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ORIGINAL ARTICLE

Designing a next generation multi-epitope based peptide vaccine candidate against SARS-CoV-2 using computational approaches

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Abstract

COVID-19 caused by SARS-CoV-2 was declared a global pandemic by WHO (World Health Organization) in March, 2020. Within 6 months, nearly 750,000 deaths are claimed by COVID-19 across the globe. This called for immediate social, scientific, technological, public and community interventions. Considering the severity of infection and the associated mortalities, global efforts are underway to develop preventive measures against SARS-CoV-2. Among the SARS-CoV-2 target proteins, Spike (S) glycoprotein (a.k.a S Protein) is the most studied target known to trigger strong host immune response. A detailed analysis of S protein-based epitopes enabled us to design a novel B-cell-derived T-cell Multi-epitope-based peptide (MEBP) vaccine candidate. This involved a systematic and comprehensive computational protocol consisting of prediction of dual-purpose epitopes and designing an MEBP vaccine construct. This was followed by 3D structure validation, MEBP complex interaction studies, in silico cloning and vaccine dose-based immune response simulation to evaluate the immunogenic potency of the vaccine construct. The dual-purpose epitope prediction protocol was designed such that the same epitope elicits both humoral and cellular immune response unlike the earlier designs. Further, the epitopes predicted were screened against stringent criteria to ensure selection of a potent candidate with maximum antigen coverage and best immune response. The vaccine dose-based immune response simulation studies revealed a rapid antigen clearance through antibody generation and elevated levels of cell-mediated immunity during repeated exposure of the vaccine. The favourable results of the analysis strongly indicate that the vaccine construct is indeed a potent vaccine candidate and ready to proceed to the next steps of experimental validation and efficacy studies.

Keywords COVID-19 · Docking · In silico · Multi-epitope · Simulation

Abbreviations

WHO	World Health Organization
MEBP	Multi-epitope-based peptide
TLRs	Toll like receptors
VLPs	Virus-like particles
ACE2	Angiotensin-converting enzyme 2
NCBI	National Centre for Biotechnological Information
ANN	Artificial neural network
PPV	Positive prediction value
BIMAS	Bioinformatics and molecular analysis section
IC_{50}	Half maximal inhibitory concentration
PDB	Protein data bank
NMA	Normal mode analysis
ENM	Elastic network model
PSSM	Position-specific scoring matrix
NTD	N-Terminal domain
CTD	C-Terminal domain
RBD	Receptor binding domain
FP	Fusion peptide
HR	Heptad repeat
MPER	Membrane proximal external region
TMD	Trans-membrane domain
CTL	Cytotoxic T-lymphocytes
HTL	Helper T-lymphocytes

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CAI Codon adaptation index
PRRs Pattern recognition receptors
PAMPs Pathogen-associated molecular patterns

Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) also known as novel Coronavirus 2019 (2019-nCoV), is a + VE sense, single-stranded RNA virus that belongs to the order *Nidovirales* and family *Coronaviridae* (Huang et al. 2020). Coronaviruses are classified into four different genera, namely α, β, γ, and δ. Among these, α and β coronaviruses infect bats and humans, respectively (Velavan and Meyer 2020). The novel coronavirus belongs to the β coronavirus genera which shares 79.5 and 96% of genome similarity with SARS-CoV and bat Coronavirus, respectively (Zhu et al. 2020; Zhou et al. 2020). The first incidence of a cluster of pneumonia-like symptoms were reported from the Wuhan city of China in December, 2019 and the disease spread rapidly in other countries in a very short period of time. The outbreak of disease probably started from a single or multiple zoonotic transmission events from wet markets in Wuhan, where meat and game animals were sold (Chakraborty et al. 2020a; Riou and Althaus 2020). On 11 March 2020, the Coronavirus disease 2019 (COVID-19) outbreak was officially declared as pandemic by the World Health Organization (WHO) (Dashraath et al. 2020). As of October 25, 2020, globally, the outbreak reached over 43 million confirmed cases with 1,157,509 reported deaths [https://covid19.who.int/]. A detailed review on history, evolution, epidemiology, pathogenesis and genome organization of SARS-CoV-2 and related human coronaviruses is published recently (Dagur 2020; Wang et al. 2020; Amawi et al. 2020). Of the seven coronaviruses (CoVs) isolated from humans so far, among them, three seem pathogenic: SARS-CoV, MERS-CoV, and the newly identified SARS-CoV-2. They cause severe infections in humans, posing significant threats to global public health. Common signs of infection include fever, cough, breathing difficulties and shortness of breath. In more extreme cases, infection can cause severe acute respiratory syndrome, kidney failure and even death.

As per reports, there is a high certainty for a second and even a third wave of COVID-19 pandemic (Wise 2020). Hence, it is very urgent and important to continue exploring means and methods of containing the infection through various interventions that include Drug development—towards a cure and the other approach to be future ready to such infections through vaccination—prophylaxis / preventive approach (Chakraborty et al. 2020b). We are still in the process of understanding the diverse modes and severity of infection of the SARS-CoV-2.

Prevention is better than cure and vaccination is undoubtedly the most effective tool to prevent infectious diseases (Li et al. 2020). To develop a safe and effective vaccine is challenging. As described elsewhere, an ideal SARS vaccine should (1) elicit highly potent neutralizing antibody responses against a broad spectrum of viral strains; (2) induce protection against infection and transmission; and (3) be safe, not inducing any infection-enhancing antibodies or harmful immune or inflammatory responses (Jiang et al. 2005). A detailed review on the first outbreak of SARS-CoV in 2003, the consequent efforts to contain the epidemic and then vaccine development efforts was published in 2008 (Enjuanes et al. 2008). Currently, several candidate vaccines belonging to various vaccine platforms are under trials against SARS-CoV-2. These include inactivated viruses, protein subunit vaccines, virus-like particles (VLPs), DNA vaccines, heterologous expression systems, and vaccines derived from SARS-CoV-2 genome by reverse genetics. As of October 29, 2020 total 45 candidate vaccines are in clinical evaluation of which 13 (29%) are using protein subunit as the vaccine platform. A total of 156 candidates are under preclinical evaluation with 55 (35%—highest among the vaccine platforms) candidates using protein subunit as respective vaccine platforms (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines). The S Protein which binds with angiotensin-converting enzyme 2 (ACE2) receptors, eliciting the immune response in the host (Human) is the primary antigen being targeted.

In the current study, immunoinformatics and structural bioinformatics tools were used to (a) predict B-cell-derived T-cell epitopes where each single epitope produces both humoral and cellular immune responses unlike earlier designs where each epitope produces only one of the immune response (b) design a Multi-epitope-based peptide (MEBP) vaccine construct using the most persuasive epitopes with suitable adjuvant and linkers (c) evaluate the vaccine construct for allergenicity, solubility and analyze various physicochemical properties (d) predict, refine and validate the 3D structure model (e) do molecular docking, calculate binding affinities, analyse intermolecular and interatomic interactions and perform molecular dynamics simulation between the peptide vaccine construct and host receptors (f) codon optimization, in silico cloning and (g) finally, carry out a dosage-vs-immune response simulation to find the immunogenic potency of the predicted vaccine construct.

Materials and Methods

The summary of the methodology followed to design the MEBP vaccine construct against SARS-CoV-2 is illustrated in Fig. 1.

