Chapter

In-Silico Approach in the Development of *Salmonella* Epitope Vaccine

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Abstract

In the case of infection control, one of our primary concerns is typhoid fever. According to WHO, typhoid prevalence in Indonesia is highly endemic. There is also the problem with the low efficacy of the available vaccine to prevent the disease. Therefore, there is an urgent need to develop a highly effective typhoid vaccine. One of the phases in vaccine development is an exploratory phase, a research-intensive phase of the vaccine development process designed to identify natural or synthetic antigens that might help prevent or treat a disease through computer in silico prediction targets. The vaccines developed through epitope peptide are designed to be safer, more efficacious, and less expensive than traditional vaccines. A thorough understanding of the disease agent, particularly critical epitopes to induce the appropriate immunological reaction, is required to achieve these aims. Mapping epitope sequences or antigenic peptides from pathogenic proteins recognized by B cells and T cells is crucial for vaccine development. Once the epitopes were identified, the polypeptide production could be produced through protein recombinant technology. The polypeptide vaccine, in the end, could be delivered using a liposomal delivery system.

Keywords: epitope, vaccine, typhoid, infection, control

1. Introduction

1.1 The urgency of infection control

Research about vaccines is urgently required because vaccinations are still the most effective way to prevent illness, disability, and death from vaccine-preventable diseases, such as Diphtheria, Pertussis, and Tetanus. WHO reported that global immunization successfully averts 2–3 million deaths of children every year. However, this achievement can still be improved, which means an additional 1,5 million deaths can be prevented. 19,4 million children worldwide are still missing out on essential vaccines. One of the critical strategies to improve global vaccine coverage is to provide the vaccine at all times and all places, in the best quality.

In the case of infection control, one of the major concerns is typhoid fever. According to WHO, typhoid prevalence in Indonesia is highly endemic. Typhoid disease still has to get serious attention because of its increasingly complex problems, making it difficult to manage, treat, and prevent [1]. This problem becomes

even more difficult with the increasing resistance to commonly used antibiotic drugs. At present, there have even been reported cases of resistance to many drugs (multidrug resistance) spread throughout the world [2]. There is also a problem with the low efficacy of the available vaccine to prevent the disease. Therefore, there is an urgent need to develop a highly effective typhoid vaccine.

2. Six stage vaccine development

According to the CDC [3], there are six vaccine development stages: exploratory, preclinical, clinical development, regulatory review, approval, manufacturing, and quality control.

Exploratory: This research-intensive phase of the vaccine development process is designed to identify "natural or synthetic antigens that might help prevent or treat a disease." Antigens might include weakened strains of a particular virus/bacteria.

Preclinical: During this phase, researchers use tissue-culture or cell-culture systems and animal testing to determine whether the candidate vaccine will produce immunity. Many candidate vaccines do not move on to the next stage of development because they fail to produce that immunity or prove harmful to test subjects.

Clinical development: At this point, a sponsor, usually a private company, submits an application for an Investigational New Drug (IND) to the U.S. Food and Drug Administration (FDA) or BPOM (*Badan Pengawas Obat dan Makanan,* National Food, and Drug Agency) in Indonesia. This step summarizes findings to date and describes how the drug will be tested and created. An institution that will host the clinical trial holds a review board for approval of the application. The FDA has 30 days to approve the application. Once the proposal has been approved, the vaccine must pass three trial stages of human testing.

Regulatory review and approval: If a vaccine passes through all three clinical development phases, the vaccine developer submits the registration documents to the regulatory board.

Manufacturing: Major drug manufacturers provide the infrastructure, personnel, and equipment necessary to create mass quantities of vaccines. They also reap the profits of successful or widely distributed drugs.

Quality control: The approval and distribution are far from the end of the line. Stakeholders must adhere to procedures that allow them to track whether a vaccine is performing as anticipated.

