MAKALAH POSTER

Parasitemic Rates, IL-4 and IFN-γ Profile on Mice Model Vaccinated by Salivary Glands from *Anopheles maculatus* for Developing TBV Against Malaria

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Parasitemic Rates, IL-4 and IFN-γ Profile on Mice Model Vaccinated by Salivary Glands from *Anopheles maculatus* for Developing TBV Against Malaria

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Abstract

Malaria is a major health problem, especially in tropical countries with mortality of one million deaths each year. Malaria is an infectious disease caused by the parasite Plasmodium and transmitted by *Anopheles* mosquito. Recent researches have shown that salivary Gland (SG) contains active compounds that play an important role in transmitting the plasmodium into the host's body. Therefore, SG of malaria vector could be used as potential target to inhibit the transmission of pathogens for the development of Transmission Blocking Vaccine (TBV). The objective of this study was to observe the potential of SG from *Anopheles maculatus* (*An. maculatus*) in modulating pathogen infection. *An. maculatus* is 1 out of 13 important malaria vectors in Indonesia. BALB-C Mice was used as model organism to examine its immune response i.e. IFN γ (Interferon- γ) and IL-4 (Interleukin-4) in relation with saliva injection as vaccine model. Furthermore, parasitemic rates of *Plasmodium berghei* was also observed to investigate the potential role of saliva in relation with the establishment of malaria infection in mouse model. Several methods were used in this research including rearing *An. maculatus* and microscopy dissection to isolate SG's mosquitoes, preparation of experimental animals and vaccination, preparation of *P. berghei* and its mice donor. Levels of IFN γ and IL-4 was analyzed by ELISA (enzyme-linked immunosorbent assay). Murine parasitemia was assessed by using thin-layer blood smears stained with Giemsa. The influence of SG vaccination model in modulating host immune response was able to detect from increasing titer of IFN γ and decreasing titer of IL-4 from vaccinated mice group compared to control groups. This could explain the reducing parasitemic rates in vaccinated mice compared to control group, since mosquito salivary components may be served as a nonspecific potentiator whose effect to induce a Th1-biased environment (represented by IFN γ in this research) that is known to be effective against malaria infection rather than Th2 (represented by IL-4 in this research). Further studies on molecular characterization of component in salivary gland that is responsible for this process in needed to understand salivary role in blocking transmission of pathogen.

Introduction

Malaria is a major health problem in the world today. Every year a 3.3billion people worldwide are estimated at risk of malaria infection (WHO, 2010). In Indonesia, malaria is a major health problem by 6 million cases and 700 people die every year (Dale *et al*., 2005). Malaria is an infectious disease caused by the Plasmodium parasite and transmitted by the *Anopheles mosquito* . From the 80 species of Anopheles in Indonesia, 24 species of potential as a vector of malaria

and *Anopheles maculatus* is one of the most on the island of Sumatra and Java (Depkes RI, 2008). Several strategies have been developed to overcome malaria disease. The last few decades it has developed a new approach in vaccine development efforts is to utilize an arthropod vector saliva components. Salivary glands of an arthropod vector has been proven to contain ingredients that are immunogenic so it can be used as a vaccine that can prevent transmission (Transmission Blocking Vaccine (TBV)) (Lavazec *et al.*, 2007; WHO, 2009). Repeated exposure of the pathogen causes the formation of protection against the occurrence of severe malaria and death, but will never achieve a sterile immunity (Taylor-Robinson, 2003;. Langhorne *et al*., 2008). This is indicated by the change of immune response from Th-2 toward Th-1. This causes is shift in the immune response of IFN-γ levels increased and decreased levels of IL-4 which acts in the activation of macrophages, followed by the production of Nitric Oxidase (NO) is very effective in inhibiting the development of the parasite in the host's body (Titus *et al*., 2006; Donovan *et al*., 2007).

Materials and Methods

This research is an experimental research laboratory with a complete randomized block design approach. There are three groups of mice, the first group be immunized with the supernatant from the saliva, the second group be immunized with saliva of pellets and a third group was a control group were given only the adjuvant. Before vaccination, each control and treatment groups dissected intrakardial for be taken as a pre-test plasma beginning. Each group is consists from the 15 mice. Interval of 3 weeks after the last immunization performed with P.berghei infection in all groups. One day and seven days after the infection is taking back for test plasma levels of IFN-γ and IL-4, using the Sandwich ELISA method. In addition two days after the infected, degree of parasitemia was calculated for seven consecutive days. All the measurement results of the measurement results either IFN-γ, IL-4 and degree of parasitemia in the control and treatment groups compared with ANOVA statistical analysis.