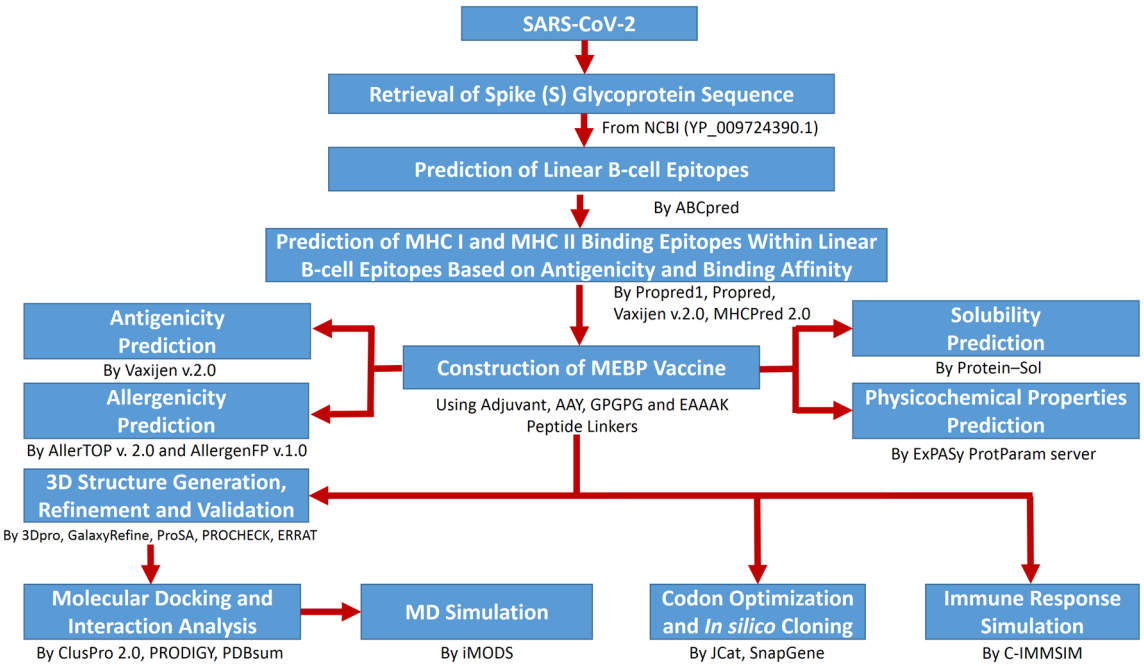


Fig. 1 Flowchart followed for the MEBP vaccine construct design against SARS-CoV-2

Retrieval of protein sequence

The complete amino acid sequence of S protein of SARS-CoV-2 was downloaded in FASTA format from the National Centre for Biotechnological Information (NCBI).

Prediction of linear B-cell epitopes

The identification of linear B-cell epitopes plays an important role in antibody production. Putative linear B-cell epitopes were predicted using Artificial Neural Network (ANN)-based server ABCpred (Saha and Raghava 2006). The prediction algorithm of ABCpred uses recurrent neural networks for a fixed length pattern of any particular epitope with fivefold cross-validation. The optimized threshold value of 0.51 was used in the current prediction of 20-mer linear B-cell epitopes, as this threshold value was demonstrated to show the highest Positive Prediction Value (PPV) of 66.51% with 64.36% accuracy of prediction (Nezafat et al. 2017).

Identification of MHC I and MHC II epitopes within B-cell epitopes—Dual-purpose epitopes

To generate strong immune response, it is critical to identify the T-cell epitopes within the B-cell epitopes. For this purpose, ProPred1 and ProPred servers were used for identification of MHC I- and MHC II-binding epitopes within the predetermined linear B-cell epitope regions (Dar et al. 2019). ProPred-1 is a matrix-based approach that uses matrices obtained from Bioinformatics and Molecular Analysis Section (BIMAS) and also from the literature (Singh and Raghava 2003). Whereas, ProPred utilizes quantitative matrices derived from the published literature (Singh and Raghava 2001).

MHC I epitopes were assessed with all the available 47 different alleles in Propred1 server. The option of proteasome and immunoproteasome filters was selected to improve the chances of finding accurate epitopes. Epitopes projected to be associated with at least five different MHC I alleles were retained. MHC II epitopes were evaluated against 51 different MHC II alleles available in ProPred server. Only epitopes predicted by at least ten different MHC II alleles were considered for further analysis. The predicted MHC I- and MHC II-binding epitopes were further subjected to VaxiJen v.2.0 server for analyzing the antigenic propensity (Doytchinova and Flower 2007). The server was run with virus as a target field at a default threshold value of 0.4.

Prediction of binding affinity with MHCPred

Predicted MHC I and MHC II epitopes with a VaxiJen score cut-off of > 1.0 were further assessed for their binding affinity against HLA A*1101 and DRB1*0101, respectively, using MHCPred version 2.0 (Guan et al. 2006). The epitopes with a half maximal inhibitory concentration (IC_{50}) value < 100 nM were shortlisted as strong candidates for construction of MEBP vaccine construct associated with strong immunogenicity.

Designing MEBP vaccine construct

We joined the predicted antigenic epitopes with specific peptide linkers, to construct a peptide vaccine construct. We selected two peptide linkers, i.e. Ala-Ala-Tyr (AAY) and Gly-Pro-Gly-Pro-Gly (GPGPG) for joining the antigenic epitopes and Glu-Ala-Ala-Ala-Lys (EAAAK) peptide linker selected for boosting the immunogenicity with the help of adjuvant—β-defensin (Kumar Pandey et al. 2018).

Prediction of antigenicity, allergenicity, solubility, and physicochemical properties

Antigenicity of the vaccine construct was predicted using VaxiJen v.2.0 server. Screening for allergenicity of any vaccine construct is crucial as it should not cause sensitization and allergic reaction inside the body. Hence, AllerTOP v. 2.0 (Dimitrov et al. 2014a) and AllergenFP v.1.0 (Dimitrov et al. 2014b) servers were used to check the allergenicity of the MEBP vaccine construct. The solubility of vaccine construct upon expression in *E. coli* was predicted using Protein–Sol (Hebditch et al. 2017). Furthermore, the various physicochemical parameters like isoelectric point (pI), half-life, instability index, aliphatic index, and others were assessed using the ExPASy ProtParam server (Wilkins et al. 1999).

Tertiary structure prediction, refinement, and validation of vaccine construct

The three-dimensional structure of the MEBP vaccine construct was predicted using the 3Dpro online server of SCRATCH suite (Cheng et al. 2005). 3Dpro uses predicted structural features, and the Protein Data Bank (PDB) knowledge-based statistical terms in the energy function. The conformational search uses a set of movements consisting of fragment substitution (using a fragment library built from the PDB), as well as random distribution for the model. Later the molecular refinement of the modelled structure was performed through GalaxyRefine web server (Heo et al. 2013). This server refines the side chains, performs side-chain re-packing followed by overall structural relaxation through molecular dynamics simulation. Refined model was validated using ProSA-web, PROCHECK server and ERRAT server for any potential structural errors (Laskowski et al. 1993; Colovos and Yeates 1993; Wiederstein and Sippl 2007).

