An in-silico approach to design a vaccine against beta lactamase enzyme of mycobacterium tuberculosis

By

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled ‘An in-silico approach to design a vaccine against beta lactamase enzyme of mycobacterium tuberculosis’ submitted by Fatiha Soumaiya Meem (ID: 17146001) of Spring 2021 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy (Hons).

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

More people die from tuberculosis (TB) than any other disease worldwide, which happens due to the presence of drug-resistant forms of Mycobacterium tuberculosis (Mtb). A vaccination against the beta lactamase enzyme of Mycobacterium TB has been developed in this study using cutting-edge computational tools and methods. Using the UniProt Knowledgebase (UniProtKB), the appropriate bacterial enzyme was chosen. Applying the Vaxijen v2.0 platform, antigenicity was anticipated. NetCTL 1.2 server was employed in order to determine the cytotoxic T lymphocytes (CTL). The MHC I alleles specific to CTL were also predicted through NetMHC Pan 4.1 server. The helper T lymphocytes (HTL) were found out with the help of the NetMHC II pan 4.0 tool. Using IFN epitope servers, IL4 pred servers, and IL10 pred servers, respectively, the productivity of interferon gamma, IL4, and IL10 of the CTL and the HTL were anticipated. Prediction of epitopes for B cells were done by employing Bepipred linear epitope prediction model 2.0. Considering their toxicity, antigenicity, and allergenicity, the B and T cells underwent additional testing. Linkers were inserted in between the adjuvant and epitopes for B and T cells to create the vaccine. To forecast the vaccine's allergenicity, antigenicity, toxicity, and other physiochemical features, various bioinformatics approaches were employed. The PROTPARAM server was used to perform biochemical analysis of the proposed vaccination. Phyre2 was utilized in order to validate the 3D structure of vaccine candidate. Patchdock tool was used to perform the docking between the obtained 3D structure with toll-like-receptor (TLR-3). To perform the Ramachandran plotting and Z scores analysis Swiss PDP plotter and Prosaweb servers were used respectively. Lastly, the immune simulations were carried out utilizing C-Immsim.

Keywords: In-silico, vaccine candidates, epitopes, beta lactamase enzyme, mycobacterium tuberculosis.

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Chapter 1 Introduction

In terms of diseases with the greatest rates of human mortality, the ancient disease throughout the history is tuberculosis (TB) that has not yet been fully addressed. The WHO estimates that 1.7 billion people, or 23% population of the world, are contaminated via the TB bacilli in 2019. The number of cases of tuberculosis is 10,000,000 and the number of people who die from the disease is 1,200,000. Of the 10,000,000 cases, 6,400,000 records of the patients are kept, while 3,600,000 go unacknowledged (Camberg et al., 2013). Use of irregular antibiotic and insufficient treatment are the consequences due to the spread of MDR-TB strains. Lower immunity makes people more resistant to infection and less able to use their immune systems effectively. As a result, greater efforts are needed to progress the development of novel TB vaccines (Bhattacharya et al., 2021). Effective T cell responses are crucial for the eradication of Mycobacterium tuberculosis. Developing effective treatments for TB requires an understanding of the disease's complex pathophysiology, Mycobacterium tuberculosis (Mtb's) slow growth, and dormant nature (Chen et al., 2020). The only recognized TB vaccine for prevention that is utilized globally is made from a weakened form of Mycobacterium bovis, also referred to as the Bacillus Calmette-Guérin (BCG) vaccine, is in use since 1923 (Rueckert & Guzmán, 2012). The effectiveness of BCG against adolescent pulmonary TB ranges from 0 to 80%, providing protection from inoculation for 10–20 years (Schneidman-Duhovny et al., 2005). Because of the dangers associated with its usage in immunocompromised people and the potential for reverting the virus to its virulent stage, BCG has a low safety rating as a live-attenuated vaccine. (Ann Detmer et al., 2006). Currently, 16 TB vaccinations are available for clinical trials in phases 1, 2, and 3 (Waterhouse et al., 2018). Considerable promise among the approaches, which has been used in generation for highly potent new vaccines for TB are due to the use of a range of epitopes for the Mtb vaccination. It has been claimed that finding vaccinations with unique immunostimulatory qualities could boost the possibility in order to discover a reliable vaccination in addition to providing helpful recommendations on the prospective development of the Mtb vaccine. (Rodo et al., 2019). This is a study where different computational methods have been used to create a vaccination based on epitopes against the Mtb antigen. Here different immunoinformatic methods were used to demonstrate the immunogenicity and effectiveness of the designed vaccine.

1.1 Mycobacterium tuberculosis and beta lactamase

Multi drug and widely tolerant TB have to be taken as key considerations to halt the spread and the prevention of TB. The main reason for this resistance is the existence of an enzyme known as beta lactamase that is responsible for the inactivation of the beta lactams and beta lactam antibiotics. This recognized beta-lactamase, designated BlaC, hydrolyzes penicillins and cephalosporins and is chromosomally encoded (Wishart et al., 2015). Beta lactamase that belongs to the Ambler class A group is sensitive to inhibition when the beta lactamase antagonists are used that are commercially available (Sebastian G Kurz et al., 2012). MTB is extremely resistant to b-lactams due to its great constitutive b-lactamase activity. Nevertheless, b-lactamase inhibitors created for different purposes have activity against the MTB b-lactamase (Giri et al., 2021). The combination therapy of B-lactam/b-lactamase inhibitor has recently seen a boost in interest due to the increase of multidrug-resistant tuberculosis. This is because, the beta lactamase enzyme is expressed genetically due to which cephalosporins and penicillins are hydrolyzed. The resistance is also observed in the action of carbapenems due to the existence of beta lactamase of Mycobacterium tuberculosis as it slowly hydrolyzes carbapenems. Recent studies have shown that beta-lactam antibiotics in particular, when combined to beta lactamase, are predicted to develop potential response towards the bacteria (Källberg et al., 2012). This study is a small approach to design a probable vaccine against the beta lactamase enzyme using advanced immunoinformatic tools and methods to observe the effectiveness and immunogenicity against M. tuberculosis. Our research proposed that the selected antigen beta lactamase enzyme [Rv2068c] could be used as a potential candidate since the epitopes for B and T cell may potentially trigger defense that is cellular and humoral (Khan et al., 2019).

Chapter 2 Methodology

Figure 1 displays a flowchart outlining the steps taken while a multi-epitope vaccine was being developed.

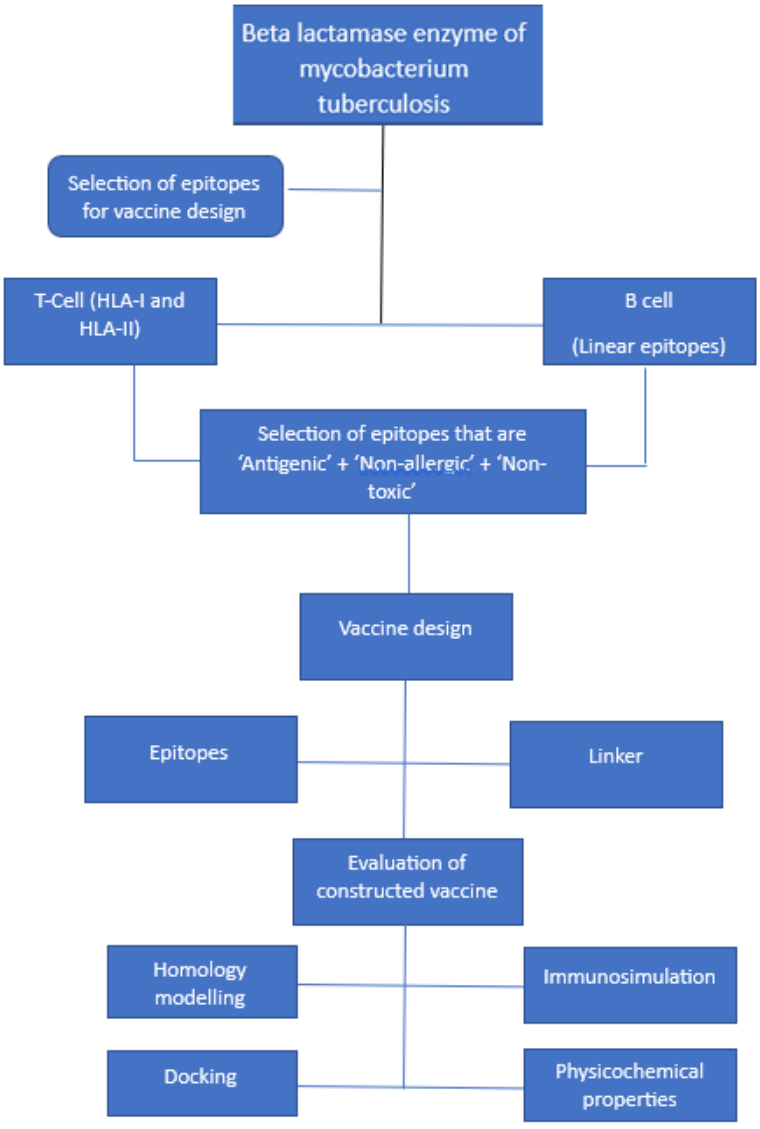


Figure 1: Schematic diagram of the designed vaccine (Camberg et al., 2013)

2.1 Selection of the Mtb antigen:

The sequence of beta lactamase of Mycobacterium tuberculosis was explored with the help of Uniprot Knowledgebase (UniProtKB) (https://www.uniprot.org/) (Mulder et al., 2007). Since the Multi drug resistant tuberculosis is creating a serious problem, the beta lactamase enzyme is chosen as the potential candidate for the vaccine preparation as it is the main reason for the resistance in different drugs. The resistance occurs due to the alteration of beta lactamase profile. Beta lactamase antagonists for example clavulanate and sulbactam when given alongside beta lactam inhibitors such as carbapenems, have somehow served to evade the resistance mechanism. The Uniprot Knowledgebase's fasta format was used to download the beta lactamase enzyme's complete sequence. To assess the protein's antigenicity, the Vaxijen 2.0 system was utilized (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (Jespersen et al., 2017). Bacteria was classified as a category for the intended microorganism and 0.5 was set as a benchmark to assess the sequence's antigenicity. Further screening was performed for the identification of the protein sequence's epitopes for B and T cells.

