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Original Article

Designing a new Vaccine Based on Multiple Epitopes Against Monkeypox Virus with the help of new Methods Based on Immunoinformatics Software

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Abstract

Monkeypox virus is a zoonotic virus belonging to the family of Poxviridae and the genus Orthopoxvirus, which is the cause of a viral disease between humans and animals, the characteristics of which are comparable to other cases of smallpox with some differences. Recently some patients caused by the monkeypox virus (MPXV) were reported to the WHO. In recent years, the widespread and rapid spread of some epidemics highlighted the need for the rapid development of effective vaccines in scientific communities**.** Although conventional therapies have played an essential role in the treatment of many diseases, emerging diseases require new methods of treatment with fewer complications. It is therefore important to develop an effective vaccine for infections caused by the Monkeypox virus to prevent mortality and the safety of the community. In this research, we have used bioinformatics to design a vaccine against the F13 envelope protein Monkeypox virus. A total epitope confined to B cells and MHC I and II alleles were structurally constructed in F13 envelope protein to stimulate immunity and antibody recognition which was used to construct a chimeric peptide vaccine. The vaccine was predicted as a stable, antigenic, and non-allergenic combination. analysis of the TRL4/vaccine docking complex and simulation indicate a sufficiently stable binding with receptor activation. The immune response simulation following hypothetical immunization indicates the potential for stimulation and production of active and memory B cells, as well as the potential for cell production of CD8 + T, CD4 + T, and the development of effective immunological responses induced by Th2 and Th1. Analysis of the in silico processes have shown that the structure of the vaccine produces high antigenicity and good cellular immunity in the host body and stimulates various immune receptors such as TLR4, MHC I and MHC II. Vaccine function was also associated with increased IgM and IgG and a set of Th1 and Th2 cytokines. But final confirmation of the effectiveness of the designed vaccine requires clinical processes.

Keywords: Vaccines, Monkeypox virus, Immunology, Bioinformatics, Envelope protein, Docking.

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1. Introduction

In recent years, the widespread and rapid spread of some epidemics such as COVID-19 highlighted the need for the rapid development of effective vaccines in scientific communities [1]. Today, the formation of new diseases caused by various factors such as mutations in

various organisms is spreading. One of these diseases is the monkeypox virus (MPV), which can become a major public health crisis around the world. Recently in May 2022, a patient's case caused by the monkeypox virus (MPXV) was reported to the WHO [2,3]. Monkeypox virus is a zoonotic virus belonging to the family of Poxviridae and the genus Orthopoxvirus, which is the cause of a common viral disease between humans and animals, the characteristics of which are comparable to other cases of smallpox with some differences [4, 5]. Smallpox vaccines immunize a person against MPV, but vaccine research must continue due to the weaknesses of older vaccines as well as the development of new methods to design more effective vaccines. On the other hand, although smallpox was eradicated in 1980, this caused many young people to never vaccinated against smallpox, so they lack adequate immunological protection against this disease [6]. Based on these statistics, about half of the world's population is not immune to Orthopoxvirus [7]. Therefore, the recent increase in this disease can be largely attributed to the cessation of vaccination since 1980. This family of poxviruses shows a great tendency to spread and spread outside its common ecological range to communities that have no previous history of dealing with this disease [8]. Monkeypox is clinically almost identical to common pox. Its clinical manifestations include a series of flu-like complications, fever, weakness, back pain, headache, and distinct complications [9]. At the molecular level, the monkeypox virus genome consists of a double-stranded linear DNA [10]. Although MPXV is a DNA virus, it spends its entire life cycle in the cytoplasm of infected cells. The MPXV genome encodes all the proteins necessary for viral DNA replication, transcription, virion assembly and egress [11]. As of 2019, there is no approved vaccine or drug to treat the monkeypox virus. The available smallpox vaccine has been used to treat other types of smallpox as well as monkeypox [12]. However,

