

REVIEW

Potential Use of Mosquito's Salivary Components as Novel Target for The Development of Transmission Blocking Vaccine (TBV)

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Mosquito-borne diseases are rampant in most tropical regions of the world, especially rural, forested, and coastal areas such as Indonesia. Despite long-standing chemotherapeutic intercession and vector control programs, mosquito-borne diseases exact a heavy burden on human health in Indonesia. Two major public health problems transmitted by mosquito in Indonesia are malaria and dengue haemorrhagic fever (DHF), causing millions of clinical episodes occurring annually. Malaria is now recognized as a serious re-emerging threat to public health. DHF cases were first observed in 1968; since then, the incidence has been constantly increasing and the disease is now one of the principal causes of child lethality. It has been widely observed that saliva of mosquito that transmits the diseases contains several factors that could enhance pathogen infection. Therefore, it should be possible to control pathogen transmission by vaccinating the host against the molecule(s) in saliva that potentiate the infection. However, specific component as a potential target for TBV in mosquito vectors of malaria & dengue, i.e. *Anopheles* and *Aedes aegypti*, has not been identified so far. This paper wanted to elaborate the potential role of salivary component from mosquitoes, particularly from Indonesian vectors as molecular target for developing TBV against two major Mosquito borne-diseases in Indonesia i.e. malaria and DHF.

Key words: *Aedes*, *Anopheles*, immunomodulators, salivary gland, TBV

Penyakit yang disebarkan oleh nyamuk banyak ditemukan di kebanyakan daerah tropis di dunia khususnya daerah pedesaan, area sekitar hutan dan perairan pantai seperti yang banyak ditemukan di Indonesia. Walaupun berbagai usaha untuk menanggulangi penyakit tersebut telah lama dilakukan, baik dengan pengendalian penyakit dengan terapi kausatif (obat/bahan kimia) maupun pengendalian vector, penyakit diperantarai nyamuk ini masih merupakan masalah kesehatan utama di Indonesia. Malaria dan Demam Berdarah Dengue (DBD) merupakan 2 masalah utama yang di transmisikan oleh nyamuk di Indonesia. Malaria masih menjadi masalah kesehatan yang serius karena pada dekade terakhir ini terjadi peningkatan prevalensi di beberapa daerah bahkan mulai muncul resistensi pada beberapa obat malaria (*re-emerging*). DBD pertama kali ditemukan pada 1968, sejak saat itu kasusnya selalu meningkat bahkan merupakan salah satu penyakit penyebab kematian utama pada anak usia sekolah. Saliva nyamuk yang bertindak sebagai vektor, telah terbukti mengandung berbagai komponen yang mampu meningkatkan transmisi patogen yang dibawanya. Oleh karena itu, transmisi patogen akan sangat mungkin untuk dihambat dengan mem"vaksinasi" inang dengan molekul "lawan" dari molekul yang meningkatkan proses transmisi tersebut sehingga dapat menghambat transmisinya (TBV). Namun demikian, komponen spesifik sebagai target potensial untuk TBV dari vektor malaria dan Dengue yaitu *Anopheles* dan *Aedes aegypti* sampai saat ini belum berhasil diidentifikasi. Selama ini, telah dilakukan eksplorasi terhadap potensi saliva vektor dari berbagai vektor nyamuk yang ada di dunia tetapi belum ada laporan tentang hal ini dari Indonesia. Paper ini akan memberikan *review* tentang potensi saliva dari vektor nyamuk di Indonesia sebagai kandidat target potensial untuk pengembangan TBV untuk 2 masalah utama kesehatan di Indonesia yang disebarkan vektor tersebut yaitu Malaria dan DBD.

Kata kunci: *Aedes*, *Anopheles*, imunomodulator, kelenjar saliva, TBV

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 hindering the control of malaria (e.g. Yadouleton *et al.* 2010). Dengue fever (DF), caused by infection with

dengue virus (DENV), is not a new disease. Today, dengue is considered as one of the most important arthropod-borne viral diseases in humans in terms of morbidity and mortality. However, life-threatening dengue cases are mostly occurred in west pacific and south east Asia such as the Philippines, Thailand, and Indonesia (Gubler 1997). In Indonesia, DF case was first observed in 1968; since then, the incidence has been constantly increasing and the disease is now one of the principal causes of child lethality. The highest

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incidence of dengue occurred between 1998-2000 in Surabaya, East Java. The highest proportion of DF related mortality occurring between 1999-2000 was associated with children aged 5-9 years (Soegijanto 2004). DKI Jakarta, the capital city, was the province with the highest number of DF cases, *i.e.* 14,071 cases with case fatality rate (CFR) 0.42% in 2003. In 2005, DF incidence increase up to 23,466 cases with CFR 0.34% (Daniel 2008).