Results and Discussion

A total of 1460 pairs of salivary glands from the *Anopheles maculatus* female have been isolated. The female salivary gland pair consist of three lobes, which each consists from the two lateral lobes and a medial lobe (Fig. 1) (Dhar and Khumar, 2003; Jariyapan et. Al, 2007).

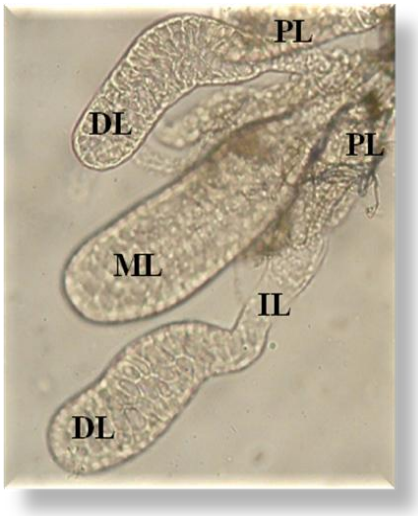

Figure 1. The results of isolation salivary glands from female *Anopheles maculatus*

Vaccination in this research conducted in three stages is primary stage as the initial trigger, and then strengthened with the booster stage I and II are effective to maximize the antibody response against the immunogen that injected into the body. first exposure on immunization (primary) with the exogenous antigen (surface) cause the primary humoral response are characterized by plasma cells producing antibodies and memory B cells. Capability for give secondary humoral responses depending on the presence of memory B cells and memory T cells (Baratawidjaja, 2010). Therefore required booster for the second memory cells are activated to cause the secondary antibody response that can maintain immunity. Additionally booster immunization purpose is to generate an immune response more specific, strong and durable (Schunk and Macallum, 2005).

Cytokines are measured in this research is Interleukin-4 and interferon-γ. IFN-γ is a cytokine produced by Th 1 lymphocytes and acts as a proinflammatory cytokine that is antagonistic. So produced immune response toward Th 2 will result in decreased levels of cytokines produced by Th 1 is IFN-γ (Baratawidjaya and Rengganis, 2009). IL-4 is a cytokine produced by Th 2 lymphocytes. Produced cytokine Th2 lymphocytes act as a major stimulus of cytokine production of IgE and Th2 development from $CD4^+$ cells.

The results of measurement IL-4 levels of before treatment showed that the levels of IL-4 sequence from the highest to lowest is the pellet, supernatant and control. These results as a pre-

test of all group will be given treatment that is used as reference in the next measurement. Levels of IL-4 on primary vaccination compared to pre-test. The results of measurement the supernatant and the pellet increased, while the control group decreased because there was no influence of salivary glands, but only influence of adjuvant. Addition of Complete Freund Adjuvant (CFA) will induce a Th1 response so that suppresses the production of IL-4, whereas is booster vaccination adjuvant on Incomplete Freund Adjuvant (IFA) induces a Th2 response areas, especially injection that stimulates antibody producing plasma cells so that suppress IFN-gamma and increased IL-4.

In the second booster vaccination decrease in the supernatant, whereas in the pellets increased from the previous hemolysis (booster vaccination I). Supernatant group which has decreased indicates a shift in the immune response of Th2 to Th1 in the presence of IL-4 decreased after repeated exposure of salivary glands. After infected by Plasmodium berghei, IL-4 levels of increase in both the supernatant and pellet. Decreased levels of IL-4 on the the pellet compared with a control group show that the vaccination post-infection affects the salivary glands of the host immune response. This suggests that the levels of IL-4 is only the adjuvant injected more improved compared with the vaccination using salivary gland whose levels are lower. Levels of IL-4 post-infection the supernatant can not be analyzed because of hemolysis, so that levels of IL-4 can only be proved with the its reduction to booster vaccination II only.

The results of measurement IL-4 in this research can not be concluded as a whole because the measure is up and down, hemolysis of samples and sampling from only a mouse in each group so that results can not be compared, the measurement of plasma of a mouse is not measured in duplicate or repetitive. The results of uncertain the average number of IL-4 and comparison with results of other groups. IL-4 levels may occur because of increased response of adjuvant used and the host immune response to the vaccination.

