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Travel Medicine and Infectious Disease 50 (2022) 102481

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Designing, characterization, and immune stimulation of a novel multi-epitopic peptide-based potential vaccine candidate against monkeypox virus through screening its whole genome encoded proteins: An immunoinformatics approach

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ARTICLE INFO

Keywords:
Monkeypox virus (MPXV)
Multi-epitope
Peptide-based vaccine
Immunoinformatics
Molecular docking

ABSTRACT

Background: The current monkeypox virus (MPXV) spread in the non-epidemic regions raises global concern. Presently, the smallpox vaccine is used against monkeypox with several difficulties. Conversely, no next-generation vaccine is available against MPXV. Here, we proposed a novel multi-epitopic peptide-based in-silico potential vaccine candidate against the monkeypox virus.

Methods: The multi-epitopic potential vaccine construct was developed from antigen screening through whole genome-encoded 176 proteins of MPXV. Afterward, ten common B and T cell epitopes (9-mer) having the highest antigenicity and high population coverage were chosen, and a vaccine construct was developed using peptide linkers. The vaccine was characterized through bioinformatics to understand antigenicity, non-allergenicity, physicochemical properties, and binding affinity to immune receptors (TLR4/MD2-complex). Finally, the immune system simulation of the vaccine was performed through immunoinformatics and machine learning approaches.

Results: The highest antigenic epitopes were used to design the vaccine. The docked complex of the vaccine and TLR4/MD2 had shown significant free binding energy (−98.37 kcal/mol) with a definite binding affinity. Likewise, the eigenvalue (2.428517e-05) from NMA analysis of this docked complex reflects greater flexibility, adequate molecular motion, and reduced protein deformability, and it can provoke a robust immune response.

Conclusions: The designed vaccine has shown the required effectiveness against MPXV without any side effects, a significant milestone against the neglected disease.

1. Introduction

During the present post-COVID-19 pandemic state, the cases of monkeypox virus (MPXV) infection started appearing in multiple non-endemic countries beyond Africa in May 2022, as reported by the WHO. Afterward, the cases of monkeypox increased to a higher rate with considerable levels. With such a continuous rapid spread, the current monkeypox outbreak has become the largest in non-African countries. Though travel links from endemic to non-endemic countries have not been found to be associated with the current MPX outbreak, the virus infection might have spread initially through a traveler—a patient with a travel history from Nigeria to the UK and back [1]. The MPXV is a viral-zoonotic disease showing similar features as observed in the smallpox virus infection, even though it is physiologically less acute [2]. The MPXV belongs to the *Orthopoxvirus* genus under the family of Poxviridae. It is also noted that the Poxviruses have a strong propensity to emerge outer of their consistent ecological range by spreading to a naive community [3]. The MPXV obtained global attention during its first appearance in the Western Hemisphere in 2003, which caused a high number of cases in the Midwest of the USA [4].

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https://doi.org/10.1016/j.tmaid.2022.102481
Received 21 July 2022; Received in revised form 11 September 2022; Accepted 13 October 2022
Available online 17 October 2022

From January 1 to September 11, 2022, 57,527 confirmed cases and 18 deaths has been reported from 103 countries/territories [5]. The Central African Republic, the Democratic Republic of the Congo, Gabon, and Ghana are the countries where the monkeypox was endemic. Gradually the MPXV infection scenario is rising, and additional cases of MPXV infection are also detected as the disease outbreak progresses [6].

During the current outbreak in African countries, the genome sequence of the MPXV was first published in NCBI online database, and several sequences from other infected countries have also followed. Subsequently, the discovered MPXV strain was not so linked to the viral strain predominant in West Africa, which causes mild symptoms with a lesser death rate. MPXV genome contains a linear dsDNA (double-strand DNA), where the ITR (inverted terminal repeats) are explicitly composed of the hairpin loops like structure, tandem repeats sequence, and the ORFs (open reading frames) at end parts. The MPXV resides in the cytoplasmic part of the virally infected cells and encodes the proteins required for DNA replication, virion assembly, RNA expression, and viral escape [7].

Currently, no specific approved drugs or vaccines are available to treat the human MPXV infection; only the Dryvax (smallpox vaccine) has been used against MPXV and smallpox virus. During the vaccination, the skin is needed to be punctured many times by a bifurcated needle holding a small quantity of vaccine element. The technique is quite painful. On the other hand, stockpiling vaccines for longer days might also cause to reduce the vaccine efficiency [8]. However, various adverse effects were noted among the vaccinated individuals [9,10]. Therefore, there is an urgent need for a next-generation vaccine for monkeypox. In this direction, we have gone through the entire genome-encoded proteins (176 protein chains) of MPXV to develop a next-generation vaccine. We have screened all the common antigenic Bcell and T cell epitopes. The common epitopes with high antigenic scores were considered for designing a next-generation peptide vaccine against the MPXV.

The principal idea for all vaccination types is the ability of a vaccine to create an effective immune response faster than the infecting virus itself. However, classical vaccines are developed on several biochemical experiments and elicit antibodies in vaccinated individuals. These vaccines are also expensive, time-taking, and allergic, and the process requires *in vitro* culture of harmful microbes and faces severe safety concerns. Conversely, peptide-based epitopic vaccine production is extremely safe and less cost-effective than the traditional vaccine candidate [11,12]. Technical requisites for effective and safe vaccines are too crucial.

In this study, intensive research was performed to identify the common (B and T cell) 9-mer antigenic epitopes from the whole genome encoded proteins of MPXV. Afterward, the high-rank epitopes with the utmost antigenic score were selected from these epitopes for vaccine construct development. Furthermore, multiple *in silico* tools characterized and validated the potential vaccine candidate, and studies were performed to understand the molecular interaction with the human TLR4/MD2 complex. Finally, we have tested the immune simulation profiling of this novel peptide-based vaccine in the mammalian immune system using a machine learning approach. This novel vaccine candidate revealed protection against MPXV without any side effects and has the potential for usage in the coming days.

2. Methods

We followed the steps of muti-epitopic peptide-based vaccine development. However, every step of this implemented methodology is important and promising for designing an effective peptide-based multi-epitopic vaccine against MPXV.

2.1. The study of the whole genome, genome-encoded proteins, and retrieval of all of the genome encoded-protein sequences from the monkeypox genome

We studied the whole genome of the dsDNA of the MPXV and its all genome-encoded proteins. We found there are about 176 gene-coding proteins in the monkeypox virus. Simultaneously, all the genome-encoded proteins of the virus were studied, and the FASTA sequences of all its genome-coded protein sequences were retrieved from the NCBI. A total number of 176 gene-coding peptide sequences were collected for epitope screening and further analysis.

2.2. Identification of the 9-mer B cell epitopes

The IEDB (Bepipred 2.0) recommended 2020.09 methods were employed to predict linear B cell (9-mer) epitopes based on sequence characteristics of the antigen using amino acid scales and Hidden Markov models (HMMs) [13]. All gene-coded proteins from the genome were submitted in the mentioned online tool for identifying the 9-mer B cell epitopes.

2.3. Identification of the 9-mer T cell epitopes

The Immune Epitope Database (IEDB) v2.24 webserver was used to predict the 9-mer T cell epitope(s) in protein sequence(s) containing MHC class I epitopes. The server is based on sequence alignment using artificial neural networks to predict T cell epitopes [14]. We have selected all the suitable parameters for identifying 9-mer T cell epitopes and each peptide sequence derived from the MPXV genome are shown in Table 1.