Despite the long-standing chemotherapeutic intercession and vector control programs, malaria and dengue fever outbreaks are always reported every year. This condition indicates that the quest for causative therapy is extraordinarily daunting. Therefore, development of a vaccine could be a more efficient strategy to overcome the epidemic. In the last decade, a new approach in the development of vaccine for arthropode-borne diseases is by using the salivary components from vectors. This approach is developed based on a hypothesis that the vector's saliva contains vasodilator and immunomodulator proteins (proposed by, *e.g.* Sack and Kamhawi 2001; Titus *et al.* 2006). The vasodilatory factors play important role to help the vector during blood feeding. There are two hypotheses concerning the function of immunomodulatory factor in saliva of mosquitoes. Many reports showed that salivary immunomodulators could enhance pathogen infection (*e.g.* Donovan *et al.* 2007) (1). However, there is also evidence that saliva appeared to directly protect dendritic cells from infection *in vitro* (Ader *et al.* 2004) (2). In relation to the first case, it should be possible to control pathogen transmission by vaccinating the host against the molecule(s) in saliva that potentiate the infection, thereby blocking the enhancing effects of saliva, thus preventing the pathogen from establishing infection in the host. In case of second condition, it could be used directly to protect host cells from infection of transmitted pathogens. These hypotheses led to closer examination of salivary factors, especially the immunomodulatory factors, as potential targets to control pathogen transmissions, *i.e.* transmission blocking vaccine (TBV) or also known as mosquito stage vaccine (Ramirez *et al.* 2009). However, specific potential target for TBV in mosquitoes such as *Anopheles* and *Ae (Ae) aegypti* has not been identified so far. Therefore, exploration of mosquito's salivary components of is an important step to find novel target for TBV development. This paper would like to elaborate the potential role of three potential malaria vectors in Indonesia, *A. aconitus*, *A. sundaicus* and *A. maculates*,

as well as a major dengue vector for, *Ae. aegypti* as TBV target model.

Reduction of Parasitemic Rates by *Anopheles* Salivary Components. The role of vector's salivary components in mediating infection is a relatively new concept in term of Arthropod borne-diseases. This is very important since mortality and morbidity caused by infectious diseases transmitted by blood sucking arthropods (haematophagous) have always been increasing in the last decade (Gubler 1998). Additionally, there are co-evolutionary functions between vectors' and their transmitted pathogens', for instance saliva plays active roles in homeostasis, inflammation and vertebrate host's immune responses (Ribeiro 1995). Mosquito's salivary components are immunogenic, that is, they induce strong immune response such as swelling and itching that accompanied mosquitoes bites (Peng and Simon 2004). The host's immune response against mosquito's saliva could reduce infectivity of transmitted pathogens (Belkaid *et al.* 1998), therefore population living in leishmaniasis endemic sites showed natural resistance against leishmania parasites (Davies & Gavgani 1999). This has also been proven in leishmaniasis murine model. Resistance against *Leishmania major* was due to exposure of mouse model to uninfected sand flies. This process was associated with a strong delayed-type hypersensitivity response and with interferon- γ production at the site of parasite delivery (Kamhawi *et al.* 2000). The hypothetical mechanism of these processes was due to the natural immunity mediated by T-helper 1 (Th1), which has protective properties and contains antibodies against sandflies saliva. Mosquito bites have shown similar effects in animal models through cytokines systemic response in host (Schneider *et al.* 2004). It may be that these immunomodulatory components has immunosuppressive properties towards effector cells such as macrophage, T lymphocytes, B lymphocytes, natural killer cells, and granulocytes (Andrade *et al.* 2005). Another evidence showed that repeated exposure to bites from uninfected *Anopheles stephensi* skewed the immune response towards Th1 phenotype as indicated by increased levels of interleukin-12, gamma interferon, and inducible nitric oxide synthase, followed by reduction of parasitaemic rates in mouse model (Donovan *et al.* 2007). Other mechanisms related to this protection were very likely due to immunogenicity of salivary protein of *Anopheles*. It has been proven with the salivary proteins from *A. gambiae* (Poinsignon *et al.* 2009),

