIRASANL	0.7082
36	TLLALHRSY	0.8009
37	GQTGKIADY	1.4019

(Table 2) that are classified as antigenic, with scores ranging from 0.5 to 1.0, and hence chose for further examination. The remaining less/non-antigenic immunogenic epitopes were eliminated.

MHC restriction cluster analysis of shortlisted epitopes

MHCclusters tool used for the identification and evaluation of MHC-I/II epitopes with respect to MHC restricted allele

Table 2 Predicted antigenicity of MHC-I epitopes (all epitopes are non-toxic, and conservancy is 100% for each)

S. no	Epitopes	Antigenicity
1	QSLIVNNATNVVIK	0.43
2	FGEVFNATRFASVYA	0.041
3	SLIVNNATNVVIKV	0.47
4	TQSLIVNNATNVVI	0.433
5	GEVFNATRFASVYAW	-0.12
6	PFGEVFNATRFASVY	0.033
7	LLIVNNATNVVIKVC	0.099
8	EVFNATRFASVYAWN	0.08
9	KTQSLIVNNATNVV	0.63
10	CPFGEVFNATRFASV	0.29
11	NCTFEYVSQPFLMDL	0.52
12	CTFEYVSQPFLMDLE	0.57
13	QQLIRAAEIRASANL	0.12
14	REGVFSVNGTHWFVT	0.44

and their appropriate peptides resulted in the confirmation of identified T-cells epitopes. The interactions between MHC-I/II and HLAs are displayed as heat map and phylogeny dynamic tree highlighting the stronger interaction in red color while yellow color represents weak interaction in terms of annotation (Fig. 1).

B-cell epitope prediction

Both humoral and cellular immunity is needed simultaneously to successfully eliminate the virus from the body. Therefore, B-cell epitopes were also identified against delta spike protein using ABCPred, BCPred, and FBCPred. These tools are resulted in the identification of 36, 21, and 39 B-cell epitopes using a threshold value of 0.51, and

75% specificity (Table S1). Furthermore, resultant B-cells epitopes were further analyzed and shortlisted based on BepiPred linear epitope prediction, Kolaskar Tongaonkar antigenicity, Parker hydrophilicity prediction, Chou-Fasman beta-turn prediction, Karplus-Schulz flexibility prediction, and Emini surface accessibility prediction. The result of predicted B-cell epitopes for delta spike proteins is highlighted in Fig. 2.

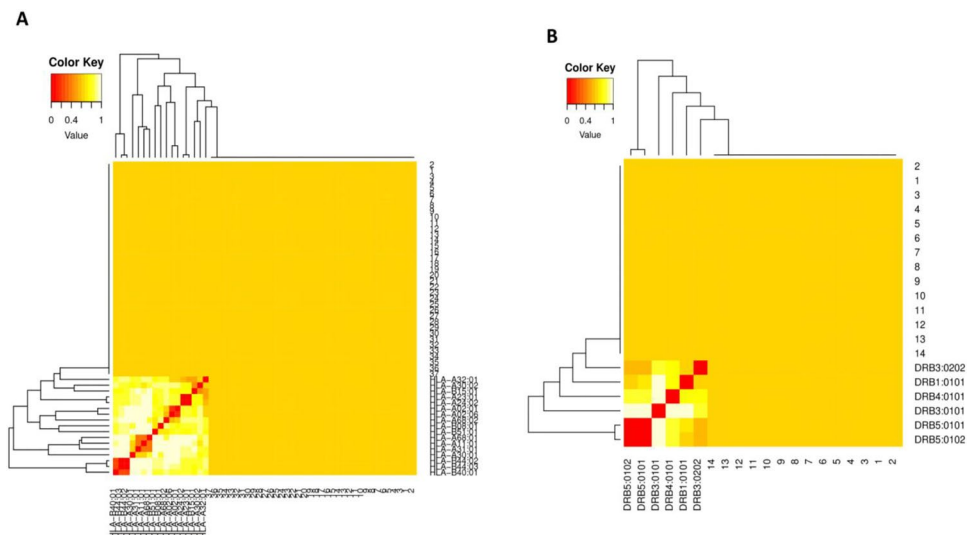
Epitope mapping and prioritization

The shortlisted B-cell epitopes were used as a template and manually compared against MHC-I, and MHC-II epitopes to screen out the overlapping epitopes. The comparative analysis was resulted in the shortlisting of 4 epitopes having common MHC-I, MHC-II, and B-cell epitopes (Table 3), i.e., TQSLIVNNATNVVIKVCEF, TRFASVYAWNRRKRI-NCV, LQELGKYEQYIKWPWYIWLG, and DQLTPT-WRVYSTGSNVFQTR, respectively.

Vaccine model construction

These four shortlisted epitopes from the above analysis were arranged in sequential manner along with the use of four adjuvants, PADRE sequences, GGGs, HEYGAEALERAG, and EAAAK linkers. This combination of different adjuvants and linkers helped to inflict strong immune response in body against viruses while PADRE sequences help to overcome the global polymorphism effect of HLA-DR molecules in various populations. Different vaccine models were constructed. The schematic diagram of vaccine construct is highlighted in Fig. 3 whereas supplementary Table S2 showed the detail of the vaccine constructs.

Fig. 1 Clustering analysis for MHC I and II epitopes. The cluster analysis of MHC molecules and HLA alleles **A** MHC I clustering allele's, **B** MHCII clustering alleles. Red color indicates strong interaction while yellow zone indicates the weaker interaction



