Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.2.2014C1219

Aedes mosquito salivary immune peptides: boost or block dengue viral infections

Natthanej Luplertlop*

Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

PEER REVIEW

Peer reviewer

Sungsit Sungvornyothin, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University. Thailand.
Tel: +6623069100 ext 1572
Fax: +6626435582
E−mail: sungsit.sun@mahidol.ac.th

Comments

This is a very good review because the author described fundamental of mosquito immmune system and raised a good point of association between bacterial respones and viral infection in mosquito. The author also mentioned the role of salivary proteins to both possibility of enhancement and inhibition of dengue virus transmission to vertebrate host.
Details on Page

ABSTRACT

Dengue virus, one of the most important arthropod−borne viruses, infected to human can severely cause dengue hemorrhagic fever and dengue shock syndrome. There are expected about 50 million dengue infections and 500 000 individuals are hospitalized with dengue hemorrhagic fever, mainly in Southeast Asia, Pacific, and in Americas reported each year. The rapid expansion of global dengue is one of a major public health challenge, together with not yet successful solutions of dengue epidemic control strategies. Thus, these dynamic dengue viral infections exhibited high demographic, societal, and public health infrastructure impacts on human. This review aimed to highlight the current understanding of dengue mosquito immune responses and role of mosquito salivary glands on dengue infection. These information may provide a valuable knowledge of disease pathogenesis, especially in mosquito vector and dengue virus interaction, which may help to control and prevent dengue distribution.

KEYWORDS

Dengue, *Aedes*, Mosquito immune, Dengue vectors

1. Introduction

Dengue fevers with its severe clinical manifestations are called dengue hemorrhagic fever (DHF) and dengue shock syndrome. This disease is now endemic in most tropical country zones and emerged as a major public health problem of worldwide concerned because of an increasing potential for mosquito vectors breeding and rapid growth of urban centers with a strain on public services like water containers. Availability of more than 100 countries are endemic for DHF and about 2.5 billion people of the world population are at risks in tropics and sub−tropics. As per estimates, over 50 million infections with about 500 000 cases of DHF have been reported annually, which leads to a cause of childhood mortality in several Asian countries[1−3].

Dengue viruses (DENV) are flaviviruses, which including four serotypes named dengue−1, 2, 3, and 4. The DENV virion is a spherical, enveloped RNA virus that has a diameter of approximately 50 nm. The dengue viral particle contains a lipid bilayer surrounding a capsid that packages the positive−single−strand RNA genome of 10.7 kb in length[4−6]. The lipid bilayer and the envelop glycoprotein shell protein

*Corresponding author: Natthanej Luplertlop, Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.
Tel: +66 894 807 337
E−mail: natthanej.lup@mahidol.ac.th
Foundation Project: Partial supported by Trop Med Grants and Dean Fund Grant, 2012, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Article history:
Received 10 Jan 2014
Received in revised form 12 Jan, 2nd revised form 19 Jan, 3rd revised form 26 Jan 2014
Accepted 6 Feb 2014
Available online 28 Feb 2014

organization had been observed by electron microscopy and image reconstruction, which provided three-dimensional structure of DENV. The structural proteins including a capsid protein (C), an envelope protein (E) play roles in receptor binding, membrane fusion, and viral assembly, which assists by transmembrane protein for proper folding and function of the E protein. The e-glycoprotein is also the principal antigen that elicits neutralizing antibodies, haemagglutination, and seven non-structural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5), which are involved in viral RNA replication[7-10].

The viruses can be transmitted from infected patient to healthy man by infective female mosquitoes, mainly *Aedes* (*Stegomyia*) spp. mosquitoes[11,12]. Dengue mosquito vectors, *Aedes* spp., which breed in domestic and peri-domestic water containers, provided high frequency of mosquitoes and humans interactions. Not only the viruses are maintained in a human-mosquito-human transmission cycle, but the infective mosquitoes can also transmit the viruses from mosquito to mosquito via transovarial transmissions[13,14]. Previously, many researchers reported that transovarial transmission of all four DENV serotypes can be occurred both in laboratory and nature[12,15-17].