Molecular docking analysis and interaction studies

Molecular docking was performed to predict the binding affinity and interaction patterns between the vaccine construct against the monomeric forms of different TLRs. The structures of TLR2 (PDB ID: 2Z7X), TLR3 (PDB ID: 2A0Z), TLR4 (PDB ID: 4G8A), TLR5 (PDB ID: 3J0A), TLR7 (PDB ID: 5GMF) and TLR8 (PDB ID: 3W3M) receptor were downloaded from RCSB PDB database and the refined 3D structure of the MEBP construct was used as a ligand (Rose et al. 2017). The protein–protein docking was carried out using ClusPro 2.0 server (Kozakov et al. 2017). The server uses three consecutive steps like rigid body docking, clustering of lowest energy structure, and structural refinement by energy minimization (Sayed et al. 2020). The best docked complex was selected based on the cluster size along with lowest energy. The binding affinity or the Gibbs free energy (ΔG) was predicted using PRODIGY web server (Xue et al. 2016). The interaction analysis and visualization of the docked complex were performed using the PDBsum and Chimera v1.14, respectively (Pettersen et al. 2004; Laskowski et al. 2018).

Molecular dynamics simulation

Molecular dynamics of the top ranked complex of the vaccine construct and TLR8 was completed through iMODS web server to elucidate the typical protein motion within the internal coordinates through normal mode analysis (NMA) (López-Blanco et al. 2014). The iMODS server calculates the dihedral coordinates of Cα atoms of docked complexes to measure the actual mobility of the proteins based on elastic network model (ENM) (Pritam et al. 2020). Furthermore, it calculates B-factor plot with structure deformation, covariance, mode variance, as well as calculates the eigenvalue.

Codon optimization and *silico* cloning

Java Codon Adaptation Tool (JCat) server was used to perform reverse translation and codon optimization for an efficient expression of the MEBP vaccine construct in *E. coli* K-12 bacterial strain (Grote et al. 2005; Bhattacharya et al. 2020a). At the same time, additional options were selected to evade rho-independent transcription terminators, prokaryotic ribosome-binding sites, and cleavage sites of restriction enzymes. Then, the optimized DNA sequence was added with XhoI and NdeI restriction sites at the N-terminal and C-terminal, respectively. Finally, the adapted DNA sequence was inserted between the XhoI and NdeI restriction sites of pET28a(+) vector using SnapGene tool (https://www.snapgene.com/).

Immune simulation analysis

The immunogenicity and dosage-vs-immune response profile of the final vaccine construct was evaluated using in silico immune simulation server C-IMMSIM (http://150.146.2.1/C-IMMSIM/index.php). It simulates the regimen and

real-time dosage response patterns of a vaccine. The server uses machine learning methods for predicting immune interactions (Rapin et al. 2010). The minimum recommended time interval between two doses is 4 weeks for most of the currently used vaccines (Castiglione et al. 2012). Therefore, total three injections four weeks apart were considered for administering with time steps set at 1, 84 and 168 (each time step equals 8 h). The simulation volume and simulation steps were set at 110 and 1100, respectively. All other parameters were kept at default before the simulation run.

Results

Protein sequence retrieval

S protein of SARS-CoV-2 is one of the most potent targets in vaccine research against COVID-19. It is a surface protein which makes the first physical interaction with host machinery triggering the immune response (Wrapp et al. 2020). The sequence from Wuhan-Hu-1 (China) isolate of SARS-CoV-2 was retrieved from NCBI with the following accession number YP_009724390.1 to carry out the in silico analysis. The monomeric S Protein is 1273 amino acids long which are further structurally divided into 2 subunits, S1 and S2. S1 subunit further is divided into N-Terminal Domain (NTD) and C-Terminal Domain (CTD). CTD contains the Receptor Binding Domain (RBD) which binds the ACE2 receptor of lung epithelial cells. Similarly, S2 subunit contains necessary structural motifs required for membrane fusion, such as fusion peptide (FP), two heptad repeats (HR), a membrane proximal external region (MPER), and a trans-membrane domain (TMD) (Li 2016). The potential structural fragments of S Protein currently used as antigens are S1 subunit, NTD, RBD and FP (Zhang et al. 2020).

Linear B-cell epitope prediction

A total of 99 linear B-cell epitopes of 20 amino acid length (window length) were identified within the S protein of SARS-CoV-2. All the epitopes along with their start position and respective scores are shown in Table S1.

Identification of B-cell-derived T-cell epitopes

T-cell epitope prediction comprises identification of MHC I- and MHC II- binding epitopes, to activate both cytotoxic T-lymphocytes (CTL)- and helper T-lymphocytes (HTL)-mediated immune response. MHC I and MHC II epitopes were scanned within the predicted B-cell epitopes to identify B-cell-derived T-cell epitopes.

A total of 39 B-cell-derived MHC I epitopes were predicted by ≥ 5 MHC I alleles available in Propred1. Out of these, 19 MHC I epitopes were found to be having VaxiJen score greater than threshold (0.4). A table containing MHC I-binding epitopes along with a number of different MHC I-binding alleles and VaxiJen scores are shown in Table S2. Similarly, a total of 52 B-cell-derived MHC II epitopes were identified by ≥ 10 different MHC II alleles available in the Propred server. Out of which, 26 of the MHC II epitopes were having a VaxiJen score above the threshold (0.4). The predicted MHC II epitopes with the number of interacting MHC II alleles and VaxiJen scores are listed in Table S3. However, to increase the precision of selection 5 MHC I- and 11 MHC II-binding epitopes showing strong antigenicity, i.e. VaxiJen scores > 1.0 were selected for screening of binding affinity.

Binding affinity prediction of T-cell epitopes

All of the predicted MHC I-binding epitopes were having strong binding affinity against HLA A*1101 allele; and five out of eleven MHC II-binding epitopes were having favorable binding affinity against DRB1*0101 in humans. These B-cell-derived T-cell epitopes were selected based on the criteria, such as the total number of binding alleles (≥ 5 for MHC I, and ≥ 10 for MHC II), VaxiJen scores > 1.0 and binding affinities (IC_{50} < 100 nM) as shown in Tables 1 and 2.

Table 1 Selected five of the B-cell derived MHC I-binding peptides based on our set of predefined criteria

Sl. No	Start Position	Sequence	No. of MHC I-binding alleles	VaxiJen 2.0 Score	MHCPred HLA A*1101 IC_{50} Value (nM)
1	109	TLDSKTQSL	12	1.0685	82.6
2	181	GKQGNFKNL	8	1.0607	22.03
3	379	CYGVSPTKL	6	1.4263	79.62
4	417	KIADYNYKL	13	1.6639	52.97
5	510	VVVLSFELL	14	1.0909	16.52

(i) Total number of different binding alleles must be ≥ 5, (ii) VaxiJen score > 1.0, and (iii) Binding affinity against HLA A*1101 (IC_{50} < 100 nM)

Table 2 Selected five out of eleven B-cell derived MHC II-binding peptides (in bold) based on our set of pre-defined criteria