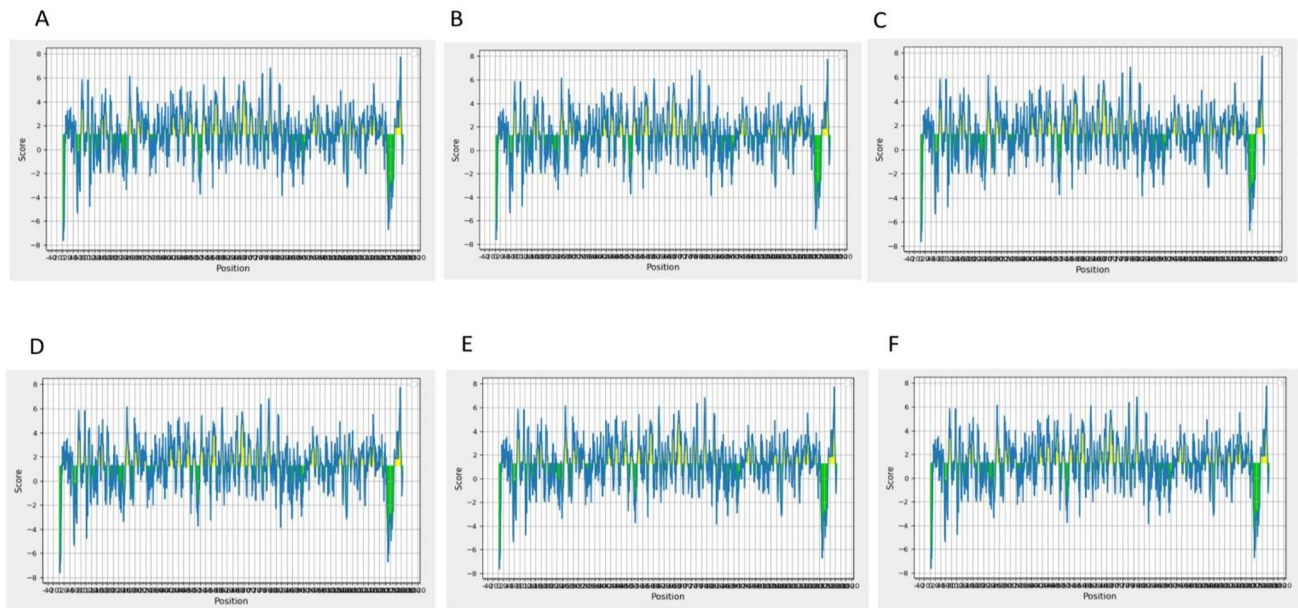
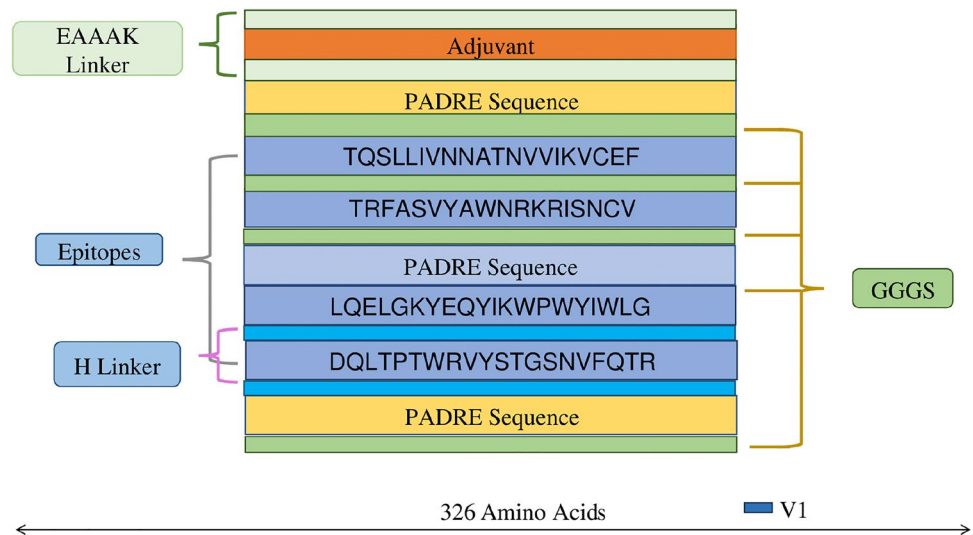


Fig. 2 B-cell epitopes analysis. **A** Bepipred linear epitope, **B** Chou & Fasman beta-turn prediction, **C** Emini surface accessibility prediction, **D** Karplus & Schulz flexibility prediction, **E** Kolaskar & Tongaonkar antigenicity, **F** Parker hydrophilicity prediction

Table 3 B cell, MHC-I, and MHC-II epitopes mapping information

S. no	Position	B-cell epitopes	MHC-I epitopes	MHC-II epitopes	Score
1	114–133	TQSLIVN NATNVVIKVCEF	NATNVVIKV	SLIVN NATNVVIKV	0.7
2	343–360	TRFASVYAWN RKRISNCV	SVYAWN RKR	FGEVFNATRFASVYA	0.8
3	1197–1216	LQELGKYEQYIKWPWYIWLG	YIKWPWYIW		0.85
4	624–644	DQLTPTWRVYSTGSNVFQTR	SVYAWN RKR		0.9

Fig. 3 Schematic presentation of the final multi-epitope vaccine peptide. The 395 amino acid long peptide sequence containing adjuvant (brown) at both N and C terminal was linked with the multi-epitope sequence through an EAAAK linker (green). B cell epitopes and HTL epitopes are linked using GGGG linkers (red) while the CTL epitopes are linked with GGGG linkers (grey)



Allergenicity, antigenicity, and solubility prediction

The twelve constructed vaccine models were further analyzed to predict their antigenicity, solubility, and allergenicity. The

AlgPred tool for allergenicity resulted in the identification of six vaccine constructs (V3, V4, V7, V8, V11, and V12) as highly allergenic in nature scoring from 0.2 to 0.3 and were excluded. However, the antigenicity predicted through ANTIGENpro

server and solubility of remaining six vaccine constructs for expression in *E. coli* vector resulted in the prediction of highly antigenicity and solubility of remaining six vaccine constructs (V1, V2, V5, V6, V9, and V10) and scores were ranging from 0.7 to 0.9, and therefore selected for further study. Table 4 showed the allergenicity, solubility, and antigenicity for all twelve vaccine constructs.

Physicochemical properties analysis of shortlisted vaccine construct

The ProtParam utilized for physicochemical characteristics' prediction for all shortlisted six vaccines constructs resulted in estimated molecular weight of vaccines model as ~35KDa, pI score of ~5, while instability index score was in the range of 28–34 whereas high aliphatic score ranges from 83 to 86. The grand average of hydropathicity, on the other hand, was calculated to be in the range of 0.2 (Table 5).