2.2 Determination of Epitopes for Cytotoxic T- Lymphocyte (CTL)

It has been demonstrated that NetCTL-1.2 has great predictive power. With strong sensitivity and specificity at 0.75, the NetCTL 1.2 was employed for the prediction of the epitopes for the CTL of the beta lactamase enzyme of Mycobacterium tuberculosis (Jensen et al., 2018). Epitopes specific to MHC class I were anticipated, and for their anticipation A1 supertype was chosen. The most promising approaches for TB vaccine development were chosen using a dosage (IC50) of 500 nm which is inhibitory half-maximum and a combined score as guidelines (Russell et al., 2017). The epitopes appear to have a considerable affinity for the receptor, as indicated by the IC50 value of 500 nm. The integrated score, TAP transport effectiveness, specificity to type I, along with the prediction for cleavage of the protein were all used to forecast each. Tap transport efficiency was assessed at 0.05 and the cleavage at the C-terminal was weighed at 0.15.

2.3 MHC I Alleles identification

The MHC I alleles unique to CTL epitopes were found by employing NetMHC Pan 4.1 tool (https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1). A lower percentile rank is associated with a stronger epitope-allele binding affinity. Epitopes with a percentile level ranging up to 0.500 were chosen as strong binders while those ranging from 0.500 to 2.00 were chosen as

the weak binders. The vaccine formulation no longer contained the epitopes with a percentile rank higher than this cutoff. Finally, input from the NetCTL server was used to ascertain the strength of the binding affinity (Paul et al., 2015).

2.4 Screening of the epitopes for Cytotoxic T Lymphocyte (CTL)

To find out whether the identified epitopes for CTL were either allergic or antigenic or toxic, they were filtered for later use. Using AllerTOP v.2 database (http://www.ddg-pharmfac.net/ AllerTOP), the allergenicity was predicted. Identified epitopes subsequently provided in basic form to the server which in turn returned the result page displaying allergen status as "Probable Allergen" or "Probable Non-allergen" (Wieczorek et al., 2017).

The epitopes with the status of "Probable Non-allergen" have been considered for the vaccine's preparation. To determine whether the CTL epitopes were antigenic or not, a server named Vaxijen 2.0 (http://www.jenner.ac.uk/VaxiJen.) was utilized. Maintaining a score of 0.5 as threshold level, the antigenicity of each of the epitopes were checked and only the probable antigens were chosen for the vaccine construction (Calis et al., 2013). In order to assess if the epitopes were toxic or not, ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) server was employed. The epitopes were submitted for virtual screening in the batch submission module of the server from which only the epitopes with "Non-Toxin" status were taken for additional vaccine design (Lund et al., 2004).

2.5 Screening of epitopes for Helper T Lymphocyte (HTL)

Protein sequence's epitopes for HTL were anticipated utilizing the server NetMHC II pan 4.0 (http://www.cbs.dtu.dk/services/NetMHCIIpan-4.0/). The primary antigen which has been submitted in server maintained a length of 15 as the baseline of the tool (Marek Wieczorek et al., 2017). It has been known that for a greater binding affinity the epitopes should have a lower percentile rank score. To predict the strong binders the percentile level was set at 2 and that for the weak binders the percentile level was set at 10. The strong binding epitopes were chosen for the vaccine construction (M. V. Larsen et al., 2005).

2.6 Cytokine inducing capability of predicted HTL Epitopes

It is essential for an antigen to induce the interferon gamma cytokine as it has a crucial impact on the development of innate and adaptive immunity. The inducing potential of the epitopes for HTL was anticipated employing IFN epitope server (http://crdd.osdd.net/raghava/ifnepitope/scan.php).

Overlapping sequences are being determined by the server and among them only the epitopes which induces IFN-gamma are anticipated, and predicted using a model named Support Vector Machine (SVM). Only the IFN-gamma inducing epitopes were finally chosen for further vaccine development (M. V. Larsen et al., 2005). The prediction of the productivity of Interleukin 4 (IL-4) was necessary as the production of IL-4 will stimulate the activity of the HTL cells and the B cells and the prediction of productivity of IL-10 was also essential as it will balance the inflammatory condition that stimulates the immune system (Gandharva Nagpal et al., 2017). IL10Pred (https://webs.iiitd.edu.in/raghava/il10pred/), IL4Pred (https://webs.iiitd.edu.in/raghava/il4pred/) were used to predict the productivity of these cytokines by the HTL epitopes. Standard variables were set in order to conduct these studies to extract the most effective ones (M. v Larsen et al., 2007).

2.7 Screening of epitopes for B-cell

For eliciting a humoral immune response, A major role in the stimulation of B cells is played by the anticipation of B cells to produce antibodies and plays a significant part in designing vaccines. The antigen was exposed to the BepiPred linear epitope prediction server (http://tools.iedb.org/bcell/result/). To identify the epitopes for B cell, 0.5 was set as a threshold and 20-mer was set as the window length (Doytchinova & Flower, 2007).

2.8 Anticipation of allergenicity, antigenicity and toxicity for selected epitopes

The vaccine candidate's need for antigenic, anti-allergen, and anti-toxin epitopes is a key component in designing a vaccination. A bioinformatic tool named Vaxijen 2.0 was utilized to anticipate the antigenicity for selected HTL epitopes and the epitopes for B cell where 0.5 was maintained as a threshold level. The VaxiJen algorithm evaluates the physiochemical characteristics of any proteins or epitopes to identify them as antigens. It is primarily focused upon sequence alignment process (Rajput et al., 2021). AllerTOP v2.0 a bioinformatic system (http://www.ddg-pharmfac.net/AllerTOP) was employed to forecast how allergenic the epitopes were. This server explores the physiochemical characteristics of proteins to build the auto cross-covariance (ACC) transformation, (kNN) k closest neighbors were the approaches to classify the allergens which also includes the machine learning approach with the amino acid E-descriptors (Ivan Dimitrov et al., 2014).

Finally, for the anticipation of toxicity of each epitope, a database named ToxinPred was utilized

(https://webs.iiitd.edu.in/ raghava/toxinpred/multi_submit.php) (W. Liu et al., 2003). For creation of the vaccine, antigenic, anti-allergenic, and anti-toxic epitopes were selected.

2.9 Construction of the vaccine:

For the final vaccine design, highly immunogenic, anti-toxic, and anti-allergenic epitopes for B cell, CTL and HTL were chosen. Specific linkers were inserted to join the epitopes with the adjuvant which will ultimately increase immunogenicity and epitope expression, which increases the efficacy of molecular vaccination. The KK linkers were utilized between the epitopes for B cells whereas, the GPGPG linkers and AAY linkers were inserted respectively between the epitopes of HTL and CTL. With the help of the EAAAK linker, the beta lactamase enzyme of Mycobacterium Tuberculosis was joined as an adjuvant. The constructed vaccine was then submitted for further evaluation (CZUB et al., 2005).

2.10 Prediction of antigenicity

A tool named VaxiJen 2.0 was utilized to determine whether the proposed vaccination was antigenic or not (http://www.ddg-pharmfac.net/Vaxijen/ VaxiJen/VaxiJen.html) where 0.5 was kept as a threshold level. The prediction of antigenicity is significant for the confirmation of the activation of defense mechanism body when vaccine is inserted inside the host body.

2.11 Solubility and physiochemical properties prediction

Evaluation of physiochemical characteristics of the finished vaccination were done by ProtParam program (http://web.expasy.org/protparam/). Theoretical isoelectric point (pI), amino acid count, formula, molecular weight, atomic and amino acid composition, extinction coefficients, instability index, approximate half-life and grand average of hydropathicity (GRAVY) were among the examined physiochemical properties. The theoretical pI and molecular weight were determined using sequences entered by the user, and the compositions for atomic and amino acid were obvious. Determination of the protein's extinction coefficient was done using its amino acid composition information (Ortiz-Prado et al., 2020).

2.12 Prediction of Toxicity and Allergenicity

The Toxin and Toxin Target Database (T3DB) (www.t3db.ca) was used to forecast the effectiveness of our proposed vaccination. This tool puts an emphasis on outlining each toxin's toxicity mechanisms and target proteins (Tomar & De, 2014).

Since the allergenic proteins cause adverse effects, the vaccination shouldn't be allergic. AllergenOnline (http://www.allergenonline.org) a bioinformatic tool was employed to evaluate the anti-allergenic character of vaccination sequence (Lu, 2020).

