Jurnal Akta Kimia Indonesia
INDONESIA CHIMICA ACTA

PAPER

View Article Online
View Journal View Issue

Cite this: *Indo. Chim. Acta.*, 2024, **17**, **2**.

Received Date:
9th October, 2024
Accepted Date:
30th December, 2024

Keywords:
Mycobacterium tuberculosis;
DNA B;
Vaccine;
Epitope.

DOI:
http://dx.doi.org/10.70561/ica.v17i2.41408

Development of a Multi-epitope Vaccine against *Mycobacterium tuberculosis* DNA Protein B: An Immunoinformatics-Driven Strategy

Asep Iin Nur Indra[1]*, Fatkhurrohmah Ashari[1], Keisha Nur Gita Aprilia[1], Putri Anggraeni[1], Veronika Silaban[1] and Egi Widiatmoko[1]

Abstract. Lead Vaccination is a primary strategy in the prevention of tuberculosis, a serious infectious disease caused by *Mycobacterium tuberculosis*. This study aims to design a novel multiepitope vaccine using the DNA B protein from *M. tuberculosis* through immunoinformatics and molecular dynamics approaches. The design process begins with the identification of potential epitopes from the DNA B protein using various bioinformatics tools to predict both B and T cell epitopes based on their immunogenic properties. After epitope identification, the selected epitopes are combined into a multiepitope vaccine construct to enhance a broad and specific immune response. The three-dimensional structural model of the vaccine construct is predicted and validated using molecular modeling techniques. Molecular dynamics simulations are performed to evaluate the stability and interactions between the multiepitope vaccine and the immune system, providing insights into the expected immune response. Simulation analysis indicates that the vaccine construct is stable and capable of eliciting a strong immune response. In silico testing was conducted to predict the vaccine's affinity for Major Histocompatibility Complex (MHC) receptors and its ability to induce T and B cell immune responses. The results of this analysis demonstrate that the designed multiepitope vaccine has high potential to trigger an effective immune response against *Mycobacterium tuberculosis*. This study provides a solid foundation for further development and evaluation of the vaccine in *in vivo* studies to determine its clinical safety and efficacy.

Introduction

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* and is one of the major global health challenges. Despite the availability of antibiotic therapy, remains a leading cause of morbidity and mortality worldwide, particularly in developing countries. The Bacillus Calmette-Guérin (BCG) vaccine is the primary vaccine used for prevention; however, its effectiveness in protecting against pulmonary tuberculosis in adults is limited. Therefore, the development of more effective vaccines is urgently needed (Lv et al., 2024). Despite the availability of antibiotic therapy, tuberculosis continues to be a major cause of morbidity and mortality in many parts of the world, particularly in developing countries (Miggiano, Rizzi and Ferraris, 2020). The Bacillus Calmette-Guérin (BCG) vaccine is the primary vaccine used for the prevention of tuberculosis. However, its effectiveness in protecting against pulmonary tuberculosis infection in adults remains limited (Fatima et al., 2020). Therefore, the development of a more effective vaccine is necessary.

According to prior research conducted by Zhang et al., DNA Binding Protein B from *Mycobacterium tuberculosis* has been identified as a promising candidate for vaccine development due to its critical role in the pathogenesis of the bacterium (Zhang et al., 2014). In vaccine development contexts, DNA B has demonstrated substantial potential as an innovative tool. Vaccines that utilize DNA fragments to encode pathogen proteins facilitate rapid production and

[1]*Department of Medical Laboratory Technology, Health Polytechnic of the Ministry of Health Bandung, Cimahi City, West Java, Indonesia;* ***Email: asepiinnurindra@gmail.com***

exhibit high stability during storage. This characteristic contributes to their capacity to elicit robust and broad immune responses without necessitating live viruses, thereby reducing the risk of adverse effects. Furthermore, the technological flexibility of DNA B allows for rapid adaptation to variations or mutations in pathogens, rendering it a highly effective and safe option in global vaccination strategies (Zhang et al., 2014).

The multiepitope vaccine designed from this protein is expected to provide broader and more effective protection by triggering a comprehensive immune response. The immunoinformatics approach has rapidly advanced in recent years and offers sophisticated methods for epitope-based vaccine design. By utilizing predictive algorithms and bioinformatics modeling, it is possible to identify immunogenic epitopes that can be integrated into a single vaccine construct. This approach enables the design of vaccines capable of stimulating immune responses from both T cells and B cells, thereby enhancing the strength and specificity of these responses (AlChalabi et al., 2022). To ensure that the designed multiepitope vaccine construct will be effective, molecular dynamics are employed to model and analyze the stability and interactions of the vaccine with immune components. This technique provides important insights into how the vaccine interacts with receptors and how the structure of the vaccine influences its ability to elicit an immune respons (Ghandadi, 2022).