Structure prediction and validation of selected vaccine construct

Phyre2 tool was used for comparative 3D structure modeling of six shortlisted model vaccine constructs. Based on modeled structure and template sequence similarities, V1 vaccine construct was selected as final vaccine model. The template identified for V1 was PDB ID: 1EQ1A, Apolipoprotein-II protein from *Manduca sexta* having sequence identity of 38% (Fig. 4).

Furthermore, the 3D structure evaluation through PROCHECK results in the stereochemical property identification

Table 4 Vaccine allergenicity, antigenicity, and solubility

Vaccine	Allergenicity	Antigenicity	Solubility
V1	-0.50794438	0.844476	0.945605
V2	-0.41786815	0.792876	0.961907
V5	-0.50794438	0.824917	0.949964
V6	-0.41786815	0.789273	0.962925
V9	-0.50794438	0.951158	0.825384
V10	-0.41786815	0.963312	0.788187

Table 5 Identified physicochemical properties of vaccine constructs

Vaccine	No of AA	MW	PI	-ive AA	+ive AA	Instability index	Aliphatic index	GRAVY index
V1	326	35435.83	5.76	40	43	29.31	83.47	-0.287
V2	317	34317.6	5.47	38	43	34.05	86.72	-0.25
V5	326	35435.83	5.76	43	40	28.83	83.47	-0.287
V6	317	34317.6	5.47	43	38	33.56	86.72	-0.25
V9	326	35435.83	5.76	43	40	29.42	83.47	-0.287
V10	317	34317.6	5.47	43	38	34.17	86.72	-0.25

of the final selected vaccine construct as, 80.9% residues in favorable region, 13.9% residue in additionally allowed region, and 0.9% residues in disallowed region, respectively (Fig. 5a). The ProSA tool predicted a Z-score value of -3.66, indicating the model is near to that of NMR/X-ray crystallography derived structures (Fig. 5b). Moreover, PSIPRED tool was used for the 2D (secondary structure) structure validation showed similar number of alpha helices, beta sheets, and beta turn as shown in Fig. 5c.

Molecular docking of vaccine construct (V1)

The interaction study of V1 model was performed with six HLAs and TLR4/MD2 complex (PDB 2Z65) to evaluate the enhancement in immune response. V1 contains the adjuvant HBHA conserved protein that is agonist to TLR4 protein which induced several immune responses. The PatchDock docking resulted in the -0.6 binding energy between V1 and TLR-4/MD2 complex (Table 6).

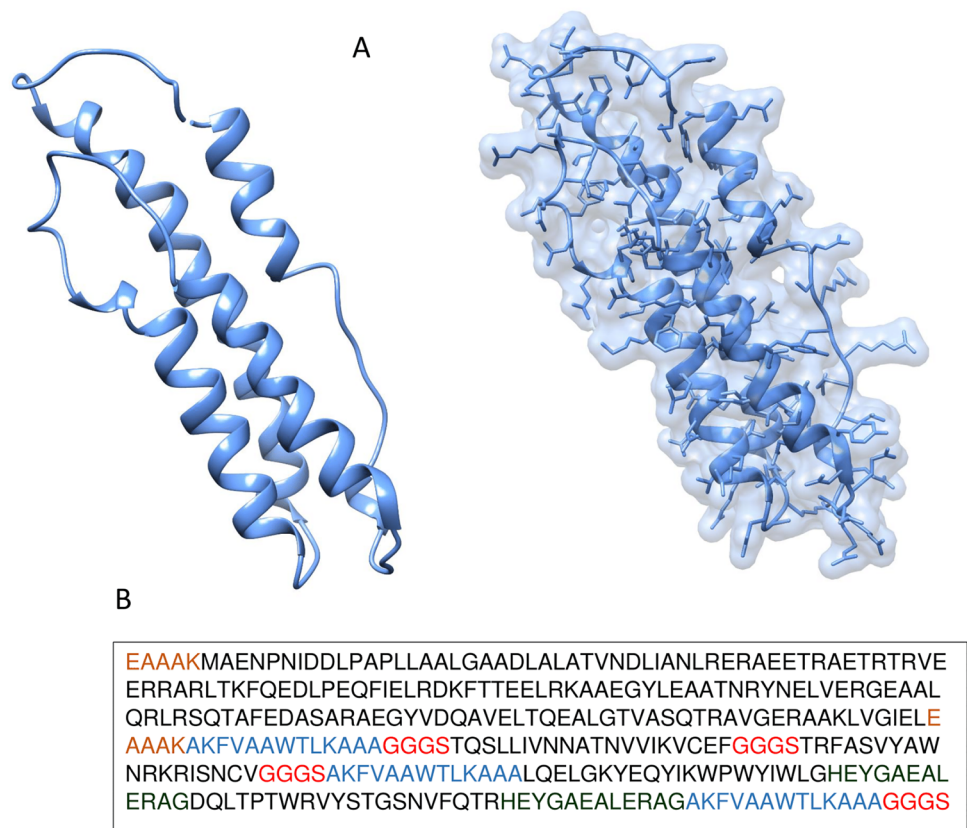
The PPI interactions of vaccine construct and TLR4/MD2 showed 179 non-bonded interactions and three hydrogen bond interactions between Ser265-Asp35, Lys224-Pro16, and Cys193-Ala17 of TLR4/MD and vaccine complex, respectively as shown in Fig. 6.

Molecular dynamics simulation study

The GROMACS simulation tool was used to determine the movements of vaccine construct in biological environment. The molecular dynamics simulation was conducted for the best-docked model to validate the complex interactions. The simulations resulted in the stability of vaccine construct at 4 ns (Fig. 7).

However, the stability of vaccine and TLR4-vaccine complex was also determined through iMOD tool. Deformability graphs were produced that illustrates the normal mode analysis (NMA) for the mobility, flexibility, and stability of vaccine-protein in terms of peaks showing the deformability found in complex as shown in Fig. 8a. The visualization and co-variance of docked complex relationship are explained by the generation of B factor (Fig. 8b). The variance association plot generated the

Fig. 4 Vaccine structure modeling and validation. **A** The 3D model of a multi-epitope vaccine was obtained by Swiss model **B** with template comparison and **C** and vaccine sequence



individual and cumulative variance represented through red and green colored highlighted in Fig. 8c. The eigenvalue of the complex was found to be $9.263151e-05$ as shown in (Fig. 8d), and co-variance map representing the correlation, non-correlation, and anti-correlation motion found between a pair of residues represented by red, white, and blue color respectively (Fig. 8e).