2.4. Identification of the common 9-mer epitopes from B cell and T cell epitopes

From the outputs of Bepipred 2.0 and IEDBv2.24 server for the identified B cell and T cell epitopes, we further characterized common 9-mer epitopes, both antigenic and non-antigenic. Afterward, we selected ten common antigenic epitopes with the highest antigenic score (VexiJen score).

2.5. Determination of epitopes population coverage

We have identified the considerable number of HLA binding antigenic epitope peptides, which provide the population coverage information by its interaction patterns. The IEDB population coverage tool is used to estimate the population coverage of the potent epitopes of MPXV that can interact with the HLA alleles (MHC-I and MHC-II) [15].

2.6. Construction of multi-epitopic peptide-based potential vaccine candidate

The complete vaccine candidate was constructed by joining common B cell and T cell-derived antigenic epitopes. Peptide linkers such as GPGPG and AAY were taken to fuse these 9-mer antigenic epitopes. As noted, the peptide linker performed a critical role in refining the epitope separation and helped epitope presentation towards MHC class I and II receptors and immunological processing purposes [16]. Additionally, for immunity boosting, the adjuvant (CTxB) was added to the N-terminal part of the developed chimeric peptide sequences by incorporating the EAAAK linker into the MPXV vaccine construct. The CTxB is considered a nontoxic piece of 124 amino acids sequence of cholera toxin, which can develop the human body's humoral and cell-mediated immune responses [17]. Lastly, 13 amino acids long chain, an extra PADRE sequence (AKFVAAWTLKAAA) was added at the construct's C-terminal end to expand the host immunogenicity [18].

Table 1
Common B cell & T cell (9-mer) epitope of MPXV with the highest antigenic score (threshold for VaxiJen score = 0.4).

SL. No.	Gene name	Protein name	Antigenic score of gene	Common B cell & T cell epitope	Start (aa)	End (aa)	Antigenic score of epitope
1.	MV-6	ankyrin-like protein	0.3705	FDLSVKCEN	127	135	2.2910
2.	MV-12	IL-1 receptor antagonist	0.4645	RFNDMTITD	76	84	1.6117
3.	MV-13	complement binding	0.6228	LDIGGVDFG	95	103	2.2501
4.	MV-20	serine protease inhibitor-like protein	0.5702	NLRKRDLGP	74	82	2.6689
5.	MV-28	major membrane protein	0.5696	VSHINYTSW	63	71	1.6572
6.	MV-42	poly polymerase large subunit	0.4419	IRYGDIDIL	198	206	2.2883
7.	MV-147	hypothetical protein	1.0183	ITIDSKIGN	6	14	1.7127
8.	MV-150	toll/IL1-receptor [TIR]-like protein	0.4593	IRHRNTISG	99	107	1.6052
9.	MV-158	ankyrin-like protein	0.4212	ANIDSVDFN	238	246	1.5435
10.	MV-170	ankyrin-like protein	0.4222	ADISLKTDD	539	547	1.5014

2.7. Prediction of antigenicity and allergenicity of the potential vaccine candidate

We have predicted the antigenicity of the vaccine candidate through the VaxiJen webserver. This server followed a new alignment-independent method specifically for antigen prediction, based on Auto Cross Covariance (ACC) transformation of targeted peptide sequences [19]. The VaxiJen server is a consistent and reliable server for assuming the protective antigens using antigenicity of peptides with a threshold value (0.4) in default mode for viruses and other organisms.

At the same time, the AllerTop 2.0 server has been applied to understand the vaccine allergenicity. This web server-based ACC method transforms the amino acids sequence as equal-length vectors [20].

2.8. Primary and secondary structure analysis

Using the ExpasyProtParam tool, we have predicted and assessed the primary structure of the proposed potential vaccine candidate [21]. We have also evaluated the set of chemical and physical features of the input peptide sequence, numbers of amino acids, isoelectric point (pI), molecular weight, grand average of hydropathicity (GRAVY), net charges, aliphatic index, instability index, total atoms number and assessed half-life [21]. Subsequently, to analyze the secondary structure of the vaccine construct, we used the PSIPRED 4.0 and SOPMA (Secondary Structure Prediction Method) tools [22,23]. We also accessed the significant properties of our vaccine construct (e.g., globular regions, transmembrane helices, random coil, bend regions, and coiled-coil regions).

2.9. Three-dimensional (3D) modeling of the potential vaccine candidate and model refinement

The protein architecture in three-dimensional (3D) shapes offer important information for understanding molecular functions at the atomic level. The 3D structure of the developed vaccine was predicted by the I-TASSER web server [24]. This server is an integrated platform for the prediction of automated protein function and structure based on the sequence-to-structure-to-function paradigm. It makes a good quality model containing accurate coordinate formation compared to other known proteins. The GalaxyWEB server was employed to refine the predicted 3D model [25].

2.10. Validation of peptide-based potential vaccine structure

Accurate validation of the predicted protein structure 3D model is also important. We applied two significant web servers to validate the peptide-based vaccine structure of MPXV (PROCHECK and ProSA). The PROCHECK server examines the stereochemical quality of any protein structure and creates several PostScript plots which analyze its overall and residue-by-residue geometry by Ramachandran plot [26]. In contrast, the ProSA server was employed to confirm the protein tertiary structure. The overall quality scoring was calculated by the 'Z' plot and local quality model [27].

2.11. Molecular docking of MPXV vaccine construct with the human TLR4/MD2 complex

The interactions of antigenic peptide-based vaccines with the targeted immune cell proteins are critical for generating an appropriate immune response. The human TLR4/MD2 complex has a significant role in eliciting immune responses against microbial infection. For the calculation of protein-protein binding interactions of the MPXV vaccine constructs with the human TLR4/MD2 complex (PDB ID: 4G8A), we used the PyMoL tool [28] to eliminate the undesired elements from the coordinates of PDB.

For molecular docking, we employed the HawkDock server [29,30]. The Hawkdock server computes its binding interactions pattern along with the basis of calculating mechanisms of MM/GBSA free energy by the top-ranked model. Hence, these molecular species were selected as per the primary structure for commencing the molecular dynamic simulation.

2.12. Normal mode analysis of peptide-based vaccine

For normal mode analysis (NMA), the iMODS server has been used to demonstrate the cooperative motion of peptide-based vaccine through normal mode within the inner coordinates [31]. We have applied the essential dynamics simulation (EDM), an added program of the iMODS, to understand the macromolecular mobility and stability of the docked complex. Here, the evaluated parameters of the docked complex are the probable motions in the specialized terms of B-factors, deformability, covariance, elastic model, and eigenvalues.

2.13. Immune simulation profiling of our peptide-based vaccine using machine learning approaches

The C-ImmSim server has been used to portray the immune response profile of the designed vaccine construct [32], which defines cellular and humoral response within the mammalian immune system in the presence of antigenic or immunogenic components of bacteria, virus, etc., within the sub-cellular level (mesoscopic scale). The specific server evaluated the specific antigen-mediated immune response using the "position-specific scoring matrix" and the machine learning approaches. Other important parameters like the simulation volume, random seed, and simulation step were adopted at 30, 12345, and 1000 correspondingly. In addition, we have applied double injection intervals of four-week to execute the proposed Immune simulation. However, all other parameters retained in the default state [33].