Sl. No	Start Position	Sequence	No. of MHC I-binding alleles	VaxiJen 2.0 Score	MHCPred DRB1*0101 IC_{50} Value (nM)
1	231	**IGINITRFQ**	35	**1.3386**	98.4
2	495	**YGFQPTNGV**	12	**1.0509**	37.33
3	511	VVLSFELLH	11	1.409	171.79
4	512	**VLSFELLHA**	20	**1.0776**	41.5
5	534	VKNKCVNFN	16	2.053	–-
6	894	**LQIPFAMQM**	17	**1.068**	6.43
7	1060	VVFLHVTYV	44	1.5122	883.08
8	1061	VFLHVTYVP	13	1.2346	488.65
9	1128	VVIGIVNNT	21	1.3063	203.24
10	1172	INASVVNIQ	18	1.1445	289.07
11	1225	**IAIVMVTIM**	15	**1.1339**	47.75

(i) Total number of different binding alleles must be ≥ 10, (ii) VaxiJen score > 1.0, and (iii) Binding affinity against DRB1*0101 (IC_{50} < 100 nM)

Designing MEBP vaccine

For designing of a MEBP vaccine construct, prioritized 5 MHC I- and 5 MHC II-binding epitopes were joined together using AAY and GPGPG linkers, respectively. Further, the N-terminal end of the first MHC I epitope was joined with β-defensin: an adjuvant of 45 amino acids length using an EAAAK linker. Whereas, the C-terminal end of the last MHC II-binding epitope was linked with 6x-His tag. The final amino acid sequence of the designed B-cell-derived T-cell-based MEBP vaccine construct is given below which has a total of 183 amino acids making it ~ 20 kDa peptide vaccine construct:

GIINTLQKYYCRVRGG RCAVLSCLP-KEEQIGKCSTRGRKCCRRKKEAAAAK-TLDSKTQSLAAYGKQGNFKNLAAY CYG VSPTKLAAYKIADYNYKLAAYVVVLSFELLGPG-PGIGINITRFQGPGPGYGFQPTNGVGPGPGVLS-FELLHAGPGPGLQIPFAMQMGPGPGIAIVMVTIM-HHHHHH.

Prediction of antigenicity, allergenicity, solubility, and physicochemical properties

As per the prediction results, the construct is antigenic, non-allergen and water-soluble in nature. Various physicochemical properties of the construct are listed in Table 3.

Tertiary structure prediction, refinement and validation of peptide vaccine construct

To elicit a strong immune response, the above 183 amino acid-long peptide construct needs to have stable and favorable interactions (non-covalent) with various B-cell and

Table 3 Antigenicity, allergenicity, solubility, and physicochemical property assessments of the primary sequence of multi-epitope-based vaccine construct

Sl. No	Features	Assessment
1	Antigenicity	0.6243 (Probable ANTIGEN)
2	Allergenicity	Probable non-allergen (AllerTOP v.2.0) Probable non-allergen (AllergenFP v.1.0)
3	Solubility	0.578 (Soluble)
4	Number of amino acids	183
5	Molecular weight	19,572.85 Dalton
6	Theoretical Isoelectric point (pI)	9.66
7	Total number of atoms	2761
8	Formula	$C_{879}H_{1386}N_{248}O_{237}S_{11}$
9	Estimated half-life	30 h (mammalian reticulocytes, in vitro) > 20 h (yeast, in vivo) > 10 h (Escherichia coli, in vivo)
10	Instability index	25.48 (Stable)
11	Aliphatic index	80.00
12	Grand average of hydropathicity (GRAVY)	− 0.111

T-cell receptors and other signaling molecules. To study the possible favourable interactions and stability of the complex structure, the knowledge about the 3D structure of the peptide is essential. 3Dpro, an ab initio 3D structure prediction programme was used to build the 3D structure model of the synthetic peptide, i.e., peptide vaccine construct. The predicted model was run for structure perturbations and relaxations to obtain a refined structure using GalaxyRefine (Fig. 2a). Ramachandran plot by PROCHECK confirms 90.8, 7.7, 0.0, and 1.4% of the residues were in most favoured regions (red), additional allowed regions (yellow), generously allowed regions (pale yellow) and disallowed regions (white), respectively (Fig. 2b). ProSA calculated Z-score of the refined model was found to be − 4.42, which falls within the vicinity of experimentally validated protein structures (Fig. 2c). ProSA also showed a valid local model quality by plotting energies as a function of amino acids present in protein structure (Fig. 2d). Overall quality factor by ERRAT was projected as 85.714, which further supports the refined structure as the high-quality model (Fig. 2e).

Molecular docking studies of MEBP vaccine construct with Human TLRs:

ClusPro 2.0 web server provided several docking models and ranked them based upon the cluster size with the lowest energy score within that cluster. The lowest energy scores for the top ranked TLR-MEBP vaccine construct complexes are as follows: (− 1126.1, TLR2), (− 995.4, TLR3), (− 977.4, TLR4), (− 1160.1, TLR5), (− 1068.9, TLR7) and (− 1149.9, TLR8) (Fig. S1). Similarly the binding affinities (in kcal/mol) obtained from PRODIGY server are as follows: (− 17.6, TLR2), (− 18.7, TLR3), (− 19.9, TLR4), (− 11.8, TLR5), (− 15.2, TLR7) and (− 18.6, TLR8). This in silico analysis reveals that the designed MEBP vaccine construct has favorable binding affinity against the TLRs. In addition, the inter-molecular interaction analysis using PDBsum revealed formation of hydrogen bonds, salt bridges and non-bonded contacts between the MEBP construct and receptors (Fig. S2). Such interactions are essential for maintaining stability of the protein complex. The detailed