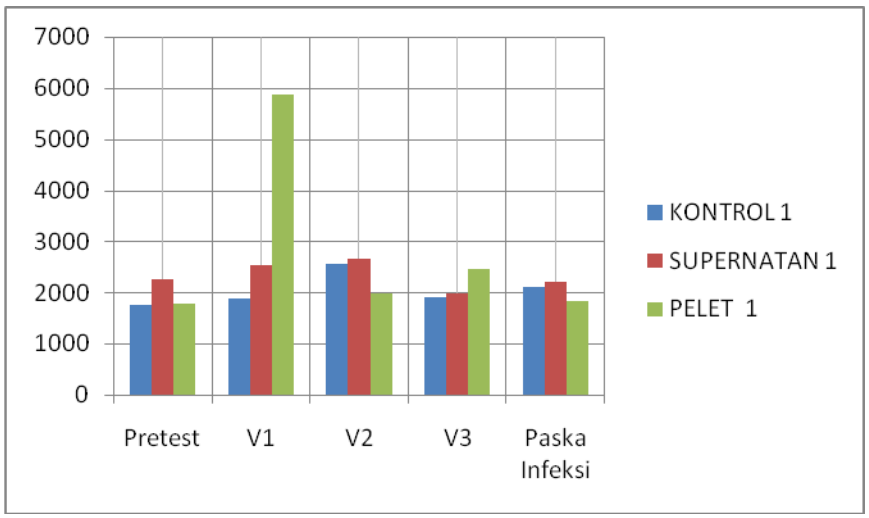

Figure 2. Charts measurement levels of IL-4

Measurement IFN-γ levels of before vaccination decrease in the pellet and supernatant. Decreased levels of IFN-γ was more likely in the control group. Decreased levels of IFN-γ pellet groups smaller than a decrease in the supernatant of mice. Measurement IFN-γ levels of on the the pellets of mice after primary vaccination decrease when compared to the prior vaccination. Levels of IFN-γ group of mice after vaccination booster pellet I and II can not be measured because of hemolysis. Levels of IFN-γ on the the supernatant of mice showed an increase after the primary vaccination and showed a decrease at 13 days after vaccination booster II (1 day before infection).

In this research after the vaccination using CFA in the control group of mice decrease IFN-γ levels of than before the vaccination. This results can occur due to "hook effect" in measurement IFN-γ levels of the control group of mice. Vaccination with the salivary gland pellet *An. maculatus* and CFA in pellet groups of mice resulted in decreased levels of IFN-γ than before vaccination. These results are consistent with the previous studies of Aedes aegypti salivary gland extracts that mosquitoes may cause suppression of IFN-γ production in splenosit (Wasserman, 2004; Zeidner et al., 1999. However, these results do not support the results of this research is showed elevated levels of IFN-γ in the supernatant after vaccination the primary (vaccination with the CFA) than before vaccination. Increased levels of IFN-γ may be caused by the use of Complete Freund's adjuvant is has the ability to stimulate an immune response toward Th 1 (Linblad, 2000; Billiau and Matthys, 2001) resulting in increased levels of IFN-γ.

Levels of IFN-γ groups of mice were pelleted and the supernatant after the vaccination booster can not be measured because of hemolysis while the levels of IFN-γ in the control groups of mice were decreased compared with the before vaccination and after the primary vaccination. Decrease may be caused by the use of IFA is has the ability to direct the immune response toward Th 2 (Billiau and Matthys, 2001).

After second booster vaccination decrease levels of IFN-γ in the supernatant and the control groups of mice were compared with the before vaccination and after the primary vaccination and booster I whereas IFN-γ levels of in the pellet can not be measured because of hemolysis. Decreased levels of IFN-γ is occurred in the supernatant was greater than the decline that occurred in the control group. This shows the supernatant salivary gland *An. maculatus* strengthen the effect of IFN-γ levels of suppression that has been produced by Incomplete Freund's adjuvant. This results consistent with previous research by Hajipirloo et al (2005) stated that vaccination with the salivary gland supernatant *An. staphensi* can stimulate the formation of antibodies. Formation of antibodies that indicate high levels of IFN-γ low. This happens because of IFN-γ has a negative effect on antibody production. Thus, when low levels of IFN-γ antibody Produced is high (Baratawidjaya and Rengganis, 2009).

