Research Article

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Health Science Journal
ISSN 1791-809X

2021
Vol. 15 No. 3: 816

Designing a Multi-Epitope Vaccine against Human Cytomegalovirus: an Immunoinformatics Approach

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Citation: Hossain I, Mimi SI, Piya UH, Sahabuddin Md (2021) Designing a Multi-Epitope Vaccine against Human Cytomegalovirus: an Immunoinformatics Approach. Health Sci J. 15 No. 3: 816.

Abstract

"Cytomegalovirus (CMV) is identified as the most frequent inborn viral contagion in people and an important matter of morbidity and destruction in immunocompromised owners". Cytomegalovirus is a typical herpes virus. Also distinguished as HCMV or human herpesvirus 5 (HHV-5). Once tainted, the human body holds the virus for life. CMV settles from person to person over body liquids, like blood, saliva, urine, semen and breast milk. "The Centers for Disease Control and Prevention organization" (CDC) predicted that around 50% of grown-ups in the United States have incurred the virus by forty years old. It afflicts men and women fairly, at any life and notwithstanding of ethnicity.

Fever, wetness, tiredness, restlessness, sore throat, enlarged glands, muscle strain, low desire and loss of weight are the obvious indications. But recurring and fundamental CMV have more dangerous symptoms and critical conditions like as jaundice, fever, pneumonia, cold, spots under the surface body, Purple color surface stains, a blemish, or all, developed liver, kidney, large spleen, low birth weight, breakdowns etc. Notwithstanding these debilitating maladies, no prescription to prevent them improvement or treatment is open till now.

That's why we proposed to express a multi-epitope vaccine upon CMV by employing an Immuno bioinformatics road. For this desire, we practised the Human CMV spike protein to ascertain the dominant. After study of the specification of these epitopes we selected only six epitopes to create the desire vaccine against the human Cytomegalovirus. All features of a perfect vaccine was present of our designed vaccine such as non-toxin, non-allergenic and highly soluble etc.

Moreover, our created vaccine showed the excellent score in all physiochemical and in lifetimein the host cell.

Keywords: HCMV; Multiepitope vaccine; Immunoinformatics; Dynamic simulation

Received with Revision February 21, 2021, **Accepted:** March 08, 2021, **Published:** March 12, 2021

Introduction

Cytomegalovirus (CMV) is in the subfamily Betaherpesviridae, in the family Herpesviridae, in the order Herpesvirales, in a genus of viruses [1]. Humans toil as common hosts. Total 8 species in this genus in sample species, Human betaherpesvirus 5 that affect people. Complications linked with HCMV-5 hold pneumonia [2,3]. Infrequent species of Cytomegalovirus recognised and selected for several vertebrates [4]. The most inquired is human affected cytomegalovirus, which is also recognised as a human (HHV-5) [5]. jaundice, fever, pneumonia, points under the body skin, Purple colour skin splotches, a rash on the body, or both, the enlarged liver in body, the enlarged spleen on the body, coarse birth weight of baby, fits and persistent hearing loss are the common consequences of CMV disease [6-8]. Like the other gut microbiota, this HCMB play an important role to create disease [7]. A huge number of CMV end-organ complications territory on human health. Pneumonia ("Cytomegalovirus pneumonia disease" is marked by the symptoms of the pulmonary infection along with the CMV in lavage liquor or lung cell specimens), Gastrointestinal disorder [9] ("Cytomegalo gastrointestinal disorder" is characterised by identity of an aggregate of medical indications from the gastrointestinal area, conclusions of macroscopic wounds area endoscopy, and showing of cytomegalovirus infection in the specimen), Hepatitis ("Cytomegalo hepatitis" is characterised by the findings raised bilirubin and/or enzyme levels during liver testing, the need of any other documented that cause for hepatitis and exposure of Cytomegalovirus germs in a liver cell biopsy sample) [10], CNS disorder ("CNS syndrome" is defined by the description of CNS indications together this the

disclosure of CMV in the sample, by production or PCR, or in brain units, by histopathologic trial, immunochemical examination, or in vitro hybridization), Retinitis (Lesions typical of of cytomegalic retinitis settled by an expert.), Cytomegalo nephritis and Cytomegalo cystitis are another which associated with CMV[11], Cystitis ("Cytomegalovirus cystitis" is exposed by the exposure of cytomegalovirus infection (by culture, in vitro hybridization) concurrently with a classification of cytomegalovirus infection, Pancreatitis is also associated with this viral infection [9]. Moreover, by these infections, cytomegalovirus is associated statistically, stimulated by bacterial superinfection, that is known as"indirect effects" of cytomegalovirus [12]. Some research showed that cytomegalovirus is associated with sensitivity [13,14], lungs organ [15], kidney organ [16-18], and innard [17] transplantings. Cytomegalovirus is responsible for Bone marrow operations [19] and liver operations [20]. For sensitive heart patient, preventive ganciclovir use lessen the fungal disease [21]. A huge randomized, placebo-controlled trial which included kidney operation patients revealed that it can decrease non-herpesvirus diseases [22]. Although disease with cytomegalovirus is usually symptomless, there is somebody at risk for cytomegalovirus disorder. There already attempts made for thirty years to produce vaccines for cytomegalovirus infections. However, in spite of these attempts, no vaccine got licensure [23]. Unfortunately, this infectious is also associated with the birth-giving child from mother [24]. Therefore, we decided a glycoprotein which has the highest antigenic average among 75 HCMV glycoproteins. Then we prophesied CTL, HTL and MHC Class-2 epitopes for forming the HCMV vaccine. After prophesied we performed Peptide modelling and molecular docking, Sketching and developing of multi-epitope vaccine, Physicochemical and immunological evaluation, Secondary structure prediction, homology modelling, 3D structure clarification and validation, docking investigations, Molecular simulation, Immune response simulation and Codon based adaptation and in silico cloning steps to the formate final vaccine. A flow chart outlining the overall procedure in **Figure 1.**