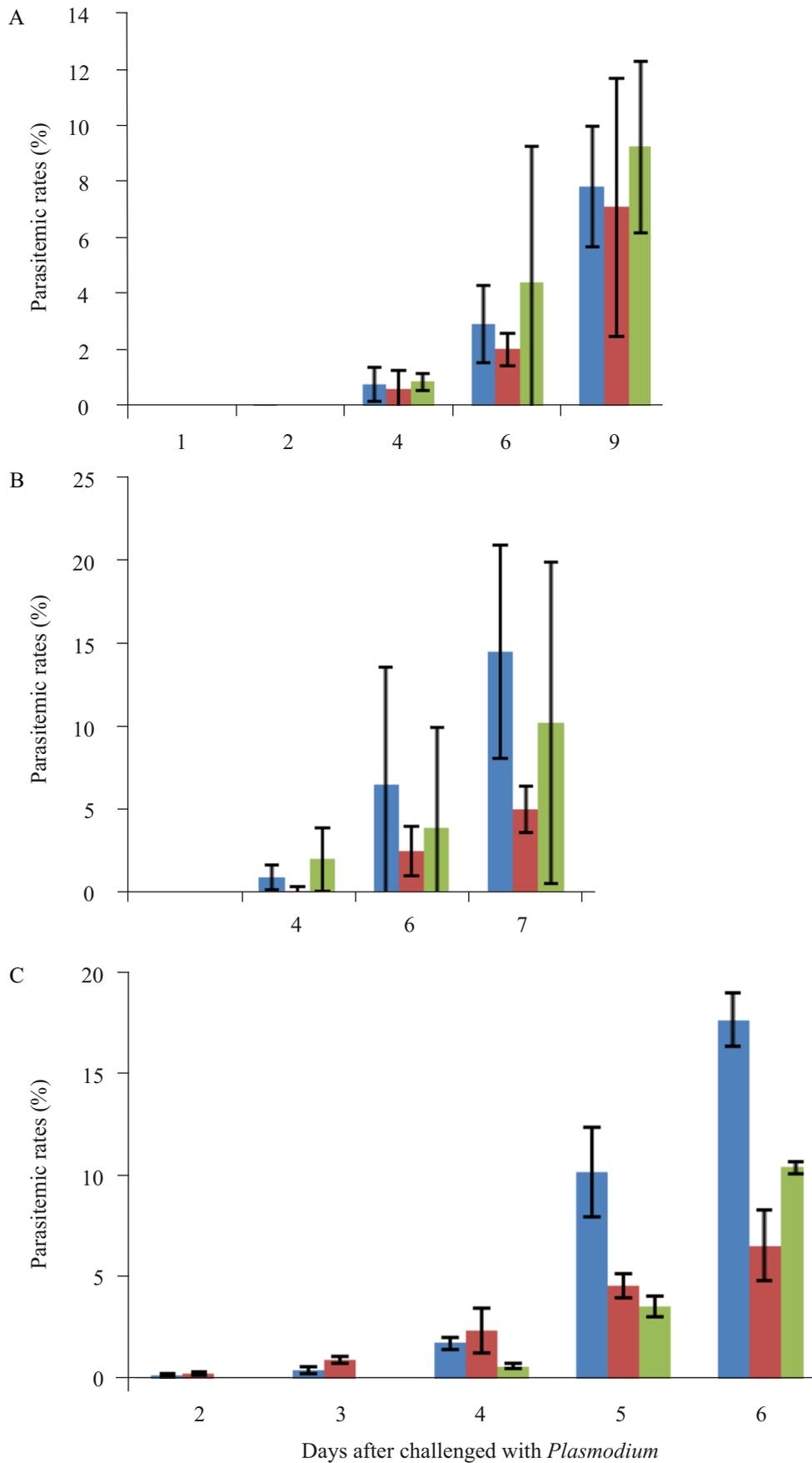


Fig 1 Average rates of parasitaemia (%) in murine model: Control (K), vaccinated with SGE from Pelet (P), and vaccinated with SGE from supernatant (S) from *A. maculatus* (A), *A. aconitus* (B), dan *A. sundaicus* (C). K = Control, P = Pellet from salivary gland extract (SGE) & S = Supernatant from SGE. X-axis represent the number of days after exposure to *P. berghei* (Armiyanti *et al.* 2012; Hanafy *et al.* 2012). ■ : K, ■ : P, and ■ : S.

