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RESEARCH ARTICLE

In Silico Approach: Beta Glucan and AdhO36 Combinations Enhance the Th1 Immune Response against *Salmonella Typhi* Infection

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Abstract

Background: Typhoid fever caused by *Salmonella* Typhi remains as an infectious disease that still becomes a health problem, especially in developing countries due to its fairly high morbidity. Vaccine development efforts, particularly about proteins that play a role in enhancing immunity, are adhesin protein (Protein AdhO36) and the addition of immunoadjuvant Beta Glucan that can improve the cellular immune system. Objective: This study was aimed to predict the ligand-receptor bonds (Dectin1-Beta Glucan; TLR2-Beta Glucan, TLR4-Beta Glucan) and the ligand-receptor bonds (Dectin1-AdhO36; TLR2-AdhO36, TLR4-AdhO36). Method: To determine the binding affinity of the bond between Beta-Glucan and Dectin1, TLR2, and TLR4, a molecular approach was specifically used using PyRx 0.8.Whereas to find out the interaction between Adh036 and target proteins Dectin1, TLR2, and TLR4, Patchdock software was used. The results of the interaction would be visualized using PyMol software. Amino acids involved in docking interactions were identified using KFC protein hotspots. Results and Conclusion: The result indicated the binding affinity between receptor Dectin1-ligand Beta Glucan and Beta Glucan TLR2 was (-6.5 kcal/mol), and TLR4-Adh036 shown the highest interaction strength with global energy -1462.6. This result has shown the potential effect of AdhO36 and Beta Glucan combinations enhanced cellular immunity against *Salmonella* Typhi infection.

Keywords: *AdhO36, Beta Glucan, TLR2, TLR 4, Dectin-1.In Silico.*

Introduction

Typhoid fever is a life-threatening infection caused by Salmonella Typhi, Nonetheless, infectious diseases are still a major cause of illness and death, especially in developing countries. World Health Organization (WHO) reported that around the world there are estimated as many as 11 to 21 million illnesses annually and 128,000 to 161,000 deaths associated with typhoid fever [1].Typhoid fever are still become health problem in Indonesia. Indonesia's hospitals reported that suspected case of typhoid fever illness of the number of typhoid fever cases in Indonesia has increased 500/100,000 people and deaths between 0.6-5% [2].At present, typhoid disease still needs serious attention because the problems are increasingly complex, thus making it difficult to manage, treat, and prevent [2, 3]. Increasing cases of carrier and relapses indicate that the current treatment method has not been effective [2].This problem becomes increasingly difficult with increasing resistance to antibiotics commonly used [4].Besides treatment procedures, efforts on prevention have been carried out, including providing vaccines especially to high-risk communities, such as those living in endemic areas.

Until now, vaccines for typhoid fever that are already available and show their safety and effectiveness from several clinical trials and is recommended by the CDC (Center for Disease Control and Prevention, USA) are oral Ty21a vaccines and parenteral ViCPS vaccine (VI capsular polysaccharide), the efficacy is only between 51% and 67%. In Indonesia, the efficacy of enteric capsule preparation use is only 43% [5]. Considering the protection capacity obtained from the provision of various types of known vaccines is still relatively low. The development of vaccines for controlling typhoid fever is still very much needed.

The research proven that *Salmonella* Typhi has AdhO36 as adhesin protein. The AdhO36 protein is proven to be a virulence factor in the process of adhesion and colonization, which has the potential to be immunogenic to stimulate the formation of protective S-IgA, so it can be a candidate vaccine to prevent the initial stages of infection.

Further research proved that immunization of AdhO36 protein via oral gave significant effect in inhibiting Salmonella Typhi attachment to mouse intestines. It was also reported that the AdhO36 Salmonella Typhi vaccine conjugated by ISCOM improved cellular immune responses, increased IgG2a opsoning antibody level (in mice), increased CD8+ T cell counts, induced delayed type hypersensitivity (DTH reactions and inhibited bacterial colonization [6].

