ICMCA _ ATM
2nd INTERNATIONAL CONFERENCE AND WORKSHOP FROM MOLECULAR TO CLINICAL ASPECTS OF HIV/AIDS, TUBERCULOSIS AND MALARIA

"The Threat of New & Re-emerging Disease in Immunocompromised persons"

MAKALAH POSTER PRESENTATION

MOSQUITO SALIVA MEDIATED INHIBITION OF PARASITES RATES ON MICE MODEL FOR MALARIA

Kartika Senjarini, Ina Soraya, Yunita Armiyanti

Malang, 23 -24 Juni 2011 – INDONESIA

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Contents

Mosquito Saliva-mediated Inhibition of Parasites Rates on Mice Model for Malaria

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INTRODUCTION

Although malaria has been virtually eradicated from Indonesia, it is currently recognized as a serious re-emerging threat to public health. Anti-malarial drug resistances as well as vector resistance against insecticides are major public health problems which hinder the control of *malaria* (e.g. Yadouleton *et al*. 2010). Therefore, development of a vaccine could be a more efficient strategy to overcome the epidemic. Because of the complexity of malaria's parasite life cycle, a vaccine will need to encompass more than a single approach to reach a high degree of efficacy i.e. pre-erythrocytic vaccine, blood-stage vaccine, and transmission-blocking vaccine (TBV) candidates. It has been widely observed that saliva of mosquito that transmits disease contains imunomodulatory factors that could enhance pathogen infection (Titus *et al.* 2006). Therefore, it should be possible to control pathogen transmission by vaccinating the host against the molecule(s) in saliva that potentiate the infection. However, salivary activities in relation with establishing parasite's infection of vectors for Malaria from Indonesia as potential target for TBV e.g. *Anopheles aconitus (A. aconitus),* has not been elaborated so far. This research wanted to test this hypothesis by using the prototypic murine model for malaria infection i.e. infection of mice via *Plasmodium berghei* after injection with mosquito SGE (Salivary Gland Extract) serving as "vaccine model". Elaborating the potential salivary activity from *A. aconitus*, as 1 out of 13 important malaria vectors in Indonesia (Stoops *et al.* 2009), could be an important step to investigate novel target for TBV against Malaria. The objective of this research is therefore to investigate the potential role of saliva in relation with the establishment of parasite infectivity in mouse model for Malaria.

MATERIALS & METHODS

Collection of Mosquitoes (*A. aconitus*) and preparation of salivary glands as vaccine model

Mosquitoes reared and maintained in an insectary at 30°C and 80% relative humidity. Mosquitoes were supplied with a cotton wool pad soaked in 10% sucrose solution ad libitum. Female *A. aconitus* mosquitoes 7–10 days-old were immobilized by chilling, surface-sterilized by brief immersion in 70% ethanol. Salivary glands (SG) were collected in PBS using *microscopy* dissection. SG were homogenized by using micropistill, and water-sonicated for 30 minute as last step (Salivary Gland Extract, SGE). They were then centrifugated at 14.000 rpm, 4°C for 15 minutes. Supernatant and pelet were separated as 2 vaccine forms i.e. SGS for supernatant and SGK for pellet vaccine respectively. SGE were diluted in Aluminium hydroxide (v/v=1:1) and incubated et least 2 h or overnight before vaccination.

Preparation of murine model

BALB/c mice aged 6 to 8 weeks were purchased as specific pathogen-free young adults and maintained in accordance with National Institutes of Health guidelines. All mice were male and were age matched for all experiments. Mice were vaccinated with SGE every 2 weeks for 4 weeks (Primary vaccination, Booster I and Booster II). A control group of age-matched mice were only vaccinated by using dilution solution for SGE i.e. PBS in Aluminium hydroxide (v/v=1:1).

Murine parasitemia

Murine parasitemia was assessed by using thin-layer blood smears. Smears were fixed in 100% methanol and stained with Giemsa. Mice were exposed to infect with *Plasmodium berghei* (i.p) 2 weeks following the last vaccination. Forty-eight hours post-exposure, blood samples were taken and thin-layer blood smears were made and stained with Giemsa. Subsequently, samples were taken and parasites were quantified each day until mice were euthanized on day 7 postinfection. For the determination of parasitemia levels, 1,000 cells from each sample were counted.