A. arabiensis and *A. funestus* (e.g. Lombardo *et al.* 2009; Rizzo *et al.* 2011) as well as from *A. barbirostris* (Jariyapan *et al.* 2012).

Our results showed that repeated exposures of salivary gland (SG) extract from 3 potential malaria vectors in Indonesia i.e. *A. aconitus*, *A. sundaicus* and *A. maculatus* were able to reduce rates of parasitaemia in murine malaria model (Fig 1.). The reduction of parasite burdens suggested the roles of host immune response in reducing the infectivity of transmitted pathogen. The data indicated that mosquito's salivary components may serve as a nonspecific potentiator that induces a Th1-biased environment known to be effective against malaria infection. Therefore, addition of *Anopheles* salivary components to antimalaria vaccines may be a suitable strategy to improve vaccine's efficacy. Molecular characterization of salivary gland's components responsible for this process is needed to understand the roles of saliva in blocking transmission of malaria parasites.

Immunogenic Proteins from SG of *Aedes aegypti*. Saliva of *Aedes aegypti* could inhibit viral infection in dendritic cells (DC) *in vitro* (Ader *et al.* 2004). Pre-sensitization of DC with saliva could enhance the inhibition of infection. Additionally, the same group also demonstrated that necrosis of DC was reduced after administration of *Aedes aegypti*'s saliva. Other effects of that application are the increase of IL-12 p70 and TNF- α production in cell culture. The data suggested the protective roles of *Aedes aegypti* salivary

components. Mosquito bites have also been reported to influence immunity and potentiate viral disease in mouse models (e.g. Limesan *et al.* 2003, Schneider *et al.* 2006), possibly through the modulation of host systemic cytokine responses by the salivary component (Schneider *et al.* 2004). This strategy may be important for the development of vaccines to combat mosquito-transmitted viral pathogens such as dengue fever.

Analyzing which protein portions of SG that can be recognized by human antibody would lead to the possibility to find SG's protein with significant role in respond to dengue infection, especially those interacting with antibody of healthy individuals from endemic area. People living in endemic area who do not get the disease must have certain immune mechanisms conferring this protection. This is similar to natural resistance built by population living in endemic areas, as previously explained in the case of leishmaniasis. Investigation of specific components important for immune response in relation to pathogen transmission started in our research group by using SG of *Ae. Aegypti* (Oktarianti *et al.* 2013). As seen in Fig 2, we were able to identify specific protein portions of *Aedes aegypti* SG with molecular weight of ~ 56 and 31 kDa, which were able to cross react only with sera of individuals from endemic area (individual response). These proteins did not seem to cross react with human sera who had never previously been exposed to mosquito bites. The ability of this protein