3. Improving safety and efficacy of typhoid vaccine using epitope vaccine

On the other hand, there are vaccine safety and efficacy issues that cannot be ignored. There are three different types of vaccination developed for *S. typhi*: live-attenuated pathogens, inactivated pathogens, and sub-unit vaccines. From the safety point of view, sub-unit vaccines provide better safety profiles because they only use specific proteins and could not be reverted into a virulent form. However, conventional protein isolation usually results in a minimal yield; thus, we need to develop an epitope vaccine. Epitope vaccine is a part of the subunit vaccine, which only uses the antigen's epitope area. The interaction of epitopes and antibodies are particular, and the peptides are well characterized. Therefore, we can produce the peptides for the epitope vaccine using the recombinant technique.

Until now, vaccines for typhoid fever that have been available and show the safety and effectiveness of several clinical trials and are recommended by the CDC

(Center for Disease Control and Prevention, USA) are oral Ty21a vaccine and ViCPS vaccine (Vi capsular polysaccharide) given parenterally [4]. Ty21a is a vaccine that uses a weakened organism (oral attenuated vaccine). This orally administered vaccine is technically more comfortable to use because it does not cause pain but can be virulent if given to an immunocompromised individual. ViCPS vaccine is a parenterally administered independent T-cell antigen that gives uncomfortable pain to the patients [5].

The development of bioinformatics tools and advances in recombinant DNA technology (rDNA) and the knowledge on the host immune response and the genetic background of the pathogen will lead to new vaccines against diseases that currently have few or no control measures in just 1 or 2 years. Through computer in silico predictions to define targets. The vaccines developed through rDNA technologies are safer, more efficacious, and less expensive than traditional vaccines. A thorough understanding of the disease agent, particularly critical epitopes to induce the appropriate immunological reaction, is required to achieve these aims [6].

The epitope is part of the antigen that would be recognized by the antibody [7]. Different epitopes of protein antigens can be identified based on sequences from amino acids or different conformational forms. Some epitopes are hidden in antigen molecules and exposed as a result of physicochemical changes. Epitope vaccine is part of the subunit/peptide vaccine. Peptide vaccines can be used to induce broad-spectrum immunity against some serological variants (serovar) or certain pathogenic strains by formulating several non-contiguous immunodominant epitopes and conserved epitopes between different serovars/pathogenic strains.

On the other hand, due to the relatively small peptides, they are often immunogenic weak on their own and therefore require carrier molecules to add chemical stability and adjuvants to induce a robust immune response. Allergenicity and molecular reactogenicity of the carrier itself increases the complexity of the peptide vaccine design. Making peptide vaccines are generally considered safe and cost-effective when compared to conventional vaccines [8].

4. Stages development of epitope vaccine

In principle, a material's antigenic nature shows how much the antigen's ability to bind to antibodies and cause different reactions in human immunity formation. Antibodies or immunoglobulins are specialized proteins that are products of differentiated B lymphocytes or plasma cells. The bond between antibodies and antigens induces systemic immunity and activates the complement to process further activate the humoral immune system [9].

Specific antibodies can be made on an individual's body by immunizing selected peptides that present epitopes of these proteins. Epitopes play an essential role in vaccine development. Mapping epitope sequences or antigenic peptides from pathogenic proteins recognized by B cells and T cells is crucial for vaccine development. Epitope mapping provides useful information for designing peptide-based vaccines and as libraries to monitor specific cellular immunity in protected individuals, patients, and vaccines [8].

B cell epitope mapping is divided into linear and nonlinear B cell epitope mapping. Although minor, linear B cell mapping has further attention in vaccine research because linear epitopes are epitopes ready to replace antigens in immunization. There are various epitope mapping method using different approaches and algorithms; for instance Kolaskar and Tongaonkar [10], Bepipred, Preditop, ABCPred, LBtope, and many others [11].

The IEDB (The Immune Epitope Database) maps experiments identify and characterize epitopes and epitope-specific receptors with related details, including host organisms, immune exposures, and induced immune responses. The genes that encode polypeptides are then cloned into pRSETA, a bacterial vector for high-level expression of proteins, plasmid vectors. Transformation of the recombinant plasmid in *E. coli* host was followed by induction with IPTG (Isopropyl ß-D-1-thiogalactopyranoside), which resulted in a polypeptide expression as observed in SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) and Western blot analysis. Purified polypeptides were subjected to step dialysis. The final concentration was quantified and adjusted to 2.5 mg/ml by Lowry's calorimetric assay against BSA (Bovine Serum Albumin) standard [12].