2. Dengue virus and mosquito vector, *Aedes* spp. interactions

The principal vector of DENV is *Aedes aegypti* (*Ae. aegypti*) mosquito, an anthropophilic species that exhibited well adaptation to the urban environment. It is a fully domesticated mosquito which can be found in both indoors and outdoors closely to human dwellings[11,18,19]. *Ae. aegypti* is believed to live originally in the jungles of Africa and spread most throughout the rest of the world via slave and trading ships. It was reported that epidemics of dengue exhibited a correlation with the spread of *Ae. aegypti* in South and Southeast Asia. While *Aedes albopictus*, the Asian tiger mosquitoes, can be a secondary vector of DENV in Southeast Asia, the Western Pacific, and increasingly in Central and South America. However, it has also been documented as the important vector in some certain dengue epidemics[20,21].

Transmission cycles of mosquito-borne viruses are depended on the intricate interrelationships that existed between virus, vector, and vertebrate host, of which each was influenced by environmental conditions[22,23]. Mosquitoes acquired the DENV by feeding on an infectious blood meal from human. During blood digestion, virus particle will escape from the blood bolus, then enter and replicate in midgut epithelial cells. Later, virus disseminates from the gut into the hemocoel and then infects the secondary sites of replication including the fat body, hemocytes, brain, and salivary glands. Finally, DENV is secreted into the salivary gland lumen. In the saliva, virus can be transmitted to a human during subsequent blood feeding. The ability of mosquitoes to become infected with an arbovirus after ingestion of an infective blood meal and subsequently virus transmission by bite or through the egg remains infected during its entire lifetime[24-26].

3. Mosquito's immune responses

Mosquitoes are exposed to a variety of infectious microorganism in their habitats throughout their life cycle and some species feed on animal or human blood infected with parasites and viruses. Mosquitoes have developed several structural barriers and a multifaceted innate immune system comprising a variety of synergistic[27,28]. The first line defense against microbes is the physical barriers which includes the outer exoskeleton, the peritrophic matrix of the midgut, and the chitinous linings of the trachea. The peritrophic matrix is a chitinous sack that facilitates digestion and also protects the midgut epithelium from direct contact with the meal and a large proportion of the microbial midgut flora, which can be boosted up to a 16 000 fold after a blood meal in some hematophagous arthropod. The midgut epithelium is served as a structural barrier for microbes and parasites. It is also an immune competent organ[29-31].

Mosquito immune system plays a key role in pathogen-vector interactions[14]. Innate immunity of mosquito has been referred to the first-line defense against the pathogens infection including melanotic encapsulation, phagocytosis, and production of anti-bacterial compounds and immune peptides. The hemocyte is a multifaceted cell that is probably involved in pathogen recognition, cell signaling, production of enzymes and immune system associated molecules (*e.g.* transferrin), and phagocytosis[32,33]. Another important organ that plays a role in mosquito immune response, which produced defensins, cecropins, and proline-rich - glycine-rich peptides, also exhibited anti-microbial activity is the fat body. Although the humoral immune response of mosquitoes does depend upon the fat body for immune system peptide production, other tissues, such as the midgut and salivary glands, do transcribe immune system peptides following activation by a pathogen[34-36].

4. *Aedes* salivary glands and their functions

Viral infection is disseminated throughout the mosquito body via hemolymph. Eventually, the dengue virus infect and possibly replicate within salivary gland before it can be shed into the lumen for final transmission in a subsequent bite[16]. Salivary gland infection barrier or escape barrier can prevent viral transmission. The virus must finally escape into the lumen of the salivary gland, where it can be transmitted

to a vertebrate host during the mosquito's normal feeding activities[37,38].

