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ORIGINAL RESEARCH

Identification of vaccine candidate against Omicron variant of SARS-CoV-2 using immunoinformatic approaches

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Abstract

Despite the availability of COVID-19 vaccines, additional more potent vaccines are still required against the emerging variations of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In the present investigation, we have identified a promising vaccine candidate against the Omicron (B.1.1.529) using immunoinformatics approaches. Various available tools like, the Immune Epitope Database server resource, and NetCTL-1.2, have been used for the identification of the promising T-cell and B-cell epitopes. The molecular docking was performed to check the interaction of TLR-3 receptors and validated 3D model of vaccine candidate. The codon optimization was done followed by cloning using SnapGene. Finally, In-silico immune simulation profile was also checked. The identified T-cell and B-cell epitopes have been selected based on their antigenicity (VaxiJen v2.0) and, allergenicity (AllerTOP v2.0). The identified epitopes with antigenic and non-allergenic properties were fused with the specific peptide linkers. In addition, the 3D model was constructed by the PHYRE2 server and validated using ProSA-web. The validated 3D model was further docked with the Toll-like receptor 3 (TLR3) and showed good interaction with the amino acids which indicate a promising vaccine candidate against the Omicron variant of SARS-CoV-2. Finally, the codon optimization, *In-silico* cloning and immune simulation profile was found to be satisfactory. Overall, the designed vaccine candidate has a potential against variant of SARS-Cov-2. However, further experimental studies are required to confirm.

Keywords Immunoinformatics · Vaccine · Docking · Immunology · Omicron · B-cell · T-cell

Introduction

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is a positive-stranded RNA virus that triggered an outbreak in Wuhan, China, in late 2019. The disease had rapidly spread over the nations by early 2020, and it had been labeled a global pandemic and a public health emergency all over the world (Abebe et al. 2020). This virus has 30 kb of nucleotides and 15 open reading frames (ORFs) that encode 29 proteins (Finkel et al. 2021). ORF1a and ORF1b are two overlapping open reading frames that are translated from positive-strand genomic RNA to synthesize continuous polypeptides that are cleaved into 16 non-structural proteins (NSPs). SARS-CoV-2, like all RNA viruses, has been developing over time due to mutations in multiple viral genes (Finkel et al. 2021; Kannan et al. 2022). To prioritize global monitoring and research, unique SARS-CoV-2 variants were classified as Variant of Interest (VOI) and Variant of Concern (VOC). Within two years of its onset, the SARS-CoV-2 epidemic had claimed the lives of almost 5 million people. With new developing strains such as Delta, Gamma, and Lambda, the world remains on high COVID-19 alert (Giandhari et al. 2021; Bian et al. 2021). Scientists from South Africa recently revealed a worrisome indication for a possible new variety (Sun et al. 2022), which has been

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classified as a Variant of Concern (Finkel et al. 2021). The Omicron variant was first spotted in Botswana on November 11, 2021, and it swiftly spread to adjacent nations, eventually being reported in more than 57 countries throughout the world (Petersen et al. 2021; Leung 2021; He et al. 2021; Meo et al. 2021; World Health Organization (WHO) 2022). Phylogenetic Assignment of Named Global Outbreak (PANGO) lineage B.1.1.529 recognized the SARS-CoV-2 virus as a VOC and assigned the Greek letter Omicron to it (World Health Organization (WHO) 2021).

The Global Initiative on Sharing All Influenza Data (GISAID database) included 127 virus genomes (VOC Omicron GR/484A) as of November 28, 2021 (Global Influenza Surveillance and Response System (GISAID) 2021). There are 60 mutations in the new Omicron variation (Table 1), several of which are unique and impair the spike protein targeted by most COVID-19 vaccines (Sun et al. 2022; Callaway 2021). These significant spike protein mutations were exploited in future analyses. The Omicron variant's spike protein has a minimum of 30 amino acids, three small deletions, one tiny insertion, and 15 of the 30 amino acid alterations occurring in the receptor-binding domain (RBD). There are also three mutations at the furin cleavage site in this variation. SARS-CoV-2 infectivity is increased by the furin cleavage site. The Omicron strain is more likely to spread than the original SARS-CoV-2 virus, based on changes in the spike protein (Zimmer 2021; Zhang et al. 2021).

The spike glycoprotein of the Omicron version of SARS-CoV-2 contains about 30 mutations. These mutations, enhance the binding of the virus to the angiotensin-converting enzyme-2 (ACE2) receptor and increase its rate of transmission. Further, the combinations of signature mutations Q498R and N501Y boost the binding affinity of the virus to ACE2 receptors (Finkel et al. 2021; Sun et al. 2022). H655Y mutation may increase spike cleavage, and aid in increasing the transmission rate. P681H has been demonstrated to improve spike cleavage, this type of mutation is found in Alpha and it's an alternate mutation at this position (P681H) found in Delta (Finkel et al. 2021). It is a cause of concern because antibodies included (vaccine) or natural infection neutralize the virus by targeting the spike protein. Certain mutations in Omicron spike protein may reduce the ACE2 binding.

Immunoinformatics (also known as computational immunology) is a sub-discipline of bioinformatics that focuses on advancing immunological research using data management and computational techniques (Bhattacharya et al. 2021; Nosrati et al. 2019a). The construction and analysis of algorithms for mapping possible B- and T-cell epitopes is a major focus of immunoinformatic research (Nosrati et al. 2019b). There are quite a number of databases that provide a lot of information for immunological research. An immunologist can use immunoinformtics techniques and expertise to analyse sequence with binding sites, and to forecast both discontinuous (conformational) and linear epitopes, as well as pathogen immunogenicity, which leads to the creation of novel vaccines. Epitope-based vaccines are a type of recombinant vaccination that has sparked a lot of attention due to their excellent

Table 1 Mutations of the Omicron variant

s no	Gene	Amino acids	References
1	ORF1ab	nsp3: K38R, nsp3: V1069I, nsp3: Δ1265nsp3: L1266I, nsp3: A1892T, nsp4: T492Insp5: P132H, nsp6: Δ105-107, nsp6: A189V, nsp12: P323L, nsp14: I42V	Finkel et al. 2021; Sun et al. 2022; Variant: 21K (Omicron) 2021; Hurst 2021
2	S	A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, **G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H,** T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F	
3	E	T9I	
4	M	D3G, Q19E, A63T	
5	N	P13L, Δ31-33, R203K, G204R	

ORF open reading frame; *S* spike protein; *E* envelope protein; *M* membrane protein; *N* nucleocapsid protein

Signature mutations **(bold text)**

accuracy, safety, stability, and low cost of manufacture. (Nosrati et al. 2017; Soltanveis and Nosrati 2021). Many researchers are working on the epitope-based vaccines for infectious diseases, such as the hepatitis C virus, influenza virus, dengue virus, and human immunodeficiency virus (Soltanveis and Nosrati 2021). They can generate a powerful immune response, both humoral as well as cell-mediated immunity against a variety of pathogenic serotypes. The amount and specificity of antigen necessary to activate T cells can be better controlled with an epitope-based vaccine and can give superior possibilities for producing either memory Helper T cells or memory Cytotoxic T cells.

Recently, various studies have designed vaccine candidates against SARS-CoV-2 infection using immunoinformatic approaches (Dong et al. 2020; Khairkhah et al. 2020; Fatoba et al. 2021; Abdelmageed et al. 2020; Bhattacharya et al. 2020). However, to the best of our knowledge, there is a dearth of studies on the design of vaccine candidates against the Omicron variant. Therefore, in this study, we have designed a vaccine candidate against the Omicron variant (B.1.1.529) using immunoinformatic approaches. The schematic representation of final construct is mentioned in Fig. 1.