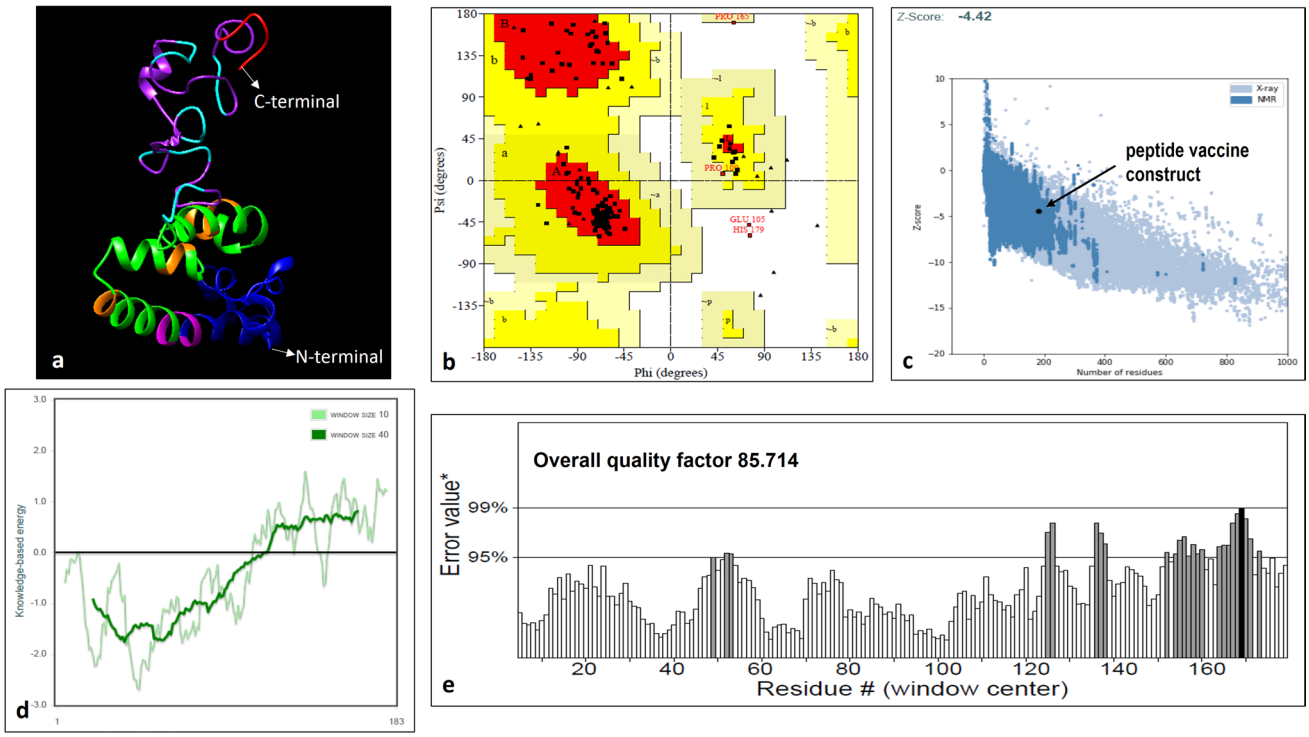


Fig. 2 3D Structure prediction, refinement and validation of MEBP vaccine construct. **a** The final 3D structure model of the vaccine construct. The colour code: Adjuvant (Blue), EAAAK linker (Magenta), MHC I-binding epitopes (Green), AAY (Orange), GPGPG (Cyan), MHC II-binding epitopes (Purple), and 6x-His tag (Red), **b** Ramachandran plot generated using PROCHECK, **c** ProSA Z-score plot. The plot contains Z-scores of all experimentally determined structures of similar sized proteins from PDB database i.e. NMR spectroscopy (dark blue) and X-ray crystallography (light blue). The Z-score of the vaccine construct is − 4.42 (black colored dot in the plot) indicates the overall model quality, **d** ProSA graphical plot (local model quality) indicates that most of the residues had a negative energy and below the threshold indicating the designed construct to have less errors or problematic parts, **e** The overall quality factor 85.714 is shown in ERRAT plot. In the plot, the regions that can be rejected at 95% confidence level are depicted with grey lines and the regions that can be rejected at 99% level are illustrated with black lines. The overall quality factor reveals the percentage of protein for which the calculated error value falls below the 95% rejection limit

overview of molecular docking and predicted interactions between MEBP vaccine construct and TLRs are given in Table 4.

Molecular dynamics simulation

Based on the predictions and properties, the designed construct seems to bind effectively with all the TLRs targeted. However, TLR8 plays an important role for generation of effective immune response in humans. TLR8 also senses the single-stranded RNA of viruses in the endosome and is predominantly expressed in lungs. For these reasons, further structure and simulation analysis was restricted to TLR8 receptors. The relative stability was analyzed for the TLR8-MEBP complex (Fig. 3). The top ranked model selected to calculate the functional mobility indicates that TLR8 and vaccine constructs move toward each other indicating stable and strong binding, represented by arrows in Fig. 4a. The deformability analysis revealed that the distortions were significantly reduced in the docked complex as compared to

Table 4 Results of the molecular docking by Cluspro 2.0, binding affinity by PRODIGY and PDBsum interactions of potential receptors (Chain A) with predicted MEBP vaccine (Chain B)

Sl. No	Component	Cluspro 2.0		PRODIGY	PDBsum Interaction Study						
		Cluster Size / Member	Lowest Energy Score	Binding Affinity ΔG (kcal/mol)	Chain wise no. of interface residues		Chain wise interface area (A^2)		No. of salt bridges	No. of hydrogen bonds	No. of non-bonded contacts
					A	B	A	B			
1	MEBP_TLR2	73	− 1126.1	− 17.6	48	36	1945	2012	7	26	284
2	MEBP_TLR3	62	− 995.4	− 18.7	36	23	1428	1553	5	24	199
3	MEBP_TLR4	76	− 977.4	− 19.9	43	32	1710	1934	2	20	242
4	MEBP_TLR5	52	− 1160.1	− 11.8	35	35	1710	1796	5	19	199
5	MEBP_TLR7	52	− 1068.9	− 15.2	37	33	1762	1672	2	15	248
6	MEBP_TLR8	80	− 1149.9	− 18.6	37	33	1897	1892	5	15	193

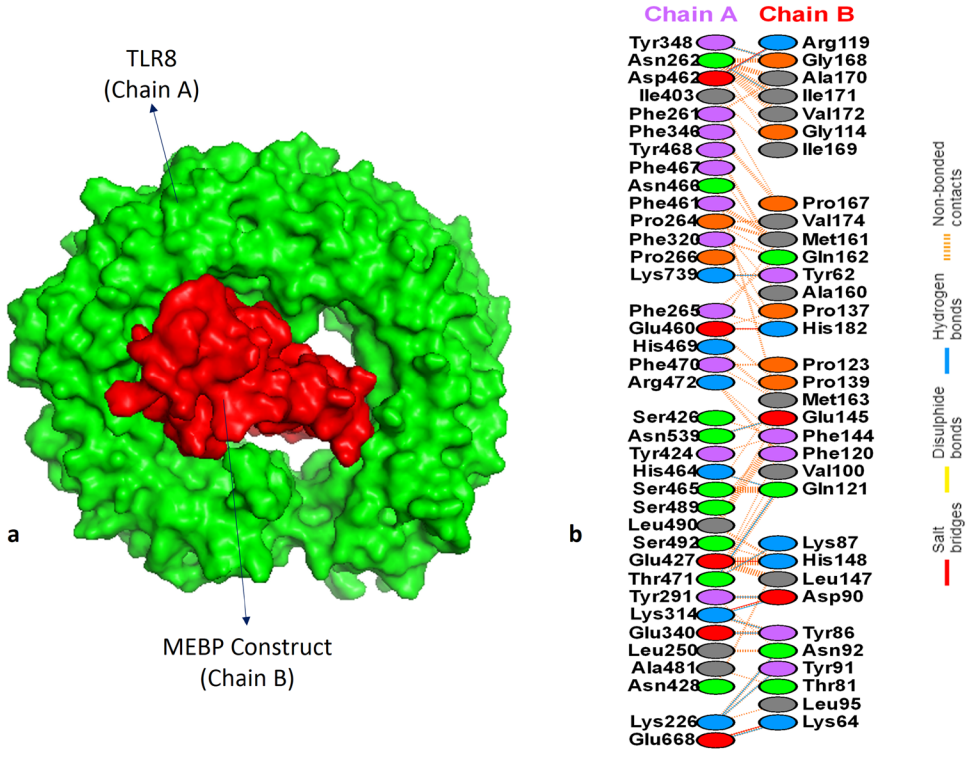


Fig. 3 Docked complex between the modeled vaccine construct and TLR8 receptor. **a** Complex showing the surface interaction between TLR8 (Green) and MEBP component (Red), **b** 2D interaction studies using PDBsum (TLR8 as chain A and Vaccine construct as chain B)