Aedes mosquito transmitted dengue viruses to vertebrate host by the infected mosquito feeding. The transmission occurs through mosquito's salivation when the female mosquito takes a blood meal. This mosquito's saliva in salivary glands seems to be a potent pharmacologically active fluid, principally proteins, which can affect vascular constriction, blood coagulation, platelet aggregation, inflammation, immunity, and angiogenesis. The key for the successful parasitism of blood-feeding arthropods is the ability to avoid host immune responses through the production of specific salivary antagonists. However, these secretions must have the effective neutralization to host hemostatic responses, making a suitable environment for parasitism. The pathogens in vector saliva must interact with both saliva components and host mediators, which take advantage of the changed host physiology to enhance the pathogens infectivity[39-41]. These secretions are secreted in salivary glands principally by only female mosquitoes and are synthesized in specialized regions especially the distal lateral and medial lobes of the salivary glands. However, male mosquitoes feed only on nectar and incapable of blood feeding due to lack these specialized regions[41,42].

Female mosquito salivary glands perform several functions for effective survival of mosquitoes. The structural design and physiology of the salivary glands makes them effective organs to overcome both physical barriers and host hemostatic and inflammatory/immunological responses for the successful location of host blood vessel and also implicated to be the important organ in transmission of pathogen[19]. The salivary glands play some important roles including facilitating blood feeding, transmission of pathogens, minimizing pathogens infection, producing chemical stimuli for completion of pathogens life cycle, and recognition of pathogens by their specific receptors[43-45].

Mosquito saliva contains many biological materials which can be classified according to their functions as follows in Table 1 enclosed (1) anti-clotting and anti-platelet factors and vasodilators which presumably increase the speed at which blood from the host is imbibed, (2) substances that affect parasite transmission by arthropod vectors, (3) enzymes which are associated with sugar feeding, (4) lysozyme, which may help to control bacterial growth in the sugar meal stored in the mosquito crop, and (5) immunomodulators.

Some of these substances in mosquito saliva have been identified as allergens, such as four recombinant salivary proteins of *Ae. aegypti*, a 68 kD apyrase, a 37 kD D7 protein, a 30 kD Aed a 3, and a 67 kD α-glucosidase[44,45]. However, these substances in the mosquito's saliva can inhibit hemostasis, vasoconstriction, and the development of inflammation and an immune response of host to them. Fortunately, not only the host hematological effects from mosquito's saliva, but it also clears that the feeding of mosquitoes has an immunomodulatory effect on their hosts[46,47].

5. Common secreted salivary proteins

5.1. D7-salivary proteins

Members of the D7 subfamily are the most abundant in salivary secretions of mosquitoes including *Aedines* and *Culicines*. There are two main types of D7 proteins: large and short sequences. Structurally, these D7 proteins related to larger family proteins of the odorant binding proteins but there showed some unusual high degree of divergence among the members of the family, even within the same mosquito subgenus[48]. Possibly, these indicated that they could have diverse functions. It was speculated that these D7-related proteins may exhibit an anti-hemostatic role by trapping agonists of host hemostasis[40,48,49].

Ae. aegypti D7 gene corresponds to a 37 kDa polypeptide presented in the saliva. Isolation and sequencing of 15 unique cDNA fragments were obtained from the salivary glands of *Anopheles gambiae* (150-550 bp). Three of these cDNAs such as D7r1 (dB1), D7r2 (iB6), and D7r3 (iC5) showed a high degree of resemblance to the D7 and apyrase genes of the *Ae. aegypti* salivary glands. Although the functions of D7 are remain to be elucidated, their location, sex- and tissue-specificity, and in the secretory cavities suggested their potential roles in blood feeding and/or pathogens transmission[50,51].

5.2. Nucleotidases

The salivary purinergic degradation machinery of *Ae.*

Table 1

Secretory products in salivary glands and its function of *Ae. aegypti* (Modified from Peng *et al*[51,63].).