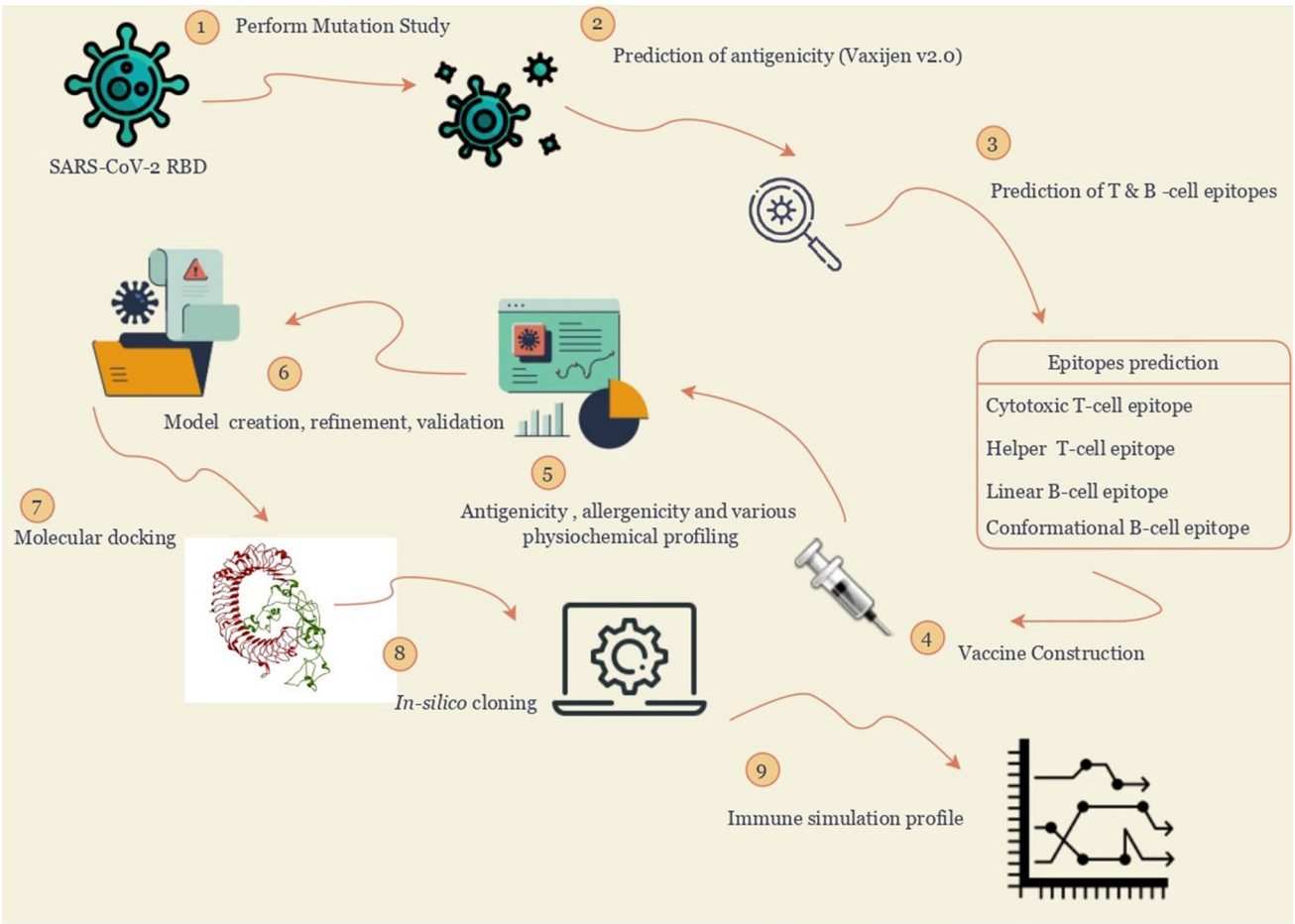


Fig. 1 Schematic representation of final construct

Materials and methods

Protein sequence retrieval and mutation study for Omicron variant of SARS-CoV-2

The mutation sites amino acid residues were identified from structural proteins (S proteins) Omicron variant of SARS-CoV-2 (Finkel et al. 2021). Then retrieved PDBID:7JMP from protein databank and processed the protein through Maestro 11.2 software by Schrodinger. We have performed the mutation study on the receptor-binding domain (RBD) sites residues as reported recently by Kannan et al. which was in the Omicron variant of SARS-CoV-2 (Finkel et al. 2021). Further, energy minimization was performed using the prime tool and extracting the spike protein part from the whole RBD complex. This mutated spike protein (As Omicron variant spike protein) was taken for our epitope design in this study.

Prediction of antigenicity of the target protein

VaxiJen v2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) was used to predict the antigenic property of sequence along with default parameters (VaxiJen v2.0 server 2022). This server system predicts the antigenicity of a protein using alignment-independent prediction.

T-cell epitope prediction and assessment

Cytotoxic (CD8 +) T-cell epitopes were predicted by submitting the Fast Alignment Sequence Test for Application (FASTA) sequence of the target protein to the NetCTL-1.2 server (https://services.healthtech.dtu.dk/service.php?NetCTL-1.2) along with default parameters (NetCTL – 1.2 server 2022). The best epitopes for the vaccine construction were selected for further prediction, based on a combined score. The selected epitopes were screened for the antigenicity by VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (VaxiJen v2.0 server 2022). The toxicity and immunogenicity of selected epitopes were determined using the toxin Pred tool (https://webs.iiitd.edu.in/raghava/toxinpred/multi_submit.php) (Toxin Pred tool 2022) and Immune Epitope Database (IEDB) analysis Resource (http://tools.iedb.org/immunogenicity/) (IEDB 2022), respectively.

Prediction of helper T lymphocyte (HTL) epitopes

The Immune Epitope Database server (IEDB; http://tools.iedb.org/mhcii/) (Immune Epitope Database server 2022) was used to predict helper T lymphocyte (HTL) epitopes. IEBD predicts epitopes based on the percentile rank or major histocompatibility complex (MHC) binding affinity. The lower the percentile rank, the stronger is the binding affinity for MHC II molecules. In the prediction method, we have selected recommended IEDB 2.22 and select species/locus as human, Human Leukocyte Antigen-DR isotype (HLA-DR), predicted for seven HLA reference set molecules (DRB3 * 01:01, DRB5*01:01, DRB1 * 03:01, DRB1 * 15:01, DRB3 * 02:02, DRB4 * 01:01, -DRB1 * 07:01.). The epitopes having a lower percentile rank were screened for allergenicity using Allergen FP v1.0 (https://ddg-pharmfac.net/AllergenFP/) (Allergen fp v1.0 server 2022). The non-allergenic epitopes were further examined for antigenicity by VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (VaxiJen v2.0 server 2022) followed by the toxicity prediction using toxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/multi_submit.php) (Fatoba et al. 2021).

Prediction of the IFN-γ inducing potency and population coverage

The cytokines IFN-γ activates macrophages and natural killer cells and stimulate both native and specific immune responses thus contributing to antiviral mechanisms. It also boosts MHC's response to antigens. To identify the IFN-γ epitope, we have submitted the selected non-toxic HTL epitopes into the IFN-γ epitope server (http://crdd.osdd.net/raghava/ifnepitope/predict.php) (IFN epitope server 2022) and sorted which predicted to be positive in the hybrid approach based on a support vector machine (SVM). Epitope population coverage is a vital parameter for developing universal vaccine. Therefore, we have checked the T-cell epitopes for population coverage using IEDB analysis resource (http://tools.iedb.org/population/).

Prediction of B-cell epitopes

Linear B-cell epitopes were predicted using the BepiPred-2.0 IEBD server (http://tools.iedb.org/bcell/) (BepiPred-2.0 IEBD server 2022). Residues with scores greater than the threshold (the default value is 0.5) are predicted to be epitopes and are labeled with "E" in the output table are selected for vaccine construction. Further, the selected epitopes were screened by the antigenicity by VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (VaxiJen v2.0 server 2022). The epitopes having antigenic properties were screened by the allergenicity by Allergen FP v1.0 (https://ddg-pharmfac.net/AllergenFP/) (Allergen fp v1.0 server 2022) and toxicity by Toxin PRED server (http://crdd.osdd.net/raghava/toxinpred/) (ToxinPred server 2022).

The conformational (discontinuous) B-cell epitope predictions were made using the 3D model, while linear B-cell and T-cell epitope predictions were generated using sequences. B-cell epitopes are generally conformational (discontinuous) and performed on multi-epitope vaccine candidates. The ElliPro server (http://tools.iedb.org/ellipro/) (ElliPro server 2022) was used to predict the conformational B-cell epitopes. The server predicts epitopes based on Protrusion Index (PI) value. Discontinued B-cell epitopes with the top PI value were further evaluated for antigenicity and allergenicity as stated above.

Multi-epitope subunit vaccine construction

The identified epitopes were merged to form a single peptide chain with the help of specific peptide linkers. The peptide linkers play an important role in protein folding, flexibility, and the separation of functional domains, resulting in a more stable protein structure. The AAY linker was used to connect CTL epitopes, the GPGPG linker was used to connect HTL epitopes, and the KK linker was used to connect B-cells. Protein adjuvants are molecular complexes that, increase an immunological response mainly cellular immune response, that can be added to the N-terminal of constructed vaccine. Adjuvants in epitope-based vaccines offer a number of benefits, such as long-term memory of the vaccine is substantially extended and regenerating a blocked immune system in an older person. The EAAAK linker was used to attach the β -defensin 2 (Homo sapiens) amino acid sequence to the vaccine's N-terminal to boost vaccine immunogenicity. The β-defensin peptides stimulate innate immune cells and recruit native T-cells via the chemokine receptor (CCR-6). A TAT sequence (GRKKRRQRRRPQ) was inserted into the C-terminal of the modeled vaccine to enable intracellular delivery.