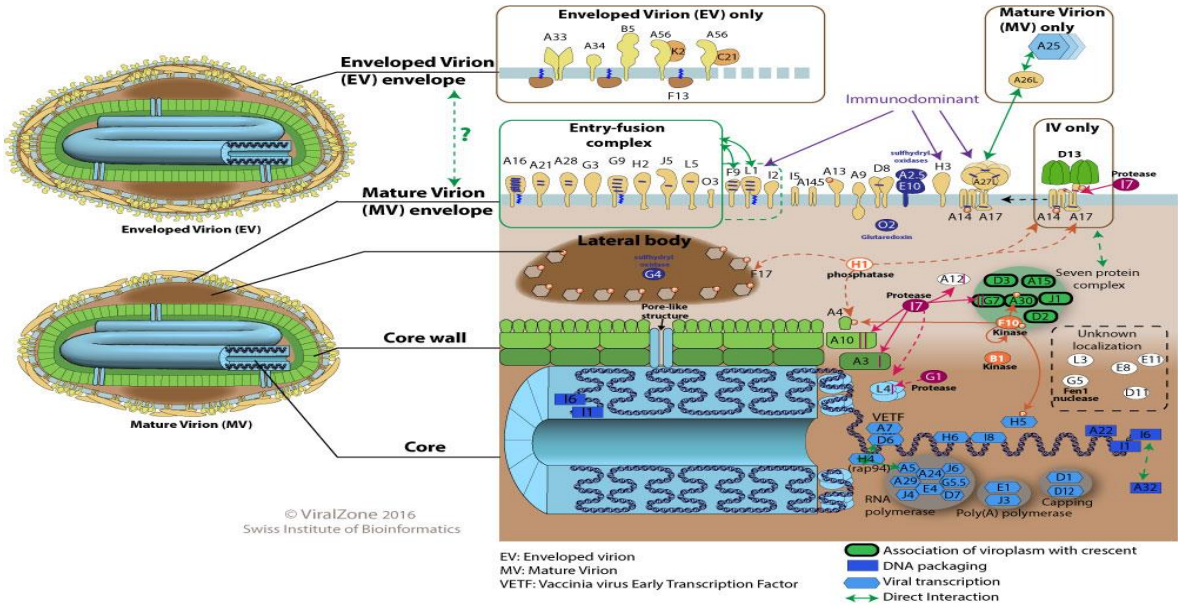

Figure 1. The structure of monkey pox virus and observing its different structural parts in order to choose the suitable structural combination to predict suitable epitopes for making vaccines.

many adverse effects affected both the vaccinated and those in contact with the vaccinated [13,14]. The main premise behind all vaccinations is the ability of the vaccine to induce an immune response faster than the virus itself. Although classical vaccines based on biochemical tests produce strong neutralizing and protective antibodies in vaccinated animals, they are expensive, sensitizing and time-consuming, and involve the cultivation of dangerous viruses in laboratory conditions and cause serious safety concerns [15, 16]. Peptide-based vaccine production is extremely safe and cost-effective, especially compared to traditional vaccinations. The need for safe and effective vaccines is critical. Therefore, in this study, we aimed to design a peptide-based vaccine based on some surface proteins of MPXV due to the availability and high recognition of these types of proteins by the host's immune system, using tools of immunoinformatics. (Figure 1).

2. Materials and Methods

2.1. Preparation of the desired protein sequence from NCBI

The F13 envelope protein sequence was retrieved from the NCBI database and checked for homology with other human genes through its accession number.

2.2. Prediction of linear B cell epitopes

F13 protein sequence was prepared in FASTA format to predict B cell epitopes and its immunogenic peptide epitopes, including continuous and interrupted epitopes, which were determined through IEDB (Immune Epitope Database) server [17].

2.3. Predicting MHC-I and MHC-II epitopes

Epitopes of MHC-I and MHC-II (Major Histocompatibility Complex class I and class II) receptors were retrieved, selected from IEDB servers (https://www.iedb.org/) and considered as final epitopes.

2.4. Design of vaccine safety structure

Selected epitopes recognizable by MHC-I and MHC-II and B cells with the help of some linkers were used to design the structure of multi-epitope vaccine. To increase the antigenicity of vaccines, adjuvants (50S ribosomal protein L7/L12) were added to the end of the vaccine sequence. Then the vaccine was made by binding adjuvants, MHC-I and MHC-II epitopes and B cell epitopes using linker compounds, namely EAAAK, GPGPG, AAY and KK [18-20].

2.5. Predicting the second structure

In this stage, the second structure of the vaccine was designed and the presence of alpha helical and beta sheets and accident coils were investigated and analyzed through PRABI (http://www.prabi.fr/) and Psipred servers (http://globin.bio.warwick.ac.uk/psipred/).

2.6. Investigation of allergy and antigenicity and toxicity of the vaccine

VaxiJen v2.0 server (http://www.ddg-pharmfac.net/vaxijen/) used to evaluate the vaccine antigenicity for validation. Vaccine allergenicity was also assessed through AllerTOP v.2.0 servers (https://www.ddg-

pharmfac.net/AllerTOP/method.html). Also, the toxicity of the epitopes of the vaccine structure was checked with the help of ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) server.

2.7. Investigation of physicochemical properties, solubility and antigenicity of vaccine

The physicochemical properties of the candidate vaccine were evaluated via ExPASy-ProtParam server. The solubility of the candidate vaccine was also evaluated and predicted through Pep-Calc (http://www.pep-calc.com) and ProteinSol servers (https://protein-sol.), as well as the surface availability of amino acids from the IEDB server in section Kolaskar and Tongaonkar antigenicity.

2.8. Investigation of the tertiary s structure of vaccine modification and validation

The Tertiary s structure of the vaccine constructed through the I-TASSER (https://zhanggroup.org/I-TASSER/) server was selected from the five models created by the same server, based on higher negative energy levels, as the superior model. Then 3D-refine servers were used to increase the quality of the selected model and structural modification of the vaccine.

2.9. Docking

Molecular docking was performed to predict the affinity between the vaccine structure and some human TLR4, MHC-I and MHC-II receptors [36]. The structure of these receptors was retrieved from the RCSB protein database. The structure was then refined and validated by SPDBV, DISCOVERY STUDIO software and used for the docking process. The protein-protein molecular docking process was performed by a ClusPro 2.0 server (https://cluspro.bu.edu/) [21]. As a result, several docking complexes with the corresponding energy weight points were obtained and the best complexes were selected in terms of energy level. Molecular docking helps to predict and select the best vaccine/receptor interaction from a group of vaccine/receptor complexes. The interactions of the selected final complexes were visualized by Paymol software and then the type of links and residues involved between the vaccine/receptor complexes were determined by the PDBSUM server (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/).