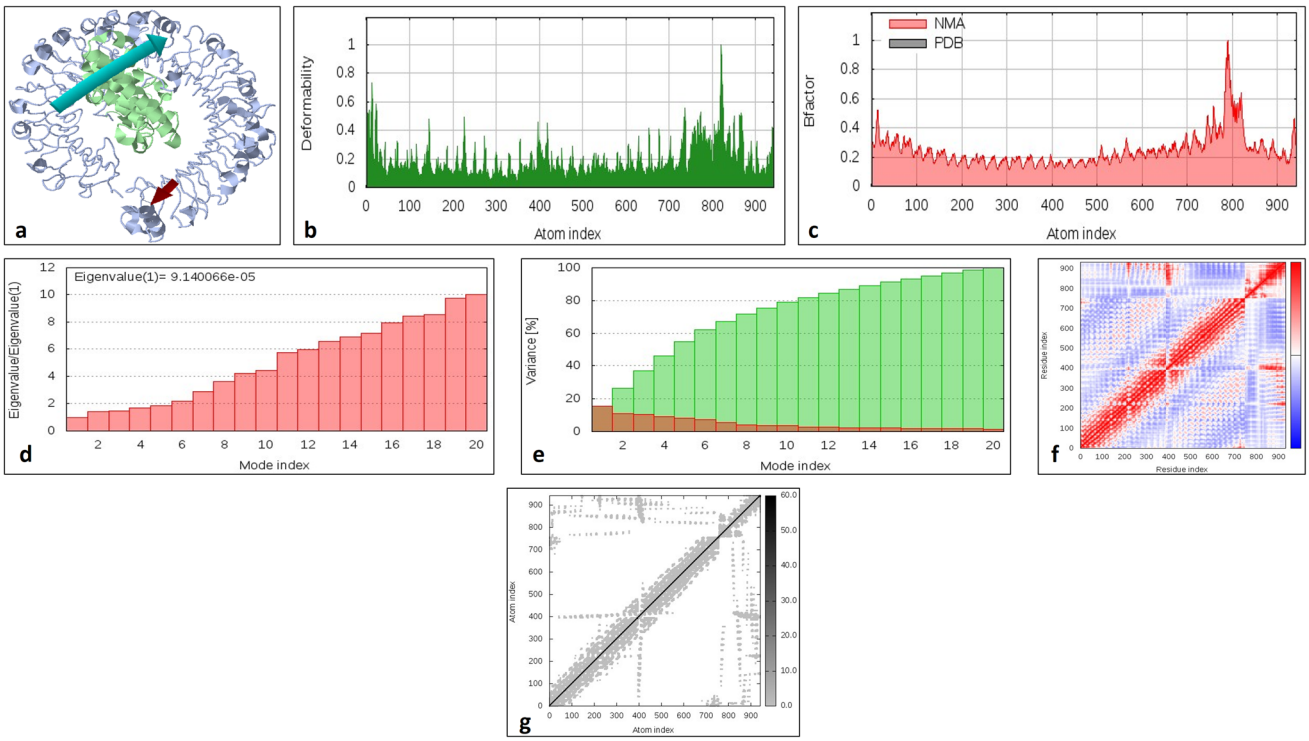


Fig. 4 Results of molecular dynamics simulation of TLR8 and Vaccine construct. **a** NMA mobility, **b** Deformability plot, **c** B-factor plot, **d** Eigenvalue plot, **e** Variance plot (red color remarks individual variances and green color indicates cumulative variances), **f** Covariance map [correlated (red), uncorrelated (white) or anti-correlated (blue) motions], **g** Elastic network (darker gray regions indicate more stiffer regions) of the complex

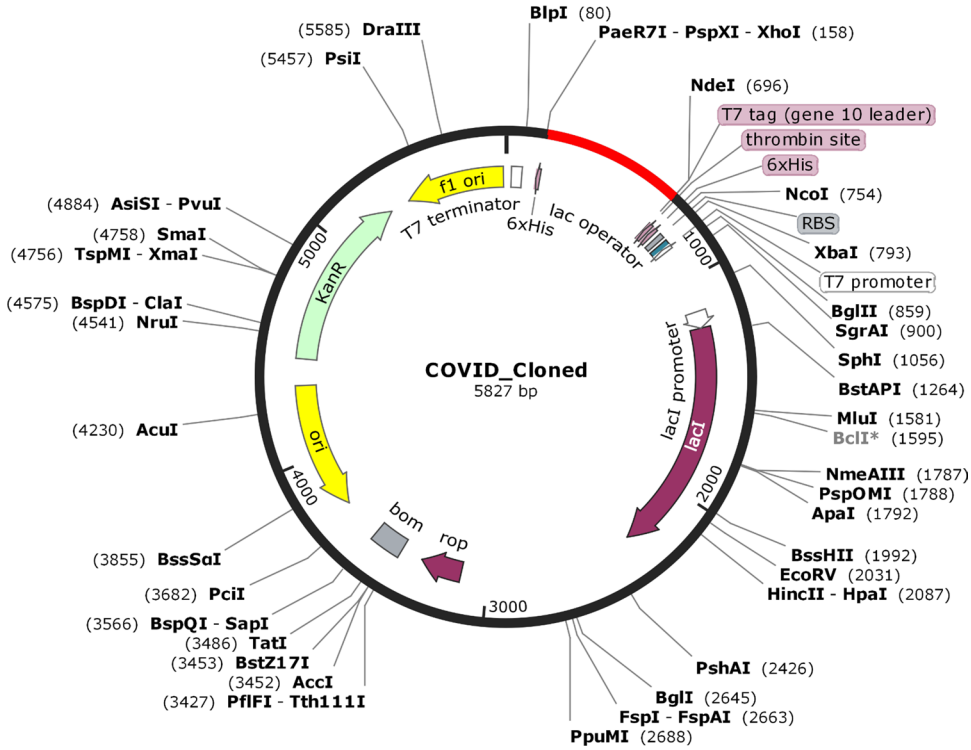
the monomeric TLR8 protein (Fig. 4b). Further, the B-factor analysis exposed the minor atomic distortions in the docked complex depicted in Fig. 4c. In addition, the eigenvalue of the docked complex was observed to be $9.140066e^{-05}$. Here, the docked complex has much higher eigenvalues, thus indicating better stability of the complex represented in Fig. 4d. In the variance analysis bar plot, which is inversely proportional to eigenvalue, the cumulative and individual variances of the complex are depicted through green bars and red bars, respectively (Fig. 4e). Covariance matrix analysis reflects the interaction between residue pairs of the proteins of a complex, where the uncorrelated, and anti-correlated motions are represented by white, red and blue colours, respectively (Fig. 4f). The protein complex stiffness study was carried out using the elastic network analysis. The darker grey dots indicate greater protein stiffness at certain portions as shown in Fig. 4g.

Codon adaptation and in silico cloning

It is important to optimize the codon bias to suit the E. *coli* expression system to ensure effective translation and maximal protein production. Therefore, in silico cloning was performed to check the expression of the synthetic peptide vaccine construct within a prokaryotic host. The length of the cDNA sequence after codon optimization was 549 nucleotides. The codon adaptation index (CAI) and GC content of the reverse-translated vaccine were found to be 0.97 and 53.73%, respectively. Ideally, the CAI value 0.8–1.0 and GC content between 30 and 70% are considered for high possibility of protein expression and reliability. These values support the efficient protein expression of the vaccine candidate in *E. coli* (strain K12). Since the optimized DNA sequence lacks the restriction sites for XhoI and NdeI restriction enzymes, it is essential to conjugate these restriction sites at N-terminal and C-terminal, respectively, before cloning. The recombinant plasmid with the adapted codon sequence was inserted between XhoI (158) and NdeI (238) restriction sites of pET28a(+) vector as shown in Fig. 5. The total length of the final clone was 5.827 kbp.