Immune response simulation

Furthermore, C-immune tool was employed for the prediction of human immune system response after the injection of vaccine at different time interval. It confirmed the consistency of immune response with real immune reactions, i.e., identification of B-cell, T-cytotoxic cells, T-helper cells, natural killer cells production, interleukins/interferons production, and antibodies production (Fig. 9). A raise in IgG1+IgG2, IgM, and IgG+IgM was observed after the induction of vaccine injection resulting in decrease antigen concentration (Fig. 9a and b). After the induction of vaccine construct injections, an increased production of Th (helper), Tc (cytotoxic) cells, and memory cells population (Fig. 9c–e) was observed. In addition, IFN-g production was also stimulated after immunization (Fig. 9f).

Codon optimization and in silico cloning of V1

The shortlisted final vaccine construct V1 was back translated into cDNA in order to be expressed in *E. coli* (strain K12) and optimized and clone codon. The Codon Optimization Index (CAI) value for V1 was predicted to be 0.9603, whereas, GC content identified for the adapted sequence was 72% explicit high rate of expression. Finally, the recombinant plasmid with inserted adapted codon sequences was performed using SnapGene tool between 5369 and 6527 bp of pET28a(+) vector (Fig. 10).

Discussion

The COVID-19 is declared as pandemic and surged to cause increase in cases often leading to deaths due to various transmissible variants of the causative virus (UK (alpha/B.1.1.7), Brazil (gamma/P.1), India (delta/B.1.1.7.2), and South Africa (beta/B.1.351)) (World Health Organization: Interim recommendations for use of the Pfizer–BioNTech COVID-19 vaccine, BNT162b2, under emergency use listing: interim guidance, first issued 8 January 2021, updated 15 June 2021 2021). Till the end of April 2021, the delta (B.1.617.2) (<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>) variant, first

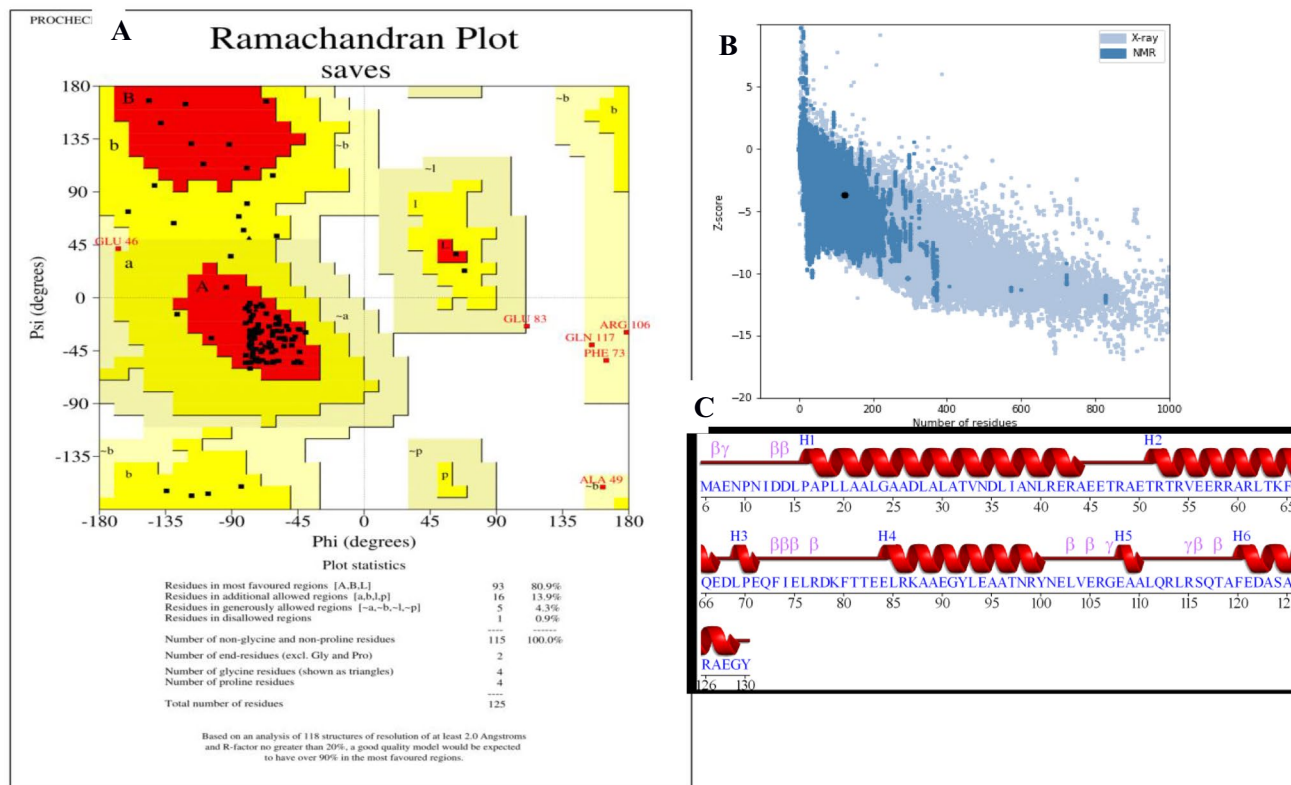


Fig. 5 Structure evaluation through PSIPRED, ProSA, and PROCHECK. **A** Modeled structure validation through Ramachandran plot using PROCHECK showing 91% residues in favored region, **B** ProSA-web modeled structure evaluation indicating the z-score

of -3.66 highlighting the protein in NMR and X-ray crystallographic region whereas, **C** shows structure confirmation for final vaccine construct generated through PSIPRED nearly same position of helices and beta-sheets as modeled structure