Biological function	Protein	Activities
Odorant-binding protein family	D7 salivary proteins	Inhibit the action of biogenic amines such as serotonin, - histamine, and norepinephrine help blood feeding- allergen
Secreted protease inhibitors	Serpins	Protease inhibitor
Enzymes	Nucleotidases apyrase adenosine deaminase purine nucleosidase serine proteases sugar hydrolases amylase, β-glucosidase	Antiplatelet aggregation, anti-inflammatory function - related to immunity, - activating anti-inflammatory pathways (such as protein C)- sugar digestion
Immunity-related proteins	Lectins lysozyme bacteolytic proteins	Opsonization, melanization - antimicrobial polypeptides - immune recognition
Antimicrobial peptides	Gambicin lysozyme defensins	Antimicrobial activity
Anticoagulant	Factor Xa	Anticoagulant
Vasodilator	Sialokinins	Vasodilator
Immunomodulator		Anti-tumour necrosis factor - anti-tumour necrosis factor
30-kDa GE-rich family	Aed a 3	Allergic reactions

aegypti comprises the enzymes apyrase (a member of the 5′ nucleotidase family), adenosine deaminase, and purine hydrolase, which may serve as an anti-hemostatic and anti-inflammatory function by removing nucleotide agonists of platelet aggregation and mast cell degranulation. In addition to these previously described enzymes, we found a second 5′ nucleotidase that may function either as an alternative apyrase or as a secreted salivary 5′ nucleotidase[52,53].

5.3. Serine proteases

Nine secreted serine proteases varying in predicted mature molecular weight between 28 kDa and 43 kDa were found in the *Ae. aegypti* sialotranscriptome. Some of these enzymes are possibly related to immunity that similar to other enzymes annotated as prophenoloxidase activators. But they could have been encompassed to function in activating anti-inflammatory pathways (such as protein C) or deactivating inflammation[54-56].

5.4. Immunity-related proteins

The mosquito's salivary glands produce various anti-microbial polypeptides and other immunity-related products such as bacterial surface-recognizing proteins and lectins that may be important in opsonization and initiates the activation of prophenoloxidase enzyme leading to pathogen melanization[57-59]. The purpose of these products may help to control microbial growth in the sugar solutions stored in the crop or in the gut following a blood meal. The salivary glands of *Ae. aegypti* have been reported the defense molecules as anti-microbial agents including gambicin, lysozyme, and defensin[60-62]. Moreover, lysozyme may prevent bacterial growth in the insect crop and exhibits anti-fungal compound called drosomycin. It is possible that the expression of immune molecules from the salivary glands might decrease microbial infection during feeding, which could be beneficial for the parasite transmission[62].

6. Summary and perspective

Dengue is now a vigorously threat to human health worldwide and is endemic or epidemic in most of tropical areas. While the solution to cure the disease such as dengue vaccine, anti-viral medicine, or well methods for diagnosis are still remain to be elucidated. Study of dengue pathogenesis should be well provided. The challenges, in which to explore the accurate mechanism and reveal the target for controlling dengue strategies are currently available. It is very interesting to reveal the mechanism of virus and mosquito interactions, also the mosquito's immune responded to the viral infection. This leaded to explore the target organ for viral transmission from mosquito to human through feeding activity. The *Aedes* salivary glands were discovered to be a final gateway for successive viral transmission to vertebrate host. Thus, this finding exhibited a new innovative approach to prevent transmission of the virus. A well understanding of the precise molecular mechanisms in interaction of dengue virus versus mosquitoes and also the disease pathogenesis would be beneficial to compete the global burden of dengue health challenge.

Conflict of interest statement

I declare that I have no conflict of interest.

Acknowledgements

The author is grateful to thanks Mr. Thitinan Kitisin for his valuable assist in this review. This review was partial supported by Trop Med Grants and Dean Fund Grant, 2012, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Comments

Background

Dengue virus is one of the most important arthropod-borne viruses which mainly prevalent in tropical countries. *Aedes* mosquito is the principal vector that plays some important roles for disease distributions and host infections especially their salivary proteins. The interaction among dengue virus-*Aedes* mosquitoes and host response still need for further elucidation.