In the development of DNA vaccines to combat *Mycobacterium tuberculosis*, the role of Human Leukocyte Antigen (HLA) class I and II is crucial, as they are involved in antigen presentation, which triggers effective cellular and humoral immune responses. Each HLA class has a specific function in mediating interactions between *Mycobacterium tuberculosis* antigens and immune cells, ultimately determining the vaccine's effectiveness (Lee and Meyerson, 2021). HLA class I mediates antigen presentation to CD8+ T cells, which are pivotal in cellular immunity. When *Mycobacterium tuberculosis* infects body cells, bacterial proteins are processed into small peptides that are then presented on the cell surface by HLA class I. CD8+ T cells recognize the HLA-peptide complex, becoming activated and producing cytokines that cause the lysis or death of infected cells. Thus, in DNA vaccine design, selecting *Mycobacterium tuberculosis* antigen epitopes that can be effectively recognized by HLA class I is crucial to ensure strong CD8+ T cell activation, which directly combats infection by destroying infected cells (Lee and Meyerson, 2021).

HLA class II, on the other hand, mediates antigen presentation to CD4+ T cells, which play a key role in humoral immunity and in regulating various aspects of cellular immunity. Antigen-presenting cells (APCs) capture antigens, process them, and then present them via HLA class II. CD4+ T cells recognize antigens presented by HLA class II, leading to the activation of T-helper cells. These T-helper cells are crucial for activating B cells to produce specific antibodies and assisting in the activation of macrophages and CD8+ T cells. In vaccine design, selecting epitopes recognizable by HLA class II ensures that the vaccine induces a broad immune response and fosters good coordination between humoral and cellular responses (Lee and Meyerson, 2021).

Additionally, in designing vaccines involving antigen presentation by HLA, choosing non-allergenic and non-toxic antigens is essential to ensure vaccine safety and efficacy. These antigens prevent allergic reactions and serious side effects while supporting an appropriate immune response without the risk of autoimmunity. Ensuring the safety of these antigens also accelerates clinical trials and reduces development costs, ultimately boosting public trust and enabling faster and more efficient vaccine distribution.

This research aims to design a novel multiepitope vaccine derived from the DNA protein of *Mycobacterium tuberculosis* by integrating immunoinformatics and molecular dynamics approaches. Through this methodology, we aspire to develop a more effective vaccine with enhanced potential for protection against tuberculosis infection, thereby contributing significantly to the advancement of more effective tuberculosis prevention strategies in the future.

Experimental

Material and Methods

The methodology employed in this study references the vaccine design research conducted by Rizarullah et al., 2024in the journal *ACS Omega*, which focuses on vaccine development for HPV. Furthermore, this research adopts the immunoinformatics-based vaccine development strategy proposed by Ghandadi, M. (2020) in the *International Journal of Peptide Research and Therapeutics*. The primary difference from previous studies lies in the various tools utilized and the target genes aimed at in the vaccine design.

Procedures

Sequence Retrieval, Alignment, and Epitope Prediction

Ten sequences of the B DNA protein from *Mycobacterium tuberculosis* were obtained from the NCBI Gene Bank database. Sequence alignment was performed using MAFFT to generate a consensus sequence, which was then refined with EMBOSS. Cytotoxic T lymphocyte (CTL)

epitopes were predicted using NetCTL 1.2, while helper T lymphocyte (HTL) epitopes were predicted using NetMHCII 2.3, focusing on binding affinity for major histocompatibility complex (MHC) class I and II molecules (Aasim et al., 2022). The antigenicity of the selected epitopes was predicted using VaxiJen v2.0, allergenicity was assessed with AllerTop, and toxicity was evaluated using ToxinPred. The potential for IL-4 induction was assessed with IL4pred, and the ability to induce IFN-γ was evaluated with IFNepitope. Epitopes were chosen based on high antigenicity, non-allergenicity, non-toxicity, and the ability to stimulate IL-4 and IFN-γ responses. Several studies suggest that a threshold of 0.7 may be chosen based on the performance analysis of diagnostic tests, including sensitivity and specificity. This threshold can help minimize inaccurate results and enhance the reliability of tests, such as in assessing the capability of tests to detect active *Mycobacterium tuberculosis* infections (Penn et al., 2018).

Prediction of B Cell Epitopes

B cell epitopes were predicted using IEDB to identify regions within the protein sequence capable of eliciting a humoral immune response. Longer peptides were prioritized due to their higher immunogenic potential, and the final epitopes were selected based on their predicted ability to bind B cell receptors and stimulate antibody production (Chen et al., 2020a).

Vaccine Construction

The selected CTL, HTL, and B cell epitopes were linked using the GPGPG linker to promote recognition by the immune system. A His-tag (HHHHHH) was incorporated to facilitate purification and expression(Obaidullah et al., 2021). The multi-epitope vaccine construct was visualized in 3D using AlphaFold software to evaluate structural integrity and folding.

Vaccine Toll-like Receptor 4 (TLR4) Interaction and Molecular Docking IIP

Molecular docking studies were conducted to assess the binding interactions between the vaccine construct and Toll-like receptor 4 (TLR4) using BIOVIA software. Binding sites and affinities were analyzed to evaluate the vaccine's potential in activating immune responses through Toll-like receptor 4 (TLR4) (Almofti et al., 2021).