Materials and Methods

Proteome retrieval and antigen collection

"For the purpose of antigen choice, we choose possible HCMV proteomes from the (https://www.viprbrc.org/) database" [28]. "The surface membrane of the HCMV is set by the spike proteins. With the cooperation of these proteins, they attach with the human host and penetrate their genome" [29]. "For the literal relationship of glycoproteins in pathogenesis, so we weighed the spike protein of the HCMV for multiepitope vaccine plan". "Firstly, the chosen protein sequences of the cytomegalovirus had downloaded in the FASTA format file". "The shielding antigens of the surface coprotein were checked by (http://www.

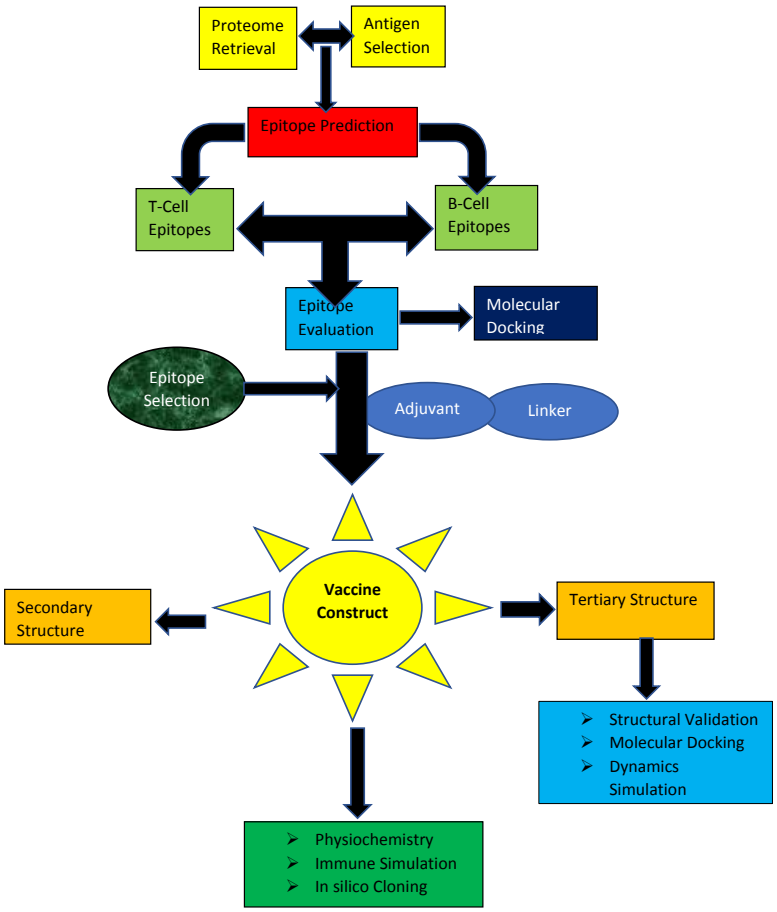

Figure 1 Schematic depiction of our workflow procedure.

ddg-pharmfac.net/vaxijen/) database [30] with a threshold value of 0.4 was set for it [31]. Conclusively, we chose the spike protein with the most powerful antigenic score for additional investigations".

Forecast and evaluation of cytotoxic T-lymphocyte epitopes

"Cytotoxic T-lymphocytes (CTLs) have the ability to kill other contagious cells efficiently with the direct process [32]. We use the NetCTL v1.2 server available at http://www.cbs.dtu.dk/services/NetCTL/ [33]. The prophesied epitopes were more valued within the VaxiJen v2.0 [34], ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) [35], and AllerTop v2.0 (https://ddg-pharmfac.net/AllerTOP/) [36] servers. The default parameters of those servers were done for all the prophecies".

Forecast and evaluation of helper T-lymphocyte epitopes

"Helper T-cells (HTLs) are an essential part of adaptive resistance that sees different antigens and begins B and cytotoxic T-cells ending in the loss of the dangerous pathogen. To learn the HTL epitopes, we did the IEDB's MHC class II necessary allele forecast tool, free at http://tools.iedb.org/mhcii/. The HTL epitopes were chosen based on a percentile level of 5% doing the Agreement method [37]. The prophesied epitopes were more assessed based on antigenicity exerting vaxijen server v2.0 (http://www.ddg-pharmfac.net/vaxijen/) [30]".

Forecast and evaluation of linear Blymphocyteepitopes

"B-cell epitopes are required to influence humoral or antibody-mediated safety [38]. Therefore, we used the iBCE-EL server, free at http://www.thegleelab.org/iBCE-EL/ with default levels"[39].

Modelling of multi-epitope vaccine

"The vaccine construct was created by applying the chosen CTL epitope, HTL epitope, and LBL epitopes as well as a perfect adjuvant that was joined by the appropriate linkers [38,40]. Here, for recognized by viral glycoproteins we used TLR4 agonist as an adjuvant [41,42]. Therefore, 50S ribosomal protein (NCBI ID: P9WHE3) was recognised as the adjuvant to enhance the immunogenicity of the vaccine applicant. The adjuvant was linked with linker EAAAK. In contrast, the elected CTL was linked with (AAY) linkers, the HTL was associated with (GPGPG) linkers also the LBL was connected with (KK) linker [38,40]. The AAY linker was used to influence protein balance, decrease more limited immunogenicity and improve epitope offering"[43,44].