Increasing the effectiveness of vaccines, especially AdhO36, is possible to do manipulations in the form of adding materials that are immunoadjuvants, which can improve the cellular immune system. At present, many known ingredients have the potential as immuno-derivatives derived from natural ingredients, one of which is Beta-Glucan which is a component of polysaccharides which has proven to be a potent immuno-immunity [7].

Beta-Glucan is considered a stimulator of cellular immunity, activating macrophages [8]. Some Beta-Glucan studies have been shown to have biological effects, such as the potentiating effects of immunity on anti-infection [9]. One source of Beta-Glucan is from *Candida albicans*. *Candida albicans* is a polymorphic fungus that is a normal intestinal flora, lives commensally, among others, in the mucous membranes of the oral cavity, respiratory tract, digestive tract, and female genital organs [10, 11, 12, 13]. The results showed that Beta-Glucan derived from wall cell extracts of *Candida albicans* could inhibit colonization of *Salmonella* Typhimurium by potentiating cellular immunity, namely an increase in CD4 + and CD8 + levels [14]. However, until recently, no studies have examined the effect of Beta-Glucan administration on individuals who have received AdhO36 immunization that can increase their protective power. Therefore, further research is needed to enhance the effectiveness of vaccination.

The initial step in designing and developing a new vaccine for typhoid fever by using in-silico method [15].The aims of our study to predict the target of a query compound to bind a new receptor-ligand target. The receptors and ligands used in this study were Beta-Glucan and Dectin1, TLR2, and TLR4 and interaction between Adh036 and target proteins Dectin1, TLR2, and TLR4.

We predict binding affinity between receptor-ligands (Dectin1-Beta Glucan; TLR2-Beta Glucan; TLR4-Beta Glucan), receptor-ligands (Dectin1-AdhO36; TLR2-AdhO36; TLR4-AdhO36) and the binding affinity between ß-glucan and Dectin, TLR2, and TLR4, a molecular approach using PyRx 0.8 was specifically carried out, while to determine the interaction between AdhO36 and target proteins dectin, TLR2, and TLR4, Patchdock software was used.

Materials Methods

Sampling

The 3D structure of beta-1,3 glucan was taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) with CID 64649, while the 3D protein structures that are Dectin-1 (ID 2CL8), TLR2 (ID 2Z7X), and TLR4-MD2 (ID 3FXI) were collected from the GDP database (https://www.rcsb.org).

Modeling of Adhesin Proteins 036 (Adh036)

The 3D Protein Adh036 structure has not been crystallized, so it must be modeled. The epitope found in the Adh036 protein is aligned using blastp (https://blast.ncbi.nlm.nih.gov/Blast.cgi), so the protein has a similarity sequence with Adh036. Following this, protein modeling was done using Swiss-Model

(https://swissmodel.expasy.org/) with sequence identity parameters of more than 20%.

Molecular Docking analysis

To find out the binding affinity between Beta-Glucan 1.3 and dectin, TLR2, and TLR4, a molecular approach was specifically used using PyRx 0.8.Whereas, to find out the interaction between Adh036 and the target protein dectin, TLR2, and TLR4, the Patch dock software (https:// bioinfo 3d.cs. tau.ac. il/PatchDock/) was used. The target areas used in Dectin, TLR2, and TLR4 docking analyzes respectively were Beta-D-Glucan (reverse docking); Pam3CSK4 (LPS agonist on TLR2) and MD2 pocket region. After docking, the results of the interaction will be visualized using PyMol software. Amino acids involved in docking interactions were identified using KFC Hotspot protein (https://mitchell-lab. biochem. wisc. edu/ KFC_ Server/index.php).

Research Results

Adh036 Protein Modeling

The results showed that the epitope alignment results had a query cover and identity as much as 100% with ID NP_454918.1 protein, namely the outer membrane adhesin [*Salmonella enterica subsp. enterica serovar* Typhi str. CT18].