Prediction of Physicochemical Properties

The physicochemical properties of the vaccine were predicted using ProtParam, including molecular weight, isoelectric point (pI), atomic composition, aliphatic index, instability index, GRAVY value, and half-life. The solubility of the vaccine was predicted using the SoluProt server to assess its potential for recombinant expression in *Escherichia coli* (Shey et al., 2022).

Immune Response Simulation

In silico immune response simulations were performed using the C-ImmSim server to evaluate the immune responses triggered by the vaccine. The dynamics of IgM, IgG, cytokine, and interleukin production were simulated to predict the vaccine's ability to induce humoral and cellular immune responses (Yin et al., 2023).

Result and Discussion

This study presents the prediction of a multiepitope vaccine design, achieved through the alignment of 10 DNA B protein sequences of *Mycobacterium tuberculosis* sourced from the NCBI Gene Bank.

Prediction and Selection of The Epitope CTL and HTL

The antigenic epitopes from a number of peptides have been identified through various prediction methods. The peptides analyzed were derived from Human T-helper Lymphocytes (HTL) prediction data. Epitopes predicted to bind to MHC class II (HLA-DRB1) were identified using several integrated prediction tools. All epitopes were then compiled, and nine epitopes were selected based on specific priority criteria. Epitopes from each peptide were chosen based on their antigenicity, allergenicity, toxicity, and ability to induce IL4 and IFN-γ.

Epitopes that fulfilled specific criteria across multiple prediction tools, such as NetCTL, NetMHCII, and VaxiJen, were prioritized for inclusion to ensure reliability and robustness in epitope selection. This approach reduces the likelihood of selecting false positives and enhances the confidence that the chosen epitopes possess strong immunogenic potential, non-allergenicity, and non-toxicity, making them suitable candidates for vaccine development. Additional considerations, such as high antigenicity, positive IFN-γ stimulation, non-allergenicity, and good conservation levels, also played a significant role in epitope selection (Ghandadi, 2022).

Each epitope was identified as a probable antigen with predicted antigen values ranging from 0.7901 to 1.3946. Epitopes with higher predicted antigen values demonstrate a greater potential to be recognized by T-helper cells, with epitopes like ‘MEIRAKARR LRQKAN IRAKARRLR’ showing a value of 1.3940, making it highly antigenic. These epitopes are crucial for stimulating the adaptive immune response by activating T-cells. Based on allergenicity

predictions, all identified epitopes are predicted to be probable non-allergens. This indicates that these epitopes have a very low likelihood of inducing allergic reactions in humans, an essential factor in vaccine development as it minimizes the risk of unwanted hypersensitivity reactions (Table 1).

Table 1. The prediction results and selection of antigenicity and allergenicity.

Peptide	Allele of HLA Class II	Antigen Prediction	Alergen Prediction
EVFRLRLASGREVEA LRLASGREV	DRB1_0101 DRB1_0901	1.1714 (Probable ANTIGEN)	Non- Allergen
LTMMEIRAKARRLRQ IRAKARRLR	DRB1_0301 DRB1_1301 DRB4_0103	1.0469 (Probable AN TIGEN)	Non- Allergen
NGQGRVYYGSTSRRL YYGSTSRRL	DRB1_0901 DRB1_0701	0.7901 (Probable ANTIGEN)	Non-Allergen
MEIRAKARRLRQKAN IRAKARRLR	DRB1_0801 DRB4_0103 DRB1_1301	1.3940 (Probable ANTIGEN).	Non-Allergen
TMMEIRAKARRLRQK IRAKARRLR	DRB4_0103 DRB1_1101 DRB1_1301	1.0496 (Probable ANTIGEN).	Non-Allergen
SPNLTMMEIRAKARR MEIRAKARR	DRB4_0103 DRB1_1301	1.3468 (Probable ANTIGEN).	Non-Allergen
NLTMMEIRAKARRLR IRAKARRLR	DRB1_1301 DRB4_0103	1.2820 (Probable ANTIGEN).	Non-Allergen
MMEIRAKARRLRQKA IRAKARRLR	DRB1_1301 DRB4_0103 DRB5_0101	1.1817 (Probable ANTIGEN).	Non-Allergen
EIRAKARRLRQKANL IRAKARRLR	DRB4_0103 DRB1_1301	0.9145 (Probable ANTIGEN).	Non-Allergen

Table 2. The prediction results and selection of Toxicity, IL4-Induser, and IFN.