Prediction of allergenicity, the antigenicity of the constructed vaccine

A vaccine should be non-allergic to avoid untoward immune responses. Hence, the allergenic potentials of the constructed vaccine were estimated using the Allergen FP v1.0 (https://ddg-pharmfac.net/AllergenFP/) (Allergen fp v1.0 server 2022) and Aller TOP v2.0 (https://www.ddg-pharmfac.net/AllerTOP/) (AllerTOP v2.0 server 2022). The antigenicity of the constructed vaccine was determined using the VaxiJen v2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (VaxiJen v2.0 server 2022). The threshold value to accept the antigenicity was fixed at 0.4. This antigenicity prediction method was based on the physicochemical properties of proteins. The prediction accuracy of the selected server ranged between 70% to 89%.

Prediction of various physicochemical properties

The vaccine candidates' physicochemical properties were assessed using the Prot Param tool (http://web.expasy.org/protparam/) (ProtPram tool-Expasy 2022). The number of amino acids, molecular weight, theoretical isoelectric point (pI), amino acid composition, atomic composition, formula, extinction coefficients, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity were some of the physicochemical properties examined (GRAVY). The stability index of a protein was used to infer the protein's stability. Proteins with an instability index greater than 40 were considered unstable. GRAVY also a measure the hydrophobic nature of protein.

Model creation, refinement, validation

The multi-epitope vaccine's 3D structure was constructed using appropriate bioinformatic tools, and a refined model of its 3D structure was created. The PHYRE2 server (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) (PHYRE2 server 2022) was used to model the 3D structure of the hypothesized vaccine. The 3D structure of the vaccine

from the PHYRE2 server was further refined by the Galaxy Refine server (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) (Galaxy Refine Server 2022) to enhance the structural quality of the protein.

The vaccine protein structure was validated using the ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) server (ProSa-web-protein structure analysis, 2022). The positive Z-score was used as a metric for the 3D protein model that was created.

Molecular docking of vaccine constructs with antigenic recognition receptor

Docking studies (https://www.uniprot.org/) were used to predict the binding affinity between the vaccine construct and the antigenic recognition domain of the toll-like receptor-3 (TLR3, UniProt id-2A0Z) (Uniprot database 2022). The TLR3 is more susceptible to the coronavirus family including SARS-CoV-2 and MERS-CoV. The PDB file of TLR3 was obtained from Protein Data Bank (https://www.rcsb.org/) (RCSB PDB 2002). The protein was prepared using protein preparation wizard of Maestro 11.2 software of Schrodinger with default parameters using OPLS3 force field. Further, protein–protein blind docking between vaccine construct and TLR3 was performed using Maestro 11.2 software of Schrodinger.

Codon optimization and in-silico cloning using SnapGene

In order to achieve maximal expression in E. coli, codon optimization was performed (strain K-12). The Java Codon Adaptation tool (http://www.jcat.de/) was employed to get the GC content and CAI score for the vector with the most expression. The parameters were calculated such as avoiding rho-independent transcription terminators and prokaryotic ribosome binding sites to generate optimal and complete protein expression, also, avoided cleavage sites of EcoRI and BamHI restriction enzymes. Restriction endonuclease sites EcoRI and BamHI were appended to N and C terminals of improved DNA, respectively.

Further, In-silico cloning was done by inserting optimized improved DNA into the pET28a (+) vector between the EcoRI and BamHI site. Finally, In-silico cloning of constructed vaccine was done using SnapGene software.

In-silico immune simulation profile

The immunological profiles of the designed vaccine candidate were obtained using the C-ImmSim server (https://kraken.iac.rm.cnr.it/C-IMMSIM/index.php?page=1). This server uses machine learning approaches to predict immunological response using position-specific scoring matrices (PSSM). Three injections of designed vaccine candidate were given over the course of four weeks at different intervals using C-ImmSim server with default parameters (simulation volume-10, simulation step-100, host HLA selection-A MHC class I-A0101, B MHC class I- B0702, DR MHC class II-DRB1_0101).

Results

The sequences of the structural proteins and prediction of their antigenicity

The sequence of the structural protein (S proteins) of SARS-CoV-2 was taken in a FASTA format to carry out further analyses (Finkel et al. 2021). FastaSeq:

> FGEVFNATRFASVYAWNRKRISSVLYSASFSTFKCYGVSPFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSKPCNGVAGFNCYFPLRSYSFRPTYGVGHQPYRVVVLSFE.

Antigenicity of sequence prediction was done using the default parameters (the threshold for this model is 0.4) of the VaxiJen v2.0 server. The predicted value of 0.4672 indicated the probable antigenic nature of the selected sequence (Probable ANTIGEN).

Prediction of T-cell epitopes

Identification of CD8 + cytotoxic T lymphocyte (CTL) epitopes is a crucial step in epitope-based vaccines. The NetCTL 1.2 server predicted CTL epitopes (9 mer) using a threshold value of 0.75, and eight epitopes were chosen based on a higher cumulative score. Further, the selected epitopes were screened by the antigenicity using VaxiJen v2.0 followed by toxicity prediction using toxinPred server. The immunogenicity was also predicted using IEBD MHC I immunogenicity server. Finally, a total of two excellent epitopes were chosen as candidates for vaccine development (Table 2).

Table 2 Predicted cytotoxic T lymphocyte (CTL) epitopes

Epitope	Comb score	Antigenicity (VaxiJen v2.0)	Toxicity (Toxin Pred)	Immunogenicity score
RSYSFRPTY	0.9550	0.9553 (Probable ANTIGEN)	Non-toxin	0.00837
VGGNYNYLY	0.7698	0.7432 (Probable ANTIGEN)	Non-toxin	−0.0148

Prediction of helper T lymphocyte (HTL) epitopes

The top epitopes with the lowest scores (highest binding capability) were sorted. The epitopes that have lower percentile rank, were screened based on the allergenicity by Allergen FP v1.0 (Allergen fp v1.0 server 2022). Non-allergen epitopes are further examined to antigenicity and toxicity by the VaxiJen v2.0 server (VaxiJen v2.0 server 2022) and toxinPred server (Toxin Pred tool 2022). Thus, a total of 10 epitopes were selected for IFN-γ inducing epitopes (Table 3).

Analysis of the IFN-γ property of CD4 + T-cell epitopes and population coverage

The 10 CD4 + T-cell epitopes were tested for their ability to induce interferon-gamma (IFN-γ) using the IFN epitope server (IFN epitope server 2022). Based on a positive result, only five epitopes (Table 4) were selected for the construction of the vaccine candidate. According to the population coverage findings, MHC class I has a high population coverage of 98.29% and the population coverage for MHC class II is 49.02%, (Fig. 2).

Prediction of B-cell epitopes

Six linear B-cell epitopes were created in total. Precisely three linear B-cell epitopes were chosen for the vaccination when they were tested for antigenicity, toxicity, and allergenicity (Table 5).

The ElliPro servers predicted discontinuous B-cell epitopes (ElliPro server 2022) and created a total of four discontinuous B-cell epitopes. Three epitopes were rejected due to the vaccine's allergenicity, but one epitope was recommended for vaccine development (Table 6).

Construction of the subunit vaccine

The epitopes discovered were utilized in the development of a vaccine. Two CTL epitopes, five HTL epitopes, three linear B-cell epitopes, and one discontinuous B-cell epitope were joined together using different linkers: CTL epitopes used the AAY linker, HTL epitopes used the GPGPG linker, and B-cell epitopes used the KK linker. The AAY linker aids epitope production of appropriate locations for TAP transporter binding and improves epitope presentation. The GPGPG linker increases HTL responses while protecting helper and antibody epitope immunogenicity. The EAAAK linker was used to attach the human β-defensin-2 amino acid sequence to the vaccine's N-terminal to boost vaccine immunogenicity. A TAT sequence (GRKKRRQRRRPQ) was added to the C-terminus of the modeled vaccine to allow it to be administered intracellularly. The final vaccination was planned to be 286 amino acids long.