Dosage-vs-immune response simulation analysis

The in silico simulated immune response was found compatible with the actual immune responses (Fig. 6). The secondary and tertiary responses were significantly higher compared to the primary response. The primary response was characterized by presence of high concentration of IgM antibodies. A marked decrease is observed in antigenic concentration during secondary and tertiary responses due



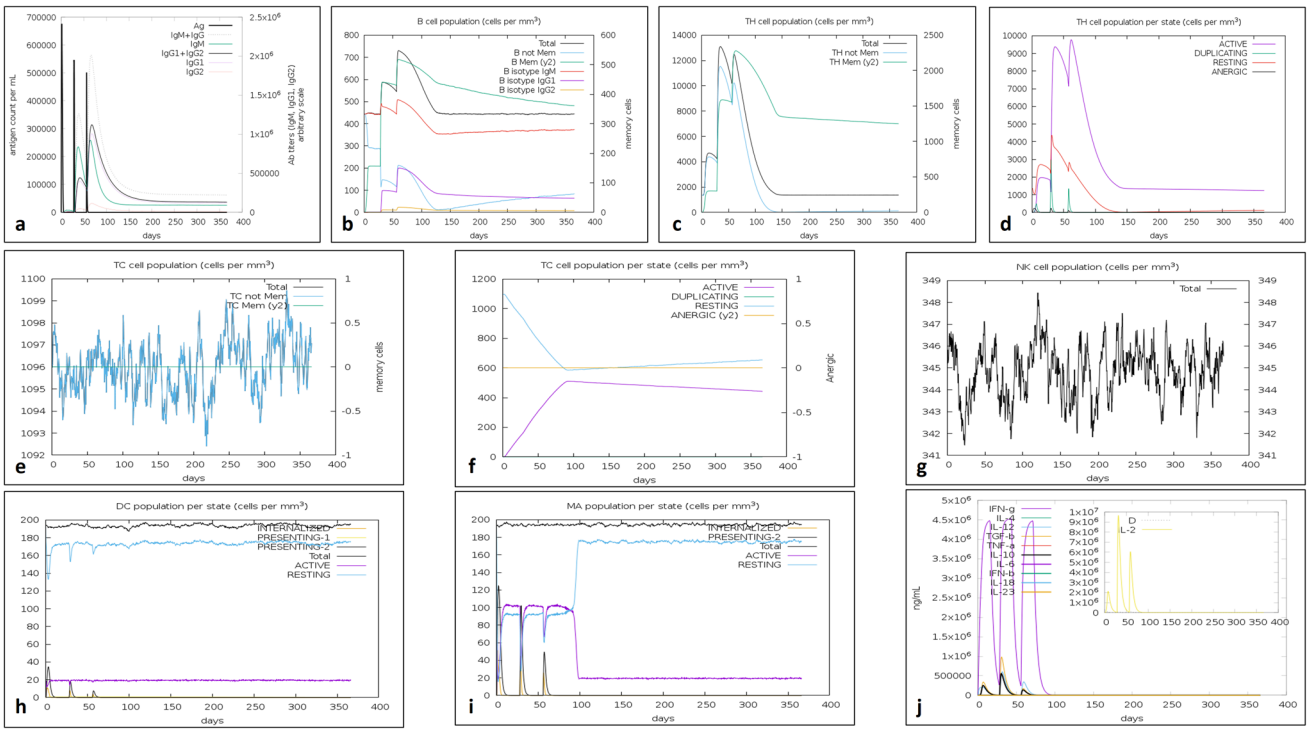


Fig. 6 In silico immune response simulation of the multi-epitope-based vaccine construct. **a** Production of immunoglobulins upon antigen exposure, **b** Population of B lymphocytes after three injections, **c** Amount of helper T lymphocytes, **d** Helper T lymphocytes population per state, **e** Amount of Cytotoxic T lymphocytes population, **f** Cytotoxic T lymphocytes population per state, **g** Population of Natural Killer cells, **h** Population of Macrophages, **i** Population of Dendritic cells, **j** Concentration of cytokines and interleukins with Simpson index [D]

to presence of elevated levels of immunoglobulin activities (i.e., IgG1 + IgG2, IgM, and IgG + IgM antibodies) as shown in Fig. 6a. Furthermore, different long-lasting B-cell isotypes indicate the isotype switching potential and memory formation (Fig. 6b). Similarly, an elevated level of active T_H (helper) and active T_C (cytotoxic) cell populations was observed along with a strong memory development (Fig. 6c–f). During exposure, the natural killer cell activity was found to be consistent along with higher macrophage and dendritic cell activity (Fig. 6g–i). An increased level of cytokines like IFN-γ and IL-2 was also detected along with lower Simpson index (D), indicating greater diversity of immune response (Fig. 6l).

Discussion

The SARS-CoV-2 has become pandemic, showing no sign of abatement (El Zowalaty and Järhult 2020). The virus is highly contagious in nature, causing respiratory distress that can lead to eventual death in susceptible individuals. Researchers across the world are fighting the challenge of finding means for halting the spread of this virus. Till now no specific therapeutic agents have been approved for treating SARS-CoV-2 infection (Saha et al. 2020). The safety issues on the use of drugs like chloroquine and hydroxychloroquine still needs to be ascertained (Pastick et al. 2020). Historically, vaccination has proved to be an effective method of protecting a large human population against viral diseases. Except for media reports, there is still no approved and licensed vaccine available in the market for combating COVID-19. There are two vaccine candidates as per WHO which are in Phase-III clinical trials. One can expect at least another 4–5 months to get approvals and reach the masses. Compared to traditional vaccines like killed, attenuated or live vaccines, epitope-based subunit vaccine platforms are increasingly being preferred and have proved to have better experimental control, reproducibility and validation. These provide better control over the immunogenic components of the pathogen responsible for causing diseases (Pourseif et al. 2019).

In recent months, several authors already reported the MEBP vaccine candidates against SARS-CoV-2 using reverse vaccinology approach. Studies published by Naz et al. (2020) and Sanami et al. (2020) predicted both B- and T-cell epitopes of S protein alone for designing a MEBP vaccine candidate against SARS-CoV-2. Sarkar et al. (2020) reported N and S proteins as target antigens for prediction of B- and T-cell epitopes. They designed MEBP vaccine constructs to increase the coverage of antigenic or immunogenicity space of protein subunit-based platforms against COVID-19 and performed bioinformatics evaluations. To increase the coverage of antigens, Kalita et al. (2020) predicted B- and T-cell epitopes by adding more target proteins, namely N, M, and S proteins of SARS-CoV-2. In all above studies, MEBP vaccine construct was designed by joining either exclusive B- or exclusive T-cell predicted epitopes. As mentioned in the introduction, an ideal vaccine should be safe and must elicit strong humoral and cellular immune response *simultaneously*. In the above mentioned studies, the focus was on the epitopes that elicit either humoral (B-cell) or cellular (T-cell) responses.