Peptide	Allele of HLA Class II	Toxicity	IL4-Induser	IFN
EVFRLRLASGREVEA LRLASGREV	DRB1_0101 DRB1_0901	Non-Toxin	Non IL4 inducer	+
LTMMEIRAKARRLRQ IRAKARRLR	DRB1_0301 DRB1_1301 DRB4_0103	Non-Toxin	Non IL4 inducer	+
NGQGRVYYGSTSRRL YYGSTSRRL	DRB1_0901 DRB1_0701	Non-Toxin	Non IL4 inducer	+
MEIRAKARRLRQKAN IRAKARRLR	DRB1_0801 DRB4_0103 DRB1_1301	Non-Toxin	Non IL4 inducer	+
TMMEIRAKARRLRQK IRAKARRLR	DRB4_0103 DRB1_1101 DRB1_1301	Non-Toxin	Non IL4 inducer	+
SPNLTMMEIRAKARR MEIRAKARR	DRB4_0103 DRB1_1301	Non-Toxin	Non IL4 inducer	+
NLTMMEIRAKARRLR IRAKARRLR	DRB1_1301 DRB4_0103	Non-Toxin	Non IL4 inducer	+
MMEIRAKARRLRQKA IRAKARRLR	DRB1_1301 DRB4_0103 DRB5_0101	Non-Toxin	Non IL4 inducer	+
EIRAKARRLRQKANL IRAKARRLR	DRB4_0103 DRB1_1301	Non-Toxin	Non IL4 inducer	+

(Table 2) All epitopes are predicted to be non-toxic, indicating that there is no evidence suggesting that these epitopes are harmful to the human body. This characteristic is essential as it ensures the safety of the epitopes for immunotherapy or vaccine applications, where toxicity could lead to adverse side effects. Regarding IL4 induction, all epitopes are predicted as Non-IL4 inducers, meaning they do not have the ability to excessively induce IL4 production, which is associated with a humoral (Th2) immune response. This observation suggests that the selected epitope has a higher possibility to enhance cellular immune response (Th1), because the candidate epitope is predicted to be positive for IFN-γ, where IFN-γ plays a role in Th1 immune response by facilitating CD4+ T cell polarization, activating myeloid cells, supporting CD8+ T cell maturation, and stimulating immune cell migration to strengthen cellular immune response (Alspach et al., 2019).

(Table 2) Furthermore, all predicted epitopes show positive predictions for IFN-γ induction. IFN-γ is a key mediator in the cellular immune response, involved in macrophage and T-cell activation, and is crucial for inhibiting viral replication. The potential for IFN-γ induction in these epitopes indicates that they have the capability to stimulate a strong protective immune response. This combination of non-toxicity, absence of IL4 induction, and positive IFN-γ induction makes the selected epitopes promising candidates for eliciting a robust and effective immune response in vaccine development.

The prediction of Cytotoxic T lymphocyte (CTL) epitopes was conducted to identify peptide candidates with the potential to induce an effective immune response. However, the analysis results revealed that no peptides met the criteria for strong binding affinity to be recognized as CTL epitopes by the MHC class I molecules used in this study. The absence of qualifying epitopes suggests that the peptides analyzed may have limitations in their ability to induce the necessary CTL response for optimal immune protection. This may be due to low binding affinity to MHC class I or insufficient interaction with T cell receptors (TCR) (Chen et al., 2020).

The failure to identify a suitable CTL epitope suggests that alternative approaches may be needed to design an effective vaccine. One potential approach is the identification and analysis of alternative peptides that may have greater potency or the use of peptide modification strategies to increase binding affinity to MHC class I molecules. Although these results do not yield ideal CTL epitope candidates, it is important to continue research into the use of adjuvants to support CD8+ T cell responses. The addition of adjuvants is a potential solution to enhance the immune response to antigens, particularly through the promotion of cross-presentation that is essential for CD8+ T cell activation via the MHC class I pathway without having to rely on strong CTL epitopes (Rapaka et al., 2021).

Prediction and Selection of The Epitope B Cell

The predicted B-cell epitope was identified as follows, "AKDQVRFLRHVGVHGAEAVAAQEMLRQLKGPVRNPNLDS APKKVWAQVRNRLSAKQMMDIQL". This epitope was selected based on the strategic choice of prioritizing longer peptides (such as Peptide 22, consisting of 62 amino acids) from the B-cell epitope prediction results. This approach aims to maximize the immunogenic potential. While shorter peptides can still play a role in detailed epitope mapping, longer sequences offer a higher likelihood of successful immune stimulation, making them more suitable for vaccine development. The variation in peptide lengths reflects the complexity of epitope prediction and emphasizes the importance of balancing specificity and immunogenicity in the selection of epitopes for vaccines (Liu et al., 2020).

Vaccine Construction, Interaction, and Stability of the Vaccine Toll-like receptor 4 (TLR4) Complex

The vaccine construct was obtained by combining multiepitope HLA and B-cell epitopes using GPGPG and HisTag linkers. The vaccine construct was visualized using AlphaFold to examine its 3D structure (Figure 1). The 3D structure of the vaccine is a crucial aspect in ensuring precise design and enhancing its immunogenic effectiveness. The 3D structure allows for the identification of specific interactions between the vaccine and the immune system, maximizing the potential immune response.

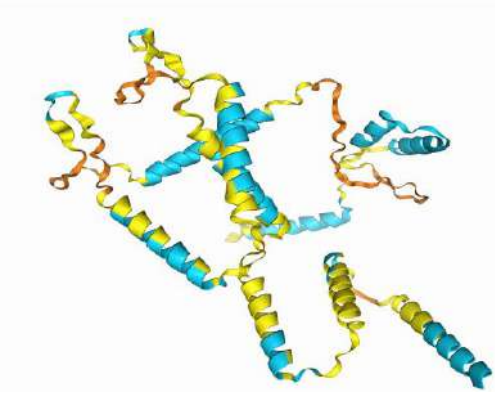


Figure 1. The vaccine construct was visualized using AlphaFold, and the tertiary structure prediction yielded a structure resembling its native form. The secondary structure of the vaccine was predominantly helical, constituting approximately 90% of the total structure.