Table 6 Docked score of HLA and vaccine model of delta variant

Vaccine construct	HLA alleles (PDB: ID)	Score	Area	Hydrogen bond energy	Global energy	Ace
V1	1A6A	15,698	2777.80	-1.06	9.52	360.52
	3C5J	16,424	2206.70	-1.32	-1.81	405.23
	1H15	18,840	3680.80	-0.30	-0.30	194.99
	2FSE	17,542	2200.40	-2.90	-55.84	493.36
	2Q6W	16,512	3323.10	-3.34	3.05	141.66
	2SEB	17,332	2355.90	-2.83	-38.16	363.88
	2Z65	18,750	2846.10	0.00	-0.06	-30.14

identified in India, has replaced the alpha (B.1.1.7) variant in usually gathered genomic data that resulted in growth of case numbers and hospitalizations (Brown et al. 2021). It currently makes up about 20% of newly diagnosed coronavirus cases in the USA alone (Farinholt et al. 2021), while 45% in Sydney declared the rise in delta cases as “worst health crisis in 120 years.” This variant of concern (VOC) (<https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html#Consequence>) is changing the current pandemic trajectory by having distinct epidemiological changes in transmission and antigenic escape, dominating in America, UK (Riley

et al. 2021), Scotland (Angelo et al. 2017; Sheikh et al. 2021), Israel, Sydney, and South Asia (<https://www.bbc.com/news/world-asia-53420537>). A successful vaccination campaign in the start of 2021 has substantially increased the immunity level whereas the advent of the B.1.617.2 lineage of SARS-CoV-2 has put the delaying dosing tactics of immunization (first dose of vaccine followed by second dose given within 12 weeks) to a new challenge (Yang and Shaman 2021). The substitution and mutation in delta variant spike protein (the main immune dominant region) reduced the binding of several antibodies suggesting the

Fig. 6 Docked vaccine construct with TLR4/MD. **A** Docked complex of vaccine (red) and TL4/MD (purple) (**B**), interaction occurs between the vaccine model and TLR4/MD protein. Interacting residues of vaccine represented in brown color, while protein interacting residues have been highlighted in orange-red color, **C** all interactions found between the docked complexes, i.e., blue lines represent hydrogen bonding, and orange lines represents non bounded interactions

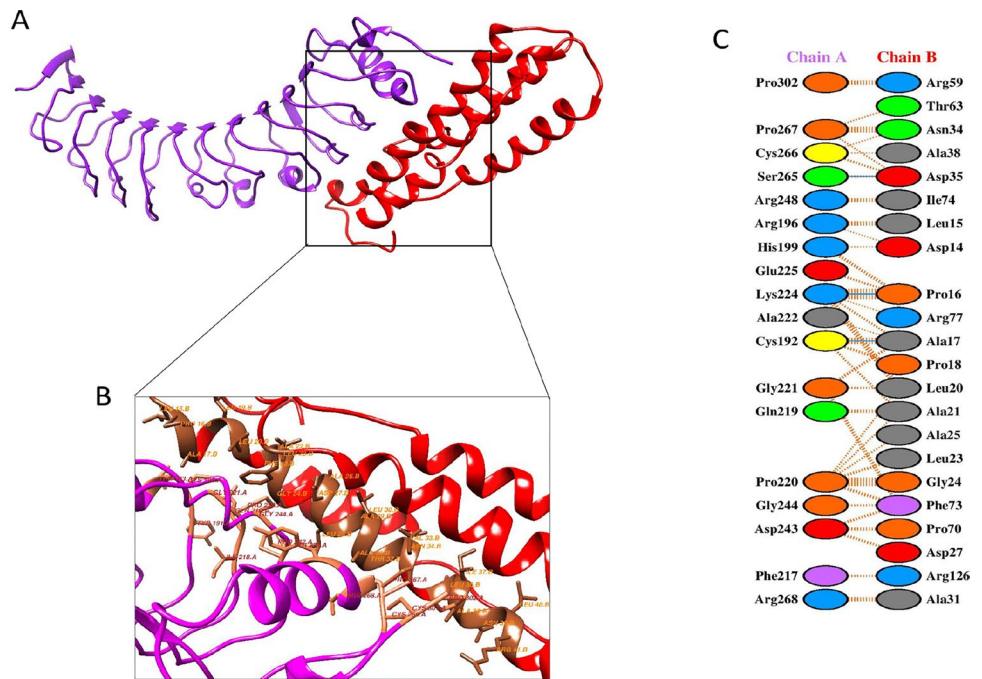
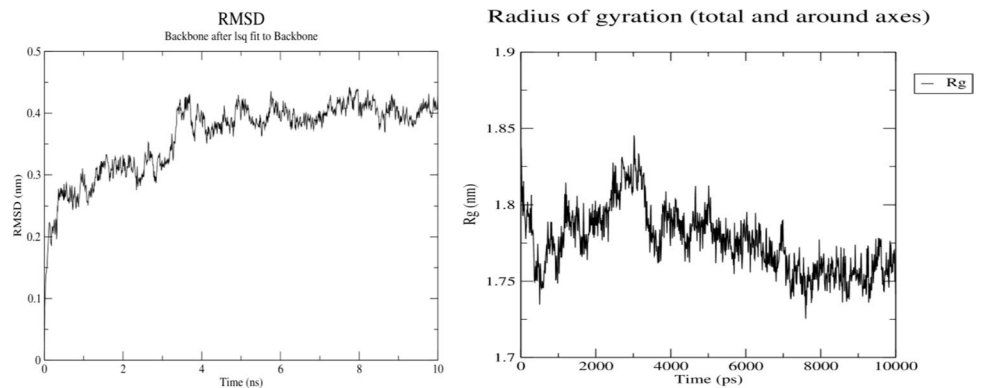