2.13 Homology modeling of vaccine to generate 3D model

The protein in the proposed vaccine had been rebuilt, and there was no identifiable homology that was known or recorded in any database. A structure-based folding simulation is used by Phyre2 to simulate portions of proteins that lack obvious homology. Using the Phyre 2 server, the anticipated vaccine's three-dimensional structure was predicted(http://www.sbg.bio.ic.ac.uk/phyre2/). Simple framework folding simulation is used by this application to model several templates in order to create a 3D model of a protein sequence which is of full length (Neuman et al., 2011).

2.14 Ramachandran Plotting and Evaluation of the Tertiary Structure for Vaccine's Quality

To assess the created vaccine's tertiary structure, the SWISS-MODEL workstation generated a Ramachandran plot. In protein structure, the amino acid residues show advantageous positions for the backbone dihedral angles with the help of Ramachandran plot. The low-quality residues in the system or model could be easily located by using the page which shows Structure Assessment, which displays the best Molprobity ratings. With the help of a program called ProSA-web (https://prosa.services.came.ac.at/prosa.php) the proteomic composition of the created vaccine was verified. A component of a 3D protein model with a high Z-score is unreliable or inaccurate whereas a negative Z-score value determines the model's overall quality (Knoops et al., 2008).

2.15 Constructed vaccine's molecular docking with immune receptors

To trigger a successful immune reaction, the interaction between the antigen and an immune receptor plays a crucial role. Protein Databank (PDB) (https://www.rcsb.org) was employed to obtain receptor TLR3 (PDB ID: 1ZIW). The recommended vaccine construct's binding affinity to these receptors was verified by utilizing the PatchDock service (https://bioinfo3d.cs.tau.ac.il/PatchDock/). For the prediction of prospective complexity, above mentioned server used 3 algorithms: depiction of the molecular form, filtering, matching of the surface patch, and scoring (L. Liu et al., 2011).

2.16: Immune Simulations

With the help of the C-ImmSim (http://www.cbs.dtu.dk/services/C-ImmSim-10.1/) web server, dynamic immune response simulations of the chosen vaccine build were carried out in order to understand profile of immunological response and the immunogenicity of the vaccine constructed (Gralinski & Menachery, 2020). Position-Specific Scoring Metrics (PSSM) are used by this system to mimic immunological response. By combining lymphocyte receptors with the amino acid composition of epitopes which are antigenic, the immune reactions are stimulated by C-ImmSim. For submitting the constructed vaccine, the values of all the settings were maintained at default in the C-ImmSim web server (Hoffmann et al., 2020). At intervals of four weeks, three doses of the preventive tuberculosis vaccine's target product were given in injectable form where 1, 84 and 168 were set as time periods (Li et al., 2003).

2.17 Remarks on the Materials and Method:

The in-silico method was used for the extensive investigation, which means that all analyses and predictions were produced using internet servers. It cannot be stated with certainty that the designed vaccine will be extremely effective at curing the tuberculosis infection. It has the potential to be a candidate for a vaccine, thus it is thought that more research is required.

Chapter 3

Results

3.1 Antigenicity prediction of Beta Lactamase enzyme (blaC)

Mycobacterium TB H37Rv's amino acid sequence of the Beta Lactamase enzyme (Blac) was downloaded in FASTA format with the help of a database known as Uniprot Knowledgebase (https://www.uniprot.org/uniprot/Q9K2Y1).

Organism: Mycobacterium Tuberculosis | Strain Name:H37Rv | Protein Name: Ambler class A beta-lactamase |Gene Symbol: **blaC,** Rv2068c.

The full protein sequence downloaded in the fasta format is given below:

```
MRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPAGADLADRFAELERRYDARLG
VYVPATGTTAAIEYRADERFAFCSTFKAPLVAAVLHQNPLTHLDKLITYTSDDIRSISPV
AQQHVQTGMTIGQLCDAAIRYSDGTAANLLLADLGGPGGGTAAFTGYLRSLGDTVSRLDA
EEPELNRDPPGDERDTTTPHAIALVLQQLVLGNALPPDKRALLTDWMARNTTGAKRIRAG
FPADWKVIDKTGTGDYGRANDIAVVWSPTGVPYVVAVMSDRAGGGYDAEPREALLAEAAT
CVAGVLA
```

A bioinformatic tool named VaxiJen v2.0 was utilized for the assessment of the antigenicity quality of the protein sequence that has been retrieved earlier (http://www.ddg-pharmfac.net/vaxijen/). Maintaining a criterion level at 0.5, obtained prediction score was 0.5478 which determines that the sequence was a probable antigen. (Figure 2).

Figure 2: Antigenicity score on the VaxiJen v2.0 server (Doytchinova & Flower, 2007).

3.2 Identification of CTL epitope

The A1 supertype of MHC I allele epitopes were found by employing Net CTL 1.2 service (https://services.healthtech.dtu.dk/service.php?NetCTL-1.2), that was used to find CTL epitopes. The total score is a crucial influence in epitope selection. The minimal limits for this combination score, which is based

Table 1: List of the selected epitopes for CTL that were successful in binding to alleles which are MHC-I A1-supertype and met the criteria for being antigenic, anti-allergen, and anti-toxin (M. V. Larsen et al., 2005).

CTL Epitope	**MHC binding affinity**	**Combined score**	**antigenicity**	**allergenicity**	**toxicity**
QLCDAAIRY	0.3207	1.6424	antigen	Non allergen	Non toxin
MSDRAGGGY	0.7565	3.4975	antigen	Non allergen	Non toxin

upon TAP transport efficiency with a value of 0.15 whereas, 0.05 was determined to set as a value of cleavage in the C terminal. Only two of these anticipated epitopes for CTL were used for the creation of vaccine because of their greater combined values and specificity for binding to MHC-I. They were also represented as antigenic, non-allergen, non-toxin, displayed in (Table 1).

3.3 MHC I alleles with CTL epitope-specificity

Previously mentioned epitopes used as input to acquire MHC I alleles by employing NetMHC Pan 4.1 algorithm (https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1). In this case, a metric called percentile rank is employed to pick epitopes; a decreased percentile rank indicates a stronger binding capacity, and vice versa. The two CTL epitopes selected with a lower percentile rank shows as the strong binder (sb) for the specific allele. In this case, a minimum threshold of 2 was specified for epitope selection. Here is a list of MHC I allele-specific binding sites for CTL epitopes, along with the related binding affinity in percentile rank.

Table 2: MHC I allele for specific epitopes along with their percentile rank and binding level (Calis et al., 2013)

Allele	**CTL Epitope**	**Length**	**Percentile Rank**	**Binding Level**
HLA- A*01:01	MSDRAGGGY	9	0.017	Sb
HLA- A*01:01	QLCDAAIRY	9	0.443	Sb

Pos	MHC	Peptide	Core	Of	Gp	Gl	Ip	Il	Icore	Identity	Score_EL	%Rank_EL	Score_BA	%Rank_BA	Aff(nM)	BindLevel
278	HLA-A*01:01	MSDRAGGGY	MSDRAGGGY	0	0	0	0	0	MSDRAGGGY	Sequence	0.9689210	0.017	0.801053	0.009	8.61	<= SB
133	HLA-A*01:01	QLCDAAIRY	QLCDAAIRY	0	0	0	0	0	QLCDAAIRY	Sequence	0.3628080	0.443	0.253061	1.058	3234.77	<= SB
141	HLA-A*01:01	YSDGTAANL	YSDGTAANL	0	0	0	0	0	YSDGTAANL	Sequence	0.3245280	0.500	0.368433	0.441	928.36	<= WB
66	HLA-A*01:01	TGTTAAIEY	TGTTAAIEY	0	0	0	0	0	TGTTAAIEY	Sequence	0.0882240	1.391	0.149264	3.020	9044.50	<= WB
54	HLA-A*01:01	RYDARLGVY	RYDARLGVY	0	0	0	0	0	RYDARLGVY	Sequence	0.0701420	1.611	0.163466	2.537	8528.02	<= WB
248	HLA-A*01:01	IDKTGTGDY	IDKTGTGDY	0	0	0	0	0	IDKTGTGDY	Sequence	0.0591970	1.788	0.116294	4.994	14207.18	<= WB
177	HLA-A*01:01	RLDAEEPEL	RLDAEEPEL	0	0	0	0	0	RLDAEEPEL	Sequence	0.0476810	2.043	0.105708	5.992	15931.30	

Figure 3: Prediction results for MHC 1 alleles specific to CTL epitopes in NetMHC pan server (Jensen et al., 2018).

3.4 Allergenicity, Toxicity and Antigenicity prediction for CTL epitopes

A bioinformatic tool AllerTOP v2.0 (http://www.ddg-pharmfac.net/AllerTOP) was utilized to assess whether selected CTL epitopes were allergen or not. A method called ToxinPred which is based on support vector machine (SVM) was utilized to anticipate whether epitopes of CTL were toxic or not (https://webs.iiitd.edu.in/ raghava/toxinpred/multi_submit.php). In order to evaluate epitope antigenicity, VaxiJen v2.0 was utilized (http://www.ddg-pharmfac.net/Vaxijen/ VaxiJen/VaxiJen.html). The predicted two CTL epitopes passed all the screening test where the epitopes represented as antigens, neither allergen nor toxic as shown in (Table 1).