After infection of Plasmodium berghei pellet groups of mice had higher levels of IFN-γ is higher compared to control mice. This showed an immune response toward Th 1. These results are consistent with the previous studies conducted by Donovan *et al*, 2007 stated that repeated exposure to salivary glands *An. staphensi* can redirect the immune response toward Th a. On malaria Th a immune response is essential to overcome the parasitaemia is occurred at the beginning of infection (Harijanto, 2010). Production of IFN-γ also plays an important role in immunity in malaria liver stagesis by stimulating macrophages is release nitric oxide can kill parasites in the liver cells (Doolan and Hoffman, 2000). Increase in is Th 1 cytokines IL-12 and IFN-γ describes an effective immune response and can significantly inhibit the progression of uncomplicated malaria to severe malaria

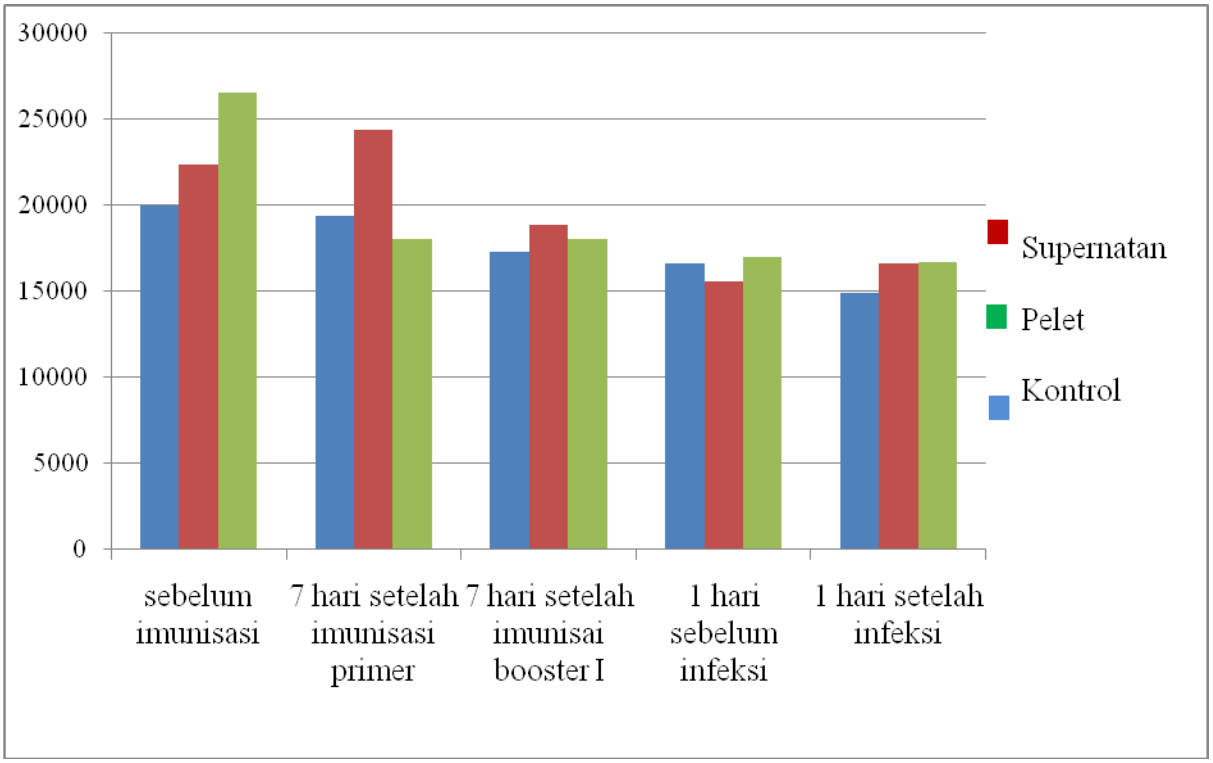

Figure 3. Charts measurement levels of IFN- γ

The results of calculation of degree of parasitemia to pellet treatment group can be seen in Figure 4. Based on the results obtained indicate that the degree of parasitemia pellet injected treatment group had a tendency vaccine pellet is lower than the control group. While the group treated supernatant supernatant was injected vaccines is likely to have a degree of higher parasitemia. This is related to the components present in the vaccine pellet Produced of centrifugation process .. The formation of sediment in the form of pellets by centrifugation process is influenced by particle mass or molecular weight, density and particle shape (Murray, 2006). Chemical complexity of in protein allows for a variety of epitopes (antibody units to stimuli) so that more likely someone will react to the one or more epitopes. Therefore, the protein is a potent immunogen (Baratawidjaja, 2010). This indicates that the immunomodulatory protein components may exist in pellet vaccine.