The typical methodologies applied to accomplish our work are also listed in a flowchart (Fig. 1).

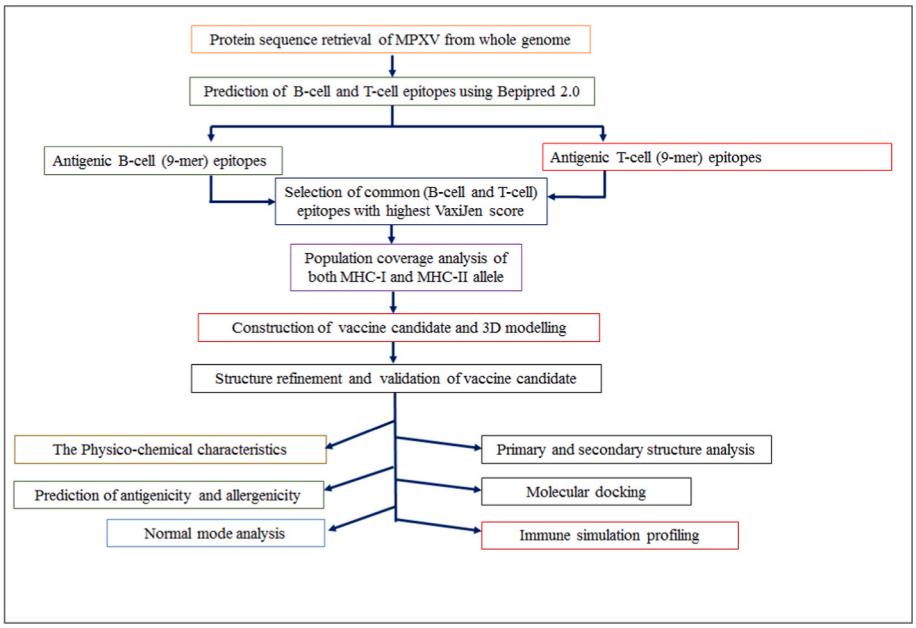

Fig. 1. Adopted methodologies (*in silico* techniques, proteome, immunoinformatics, and machine learning approaches) for designing next-generation multi-epitopic potent vaccine candidate screened from whole-genome encoded proteins of MPXV.

3. Results

3.1. Identification and prediction of the 9-mer common B cell and T cell epitopes from monkeypox whole genome encoded protein

In an antigen, the epitopic part is considered to be the key stimulator of the immune system. T cells or B cells, the shared peptide-based epitopic vaccine construct, may achieve a dual purpose of boosting the host immune system. Therefore, we designated 75 common 9-mer B cell and T cell epitopes from MPXV genome-encoded proteins. In contrast, the ten significant 9-mer common B cell and T cell epitopes having maximum antigenicity with highest VaxiJen score were selected for the final vaccine development (Table 1).

3.2. Population coverage analysis

We have interpreted the population coverage based on the population coverage (epitope-based) analysis of the parallel alleles of MHC-I and MHC-II. This web server clearly defined the population coverage for MHC-I and MHC-II epitopes.

The population coverage in respect to MPXV whole genome encoded protein-derived filtrated epitopes for both the MHC-I and MHC-II alleles is shown in Fig. 2.

3.3. Prediction of antigenicity and allergenicity of MPXV vaccine candidate

The exploration of vaccine construct antigenicity by the VaxiJen v2.0 tool exhibited that it is a potent antigen having an antigenicity score of 1.1013 (Table 2). This score also proposes that the designed peptide construct of multi-epitopes, along with the PADRE sequence and adjuvant, might enhance the immune response in the host body. The diagram shows the constructed vaccine in Fig. 3A.

The AllerTOP 2.0 webserver was applied for the allergenicity calculation of the potential vaccine candidate for safe future use.

This server calculated the significant physicochemical properties of the MPXV vaccine candidate (Table 2), which was likely to be basic. The developed vaccine's theoretical Isoelectric point (pI) was 6.33, with molecular weight (MW) of 18108.33 Da. The Grand average of hydropathicity (GRAVY) was calculated at 0.069, the positive GRAVY score specifying the potential vaccine candidate protein might be hydrophobic. The hydrophobic nature of the peptide chain should help in the smooth purification and better design of the vaccine.

The Aliphatic index (thermo-stability) of the designed MPXV vaccine was found to be 88.08. Additionally, the half-life of the potential vaccine candidate was calculated as 1 h in mammalian reticulocytes (*in vitro*), 30 min in yeast (*in vivo*), and >10 h in *E. coli.* At the same time, the instability index was calculated as 18.96. It specifies the stable MPXV vaccine construct of protein.

3.4. Primary and secondary structure analysis of MPXV vaccine construct

The SOPMA study projected the secondary structural architecture values such as the alpha helix (47.90%), extended strand (11.98%), beta-turn (0.60%), and random coil (39.52%) in the case of our vaccine (Fig. 4A and B). This server considered the window width value as 17. The similarity threshold value was fixed as 8 and measured the four numbers of states. Additionally, the feature of the vaccine secondary structure was also predicted through the PSIPRED server. This result indicates a higher ranking quality of the peptide vaccine secondary structure (Fig. 5).

Furthermore, the Protein-Sol server was used to calculate the solubility of our MPXV vaccine construct and was found as soluble (0.549). The scaled solubility value (QuerySol) is shown in Fig. 4C.

3.5. Validation of peptide-based vaccine structure

The validation of protein structure was completed by ProSA and PROCHECK web tools. These assess the overall quality of MPXV vaccine protein structure. The refined vaccine model from the Ramachandran plot showed that the residues exist in four specific states as favored (42.7%), additionally allowed (33.1%), generously allowed (19.1%), and disallowed (5.1%) (Fig. 6A Table 3). The 'Z-score' of the MPXV vaccine advanced model was calculated as −2.78 (Fig. 6B). At the same time, the negative score specifies that it is a superior 3D protein model. Subsequently, we generated an additional local quality model using the ProSA server. It is portrayed by a substantial plot, whereas it showed the overall quality of the peptide model as acceptable (Fig. 6C). The 3D