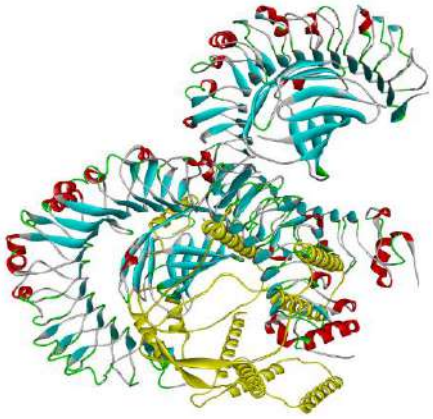
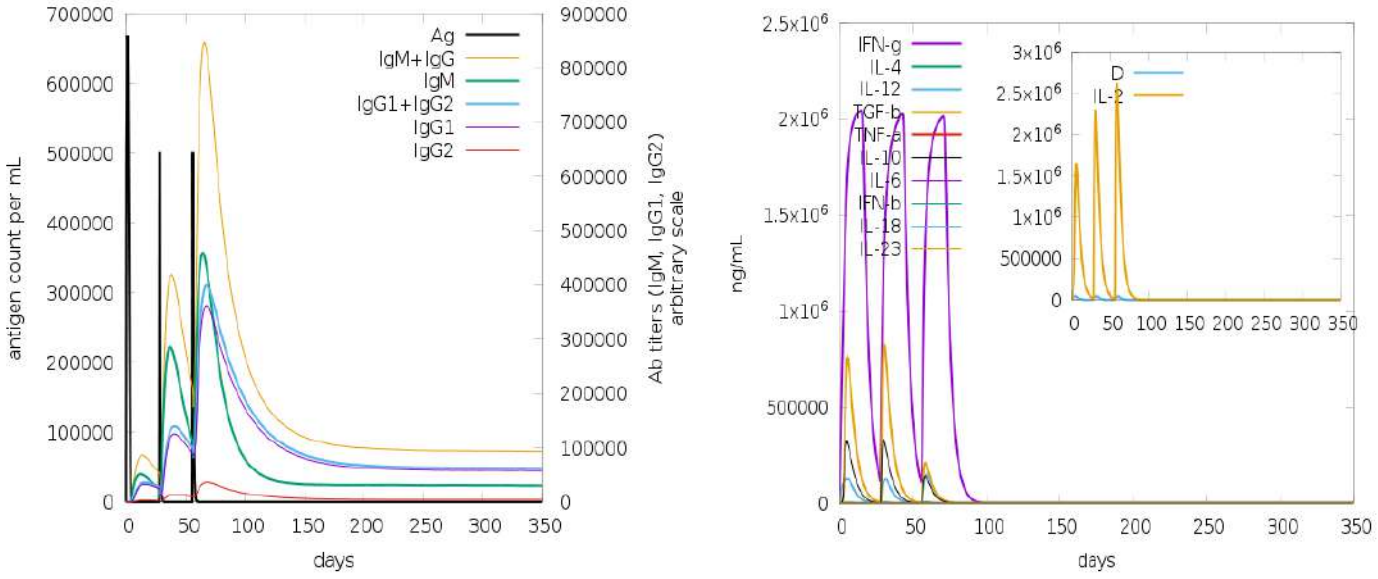


Figure 2. Molecular docking simulations using BIOVIA revealed the binding interactions between the vaccine (ligand) and the Toll-like receptor 4 (TLR4) The cyan and red structures represent the receptor, while the yellow structure corresponds to the vaccine.

The docking results, visualized using BIOVIA software, depict the interaction between the vaccine, shown in yellow, and Toll-like receptor 4 (TLR4), represented by a combination of three colors (Figure 2). These findings demonstrate a specific interaction between the vaccine and the target, which is essential for assessing binding potential and immune response activation. The color scheme and ribbon structures represent secondary structural elements such as alpha-helices and beta-sheets. The proximity of the molecules highlights critical interaction regions that contribute to the determination of the vaccine's immunogenic efficacy.

The Predicted Physicochemical and Solubility Properties

Based on the ProtParam analysis, the designed protein has a molecular weight of 40,060.25 Daltons and consists of 358 amino acids. The protein has an isoelectric point (pI) of 12.26, which indicates that the protein is basic. The atomic composition includes 1,714 carbon atoms, 2,599 hydrogen atoms, 637 nitrogen atoms, 443 oxygen atoms, and 15 sulfur atoms, totaling 5,708 atoms. The protein's aliphatic index is recorded at 73.77, suggesting relatively good thermal stability. However, the instability index of 51.36 classifies the protein as unstable, meaning that it may tend to denature or degrade more quickly under certain conditions. Factors contributing to this instability could include the amino acid composition, structural arrangement, and the protein's tendency for modification or degradation. In practical applications, unstable proteins may require additional engineering or special storage conditions to maintain functionality (Niazi, Mariam and Paracha, 2024).