Peptide ID	Peptide Sequence	SVM Score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol wt
seq1	MSDRAGGGY	-0.93	Non-Toxin	-0.17	-0.76	0.22	0.00	1076.28
seq2	QLCDAAIRY	-0.36	Non-Toxin	-0.13	0.16	-0.17	0.00	1215.52

Figure 4: Prediction of Toxic peptides on ToxinPred server(Bhattacharya et al., 2021)

3.5 MHC II alleles specific to HTL epitopes

The core antigen is used as an input by the NetMHCIIpan 4.0 server to identify MHC II alleles. Percentile rank can be used to distinguish between MHC II alleles; a low percentile rank denotes a higher binding affinity, and vice versa. Only strong binding peptides were chosen for the investigation. The Strong binding peptides threshold (%Rank) is 2% and the Weak binding peptides threshold (%Rank) is 10%.

3.6 Capability of HTL epitopes of inducing cytokine

Initially, the interleukin-producing ability of HTL epitopes, specifically the IFN epitope prediction, IL-4 and IL-10 productivity were discovered. Utilizing the servers IFN epitope, IL-4pred, and IL-10pred, these predictions were made. For IL4 and IL10 pred servers, the SVM technique was applied with default thresholds of 0.2 and -0.3 respectively. The HTL epitopes were

found to give a positive score for the IFN productivity prediction and were inducers for both the IL10 and IL4 inducing predictions as presented in (Table 3).

Table 3: IFN, IL4 and IL10 prediction for HTL epitope(M. V. Larsen et al., 2005)

HTL epitope	IFNγ	IL4	IL10
GVYVPATGTTAAIEY	POSITIVE	INDUCER	INDUCER
GFPADWKVIDKTGTG	POSITIVE	INDUCER	INDUCER
LADRFAELERRYDAR	POSITIVE	INDUCER	INDUCER
LCDAAIRYSDGTAAN	POSITIVE	INDUCER	INDUCER

3.7 B-cell epitope prediction

The BepiPred linear epitope identification 2.0 (http://tools.iedb.org/bcell/) was put in use to locate linear B-cell epitopes, which were discovered at threshold of 0.5. The table below displays various B-cell epitopes' beginning and ending positions as well as their lengths. Only the epitopes with a length of more than 20 mer were chosen for the further screening and vaccine construction.

Table 4: Predicted peptides with start, end, and length(Paul et al., 2015)

No	Start	End	Peptide	Length
1	5	8	GFGR	4
2	26	47	RHASGARPASTTLPAGADLADR	22
3	49	51	AEL	3
4	73	79	EYRADER	7
5	100	103	LTHL	4
6	108	129	TYTSDDIRSISPVAQQHVQTGM	22
7	156	159	GPGG	4
8	171	195	LGDTVSRLDAEEPELNRDPPGDERD	25
9	214	220	ALPPDKR	7
10	228	242	ARNTTGAKRIRAGFP	15
11	281	291	RAGGGYDAEPR	11

3.8 Screening of the B cell epitopes:

Determination for the antigenicity was done with the employment of VaxiJen v2.0

(http://www.ddg-pharmfac.net/Vaxijen/ VaxiJen/VaxiJen.html). Prediction of being allergen was completed utilizing AllerTOP v2.0 server (http://www.ddg-pharmfac.net/AllerTOP). Further screening was accomplished by predicting the toxicity using a tool named ToxinPred (https://webs.iiitd.edu.in/ raghava/toxinpred/multi_submit.php). Screened epitopes for B cell were found as antigen, anti-allergen, anti-toxin respectively as shown the table below:

http://www.ddg-pharmfac.net/AllerTOP). Further screening was accomplished by predicting the toxicity using a tool named ToxinPred (https://webs.iiitd.edu.in/ raghava/toxinpred/multi_submit.php). Screened epitopes for B cell were found as antigen, anti-allergen, anti-toxin respectively as shown the table below:

Table 5: Detection of epitopes for B cells

B cell epitope	**Antigenicity**	**Allergenicity**	**Toxicity**
RHASGARPASTTLPAGADLADR	Antigen	Non-allergen	Non-toxin
TYTSDDIRSISPVAQQHVQTGM	Antigen	Non-allergen	Non-toxin
LGDTVSRLDAEEPELNRDPPGDERD	Antigen	Non-allergen	Non-toxin

3.9 Construction of Final proposed vaccine:

In order to create a unique vaccine, three epitopes for B cell, four epitopes for HTL, and two epitopes for CTL were proposed that meet the requirements of a stronger affinity for binding, antigenicity, nontoxicity, and non-allergenicity, (Walls et al., 2020). The Beta lactamase enzyme (blaC) of Mycobacterium Tuberculosis that has been extracted was considered as the adjuvant. The adjuvant was fused with the CTL epitopes using the “EAAK” linker. In between the CTL epitopes “AAY” was used. The epitopes for HTL were connected with the CTL epitopes and within themselves using the “GPGPG” linker. In the end of the construction of vaccine, the “KK” linker was inserted for fusing epitopes for B cell with the epitopes of HTL and within themselves (Ilyicheva et al., 2022). The linkers will aid in maintaining the designed vaccine’s stability and will also increase the vaccine’s immunogenicity (Gruca et al., n.d.). The vaccine constructed is represented below:

MRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPAGADLADRFAELERRYDA
RLGVYVPATGTTAAIEYRADERFAFCSTFKAPLVAAVLHQNPLTHLDKLITYTSDDIRSIS
PVAQQHVQTGMTIGQLCDAAIRYSDGTAANLLLADLGGPGGGTAAFTGYLRSLGDTVS
RLDAEEPELNRDPPGDERDTTTPHAIALVLQQLVLGNALPPDKRALLTDWMARNTTGA
KRIRAGFPADWKVIDKTGTGDYGRANDIAVVWSPTGVPYVVAVMSDRAGGGYDAEPR
EALLAEAATCVAGVLA**EAAAK**MSDRAGGGY**AAY**QLCDAAIRY**GPGPG**GVYVPATGT
TAAIEY**GPGPG**GFPADWKVIDKTGTG**GPGPG**LADRFAELERRYDAR**GPGPG**LCDAAIR
YSDGTAAN**KK**RHASGARPASTTLPAGADLADR**KK**TYTSDDIRSISPVAQQHVQTGM**K**
KLGDTVSRLDAEEPELNRDPPGDERD

3.10 Anticipation of antigenicity, allergenicity and toxicity of the constructed vaccine

Constructed vaccine's ability to be an antigen was anticipated using a tool named VaxiJen v2.0 (http://www.ddg-pharmfac.net/Vaxijen/ VaxiJen/VaxiJen.html) where a limit of 0.5 was maintained where the vaccine came out to be as probable antigen with an increase in its antigenicity. The AllergenOnline server (http://www.allergenonline.org) was put in use to determine vaccine's sensitivity towards the allergy. To effectively assess the efficiency of the protein, a value of 0.5 was chosen based on z-score analysis, and in this case, the Full FASTA 35 technique was used. This vaccination was discovered for being allergen-free. Furthermore, to determine whether the vaccination was toxic or not a tool named T3DB was utilized (http://www.t3db.ca/). The result in the server web page showed that the vaccine was non-toxic.

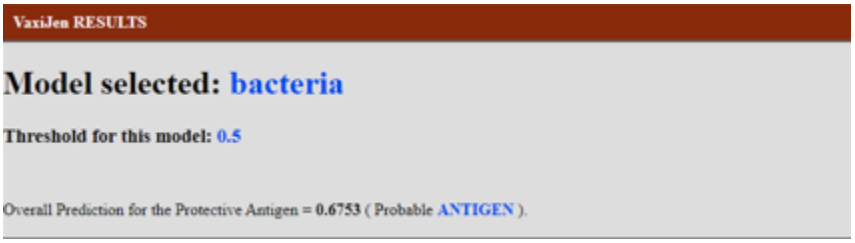


Figure 5: Results of antigenicity prediction (Walls et al., 2020)

```
# fasta36.exe -q -B -m 9i -w 80 -E 0.5 -d 20 C:\Windows\Temp\all4DB5.tmp version2136.fasta
FASTA searches a protein or DNA sequence data bank
 version 36.3.8g Oct, 2018
Please cite:
 W.R. Pearson & D.J. Lipman PNAS (1988) 85:2444-2448

Query: C:\Windows\Temp\all4DB5.tmp
  1>>>query - 487 aa
Library: version2136.fasta
   540227 residues in  2233 sequences

Statistics:  Expectation_n fit: rho(ln(x))= 9.4224+/-0.00747; mu= 5.1176+/- 0.370
 mean_var=63.5415+/-16.095, 0's: 0 Z-trim(84.0): 18  B-trim: 21 in 1/35
 Lambda= 0.160896
 statistics sampled from 557 (557) to 557 sequences
Algorithm: FASTA (3.8 Nov 2011) [optimized]
Parameters: BL50 matrix (15:-5), open/ext: -10/-2
 ktup: 2, E-join: 1 (0.575), E-opt: 0.2 (0.249), width:  16
 Scan time:  0.000
!! No sequences with E() < 0.5
>>>///
```

Figure 6: Prediction of the constructed vaccine (Gendel & Jenkins, 2006)