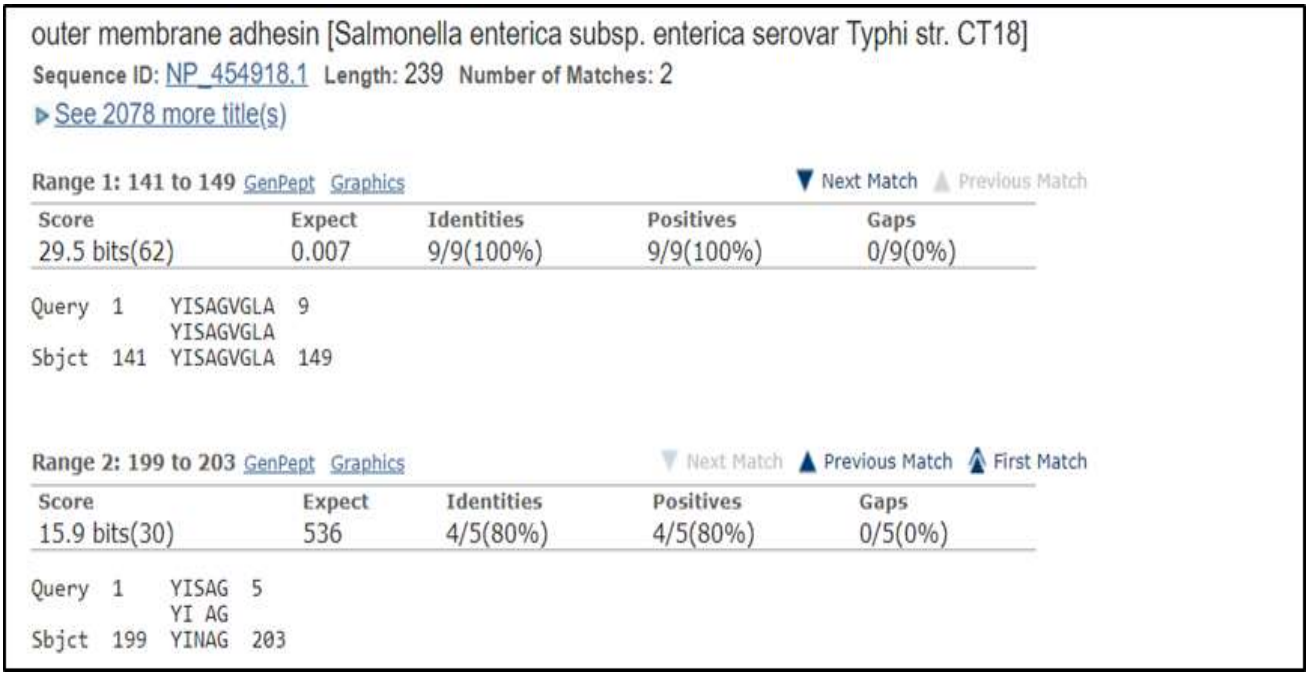


Figure 1: Epitope alignment results of AdhO36 protein modeling

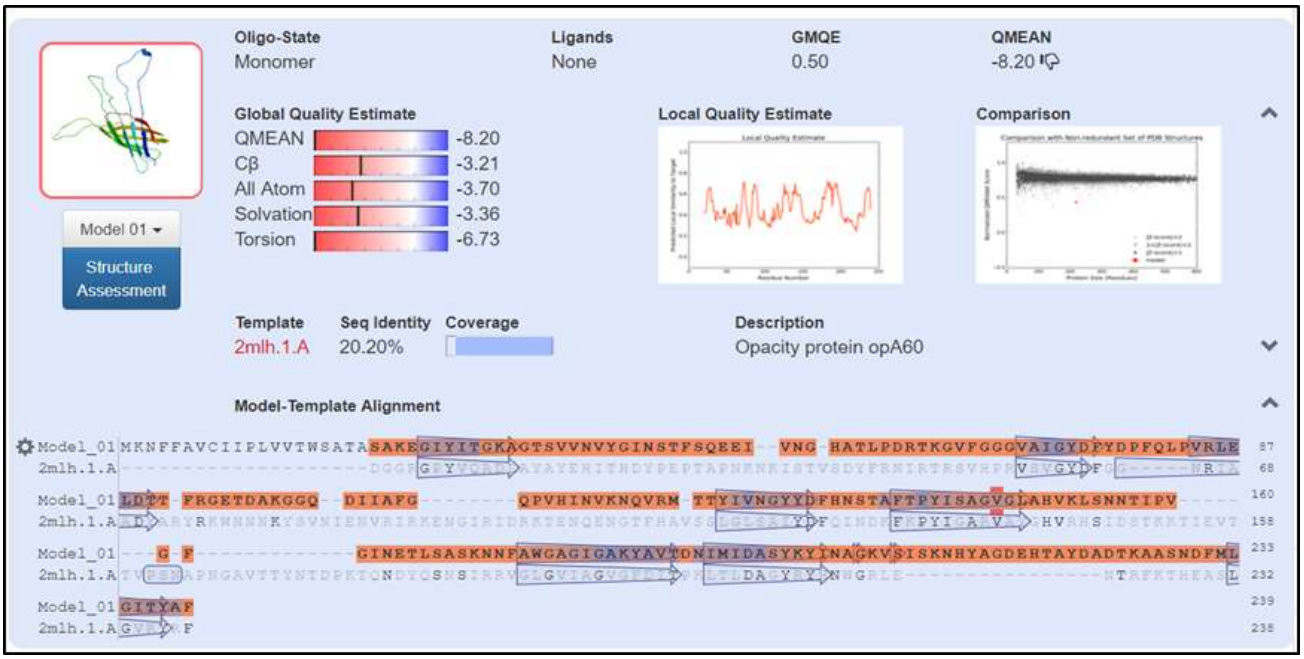


Figure 2: 3D structure of the NP_454918.1 sequence the 3D structure of the NP_454918.1 sequence has a sequence identity as much as 20.20% with a template 2mlh.1.A

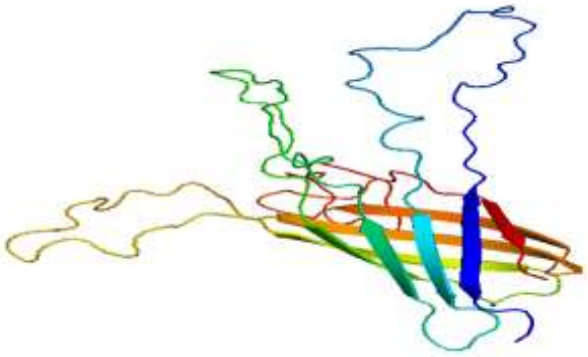
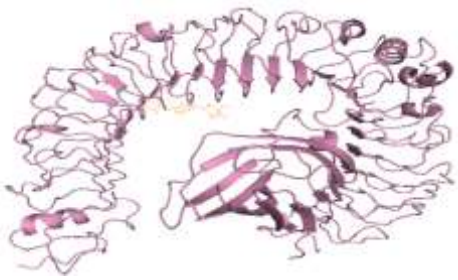