Physicochemical and immunological evaluation

"The physicochemical characteristics of the vaccine were predicted by applying the ProtParam database free at https://web.expasy.org/protparam/ to know the functional characteristics of the vaccine [45]. We further assessed the immunological attributes within VaxiJen v2.0 [34], MHC-I immunogenicity [33], AllerTop [36], Biosoland SOLpro (https://protein-sol.manchester.ac.uk/) [31] website".

Secondary construction forecast: "The two-dimensional basic peculiarities such as alpha-helix and random coils of the construct were recognised by SOPMA server at https://npsa-prabi.ibcp.fr/NPSA/npsa_ seccons.html [46] and PSIPRED v4.0 server at http://bioinf.cs.ucl.ac.uk/psipred/ [47] with default value. SOPMA has more than eighty per cent prophecy correctness [46]. The 2D fundamental specialities were recovered and decided to agree with the construction nature of the vaccine".

Homology modelling, 3D construction clarification and validation: "The created vaccine was offered into Galaxyweb online network portal (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=TBM) for (3D) structure prophecy [48]. The recognised 3D building was presented into the Galaxy Refine (http://galaxy.seoklab.org/refine) online web-based site for the breeding of the vaccine composition [49]. The Galaxy Refine site presents an overall full point average. The elegant building was downloaded, and the chosen structure was named depending on the effectiveness number of the quietest and highest RMSD rate. The elegant and distinguished formation was conceived practising the PyMOL v2.3.4 software [50]. The Ramachandran plot and Z-point signified was examined by the ProSA-web (https://prosa.services.came.sbg.ac.at/prosa. php) accessory and Procheck (https://servicesn.mbi.ucla.edu/PROCHECK/)" [51].

Molecular docking investigations: It exposes the necessary communications between modelled protein and receptor units. For this commission, we resigned the elegant vaccine model as ligand and TLR4 protein as a receptor molecule into the ClusPro v2.0 site(https://cluspro.bu.edu/), for docking study [52]. The TLR4 receptor (PDB ID: 3W3M) was chosen and took from the PDB site[53].

Dynamics simulation study: For molecular dynamics simulation, we took server-based instruments to estimate the dynamics and security of the vaccine–receptor fear critically. The obsession was resigned to the iMODS site, open at http://imods.chaconlab.org/ [54].

Protected rejoinder simulation: To assess the pleasant safe reply of the vaccine, the good construct was resigned on C-IMMSIM v10.1 site free at http://150.146.2.1/C-IMMSIM/ and the created replies were recovered for accurate pronouncement [55]. We admitted the smallest interlude point of 30 days between two applications, as reported hardy [56].

Codon adaptation and in silico cloning Technique

Concerning the appearance of an alien gene in an organism, codon optimization more needed according to the special organism [57]. So, the construct was resigned on JCat server (http:/jcat.de/) as the codon change. The modified course was assessed based on the codon adaptation ratio (CAI) preference and guanine-cytosine content [57]. The body in silico cloning plan was accomplished in SnapGene v4.2 [58].

Results

Best antigenic protein selection

"We got 75 S-proteins from India region. By antigenicity, we designed a protein with an antigenic point of 0.5350 (VaxiJen)the GenBank id was D7PI78".

Possible CTL epitopes

"Amidst the 115 epitopes we took the top five CTL epitopes for the main vaccine building based on the antigenicity value **(Table 1)".**

Possible HTL epitopes

"Total of 219 HTL epitopes, each was selected originally practising the IEDB site. We thought the highest fiver HTL epitopes for combining into the last vaccine assemble based on antigenicity **(Table 2)".**

Possible LBL epitopes

"Amidst all direct B cell epitopes we decided two epitopes those are antigenic, nonallergen and Grand average of hydropathicity point are negative **(Table 3)".**

Vaccine construct and fundamental premises

The vaccine was formulated using the chosen 6 epitopes based on antigenicity, allergenicity and toxicity belonging to three separate groups (2 CTL, 2HTL, and 2 LBL). The last vaccine Formed was 316 amino acids long **(Figure 1).**

Physicochemical characteristics and immunological assessment

The physicochemical characteristics of the vaccine built were evaluated as given in **Table 4**. The molecular weight of it 28111.58 Da. Other features such as (pI) were 6.19, Number of amino acids:

Table 1 The decided CTL epitopes for final vaccine building.

CTL	Epitope	C. Score	Antigenicity	Allergenicity	Toxicity
	KAHCTSHMY	1.2081	0.6249	NON-ALLERGEN	Non-Toxin
	STSVSTTKL	0.8355	0.7135	ALLERGEN	Non-Toxin
	WTMLNALIL	0.7654	0.3918 NON	NON-ALLERGEN	Non-Toxin
	SSFAAWWTM	0.7589	0.1287	ALLERGEN	Non-Toxin
	TTSRSSTSV	0.7531	0.5193	ALLERGEN	Non-Toxin

Table 2 The decided HTL epitopes for final vaccine building.