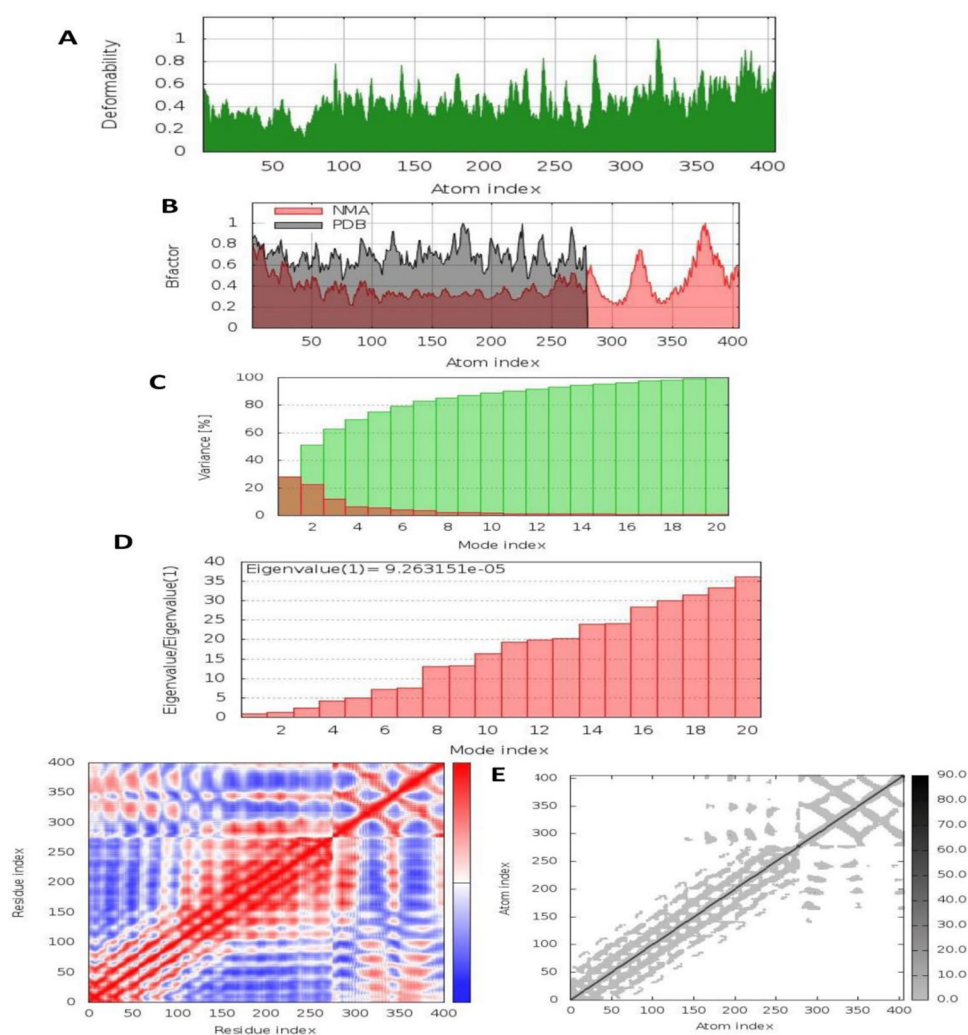
Fig. 7 Molecular dynamic simulation of construct V1. **A** Root mean square deviation (RMSD) of protein backbone, **B** plot of radius of gyration vs time during MDS



role of these mutations in immune evasion (Planas et al. 2021). Recently, it was reported by Wall et al. (2021) that they identified the resistance of delta variant fourfold less potent compared to variant alpha (B.1.1.7) to neutralization of sera from convalescent patients whereas, sera from people who received a single dose of AstraZeneca or Pfizer vaccines hardly inhibited delta variant (Mlcochova et al. 2021; Planas et al. 2021). Nevertheless, some studies also suggested the use of multiple (three) dose regimes of vaccine to minimize delta variant effects and induce immunity (<https://edition.cnn.com/2021/07/08/health/us-coronavirus-thursday/index.html>). It has been reported that delta variant infected individuals on average having up to 1000 times more copies of the virus in their respiratory system compared to those infected by alpha strain (Mlcochova et al. 2021). Due to high transmission and immune evasion of ongoing delta variant, the development of new vaccines is urgently needed to overcome the spread of fourth wave of the pandemic.

The traditional methods of vaccine designing utilize large proteins or the complete organisms that produced an unnecessary antigenic load and increased allergenic responses (Chauhan et al. 2019; Sette and Fikes 2003). On the other hand, the immunoinformatic approaches are cost effective and time saving that can address this problem through peptide-based vaccine construction and stimulates the strong but targeted immune response (He et al. 2018; Lu et al. 2017). Therefore, the current study employed the in silico approach-based reverse vaccinology to attain a multi-epitope vaccine against spike protein of delta variant that can induce the activation of immune cells (Abraham Peele et al. 2020). The design of multi-epitope-based vaccine is an emerging field that produces the vaccine models having not only protective immunity (Cao et al. 2017; Guo et al. 2014; Zhou et al. 2009) but also has been characterized in phase-I clinical trials (Jiang et al. 2017; Lennerz et al. 2014; Slingluff et al. 2013; Toledo et al. 2001).

Fig. 8 The results of molecular dynamics simulation of vaccine construct and TLR4/MD docked complex. **A** Deformability, **B** B-factor, **C** variance (red color indicates individual variances and green color indicates cumulative variances), **D** eigenvalues, and **E** co-variance map (correlated (red), uncorrelated (white), or anti-correlated (blue) motions)



The present study resulted in the identification of probable immunogenic MHC-I, MHC-II, and B-cell epitopes to construct the multi-epitope vaccine using various filters such as (i) the epitopes must be non-toxic, antigenic, non-allergenic, highly conserved (Table 1) and (ii) have the ability to bind to MHC-I/II alleles, and should be overlapping to CTL, HTL, and B-cell epitopes (Table 2).

Bazhan et al. applied the similar approach to design T-cell multi-epitope vaccine model against *Ebola virus* that was significantly immunogenic in mice (Bazhan et al. 2019). In the current study, twelve different vaccine constructs were modeled using four adjuvants, i.e., HBHA protein, HBHA conserved sequence, beta-defensin, and L7/L12 ribosomal protein along with GGGs, PADRE sequences, HEYGAE-ALERAG, and EAAAK linkers (Table S2). These twelve-vaccine models were further subjected to filtration steps, i.e., allergenicity, antigenicity, solubility, and physicochemical property analysis to shortlist a most promiscuous vaccine construct against the delta variant. The filtration resulted in the identification of V1 as the most potent vaccine constructs

against delta variant as non-allergenic, most antigenic, highly soluble over expressing in *E. coli* and having suitable physicochemical properties (Tables 4 and 5). Similar in silico strategies were also applied by Foroutan et al. against *Toxoplasma gondii* to evaluate the allergenicity and physicochemical properties of their model vaccine and through laboratory validation. It was validated that this vaccine model design approach was able to trigger immune response in mice (Foroutan et al. 2020). Hence, the shortlisted V1 vaccine in this study was modeled through Phyre2 tool and validation of modeled structure was performed through PROCHECK. The Ramachandran plot analysis showed 80% of residues classified in favored region, validating the tertiary structure of the vaccine. Z-score assessment by ProSA web server, i.e., -3.66 indicated that the protein falls in experimentally approved structures of proteins solved by NMR and X-ray crystallographic methods.