Table 3 Predicted helper T lymphocyte (HTL) epitopes

Epitope	Percentile rank	Allergenicity (Allergen FP v1.0)	Antigenicity (VaxiJen v2.0)	Toxicity (Toxin Pred)
SFVIRGDEVRQIAPG	0.51	PROBABLE NON-ALLERGEN	0.5882 (Probable ANTIGEN)	Non-toxin
TRFASVYAWNRKRIS	0.52	PROBABLE NON-ALLERGEN	0.4963 (Probable ANTIGEN)	Non-toxin
FVIRGDEVRQIAPGQ	0.54	PROBABLE NON-ALLERGEN	0.4940 (Probable ANTIGEN)	Non-toxin
NATRFASVYAWNRKR	0.83	PROBABLE NON-ALLERGEN	0.4062 (Probable ANTIGEN)	Non-toxin
RFASVYAWNRKRISS	0.90	PROBABLE NON-ALLERGEN	0.4329 (Probable ANTIGEN)	Non-toxin
VLYSASFSTFKCYGV	1.70	PROBABLE NON-ALLERGEN	0.4243 (Probable ANTIGEN)	Non-toxin
GCVIAWNSNNLDSKV	1.80	PROBABLE NON-ALLERGEN	0.4585 (Probable ANTIGEN)	Non-toxin
KSNLKPFERDISTEI	1.90	PROBABLE NON-ALLERGEN	0.4014 (Probable ANTIGEN)	Non-toxin
TGCVIAWNSNNLDSK	2.10	PROBABLE NON-ALLERGEN	0.6531 (Probable ANTIGEN)	Non-toxin
CVIAWNSNNLDSKVG	2.20	PROBABLE NON-ALLERGEN	0.9531 (Probable ANTIGEN)	Non-toxin

Table 4 IFN-γ property of CD4 + T-cell epitopes

Epitope name	Sequence	Method	Result	Score
seq2	TRFASVYAWNRKRIS	SVM	POSITIVE	0.73155567
seq4	NATRFASVYAWNRKR	SVM	POSITIVE	0.47646374
seq5	RFASVYAWNRKRISS	SVM	POSITIVE	0.64581077
seq6	VLYSASFSTFKCYGV	SVM	POSITIVE	0.1056124
seq7	GCVIAWNSNNLDSKV	SVM	POSITIVE	0.022799683

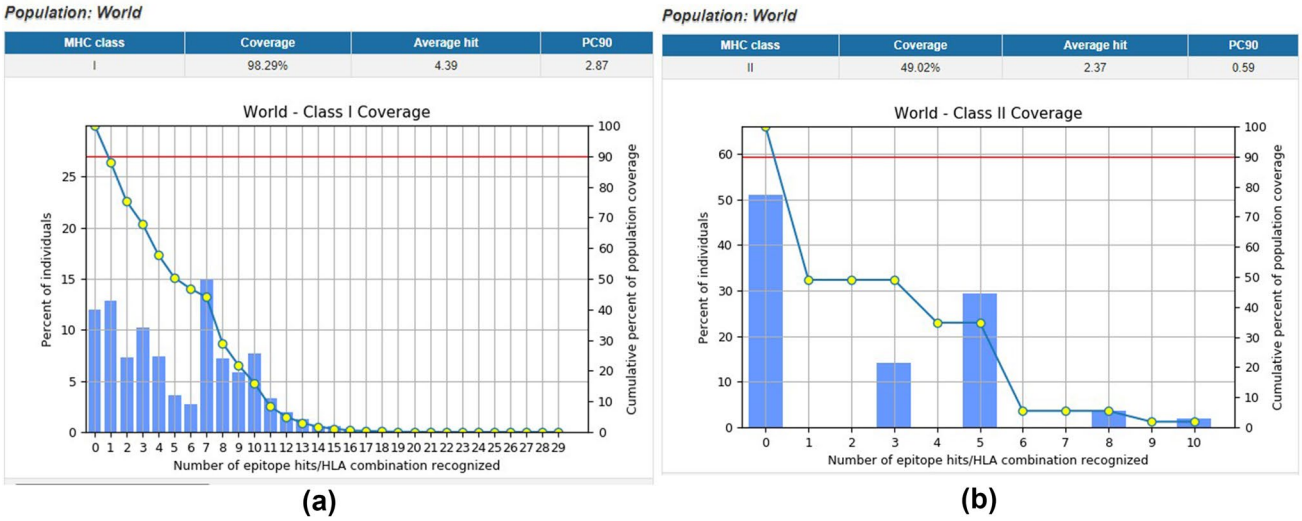


Fig. 2 Population coverage for T-cell epitopes. **a** Binding of MHC class I molecules to the world population coverage. **b** Binding of MHC class II molecules to the world population coverage

Table 5 Predicted linear B-cell (BCL) epitopes utilized for construction of a multi-epitope subunit vaccine

Epitope	Allergenicity (Allergen FP v1.0)	Antigenicity (VaxiJen v2.0)	Toxicity (toxinpred)
FNATRFASVYAWNRKRISSVLYSASFST	PROBABLE NON-ALLERGEN	0.4277 (Probable ANTIGEN)	Non-toxic
GDEVRQIAPGQTGKIADYNYKLPD	PROBABLE NON-ALLERGEN	1.1472 (Probable ANTIGEN)	Non-toxic
YSFRPTYGVGHQ	PROBABLE NON-ALLERGEN	0.9174 (Probable ANTIGEN)	Non-toxic

Table 6 Predicted discontinuous B-cell (BCL) epitopes used for construction of a multi-epitope subunit vaccine

Epitope	Numbers of residues	Allergenicity (Allergen FP v1.0)	Antigenicity (VaxiJen v2.0)
B:Y122, B:Q123, B:A124, B:G125, B:S126, B:K127, B:P128, B:C129, B:N130, B:G131, B:V132, B:A133, B:G134, B:F135, B:N136, B:C137, B:Y138	17	PROBABLE NON-ALLERGEN	(Probable ANTIGEN)

Prediction of allergenicity, antigenicity, and various physicochemical properties of the constructed vaccine

The vaccine's allergenic character was assessed using the Allergen FP service, and it was found to be non-allergenic. Similarly, the 0.6433 (Probable antigen) estimated antigenicity score was acceptable. In the virus model, the antigenicity threshold was set at 0.4 by default. Prot Param server (ProtPram tool-Expasy 2022) was used to determine the physicochemical properties of the constructed vaccine. The vaccine was made up of 286 amino acids and had a molecular weight of 31,897.77. The hypothetical isoelectric point (pI) was calculated to be 10.47. There were seven negatively charged residues and 50 positively charged residues in the vaccination. The vaccine construct was composed of 4477 atoms, and its chemical formula was $C_{1452}H_{2219}N_{419}O_{377}S_{10}$. Instability was calculated to be 39.82. The half-life in vitro was calculated to be 30 h. In vivo, yeast and *Escherichia coli* have estimated half-lives of greater than 20 and 10 h, respectively. The vaccine construct had an aliphatic index of 55.94, indicating that it is quite thermostable. The vaccine design had a GRAVY value of -0.520, indicating that the protein is hydrophobic.

3D structure prediction

PHYRE2 server (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) (PHYRE2 server 2022) was used for modeling the 3D structure of designed constructs which shows 168 residues (59%) modeled at > 90% confidence. The secondary structure of the predicted vaccine candidate

shows 14% Alpha helix, 38% Beta strands, 6% TM helix, and 29% disorders. The first model was improved in the Galaxy-Refine server (Galaxy Refine Server 2022) to refine the 3D structure of the projected vaccine. Based on the root mean square deviation (RMSD) and the MolProbity method, the GalaxyRefine server created five models. We chose Model 1 because it is the most popular Ramachandran model and thus the best for docking. The SWISS-MODEL workspace was used to analyze the initial model generated by the PHYRE2 server and the refine model provided by GalaxyRefine. The Ramachandran preferred region has 65.14% residues, the Ramachandran outliers' region has 15.14% residues, and the rotamer region has 0.00% residues. The refined model was 86.62% of residues in the Ramachandran favored region, 2.46% in the Ramachandran outlier's region, and 1.30% in the rotamer region (Fig. 3). ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) was used to check the quality of the final vaccine 3D model (ProSa-web-protein structure analysis 2022). The Z-score represents the overall quality of the model; the model with a lower Z-score was considered to be of greater quality. The first model has a Z-score of 4.19, whereas the refined model had a Z-score of 4.47 (Fig. 4).