HTL	Epitope	Percentile Rank	Adjusted Rank	Antigenicity	Allergenicity	Toxicity
	CTSHMYELSLSSFAA	4.40	4.40	0.4497	NON-ALLERGEN	Non-Toxin
	TSHMYELSLSSFAAW	4.60	4.60	0.5164	NON-ALLERGEN	Non-Toxin
	AAWWTMLNALILMGA	4.90	4.90	0.2066	NON-ALLERGEN	Non-Toxin
	AWWTMLNALILMGAF	4.90	4.90	0.1731	NON-ALLERGEN	Non-Toxin
	FAAWWTMLNALILMG	4.90	4.90	0.1351	ALLERGEN	Non-Toxin

Table 3 The decided LBL epitopes for final vaccine building.

B cell epitope	Epitope	Start	End	Peptide length	Antigenicity	Allergenicity	Toxicity
	VAESSGNNSSASTSATTSRSSTSVSTTK	11	38	28	0.5030	Non ALLERGEN	Non-Toxin
	TSATTTTTTTLSTTSTKLSSTTHDPNVMRRHANDDFYKAHCTS	45	87	43	0.5561	NonALLERGEN	Non-Toxin

Table 4 Physicochemical characteristics of the construct.

Characteristics	Findings	Remarks
Amino acids	271	suitable
Weight	28111.58	average
Theoretical pI value	6.19	Slightly basic
Total value of negatively charged residues	32	
Total value of positively charged residues	29	
Formula	$C_{1221}H_{1972}N_{328}O_{411}S_9$	
Total value of atoms	3941	
The predicted half-life	30 Hours	
The instability index	29.89	thermostable
Aliphatic index	70.04	stable
Grand average of hydropathicity	-0.199	Hydrophilic
Antigenicity	0.4638	
Allergenicity	No	
Solubility	0.620	

271, the chemical formula was C1221H1972N328O411S9, a total number of atoms: 3941, The volatility index (II) is calculated to be 29.89, aliphatic point was 70.04 and grand average point of hydropathicity was -0.199(which refers that this vaccine is easily accessible to cell). Physicochemical comments, the immunological control of the construct were estimated. For instance, the antigenicity of the construct was 0.4638 and non-toxin. Again we predicted its solubility (https://protein-sol.manchester.ac.uk/results/solubility/run-dc634d71e4e905f20faa/results.html) scale is 0.620 **(Figure 3)** that meant this is higher soluble, and windowed charge score and fold propensity score are shown in **Figures 4,5**.

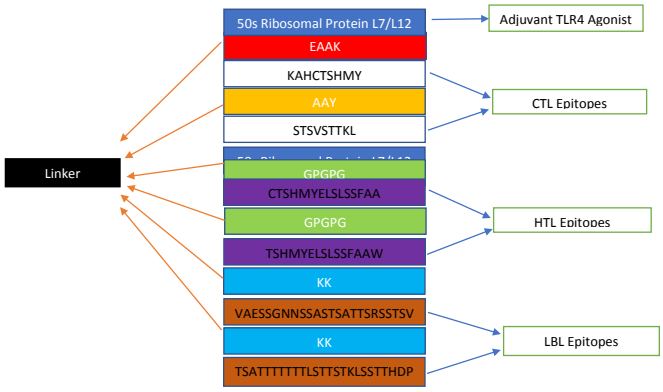

Figure 2 Graphical outline of the expressed multi-epitope vaccine assemble.

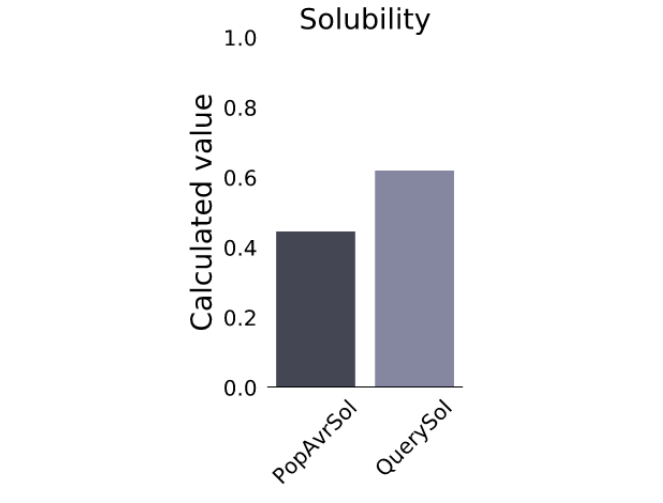

Figure 3 Calculated value of solubility using protein sol server.

Again, the vaccine was not allergies. The secondary structural figure includes a-helix and arbitrary turns that were assessed using SOPMA site prediction is given in **Table 5** and Prispred server prediction are shown in **Figure 6**.

Tertiary structure, sophistication and evaluation

"For this we used (http://galaxy.seoklab.org/index.html) to generate the highest five models. Among the all models, we decided the model **(Figure 7)** with the minimum value C-score as recommended by the site.

After refinement step, the vaccine model found 5 **(Figure 8)** showed that 94.4% residues in the considerable region in the Ramachandran grapgh, with GDT-HA score 0.9917, RMSD 0.274, MolProbity 2.099, Clash 12.4 and rotamers score 1.4".

Then we used the Procheck online site (https://saves.mbi.ucla.edu/) provided a **Table 6** and supplementary file with all findings and ProSA-web server(https://prosa.services.came.sbg.ac.at/prosa.php). The total average quality and z score average is -4.55 **(Figures 9 & 10).**

Molecular docking incestigation

Between them on the Clus Pro v2.0 server provided 29 docked models with different positions. Among these, we took the model with the least energy value (Supplementary file) So, model 1 placed the inclined roles and that was energy score of –1148.6 (supplementary pdf file).