Furthermore, the spike protein of delta variant should interact with Toll-Like Receptor 4 (TLR4) expressed in immune cells to induce CTB (Boehme and Compton 2004;

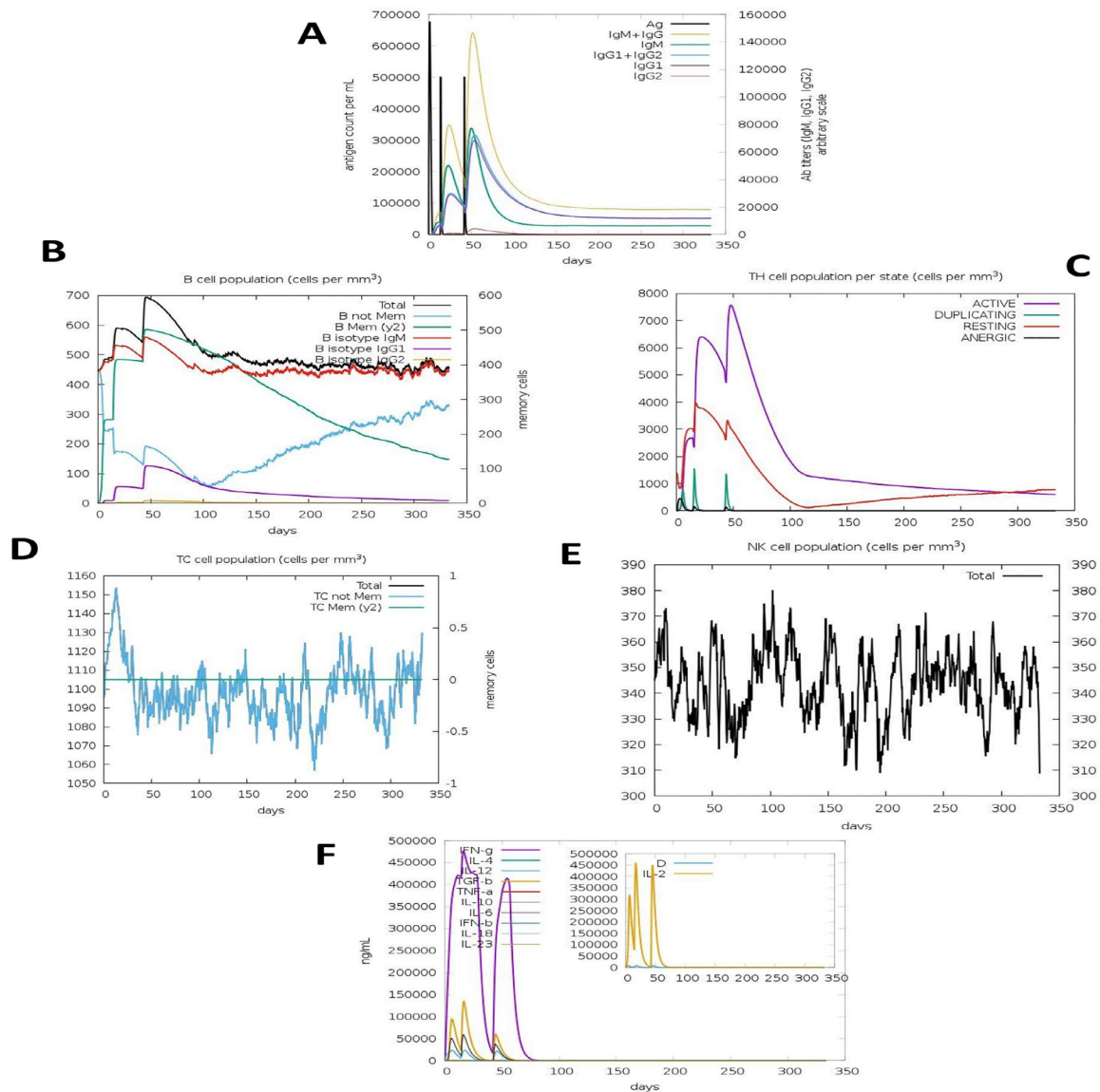


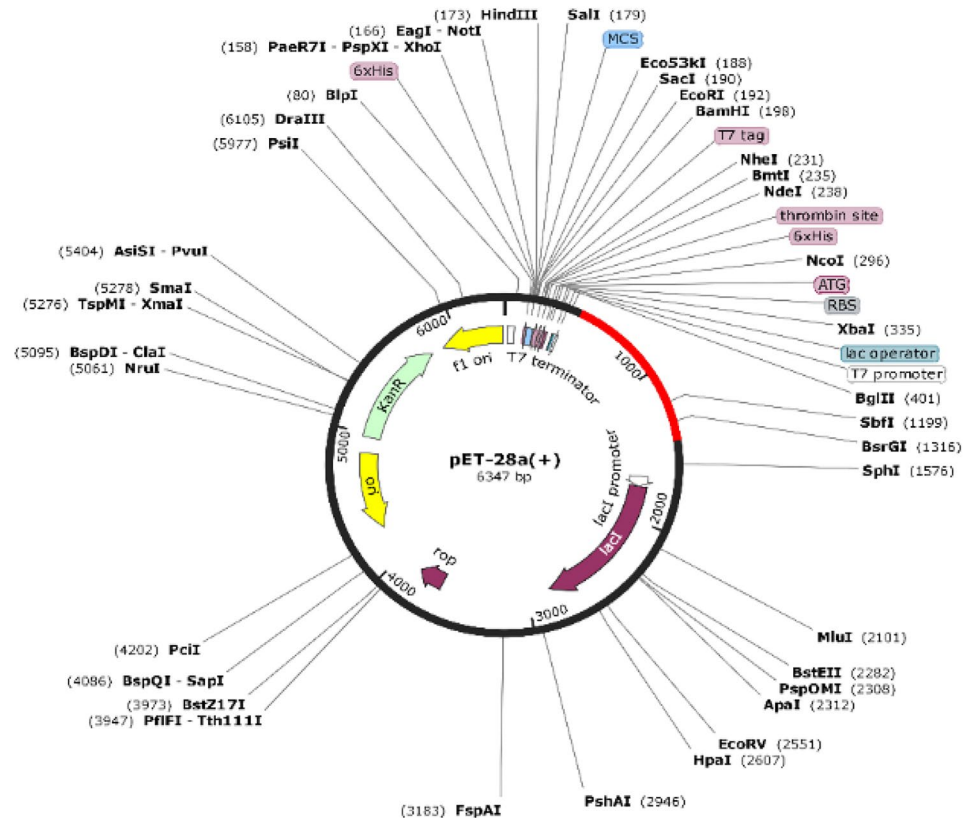
Fig. 9 C-ImmSim presentation of an in silico immune simulation with the construct. **A** Immunoglobulin production in response to antigen injections (black vertical lines); specific subclasses are shown as colored peaks and **B** the evolution of B-cell populations after the three injections. **C** T-helper cell populations per state after the injections, **D** the evolution of T-cytotoxic, and **E** highlights the production of natural killer cells. The resting state represents cells not pre-

sented with the antigen while the anergic state characterizes tolerance of the T-cells to the antigen due to repeated exposures. **F** The main plot shows cytokine levels after the injections. The insert plot shows IL-2 level with the Simpson index, D shown by the dotted line. D is a measure of diversity. Increase in D over time indicates emergence of different epitope-specific dominant clones of T-cells. The smaller the D value, the lower the diversity

Carty and Bowie 2010; Xagorari & Chlichlia, 2008). It has been reported that the CTB lost ability to trigger inflammatory response in TLR4-deficient macrophages (Vaure and Liu 2014). It was demonstrated through ELISA-based assays that the direct binding of CTB with TLR4 inflicts the activation of NF- κ B (Phongsisay et al. 2015). Hence, the interaction of modeled vaccine construct with human leukocyte antigen (HLA) and TLR4 to elucidate effective immune response was studied using molecular docking simulation studies. The docking study of TLR4 and the vaccine model

resulted in the formation of three hydrogen bonds between Ser265-Asp35, Lys224-Pro16, and Cys193-Ala17. Several studies highlighted the importance of interaction of vaccine with TLR4 such as, Totura et al. demonstrated that the susceptibility of mice to SARS-CoV infection is relatively high in TLR4 deficient mice compared to wild type (Totura et al. 2015), similarly Hu et al. observed that upregulation in expression of TLR4 when exposed to SARS-CoV infection, suggesting the importance of TLR in immune response stimulation (Hu et al. 2012).

Fig. 10 Codon optimization and in silico cloning of vaccine model. In silico restriction cloning of the multi-epitope vaccine sequence into the pET30a (+) expression vector using SnapGene software, the red part represents the vaccine's gene coding, and the black circle represents the vector backbone