Protein–protein docking analyses of a multiepitope-based vaccine against TLR3

To activate immunological responsiveness, a suitable connection between immune receptor molecules and the antigen molecule is required. The molecular docking investigations of the MEV with human immune receptors TLR3 were performed using Maestro11.2 software. Following virus detection, TLR3 can effectively trigger an immune response. The molecular docking studies revealed that MEV and TLR3 have good binding interactions. Following protein docking, we have thirty models based on their binding affinity for TLR3. We looked at the top-ranked poses for additional investigation and discovered that MEV established 7 hydrogen bonds with TLR3 in the range of 3.00 Å. MEV-interacting amino acid sequences that hydrogen bond to TLR3 is depicted as cyan-colored stick representations, whereas TLR3 amino acids that hydrogen bond to MEV is depicted as green-colored stick representations, with all hydrogen bonding displayed as a yellow-colored dashed line (Fig. 5). The data of interacting amino acid residues are presented in (Table 7). After the protein–protein docking analysis sitemap study was also conducted by using top complex and we analyzed that all the interacting amino acid residues lie on the active catalytic domain of TLR3 protein (Fig. 6). This hypothesis confirmed the above residues will be important for interaction against Omicron.

Codon optimization and in-silico cloning using SnapGene

The codon adaptation index value (CAI-Value) of improved sequence was found to be 0.93874 which indicate optimization of codon. The GC-Content of improved

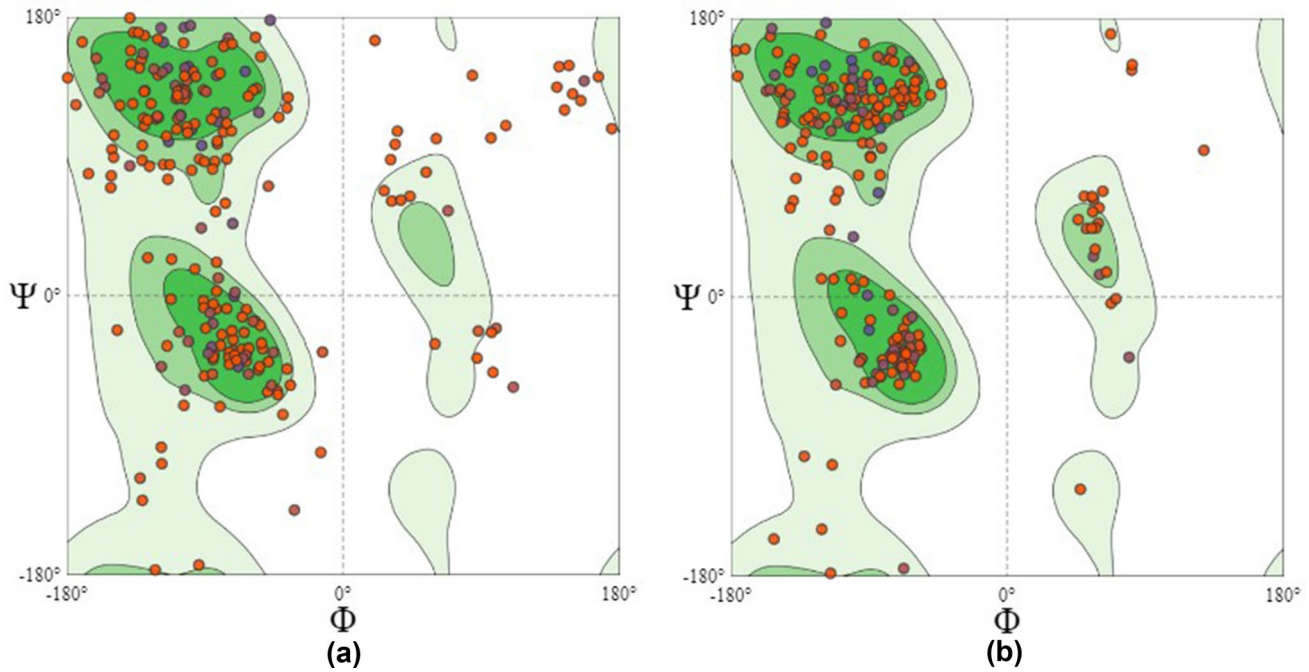


Fig. 3 Ramachandran Plot a) 65.14% residues in the favored region (initial model) b) 86.62% of residues in the favored region (refined model)

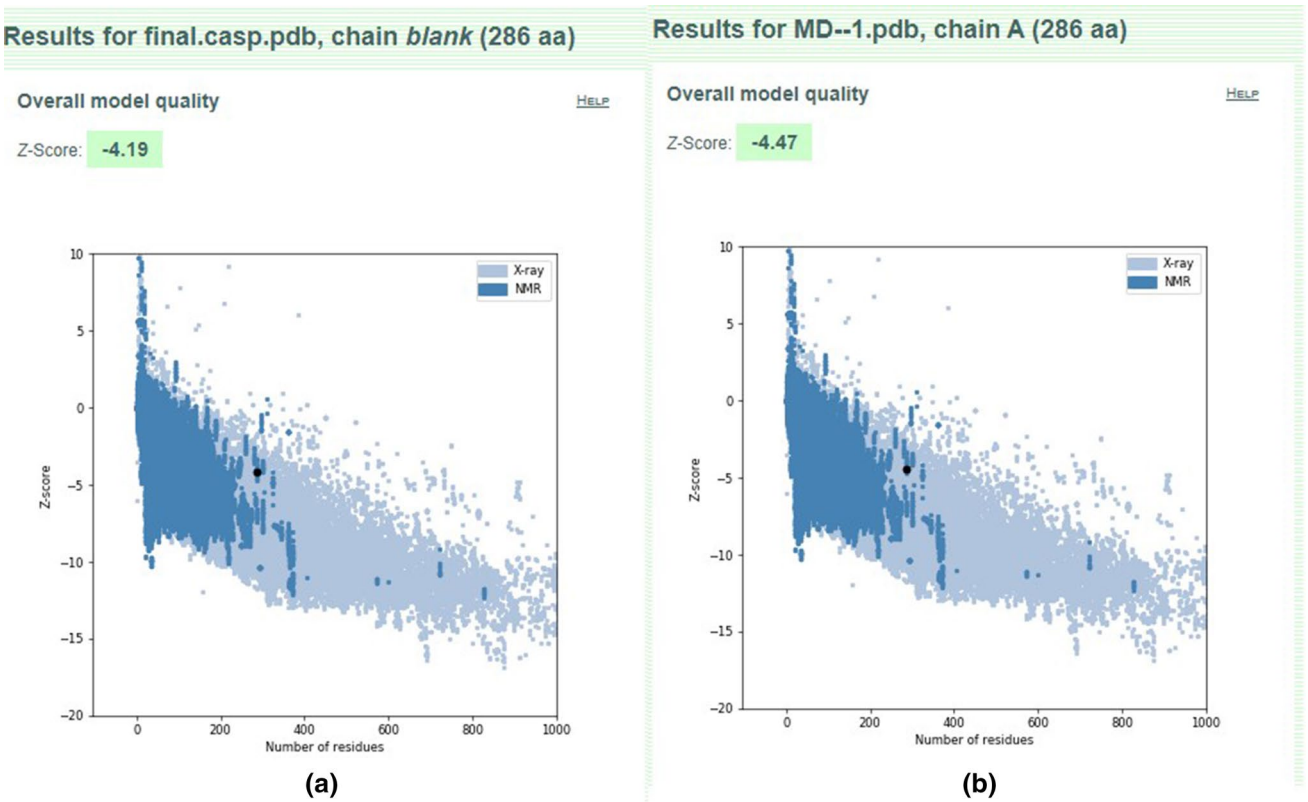


Fig. 4 **a** The z-score of the initial model was − 4.19. **b** The z-score of the refined model is − 4.47