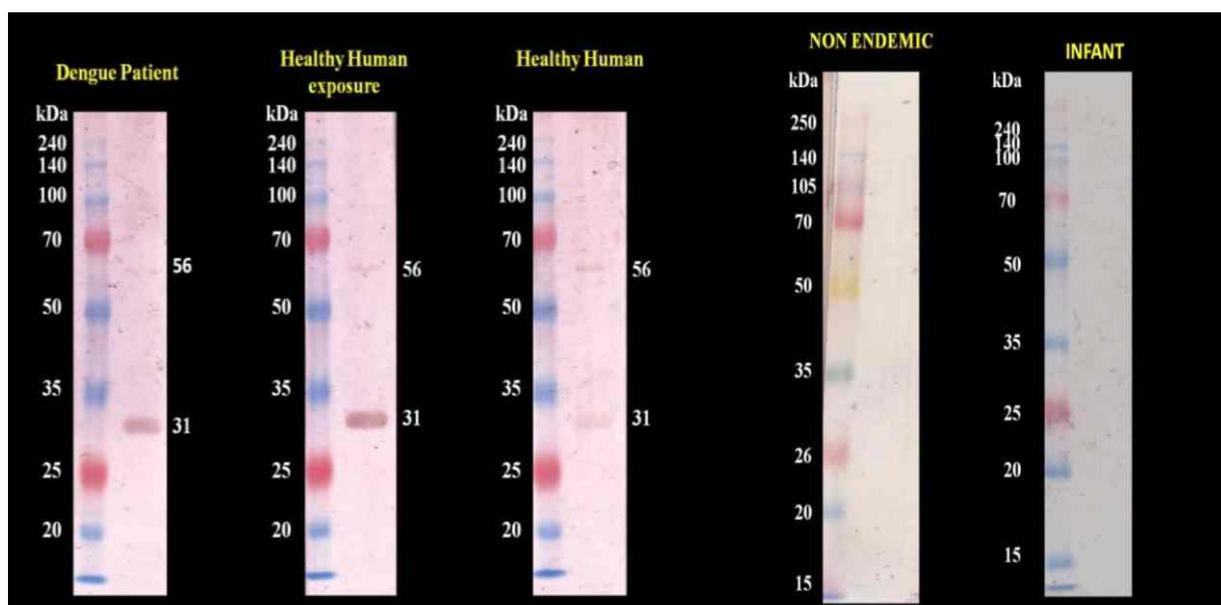


Fig 2 Results of Western Blot analysis showed two specific proteins from SG *Ae. aegypti* (31 & 56 kDa) recognized by only human antibody from sera of people living in endemic area or sera of dengue patient. Sera from people living in non endemic area were taken from Japanese who had never traveled to tropical countries (Oktarianti *et al.* 2013; 2014).

to response against human antibody (IgG) of healthy person from endemic area may serve as an indicator for human resistance against dengue. In accordance to this result, further characterization focusing on these specific proteins and their function must be performed. Our results show similarity with the previously published immunogenic proteins from SG of *Aedes*, i.e. putative secreted 30 and 37 kD Protein, Putative DenV binding Protein (54-58 kD) and SGS1 Protein (387 kD, consist of 2 fragmen ranging from 120-220 kD). These protein have been identified as a potential proteins conferring immunomodulatory activities (King *et al.* 2011; Wasinpiyanmonkol *et al.* 2010). These evidences strongly support the possible development of TBV against DF based on *A. Aegypti* salivary components. Therefore, vaccination using combination of salivary components known to inhibit virus infection and human antibodies against salivary immunosuppression factors would be an effective and efficient strategy to combat malaria, DF and possibly also other mosquito borne-diseases in tropical countries such Indonesia.

CONCLUSION AND OUTLOOK

To initiate eradication of two major mosquitoes borne-diseases in Indonesia such as malaria and DF, a comprehensive strategy is needed. Chemical intercession and pathogen based vaccine development may not be sufficient to halt transmission. Vector-based strategy could be another alternative, because it targets not only an essential step in the transmission process, but also blocking the spread of pathogens, thus preventing rather than treating the illness. Reduction in the rates of parasitaemia in mouse model vaccinated by salivary extract from *A. aconitus* and *A. maculatus* indicated the potential role of vector's saliva as a new vaccine target and novel strategy against malaria. Many reports suggested that any measure that limits parasite densities will reduce the morbidity and mortality associated with malaria infection (McErroy *et al.* 1994). The existence of specific proteins with MW 31 and 56 kDa that may serve as important factors to confer resistance to dengue infection, suggesting their important role in human-virus-pathogen interaction. These are the first reports on exploration of salivary glands from Indonesian mosquito's vector. Therefore, to further elucidate the role of host's salivary components in establishing and/or inhibiting infection, the predominant effectors mechanism in host immune response and molecular characterization of those specific SG's protein should be further

investigated.

ACKNOWLEDGMENT

We are very grateful for the financial supports in Research on the Development of TBV for Malaria and Dengue from RISBIN IPTEKDOK DEPKES, L'oreal UNESCO FWIS, Department of Higher Education (DIKTI), Ministry of Research and Technology (Insentif RISTEK) as well as Lembaga Penelitian Universitas Jember

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