Research frontiers

This review aimed to highlight the current understanding of dengue mosquito immune responses, and general role of mosquito salivary glands on dengue infection.

Related reports

Entomologists try to present the relationship among mosquitoes or arthropods salivary proteins, their specific pathogens and definite host to explain their pathogenesis and disease severity.

Innovations and breakthroughs

This review may provide a valuable knowledge of dengue pathogenesis especially in mosquito vector and dengue virus interaction, which may guide further experimental design and help to control and prevent dengue distribution.

Applications

This review may guide the further scientific research to study about dengue pathogenesis and their important role

of mosquito salivary proteins. Not only their pathogenesis, but also some diagnostic tools, inhibitors or vaccine development and disease control correlated to their specific properties of mosquito's saliva.

Peer review

This is a very good review because the author described fundamental of mosquito immmune system and raised a good point of association between bacterial respones and viral infection in mosquito. The author also mentioned the role of salivary proteins to both possibility of enhancement and inhibition of dengue virus transmission to vertebrate host.

References

[1] Lam SK. Challenges in reducing dengue burden; diagnostics, control measures and vaccines. *Expert Rev Vaccines* 2013; **12**(9): 995-1010.

[2] Murray NE, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. *Clin Epidemiol* 2013; **5**: 299-309.

[3] Chen LH, Wilson ME. Dengue and chikungunya in travelers: recent updates. *Curr Opin Infect Dis* 2012; **25**(5): 523-529.

[4] Iglesias NG, Gamarnik AV. Dynamic RNA structures in the dengue virus genome. *RNA Biol* 2011; **8**(2): 249-257.

[5] Stiasny K, Fritz R, Pangerl K, Heinz FX. Molecular mechanisms of flavivirus membrane fusion. *Amino Acids* 2011; **41**(5): 1159-1163.

[6] Perera R, Kuhn RJ. Structural proteomics of dengue virus. *Curr Opin Microbiol* 2008; **11**(4): 369-377.

[7] Noble CG, Shi PY. Structural biology of dengue virus enzymes: towards rational design of therapeutics. *Antiviral Res* 2012; **96**(2): 115-126.

[8] Alvarez DE, Lodeiro MF, Filomatori CV, Fucito S, Mondotte JA, Gamarnik AV. Structural and functional analysis of dengue virus RNA. *Novartis Found Symp* 2006; **277**: 120-132.

[9] Padmanabhan R, Mueller N, Reichert E, Yon C, Teramoto T, Kono Y, et al. Multiple enzyme activities of flavivirus proteins. *Novartis Found Symp* 2006; **277**: 74-84.

[10] Harris E, Holden KL, Edgil D, Polacek C, Clyde K. Molecular biology of flaviviruses. *Novartis Found Symp* 2006; **277**: 23-39.

[11] Kuno G. Early history of laboratory breeding of *Aedes aegypti* (Diptera: Culicidae) focusing on the origins and use of selected strains. *J Med Entomol* 2010; **47**(6): 957-971.

[12] Wiwanitkit V. Unusual mode of transmission of dengue. *J Infect Dev Ctries* 2009; **4**(1): 51-54.

[13] Jansen CC, Beebe NW. The dengue vector *Aedes aegypti*: what comes next. *Microbes Infect* 2010; **12**(4): 272-279.

[14] Rohani A, Zamree I, Joseph RT, Lee HL. Persistency of transovarial dengue virus in *Aedes aegypti* (Linn.). *Southeast Asian J Trop Med Public Health* 2008; **39**(5): 813-816.

[15] Thongrungkiat S, Wasinpiyamongkol L, Maneekan P, Prummongkol S, Samung Y. Natural transovarial dengue virus infection rate in both sexes of dark and pale forms of *Aedes aegypti* from an urban area of Bangkok, Thailand. *Southeast Asian J Trop Med Public Health* 2012; **43**(5): 1146-1152.