RESULTS & DISCUSSION

The salivary glands of adult mosquitoes are present in the thorax flanking the oesophagus. They are sexually dimorphic which is related with their ability in hematophagy. Structural differences in female salivary glands reflect their function to engage succesfully in hematophagy (Stark & James 1996). The glands are paired stuctures and are much larger in female than in males. Each gland consists of three lobes, two lateral and one median (Fig. 1). The medial and distal-lateral lobes express genes whose products such as apyrases, anticoagulants and vasodilatory agents are involved in hematophagy (e.g. Arcá et al., 1999).

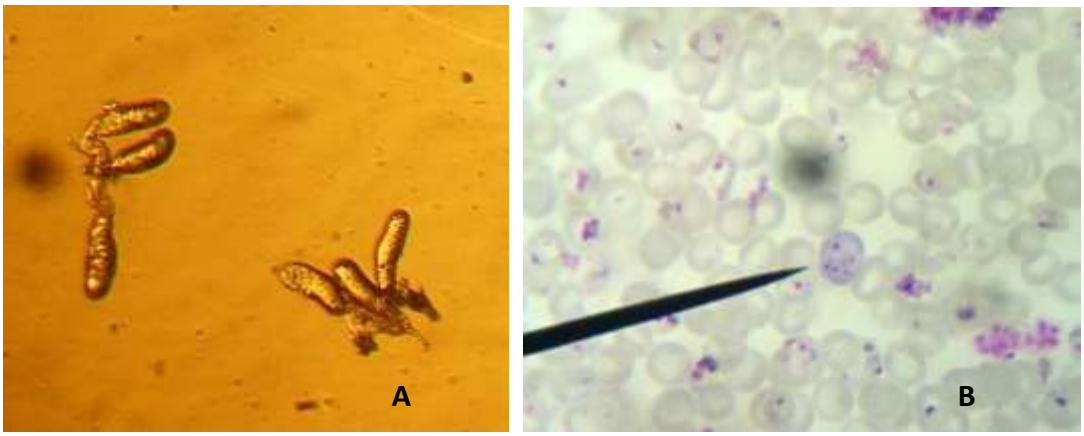

Figure 1. Female salivary gland of *A. aconitus*, stereo microscopy Nikon, 8x magnified (Cropping and Editing Closed Up, Camera: NOKIA N73) (A). Blood-stage infection was monitored each day for 7 days via blood smears from both control and SG-vaccinated mice. Picture showed several parasite stadia in red blood cell (B).

In sum, 1500 pairs of SG has been isolated and diluted in Aluminium hydroxide serving as adjuvant for "SG's vaccine model" which is injected sub-cutaneously to mimic mosquito probing during blood meal. SG-vaccinated mice exhibited reduced in *P. berghei* burdens 4 – 7 days post-infection compared to control when blood parasitemia levels were assessed (Fig. 1B & 2). These reduced parasite burdens suggest the function of host immune response towards saliva to reduce infectivity of transmitted pathogen. This could be explained by the following hypotheses. Host immune response against mosquito's saliva could decreased infectivity of

transmitted pathogen (Belkaid et al, 1998). Population living at endemic of leishmaniasis sites showed natural resistency against laishmania parasites (Davies and Gavgani, 1999) because of its natural immunity mediated by Th1 that has protective properties and contains antibodies against sandflies's saliva (Kamhawi *et al.* 2000) (1). Furthermore, mosquitoes bites have shown similar effects in animal models through modulating in host cytokines systemic response (Schneider *et al*. 2004). Further studies suggest that mosquito salivary components may be served as a nonspecific potentiator whose effect to induce a Th1-biased environment that is known to be effective against malaria infection (Donovan 2007) (2).