5. In silico experiments as tools to design epitopes for vaccine

Epitope-based vaccine design using this computational method silico is an effective strategy that can lead to vaccine development to induce the necessary immunogenicity without the emergence of a cytokine storm or immune tolerance. Based on several in vitro studies and in vivo, if scientifically and critically designed, epitope-based vaccines offer several advantages over other types of vaccines, including their fast design and accurate, time/cost effective formulations, and desired immunogenicity with minimal side effects [13].

There is no denying that vaccination is beneficial in promoting a healthy global population. This act has saved countless lives, reduced healthcare costs, and improved the quality of human life. Accidental discoveries in immunology are augmented by knowledge about bioinformatics tools for epitope prediction, resulting in the emergence of a pattern new vaccine design. The art and science of efficient and comprehensive information extraction and analysis of data stored in relevant databases is of increasing importance in research related to immunology [14].

Fortunately, although research in experimental immunology are expensive and highly intensive, usually large amounts of data are generated. Such data can only be analyzed with high precision and high-speed using bioinformatics tools. For example, genome sequencing as well as in vitro T-cell confirmation takes place within a few months. With conventional vaccine designs, computational immunology methods drastically reduce time and labor requirements in epitope screening. With computational immunological techniques, it is possible to find vaccine candidate epitopes only by scanning deep protein sequences of the desired pathogen. Many of these proteins have not been isolated or at least cloned into specific and unique pathogens, and they present a ready candidate in vaccine construction [14].

This in silico strategy also helps in selecting better molecules before testing conditions in vitro or in vivo. In this early stage, the use of in silico methods can direct and thus significantly shorten the next experimental work. Besides, proper use of the silico method can replace, reduce and improve the usage of animal experiments that are often misleading and time-consuming [15].

The position of the in-silico method in the vaccine development process lies at the preclinical development stage. In vaccine development, the first steps are to do is identify a vaccine candidate. The preclinical stage aims to determine the safety profile of the vaccine. During this stage, the researchers will carefully select the appropriate antigen and technology, and in vitro and in vivo will do. The information gathered from this study will be essential to continue with the subsequent clinical trials in humans [16].

There are some advantages of using the In silico method [17]. This technique offers an advantage in giving new drug candidates faster and at a lower cost. It is

also Increases the chances of success in the many stages of the discovery process and facilitates access to the large amount of data generated. In silico experiments, it was also turning massive complex biological data into useful knowledge.

Revolution in information technology and molecular biology, together with growth in genome data storage, has provided the basis for vaccine design using computational and bioinformatics tools. These tools are used for silico mapping of the most precise and immunogenic components for the manufacture of a hypothetical protein. The vaccine that is designed can then be simulated and evaluated prior to experimental validation, enabling the process research and development (R&D) to be carried out efficiently, and leads to the development of vaccines with few adverse effects [13]. Here is some software that can be used to help in silico design of epitope vaccine.

5.1 Mega x™

Over the last decade, genome sequencing has become an efficient and efficient way to investigate a wide variety of biological systems, ranging from diversity studies large-scale biology to tracking the evolution and origin of pathogenic microbes. The steps needed to gather interpretable and interpretable results actionable from raw sequence data always require a comparative analysis of molecular sequences to find differences in functional and adaptive genomes. The Molecular Evolutionary Genetics Analysis (MEGA) software provides the tools to do that analysis. MEGA includes many programs for assembling sequence alignments, inferring evolutionary trees, estimating genetic distances and diversities, inferring ancestral sequences, computing time trees, and testing selection [18]. MEGA software or other software allows alignment of *salmonella* typhi genome sequences from various countries to determine which sequences are considered sustainable. The selection of sustainable sequences is vital for the manufacture of vaccines so that later the vaccine can be used in many countries.