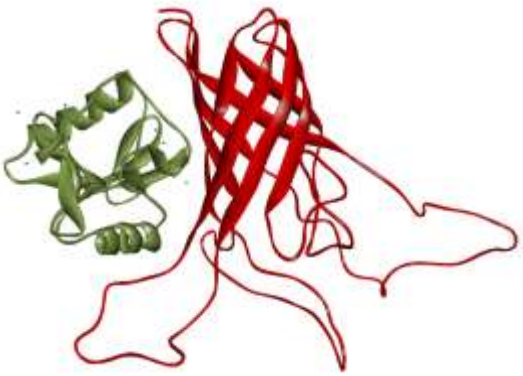
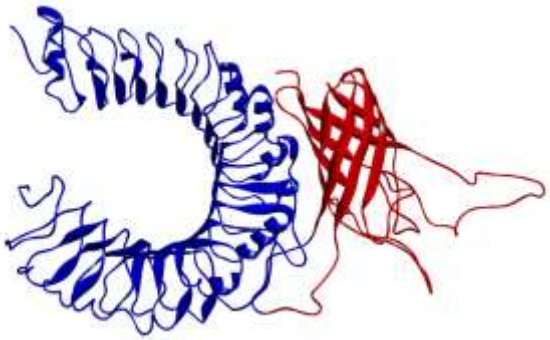
Figure 3: Model results of NP_454918.1 with SWISS-MODEL

Results of Docking

The results show that the bond strength between Dectin-beta glucan and TLR2-beta glucan is the same, i.e. 6.5 kcal/mol. Whereas TLR4 bond via pocket0 beta glucan MD2 has a lower strength, i.e. -6.0 kcal/mol. Blind docking analysis between TLR4-Adh036 shows the highest interaction strength with global energy -1462.6; followed by the TLR2-Adh036 complex with a score of -1324.5, and the lowest bond between Dectin-Adh036 with a score of -1112.7.

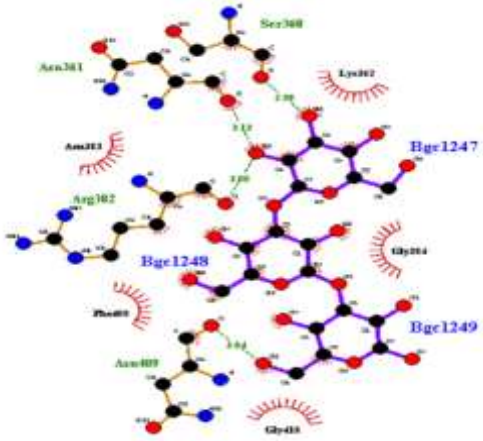
Table 1: Results of Docking Receptors Dectin1, TLR2, and TLR4 with Beta Glucan Ligands and Docking Receptors Dectin 1, TLR2 and TLR4 with AdhO36 Ligands

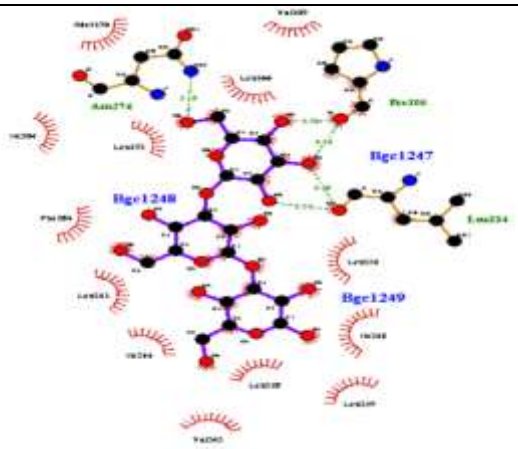
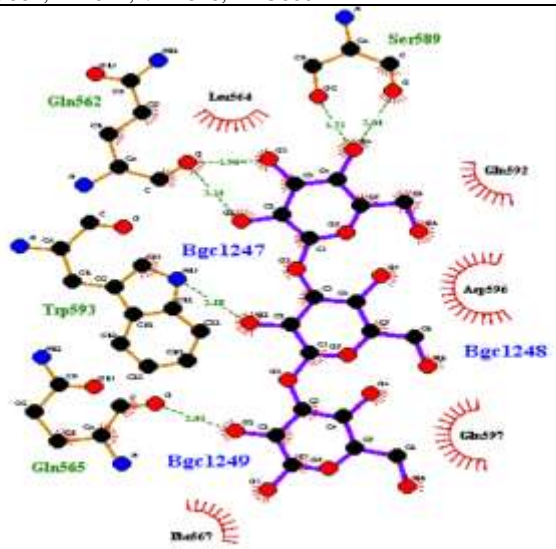
Receptor	Ligand	Binding affinity (kcal/mol)
Dectin 1 (cartoon; blue)	Beta-glucan 1,3 (line; orange)	-6.5 
TLR2 (cartoon; green)		-6.5 
TLR4-MD2 (cartoon; pink)		-7.0 
Receptor	**Ligand**	**Global energy (lowest)**