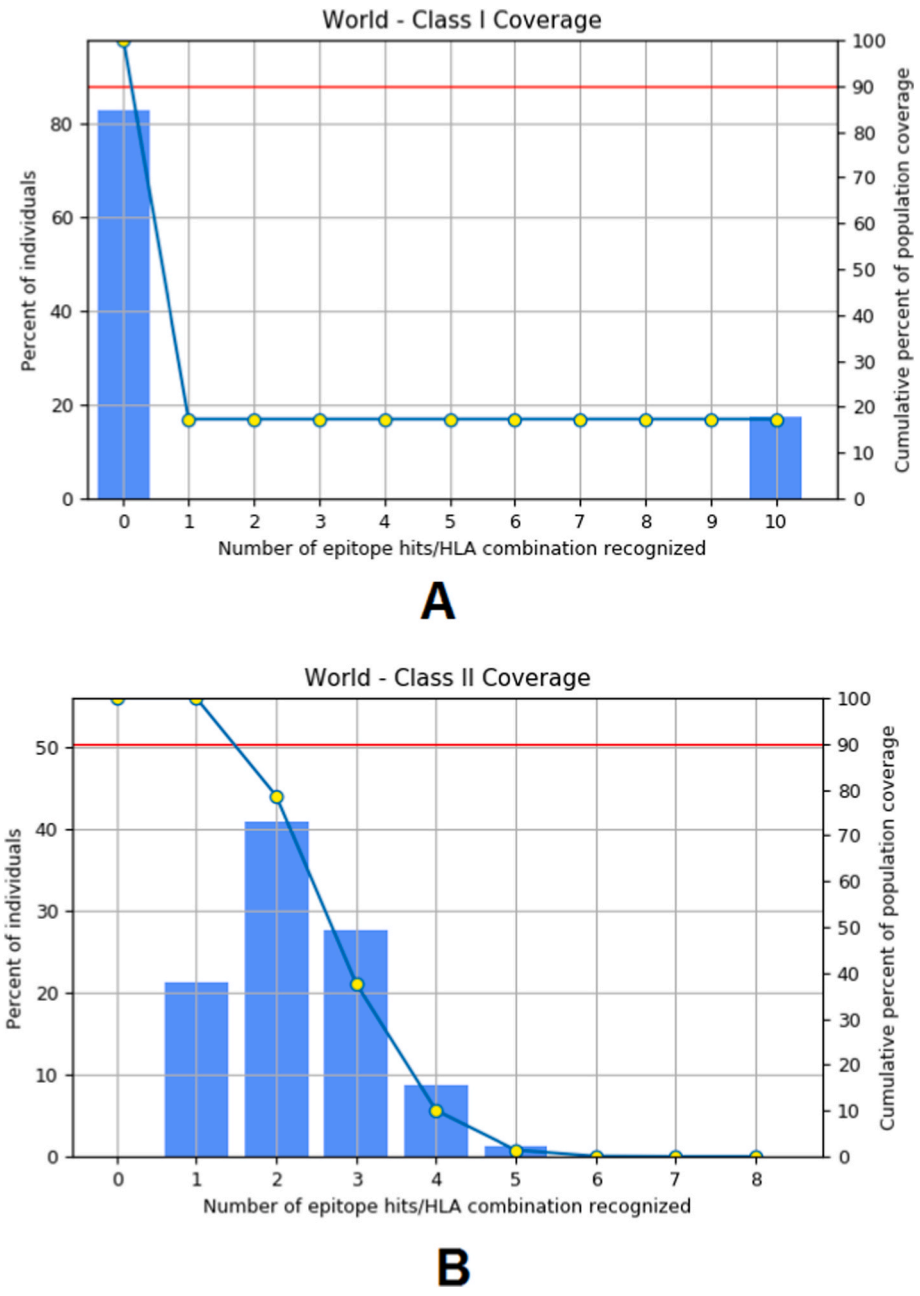

Fig. 2. Population coverage by selected CTL epitopes and their respective MHC binding alleles of our vaccine construct. We consider two alleles for our work (MHC-I and MHC-II) (A) It shows the worldwide population coverage of the MHC-I allele, (B) It shows the worldwide population coverage of the MHC-II allele.

structure of the MPXV vaccine protein is shown in Fig. 3C (ribbon model) and 3D (surface model).

3.6. Molecular docking of vaccine construct with the human TLR4/MD2 complex

Molecular docking was executed by the HDOCK server with vaccine construct and human TLR4/MD2 complex (PDB code: 4G8A). This TLR4/MD2 complex can activate proinflammatory cascades within the human body. Within the protein-protein docked complex among the top 10 models, the top-rank docking cluster with the highest binding score (−309.78) was selected to visualize binding interactions. Furthermore, the HawkDock web tool was employed to provide a molecular docking complex by machine learning approach, followed by the MM/GBSA. We also found the binding free energy of the docked complex was −98.37 kcal/mol. This score supports a superior binding affinity between the MPXV vaccine model as a ligand molecule and TLR4/MD2 as the receptor (Fig. 7A).

3.7. Normal mode analysis of peptide-based MPXV vaccine and TLR4/MD2 complex

Outputs of the normal mode analysis of the MPXV vaccine construct and the TLR4/MD2 are shown in Fig. 7B. The peak points of the deformability graph represent the parallel regions having deformability in the docked protein complex (Fig. 7C). The B-factor plot in the docking complex reflects an accurate visualization of the assessment among the PDB model and NMA (Fig. 7D). Fig. 7E shows the eigenvalue ($2.428517e\text{-}^{05}$) of the designed vaccine and TLR4/MD2 complex. Furthermore, the covariance map of the complex offers the interacting motion between the two molecules. In the present study, the correlated motion among the pair of amino acid residues is specified by red color,

Table 2
Antigenicity, allergenicity, solubility, and other physicochemical property evaluations of the primary protein sequence of the multi-epitopic potent vaccine candidate.

Sl. No.	Features	Assessment	Remark
1.	Antigenicity	1.1013 (antigenic score)	Probable antigen
2.	Allergenicity	Confirmed by AllerTOP v.2.0 server	Probable non-allergen
3.	Solubility	0.549	Soluble
4.	Number of amino acids	167	Suitable
5.	Molecular weight	18108.33 Da	Average
6.	Theoretical Isoelectric point (pI)	6.33	Slightly acidic
7.	Total number of atoms	2524	Suitable
8.	Formula	$C_{825}H_{1240}N_{214}O_{243}S_2$	–
9.	Estimated half-life	1 h (mammalian reticulocytes, *in vitro*) 30 min (yeast, *in vivo*) > 10 h (*Escherichia coli*, *in vivo*)	–
10.	Instability index	18.96	Stable
11.	Aliphatic index	88.08	Thermostable
12.	Grand average of hydropathicity (GRAVY)	0.069	Hydrophilic

and uncorrelated motion is marked as white color. In contrast, the anti-correlated motion is denoted as blue (Fig. 7F). The specialized elastic map of the vaccine and TLR4/MD2 complex signifies the assembly between the atoms of the larger molecule, and the stiffer regions are indicated by the darker grey color (Fig. 7G).

3.8. Immune simulation profiling of peptide-based potential vaccine

The immune stimulation by *in silico* technique of the potential vaccine candidate was carried out through primary and secondary immune responses by activating the immune system, containing HTL, CTL, viable memory cells, and other associated immune cells (DC cells, NK cells, etc.). Levels of the immunoglobulins (IgM + IgG) were observed to be drastically increasing after administering the vaccine construct. Subsequently, extended immune responses were reported against the MPXV through the high titers value of IgM and IgG (Fig. 4A). In the acting and resting phase, the innate immunity within the host body and B cell population was augmented with IgM, B isotype, and B-memory cells, which might be scaled up at 650–750 cells/mm^3 (Fig. 8A–C). Likewise, CTL cells were elevated and reached a maximum of 1117 cells/next ~8 days of immunization by vaccine and progressively reduced after 24 days (Fig. 8D).

Moreover, during the resting and active phase, the T cells exhibited abundant diversity within the T cell population (Fig. 8E). It was also noted that the raised HTL cells (in resting and active phases) performed a crucial role in creating adaptive immunity against the infections of MPXV. The raised cells produce the highest memory cells (Fig. 8F–G).