(a) **(b)**

Figure 3. (a) Antigen count per mL and antibody titers (IgM, IgG1, IgG2) over time. The graph shows the antigen (Ag) curve alongside various antibody responses, including IgM, IgG1, and IgG2. Each line represents the dynamics of the immune response, with notable peaks for IgM and IgG occurring at different points. The x-axis represents days, while the y-axes represent antigen count per mL and antibody titers (in arbitrary scale); (b) Cytokine response over time. The panel shows the levels of several cytokines such as IFN-γ, IL-4, and TNF-α, measured in ng/mL. The graph highlights sharp spikes in cytokine levels shortly after antigen exposure, followed by a rapid decline. An inset shows specific cytokine data (IL-2 and D) for a detailed view of their dynamics over time.

The hydrophilic nature of the protein is indicated by a GRAVY value of -0.838. Additionally, the protein has a half-life of 30 hours in mammalian reticulocytes (in vitro), more than 20 hours in yeast (in vivo), and over 10 hours in *Escherichia coli* (in vivo). Based on the predicted physicochemical properties using ProtParam, the extinction coefficient of 114,866 M^{-1} cm^{-1} at a wavelength of 280 nm reflects the protein's strong ability to absorb light at this wavelength, which can be utilized to determine its concentration in solution. This analysis provides essential insights into the stability and potential applications of the protein in various environmental conditions.

The analysis results using the SoluProt server indicate that the developed vaccine protein has a solubility value of 0.847, demonstrating a high potential for solubility in biological systems such as *Escherichia coli*. A value above 0.5 is considered a positive indicator of protein solubility, which is crucial for facilitating vaccine production, purification, and stability. Well-soluble proteins will support the vaccine formulation process and ensure effectiveness in both in vitro and in vivo tests, thereby enhancing the efficiency of recombinant protein-based vaccine development.

Results of Immune Response Simulation

IgM, as the first immunoglobulin to respond to antigens, exhibits a sharp increase immediately following antigen (Ag) detection, peaking around day 50, followed by a gradual decline. IgG, which appears after the IgM response, begins to rise after IgM has reached its peak. The IgG response tends to peak more slowly; however, its concentration persists longer in the bloodstream, providing long-term immunity (Figure 3 (a)). The combination of IgM and IgG (IgM + IgG) reflects the total immune response to the antigen, with the early phase dominated by IgM followed by a more sustained response from IgG. This pattern illustrates the body's immune response to a vaccine, where IgM offers rapid early protection, while IgG maintains protection over a longer duration(Jing et al., 2022).

The dynamics of cytokine concentrations, including interleukins, in the immune response following antigen exposure show that cytokines such as IFN-γ and TNF-α exhibit a sharp increase immediately after antigen detection, signaling a strong inflammatory response essential for effectively combating the antigen. The peak cytokine concentration occurs in the early days, followed by a decline as the antigen diminishes (Figure 3 (b)).

Interleukins, such as IL-2 and IL-4, also exhibit significant increases. For instance, IL-2 plays a crucial role in T cell proliferation, enhancing the adaptive immune response. After reaching their peak, the concentrations of these interleukins decline, indicating a transition from the inflammatory phase to immune resolution and the body's return to a state of homeostasis. Overall, this diagram illustrates the critical roles of cytokines and interleukins in regulating the immune response, from the active early phase to the recovery phase (Xia et al., 2023).

Limitations and Future Directions

This multiepitope vaccine design overcomes the major drawbacks of traditional tuberculosis vaccines, such as BCG, which has limited efficacy in protecting adults against pulmonary tuberculosis (Fatima et al., 2020). Unlike BCG that uses live attenuated mycobacteria with low immunogenicity, this vaccine targets the DNA B protein of *Mycobacterium tuberculosis*, a highly conserved essential molecule in the pathogen life cycle (Zhang et al., 2014). With an immunoinformatics-based approach, the selected epitopes have high antigenicity, non-allergenicity, and non-toxicity, thus enhancing its safety and efficacy (Chen et al., 2020a). Molecular dynamics simulations also showed a stable interaction with Toll-like receptor 4 (TLR4), which is important in innate immune activation, supporting the potential of this vaccine to trigger a strong immune response (Almofti et al., 2021).

Despite its promising design, this multiepitope vaccine faces certain limitations that require further optimization. In particular, the lack of a strong cytotoxic T lymphocyte (CTL) epitope limits its ability to potently activate CD8+ T cells, which are critical for eliminating intracellular pathogens such as *Mycobacterium tuberculosis* (Chen et al., 2020). Further research could begin by optimizing the CD8+ response by conducting research on the addition of suitable adjuvants to this vaccine. This process begins with the selection of an adjuvant that supports this mechanism, such as saponins (e.g. QS-21), which enhance antigen uptake by dendritic cells. The antigen is then formulated with the selected adjuvant. Next, its effectiveness was tested in vitro using dendritic cell cultures to observe antigen presentation and T cell activation, followed by in vivo testing in animal models to evaluate the immune response and resulting protection (Rapaka et al., 2021).

