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Original article

In Silico Design of a Multi-Epitope Vaccine Candidate Against Lassa Virus Using Immuno-informatics and Molecular Modeling Approaches

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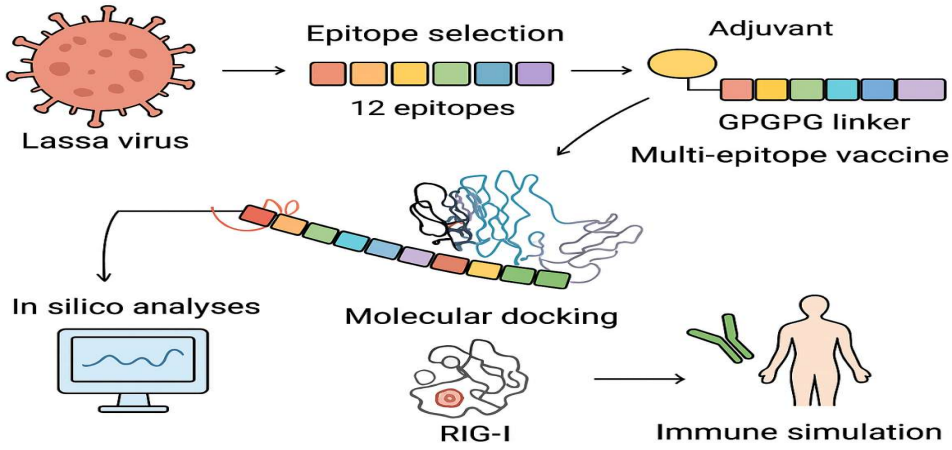
Abstract: Lassa hemorrhagic fever is a viral acute zoonotic disease caused by Lassa mammarenavirus (LASMV) that is part of the Arenaviridae family. With rising global infection and mortality, developing an effective LV vaccine remains an urgent worldwide necessity. In this research, utilizing immunoinformatics methods, we developed a multi-epitope vaccine for the prevention and treatment of LV. Experimentally determined epitopes of the LV were retrieved from ViPR and IEDB databases and subsequently analyzed for various immunological and physicochemical properties. The finalized 12 epitopes were used for vaccine constructions [(LV-1 (300 aa) and LV-2 (353 aa)] with the help of adjuvant (beta-defensin-1 and ribosomal protein) linked to the N-terminal by an EAAK linker. To make the immunogenicity of the vaccine, a pan-HLA DR binding epitope (13aa) was also introduced, and selected epitopes were separated with GPGPG linkers. Predicted vaccine constructs were safe, antigenic, and stable with modeled secondary and 3D structures against LV. The complex between the final vaccine and immune receptors (RIG-I) was evaluated by molecular docking and showed a favorable interaction pattern, including hydrogen bonding and high binding affinity of -10.99 (LV-1) and -29.22 (LV-2) kcal/mol. Codon optimization and in-silico cloning verified the suggested vaccines' effective expression in E.Coli. Finally, in-silico immune simulation demonstrated that LV-1 and LV-2 would also strongly stimulate the immune system against LV. The final vaccines need experimental confirmation of their safety and efficiency in the control of LV infections.

Keywords: Lassa virus, multi-epitope vaccine, ribosomal protein, beta-defensin-1, Rig-1

Graphical Abstract:



Introduction

The Lassa virus LASV, a newly discovered virus of the Arenaviridae family, can cause severe viral hemorrhagic fever, also referred to as Lassa fever, which has a 20% chance of death (Lukashevich et al., 2020). Lassa fever is a viral acute zoonotic illness that affects around 500,000 individuals and causes 5000 fatalities each year in Western Africa (Aloke et al., 2023). Lassa fever is believed to impact around 10% of the population in Ghana, 50% in Guinea, 30% in Côte d'Ivoire, 80% in Sierra- Leone and Liberia, 40% in Nigeria, and a few regions in Mali (Mylne, 2015; Safronetz, 2010). Furthermore, it affects several regions of Europe, including the Netherlands (Overbosch et al., 2019), Germany (Lehmann et al., 2016) and United Kingdom (Wolf et al., 2020). Even though the virus mostly attacks endothelium and antigen-presenting cells and prevents them from fully developing and activating, the pathophysiology of Lassa fever is still not apparent (Medugu, 2023; Klitting, 2020) However, there is an urgent need to createa useful LASV vaccine due to the high annual incidence and death rate. The large (L) and small (S) parts of the Lassa virus's single-stranded RNA genome are enclosed, ambisense, bisegmented, and negative sense (Klitting et al., 2020). The 200 kDa RNA polymerase (L) protein and the 11 kDa matrix protein (also known as Z-protein), which regulate transcription and replication, are written in code by the large part of the RNA (Liu, 2023; Lan, 2022). The tiny segments encode the nucleoprotein (NP, 63 kDa) and the surface glycoprotein precursor (GP, 75kDa), which are broken down by proteases into GP1 and GP2 (envelope glycoprotein), which attach to the alpha-dystroglycan receptor and facilitate entrance into the host cell (Arefin, 2021; Klitting, 2020) LASV is spread to humans by the rodent reservoir Mastomys natalensis, a common African rat that lives in village dwelling (Ramzan et al., 2024). However, recent research suggests that additional rodent species, such as Guinea mouse M. erythroleucus, and African wood mouse Hylomyscus pamfi, may also be LASV recipients (Medugu et al., 2023). When a healthy individual encounters an infected person's blood, secretions, tissue, or excretions, or with food tainted by the host's waste, LASV exchange takes place. The virus cannot transmit through skin-to-skin contact, which excludes the interchange of bodily fluids (Olayemi et al., 2024). Children under the age of 10 are considered particularly prone to LASV (Tiamiyu et al., 2024). Furthermore, pregnant women who have Lassa fever experience miscarriages (Kayem et al., 2020). Ribavirin, an antiviral medicine, has been demonstrated to be beneficial in the early stages of Lassa fever and can lower the mortality rate (Salam, 2022; Ogbaini-Emovon, 2024). Nonetheless, the emergence of potential toxicity and teratogenicity when provided at the advanced phases of the disease suggest that Ribavirin is not an effective treatment for Lassa fever (Cheng et al., 2022). Peptide vaccines serve as immune stimulants in which bits of virus-derived proteins imitate natural infections, making them more impactful in safety regulations, effectiveness, and specificity (Sayed et al., 2019). The 1st epitope-based vaccine against *E. coli* was created in 1985 by employing cholera toxin (Jacob et al., 1985). This study involves the comprehensive screening of the full LASV proteome and the subsequent classification of the viral proteins. For antigen A, each protein group was analyzed individually for T-cell and B-cell epitopes and their associated MHC alleles using the vaccine technology. A vaccine was then formulated based on the most dominant epitopes of each protein and relevant adjuvant and linkers. Following immunogenic profile and physiochemical investigation, the sequence was used to predict tertiary and secondary structures. The model being suggested was used for a coarse-size refinement and validation. Additionally,

disulphide bridging was carried out to enhance its structural integrity. Binding activity of the vaccine protein and the receptor was assessed by molecular docking and dynamic modeling. Codon adaptation and *In-silico* cloning were performed to check the expression of chimeric protein in a suitable host. Lastly, an immune simulation was conducted to simulate the real-life immunity potential.

Material and methods

Target Epitopes Retrieval

Experimentally determined epitopes of Lassa virus were obtained from the ViPR database (https://www.viprbrc.org/brc/home.spg?decorator=vipr) and IEDB Database (https://www.iedb.org/). The surface exposer of the retrieved peptides was predicted using the TMHMM server (https://dtu.biolib.com/DeepTMHMM). Peptides that were located outside of the cell membrane were considered for further screening. Using the IFNepitope server (http://crdd.osdd.net/raghava/ifnepitope/), the retrieved epitopes were screened for the capacity to induce IFN-gamma production; peptides showing positive IFN-gamma inducer were tested for allergenicity using the AllerTop tool (https://www.ddg-pharmfac.net/AllerTOP/). The non-allergen epitopes were screened for their antigenicity via VaxiJen 2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) with a threshold greater than 0.5 and model set as "virus". The non-allergen epitopes were further tested for toxicity using the Toxinpred tool (http://crdd.osdd.net/raghava/toxinpred/) and the number of epitopes was further narrowed down by discarding those exhibiting toxicity. Finally, to evaluate the retrieved epitopes solubility, the Innovagen webserver (http://www.innovagen.com/proteomics-tools) was employed with default parameters. Peptides predicted as soluble were retained, and non-soluble were discarded.