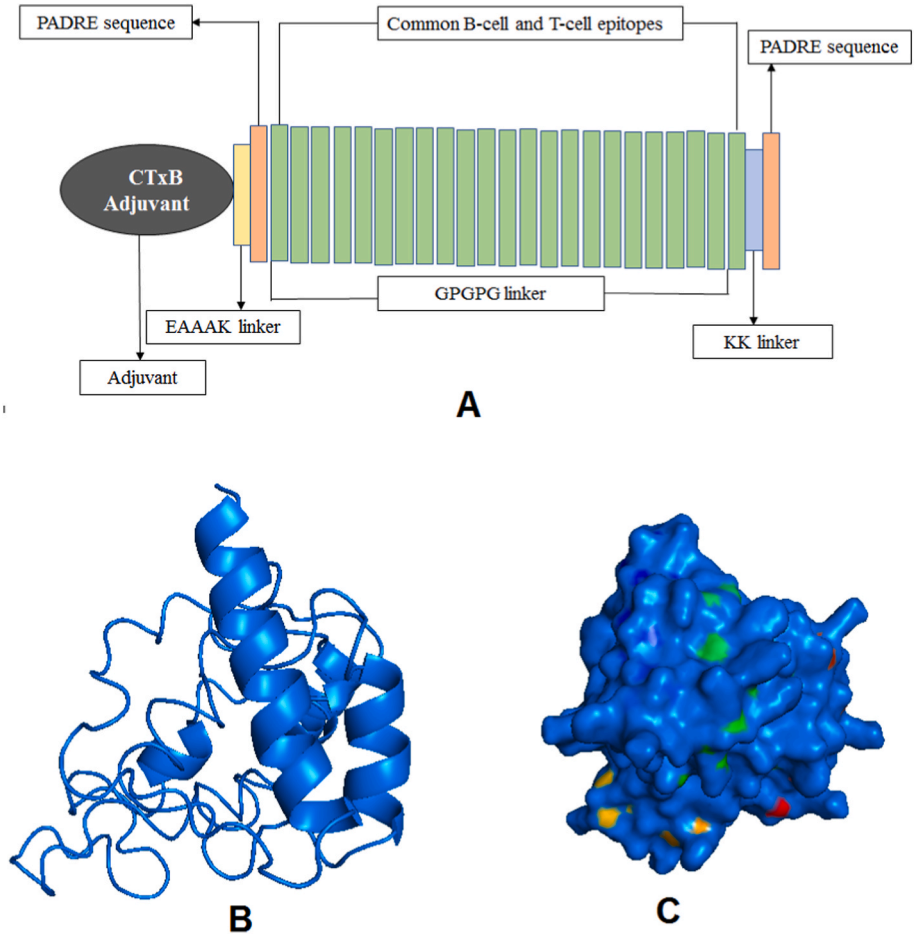

Fig. 3. Vaccine construct and its refined 3D structure from the MPXV full genome encoding proteins. (A) The graphical diagram depicts the final vaccine construct from antigenic epitopes of MPXV full genome encoding proteins using different linkers such as GPGPG and AAY (B) Ribbon model, (C) The model shows the Surface structure of the vaccine construct.

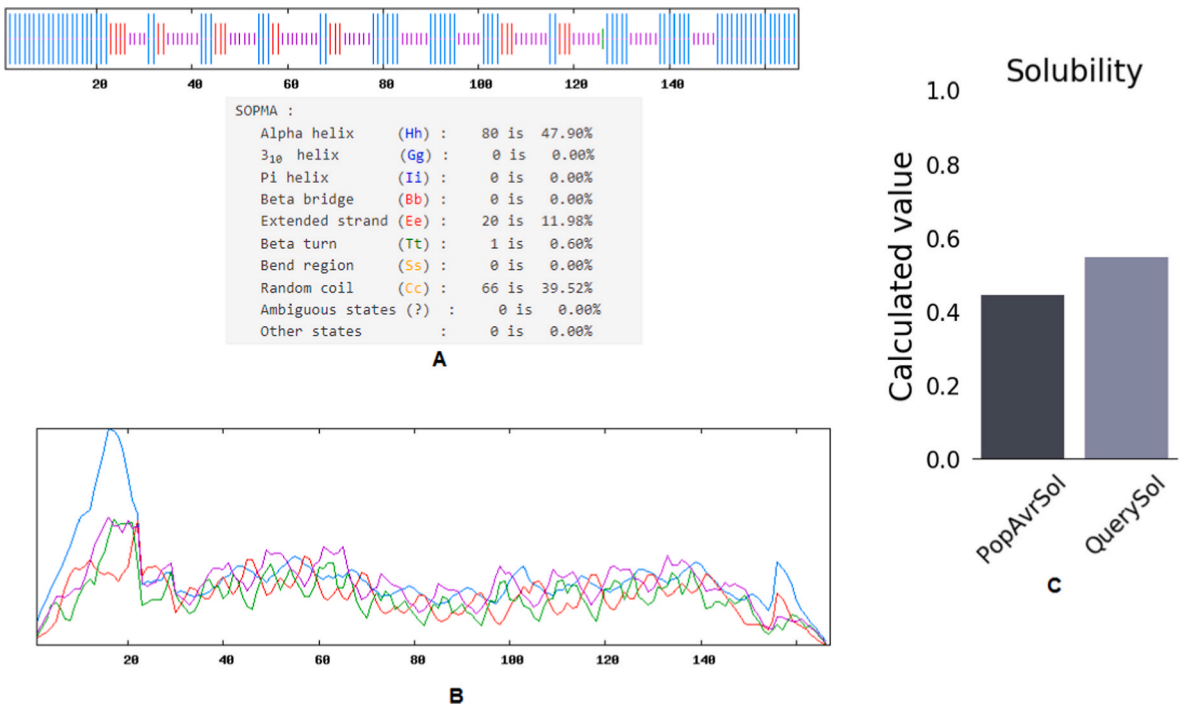

Fig. 4. Secondary structure prediction plot of the MPXV vaccine construct. Here the alpha helices were shown in blue color, while extended strands and beta turns were shown in red and green colors, correspondingly. (A) It shows the visualization of the result of the study and (B) The score curves for each predicted state of study (C) Protein-Sol predicted water solubility test of the potent vaccine candidate. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