Conclusion

Mycobacterium tuberculosis remains a global problem, especially in developing countries, as the existing BCG vaccine is less effective in protecting adults from pulmonary tuberculosis. This study developed a multiepitope vaccine targeting the DNA B protein of Mycobacterium tuberculosis, consisting of nine peptide sequences with high antigenicity, non-allergenicity, and

non-toxicity, ensuring its safety and immunogenicity. Molecular docking results showed that the vaccine stably binds to Toll-like receptor 4 (TLR4), an important receptor in the innate immune system, highlighting its ability to trigger immune activation. The vaccine also showed good thermal stability, supported by a high aliphatic index, indicating its resistance to temperature variations and suitability for storage and application. However, the lack of a strong CTL epitope limits its ability to potently activate CD8+ T cells. This limitation can be overcome by exploring adjuvants to enhance cellular immunity. Future studies will focus on in vitro and in vivo validation to ensure the safety and efficacy of the vaccine, paving the way for its advancement towards clinical application.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgements

Sincere gratitude is extended to the supervisor for their invaluable guidance and support throughout the duration of this research project. Their expertise and insights significantly contributed to the understanding of bioinformatics and molecular biology. This research was conducted through the collaborative efforts of all parties involved in the project as part of the bioinformatics learning within the Molecular Biology program. Appreciation is also expressed to the Department of Medical Laboratory Technology (MLT), Health Polytechnic of the Ministry of Health Bandung, for providing the essential facilities and resources that facilitated this research.

References

Aasim, Sharma, R., Patil, C. R., Kumar, A., & Sharma, K. (2022). Identification of vaccine candidate against Omicron variant of SARS-CoV-2 using immunoinformatic approaches. *In Silico Pharmacology*, *10*(1), 12. https://doi.org/10.1007/s40203-022-00128-y

AlChalabi, R., Al-Rahim, A., Omer, D., & Suleiman, A. A. (2022). Immunoinformatics design of multi-epitope peptide-based vaccine against Haemophilus influenzae strain using cell division protein. *Network Modeling Analysis in Health Informatics and Bioinformatics*, *12*(1), 1. https://doi.org/10.1007/s13721-022-00395-x

Aasim, Sharma, R., Patil, C. R., Kumar, A., & Sharma, K. (2022). Identification of vaccine candidate against Omicron variant of SARS-CoV-2 using immunoinformatic approaches. *In Silico Pharmacology*, *10*(1), 12. https://doi.org/10.1007/s40203-022-00128-y

AlChalabi, R., Al-Rahim, A., Omer, D., & Suleiman, A. A. (2022). Immunoinformatics design of multi-epitope peptide-based vaccine against Haemophilus influenzae strain using cell division protein. *Network Modeling Analysis in Health Informatics and Bioinformatics*, *12*(1), 1. https://doi.org/10.1007/s13721-022-00395-x

Almofti, Y. A., Abd-elrahman, K. A., & Eltilib, E. E. M. (2021). Vaccinomic approach for novel multi epitopes vaccine against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *BMC Immunology*, *22*(1), 22. https://doi.org/10.1186/s12865-021-00412-0

Alspach, E., Lussier, D. M., & Schreiber, R. D. (2019). Interferon γ and Its Important Roles in Promoting and Inhibiting Spontaneous and Therapeutic Cancer Immunity. *Cold Spring Harbor Perspectives in Biology*, *11*(3), a028480. https://doi.org/10.1101/cshperspect.a028480

Chen, H.-Z., Tang, L.-L., Yu, X.-L., Zhou, J., Chang, Y.-F., & Wu, X. (2020a). Bioinformatics analysis of epitope-based vaccine design against the novel SARS-CoV-2. *Infectious Diseases of Poverty*, *9*(1), 88. https://doi.org/10.1186/s40249-020-00713-3

Chen, H.-Z., Tang, L.-L., Yu, X.-L., Zhou, J., Chang, Y.-F., & Wu, X. (2020b). Bioinformatics analysis of epitope-based vaccine design against the novel SARS-CoV-2. *Infectious Diseases of Poverty*, *9*(1), 88. https://doi.org/10.1186/s40249-020-00713-3

Fatima, S., Kumari, A., Das, G., & Dwivedi, V. P. (2020). Tuberculosis vaccine: A journey from BCG to present. *Life Sciences*, *252*, 117594. https://doi.org/10.1016/j.lfs.2020.117594

Ghandadi, M. (2022). An Immunoinformatic Strategy to Develop New *Mycobacterium tuberculosis* Multi-epitope Vaccine. *International Journal of Peptide Research and Therapeutics*, *28*(3), 99. https://doi.org/10.1007/s10989-022-10406-0

Jing, X., Xu, M., Song, D., Yue, T., Wang, Y., Zhang, P., Zhong, Y., Zhang, M., Lam, T. T.-Y., Faria, N. R., De Clercq, E., & Li, G. (2022). Association between inflammatory cytokines and anti-SARS-CoV-2 antibodies in hospitalized patients with COVID-19. *Immunity & Ageing*, *19*(1), 12. https://doi.org/10.1186/s12979-022-00271-2