Chimeric Vaccine Designing

In contrast to peptide vaccines, a multi-epitope peptide vaccine is a much more efficient immunogen, and the underlying reason is that it contains multiple immunodominant epitopes fused together (Ismail et al., 2022). The shortlisted epitopes of Lassa virus were used for multiple epitopes-based vaccine construction. Two different types of adjuvants were used herein: (1) β-defensin-1 (Omoniyi et al., 2021) adjuvant, (2) ribosomal protein (Sayed et al., 2020) for boosting of immune reactions, which were linked with PADRE sequence via the EAAAK linker, followed by a GGGS linker that links the PADRE sequence to the multi-epitope sequence. In addition, the Glycine (G) and proline (P) rich GPGPG linker was inserted between epitopes (Ismail et al., 2022).

Evaluation of Human Homology

Non-overlapping epitopes and vaccination constructs were subjected to BLASTp analysis against the human proteome (taxid: 9606), accessible at https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins.

Secondary Structure Prediction

The PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/) was deployed using the vaccines' primary sequence to predict the secondary structure features, such beta-sheets, alpha-helix, and coil.

Physicochemical and Immunological Properties

The ExPASy ProtParam server was employed to investigate the vaccine constructions' physiochemical characteristics (Gasteiger et al., 2005). Molecular weight, Instability index, extinction coefficient, aliphatic index, theoretical isoelectric point (pI), half-life, and Grand average of hydropathicity (GRAVY) are some of these. The solubility of the vaccine designs was evaluated using Protein-sol (Hebditch et al., 2017) and SOLpro (Magnan et al., 2009) servers. The Vaxijen and AllerTop servers were used to assess immunological characteristics for both vaccinations, including antigenicity and allergenicity.

Three-Dimensional Structural Modelling of the Vaccine Construct

The sequence of Lassa virus vaccine constructs (LV-1 and LV-2) was submitted to the RoseTTAFold tool (https://robetta.bakerlab.org/) for tertiary structure prediction; the server generates the five models from the input amino acid sequence. Then, top predicted 3D structure model was submitted for structural refinement to the GalaxyRefine server (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE). The GDT-HA score, clash score, Molprobity score, Ramachandran plot score and RMSD score were employed to assess the quality of the modified model. Lastly, our improved 3D model of the vaccine design was validated utilizing the QMEAN SWISS MODEL and ERRAT systems (Colovos and Yeates, 1993, Wiederstein and Sippl, 2007).

Molecular Docking

An antigenic molecule's contact with a particular immune receptor is necessary for the molecular beginning of a suitable immune response. A computer method called "molecular docking" uses the interaction between a ligand and a receptor resulting in the formation of a stable adduct with a score that indicates the strength of the binding contact (Omoniyi et al., 2021). Gene induced by retinoic acid High pro-inflammatory reactions against Lassa virus infection can be mediated by RIG-I-like receptors (RLRs) (Omoniyi et al., 2021). Hence, molecular docking between the best LV-1 and LV-2 vaccine refined structures and RIG-1 receptor (PDB ID: 2QFB) was executed through the internet web server ZDock (https://zdock.wenglab.org/). The graphical depiction of the interaction involving the docked complex was created using PDBsum (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) and PDBePisa (https://www.ebi.ac.uk/pdbe/pisa/) (Krissinel and Henrick, 2007).

Reverse Translation, Codon Optimization and In-Silico Cloning

The vaccine construct's sequence was reverse translated and optimized for codon use using the Java Codon Adaptation Tool (JCat) tool to increase the expression of the cloned sequence within the expression system (Escherichia coli) (Grote et al., 2005). The expression of the cloned sequences was assessed using the codon adaptation index (CAI) and the percentage of GC. The revised sequences of GC content should ideally be between 30 and 70%, indicating good transcriptional and translational efficiency, and the CAI value should be 1. To stop rho independent transcription termination, additional input parameters were also considered. Additionally, cleavage sites for the HindIII and BAMHI enzymes were inserted at the N- and C- terminus of the LV-1 vaccine codon sequence and cleavage sites XhoI and BAMHI for LV-2 vaccine sequence. Lastly, the improved vaccine design was cloned in the pET-28a (+) expression vector using SnapGene (https://www.snapgene.com) software.

Computational Immune Simulation

The C-ImmSim server (https://kraken.iac.rm.cnr.it/C-IMMSIM/) utilizes a position-specific score matrix (PSSM) alongside several machine learning methodologies to forecast and analyze epitope and immunological interactions was used to conduct computational immune simulations to evaluate the immunogenic potential of the vaccine (Rapin et al., 2010). The following parameters were used in the immunological simulation test of the vaccines: dosage interval of four weeks; the three injections' time steps were set at 1, 84 and 168; the total time steps were set at 1000, or roughly a year. Every timestep in the simulation corresponds to eight hours of daily living (Sami et al., 2021).

RESULTS

Epitopes Retrieval and Analysis

Antigenicity, toxicity, transmembrane helices, allergenicity, IFN-positivity, solubility, and virulence were among the criteria used to screen epitopes. For designing the vaccine construct, 497 epitopes from the ViPR database and 63 epitopes from the IEDB database were retrieved from empirically confirmed immune protectors. Out of 560 epitopes, 160 allergens, 172 nonantigenic (<0.8 antigenicity score), 78 poorly soluble, and 98 epitopes topology were predicted inside the cell and were discarded. Of the 40-IFN positive epitopes, 30 non-virulent epitopes were eliminated, while 12 were identified as virulent. Ultimately, twelve epitopes were selected. PVIQDQDLEMFVREV, GYL-GLLSQRTRDIYISRRLL, ELLGVEPPSESDLEF, GYAWIDFDIEPARFN, PSPIGYLGLLSQRTR, SLAHVSYSMDHSKWG, FPAQPGLTSA, VDINLIPLI, FSRPSPIGYL, GYLGLLSQRTRDIYISRRLL, MAWGGSYIALDSGRGNWDC, and RPSPIGYLGL. Every chosen epitope was made visible. The chosen epitopes' antigenicity exceeded the default threshold of 0.8, ranging from 0.5997 to 2.346. additionally, Table 1 shows that the pathogenicity score was between 1.0543 and 1.0852, which was higher than the normal threshold of 0.0. The construction of vaccine constructs was then based on these 12 filtered epitopes.

Table 1: Selected epitopes for the designing of vaccine construct.

Epitopes (Model)	Allergenicity	Antigenicity	Virulentpred	Solubility	TMHMM	IFN-Gemma	Toxinpred
PVIQDQDLEMFVREV (Human)	Non-allergen	0.5597	Virulent (1.0575)	Good soluble	outside	+ve	NT
GYL-	Non-allerge	1.414	Virulent	Good	outside	+ve	NT

GLLSQRTRDIYISRRLL (Human)	n		(1.0638)	soluble			
ELLGVEPPSESDLEF (Human)	Non-allergen	0.988	Virulent (1.0606)	Poor Soluble	outside	+ve	NT
GYAWID-FDIEPARFN (Human)	Non-allergen	1.6445	Virulent (1.0602)	Poor Soluble	outside	+ve	NT
PSPIGYL-GLLSQRTR (Human)	Non-allergen	1.7761	Virulent (1.0799)	Poor Soluble	outside	+ve	NT
SLAH-VSYSMDHSKWG (Human)	Non-allergen	1.1472	Virulent (1.0633)	Poor Soluble	outside	+ve	NT
FPAQPGLTSA (Human)	Non-allergen	1.1545	Virulent (1.0549)	Poor Soluble	outside	+ve	NT
VDINLIPLI (Human)	Non-allergen	1.6831	Virulent (1.0606)	Poor Soluble	outside	+ve	NT
FSRPSPIGYL (Human)	Non-allergen	1.2884	Virulent (1.0589)	Poor Soluble	outside	+ve	NT
GYL-GLLSQRTRDIYISRRLL (Human)	Non-allergen	1.414	Virulent (1.0638)	Good soluble	outside	+ve	NT
MAW-GGSYIALDSGRGNWDC (Human)	Non-allergen	1.0893	Virulent (1.0852)	Poor Soluble	outside	+ve	NT
RPSPIGYLGL (Human)	Non-allergen	2.346	Virulent (1.0619)	Poor Soluble	outside	+ve	NT

Construction of Multi-Epitope-Based Vaccine

Table 2 displays the finished sequences for our multi-epitope vaccines. All the selected epitopes were linked to one another using a particular linker called GPGPG to create a multi-epitope vaccine. Two different adjuvants were also added at the LV-1 and LV-2 construct N-terminal of the construct was added, including β-defensin-1 (accession no: P60022) and 50S ribosomal protein L7/L12 (accession no: P0A7K2) respectively. Every adjuvant was associated with the PADRE sequence using EAAAK linkers. Also, the PADRE and epitope sequences was separated using GGGS linker. Total amino acid residues count for the final LV-1 and LV-2 construct was 300 and 353, respectively.