Molecular dynamics simulation experiment

This was carried out in the iMODS site, where NMA evaluations was submitted to the internal complex. Along with B-factor average, Eigenvalues average, Variance average, Covariance map average, Elastic network average are shown in figure 10A,10B,10C,10D,10E,10F.

Exempt rejoinder simulation

The safe reply determined comparable to actual immunological aspects produced by special pathogens as confirmed in 11A to 11G. Antigen and immunoglobulins 11A, B lymphocytes cell: these are IgM and IgG2 in 11B, Plasma B lymphocytes count sub-divided per isotype (IgM, IgG1 and IgG2) in 11C, CD4 T-helper cells total. Rest values showed in 11C to 11G.

Codon evolution and in silico cloning

We optimized the codons now in the vaccine create according to the E. coli K12 by the JCat site(http://jcat.de/) to develop their key facility. The final constructed size of the vaccine cloning

Figure 4 Windowed charge score per amino acid.

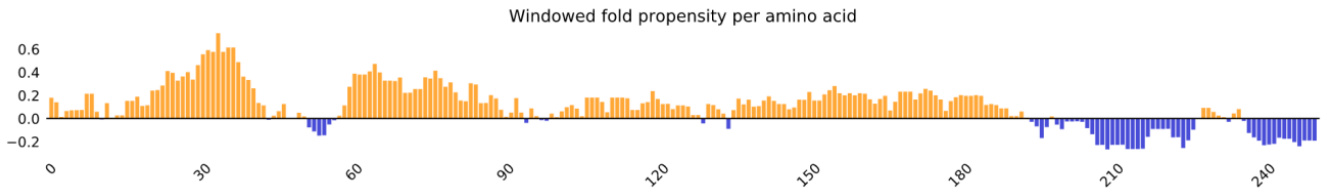

Figure 5 Windowed fold propensity score per amino acid.

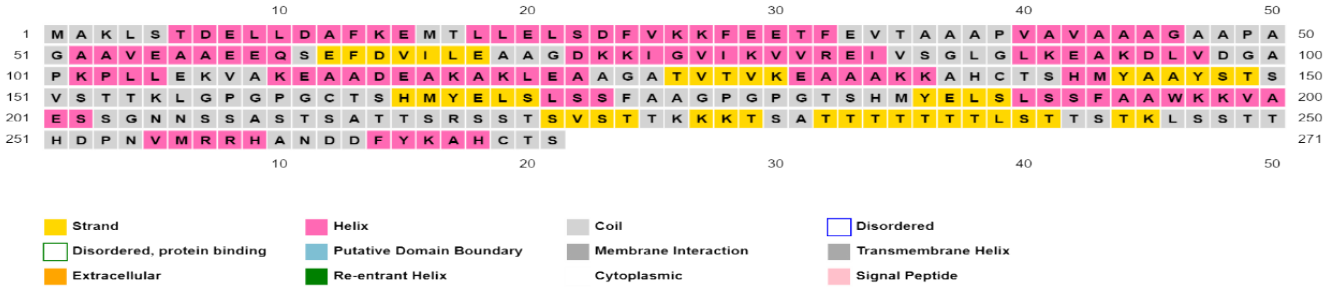

Figure 6 Secodary structure prediction report.

Table 5 The secondary structural features of designated vaccine.

Features	Findings
Alpha helix	113 is 41.70%
310 helix	0 is 0.00%
Pi helix	0 is 0.00%
Beta bridge	0 is 0.00%
Extended strand	44 is 16.24%
Beta turn	0 is 0.00%
Bend region	0 is 0.00%
Random coil	110 is 40.59%
Ambiguous states	4 is 1.48%
Other states	0 is 0.00%

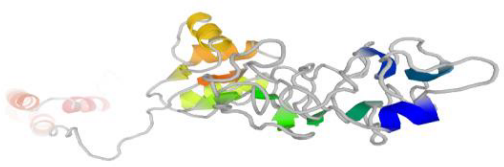

Figure 7 Tertiary structure of designated vaccine.

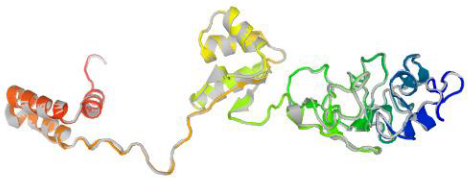

Figure 8 Refined Tertiary structure of designated vaccine.

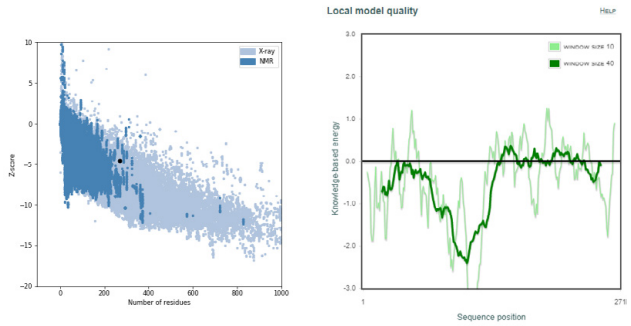

Figure 9 Finalisation of the tertiary formation of the vaccine.

Table 6 The protein structure and overall structure geometry.

Features	Findings
Ramachandran	90.4% core 6.3% allow 1.7% gener 1.7%
All Ramachandrans averages	10 labelled residues (out of 263)
Chi1-chi2	0 labelled residues (out of 115)
Side-chain	5 better 0 inside 0 worse
Residue	Max.deviation: 17.9 Bad contacts: 0
Bond	6.8 Morris et al class: 1 1 2
G-factors	0.29 Covalent: -0.25 Overall: 0.08

product is 5843 bp where the vector was 5363 and insert 480 bp nucleotide base pairs (bp).