5.2 IEDB

The Immune Epitope Database (IEDB, iedb.org) [19] captures experimental data limited in figures, texts, and tables of the scientific literature, making them available online free and easily searchable by the public. The scope of the IEDB includes data on immune epitopes associated with all studied species and includes the antibody binding context, T cells, and MHCs associated with infection, allergy, autoimmune, and related diseases transplant [20]. The IEDB is a website that provides tools computations that focus on the prediction and analysis of B and T cell epitopes. The IEDB maps experiments identify and characterize epitopes and epitope-specific receptors with related details, including host organisms, exposures immunity, and induced immune response. The site associated with the IEDB is the ISDBA Analysis Resource, which is a predictor of various B cell and T cell epitopes. Using a trained and validated algorithm [21]. An antigenicity analysis was carried out using the Kolaskar and Tongaonkar principles followed by epitope mapping using Bepipred. Both are done based on a database owned by IEDB (The Immune Epitope Database).

IEDB web server using some parameters, including Kolaskar-Tongaonkar as the antigenicity scale parameter, which is the standard for epitope prediction [22]. The Kolaskar and Tongaonkar antigenicity scales based on the amino acid residues' physicochemical properties and the known tendency frequency as an experimental epitope have an accuracy of 75% [10]. The BepiPred-2.0 server predicts B cell epitopes from protein sequences, using a Random Forest algorithm trained on epitopes and amino acids non-epitope determined from the crystal structure [21, 23]. The Immune Epitope Database (IEDB) retrieves experimental data through pictures,

texts, and tables of scientific literature, even the scope of the IEDB extends to the immune epitope, where data are linked to all studied species including antibodies, T cells, and the binding context MHC is associated with infections, allergies, autoimmune, and transplant-related diseases [20].

5.3 VAXIJEN 2.0™

VaxiJen is the first server for the prediction of protective antigens, antigens tumor, and vaccine subunits. It is also a grade-free bioinformatics tool first for silico immunogen identification. VaxiJen uses the Z-scale Wold to explain the Physicochemical properties of the primary amino acids building protein. The Amino acids are tested, converting the derived string to a uniform vector with auto cross-covariance (ACC). The next step is to select the relevant variable with a genetic algorithm (G.A.) or gradual regression and classify protein as a protective or non-antigen antigen by discriminant analysis least-squares based (PLS). Initially, Algorithm files are trained to identify the protective immunogens of bacteria. A model for immunogens viruses and tumors was then included, and VaxiJen was developed to provide access free to the model. The latest version of VaxiJen (VaxiJen 2.0) also includes a model for identifying parasitic and fungal immunogens [15]. Vaxijen used to validate the results of the epitope sequences found from the IEDB whether they are immunogenic or not.

6. Epitope mapping model using IEDB

The IEDB was first published in 2004 using data that limited numbers, text, and tables of scientific literature. Finally, in 2015, the IEDB experiments' number increased by 140% to exceed 1.6 million, and receptor sequence data in the IEDB scheme. Previously, this device could only capture antibodies and the T cell receptor (TCR) but can now capture antibodies and complementarity determining regions (CDR), which is essential for the antigen's specificity of its diversity. The scope of this device extends throughout the epitope for data relating to all species includes immunity body/antibody, the context of T cell binding, and MHC-related infections, allergies, autoimmune, and transplantation of certain diseases and features to access also summarize data in terms of quantity and complexity [20].

An antigenicity analysis is used, and epitope mapping is also carried out (**Figure 1**) to analyze areas that have antigenicity potential against B-cell, for example, using Bepipred and Emini methods. Both are done based on a database owned by IEDB (The Immune Epitope Database). Selection of epitopes according to the score above the threshold. Epitope mapping was performed using Bepipred software from the immune epitope database (HTTP://toolsiedb.ofg/bcell/) to find linear B cell epitopes from a sustainable region with an average threshold value of 0.030. This method is classically used to measure propensity.