Additionally, the vaccine model was simulated under the in vivo conditions to check its stability using GROMACS. The molecular dynamics simulation of the vaccine for 10 ns displayed the stability of vaccine model at 4 ns (Fig. 8). The codon optimization of V1 model was reverse translated to its cDNA to ensure a successful expression in *E. coli* pET-28a(+) expression vector. The GC and CAI values predicted for V1 were 72% and 0.9603, respectively resulting in the successful expression of vaccine (Fig. 9). Comparably, Foroutan et al. performed in silico codon optimization before expressing it in mice (Foroutan et al. 2020). The immune simulation of vaccine models showed that the constructed vaccine model against delta variant significantly elicited immune response (Fig. 10). Correspondingly, the immune-simulation studies have been widely used for the construction of chimeric vaccine model against *Klebsiella pneumoniae* (Solanki et al. 2021), *Mycobacterium tuberculosis* (Bibi et al. 2021), *Acinetobacter baumannii* (Solanki and Tiwari 2018), *Ebola virus* (Ullah et al. 2020), as well as against cancerous antigens (Zhang 2018). Current study's pipeline can be used further for the identification and designing a vaccine model against other SARS-CoV-2 variants too.

Conclusion and future perspectives

Briefly, the efforts to tackle the new mutated variants of SARS-CoV-2 are ongoing. However, low progress observed due to the immune evasion and high transmissibility of such variants. Although vaccines are being introduced, yet no effective results are reported. The elimination of SARS-CoV-2 and its variants will not be achieved without the innovative control strategies. Given that the spike proteins are the main source of viral infection, the peptide vaccine expressed from spike proteins might possibly provide therapeutic and prophylactic advantages. The chimeric vaccine models developed in this work could be utilized as a supplement to other approaches to eliminate the delta variant. However, the modeled V1 vaccine needs to be validated by in vitro as well as animal models and if successful then pre-clinical studies are required before administration. For top selected V1 construct, several ways to explore or validate in vivo cellular localization (e.g., immunofluorescence and western-blot tests), protein structure (e.g., crystallography), and protein–protein interactions (e.g., yeast two-hybrid) may be the future interests. Pilot vaccination trials are required to validate in vivo

immunogenicity of peptides. For that various factors must be considered including antigen design/production (such as peptide, native protein, synthetic, polymers, type of host expression system, recombination with other promising antigens, and linkers), antigen administration (for example route/system, dose, adjuvant), host response (such as humoral and cellular immune response, physiological and clinical responses).

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Author contribution KJ and KK have done the experiments and written the original draft. MNA and SSH have rechecked the manuscript for any errors. ZB has analyzed the data. FA and SAK have done the computational studies. RU has designed and supervised an experiment.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All the authors have approved the manuscript for publication.

Competing interests The authors declare no competing interests.

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