Discussion

Difficulty of CMV disease is the point for developing the vaccine against this viral infection. In this world, CMV is the most prevalent fundamental viral plague [59]. Various examinations

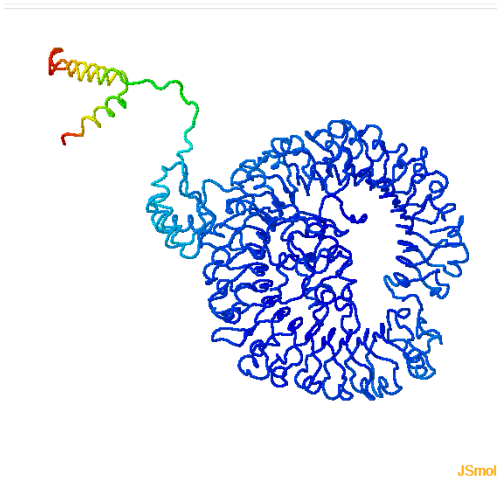

Figure 10 Constructed vaccine after molecular docking.

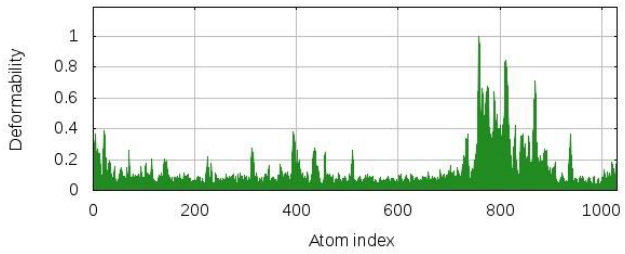

Figure 10A B-factor/Mobility on molecular dynamic simulation.

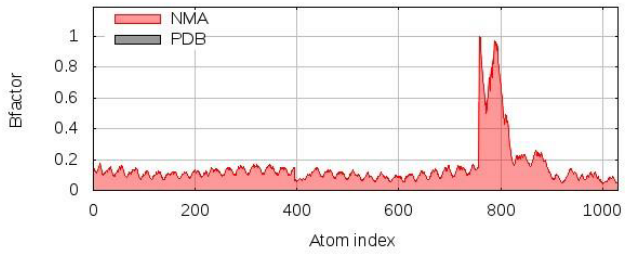

Figure 10B B-factor/Mobility on molecular dynamic simulation.

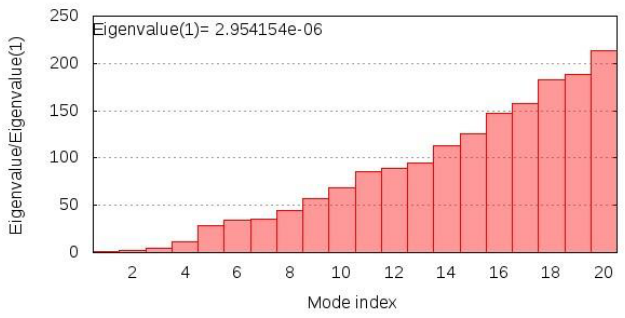

Figure 10C Eigenvalues on molecular dynamic simulation.

Figure 10D Variance on molecular dynamic simulation.

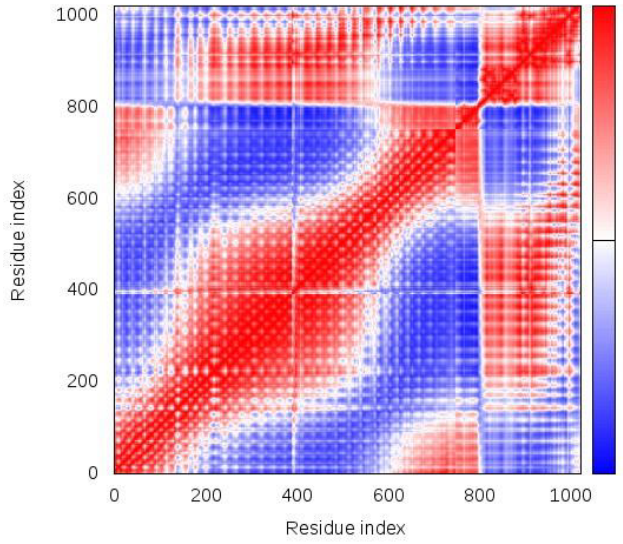

Figure 10E Covariance map on molecular dynamic simulation.

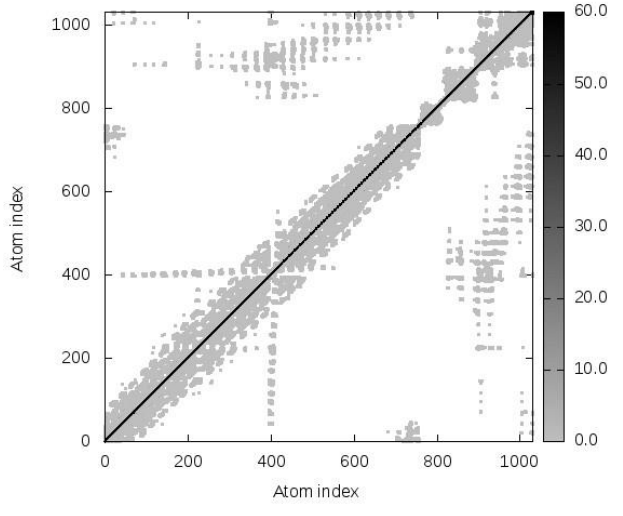

Figure 10F Elastic network on molecular dynamic simulation.