[16] Thongrungkiat S, Maneekan P, Wasinpiyamongkol L, Prummongkol S. Prospective field study of transovarial dengue-virus transmission by two different forms of *Aedes aegypti* in an urban area of Bangkok, Thailand. *J Vector Ecol* 2011; **36**(1): 147-152.

[17] Wasinpiyamongkol L, Thongrungkiat S, Jirakanjanakit N, Apiwathnasorn C. Susceptibility and transovarial transmission of dengue virus in *Aedes aegypti*: a preliminary study of morphological variations. *Southeast Asian J Trop Med Public Health* 2003; **34**: 131-135.

[18] Khin MM, Thank A. Transovarial transmission of dengue 2 virus by *Aedes aegypti* in nature. *Am J Trop Med Hyg* 1983; **32**: 590-594.

[19] Urdaneta-Marquez L, Failloux AB. Population genetic structure of *Aedes aegypti*, the principal vector of dengue viruses. *Infect Genet Evol* 2011; **11**(2): 253-261.

[20] Hidari KI, Suzuki T. Dengue virus receptor. *Trop Med Health* 2011; **39**(Suppl 4): S37-S43.

[21] Doolittle JM, Gomez SM. Mapping protein interactions between dengue virus and its human and insect hosts. *PLoS Negl Trop Dis* 2011; **5**(2): e954. doi: 10.1371/journal.pntd.0000954.

[22] Gratz NG. Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol* 2004; **18**(3): 215-227.

[23] Sanchez-Vargas I, Travanty EA, Keene KM, Franz AW, Beaty BJ, Blair CD, et al. RNA interference, arthropod-borne viruses, and mosquitoes. *Virus Res* 2004; **102**(1): 65-74.

[24] Martins GF, Serrão JE, Ramalho-Ortigão JM, Pimenta PF. Histochemical and ultrastructural studies of the mosquito *Aedes aegypti* fat body: effects of aging and diet type. *Microsc Res Tech* 2011; **74**(11): 1032-1039.

[25] Acosta EG, Castilla V, Damonte EB. Functional entry of dengue virus into *Aedes albopictus* mosquito cells is dependent on clathrin-mediated endocytosis. *J Gen Virol* 2008; **89**: 474-484.

[26] Pandey B, Ichinose A, Igarashi A. Electron microscopic examination of *Aedes albopictus* clone C6/36 cells infected with dengue virus 2 at elevated incubation temperature. *Acta Virol* 1998; **42**(1): 35-39.

[27] Fallon AM, Sun D. Exploration of mosquito immunity using cells in culture. *Insect Biochem Mol Biol* 2001; **31**(3): 263-278.

[28] Lowenberger C. Innate immune response of *Aedes aegypti*. *Insect Biochem Mol Biol* 2001; **31**(3): 219-229.

[29] Dimopoulos G. Insect immunity and its implication in mosquito-malaria interactions. *Cell Microbiol* 2003; **5**: 3-14.

[30] Fiandra L, Casartelli M, Cermenati G, Burlini N, Giordana B. The intestinal barrier in lepidopteran larvae: permeability of the peritrophic membrane and of the midgut epithelium to two biologically active peptides. *J Insect Physiol* 2009; **55**(1): 10-18.

[31] Suwanmanee S, Chaisri U, Wasinpiyamongkol L, Luplertlop N. Peritrophic membrane structure of *Aedes aegypti* (Diptera: Culicidae) mosquitoes after infection with dengue virus type 2 (D2-16681). *Appl Entomol Zool* 2009; **44**(2): 257-265.

[32] Hillyer JF. Transcription in mosquito hemocytes in response to

pathogen exposure. *J Biol* 2009; **8**(5): 51. doi: 10.1186/jbiol151.