Table 2: Final sequence of the vaccine constructs for the Lassa virus in this study. Letter in bold indicated the PADRE sequence)

Vaccine	Sequence
LV-1 (Beta-defensin-1) (300aa)	MRTSYLLLFTLCLLLSEMASGGNFLTGLGHRSDHYNCVSSGGQCLYSAC-PIFTKIQGTCYRGKAKCCKEAAAKPVIQDQDLEMFVREVGGGSGYL-GLLSQRTRDIYISRRLLGPGPGELLGVEPPSESDLEFGPGPGGYAWIDFDIEPAR-FNGPGPGPSPIGYLGLLSQRTRGPGPGSLAHVSYSMDHSKWGG-PGPGFPAQPGLTSAGPGPGVDINLIPLIGPGPGFSRPSPIGYLGPGPGGYL-GLLSQRTRDIYISRRLLGPGPGMAWGGSYIALDSGRGNWDCGPGPGRPSPIGYLGL
LV-2 (Ribosomal protein)	MSITKDQIIEAVAAMSVMDVVELISAMEEKFGVSAAAAVAVAAGPVEAAEEKTEFD-VILKAAGANKVAVIKAVRGATGLGLKEAKDLVESAPAALKEGVSKDDAEALK-KALEEAGAEVEVKEAAAKPVIQDQDLEMFVREVGGGSGYL-

(353aa)	GLLSQRTRDIYISRRLLGPGPGELLGVEPPSESDLEFGPGPGGYAWIDFDIEPAR-FNGPGPGPSPIGYLGLLSQRTRGPGPGSLAHVSYSMDHSKWGG-PGPGFPAQPGLTSAGPGPGVDINLIPLIGPGPGFSRPSPIGYLGPGPGGYL-GLLSQRTRDIYISRRLLGPGPGMAWGGSYIALDSGRGNWDCGPGPGRPSPIGYLGL

Human Homology Evaluation

The selected epitopes as well as BLASTp homology search was performed on the final vaccine constructions against the human proteome (taxid:9606). Human protein sequences were not substantially like the query coverage of the vaccine construct and selected epitopes.

Secondary Structure Prediction

The LV-1 design included 23% (69 residues) of alpha-helix, 17.3% (52 residues) of beta-sheets, and 59.6% (179 residues) of coil, according to secondary structure prediction using the PSIPRED website. However, the LV-2 construct had 56.9% (201 residues) of coiled structures, 13% (46 residues) of beta-sheets, and 30% (106 residues) of alpha-helices (Figure 1).

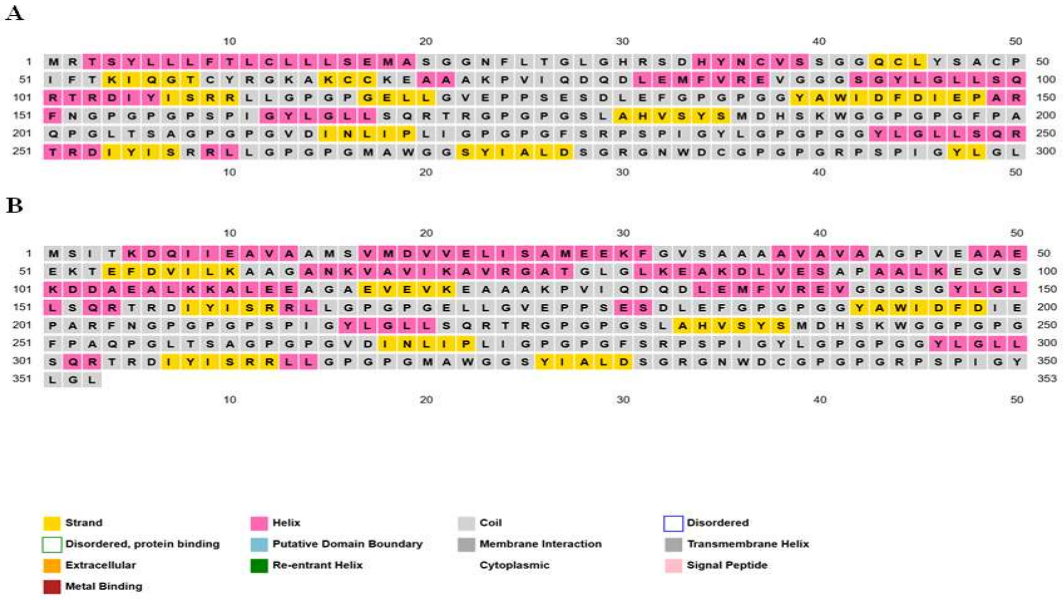


Figure 1: Predicting the secondary structure of a multi-epitope vaccine for Lassa virus (A) LV-1 (B) LV-2 (PSIPRED)

Predication of Antigenicity, Antigenicity, and Toxicity

The analysis showed that the LV-1 and LV-2 multi-epitope vaccines were non-toxic. AllerTop verified that there was absolutely no allergenicity in the construct, with antigenicity values of 0.7562 and 0.6923, respectively. There are adequate open surfaces on both constructs to trigger an immunological response, according to their surface associability as determined by TMHMM (Table 3).

Assessment of Physicochemical Parameters and Solubility

Following the designing of vaccines, their physicochemical qualities were identified and are summarized in Table 3. Since the molecular weight of LV-1 (31294.69 KDa) and LV-2 (36170.18 KDa) is less than 110 KDa, both vaccine designs were anticipated to be water soluble. As can be seen, the construct's tiny size makes it easy to handle during experimental examination and extremely thermally stable. The hydrophilic character of the beta-defensin-1 and ribosomal protein vaccine constructs was shown by their negative GRAVY values (-0.167 and -0.071). For LV-1 and LV-2, the theoretical pI values were 8.39 and 4.92, respectively, suggesting that they are alkaline. The protein structures were classified as stable (score > 40 indicates instability) based on the instability index prediction for LV-1 and LV-2, which showed values of 39.00 and 38.27. The vaccine designs' thermostability was further demonstrated by an aliphatic index

of 77.40 (LV-1) and 87.39 (LV-2). Both constructs were shown to have half-lives of 30 hours in mammalian systems, >20 hours in vivo in yeast, and >10 hours in vivo in E. coli. The construct structures' total amino acid composition was comparable, apart from a few adjuvants. Consequently, no significant changes in physiochemical characteristics were noted.