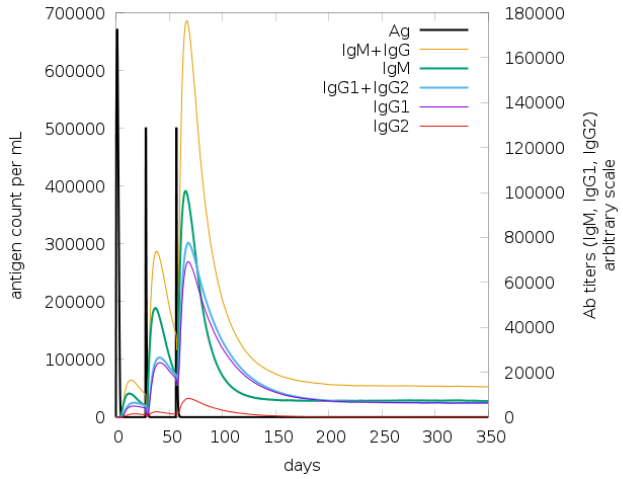

Figure 11A Antigen value for immunoglobulin.

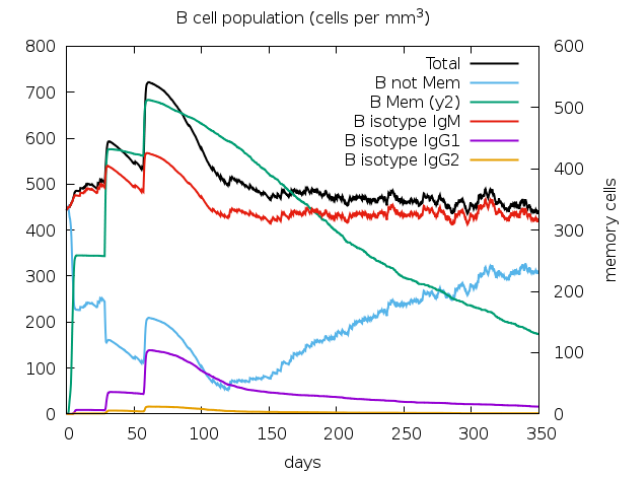

Figure 11B Total B lymphocytes.

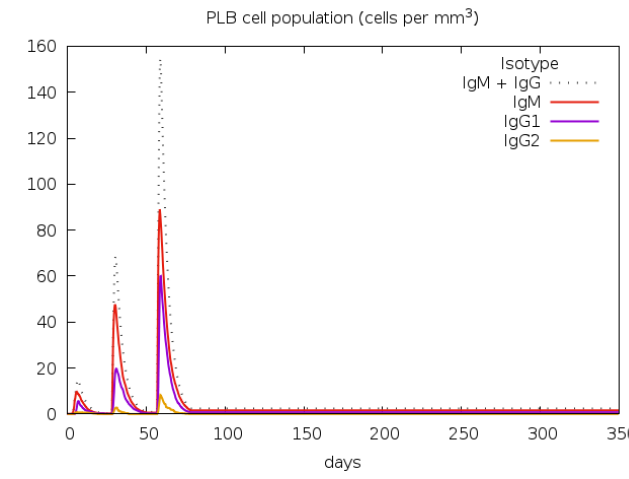

Figure 11C Total Plasma B lymphocytes.

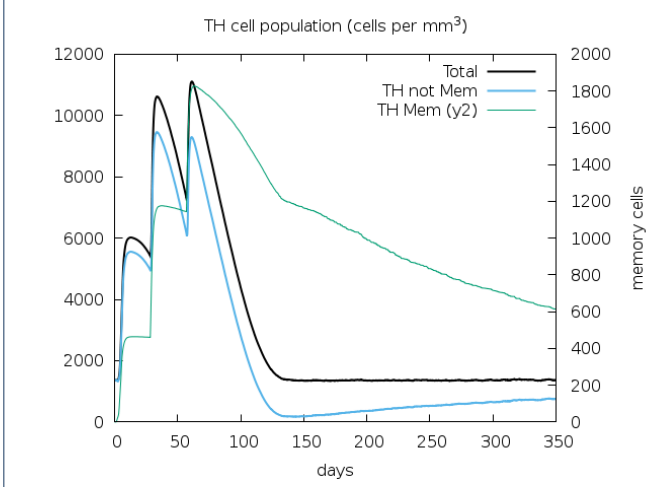

Figure 11D Total CD4 T-helper lymphocytes count.

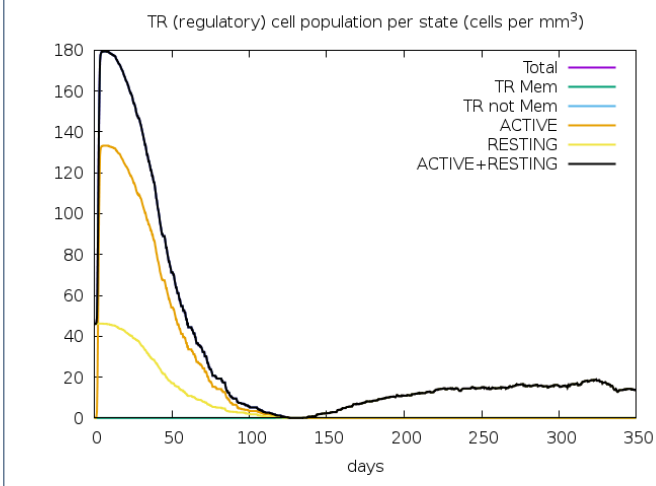

Figure 11E Total CD4 T-regulatory lymphocytes count.