2.10. Normal state analysis for structural stability analysis of vaccine/receptor complex

Normal analysis was performed to analyze the structural stability of the selected binding complex by the iMODS web server (https://imods.iqfr.csic.es/). The server displays NMA mobility with arrows that indicate mobility dynamics. The server also provides the B factor, the variance, the covariance map, and the link matrix of the desired structure.

2.1. Immunological simulation

In silico Immune Simulation was performed using a C-ImmSim server (http://150.146.2.1/C-IMMSIM/index.php) to confirm immunization and immune response against the selected vaccine. This server uses a machine learning base to predict epitopes and related security interactions. In this process,

three injections containing the designed peptide vaccine were simulated at intervals of four weeks. The initial amplifier approach for our simulation was used at 4-week intervals to achieve a long-term preservation safety response based on the evaluation of safety readings. One of the default parameters is that each time-step was set to 1, 84 and 168, meaning that each time-step was 8 hours and each time-step was also 1 injection at time zero. Therefore, three injections were performed four weeks apart. However, eight injections were performed four weeks apart to stimulate repeated exposure to the antigen. In this scenario, T cell memory was continuously evaluated. And Simpson index was graphically interpreted from the analysis of this design [22].

2.12. Codon optimization and preparation and cloning process

Codon optimization was performed to predict the expression of the modeled vaccine in an expression vector using the codon optimization tool (JCat) (http://www.jcat.de/). Here, E. coli (strain K12) was used to optimize the codon. The server provides a codon matching index (CAI) which indicates the bias of codon usage and GC content. The pET28a (+) expression vector was also used to simulate in silico gene sequencing using SnapGene software.

3. Results and Discussion

3.1. Protein sequencing from NCBI

Envelope protein F13 sequences were retrieved from the NCBI database with access number P33815.

3.2. Prediction results of linear cell epitopes of B, MHC-I and MHC-II and their analysis

In this work, IEDB servers were used to predict epitopes by B and T cells. After determining the appropriate epitopes, they were used in vaccine design and their sequence and antigenic score are presented in table (Table 1).

Table 1: B cells linear, MHC-I T cell and MHC-II T cell epitopes of F13 protein monkeypox virus and their immunogenic properties.

B cell epitope peptide	Antigenicity score	Allergenicity&Toxicity	MHC-I epitope peptide	Antigenicity score	Allergenicity&Toxicity	MHC-II epitope peptide	Antigenicity score	Allergenicity&Toxicity
ENMDFRSDHLT	1.3784	Non allergen& toxic	LPVSTAYHI	0.6632	Non allergen& toxic	ENMDFRSDHLTTFE	0.9371	Non allergen& toxic
RGKRNLGELQSHCPD	0.4990	Non allergen& toxic	NEIITLAKKY	0.6788	Non allergen& toxic	FNEIITLAKKYIYIA	0.6332	Non allergen& toxic
TLAKKYIYI	0.5610	Non allergen& toxic	TSAPAGAKCR	0.8095	Non allergen& toxic	VLLDERGKRNLGELQ	0.5662	Non allergen& toxic
EIITLAKKY	0.9377	Non allergen& toxic	NPLSTTRGAL	0.9105	Non allergen& toxic	ETLPENMDFR	1.1189	Non allergen& toxic
-	-	-	APAGAKCRL	0.5468	Non allergen& toxic	-	-	-

MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILEAAGDKKIGVIKVVRE
IVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKEAAAKENMDFRSDHLTKKRGKRNGELQSHCPDKK
TLAKKYIYIKKEIITLAKKYGPGPGAPAGAKCRLKKLPVSTAYHIKKNEIITLAKKYKKTSAPAGAKCRKKNPLSTTRGALAAYE
NMDFRSDHLTTFEKKFNEIITLAKKYIYIAKKVLLDERGKRNLGELQKKETLPENMDFRHHHHHH

Figure 2. Graphic representation of the structure of multi-epitope vaccine. The vaccine construct consists of an adjuvant (red) at the N-terminal end linked to the entire multi-epitope sequence via the EAAAK linker (blue). BL (green), HTC (yellow) and HTL (orange) epitopes are combined with the help of GPGPG (blue), KK (blue) and AAY (blue) linkers, respectively.

3.3. Vaccine design results

At this stage, the chimeric protein of the vaccine was generated from the epitopes we screened in the previous steps. To do this, the B cell epitopes were first put together and linked up by linker structures called EAAAK and KK. Then, with the help of GPGPG linker, they were connected to MHCI-derived epitopes, which were also connected with KK linker and finally, by AAY linker. A connection was established between the section containing MHC-I and MHC-II epitopes. After completing the structural sequences, an adjuvant (50S ribosomal protein L7/L12) compound was added to the end of the generated chimer sequence (Figure 2). Selected antigenic epitopes were scrutinized to determine which could potentially induce different Th1 and Th2 cytokines. Those with this attribute were selected for the vaccine construct. To construct a multi-epitope vaccine, we finally selected CTL, HTL and B cell linear epitopes, linked together with the help of EAAAK, GPGPG and KK linkers, respectively. To boost the immunogenic profile of the selected profile epitopes, an adjuvant would usually be required. The adjuvant (50S ribosomal protein L7/L12) was putatively added through the EAAAK linker, with the B and HTL epitopes linked together through the GPGPG linkers. These complexes were subsequently added to the CTL epitopes through the AAY linkers. The tag (6xHis-tag) was added at the C terminal end of the vaccine construct. The 6xHis-tag is one of the simplest and most widely used purification tags, with six or more consecutive histidine residues (Figure 2).

