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Expression of Toll-like Receptor-4 (tlr-4) in Balb/c Mice Induced by Salmonella Typhi

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Abstract

Salmonella typhi is a gram negative bacteria that causes systemic infections and typhoid fever in humans. It has caused many deaths in developing countries. WHO (World Health Organization) estimated the incidence rate worldwide approximately 17 million per year with 600,000 deaths and 70% of them occurred in Asia (WHO, 2008). By Toll-like receptor 4 (TLR-4), macrophages can recognize lipopolysaccharides (LPS) present in *S. typhi*. The bond of LPS and TLR-4 that activates MyD88 plays an important role in controlling bacterial exponential growth. This study aimed to analyze the TLR-4 mRNA in Balb/c mice induced by *S.typhi*. This study was a laboratory experimental research on Balb/c mice with simple randomized design. Mice blood was taken before and after induction by 10^3 CFU of *S.typhi*. Then the blood samples were examined at Microbiology Laboratory in Faculty of Medicine, Hasanuddin University to measure expression of Toll-like receptor-4 (TLR-4) mRNA using Real Time Polymerase Chain Reaction (RT-PCR). The result of this study showed that there were enhancement of TLR-4 expression from 20 samples obtained. The data indicated that *S.typhi* induction can increase TLR-4 expression.

Keywords: Toll-like receptor-4 (TLR-4); *Salmonella typhi*; Real Time- Polymerase Chain Reaction (RT-PCR).

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1. Introduction

Typhoid fever is an acute systemic febrile infection caused by *Salmonella typhi*, a serotype of *Salmonella enterica* [1,2]. Based on World Health Organization (WHO) data, incidence rates of typhoid fever worldwide were approximately 17 million per year with 600,000 deaths due to typhoid fever and 70% of deaths occurring in Asia [2]. One way to kill these germs is by spurring the function of macrophages to destroy and eliminate bacteria. With Toll-like receptor 4 complex TLR-4/MD2/CD-14, macrophages recognize pathogenic molecular patterns (PAMPs) such as lipopolysaccharide (LPS). The bond between LPS and TLR4 that activates MyD88 plays an important role in controlling exponential growth of *S. typhi* [3,4,5,6,7]. *S.typhi* infection in mice shows symptoms of the same disease as in humans infected by *S.typhi*. Balb/c mice was considered an experimental model for typhoid fever in humans.

2. Materials and Methods

2.1. Bacterial preparation

S. typhi bacteria was from the Biomolecular and Immunology Laboratory, Faculty of Medicine, University of Hasanuddin. The amount was 10^3 CFU/mL (Mc Farland Standard).

2.2. Balb/c mice

BALB/c mice were 25-29 grams which obtained from maintenance in Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Mice were adapted for 7 days, stored in a standard cage (n = 20).

2.3. RNA Nucleic Acid Extraction

The sample volume of about 100 µg/ul blood was fed into 900 µl of "L6" solution consisting of 120g of Guanidium thyocyanate (GuSCN) in 100 ml 0.1 M Tris HCl , PH 6.4, 22 ml 0.2 M Ethylene Diamine Tetra Acetate (EDTA) pH 8.0 and 2.6g Triton X-100 (Packard, Instrumens) with final concentration 50 mM Tris HCl, 5 M GuSCN, 20 mM EDTA, 0.1% Triton X- 100. Next played at 12,000 rpm. The sediment added a 20 µl diatom suspension consisting of 50 ml of H2O and 500 µl of 32% (w / v) "Celite" (Jansen Chimica, Beerse, Belgium, 10,846.79). Where 20 µl of this diatom suspension can bind 10 µg RNA/tissue/blood DNA, then vortex and centrifuged in a 1.5 ml eppendorf tube at 12,000 rpm for 15 min. The supernatant was removed and the sediment was washed with a solution of "L2" consisting of 120 g of GuSCN in 100 ml 0.1 M Tris HCl, pH 6.4 by adding 1 ml of "L2" solution. Then divortex and centrifuged at 12,000 rpm for 15 min, then washing repeated 2 times using "L2" solution, followed by washing with 1 ml of 70% ethanol twice and 1 ml of acetone. The result was then heated in a waterbath at a temperature of 56oC for 10 min and added 60 µl of "TE" solution comprising 1 mM EDTA in 10 mM Tris HCL pH 8.0, then vortex and centrifuge followed at 12,000 rpm for 30 s, then incubated in Oven for 10 minutes at a temperature of 56oC. Then performed vortex and centrifuse again for 30 seconds at a speed of 12,000 rpm and taken supernatannya. The supernatant of this process will be obtained by nucleotide extraction and stored at -80 ° C before PCR analysis.