To produce an ideal immune response, the epitopes must be accessible to both MHC I and MHC II molecules along with the B-cell which inspired our design (Patra et al. 2020; Sajjad et al. 2020). Hence, our focus has been on B-cell-derived T-cell epitopes—such epitopes which can elicit simultaneous humoral and cellular immune response. Essentially, it means the same epitope of 9 amino acids length will elicit both humoral and cellular immune response simultaneously. Bhattacharya et al. (2020b) followed a similar design and predicted different B-cell-derived T-cell epitopes and designed a MEBP vaccine against SARS-CoV-2. However, our study reveals a) that eight out of ten predicted epitopes found to be novel, b) had better VaxiJen scores i.e. > 1.0 and c) which have not been reported earlier. We were able to identify 5 MHC I and 5 MHC II epitopes with high antigenic potential and strong binding affinity. The binding affinities of the selected epitopes were predicted against HLA A*1101 and DRB1*0101 alleles, as these are the most common MHC class I and MHC class II alleles, respectively, in the human population (Southwood et al. 1998; Li and Bouvier 2004).

For designing of a MEBP vaccine construct, predicted epitopes were joined together using different linkers for adequate separation of the epitopes (Khan et al. 2019). A suitable adjuvant was added at the N-terminus end to boost the immunogenicity within the human body and 6x-His tag was added to the C-terminus end for identification and purification purposes. The vaccine construct was predicted as probable antigen (VaxiJen score 0.6243), non-allergen and water-soluble in nature. These predicted values provide the first level of confidence that this construct has the potential to become a vaccine candidate.

The analyses of the physicochemical parameters of the peptide vaccine construct are found to be favourable. Generally, protein with molecular weight less than 110 kD is considered as good vaccine candidate (Naz et al. 2015). The computed molecular weight of our MEBP construct is 19.573 kD. A pI of 9.66 indicates that the peptide vaccine construct is basic in nature and stable at this pH range. The aliphatic index 80.00 indicates the thermostable nature of the construct at various temperatures. Similarly, an instability index of 25.48 (< 40) indicates that the construct is stable after expression. The Grand average of hydropathicity (GRAVY) value was computed to be negative (− 0.111),

revealing that the vaccine is hydrophilic in nature and likely to interact with other protein molecules. The above observations that all the physicochemical properties of our MEBP construct are within the optimum range indicate that our vaccine construct has the properties of a good vaccine (Solanki et al. 2019; Ahmad et al. 2020).

Further, the 3D structure model of vaccine construct was validated and found suitable for molecular docking experiments. ProSA Z -score represents the overall quality of the protein model which was found within the range of the native protein structures. A ProSA Z-score of -4.42 indicates that the peptide vaccine candidate has features characteristic to native protein structures. The PROCHECK Ramachandran plot used to find out energetically allowed and disallowed psi (ψ) and phi (ϕ) dihedral angles of amino acids, which was calculated based on van der Waal radius of the side chain. Our peptide vaccine construct has less than 1.5% of the residues in disallowed regions in the Ramachandran plot, indicating negligible number of steric clashes between the side-chain atoms and main chain atoms. Again, ERRAT server was used to find out the pattern of non-bonded atomic interactions. The overall quality factor (ERRAT score) was > 50.0 i.e. 85.714, indicating the high-quality model (Jain et al. 2015).

While docking in Cluspro 2.0 server, the top ranked model of the docked complex does not signify the actual binding affinities of the vaccine construct against the target. Hence, the prediction of ΔG (kcal/mol) and interaction patterns of the top docked complexes were carried out to evaluate the actual binding potential of the MEBP construct with different TLRs in humans. All the docked complexes with various TLRs were found energetically favourable, indicated by negative values of ΔG. Current study utilizes the TLRs located on cell surfaces (TLR2, TLR4 and TLR5) as well as TLRs located in endosomes (TLR3, TLR7 and TLR8) (Sakaniwa and Shimizu 2020). TLRs are the pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) of various microbes and play a vital role in innate immune responses. It triggers the downstream signaling pathways for production of cytokines, interferons and chemokines. These processes ultimately activate the immediate host defense responses as well as antigen-specific adaptive immune responses (Kawasaki and Kawai 2014).

In addition, the iMODS server was used to assess the structural stability and atomic level movements of the MEBP_TLR8 complex. TLR8 is predominantly expressed in the lung and recognizes ssRNA of the virus to initiate the innate immune response (Sallenave and Guillot 2020; de Groot and Bontrop 2020). Also it induces the sequential production of various pro-inflammatory cytokines like TNF-α, IL-6 and IL-12 against SARS-CoV infections (Li et al. 2013). Recognition of MEBP via TLR8 can ultimately augment the strong innate and adaptive immune responses against SARS-CoV-2. The analysis of B-factor and deformability plots revealed that the docked complex had insignificant deformability for each individual residue and thereby strengthening our prediction of eigenvalues for $9.140066e^{-05}$.

Further to ensure the higher expression of the designed vaccine construct in prokaryote host, codon optimization and in silico cloning were performed. The CAI (0.97) and GC content (53.73%) were promising and within the acceptable range for high level expression of protein *E. coli* K12 (Kar et al. 2020). Further, the immune simulation study of the designed vaccine construct revealed promising outcomes in terms of both humoral and cellular immune response. Repeated exposure of the construct led to an overall increase in the immune response and subsequent decrease in the antigenic load. Generation of long-lasting memory B- and T-cells indicates the suitability of our construct to be a high-potency vaccine candidate. Another interesting finding is that the level of IFN-γ and IL-2 was increased and maintained at peaks during the repeated antigen exposure. This indicates higher concentration of T_H Cells and effective production of antibodies, which support an effective humoral response. Nain et al. (2019) also reported similar kinds of immune response simulation patterns with a MEBP construct against *Elizabethkingia anopheles*. The above bioinformatics analysis and evaluation has shown that the MEBP vaccine construct has all the parameters within the optimal and recommended ranges for it to be considered as a potent vaccine candidate and needs to be experimentally validated to assess the actual efficacy in humans.

Conclusion

SARS-CoV-2 is a new virus which has become a serious Public Health Emergency of Global Concern. In the current study, an in silico methodology is described to design an MEBP vaccine candidate, wherein each predicted epitope may elicit strong cellular and humoral response simultaneously, against SARS-CoV-2 infection. This is achieved by predicting T-cell epitopes within the predicted B-cell epitopes. The peptide vaccine candidate was carefully analysed and evaluated for various features and properties using immunoinformatics approaches and other computational methods. The designed vaccine construct had suitable structural, physicochemical and immunological properties. The construct had all parameters within the recommended ranges of respective tasks. The interactions, the binding affinities and energies of MEBP-TLR complexes in general and TLR8-MEBP in particular are very encouraging. The results of immune dosage response simulation study were also very encouraging and realistic. The detailed analysis

of the S protein-based B-cell-derived T-cell MEBP vaccine construct is found successfully passing various stringent in silico criteria and thorough validation. This proposed recombinant vaccine construct is now ready and must be validated through in vitro and in vivo bioassays to prove its actual safety, efficacy, and immunogenicity against COVID-19.

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Author contributions RS initiated the study, performed the various tasks to generate the relevant data and wrote the MS. PG contributed to the modeling and simulation part on the vaccine construct. BVLS contributed to the overall design, discussion, execution of the protocols, critical evaluation and final MS preparation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical statements This article does not contain any studies with human participants or animals performed by any of the authors.

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