Moreover, using hidden Markov programming [21], we show the epitope mapping of the OMP28 protein, a typhus vaccine candidate researched by Saxena et al. (2012). OMP28 is outer membrane protein 28 from *Salmonella enterica subsp. enterica* serovar Typhi with Accession number NCBI: ACX42427. The OMP28 protein sequence is MNKFSLATAGIIVAALVTSVSVN AATDTTKTNVTPKGMSCQEFVDLNPQTMAPVAFWVLNEDEDFKGGD YVDFQETETTAVPLAVELCKKNPQSELSKIKDEIKKELSK. Preferably, before starting epitope mapping, we need to make sure that the sequence that will be

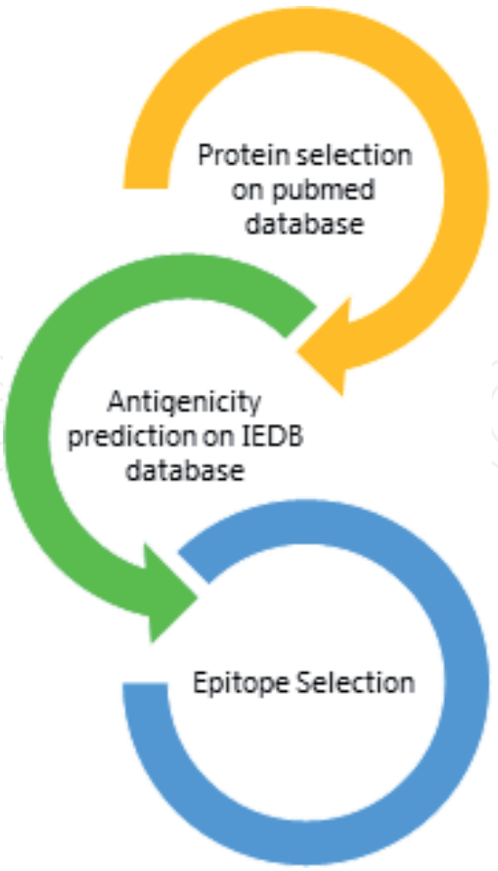

Figure 1.
Steps of epitope mapping.

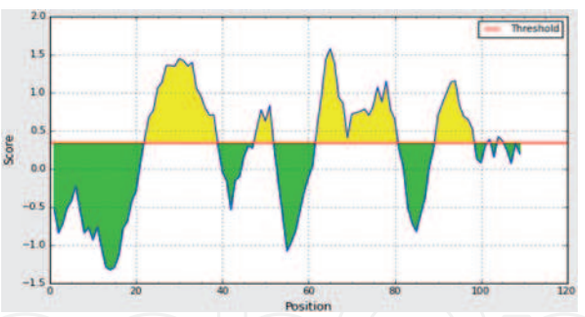

Figure 2.
B cell epitope mapping using the IEDB Bepipred method (IEDB.org) [19].

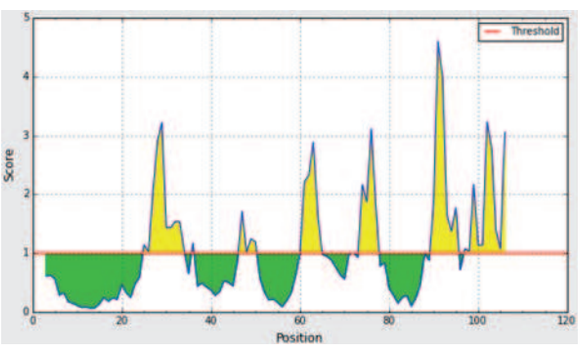

Figure 3.
B cell epitope mapping using the IEDB Emini method (IEDB.org) [19].

analyzed is a conserved region. This step can be done by aligning many of the same protein sequences from different strains of *salmonella*.

The epitope mapping results are shown in **Figures 2** and **3**; the yellow area is considered to have high antigenicity potential. In bepipred linear epitope prediction, a value equal to or greater than the threshold value of 0.030 is said to have a strong potential to bind to B cells. This is also done for other methods, such as the emini surface method with a threshold limit of 1.