Lee, M. N., & Meyerson, M. (2021). Antigen identification for HLA class I– and HLA class II–restricted T cell receptors using cytokine-capturing antigen-presenting cells. *Science Immunology*, *6*(55). https://doi.org/10.1126/sciimmunol.abf4001

Liu, T., Shi, K., & Li, W. (2020). Deep learning methods improve linear B-cell epitope prediction. *BioData Mining*, *13*(1), 1. https://doi.org/10.1186/s13040-020-00211-0

Lv, H., Zhang, X., Zhang, X., Bai, J., You, S., Li, X., Li, S., Wang, Y., Zhang, W., & Xu, Y. (2024). Global prevalence and burden of multidrug-resistant tuberculosis from 1990 to 2019. *BMC Infectious Diseases*, *24*(1), 243. https://doi.org/10.1186/s12879-024-09079-5

Miggiano, R., Rizzi, M., & Ferraris, D. M. (2020). *Mycobacterium tuberculosis* Pathogenesis, Infection Prevention and Treatment. *Pathogens*, *9*(5), 385. https://doi.org/10.3390/pathogens9050385

Niazi, S. K., Mariam, Z., & Paracha, R. Z. (2024). Limitations of Protein Structure Prediction Algorithms in Therapeutic Protein Development. *BioMedInformatics*, *4*(1), 98–112. https://doi.org/10.3390/biomedinformatics4010007

Obaidullah, A. J., Alanazi, M. M., Alsaif, N. A., Albassam, H., Almehizia, A. A., Alqahtani, A. M., Mahmud, S., Sami, S. A., & Emran, T. Bin. (2021). Immunoinformatics-guided design of a multi-epitope vaccine based on the structural proteins of severe acute respiratory syndrome coronavirus 2. *RSC Advances*, *11*(29), 18103–18121. https://doi.org/10.1039/D1RA02885E

Penn, B. H., Netter, Z., Johnson, J. R., Von Dollen, J., Jang, G. M., Johnson, T., Ohol, Y. M., Maher, C., Bell, S. L., Geiger, K., Golovkine, G., Du, X., Choi, A., Parry, T., Mohapatra, B. C., Storck, M. D., Band, H., Chen, C., Jäger, S., … Krogan, N. J. (2018). An *Mycobacterium tuberculosis* - Human Protein-Protein Interaction Map Identifies a Switch between Host Antiviral and Antibacterial Responses. *Molecular Cell*, *71*(4), 637-648.e5. https://doi.org/10.1016/j.molcel.2018.07.010

Rapaka, R. R., Cross, A. S., & McArthur, M. A. (2021). Using Adjuvants to Drive T Cell Responses for Next-Generation Infectious Disease Vaccines. *Vaccines*, *9*(8), 820. https://doi.org/10.3390/vaccines9080820

Rizarullah, Aditama, R., Giri-Rachman, E. A., & Hertadi, R. (2024). Designing a Novel Multiepitope Vaccine from the Human Papilloma Virus E1 and E2 Proteins for Indonesia with Immunoinformatics and Molecular Dynamics Approaches. *ACS Omega*, *9*(14), 16547–16562. https://doi.org/10.1021/acsomega.4c00425

Shey, R. A., Ghogomu, S. M., Nebangwa, D. N., Shintouo, C. M., Yaah, N. E., Yengo, B. N., Nkemngo, F. N., Esoh, K. K., Tchatchoua, N. M. T., Mbachick, T. T., Dede, A. F., Lemoge, A. A., Ngwese, R. A., Asa, B. F., Ayong, L., Njemini, R., Vanhamme, L., & Souopgui, J. (2022). Rational design of a novel multi-epitope peptide-based vaccine against Onchocerca volvulus using transmembrane proteins. *Frontiers in Tropical Diseases*, *3*. https://doi.org/10.3389/fitd.2022.1046522

Xia, T., Fu, S., Yang, R., Yang, K., Lei, W., Yang, Y., Zhang, Q., Zhao, Y., Yu, J., Yu, L., & Zhang, T. (2023). Advances in the study of macrophage polarization in inflammatory immune skin diseases. *Journal of Inflammation*, *20*(1), 33. https://doi.org/10.1186/s12950-023-00360-z

Yin, Z., Li, M., Niu, C., Yu, M., Xie, X., Haimiti, G., Guo, W., Shi, J., He, Y., Ding, J., & Zhang, F. (2023). Design of multi-epitope vaccine candidate against Brucella type IV secretion system (T4SS). *PLOS ONE*, *18*(8), e0286358. https://doi.org/10.1371/journal.pone.0286358

Zhang, H., Zhang, Z., Yang, J., & He, Z. (2014). Functional characterization of DnaB helicase and its modulation by single-stranded <scp>DNA</scp> binding protein in *Mycobacterium tuberculosis*. *The FEBS Journal*, *281*(4), 1256–1266. https://doi.org/10.1111/febs.12703