[33] Bartholomay LC, Mayhew GF, Fuchs JF, Rocheleau TA, Erickson SM, Aliota MT, et al. Profiling infection responses in the haemocytes of the mosquito, *Aedes aegypti*. *Insect Mol Biol* 2007; **16**(6): 761-776.

[34] Zhang M, Zheng X, Wu Y, Gan M, He A, Li Z, et al. Differential proteomics of *Aedes albopictus* salivary gland, midgut and C6/36 cell induced by dengue virus infection. *Virology* 2013; **444**(1-2): 109-118.

[35] Mc Elroy KL, Girard YA, McGee CE, Tsetsarkin KA, Vanlandingham DL, Higgs S. Characterization of the antigen distribution and tissue tropisms of three phenotypically distinct yellow fever virus variants in orally infected *Aedes aegypti* mosquitoes. *Vector Borne Zoonotic Dis* 2008; **8**(5): 675-687.

[36] Platt KB, Linthicum KJ, Myint KS, Innis BL, Lerdthusnee K, Vaughn DW. Impact of dengue virus infection on feeding behavior of *Aedes aegypti*. *Am J Trop Med Hyg* 1997; **57**(2): 119-125.

[37] Tchankouo-Nguetcheu S, Bourguet E, Lenormand P, Rousselle JC, Namane A, Choumet V. Infection by chikungunya virus modulates the expression of several proteins in *Aedes aegypti* salivary glands. *Parasit Vectors* 2012; **5**: 264. doi: 10.1186/1756-3305-5-264.

[38] Chen WJ, Wei HL, Hsu EL, Chen ER. Vector competence of *Aedes albopictus* and *Ae. aegypti* (Diptera: Culicidae) to dengue 1 virus on Taiwan: development of the virus in orally and parenterally infected mosquitoes. *J Med Entomol* 1993; **30**(3): 524-530.

[39] Mizurini DM, Francischetti IM, Monteiro RQ. Aegyptin inhibits collagen-induced coagulation activation *in vitro* and thromboembolism *in vivo*. *Biochem Biophys Res Commun* 2013; **436**(2): 235-239.

[40] Calvo E, Mans BJ, Andersen JF, Ribeiro JM. Function and evolution of a mosquito salivary protein family. *J Biol Chem* 2006; **281**(4): 1935-1942.

[41] Stark KR, James AA. Salivary gland anticoagulants in culicine and anopheline mosquitoes (Diptera: Culicidae). *J Med Entomol* 1996; **33**(4): 645-650.

[42] Tu WC, Chen CC, Hou RF. Ultrastructural studies on the reproductive system of male *Aedes aegypti* (Diptera: Culicidae) infected with dengue 2 virus. *J Med Entomol* 1998; **35**(1): 71-76.

[43] Mueller AK, Kohlhepp F, Hammerschmidt C, Michel K. Invasion of mosquito salivary glands by malaria parasites: prerequisites and defense strategies. *Int J Parasitol* 2010; **40**(11): 1229-1235.

[44] Stanley D. Prostaglandins and other eicosanoids in insects: biological significance. *Annu Rev Entomol* 2006; **51**: 25-44.

[45] Beerntsen BT, James AA, Christensen BM. Genetics of mosquito vector competence. *Microbiol Mol Biol Rev* 2000; **64**(1): 115-137.

[46] Schneider BS, Higgs S. The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response. *Trans R Soc Trop Med Hyg* 2008; **102**(5): 400-408.

[47] Olson KE, Adelman ZN, Travanty EA, Sanchez-Vargas I, Beaty BJ, Blair CD. Developing arbovirus resistance in mosquitoes. *Insect Biochem Mol Biol* 2002; **32**(10): 1333-1343.

[48] Reagan KL, Machain-Williams C, Wang T, Blair CD. Immunization of mice with recombinant mosquito salivary protein D7 enhances mortality from subsequent West Nile virus infection via mosquito bite. *PLoS Negl Trop Dis* 2012; **6**(12): e1935. doi: 10.1371/journal.pntd.0001935.