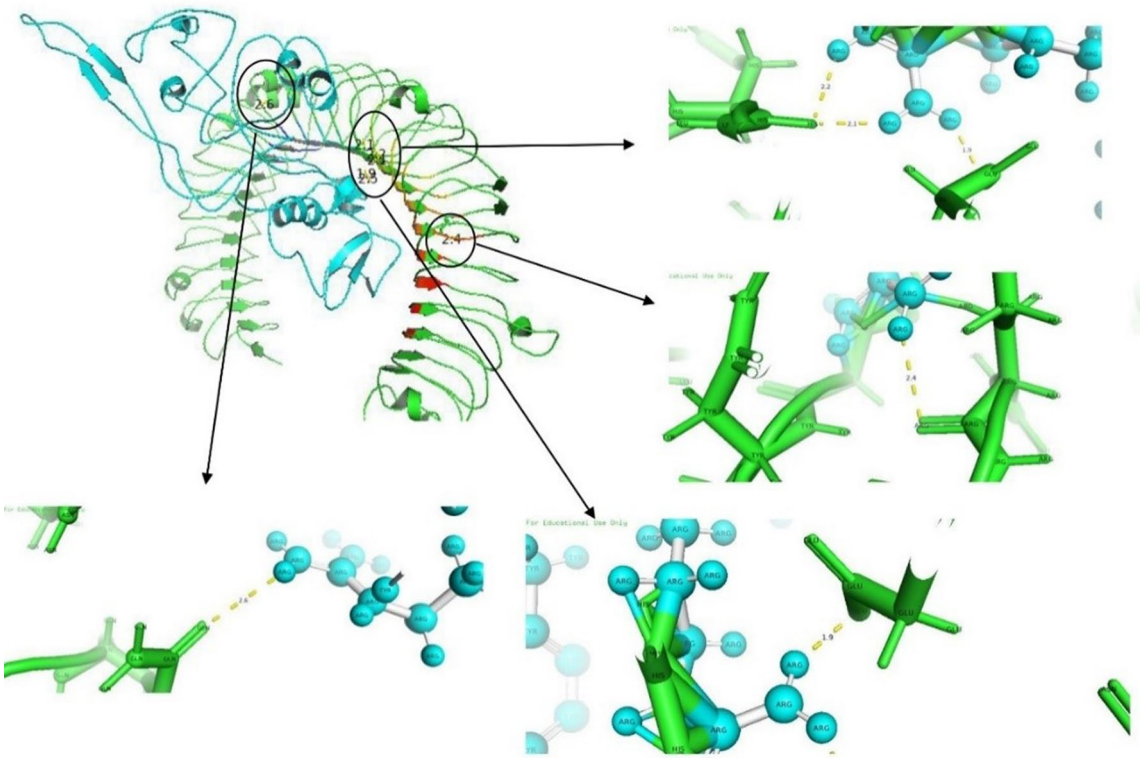


Fig. 5 Protein–protein docking top-ranked pose interaction: Participating residues from MEV are shown in yellow color

Table 7 Interacting residues of vaccine candidate with TLR3

Measurement no	Residue with location (TLR3)	Residue with location (vaccine candidate)	Angstrom (Å)
1	Glutamine299	Arginine147	2.6
2	Tyrosine382	Arginine70	2.5
3	Arginine488	Arginine45	2.4
4	Glutamic acid434	Arginine267	1.9
5	Histidine432	Arginine267	2.1
6	Histidine432	Arginine267	2.2
7	Proline408	Arginine267	2.1

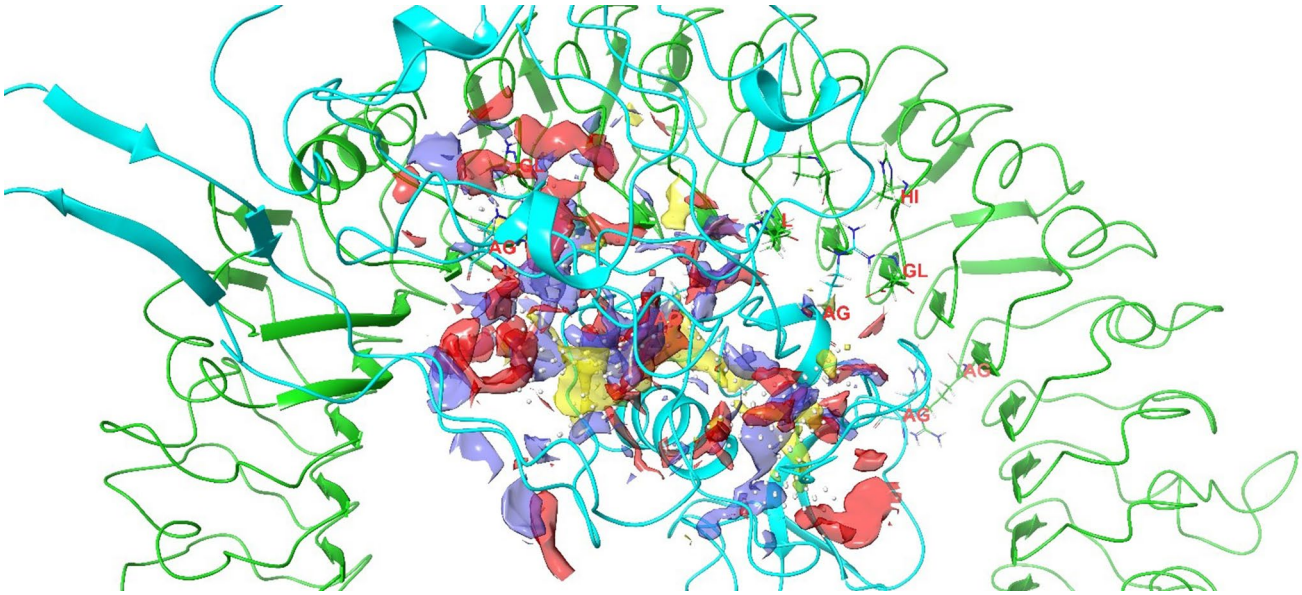


Fig. 6 Sitemap analysis study: highlighted residues lie in active sites of the TLR3 protein

sequence was found to be 51.5151 which was in normal range (40–60).

The SnapGene results have shown expression of designed vaccine candidate in *E. coli* system as shown in Fig. 7.

In-silico immune simulation

Immune simulation profile shows primary response was lower as compared to secondary and tertiary immune response. The In-silico immune profile has shown increase in the IgM as well as IgG level. The antigen count was found to be decreased (Fig. 8a). The memory cell proliferation shows a tertiary response, which is linked to a high level of B cell activation (Fig. 8b). Following vaccination, the B cell population was greatly boosted. The number of cytotoxic and helper T cells were found to be increased which indicates the secondary and tertian immune responses (Fig. 8c, d). The natural killer (NK) cells were also significantly increased (Fig. 8f). The large quantities of cytokines (IFN-gamma and IL-2) were produced as a result of injections which confirms the simulation of immune system through designed vaccine candidate (Fig. 8e).

Discussion

Emerging and pandemic zoonotic illnesses (viruses, bacteria, and other pathogens) offer a significant challenge to medicine and research because little is known about them before their appearance (Salyer et al. 2017). Infectious diseases account for 15.8% of deaths worldwide and 43.7% of deaths in low-income nations. Each year, 2.7 million people die as a result of zoonotic illnesses around the world. In addition to human deaths, zoonotic illnesses cause significant economic losses around the world, particularly in low-income nations. For example, the previous Ebola pandemic in 2014 was too responsible (Huber et al. 2018).

Immunoinformatics is an excellent tool for developing vaccines and diagnostics for newly discovered viruses. This method has the potential to save time and money. Several researchers are currently using immunoinformatic methodologies to identify and characterize T-cell and B-cell epitopes, as well as produce vaccines. We identified and described T-cell and B-cell epitopes against Omicron SARS-CoV-2 using existing immunological information and the genetic similarity of SARS-CoV-2 with SARS-CoV surface glycoproteins. To the best of our knowledge, there is a dearth of study so far against the Omicron variant using immunoinformatic. Thus, in this current study, we have designed vaccine candidates against Omicron, a variant of SARS-CoV-2.

Fig. 8 Immune simulation profile. **a** Antigen and immunoglobulins. Antibodies are sub-divided per isotype. **b** B lymphocytes population per entity-state (i.e., showing counts for active, presenting on class-II, internalized the Ag, duplicating and anergic. **c** CD4 T-helper lymphocytes count sub-divided per entity-state (i.e., acctive, resting, anergic and duplicating). **d** CD8 T-cytotoxic lymphocytes count per entity-state. **e** Cytokines. Concentration of cytokines and interleukins. D in the inset plot is danger signal. **f** Natural Killer cells (total count)

During the virus replication cycle, the surface glycoprotein is primarily engaged in the virus's entry into the cell (Li 2016). Coronaviruses' surface glycoproteins can create neutralizing antibodies, which can impede virus entrance or neutralize the infection. In the present study, we have repossessed the coding sequences for structural proteins