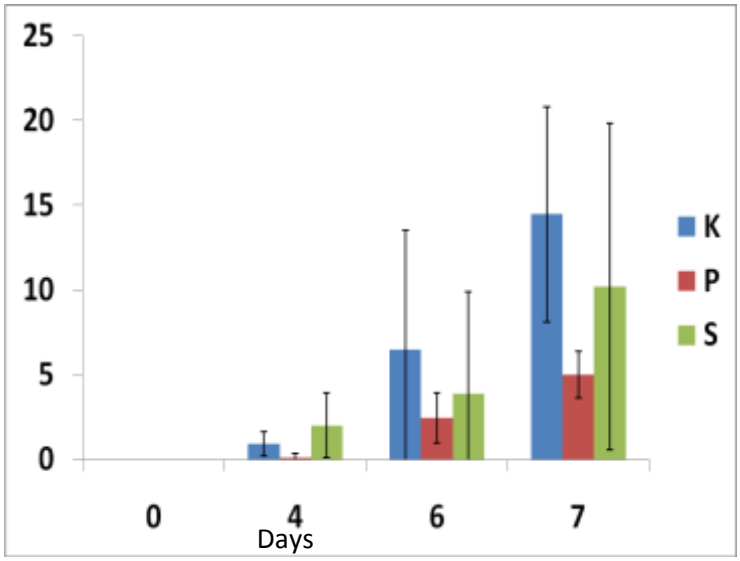

Figure 2. Average of parasitemic rates (%) in murine model: Control (K), vaccinated with SGE from Pelet (P), and vaccinated with SGE from supernatant (S), Numbers represent days after exposing murine model with *P. berghei*.

CONCLUSION & OUTLOOK

Reduction in parasitaemia rates in mouse model which is previously vaccinated by salivary extract from *A. aconitus* in this research indicated the potential role this vector's saliva to serve as novel target in developing new vaccine targets and novel strategies against malaria. Many reports suggest that any measure which limits parasite densities will reduce the morbidity and mortality associated with malaria infection (McErroy *et al*. 1994). Therefore, to elaborate its

potential role, the predominant effectors mechanism in host immune response should be further investigated.

Acknowledgment

We are very grateful for the financial support from HIBAH STRATEGIS NASIONAL DIKTI 2010 for this research.

References

Arcá, B., Lombardo, F., de Lara Capurro Guimarães, M., della Torre, A., Dimopoulos, G., James, A. A. and Coluzzi, M. 1999. Trapping cDNAsencoding secreted proteins from the salivary glands of the malaria vector Anopheles gambiae. Proc. Natl. Acad. Sci. USA 96: 1516-1521.

Donovan, M.J., Messmore, A.S., Scrafford, D.A., Lacks, D.L., Kamhawi,S., McDowell, M.A. 2007. Uninfected mosquito bites confer protection against infection with Malaria parasite. Infection and Immunity. 75(5) : 2523-2530.

Kamhawi, S., Belkaid, Y., Modi, G., Rowton, E. Sacks, D. 2000. Protection against cutaneous Leishmaniasis resulting from bites of uninfected sand flies. Science. 290: 1351-1354.

McElroy, P. D., Beier, J. C., Oster, C. N., Beadle, C., Sherwood, J. A., Oloo, A. J., Hoffman, S. L. 1994. Predicting outcome in malaria: correlation between rate of exposure to infected mosquitoes and level of Plasmodium falciparum parasitemia. Am. J. Trop. Med. Hyg. 51:523–532.

Schneider, B.S., Soony, L., Ziednen, N.S., Higgs, S. 2004. Aedes aegypti salivary gland extracs modulate anti-viral and Th1/Th2 cytokine responses to sindbis virus infection. Viral Immunol. 17: 565-573.

Stark, K. & James, A. A. 1996. The salivary glands of disease vectors. In The Biology of Disease Vectors (ed. W. C. Marquardt and B. Beaty), pp. 333-348. Niwot: University of Colorado Press.

Stoops, C.A., Rusmiarto, S., Susapto, D., Munif, A., Andris, H., Barbara, K.A., Sukowati, S. 2009. Bionomics of *Anopheles* spp. (Diptera: Culicidae) in a malaria endemic region of Sukabumi, West Java, Indonesia. Journal of Vector Ecology. 34: 200-207

Titus, R.G., Bishop, J.V., Mejia, Z.S. 2006. The immunomodulatory factors of arthropod saliva and the potential for these factors to serve as vaccine targets to prevent pathogen transmission. Parasite Immunology, 28: 131-141.

Yadouleton, A.W., Padonou, G., Asidi, A., Moiroux, N., Bio-Banganna, S., Corbel, C., N'guessan, R., Gbenou, D., Yacoubou, I., Gazard, K., Akogbeto, M.A. 2010. Insecticide resistance status in *Anopheles gambiae* in southern Benin. Malar J. 9: 83-89.