3.4. Secondary structure prediction

At this stage of the evaluation of the chimer composition produced in terms of two-dimensional and three-dimensional structure, it was examined that when undesirable folds are created in its structure at the very beginning of the work, the disruptive epitope parts of the chimer structure were removed from its original structure. With the help of softwires - PSIPRED and Prabi, the secondary structure of the chimera was investigated. The results of this study were that the second structure of the chimer composition in terms of alpha helix percentage with 48.96%, 44.81% random coil and 6.22% extended strand, have had favorable conditions (Figure 3).

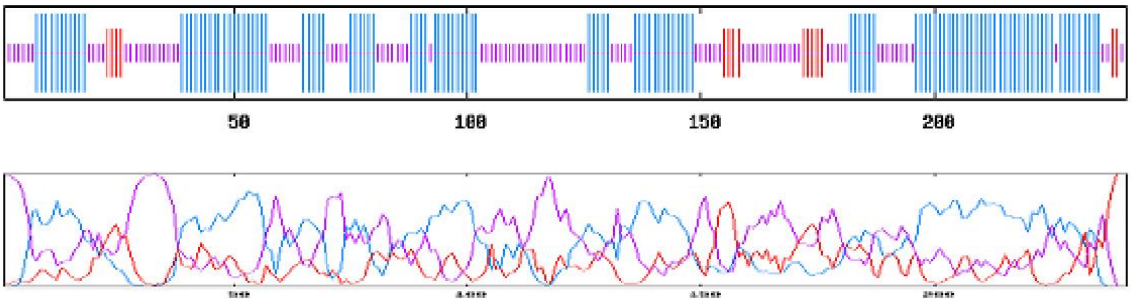

Figure 3. Graphical representation of the secondary structure of fusion construct. Blue, purple and red represent the Helix, extended strand and random coiled structures, respectively.

3.5. Allergy and antigenicity

By examining the antigenicity of epitopes selected by IEDB server from Kolaskar and Tongaonkar antigenicity section (Figure 4) and determining the antigenicity score (Table 1), A threshold score 0.4 of selected epitopes for vaccine structure was derived by VAXIJEN server and ensured that all selected epitopes were antigenic in use. It was then time to check their allergenicity, which at this stage was evaluated with the help of ALLERTOP software, where those that had an allergic risk were removed and those that did not have allergenic properties were isolated and used to make the vaccine structure [23, 24].

3.6. Physicochemical properties and solubility

The evaluation of the vaccine with a number of physical and chemical properties was performed by using the ProtParam, PepCalc and Prosol softwares, which resulted in a vaccine composition with an isoelectric pH of 10.480 and a stable composition of 33.42 in the body. Its predictable half-life in mammals was one hour. The composition of the case, was found to have a molecular weight of 27503.10 Da, a GRAVY of -0.868 and an aliphatic coefficient of 68.59. The case composition was also in good condition in terms of hydrophobicity and extinction coefficient.

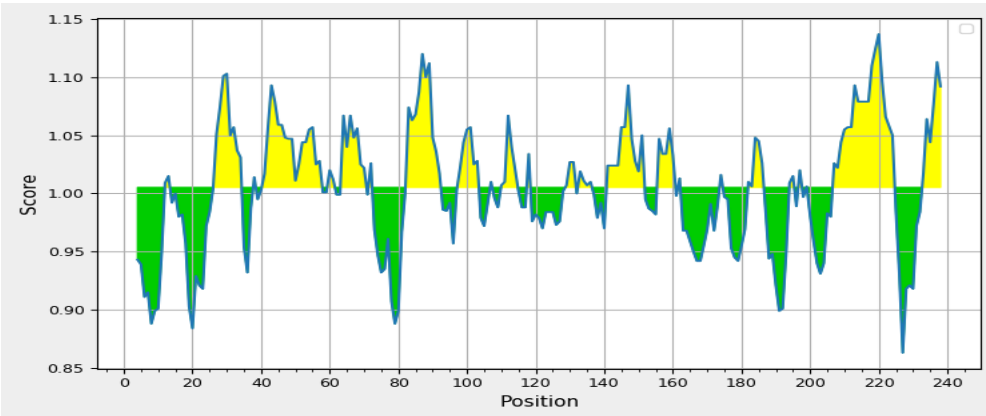

Figure 4. In the diagram above, the antigenicity of the initial sequences of the vaccine structure shows the high power of the B cell epitopes selected by the Kulascar and Tongaonkar algorithm of the IEDB server, and the yellow sections indicate the high antigenic power of these regions in the vaccine structure.

According to the results obtained from the Pepcalc and Prosol servers with a score of 0.692, there was good solubility in water, which allows it to be introduced in laboratory phase processes [25, 26].

3.7. The tertiary structure of vaccine modification and validation

To obtain our three-dimensional structure, modeling methods of the I-TASSER servers were used. RMSD was selected as the best model in terms of negative energy level among the models prepared from I-TASSER server (C-score=-4.76 , TM-score = 0.27±0.08), in terms of these three indicators [27, 28]. The structure of the vaccine in the ProSA server received a Z-score -1.51, which indicated that the model obtained was in the range of compounds obtained from the NMR technology. The model obtained after modification by the 3DRefine server was placed in a favorable structural position in terms of its amino acids. According to the results of PROCHECK server and through Ramachandran diagram, about 94.9% was in an acceptable and desirable position, which indicates the appropriateness of the structure of the obtained vaccine model (Figure 5).