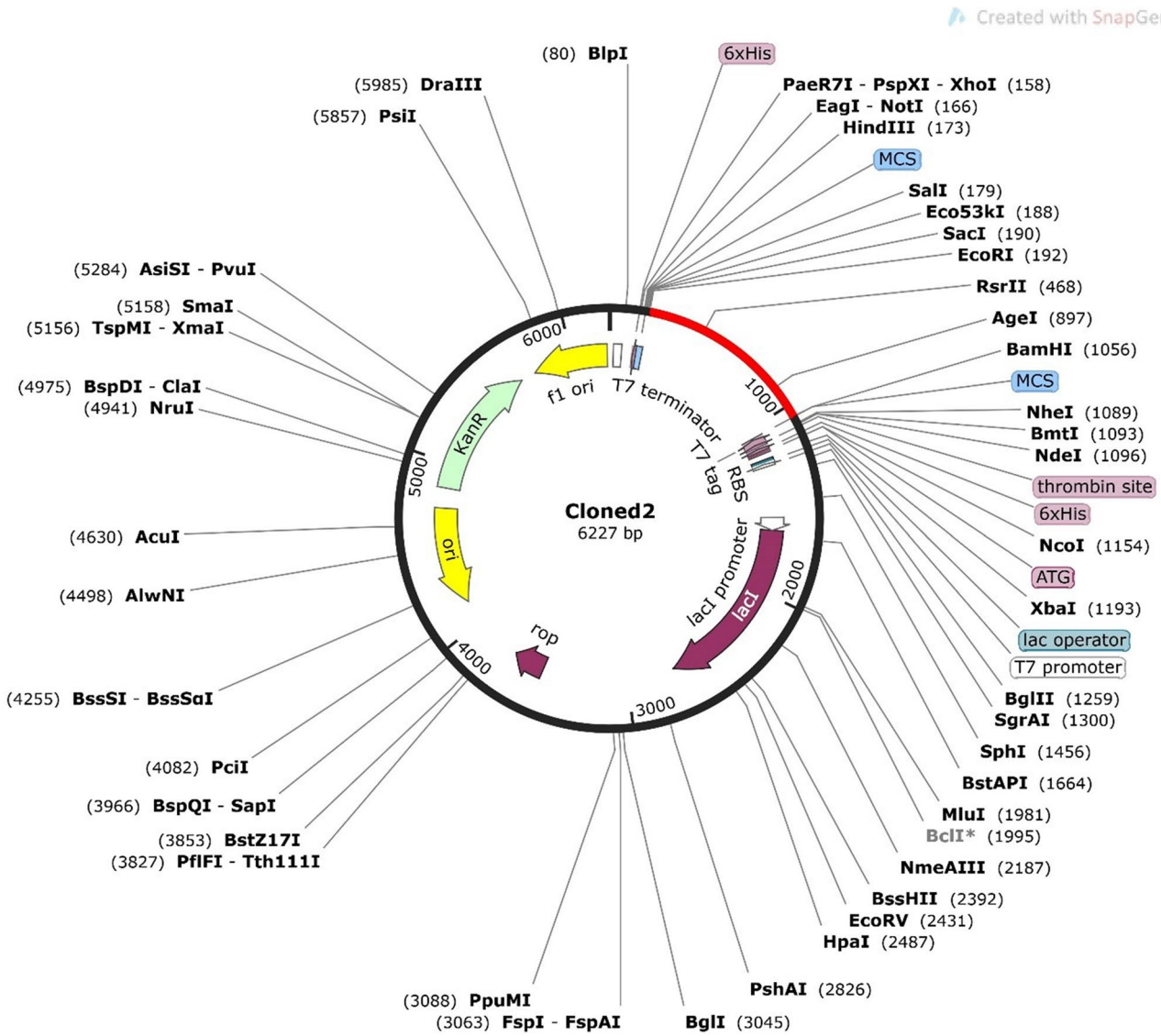
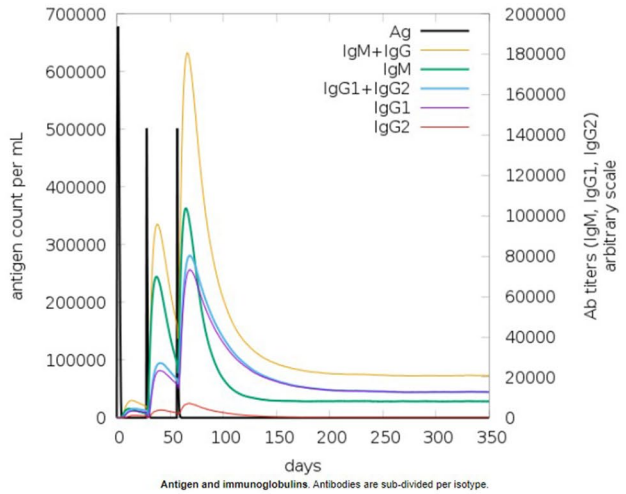
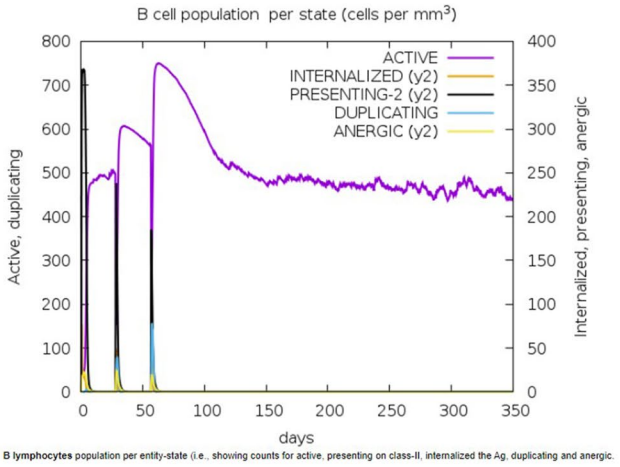


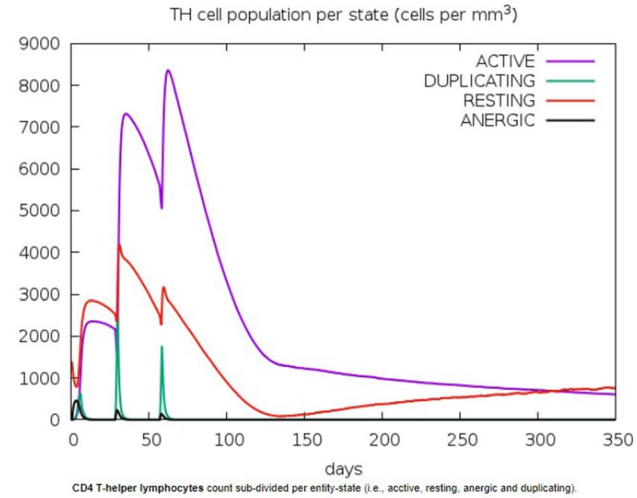
Fig. 7 In-silico cloning of the final designed vaccine candidate. The vector was shown in black color, while the red color provided the gene coding


Ag
IgM+IgG
IgM
IgG1+IgG2
IgG1
IgG2
antigen count per mL
Ab titers (IgM, IgG1, IgG2) arbitrary scale
days
Antigen and immunoglobulins. Antibodies are sub-divided per isotype.

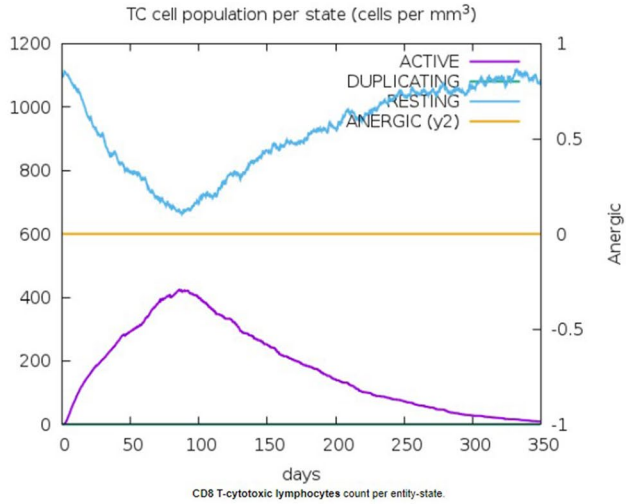
(a)


B cell population per state (cells per mm3)
ACTIVE
INTERNALIZED (y2)
PRESENTING-2 (y2)
DUPLICATING
ANERGIC (y2)
Active, duplicating
Internalized, presenting, anergic
days
B lymphocytes population per entity-state (i.e., showing counts for active, presenting on class-II, internalized the Ag, duplicating and anergic.