[49] Calvo E, Mans BJ, Ribeiro JM, Andersen JF. Multifunctionality and mechanism of ligand binding in a mosquito antiinflammatory protein. *Proc Natl Acad Sci U S A* 2009; **106**(10): 3728-3733.

[50] Doucoure S, Cornelie S, Patramool S, Mouchet F, Demettre E, Seveno M, et al. First screening of *Aedes albopictus* immunogenic salivary proteins. *Insect Mol Biol* 2013; **22**(4): 411-423.

[51] Peng Z, Yang J, Wang H, Simons FE. Production and characterization of monoclonal antibodies to two new mosquito *Aedes aegypti* salivary proteins. *Insect Biochem Mol Biol* 1999; **29**(10): 909-914.

[52] Francischetti IM, Valenzuela JG, Pham VM, Garfield MK, Ribeiro JM. Toward a catalog for the transcripts and proteins (sialome) from the salivary gland of the malaria vector *Anopheles gambiae*. *J Exp Biol* 2002; **205**: 2429-2451.

[53] Champagne DE, Smartt CT, Ribeiro JM, James AA. The salivary gland-specific apyrase of the mosquito *Aedes aegypti* is a member of the 5′-nucleotidase family. *Proc Natl Acad Sci U S A* 1995; **92**(3): 694-698.

[54] Saboia-Vahia L, Borges-Veloso A, Mesquita-Rodrigues C, Cuervo P, Dias-Lopes G, Britto C, et al. Trypsin-like serine peptidase profiles in the egg, larval, and pupal stages of *Aedes albopictus*. *Parasit Vectors* 2013; **6**: 50. doi: 10.1186/1756-3305-6-50.

[55] Rascón AA Jr, Gearin J, Isoe J, Miesfeld RL. *In vitro* activation and enzyme kinetic analysis of recombinant midgut serine proteases from the dengue vector mosquito *Aedes aegypti*. *BMC Biochem* 2011; **12**: 43. doi: 10.1186/1471-2091-12-43.

[56] Mesquita-Rodrigues C, Saboia-Vahia L, Cuervo P, Levy CM, Honorio NA, Domont GB, et al. Expression of trypsin-like serine peptidases in pre-imaginal stages of *Aedes aegypti* (Diptera: Culicidae). *Arch Insect Biochem Physiol* 2011; **76**(4): 223-235.

[57] Hillyer JF. Mosquito immunity. *Adv Exp Med Biol* 2010; **708**: 218-238.

[58] Zou Z, Shin SW, Alvarez KS, Kokoza V, Raikhel AS. Distinct melanization pathways in the mosquito *Aedes aegypti*. *Immunity* 2010; **32**(1): 41-53.

[59] Christensen BM, Li J, Chen CC, Nappi AJ. Melanization immune responses in mosquito vectors. *Trends Parasitol* 2005; **21**(4): 192-199.

[60] Vizioli J, Bulet P, Hoffmann JA, Kafatos FC, Müller HM, Dimopoulos G. Gambicin: a novel immune responsive antimicrobial peptide from the malaria vector *Anopheles gambiae*. *Proc Natl Acad Sci U S A* 2001; **98**(22): 12630-12635.

[61] Kajla MK, Shi L, Li B, Luckhart S, Li J, Paskewitz SM. A new role for an old antimicrobial: lysozyme c-1 can function to protect malaria parasites in *Anopheles* mosquitoes. *PLoS One* 2011; **6**(5): e19649. doi: 10.1371/journal.pone.0019649.

[62] Wilmes M, Sahl HG. Defensin-based anti-infective strategies. *Int J Med Microbiol* 2014; **304**(1): 93-99.

[63] Peng Z, Simons ER. Mosquito allergy: immune mechanisms and recombinant salivary allergens. *Int Arch Allergy Immunol* 2004; **133**: 198-209.