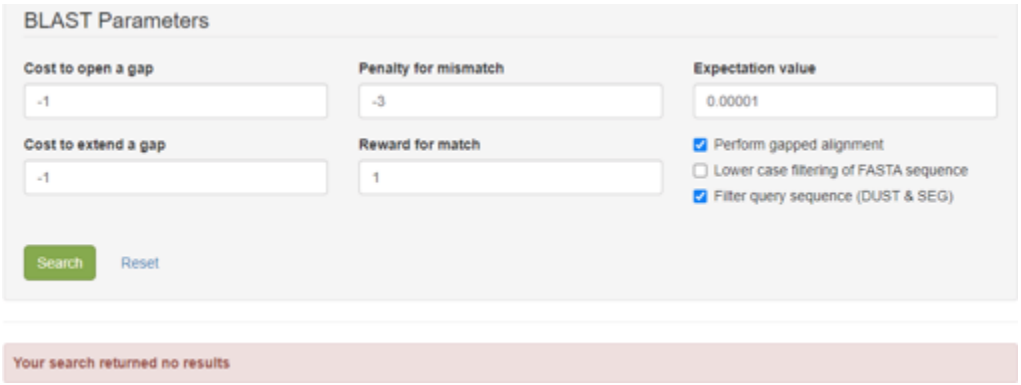
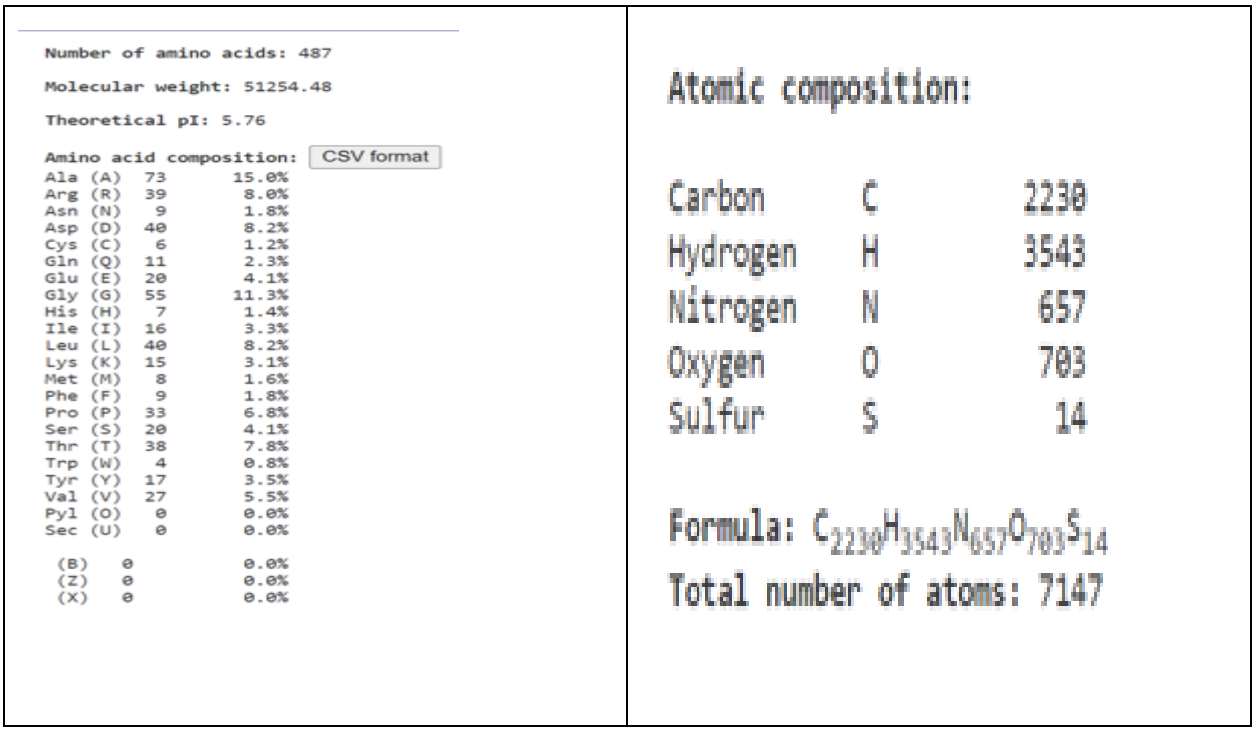


Figure 7: Toxicity result of the constructed vaccine (Wishart et al., 2015)

3.11 Validation of the Proposed Vaccine's physiochemical properties

The PROTPARAM application on the Expasy algorithm was used to carry out biochemical tests which represents a protein's structural and functional qualities (https://web.expasy.org/protparam/). The findings are provided by the server in accordance with the molecular formula, molar mass, instability index, aliphatic index, theoretical PI, GRAVY, and other factors (Fig 8-9). The final protein's molecular weight was calculated to be 51254.48Da and 5.76 was obtained as a score for the theoretical isoelectric point. Reticulocytes in mammals have showed a half-life of 30 hours when tested in laboratory. It was estimated to take over 20 hours in yeast and in E. coli it took about 10 hours when tested in cells. The protein's incredible stability was demonstrated by a score of 36.09. The thermostability is indicated by a score of 75.91 which

showed high aliphatic index (Atsushi IKAI et al., 1980). The vaccine constructions' hydrophilic character was obtained by a score of -0.319 which may be seen by the grand average of hydropathicity (GRAVY).



Number of amino acids: 487

Molecular weight: 51254.48

Theoretical pI: 5.76

Amino acid composition: CSV format

Ala (A)	73	15.0%
Arg (R)	39	8.0%
Asn (N)	9	1.8%
Asp (D)	40	8.2%
Cys (C)	6	1.2%
Gln (Q)	11	2.3%
Glu (E)	20	4.1%
Gly (G)	55	11.3%
His (H)	7	1.4%
Ile (I)	16	3.3%
Leu (L)	40	8.2%
Lys (K)	15	3.1%
Met (M)	8	1.6%
Phe (F)	9	1.8%
Pro (P)	33	6.8%
Ser (S)	20	4.1%
Thr (T)	38	7.8%
Trp (W)	4	0.8%
Tyr (Y)	17	3.5%
Val (V)	27	5.5%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

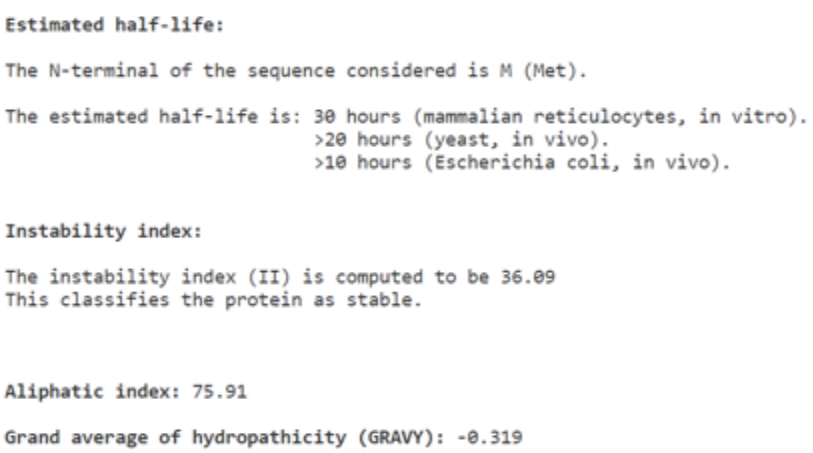
Atomic composition:

Carbon	C	2230
Hydrogen	H	3543
Nitrogen	N	657
Oxygen	O	703
Sulfur	S	14

Formula: $C_{2230}H_{3543}N_{657}O_{703}S_{14}$

Total number of atoms: 7147

Figure 8: Constructed Vaccine's number of molecular weight, amino acids, and theoretical Pi and its atomic composition (Gendel & Jenkins, 2006)



Estimated half-life:

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 36.09
This classifies the protein as stable.

Aliphatic index: 75.91

Grand average of hydropathicity (GRAVY): -0.319

Figure 9: Constructed vaccine's GRAVY value, half-life, instability index and aliphatic index (Gralinski & Menachery, 2020)

3.12 Homology modeling of vaccine:

Obtaining a 3D structure of our vaccine is essential to advancing this research. With the in-silico method, PDB file containing a 3D design was obtained. The homology modelling of the constructed vaccine was carried out utilizing Phyre2 system (http://www.sbg.bio.ic.ac.uk/phyre2/). This system used c3cg5A, a top scoring template to model 265 residues which is 54% of the sequence with a confidence of 100% (Figure 10).