3.8. Docking results

In this study, receptors used in the docking process such as TLR4, HLA-A0201 and DRB.0101 were selected due to their immunomodulatory ability to stimulate IFN-g as well as activation of IFN type I responses [29]. Epitopes selected as CD4+T epitopes were able to stimulate both Th1 and Th2 cytokines. The docking process was thus performed between the vaccine construct and TLR4, HLA-A0201 andDRB.0101, using the ClusPro server. The server created about 25 to 30 possible docking structures with equivalent energy values from which complexes with the lowest energy scores were selected. The complex between the TLR4 vaccine receptor and two human HLA receptors belonging to

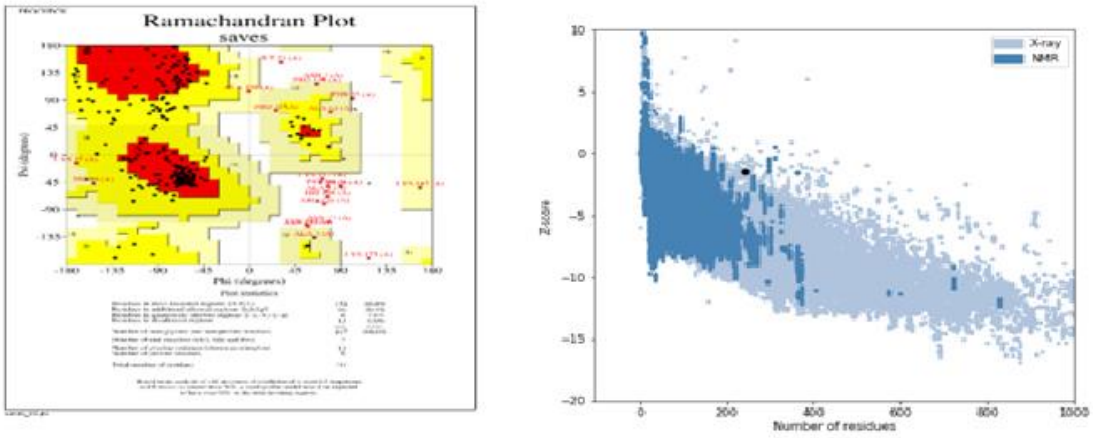

Figure 5. Post-modeling analyses of the 3D structure of VACCINE by PROSA and PROCHEK servers. A. Ramachandran plot depicting the stereochemical quality of VACCINE. B. Analysis of stability of the 3D structure of VACCINE.

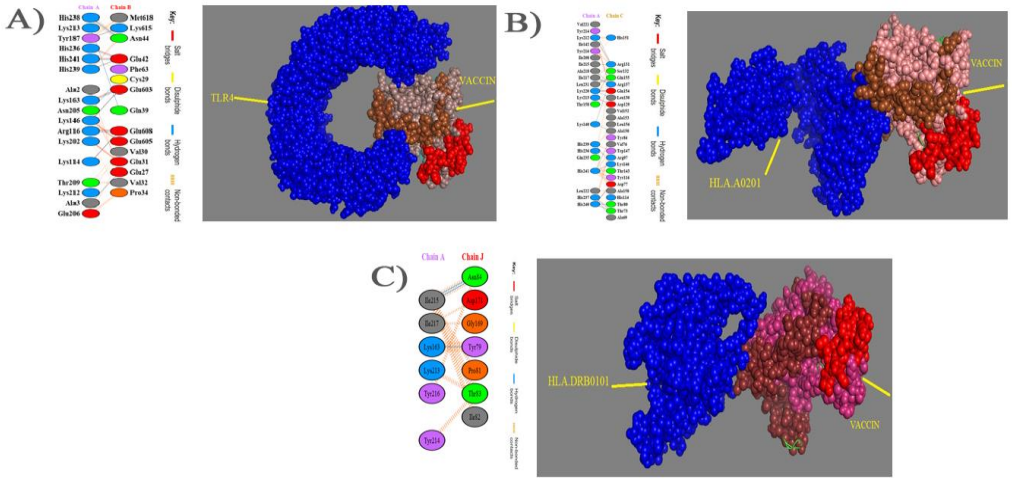

Figure 6. Interactions between vaccines designed with receptors, TLR4(figA), HLA-A0201(figB), DRB1.0401(figC).

MHC-I and MHC-II classes, were analyzed in terms of energy and named as HLA-A0201 andDRB.0101, respectively. The docking structures were then visualized using PyMOL software. The interactions between the vaccine designed with different receptors and residues, as well as the type of links involved in these interactions were all analyzed and obtained by the PDBsum server (Figure 6).