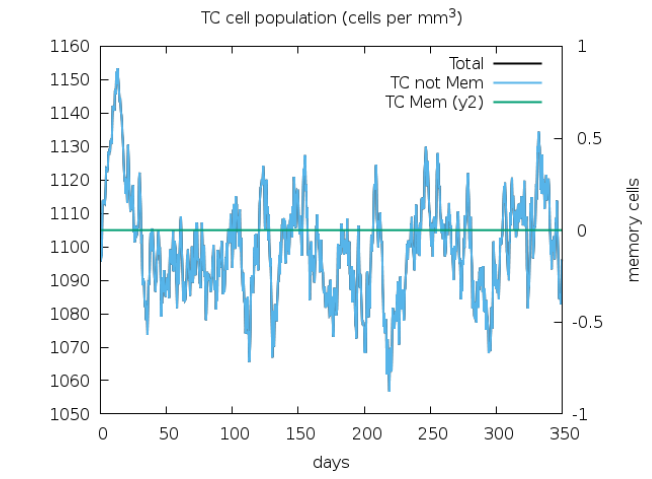

Figure 11F CD8 Total and memory shown.

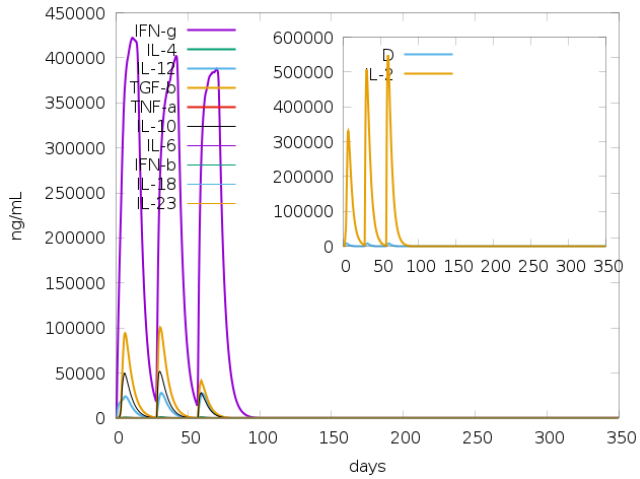

Figure 11G Cytokines and interleukins.

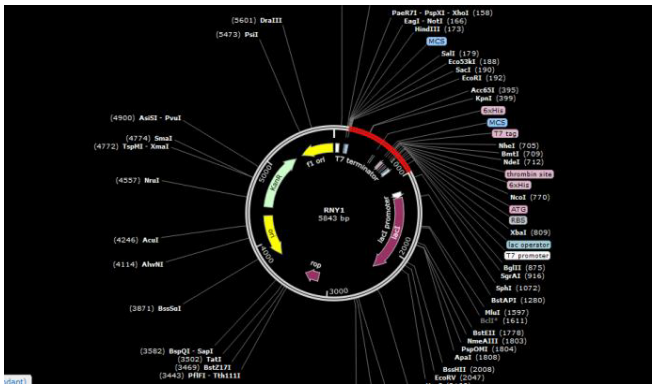

Figure 12 The in silico cloning using pET-28a (þ) vector.

say that this virus is also affecting the new borne individual [60]. There are 40,000 contaminated newborn baby shown yearly with CMV disease USA [61]. This viral infection also affects the human brain disease along with neurological disorder [62]. In the 1990s, the total cost to the USA health centre system associated approximately $1.9 billion regularly, with a normal charge per daughter of up $500,000 [63]. This is the best time to take the vaccine against these HCMV viral diseases [64]. In this study, we abled to formulate an effective vaccine against this cytomegalovirus infection. This manufactured can prevent imminent revolutions [65].

As the spike protein takes the major role to infect human by this virus to transmit into the host, thus we targeted this protein. We took LBL, HTL, CTL epitopes to construct the vaccine [66]. The CTL epitope linked by EAAAK linker that helps them to communicate with the target than any others [67,68]. In the last step we got 316 amino acid residues long vaccine [69,70]. The solubilityvalue of this vaccine is excellent that meant it is able to soluble easily in the host cell and will be able to work out properly. In our studied we got the Zscore (–4.55) and overall good physiochemical characteristics.

This vaccine has a great ability to generate antibody against the HCMV as its antigenicity is 0.4638 along with the nonallergenic and nontoxicity. Our built vaccine is hydrophilic as its Gravy value is -0.199. Therefore this vaccine is simple will intrude into our cell.

Conclusion

Through our investigation, we got the well structured and good physiochemical featured vaccine that can fully prevent the HCMB infection in the human host. According to this process with proteins epitope constructed vaccine will be able to completely eradicate the CMV infection from the human body. So, In wet lab should construct according to this formula for India region.

Acknowledgments

“The authors would like to thank the department of Biotechnology and Genetic Engineering, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, Bangladesh for supporting and discussing about this research article”.

Conflict of Interest

The authors declare no conflict of interest.

Author contributions

MIH, SIM, UHP and MS designed the project; MIH, SIM, UHP and MS performed the experiments; MIH and MS evaluated and interpreted the data; MIH, SIM, UHP and MS prepared the draft manuscript; MIH, SIM, UHP and MS finalized the manuscript. All authors approved the final version of the manuscript.

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