2.4. Real Time Polymerase Chain Reaction

Quantitative Real-Time PCR analysis total RNA was extracted from blood using L6 buffer according to the Boom methods. RNA quality and concentration were detected by a NanoDrop 2000 device (Thermo Scientific, Wilmington, DE, U.S.A.). In a reaction volume of 20 µL using M-MLV reverse transcriptase, 2 µg RNA was then reverse transcribed to cDNA using a RT-PCR kit. The mRNA level of the target gene was quantified by real-time PCR using a SYBR® Premix E x Taq kit on a CFX Connect system, Biorad Laboratories, Real Time PCR 96 well 0.1 ml, USA. The standard PCR conditions were as follows: 95ºC (10 min), 40 cycles of 95ºC (15 s) and 60ºC (1 min), followed by a standard denaturation curve. mRNA expression levels of the relevant genes and β-actin were determined using relative quantification by comparison with a standard curve for each gene, which was included in each PCR run generated from the serial dilution of a cDNA pool from theblood samples in the study. The primer pairs TLR-4 Forward: 5´-TGA CAG GAA ACC CTA TCC AGA GTT-3´ and Reverse: 5´-TCT CCA CAG CCA CCA GAT TCT-3´ dan β-actina Forward: 5´-AGA GGG AAA TCG TGC GTG AC-3´ and Reverse: 5´-CAA TAG TGA TGA CCT GGC CGT-3´. Relative mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method with data normalized to the Beta actin housekeeping gene.

3. Results and Discussion

The study was conducted by using samples of twenty Balb/c mice that were approximately 1 year old and 35-50 gram. Mice will be adapted first for 7 days. After adaptation, the mice blood were taken before (H0) and after (H1) induction by S.typhi for TLR measurement using real time polymerase chain reaction (RT-PCR).

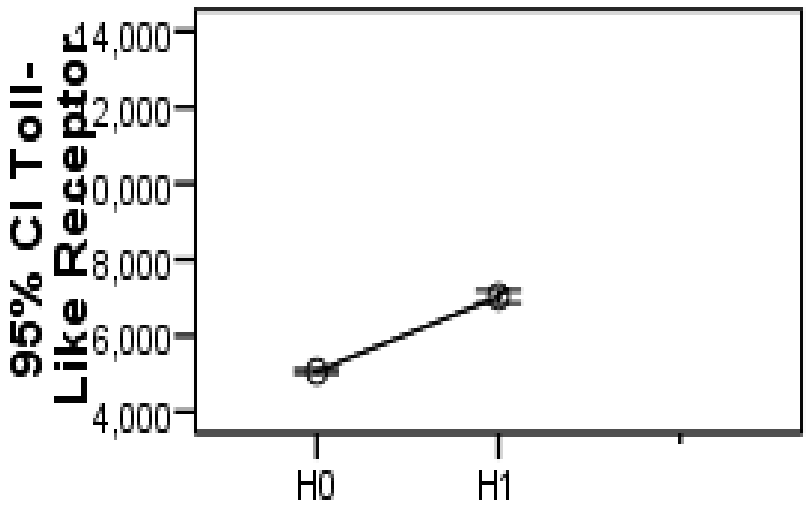

Figure 1: Graph pattern of mRNA TLR-4 expression before (H0) and after (H1) induction by S.typhi

There was an increase of expression mRNA TLR-4 in Balb/c mice linearly (by mean), before and after intervention.

Table 1: Expression of mRNA TLR-4 before and after induction by S.typhi

Mice (n=20)	**Expression of mRNA TLR-4**	
	Before Induction by S.typhi	After induction by S.typhi
Means	5.065	7.064
Means±SD	5.064 ±0.07	7.064 ±0.157
P*	*.000*	

**Paired-sample T-test*

Paired-sample T-test analysis test showed that there was significantly difference between before and after induction (H0 and H1) while p= 0.000. The results of this study were in accordance with those expressed by Suzanne Talbot and his colleagues 2009 that Toll-like receptor-4 (TLR4) is important in protection against lethal *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) infection. In the mouse typhoid model showed that TLR4 and MyD88, but not Mal or TRIF, were essential for the control of exponential *S.* Typhimurium growth [3].

4. Conclusion

Salmonella typhi induction increase expression of mRNA TLR-4 in *Salmonella typhi*-induced Balb/c mice.

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Competing Interest

The authors declare that they have no competing interests.

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