Table 3: Prediction of physiochemical properties, solubility, allergenicity, antigenicity, toxicity, and transmembrane helices of the vaccine constructs

Property	LV-1	LV-2
Sol-pro	0.900449 (soluble)	0.904520 (soluble)
Protein Sol	0.294 (soluble)	0.559 (soluble)
Molecular weight	31294.69 Da	36170.18 Da
Formula	$C_{1401}H_{2161}N_{385}O_{405}S_{13}$	$C_{1617}H_{2555}N_{435}O_{489}S_{8}$
Theoretical pI	8.39	4.92
Ext. coefficient	42860	36900
Instability index	39.00	38.27
Aliphatic index	77.40	87.39
Grand average of hydropathicity (GRAVY)	-0.167	-0.071
Half-Life	30 hours (mammalian reticulocytes, in vitro) >20 hours (yeast, in vivo) >10 hours (Escherichia coli, in vivo).	30 hours (mammalian reticulocytes, in vitro) >20 hours (yeast, in vivo) >10 hours (Escherichia coli, in vivo).
Allergenicity	0.7562 (VexiJen)	0.6923 (VexiJen)
Antigenicity	Non-Allergen (Aller Top)	Non-Allergen (Aller Top)
Toxicity	Non-toxic	Non-toxic
Surface accessibility	Outside	Outside

Prediction, Refinement, and Validation of the Vaccine Sequence's Tertiary Structure

RoseTTAFold and the GalaxyRefine server were employed to predict and enhance the three-dimensional structure of the vaccine construct. Based on the model quality ratings of the five refined models that GalaxyRefine predicted, we selected model 1 for LV-1 and model 3 for LV-2 (Table 4). The improved vaccine structural model's overall model quality was confirmed using the ERRAT quality score, ProSA Z-score, and Ramachandran plot analysis. LV-1 and LV-2 had overall quality scores of 81.91 and 81.52, respectively, according to evaluation by the ERRAT server. High overall model quality is shown by the ProSA projected Z-scores of LV-1 and LV-2, which were -5.5 and -7.57, respectively, within the scoring range of natural proteins of comparable sizes. We also performed Ramachandran plot analysis using the PROCHECK website. For LV-1 refined modeled structure, 80.6%, 13.6%, and 1.0% of residues were in most favored, additionally allowed, and generously allowed regions, respectively, whereas 4.9% were in disallowed regions. For LV-2 refined modeled structure, 88.4%, 10.9%, and 0.4% of residues were in most favored, additionally allowed, and generously allowed regions, respectively, whereas only 0.4% were in disallowed regions (Figure 3 and 4). The final 3D structural model of vaccine constructs is represented in Figure 2.

Table 4: Physical characteristics for the final subunit vaccine construct's validation (GalaxyRefine).

Vaccine	Model	GDT-HA	RMSD	MolProbity	Clash score	Poor rotamers	Ramachandran score

LV-1	Initial	1.0000	0.000	3.128	115.8	0.0	87.9
	MODEL 1	0.9867	0.297	2.163	14.8	0.0	91.9
	MODEL 2	0.9900	0.291	2.191	16.4	0.9	92.3
	MODEL 3	0.9908	0.289	2.215	16.4	0.9	91.6
	MODEL 4	0.9908	0.289	2.275	20.2	0.9	92.3
	MODEL 5	0.9867	0.297	2.298	16.6	1.3	91.9
LV-2	Initial	1.0000	0.000	1.728	4.7	0.0	92.0
	MODEL 1	0.9922	0.267	2.094	14.7	1.2	94.6
	MODEL 2	0.9873	0.281	2.161	18.9	1.2	95.2
	MODEL 3	0.9858	0.296	2.138	14.1	1.6	95.2
	MODEL 4	0.9887	0.292	2.187	19.3	1.2	94.9
	MODEL 5	0.9858	0.285	2.321	17.0	1.9	94.6

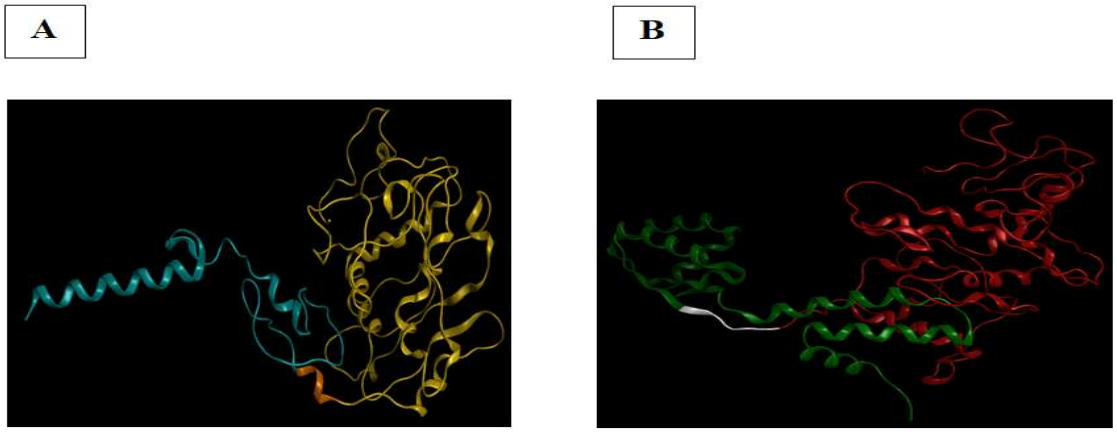


Figure 2: Final 3D structural model of (A) LV-1 and (B) LV-2 vaccine construct

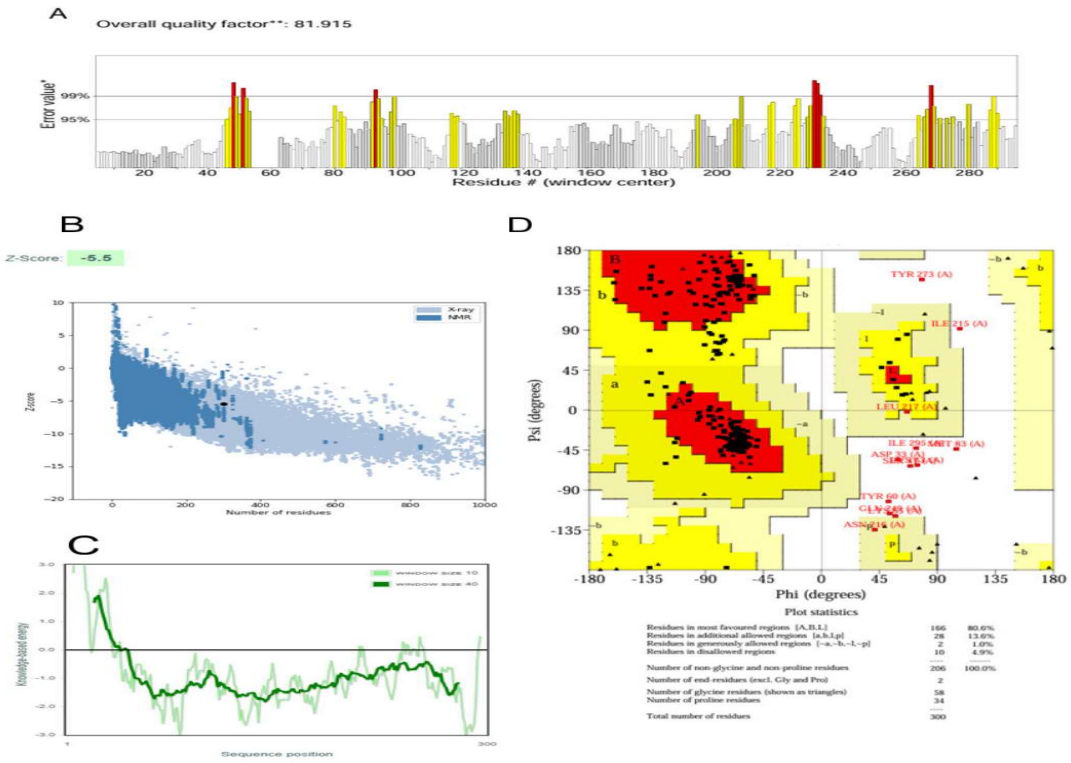


Figure 3: Validation of the predicted 3D structure model of LV-1 vaccine construct with **(A)** ERRAT overall quality factor **(B)** ProSA-webserver Z-score **(C)** Local quality model **(D)** Ramachandran plot analysis with PROCHECK.