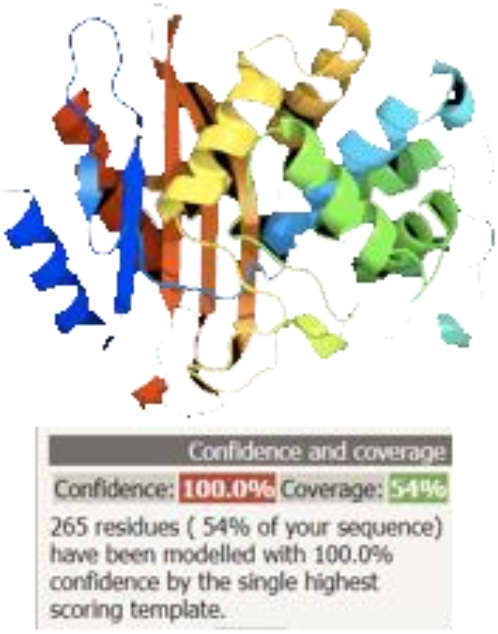


Figure 10: A 3D vaccination model created using Phyre2 server (Kelley et al., 2015)

3.13 Homologous vaccine model's analysis:

The 3D model of the vaccination got to be revealed to perform the Ramachandran plot analysis with the help of SWISS PDB plotter (https://swissmodel.expasy.org/). Region that was favored in between the torsional angles in which the residues reside was revealed in the Ramachandran plot. It also reveals the Ramachandran Favored Region which is 96.58% and 0.00% as in outlier portion reflecting that the model's general quality was good as shown in figure 11(a) and 11(b). Moreover, an analysis curve of Z-score versus residue was produced employing PROSA website (https://prosa.services.came.ac.at/prosa.php). A value of -8.03 was found as the Z-score which indicated that the 3D structure was validated with an outstanding quality as demonstrated in figure 12.

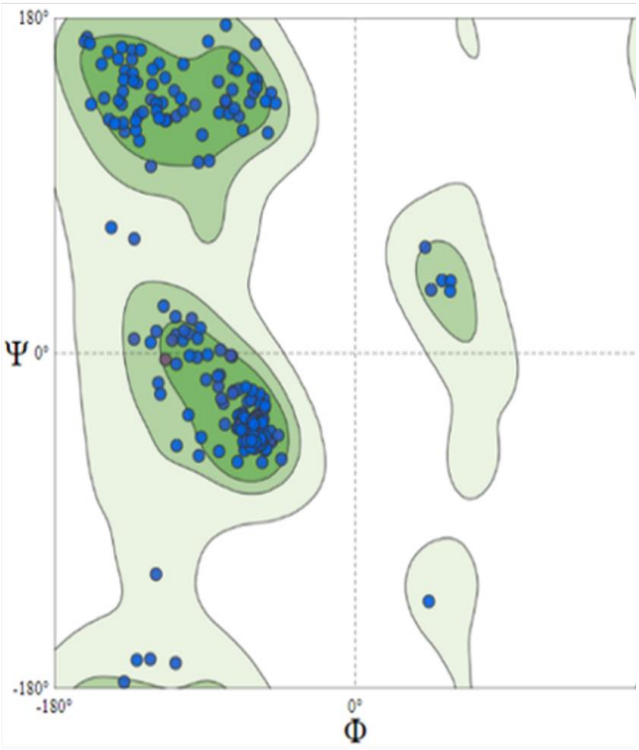


Figure 11: (a) Ramachandran plot using SWISS PDB plotter (Rueckert & Guzmán, 2012)

MolProbity Results

MolProbity Score	2.13	
Clash Score	26.38	(184 GLU- 187 ARG), (82 PHE- 197 THR), (52 GLU- 74 TYR), (83 CYS- 86 PHE), (281 ARG- 291 ARG), (109 TYR- 121 ALA), (237 ILE- 241 PHE),
Ramachandran Favoured	96.58%	
Ramachandran Outliers	0.00%	
Rotamer Outliers	0.00%	
C-Beta Deviations	0	
Bad Bonds	16 / 2027	96 HIS, 102 HIS, 124 HIS, 200 HIS, 217 PRO, 199 PRO, 84 SER- 85 THR, 190 PRO, 235 LYS- 236 ARG, 183 PRO, 293 ALA- 294 LEU, 272 PRO
Bad Angles	6 / 2767	(235 LYS- 236 ARG), 200 HIS, 96 HIS, 124 HIS, 102 HIS, (84 SER- 85 THR)
Cis Prolines	1 / 15	(182 GLU- 183 PRO)

Results obtained using MolProbity version 4

Figure 11: (b) MolProbity results of Ramachandran plotting (Rueckert & Guzmán, 2012)

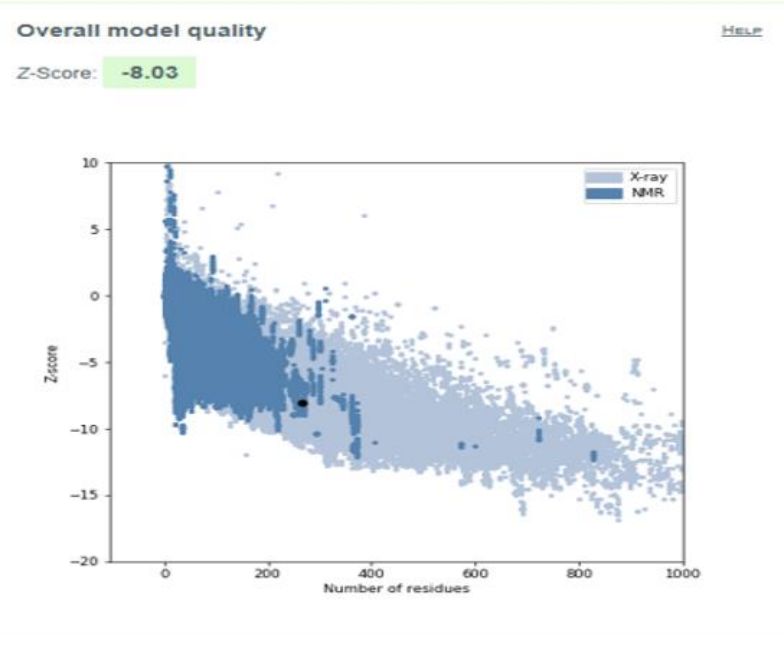


Figure 12: Z-score analysis of the 3D model (Camberg et al., 2013)

3.14 Receptor Molecular Docking with the Complete Vaccine Design

The proposed vaccine design's affinity for the relevant human "Toll-Like Receptor (TLR3)," a member of the toll-like receptor family, was analyzed by performing the molecular docking. This receptor family, which includes protein-rich receptors, stimulates the innate immune response. They are frequently found in cells, which are primarily in charge of eliminating pathogens that are invading the body, due to their physical designation as single-pass membrane-spanning receptors. Our study employed TLR3 (PDB ID: 1WIZ) as the receptor and (PDB file downloaded from the phyre2 service) as the ligand. In order to perform the docking a server named Patchdock was employed (https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php), which also offers a large number of specialized docked complexes (Bhattacharya et al., 2021).

The results show that the best complex between TLR3 and the proposed vaccine gave the highest score of 16070 with a transformation of (-2.60 -0.86 1.58 10.10 4.29 37.6)

Solution No	Score	Area	ACE	Transformation	PDB file of the complex
1	16070	2366.10	331.53	-2.60 -0.86 1.58 10.10 4.29 37.61	result.1.pdb

Figure 13: Molecular Docking results Based on Shape Complementarity Principles (Bhattacharya et al., 2021)

331.53 KJmol-1 was the value of ACE, which covered the area of 2366.10 square angstroms. With the 64-bit client version of Discovery Studio 2016, the PDB structure of the produced protein-ligand combination can be viewed.

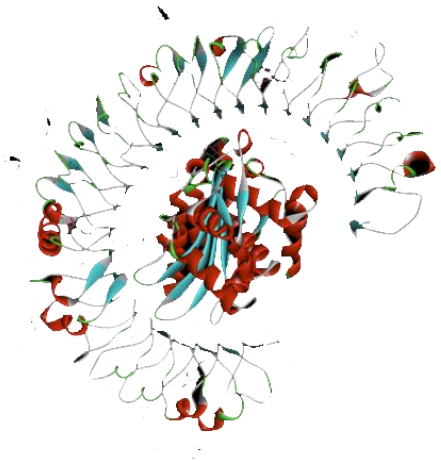
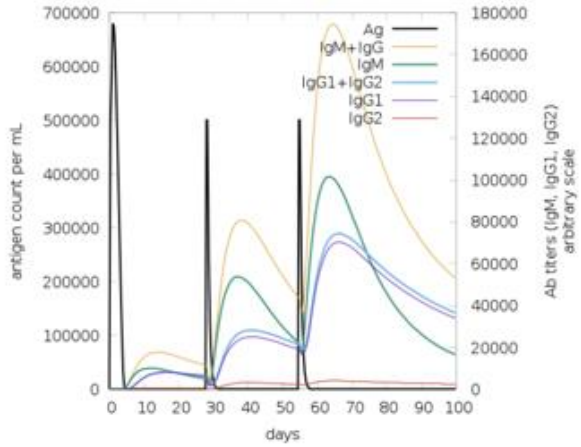


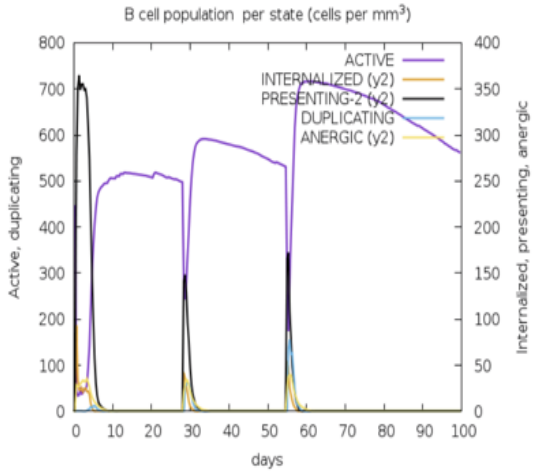
Figure 14: The docked complex between the TLR3 receptor and the proposed vaccination in 3D (Waterhouse et al., 2018)