3.9. Normal state analysis for structural stability of vaccine / receptor complex

In order to analyze the biophysical stability and changes of the VACCINE-TLR4 complex, molecular dynamic simulations were performed through the iMODs server [30]. The resulting iMOD original chain deformation is shown in Figure 7A. The area where the hinges are located has a strong tendency to deform. The values of factor B calculated by normal state analysis are proportional to the square root of the mean, and factor B shows the fluctuations of the atomic position, which measures the unpredictability of each atom, as shown in Figure 7B. Figure 7C shows the eigenvalues that were closely correlated with the energy required to smooth the structure, and in general this figure reflects the stability of the dock complex by the eigenvalue shown. The eigenvalue of the VACCINE-TLR4 complex is 1.249706e-04, which indicated less energy required for deformation of the structure and also indicated the stability of the complex. The immune cascades were activated to remove antigens. The variance is inversely related to the eigenvalue. In Figure 7D, the individual variance with red and green represents cumulative variance. The covariance map shows the relationship between the pairs of residues, where the red, blue, and white colors indicate the correlated, anti-correlated, and unrelated pairs of residues, as shown in Figure 7E. The elastic lattice diagram shows the pair of atoms attached to the spring (springs) and each point on the diagram represents a spring between the corresponding pair of atoms. Also in the diagram, the darker gray color indicates the stiffer springs, as shown in Figure 7F.

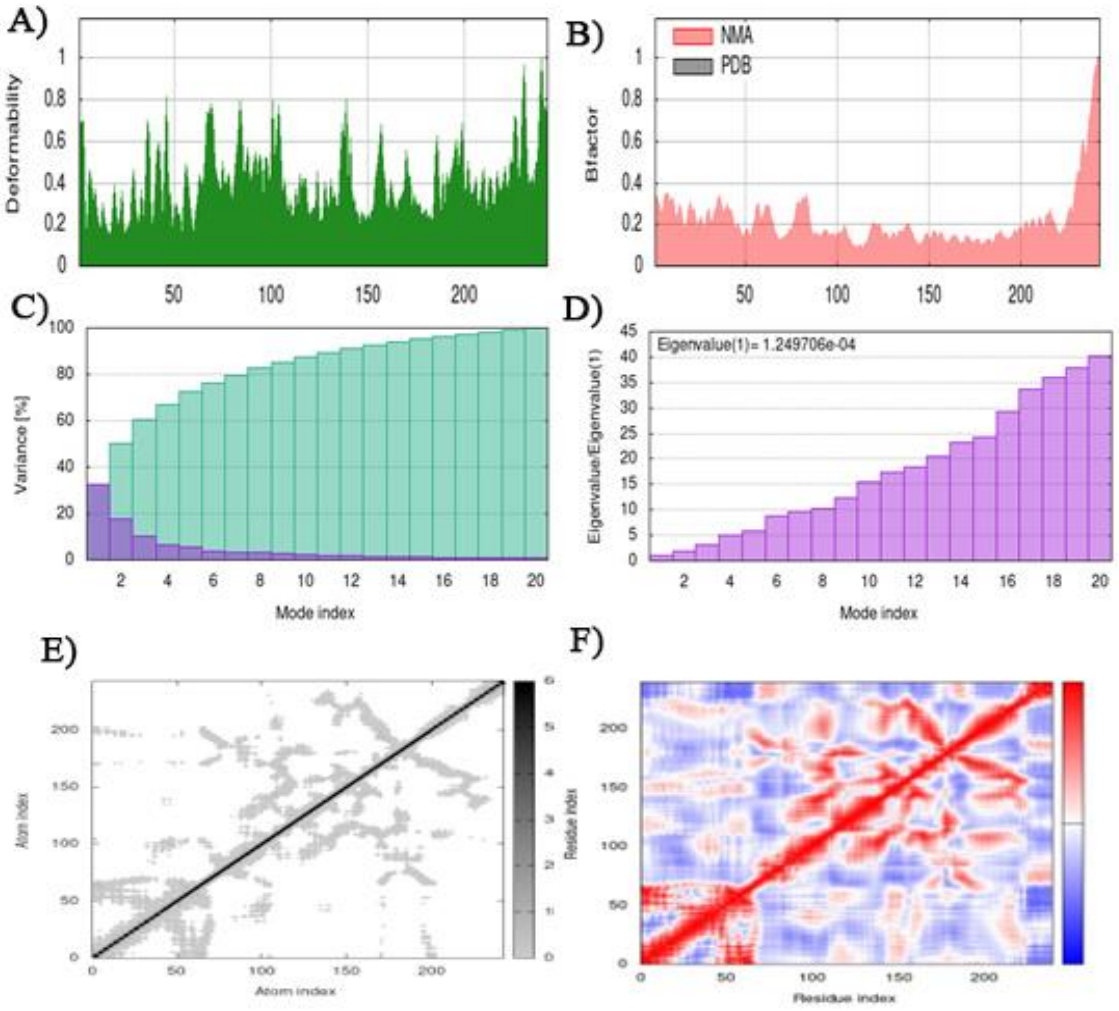

Figure 7. Molecular dynamics simulation of the VACCEN/TLR4 complex. The stability of the protein-protein complex was investigated through deformation (A), factor B (B), eigenvalue (C), variance (D), covariance matrix (E) and elastic lattice (F).

3.10. Immunological simulation

The results provided by the C-ImmSim server were consistent with the actual safety responses, as evidenced by the increased production of secondary responses. The initial response to the vaccine was demonstrated by increasing high IgM levels. In the secondary and tertiary responses, the increase in B cell population as an increase in IgG1+IgG2, IgM, and IgG+IgM levels is shown in Figure 7. In addition, we found that there was an increase in helper T cell populations and cytotoxic T cell populations with memory cell proliferation. We also noticed higher levels of cytokines such as IFN-γ, IL-10, TGF-B, IL. -12, as shown below, indicating the observations to have showed that the designed vaccine elicited promising anti-Quid-19 immunogenic reactions (Figure 8).