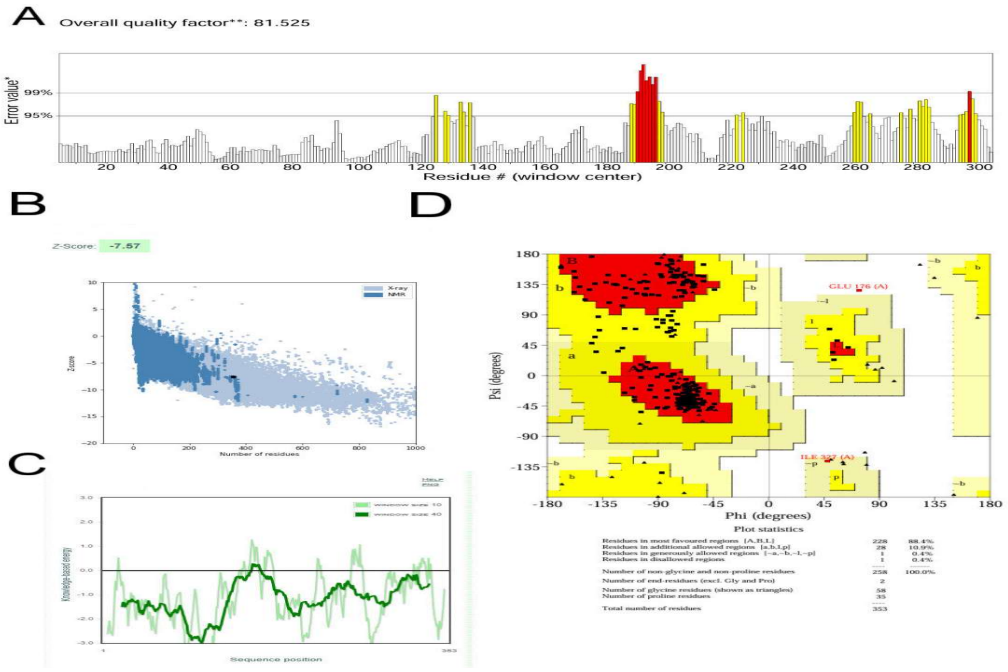


Figure 4: Validation of the predicted 3D structure model of LV-2 vaccine construct with (A) ERRAT overall quality factor (B) ProSA-webserver Z-score (C) Local quality model (D) Ramachandran plot analysis with PROCHECK

Molecular docking of Vaccine Construct with RIG-I

The binding relationship between vaccines and human immune cell receptors is essential in computational vaccine design research to ensure the generation of certain cellular and antibody immunological responses (Mehmood et al., 2019). One method for predicting the optimal binding confirmation between vaccine constructs and receptors is molecular docking. The RIG-I immune receptor's surface was made available for vaccine construct binding using ZDock (a blind docking approach) (Mehmood et al., 2019; Mehmood et al., 2021; Qamar et al., 2021). The docking was performed with 128 angular steps and a grid spacing of 1.2 Å. Deep binding and strong interactions between the two vaccines and the receptor molecules were noted. Figures 5 and 6 show the vaccines' binding mechanism and interactions with RIG-I. The PDBsum site provided useful information regarding intended vaccine binding interactions with RIG-I. It was shown that the stable binding of the docked complexes depends on hydrophobic interactions and hydrogen bonds.

Table 5: Top 10 LV-1 vaccine design docked complexes with RIG-I. Kcal/mol can be used to understand the data.

Rank	Model	ZDock Score
1	1	1560.416
2	14	1405.280
3	10	1375.579
4	105	1351.615
5	3	1342.477
6	123	1336.472
7	109	1336.297
8	113	1327.439
9	104	1309.272
10	11	1305.517

Table 6: Top 10 LV-2 vaccine design docked complexes with RIG-I. Kcal/mol can be used to understand the data.

Rank	Model	Z-Dock Score
1	1	1424.855
2	2	1384.154
3	3	1254.449
4	4	1250.767
5	5	1218.223
6	6	1217.005
7	7	1195.578
8	8	1190.159
9	9	1185.170
10	10	1178.781

The number of hydrogen bonds between the LV-1 vaccine constructs and the RIG-1 receptor is ten. Vaccine residues (ASP78, PRO174, GLY173, GLY192, GLY237, PRO199, SER39, TYR35, SER228, GLY226) were seen in hydrogen bonding with the receptor in a range of 2Å-3Å. In addition, vaccine residues were not having salt bridges with RIG-I, respectively within a range of 4Å. The number of hydrogen bonds between the LV-2 vaccine constructs and the RIG-1 receptor is six. Vaccine residues (GLN902, THR899, LYS807 (2X), ARG866, ALA865) were seen in hydrogen bonding with the receptor within a range of 3Å. In addition, vaccine-RIG-I residues did not show salt bridge interaction. In Figure 5 and 6, to illustrate intermolecular interactions, bond interaction maps are displayed next to each complex.

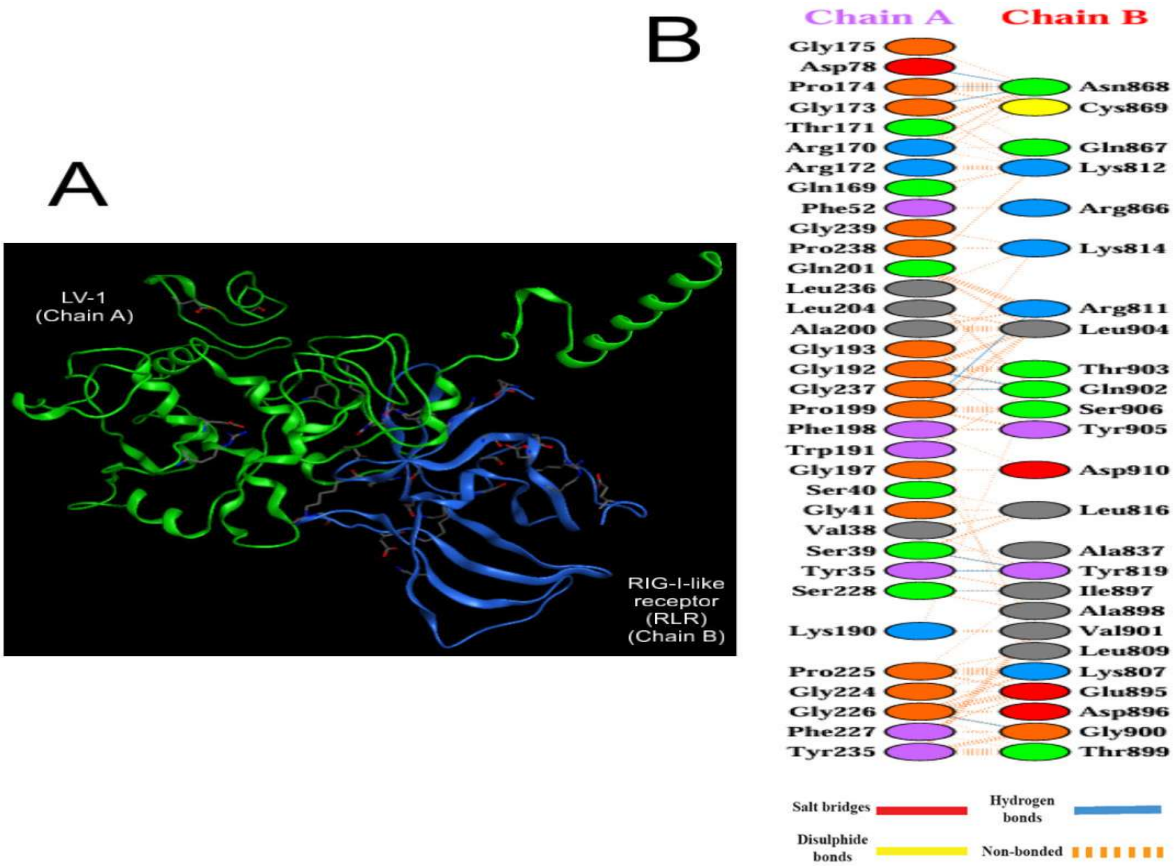


Figure 5: Lassa virus vaccine constructs docking with human RIG-1-like receptor (RIG): (A) LV-1-RIG docked complex in cartoon representation. LV-1 vaccine construct is represented with green color, and RIG is shown in blue color. (B) Every interaction residue between RIG and LV-1. 10 Hydrogen bonds with blue lines are indicated. The colors of the residues interacting reflect amino acid properties (Negative: Red, Positive: Blue, Neutral: Green, Aromatic: Pink, Pro&Gly: Orange, Cys: Yellow, and Aliphatic: Grey).