This indicates the presence of immunomodulatory proteins in saliva pellet vaccine vector may provide a stimulus to the host's immune, so that host more resistant to pathogens as indicated by the decrease in degree of parasitemia. Decrease degree of parasitemia can be described as a process that leads to the induction of Th1 immune response to one of expression

of is IFN-γ sitokainnya that activates macrophages to produce NO to inhibit parasite development.

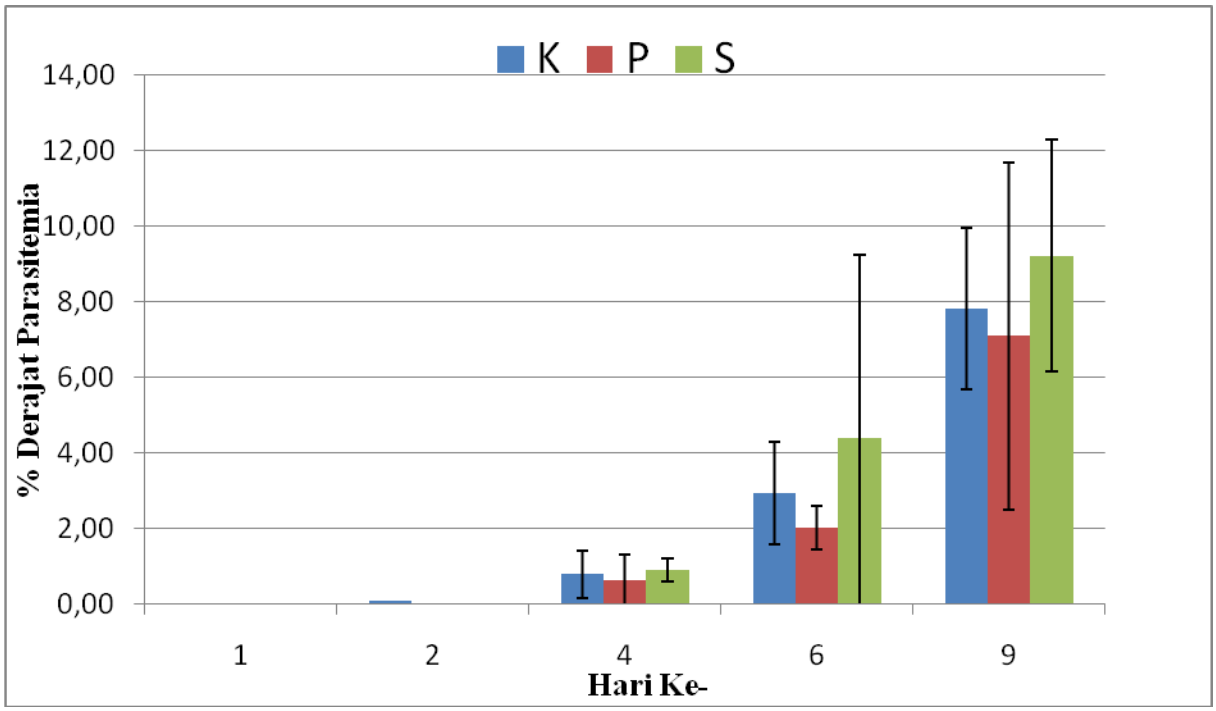

Figure 4. Charts measurement levels degree of parasitemia

Conclusion and Outlook

The measurement IL-4 levels of in mice showed the influence of salivary gland vaccination in modulating response IL-4, decreased levels of IL-4 mice occurred in the supernatant after the second booster vaccination and the pellet after *Plasmodium berghei* infection. Decrease IFN-γ profiles occur in groups of mice were pellets and raising the profile of IFN-γ in the supernatant of mice. After second booster vaccination decrease IFN-γ profile in the supernatant compared to mice before vaccination. *Plasmodium berghei* infection post IFN-γ profile of pellet groups of mice was higher than the control group. The measurement degree of parasitemia of mice vaccinated with salivary gland post-infection of *An. maculatus Plasmodium berghei* in the group treated pellets have a tendency to a lower degree of parasitemia when compared with a control group. Supernatant while the treatment group tended to have a degree of parasitemia is higher when compared with a control group.

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