3.15 Immune Simulation in silico for the immune response

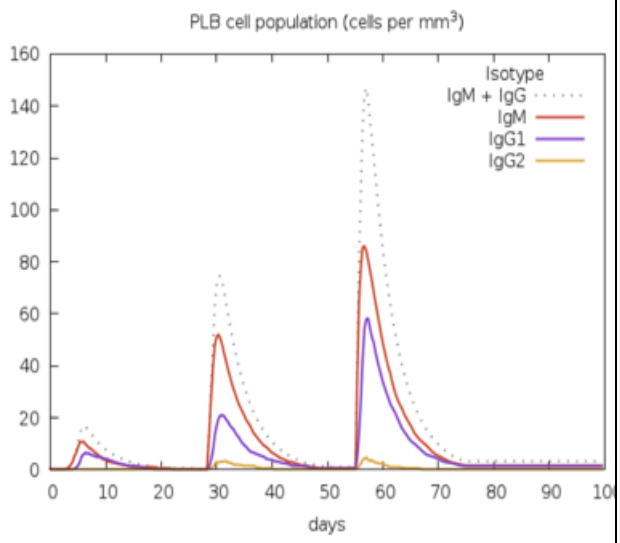
Through a technique that lengthens their half-life, C-ImmSim investigates effective and repeated immunological responses of the cell's condition as well as immune cells' memory (http://www.cbs.dtu.dk/services/C-ImmSim-10.1/). The strategy's impact shows significant extension in half-life of few selected cells and outlive rest of the cells. C-ImmSim Immunological simulation results confirmed congruence with actual immune reactions as presented in figure 15 (a-k). High IgM levels served as an illustration of the main response. Additionally, a rise with immunoglobulin's expression (IgG1+IgG2, IgM, and IgG+IgM) was linked with increased B-cell population, which led to a drop in the antigen concentration in figure (a). Additionally, there is a noticeable rise in the number of T cells in association with growth of the memory cells in figure (e). Figure (b) shows the rise of the active B lymphocytes in correspondence with the number of days after vaccination.



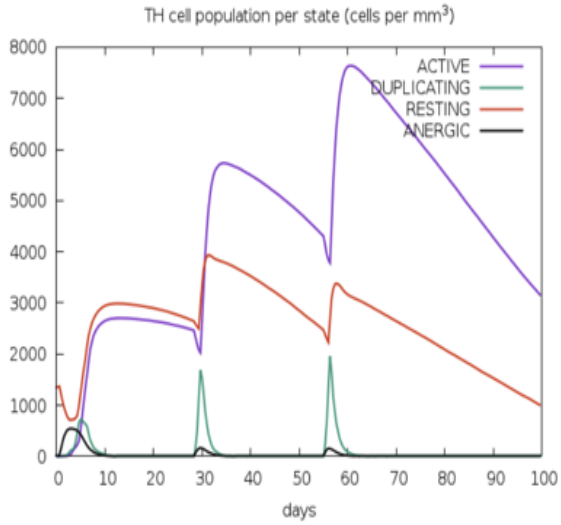
(a) The virus, the immunoglobulins, and the immunocomplexes (Jensen et al., 2018)



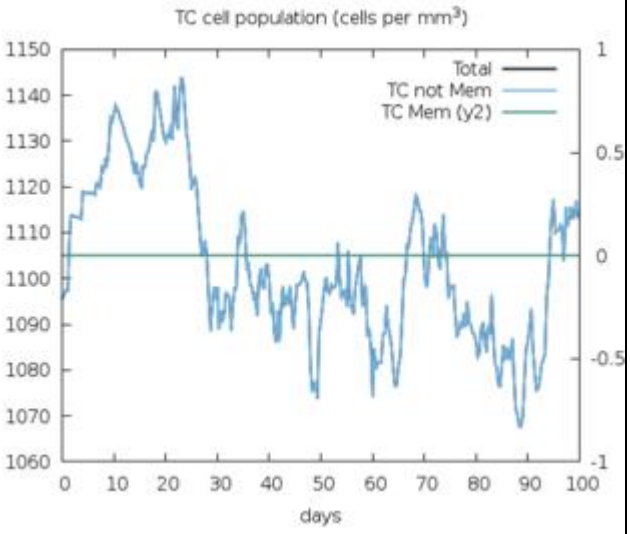
(b) Graph comparing the entity-state of B lymphocytes with the number of days following vaccination (Jensen et al., 2018)



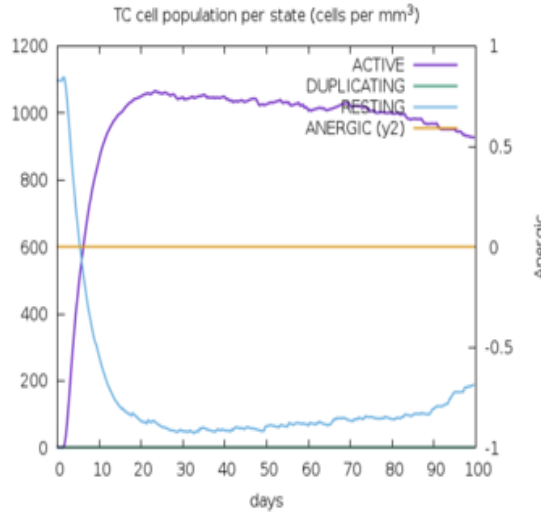
(c) Plasma B cell population expansion vs. vaccination treatment day (Jensen et al., 2018)



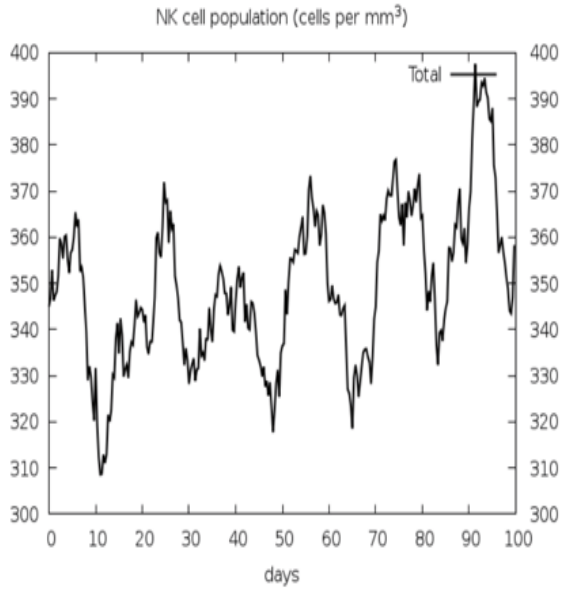
(d) Count of CD-4 HTL cells divided by entity-state (Jensen et al., 2018)



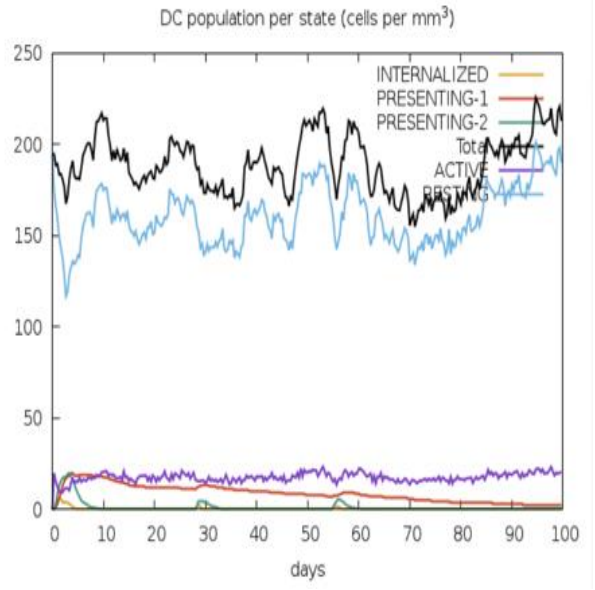
(e) CTL total count (Total and Memory) (Jensen et al., 2018)



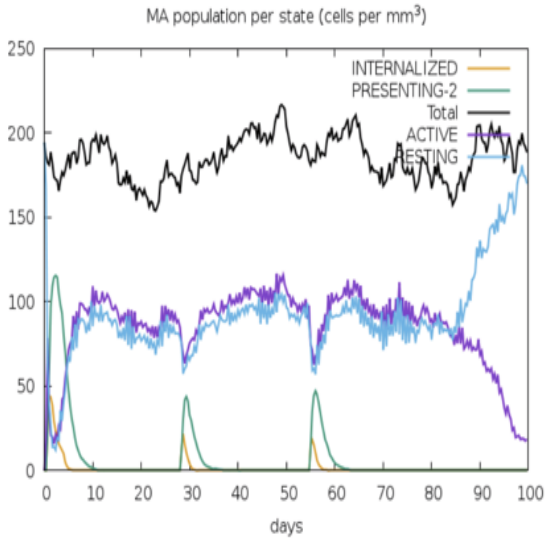
(f) CTL count per entity state (Jensen et al., 2018)