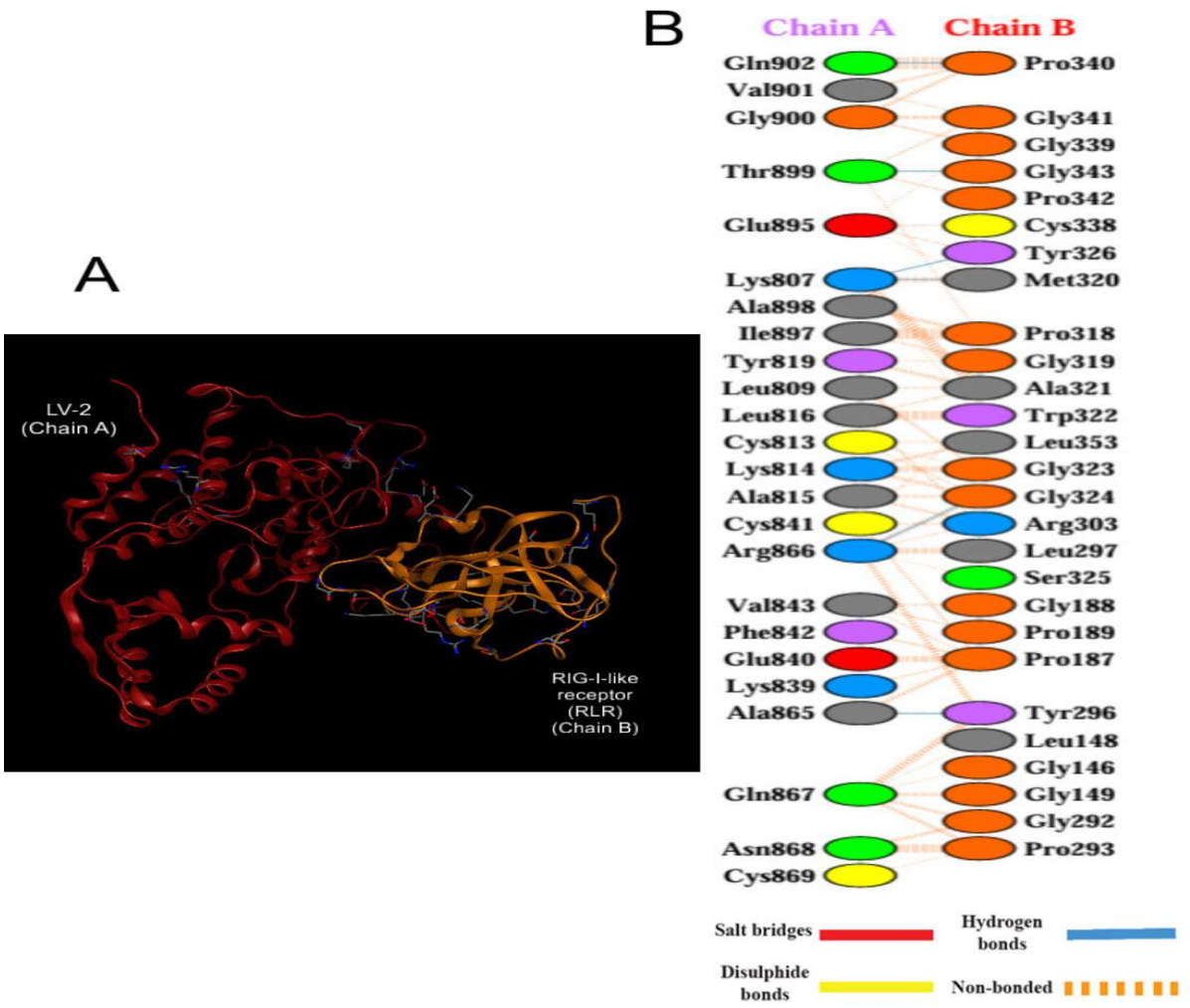


Figure 6: Lassa virus vaccine construct docking with human RIG-1 like receptor (RIG): (A) LV-2-RIG docked complex in cartoon representation. LV-2 vaccine construct is represented with red color, and RIG is shown in orange color. (B) Every interaction residue between RIG and LV-2. Four Hydrogen bonds with blue lines are indicated. The colors of the residues interacting reflect amino acid properties (Negative: Red, Positive: Blue, Neutral: Green, Aromatic: Pink, Pro&Gly: Orange, Cys: Yellow, and Aliphatic: Grey).

Reverse Translation and Codon Optimization

Vaccine construct sequences were reversely translated into DNA sequences using the JCaT sever to obtain high expression in E. coli. The E. coli expression system was selected to generate recombinant proteins. We performed codon optimization to guarantee that the E. coli K12 system generated our recombinant vaccine proteins at a high level. LV-1 and LV-2 had GC content values of 59.0% and 55.80%, respectively. Both LV-1 and LV-2 have CAI values of 1.0. The strong expression potential of the vaccines was shown by the fact that all these values fell within an acceptable range.

In Silico Cloning of the Final Vaccine Construct

The successful expression of vaccines in the E. coli expression system is a crucial step in in silico cloning. Two vaccines (LV-1 and LV-2) were designed with the prioritized RIG-1 receptor anticipated epitopes with proper linkers and adjuvants. To adapt the codon usage for most sequenced prokaryotic species, the sequences of these vaccines were fed (separately) into JCaT. This analysis's outcomes demonstrated that the DNA sequences of LV-1 and LV-2 were 850 and 1050 nucleotides, respectively. After the restriction recognition sites of the restriction enzymes XhoI and BamHI were conjugated at the N and C-terminals of reverse translated nucleotides of LV-1 and HindIII and BamHI for LV-2, the final optimized sequences were inserted into the pET28a (+) vector to clone the intended vaccines using SnapGene

software. The cloned plasmids' ultimate lengths were 6238bp for LV-1(Figure 7) and 6386bp for LV-2, respectively (Figure 8).

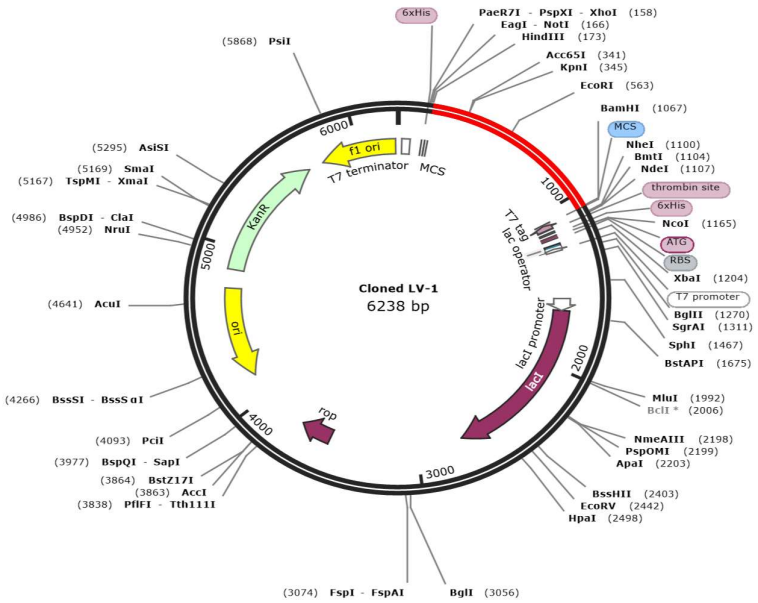


Figure 7: Cloning the LV-1 vaccine construct through computational restriction. Using SnapGene software, the final vaccine codon-optimized sequence (red color) was cloned into the pET-28a (+) expression vector between the HindIII (173) and BamHI (1067) cutting sites (black color). E. coli (strain K12) may be utilized to efficiently express the necessary structures, which makes it possible to make vaccines quickly.