From the **Tables 1** and **2**, it can be seen that the highest epitope potential is in positions 62th–80th because it has the highest score 1.581 with a long sequence of 19 amino acid sequences, and 22–38 positions with a score of 1.449 along with

Bepipred Method Analysis (Treshold = 1,030)			
Score Epitope Prediction			
Position	**Residue**	**Score**	**Assignment**
65	F	1.581	E
30	K	1.449	E
64	D	1.436	E
31	T	1.422	E
33	V	1.399	E

Sequence Epitope Pediction				
No.	**Start**	**End**	**Peptide**	**Length**
1	22	38	VNAATDTTKT NVTPKGM	17
2	48	51	PQTM	4
3	62	80	DEDFKGGDYV DFQETETTA	19
4	90	98	KNPQSELSK	9
5	102	102	E	1
6	104	105	KK	2

Table 1.
Resume of Bepipred analysis on OMP28 sequence.

Emini Analysis (Treshold = 1) Score Epitope Prediction					
Position	**Residue**	**Start**	**End**	**Peptide**	**Score**
91	N	89	94	KKNPQS	4.605
92	P	90	95	KNPQSE	3.988
102	E	100	105	KDEIKK	3.235
29	T	27	32	DTTKTN	3.221
76	T	74	79	QETETT	3.115

Sequence Epitope Prediction				
No.	**Start**	**End**	**Peptide**	**Length**
1	25	34	ATDTTKTNVT	10
2	90	95	KNPQSE	6

Table 2.
Resume of Emini analysis on OMP28 sequence.

the 17 amino acid sequences. As a comparison, the antigenicity analysis was also performed using the emini surface technique, and the most significant potential was obtained at positions 25–34 with a sequence length of 10 amino acids, namely ATDDTKTNVT.

Based on the analysis of bepipred and emini surface, the sequences that have the potential to provide the greatest immunogenicity can be identified at positions 22–38 and positions 62–80. Furthermore, this peptide can be produced by the recombinant protein method. The multi-epitope candida vaccine is considered more promising than the single epitope vaccine.

7. Epitope vaccine delivery

One of the drawbacks of using peptide fragments as vaccine antigens is the weak immunogenicity generated compared to inactive and live-attenuated vaccines [24]. A formulation with the addition of adjuvant ingredients is needed to increase the immune response in the subjects. One example of adjuvants that have been used commercially for a long time in vaccine formulations is aluminum salts or "alum" [25]. Several publications report alum mechanisms such as creating a gel depot that prolonged exposure of the immune system to antigen, forming particulate structures that promote antigen uptake by APCs (Antigen Presenting Cells) via phagocytosis, and inducing inflammation and secretion of chemokines. However, its inability to induce Th1 cell-mediated immune responses also becomes a limitation for this adjuvant [26], Since the previous study indicated that the typhoid sub-unit vaccine-induced cellular immunity through TCD4+ [27].

The benefit of liposome as carrier and adjuvant for antigen has been known for quite a long time [28]. Liposomes can boost immune response due to its mechanism to create depot effect by causing antigen retention and slowly releasing them to the immune cells [29]. Liposome delivery of antigen is influenced by lipid bilayer components such as lipid choice and the role of cholesterol [30]. We can either choose neutral lipid or charged lipid as the membrane constructing unit. However, previous research showed that positively charged lipid like DDA (Dimethyldioctadecyl ammonium) has an advantage over neutral lipid [31–33]. Cationic DDA can effectively interact with the APC cell membrane and increase the number of antigens delivered into the cell [34]. To put it simply, the more antigens delivered, the more significant immune responses are. However, DDA could not produce a stable vesicle due to its positive charge due to electro-repulsive force. Therefore, previous publications mentioned that DDA should be formulated with phosphatidylcholine as a major lipid component and cholesterol to maintain membrane integrity [35, 36].

8. Conclusion

There is an urgent need to develop a highly effective typhoid vaccine, especially in a highly endemic region. One of the reasons above is that this paper's rationale is to find a faster, cheaper, and more efficient vaccine candidate design. The vaccine design used is the Peptide vaccine, and the design is done in silico. Peptide vaccine has several advantages when compared to conventional vaccines. The advantages of peptide vaccines can be seen in specifications, disease, purity, production capacity, and production cost efficiency.

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Conflict of interest

The authors declare no conflict of interest.

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