Dectin 1 (cartoon; green)	Adh036 (cartoon; red)	 -24.84
TLR2 (cartoon; blue)		-25.51 
TLR4-MD2 (cartoon; yellow)		-18.80 

Amino Acids that Interact in the Docking Process

Table 2: The amino acids that interact in the Docking process

Receptor	Ligand	Binding affinity (kcal/mol)
Dectin 1	Beta-glucan 1,3	 SER360, ASN361, LYS362, ARG382, ASN383, GLY384, PHE408, ASN409, GLY410

TLR2		 LEU266, MET270, LEU273, ASN274, PHE284, ILE304, PRO306, VAL309, LEU312, ILE314, LEU328, LEU331, LEU334, ILE341, VAL343, LEU359
TLR4-MD2		 LEU564, GLN562, GLN565, GLN565, PHE567, ASP596, GLN597, GLN592, TRP593, SER589
Dectin 1	Adh036	**Dectin:** GLN117, LEU120, GLY127, LYS128, SER129, SER136, ASN138, LYS144, ARG145, HIS146, SER148, GLN149, LEU150, GLY151, ALA152, HIS153, LYS156, ASP158, ASN159, SER160, LYS161, GLU162, TRP193, GLU194, ASP195, GLY196, SER197, ALA198, PHE200, PRO201, ASN202, GLU241, LYS242, GLU243, LEU244, LYS245 **Adh036:** GLU24, GLY25, ILE26, ILE72, GLY73, TYR74, PHE76, PHE80, GLN81, LEU82, PRO83, VAL84, ARG85, LEU86, LEU88, THR90, ARG93, TYR124, VAL126, ASN127, GLY128, TYR130, SER143, ALA144, VAL146, LEU148, ALA149, HIS150, VAL151, LYS152, LEU153, SER154, ASN155, SER171, PHE175, TRP177
TLR2		**TLR2:** **Adh036:**
TLR4-MD2		**TLR4-MD2:** TYR42, ILE66, PRO67, ARG68, ARG96, SER98, ASP99, ASP100, ASP101, TYR102, SER103, PHE104, ARG106, LEU108, LYS109, GLY110, GLU111, THR112, THR115, THR116, ILE117, SER118 **Adh036:** GLY30, ALA32, GLY33, THR34, SER35, PHE66, PHE80, GLN81, LEU82, PHE132, HIS133, ASN134, PHE138, PRO140, ILE142, ILE181, GLY182, ALA183, TYR185, ALA186, VAL187, ASN190, ILE191, MET192, ILE193, ALA195, TYR197, ASN210, HIS211, TYR212, ALA213, GLY214, ASP215, GLU216, THR218, MET232, LEU233, ILE235, TYR237

Discussion

Drug development with Molecular Docking has been growing rapidly to design specific,

fast, and inexpensive drugs. Drugs are usually made of small compounds that can bind specifically to the surface or inside a protein. The bond can affect the protein function, both inhibiting and triggering its activity.

The docking results carried out using the Auto dock vina program are sorted using a scoring function, which is a method of assessing the quality of ligand binding and receptors based on the calculation function of bond energy between ligands and receptors. Components that contribute to the final calculation of bond free energy are ΔG Gaussian value, ΔG repulsion value, ΔG hydrogen bond value, ΔG hydrophobic interaction value, and ΔG value of rotating bond number effect. Thus, the more binding energy value that contributes to these effects, the more negative the binding energy will be [15, 16, 17].