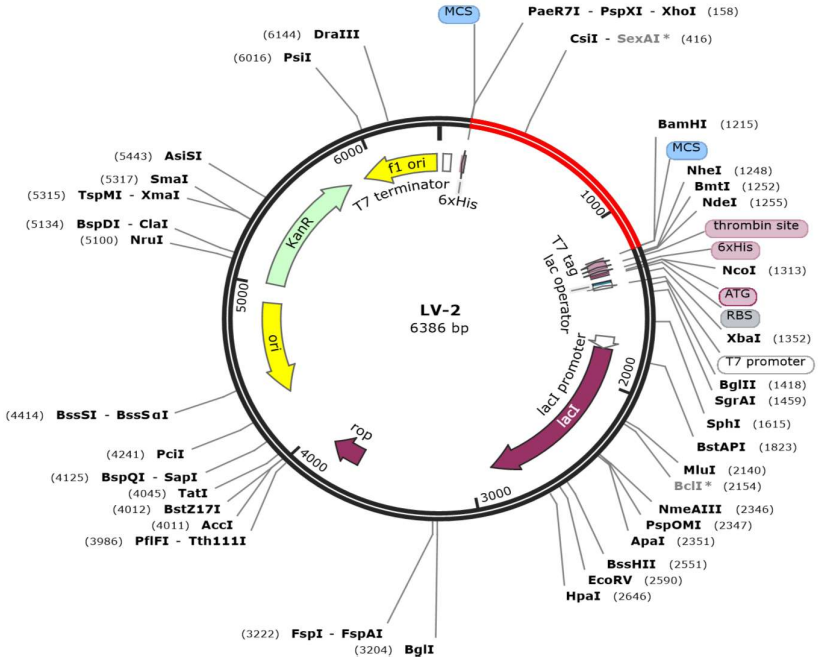


Figure 8: Computational restriction cloning of the LV-2 vaccine construct. The final vaccine codon-optimized sequence (red color) was cloned into the pET28a (+) expression vector using the SnapGene program. The XhoI (158) and BamHI (1215) cutting sites are shown in black. *E. coli* (strain K12) may be utilized to efficiently express the necessary structures, which makes it possible to make vaccines quickly.

In silico Immune simulation

Figures 9 and 10 show the outcome of the immunological simulation following the injection of LV-1 and LV-2. When compared to primary antibodies, a much higher amount of tertiary and secondary antibodies was observed in response to several exposures of LV-1 and LV-2 to the host immune system. As a result, fast antigen clearance (a drop in antigen concentration) was observed (Figure 9A and 10A). Figures 9 and 10B-H show the various immune cell populations and state counts. Considerable memory cell formation was accompanied by a considerable rise in several immune cells, including as B-cells, plasma B-cells, cytotoxic T-cells, helper T-cells, epithelial cells, macrophages, and dendritic cells; nevertheless, T-cells eventually declined following their initial increase. Similarly, IFN-ϒ (>400,000 ng/ml) cytokine levels were found to be elevated (Figure 9I and 10I). These findings imply that protection against the Lassa virus may be conferred via immunological memory formation and vaccine designs.

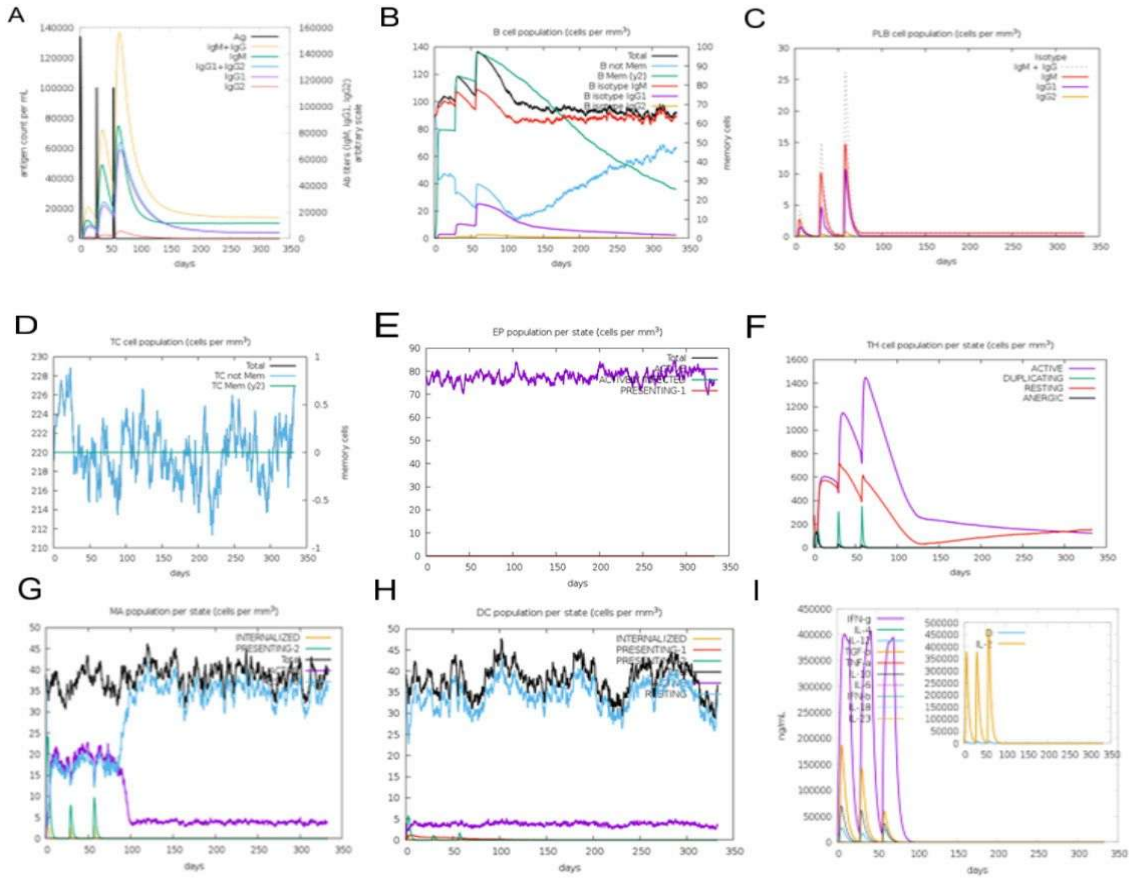


Figure 9: Results of immune simulation for the LV-1 vaccine construct after three consecutive doses: (A) immunoglobulin elevation at various antigen concentrations, (B) population of B-cell and their isotypes in response to the antigen, (C) population of plasma B-cells, (D) cytotoxic T-cells quantity, (E) population of epithelial cells, (F) total count of helper T-cells in distinct states (active and resting), (G) total count of macrophages, (H) total count of dendritic cells, (I) cell population of cytokines and interleukins in the active and resting states. Cells/mm3 is the unit of measurement for all units.