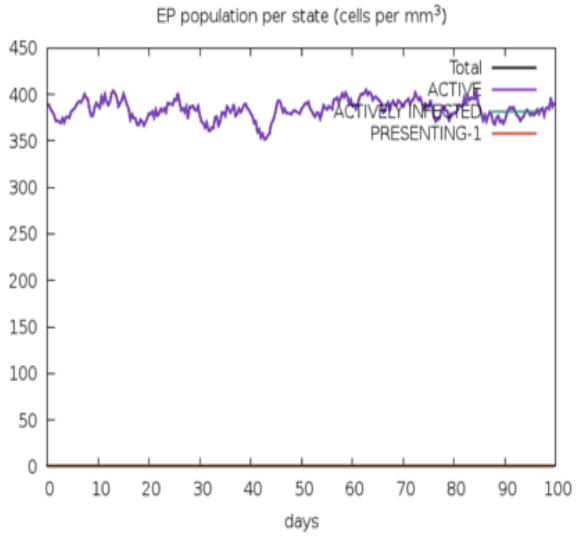
(g) Following vaccination, the overall NK cell population was counted (Jensen et al., 2018)



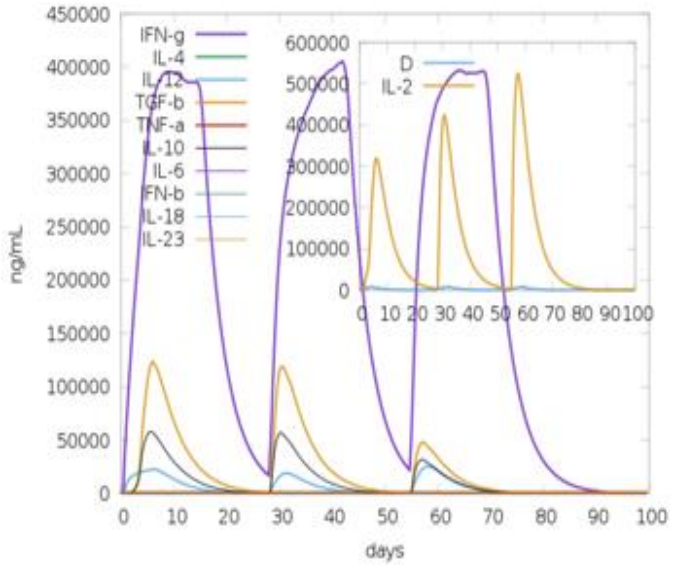
(h) DC population contain antigenic peptides (Jensen et al., 2018)



(i) Growth in the number of macrophages by entity state (Jensen et al., 2018)



(j) Epithelial cells total population count per entity-state (Jensen et al., 2018)



(k) interleukins and cytokines are concentrated. The danger signal is represented by the leukocyte growth factor IL-2 in the inset

Figure 15 (a-k): Immune simulations of vaccine via C-IMMSIM

Figure (c) showing B cell concentrations were created in the same way that graphs showing CTL and HTL epitope concentrations were obtained from the server in figure (d). Let's examine the evolution of putative CTL epitopes in response to immunization. In both memory-inducing and non-memory-inducing stages, the CD-8+ concentration of the CTL epitope was more significant as shown in figure (f). Moreover, it was determined that vaccination enhanced the production of IFN-γ after. A bacterial infection can propagate well in environments where interferon, interleukin, and other chemicals that make the host swell are present in figure (k). Graphs were made to show the evolution of cytokines and epithelial cells following immunization. After vaccination, the host's WBC, DC, epithelial, NK, and macrophage counts are also displayed in figure (g), (h), (i) and (j) by the C-Immsim server which showed evolution of each of the cells following immunization.

Chapter 4 Discussion

The BCG vaccine, which is the only TB vaccine now in use internationally and accounts for the majority of TB infections worldwide, provides only modest protection against the life-threatening disease tuberculosis (TB) (Antonella Riccomi et al., 2019). Therefore, there is an urgent necessity of novel TB candidate vaccines, some of which are now being tested in clinical trials. Designing vaccines is aided by advances in bioinformatic tools and technology along with the genomic and proteomic data availability. Additionally, successful application of bioinformatics technologies is advantageous in comparison to conventional vaccine design. (Kavita Reginald et al., 2018). A crucial stage for designing a vaccination since, it may be used to predict epitopes in silico is to identify the antigens which are immunogenic. (Armina Alagheband Bahrami et al., 2019). Immunogenic antigens are distinct in that they can bind to immune cells, elicit a response, and exert immunodominant activity (Lorenzo Galluzzi et al., 2017). Using computational method, the Mtb antigen which is the beta lactamase enzyme (blaC) was extracted and its antigenicity was predicted. The enzyme that has been retrieved was found to be an antigen which indicated that further procedures can be carried out to design the vaccine. Through the use of bioinformatics technologies, epitopes for B and T cells were predicted which might boost humoral and cellular response (Zahra Yazdani et al., 2020). Since immune defence against Mtb is based on the immune reaction mediated by the CTL and HTL cells, immunoinformatic databases were utilized for anticipating the MHC class-I and class-II specific peptides only. The epitopes for CTL and HTL were specific to production of cytokines which are IFN-γ, IL-4 and IL-10. Screening of predicted epitopes for CTL and HTL were done by taking their nature as an antigen, anti-allergen and anti-toxin in consideration. The antigenic, anti-allergenic and anti-toxin epitopes were picked to create the vaccination. The ability to predict B-cell epitopes is important for vaccine development (Kashyap Kumar Dubey et al., 2018) and to serve as a location for the antigen-antibody interactions. (Zuzana Krocova et al., 2020). In order to stimulate the adaptive immune system and work with MHC molecules, B-cell epitopes are necessary. (Sunita et al., 2020). Specific algorithm was employed to determine the epitopes for B cell from which, epitopes more than 20 mer were chosen. The specific epitopes for B cell which were revealed to be antigenic, anti-allergenic and anti-toxic when checked in a specific tool were selected. Selected epitopes for B and T-cell that were combined to the right linkers where sequence of the beta lactamase enzyme was considered to be an adjuvant which would increase the vaccine's immunological actions and bioactivity. The

constructed vaccine showed increased antigenicity when its antigenicity was checked. The vaccine was also non-allergen and non-toxin which has boosted its effectiveness to become a candidate for vaccination. The vaccine that has been constructed had a molecular weight of 51254.48Da. The physiochemical properties of the proposed vaccine showed that, its instability score was below 40 and a GRAVY score of -0.319 which represented that the vaccine was stable and hydrophobic. The homology modelling of the vaccine showed 54% coverage with a confidence of 100%. The vaccine candidate's 3D complex significantly enhanced and displayed requisite characteristics in accordance when combined with the Ramachandran plot's findings. The overall quality of the vaccination was found to be satisfactory which was revealed by the results shown in the Ramachandran plot where, in the preferred area there were 96.58% residues and that of outlier area contained 0.00% residues. Docking of the vaccination was done with TLR-3 in order to further test its capacity to connect with TLR (Toll like Receptors) within immune system. Interaction with TLR-3 was seen to be high by the demonstrated results of the new vaccination. A chance for the vaccine to trigger both an innate and an adaptive immune response was indicated by this interaction. Results from immunological simulation were in line with usual immune responses as presented in figure 15 (a-k). After continuous interaction with antigenic substance, an overall increase was observed in the induced immunological reactions. There was no doubt about the B-cell memory would remain for several months with a development in both T and memory B cells. In particular, helper T cells were activated. There was rise in the immunoglobulins in response to the antigen at interval of 28 days as shown in figure (a). Figure (b) shows increased B lymphocytes with the number of days following vaccination. The B cell population in the plasma was seen to expand in correspondence with the days of vaccination in figure (c). The concentration of active HTL was observed in figure (d). Additionally, memory development is associated with a discernible increase in T (cytotoxic) cell numbers which is observed in figure (e). Increased concentration of the CTL cells was seen in figure (f). Growth in the number of macrophages, NK cells, DC and epithelial cells was observed in figure (g-i). Another intriguing finding was observed in the quantity of IFN-γ and IL-2 increased just after initial exposure which maintained their peaks followed by several doses of the antigen in figure (k). This points to strong TH cell counts and effective Ig generation, both of which are supportive of a humoral response.

Chapter 5 Conclusion

The computational methods described in the current study could result in new understanding of Mtb vaccine antigens and probable candidates for vaccination which would be difficult to obtain just by performing preliminary, in vitro, and tissue sample investigation. An immuno-informatics method was utilized in this study to identify a new vaccination with many epitopes for tuberculosis that is quite immune-stimulating and contains the necessary characteristics so that it can function as a vehicle vaccine. Numerous epitopes for B and T-cells combined to the right linkers along with the bacterial enzyme to boost the vaccine's immunogenicity were analyzed by utilizing epitope-prediction methods. The study of vaccine's tertiary structure along with its physiochemical characteristics, solubility, allergenicity, and antigenicity were all found to be highly excellent. TLR-3 and vaccination underwent molecular docking, which allowed for estimation of the complex's binding strength and stability. The immunological simulation that was conducted confirmed the clearance rate of immune cells from antigens. There was an overall increase in the elicited immunological reactions when exposed continuously to the antigen. To investigate the many features of the vaccination in this study, a variety of immune-informatics methods were used. Furthermore, the evaluation of the anticipated subunit vaccination based on epitopes might be highly acceptable to demonstrate that they are an immunogenic and prospective vaccine candidate against tuberculosis.

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