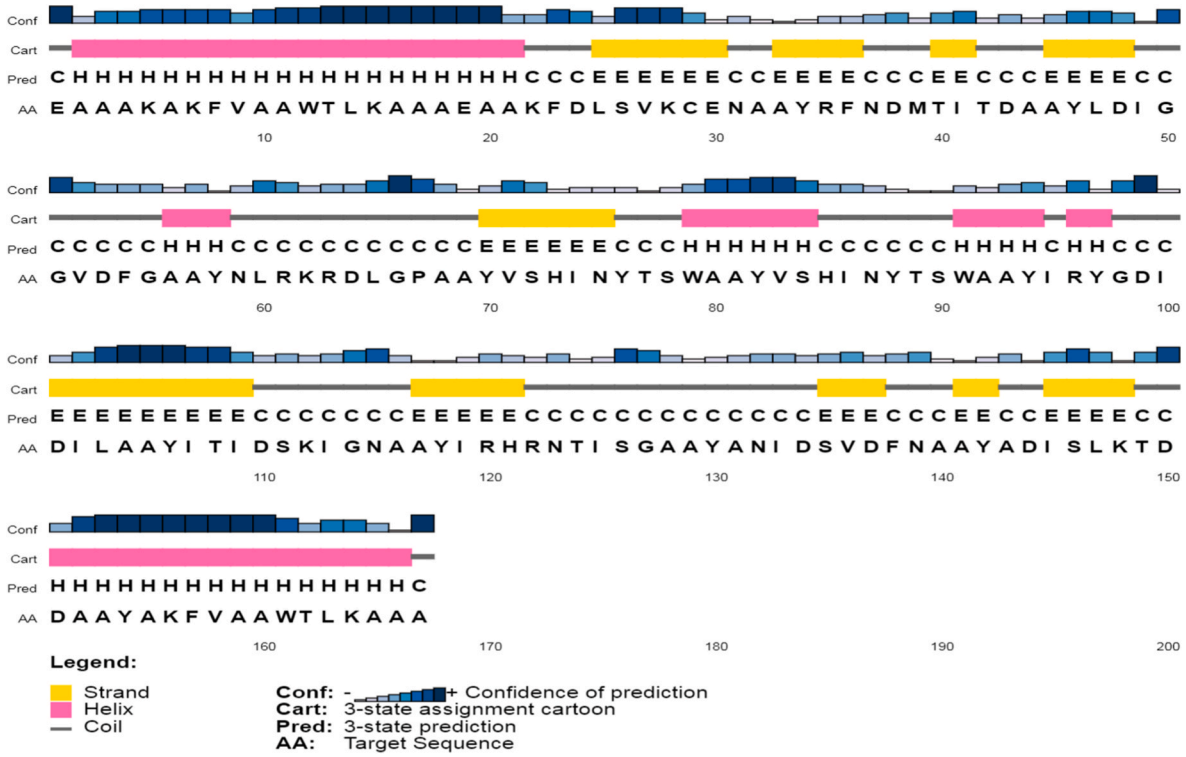

Fig. 5. The secondary structure analysis of the result of the multi-epitopic vaccine by the PESIPRED webserver.

Memory cells play a curial role in preventing and regulating viral infection and reinfection over the self-memorization by encountering pathogens. The successful administration of the vaccine candidate effectively elevated added regulatory elements of the immune system (e.g., interleukins, cytokines, and NK cells) (Fig. 8F-L). These consequences indicate that the designed MPXV vaccine could be considered a potent peptide-based next-generation vaccine for provoking a robust immune response to counteract MPXV infection.

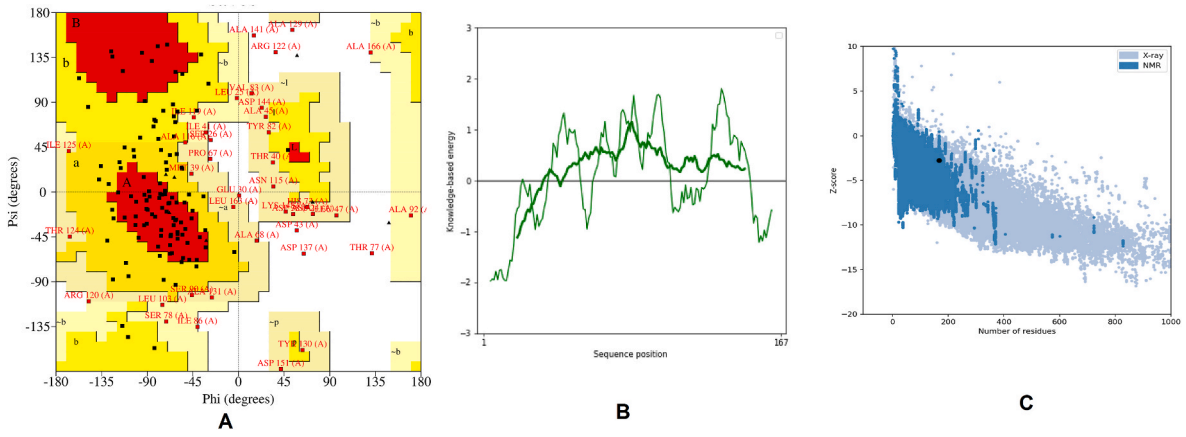

Fig. 6. Structural validation of MPXV vaccine candidate (A) The structure was validated through a Ramachandran plot for all residues of vaccine construct, and it shows the result of the validation, (B) It shows the energy plot of amino acid of vaccine component, (C) The 'Z' score of vaccine construct (black dot) within the 'Z' score range of experimentally proved structure.

Table 3
Distribution of amino acid residues showing in Ramachandran plot of the multiepitopic peptide-based potent vaccine candidate.

Amino acid position	Residue number	Percentage (%)	Total
Favored region	67	42.7%	157
Additional allowed region	52	33.1%	
Generously allowed region	30	19.1%	
Disallowed region	8	5.1%	
End-residues			2
Glycine residues			7
Proline residues			1
Total residues			167

4. Discussion

Due to the sudden surge of MPXV infection cases within a short time, global public health is under significant threat. Till today no specific vaccine is available that could resist the infection of MPXV, even though the vaccines for smallpox virus are also used against MPXV. But the administration of these vaccines are quite painful, and the vaccine's efficacy is also reduced due to the longer stockpile. Therefore, the present work was strategically planned for designing a peptide-based multiepitope subunit vaccine candidate from the whole genome encoded protein by computational tools and techniques that can induce an immunological response against the MPXV. We have planned to screen out all the potent antigenic epitopes (common B cell and T cell) from the whole genome encoded protein sequences of the MPXV. Subsequently, ten common epitopes with highest antigenic score with suitable peptide linkers and adjuvants were employed for developing the final vaccine construct. This proposed novel vaccine should operate on TLR4/MD2 complex (cell surface protein) as a target and be safe with the required functions. This peptide-based vaccine should be developed by synthesizing antigenic B cell and T cell epitopic sequences in a chemical process that stimulates specific antibody production [34,35].

The distribution and expression profile of HLA (MHC-I and MHC-II) alleles may differ worldwide between ethnicities and regions. Subsequently, for developing an effective vaccine, it is essential to conduct the baseline assessment of HLA allelic distribution within the whole world population [36,37]. Our study has shown the worldwide population coverage of the MHC-I and MHC-II alleles in respect to ten highest antigenic epitopes from the whole genome encoded protein of MPXV. The cumulative percentage (%) coverage for MHC I allele is 19.8% of ten epitopes for the entire world population. Whereas two epitopes have 70% coverage, three epitopes have 28% coverage, one epitope has 21% coverage, four epitopes have 9%, and five epitopes have 2% coverage, respectively (Fig. 2).

The B cell-derived T cell epitopes (9-mer) and common antigenic epitopes were characterized for the formulation of a multi-epitope peptide-based vaccine. The antigenic epitopes can provide a robust immunological response against MPXV in the host body. Our vaccine construct contains ten epitopes (9-mer) with 147 amino acids, PADRE sequence, CTxB adjuvant, and required peptide linker. Within the N-terminal end of the vaccine, the adjuvant is joined with the EAAAK peptide linker. It was merged for the dynamic separation of the bifunctional fusion protein [38]. In addition, the EAAAK linker was also joined by the PADRE sequence, which acts as a helper T cell epitope for uprising the CTL response in diverse antigens.