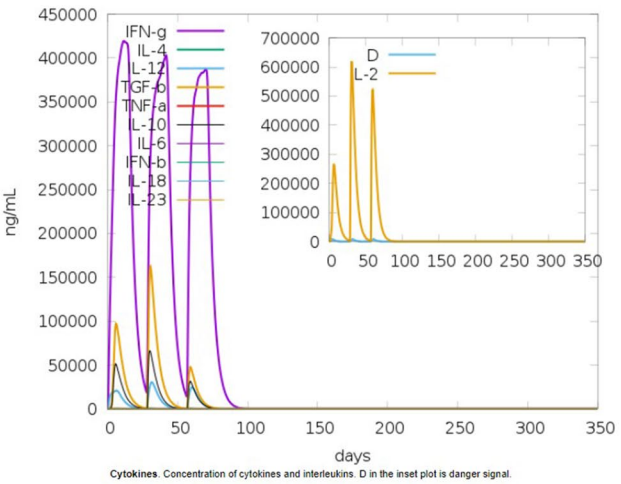
(b)


TH cell population per state (cells per mm3)
ACTIVE
DUPLICATING
RESTING
ANERGIC
days
CD4 T-helper lymphocytes count sub-divided per entity-state (i.e., acctive, resting, anergic and duplicating).

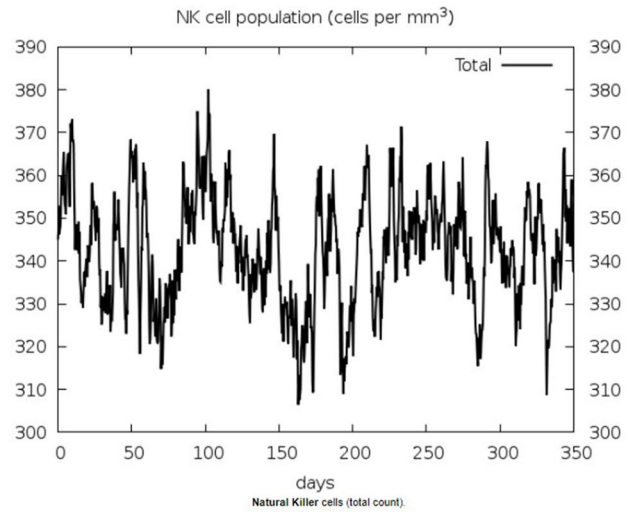
(c)


TC cell population per state (cells per mm3)
ACTIVE
DUPLICATING
RESTING
ANERGIC (y2)
Anergic
days
CD8 T-cytotoxic lymphocytes count per entity-state.

(d)


IFN-g
IL-4
IL-12
TGF-b
TNF-a
IL-10
IL-6
IFN-b
IL-18
IL-23
D
IL-2
ng/mL
days
Cytokines. Concentration of cytokines and interleukins. D in the inset plot is danger signal.

(e)


NK cell population (cells per mm3)
Total
days
Natural Killer cells (total count).

(f)

(S proteins) of SARS-CoV-2 from the former study (Finkel et al. 2021). The sequence was extracted in the FASTA format having 165 amino acids that code for spike surface glycoprotein of SARS-CoV-2. From the previous study, it was observed that the S protein region of the Omicron variant has several mutations, which affect the binding affinities of antibodies to the S protein. Massive efforts are being done around the world to produce an S protein-based vaccination or treatment. Although there is a scarcity of information on the immunological characteristics of COVID-19 patients, accumulating experimental evidence suggests that B-cells and T-cells are normally activated during infection and contribute significantly to the resolution of SARS-CoV-2. In a similar vein, Ahmed et al., used immunoinformatic analysis to produce SARS-CoV-2 vaccines. The shared epitopes of B-cell and T-cell were predicted using the surface glycoprotein of SARS-CoV-2 (Ahmed et al. 2020). The immunoinformatic approaches were also used by Baruah and Bose group to identify vaccine candidate against SARS-CoV-2 (Baruah and Bose 2020). The epitope identification of the COVID-19 vaccine was also done using surface glycoprotein. The antigenicity of common B-cell and T-cell epitopes were investigated in this work. They discovered three B-cell epitopes that are sequential and five that are discontinuous.

Identification of CD8 + cytotoxic T lymphocyte (CTL) epitopes is a crucial step in epitope-based vaccine design. The prediction of CTL epitopes was performed using the NetCTL 1.2 server at the threshold value of 0.75, using MHC supertype A1. Based on a higher combined score, we got eight CTL epitopes, out of which two were selected for the construction of vaccine candidate. Similarly, Singh et al., also used the VaxiJen server for the determination of the antigenicity (Singh et al. 2021). Grifoni et al., employed immunoinformatics to construct a multi-epitopic vaccination and predicted a shared epitope from B- and T-cells (Grifoni et al. 2020). Kumar et al., used immunoinformatics techniques to show the antigenic diversity of S glycoprotein of SARS-CoV-2 in another investigation. Using NetCTL 1.2 and IEDB resources, the researchers predicted Cytotoxic T lymphocyte (CTL) epitopes (Kumar et al. 2021).

In the current study, we have used Epitopes Immune Epitope Database server (IEDB) to predict helper T lymphocyte (HTL) epitopes. A total of 10 epitopes were selected for IFN-γ Inducing Epitopes. Similarly, Grifoni et al., have also used an IEDB server for the perdition of the epitopes and designing of the vaccine (Grifoni et al. 2020). In addition, we also tested CD4 + T-cells epitopes to check their ability to induce interferon-gamma (IFN-γ) and five epitopes were selected based on positive results.

Further, BepiPred 2.0 server and ElliPro severs for linear and discontinuous B-cell epitopes were used. Linear B-cell epitopes were predicted with scores greater than the threshold (the default value is 0.5), discontinues B-cells epitopes predicted with the top PI value (Protrusion Index). From the results, three linear B-cell epitopes and only one discontinuous epitope found a potential candidate for vaccine construction was selected and the rest of the epitope was removed because it shows the allergenicity of the vaccine. Similarly, Panda et al., used immunoinformatics to discover epitopes from spike protein (structural protein) and Mpro (nonstructural protein). They used the VaxiJen server to discover that both the spike proteins and Mpro are naturally antigenic and have antigenicity. Several T-cell and B-cell epitopes have been discovered. They discovered both discontinuous and linear epitopes from B-cell epitopes (Panda et al. 2020). Likewise, for the 11 B-cell epitope perdition, Singh et al. used the BepiPred 2.0, Sequential B-Cell Epitope Predictor, and ABCpred (Singh et al. 2021). Ahmed et al., used immunoinformatics analysis to create vaccines against SARS-CoV-2 in another investigation. The residues of B-cell discontinuous and linear epitopes have been mapped by researchers. SARS-CoV-2 was observed to elicit an adequate immune response against all of these B-cell and T-cell epitope sets (Ahmed et al. 2020). The key role of selected epitopes is the stimulation of individual's adaptive immune system. As a result, only the best epitopes were used in the vaccine development. Two CTL epitopes, five HTL epitopes, three linear B-cell epitopes, and one discontinuous B-cell epitope were linked together using different linkers. CTL epitopes were linked using the AAY linker, HTL epitopes with the GPGPG linker, and B-cell epitopes with the KK linker. The human β-defensin-2 amino acid sequence was also attached to the vaccine's N-terminal with the use of the EAAAK linker to boost vaccine immunogenicity. A TAT sequence (11aa) was inserted into the C-terminus of the modeled vaccine to allow it to be administered intracellularly. The final vaccination was planned to be 286 amino acids long. The PHYRE2 server was used to create the 3D model of the vaccine candidate, which was then refined using the Galaxy Refine server and validated using the ProSA-web server to improve the structural quality. Similarly, Bhattacharya et al., used spike protein to build an immunoinformatics-based SARS-CoV-2 vaccine (Bhattacharya et al. 2020). They chose 13 MHC-I epitopes and three MHC-II epitopes. To develop a multi-epitope-based peptide vaccination, these peptides were connected with an (EAAAK) linker and a 3D model was generated using the PHYRE2 server.

Docking studies are one of the important studies which predict energetically favored interaction (Gupta et al. 2020). The protein–protein docking is useful to check the interaction between the proteins. In the current investigation, we have found the interaction of the designed vaccine candidate with Toll-like receptor 3 (TLR3). Further, the codon optimization followed by In-silico cloning was performed and

found satisfactory results. Finally, immune simulation profile was also found to be satisfactory.

Conclusion

In conclusion, the current study has identified a promising vaccine candidate (probable antigenic and non-allergenic) using immunoinformatic tools against omicron, a variant of SARS-CoV-2.

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Author contributions A; conducted the designed experiments; RS; drafted the manuscript; CRP; revision of the manuscript; KS; conducted protein–protein docking studies, AK: design of study, analysis of data and revision of the manuscript.

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Data availability statement All data has been included inside the manuscript.

Declarations

Conflict of interest The authors declared that they have no conflict of interest.

Ethical approval Not applicable.

Patient consent statement Not applicable.

Permission to reproduce material from other sources No material has been reproduced from other sources.

Clinical trial registration Not applicable.

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