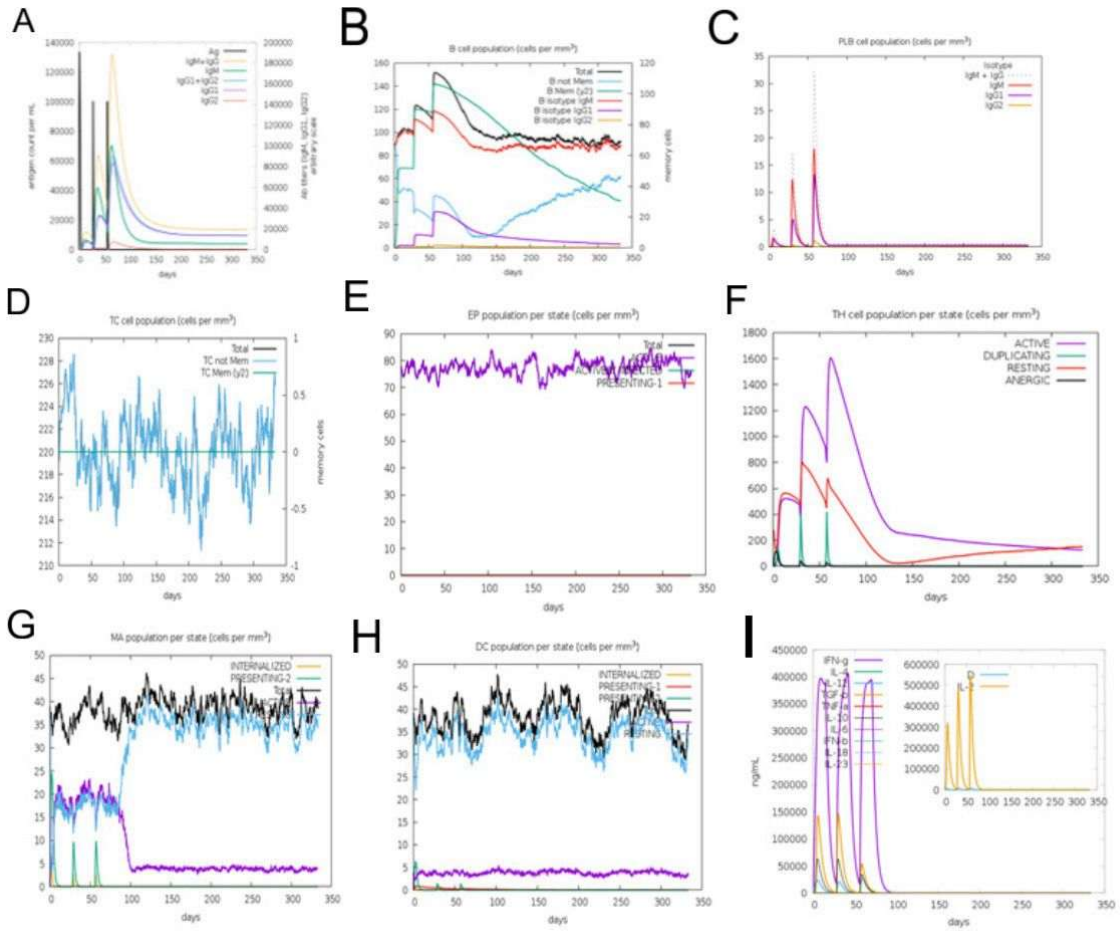


Figure 9. Results of immune simulation for the LV-1 vaccine construct after three consecutive doses: (A) immunoglobulin elevation at various antigen concentrations, (B) population of B-cell and their isotypes in response to the antigen, (C) population of plasma B-cells, (D) cytotoxic T-cells quantity, (E) population of epithelial cells, (F) total count of helper T-cells in distinct states (active and resting), (G) total count of macrophages, (H) total count of dendritic cells, (I) cell population of cytokines and interleukins in the active and resting states. All units are given in cells/mm3.

Discussion

The first line of vaccine research is increasingly acknowledged to be the application of immunoinformatic techniques to create potential vaccines against a variety of microorganisms, especially viruses (Dar, 2019). Due to their potential advantages over conventional vaccine, epitope-based vaccines have attracted a lot of attention (Dong, 2020). Epitope-based subunits for SARS-CoV-2 (Dong, 2020; Jyotisha, 2020) and MERS-CoV (Mahmud, 2021) have recently been created utilizing techniques guided by immunoinformatics. Despite the high mortality and morbidity associated with Lassa virus infection in human, there is no approved vaccine against this virus. Therefore, the goal of the current work was to develop multi-epitope Lassa virus vaccine candidates employing human immune system activating epitopes against Lassa disease that were discovered through experimentation. When compared to monovalent vaccines, the multiepitope-based subunit vaccine has the benefit of eliciting humoral, innate, and cellular immune responses simultaneously (Amanna, 2011). Herein, experimentally determined epitopes of Lassa virus were retrieved from ViPR and IEDB database for the vaccine designing. To shortlist the potential epitopes, several properties of retrieved epitopes were analyzed, including antigenicity, non-allergenicity, non-toxicity, water solubility, and virulence. The synthesis of interferon-gamma (IFN-γ) is essential for viral elimination and the stimulation of the host immune response (Kak et al., 2018). Thus, IFN-γ induction potential of epitopes were also assessed and only IFN-γ positive epitopes were chosen for the next steps of the analysis. For proper component selection, the transmembrane helices and localization of the obtained epitopes were evaluated and those predicted inside were retained due to their expo-

sure potential for immune cells. During the vaccine formulation process, this technique establishes if a potential vaccine is appropriate for experimental validation (Kumar Jaiswal, 2017; Naz, 2019). The results of epitope analyzed revealed 12 epitopes satisfying all selected criteria and were deemed as potential epitopes of Lassa virus for the vaccine construction.

Peptide multi-epitope vaccines can be made more immunogenic by adding an adjuvant (Ismail et al., 2022). Like Saba Ismail et al. study, all the chosen epitopes were connected to one another using GPGPG linkers to create a multi-epitope vaccine construct. At the N-terminal of the construct, adjuvant (ribosomal protein and beta-defensin) was added with the aid of EAAK linker. The PADRE sequence was also added, it is a pan-HLA-DR epitope peptide that reduces toxicity while increasing immunizing effectiveness. The PADRE sequence allows CTL epitopes to interact with different MHC class-II molecules with high affinity, making it a potential immunogen (Fadaka et al., 2021).

ERRAT server, PROCHECK, and ProSA-web were used to verify the multi-epitope vaccines' predicted 3D structures. A Ramachandran plot is the PROCHECK server's predicted structural analysis output. The graph depicts the amino acids' phi (φ) and psi (ψ) torsional angles in the input PDB file. A local and an overall model quality graphical output is generated based on the Z-score and input of the projected model by this program. Yadav et al. (Yadav, 2014) revealed that the template and target protein structures were -5.98 kcal/mol and -6.66 kcal/mol, respectively. Also, using an immunoinformatic technique to investigate the immunogenic features of Hemolin, it was discovered that the modeled protein's Z-score was -7.08 kcal/mol (Aathmanathan, 2018). Droppa-Almeidaet al. (Droppa-Almeida, 2018), Rekik et al. (Rekik, 2015), and Hashemzadeh et al. (Hashemzadeh, 2020) found the modeled 3D structures with Z-score ranged from -2.11 kcal/mol and -9.5 kcal/mol as accurate and of good resolution. With Z-scores of -5.5 kcal/mol and -7.57 for the predicted LV-1 and LV-2 vaccines, respectively, X-ray crystallographic characteristics are reflected in the overall model quality. The overall findings of the validation tools indicate that the anticipated vaccine structures are of excellent quality. As a result, the 3D structures of the anticipated vaccines are trustworthy and suitable for further assessment.

An essential in silico technique for examining interaction patterns and ligand-receptor binding affinities using a lock-and-key approach is molecular docking (Meng et al., 2011). In immunoinformatics, protein-protein docking is frequently used to examine the binding modes, interacting atoms, and binding energies of protein to determine the optimal, stable, and successful vaccine (Fadaka et al., 2021). Following the same method, the constructed vaccines were docked individually with RIG-I-like receptors (RLRs). High pro-inflammatory reactions against Lassa virus infection can be mediated by the retinoic acid-inducible gene RIG-I. The proposed constructions (LV-1 and LV-2) and receptors (RIG-I) docked well, demonstrating that the vaccine designs can produce long-lasting immune responses because the proposed vaccines fit neatly into the binding sites of the receptors. In addition, negative docking score indicated favorable binding of the constructed vaccine with the receptor. Interaction analysis revealed several hydrogen bonds between the vaccine-RIG-I complexes, hence showing their role in stability of the complexes.

After codon optimization using the JCAT online service, computational cloning was carried out using a pET28a (+) vector to lessen codon bias (Marín, 2003). The GC content and CAIs of the optimized nucleotides fell within the permissible ranges of 30-70% and 0.8-1.0, respectively. These findings showed that the suggested designs are robust and effectively expressed in E. coli (strain K12). The C-ImmSimm server, an immune response stimulator, was used to test how well the predicted vaccine would make the immune system work (Rapin et al., 2010). This approach simulates the thymus, bone marrow, and lymph node, the three key parts of a healthy mammalian system. Since a successful vaccine must provide a lifetime adaptive immunity that mimics the antigen-induced natural immunity, researchers studied how immune cells, such as CTL, antibodies, HTL, B-cells, macrophages, dendritic cells and cytokines, reacted to the vaccines that were made. Immune simulation employing the LV-1 and LV-2 vaccine design was shown to induce primary antibodies (IgG and IgM), T-cells, B-cells, and cytokines. Like other multi-epitope vaccine design research utilizing immunoinformatics methods, the produced vaccines can provide protection against Lassa virus infection (Devi, 2021; Devi, 2021; Ismail, 2020; Tahir ul Qamar, 2020). It is necessary to conduct additional experimental validation of the safety and effectiveness of the developed vaccines against NeoCov.

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