The outcomes of different bio-computational tools and servers also established that the final vaccine construct was slightly acidic and stable in the human physiological pH range. Furthermore, the assessed aliphatic index score specified that the construct is also thermostable [39]. Consequently, the positive value of GRAVY indicated its hydrophobic nature, with no such viable interaction with the water or non-soluble in water [40].

In the present study, the I-TASSER server was used for the development of the 3D structure of this MPXV whole genome encoded protein-derived peptide vaccine candidate. The 3D structure was also critically validated by ProSA and PROCHECK web servers, where the local energy plot and 'Z' score were accessed to understand the proper folding of the protein. The ProSA server predicted a 'Z' score negative value (−2.78), signifying an upright signal for the structural quality assessment [41]. Along with the analysis of the local quality model, this model has been considered as a reliable model.

The subsequent analysis also showed that the peptide vaccine constructs strongly interact with TLR4. It was also noted that the TLRs played an important role in recognizing pathogens and stimulating the proper innate immune response against infectious agents [11]. In the current study, we have executed the molecular docking between the TLR4/MD2 complex and the peptide vaccine construct using the HwkDock and HDOCK web servers. The negative docking score demonstrated that the vaccine construct contains high binding interaction characteristics. It supports that the potential vaccine candidate can trigger the TLR4 activation and boosts the immune response against the MPXV. The TLR4/MD2 and vaccine candidate docked complex bear the free binding energy of −98.37 kcal/mol. This value specifies a reliable binding affinity between the immune sensing (TLR4) cell receptor protein and our potential vaccine candidate.

The normal mode analysis (NMA) study was carried out to analyze the comparative deformability, molecular motion, and the B factor from the protein PDB. Our analysis has shown the eigenvalue of the studied

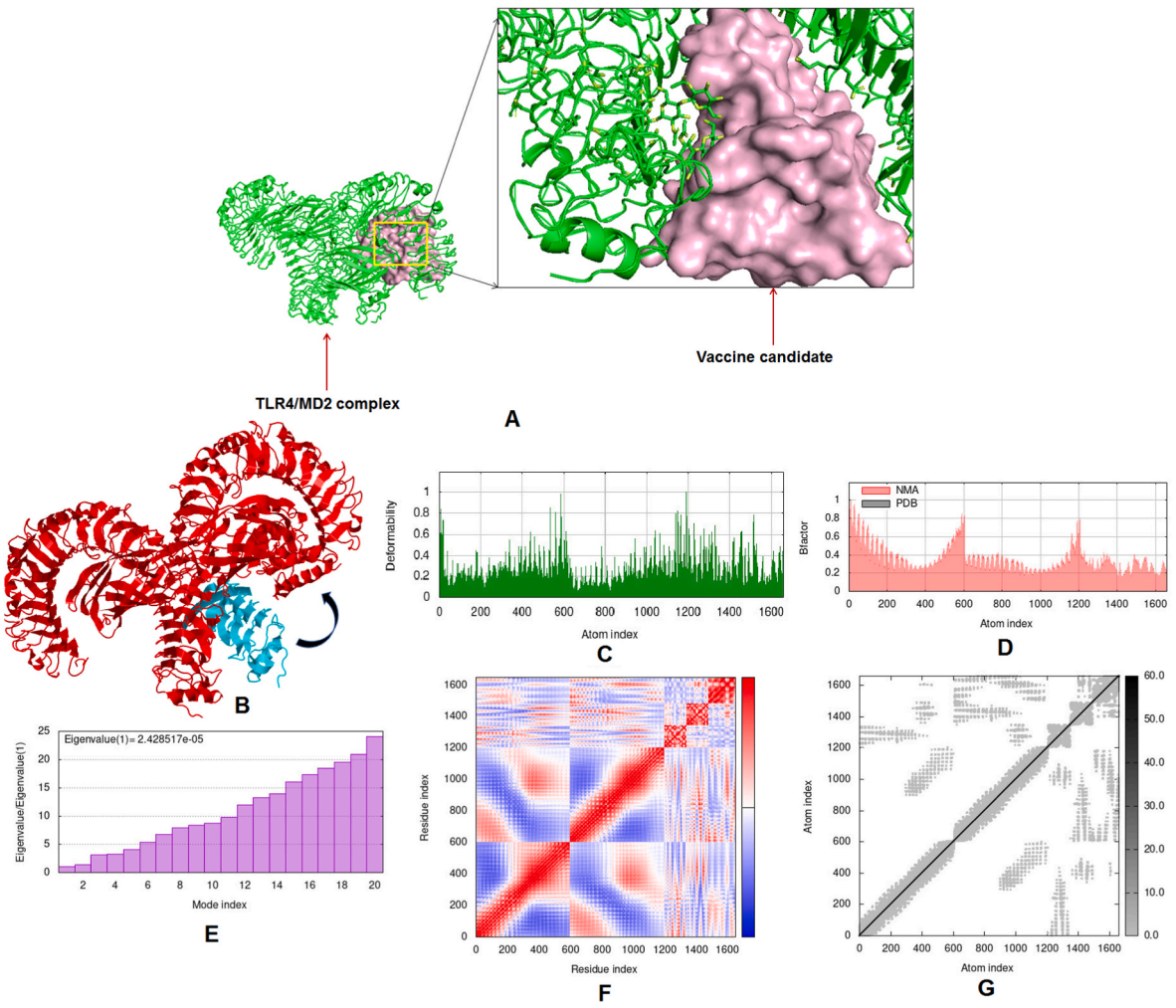

Fig. 7. The structure shows the docked complex and the outcome of our vaccine construct's NMA (Normal Mode Analysis). our vaccine construct was docked with TLR4/MD2 (A) docked complex (vaccine construct docked with TLR4/MD2) illustrates the ribbon model showing TLR4/MD2, and surface model shown vaccine construct, (B) Mobility of the docking complex of our vaccine and TLR4/MD2 indicated with arrows, (C) Deformability plot of the docking complex, (D) Calculated B-factor of NMA and PDB B-factor of our vaccine construct, (E) The eigenvalue for the docking complex, (F) Covariance matrix map of atomic pair of amino acid residues of docked complex, (G) Connection spring map of the elastic network model of the docked complex.

peptide sequence as 2.428517e-^{05}. This calculated eigenvalue designated the superior flexibility of the final construct of the vaccine.

The immune simulation profiling of the vaccine construct confirmed that the vaccine candidate could trigger both types (humoral and cellular) of immune responses against MPXV infection. Moreover, the secondary immune response produced by the designed vaccine was expressively higher than the primary immune response.

Finally, our vaccine construct showed a superior result from its structural and functional properties as evaluated during *in silico* studies. The structural validation shows a 'Z' score in negative value (−2.78), indicating a reliable protein model, docked complex of TLR4/MD2, and a vaccine candidate with a free binding energy of −98.37 kcal/mol specifies a reliable binding affinity for immune sensing by molecular interactions. Furthermore, in NMA analysis, the eigenvalue of the studied docked peptide sequence (2.428517e-^{05}) offered greater flexibility, good molecular motion, and lesser protein deformability. The immune simulation profiling of vaccine candidates also supports the production of abundant diversity in the T cell population and the rise of HTL cells in the mammalian immune system. Therefore, all the observed properties indicate that our vaccine construct is an ideal next-generation vaccine against MPXV.