This study indicated the bond strength between the receptors between Dectin-beta glucan and TLR2-beta glucan was the same, i.e. -6.5 kcal/ mol. While TLR4 bond via MD2 pocket0 beta glucan has a lower strength of -6.0 kcal/mol. Blind docking analysis between TLR4-Adh036 shows the highest interaction strength with global energy of -1462.6; followed by the TLR2-Adh036 complex with a score of -1324.5, and the lowest bond between Dectin-Adh036 has a score of -1112.7.

Analysis of 3D molecular interactions is important for understanding the relationship between structure and function, the effect of a mutation, the mechanism of ligand interactions, and also in the drug design process [18]. Recognition of microbial infections and initiation of host defense responses is controlled by several mechanisms. Toll like Receptors (TLR) is a key component of the innate immune system to detect microbial infections and trigger the response of antimicrobial formation in the body.

TLR activates several steps in inflammatory reactions which help in eliminating pathogenic invasion and in coordinating the defense system in the host. TLR controls several dendritic functions of cells and activates signals involved in the initiation of the adaptive immune response [19]. *Salmonella* Typhi, which is a Gram-negative bacterium, has a bacterial cell envelope which structurally consists of three layers, namely cytoplasmic membrane, peptidoglycan or murein, and outer membrane. The outer membrane consists of phospholipid, lipopolysaccharide (LPS), and protein. LPS has a very important role in the structure and function of the external membrane of cell walls of gram-negative bacteria.

LPS plays a role in the transport of hydrophobic molecules to the interior of bacterial cells and the interaction of bacteria with hosts. The existence of LPS will activate TLR4 [20, 21]. *Salmonella* Typhi besides having a fimbria adhesion protein called AdhF36, which has fimbriae type I characteristics, also has a new adhesion protein which is later called AdhO36 (afimbrial adhesin).

Both AdhF36 and AdhO36 proteins are proven to be virulence factors in the adhesion and colonization process, which have the potential to be immunogenic to stimulate the formation of protective S-IgA, so they can be the candidates for vaccines to prevent the initial stages of infection [6]. From the in-silico results, a blind docking analysis between TLR4-Adh036 showed the highest interaction strength with global energy -1462.6.

This could be a prediction that LPS in *Salmonella* Typhi will compete with AdhO36 in activating TLR4 and initiating adaptive immunity [22-30].The research results showed that ß-glucan derived from wall cell extracts of *Candida albicans* could inhibit colonization of *Salmonella* Typhimurium by creasing the number of $CD4^+$ and $CD8^+$ [14]. The increasing of CD4+ and CD8+ co-receptors in T cells to recognizes MHC molecules and helps the T cell complex receptor (TCR) to activated T cells effector that have acquired the capacity to kill pathogen.

The functions of CD4+ are activating macrophages, B cells, other cells; the functions of CD8+ are killing infected "target cells" and macrophage activation [31]. The results of docking in-silico studies can be concluded that the bond strength between receptors-ligands between Dectin-beta glucan and TLR2-beta glucan is the same, at -6.5 kcal /mol, while TLR4 bond via MD2 pocket0 beta glucan has a lower strength, at -6.0 kcal /mol. This predicts that beta-Glucan binds to

TLR2, so it can stimulate Antigen Presenting Cell (APC) to increase lymphocyte T cell activation and increase signaling in the adaptive immune response [19].

The difference in receptor-ligand bonds between TLR-2-beta Glucan and TLR4-Adh036 will have a potentiating effect in enhancing cellular immunity and increasing stimulation of adaptive immunity.

Conclusion

Based on the results of the docking, it can be concluded that there is a strong bond between ligands-receptors and Dectin1-Beta Glucan and TLR2-beta Glucan that is equal to -6.5 kcal/ mol, and TLR4-Adh036 shows the highest interaction strength with global energy -1462.6. This shows the potentiation effect of the combination of AdhO36 and Beta Glucan by enhancing of cellular immunity toward *Salmonella* Typhi infection.

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