3.11. Codon optimization and vaccine cloning

Nucleotide sequences for VACCINE were obtained after reverse translation and codon optimization on the JCat server. The CAI value of the optimized sequences was 1.00 and the

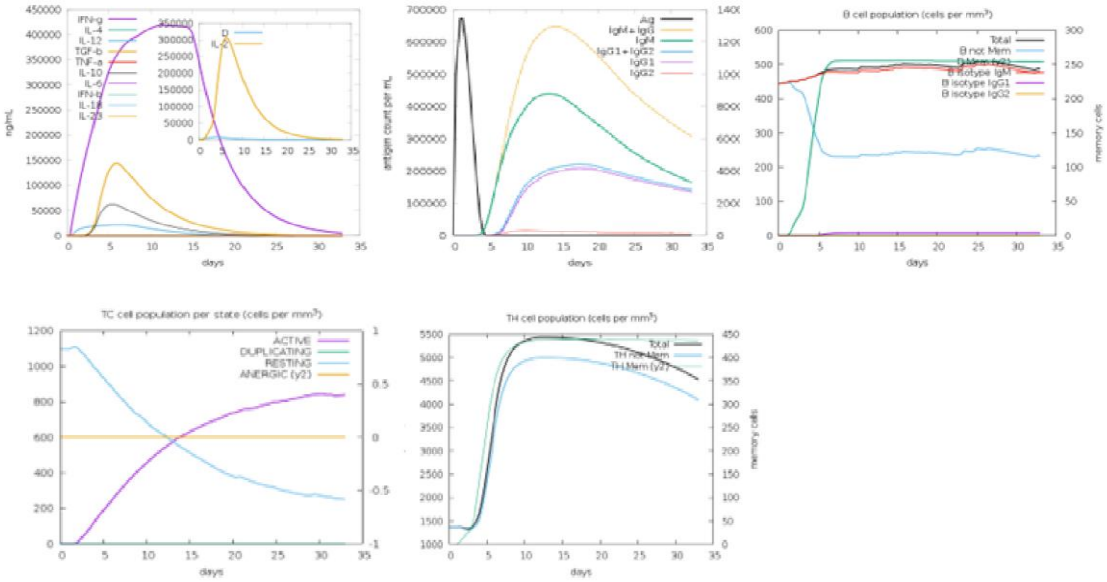

Figure 8. Immunological simulation results vaccine-induced immune response. provided by C-ImmSim server. The images above show the production of several immunoglobulin subclasses (colored lines), the enrichment of B cells, the development of TH cells, the development of TC cells, and the production of cytokines and interleukins after vaccine injection in the host.

GC content was 44.67496542185339, thereby indicating good expression of vaccines in the *Escherichia coli*. A recombinant plasmid was created by inserting the designed vaccine sequence into the pET28a (+) vector, after adding the two restriction sites of *EcoRI* and *BamHI* restriction enzymes, at the N and C terminals of the nucleotide sequence. The vaccine cloning process was conducted using the SnapGene software. The accuracy of the cloning process was also investigated using the same SnapGene software, with the gene sequence structure of the designed vaccine, the vector and recombinant structure of the clone. These resulted in having the vector and the gene sequence of the vaccine being designed after digestion by two enzymes *EcoRI* and *BamHI*, using electrophoresis (Figure 9).

Epidemics of new viral origin are a serious threat and the cause of death of many people around the world. Therefore, there is a serious need for further therapeutic and preventive measures in this area, such as the vaccine production. Monkeypox is a viral disease between humans and animals, which also poses a serious threat to public health [31], which has in fact recently spread worldwide. The use of immunoinformatic approaches in the development of strong vaccines against various microorganisms, especially viruses, as the first line of vaccine production, is becoming increasingly acceptable. In recent years, the immunoinformatics-driven approaches have been used to design epitope-based subunits for various diseases. Therefore, our study focused on the design of peptide-based vaccines from different epitopes based on the F13 envelope

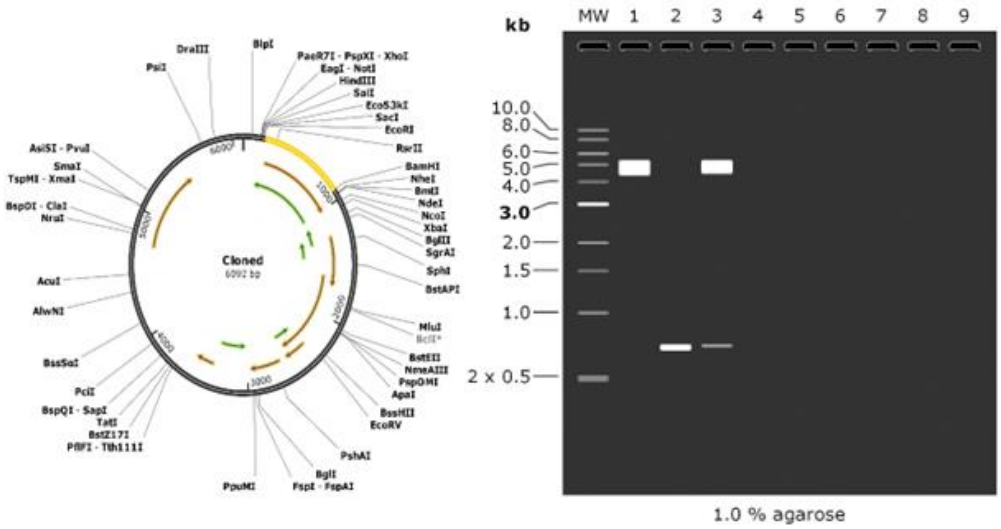

Figure 9. The resulting image and the path of the vaccine cloning and electrophoresis process.