5. Conclusion

The present work aimed to design a novel, peptide-based multi-epitopic next-generation potential vaccine candidate against the MPXV by employing various bioinformatics approaches. The vaccine was developed from the whole-genome encoded proteins, and it was found that the MPXV epitopic vaccine construct showed superior characterized properties in terms of antigenicity, non-allergenicity, and physico-chemical properties. Therefore, it might be concluded that our designed vaccine construct is not only ideal but also effective and safe to be used against MPXV.

This designed vaccine candidate might serve as a significant milestone and aid in developing an antiviral vaccine against MPXV. It might be administered to the host body (human) of MPXV next to the further outcomes of the fruitful results from the pre-clinical and clinical trials. In addition, the structural validation of our vaccine candidate and the binding affinity to immune receptor were performed, followed by the protein-protein molecular docking and normal mode analysis. Even though immune system simulation through the immunoinformatics approaches confirmed that the vaccine construct could elicit a good immune response, this vaccine candidate can deliver an extensive range of protection against MPXV. However, successive validation (*in vivo* and *in vitro*) is required to assure the vaccine candidate's effectiveness before

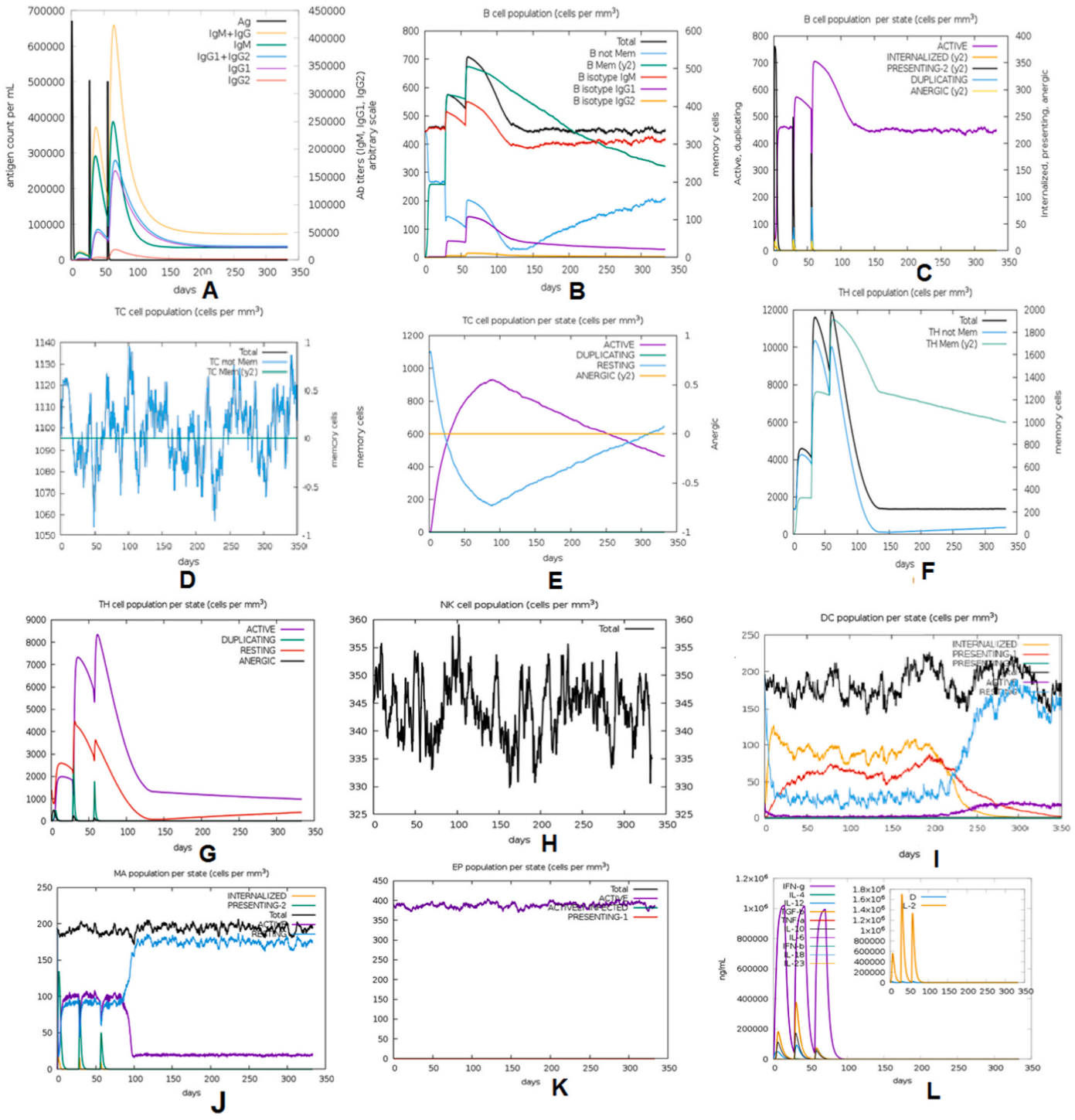

Fig. 8. The immune response of humans after injection of our MPXV vaccine construct. The study noted different results from the *In silico* simulation and machine learning approaches. (A) The simulation shows the contract can elevate immunoglobulins. The noted elevation of immunoglobulins at different concentrations of antigen, (B) The study indicates the population of B lymphocytes (IgM, IgG1, and IgG2) after three injections of our vaccine construct (C) The figure depicts the analysis outcome of the population per entity-state (i.e., showing counts for active, presenting on class-II, internalized the Ag, duplicating and antigenic by the different color variant, (D) The figure informs the cytotoxic T lymphocyte population in the time (days) after injection of our MPXV vaccine construct, (E) The figure illustrates the Cytotoxic T lymphocytes population in different states; resting and active, in the time (days) after injection of our MPXV vaccine construct (F) It shows the total count of TH cell population along with memory cells and sub-divided in isotypes IgM, IgG1 and IgG2 after injection of our MPXV vaccine, (G) It shows the population per entity-state of Helper T cell count in the resting and active states after injection of our vaccine, (H) The behaviour of the Population of Natural Killer cells. (I) The behaviour of the population of Dendritic cells in the active and resting states after injection of our vaccine, (J) Its shows the population of macrophages after MPXV vaccination, (K) Concentration of cytokines and interleukins with Simpson index [D], (L) Total count of EC cells that is broken down to active, virus-infected and presenting on class-I MHC molecule. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

its administration in the human body.

Ethical approval and consent to participate

Not applicable.

Source of funding

No funding received.

Data availability statement

All data included within the manuscript.

CRediT authorship contribution statement

Manojit Bhattacharya: Writing – original draft, Methodology, Investigation. **Srijan Chatterjee:** Data curation, validation, Formal analysis. **Sagnik Nag:** Data curation, validation, Formal analysis. **Kuldeep Dhama:** Writing – review & editing, Visualization. **Chiranjib Chakraborty:** Conceptualization, Writing – review & editing, Supervision, Project administration, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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