protein of monkeypox virus. We successfully developed a peptide vaccine after various in silico processes and analyses. Epitopes were considered using a set of immunoinformatics tools and based on criteria such as stimulation and activation of the immune response without toxicity and allergy in the host body. Both B and T cell epitope groups were predicted and recovered in this study to design and manufacture the desired immunogen, because we have combined the B cell epitopes due to their role in antibody production. Although antigens can eventually overcome the humoral response of memory B cells over time, T-cell or cell-mediated immunity usually results in longer-lasting protection [32]. B-cell epitopes were therefore selected based on surface availability and Kolaskar&Tongaonkar's antigenic determination methods through the IEDB server. These peptides are also non-toxic, making them safe vaccines. T cell antigen epitopes that were able to present to a large number of MHC-I and MHC-II alleles were also predicted and selected using the IEDB server [33]. CD8 + T cells recognize the antigen of a pathogen after it is presented by MHC-I molecules, so cytotoxic T lymphocytes fight pathogens by recognizing the virus and killing the infected cells and by releasing specialized cytokines that fight the viruses [34, 35]. Various studies have shown that specific antibodies against monkeypox disease are produced and that these antibodies are relatively persistent in patients who have recovered from monkeypox infection [36, 37]. This suggests that specific antibodies produced against smallpox infection provide long-term immunity in patients. The binding results between the immunogenic vaccine constructs with MHC-II molecules and other immune system receptors demonstrate their ability to activate the response of specific T + CD4 cell subsets. In addition, the results of immunological simulations have showed that both Th1 and Th2 responses can stimulate our vaccine construct by inducing responses from

different cytokines in vivo. The results of immunological simulations were consistent with responses often seen in the immune system after repeated exposure to antigen, where there was a significant increase in the number of immune responses in general. It was very clear that memory B cells were generated in addition to the memory T cells and helper T cells, that were also generated. After the first injection, a significant increase in the level of IL-2, INF-G, and IL10 was seen, the results of which were very similar to the results of the research work of Bhattacharya et al. [40]. In fact, it is clear that designing our vaccine as a multi-epitope construct has several advantages, that it can achieve both cellular immunity and humoral immunity in the long term, provided that the subject is induced to have received 3 injected doses of the vaccine. The results obtained from the binding energy level scores between the predicted vaccine structure and MHC-II (-774.2), MHC-I (-1276.9) and TLRS (-770.1) molecules, which were evaluated comparatively, have all showed that the vaccine structure had good affinity. It has a significant connection with the active area of receptor proteins and leads to the formation and establishment of diverse and stable interactions and relationships with the receptors [34, 35]. These specific interactions with the high-affinity epitope of the HLA class II molecule caused an adaptive and specific immune response, the results of which were very similar to the results of the work of Bhattacharya et al. [40], and were in confirmation with other researchers too [38]. It was also shown that the target vaccine is a stable compound (instability index = 33.42). It has a solubility index (0.69) higher than 0.45 with optimal solubility in water. The molecular weight of the vaccine and its high pI value indicate the efficacy as well as the stability of the vaccine structure, because these types of proteins are usually good vaccine candidates [39]. Validation of a vaccine candidate begins with serological evaluation. This is one of the first steps in the process. To do this, it is necessary to express the recombinant protein in a suitable host. E. coli-based expression systems are suitable for the production of recombinant proteins. The results of our work showed that the indices obtained from the designed vaccine such as CAI content (1) and GC (44.67) have achieved high level protein expression in the E. coli host after optimization and had appropriate values for simulation of these indices. The above-mentioned findings have showed that our designed chimeric construct can be well cloned and expressed in E. coli. Finally, based on the immunoinformatic evaluations of the above vaccine, it was found that this chimeric structure is indeed a safe, soluble, stable compound at different temperatures and hydrophilic levels. Therefore, this peptide combination could be a new candidate for monkeypox immunity. However, further confirmation of the effectiveness of the above vaccine requires further evaluation, using in vitro and in vivo studies.

4. Conclusion

A new approach to predicting vaccines based on epitopes of some membrane proteins of monkeypox virus using bioinformatics tools, can indeed target the immune system in the host body. These antigen-predicting antigens could

accelerate the development of a protective vaccine for patients whose immune systems are compromised worldwide. In this research, our vaccine was able to stimulate the production of neutralizing antibodies as well as cellular response components after the last dose administration. Our selected epitopes in the form of a vaccine were able to be properly identified by the B and T cells and have elicited a favorable response against the monkeypox membrane proteins, that this work model in the future can thus promise various and useful treatment methods with less risk and higher efficiency, all of which requires clinical phase processes for the final approvals.

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Conflict of interest

None

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