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Insect anionic septapeptides suppress DENV replication by activating antiviral cytokines and miRNAs in primary human monocytes

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ABSTRACT

Dengue viruses (DENVs) have threatened 2/3 of the world population for decades. Thus, combating DENV infection with either antiviral therapy or protective vaccination is an urgent goal. In the present study, we investigated the anti-DENV activity of insect cell-derived anionic septapeptides from C6/36 mosquito cell cultures persistently infected with DENV. These molecules were previously shown to protect C6/36 and Vero cells against DENV infection. We found that treatment with these septapeptides strongly and rapidly downregulated the multiplication of DENV-1 16007, DENV-3 16562, and DENV-4 1036 but not that of DENV-2 16681 in primary human monocytes. This inhibitory effect was likely mediated through various routes including the increased production of antiviral cytokines (IFN–I), activation of mononuclear cell migration, and upregulation of the expression of antiviral miRNAs (has-miR-30e*, has-miR-133a, and has-miR-223) and inflammation-related miRNAs (has-miR-146a and has-miR-147). In conclusion, anionic septapeptides exerted anti-DENV activity in human monocytes through the upregulation of innate immune responses and the activation of several previously reported antiviral and inflammation-related miRNAs.

1. Introduction

Dengue virus (DENV) belongs to the genus *Flavivirus* and family *Flaviviridae*. With the driving forces of climate change and global warming, DENV and its mosquito vector, *Aedes* sp., have spread progressively from endemic tropical zones to temperate regions of the world. Indeed, DENV is accepted as the most rapidly spreading arbovirus, with more than 120 countries where it has become endemic (Banu et al., 2014). All four serotypes of DENV can either cause asymptomatic or symptomatic infections, which include a febrile illness, dengue fever or dengue fever that evolves toward a severe hemorrhagic manifestation. Severe dengue can be fatal without appropriate care. Unfortunately, specific treatment for DENV infection is not yet available. Prevention relies mainly on vector control and is only partially successful (Achee et al., 2019). One type of vaccine has recently been licensed in a few countries. This vaccine provides unsatisfactory protection in seronegative individuals (Aguiar et al., 2016). Moreover, there is no anti-DENV drug in clinical use. Thus, the development of effective and tolerable medication for dengue therapy remains of utmost importance.

In the successful infection of host cells, both viral and host factors are crucial players (Lim, 2018; Mahmud-Al-Rafat et al., 2019). Therefore, potential therapeutic compounds should target these crucial components. Among these components, the RNA polymerase and proteases of DENV have received the most attention due to their virus specificity. Several compounds that inhibit these sets of DENV enzymes have been identified and tested in *in vivo* models (Julander et al., 2010; Koff et al., 1983; Pelliccia et al., 2017; Yin et al., 2009). During the past few years, research on repurposing medicine has been introduced and become an attractive approach. This approach can shorten the timeline and reduce the effort spent on anti-DENV drug development. Drugs such as corticosteroids, chloroquine, and prochlorperazine and antibiotics such as minocycline have been evaluated for their anti-DENV activity. Minocycline, a second-generation semisynthetic tetracycline, exerts anti-DENV activity by decreasing ERK1/2 phosphorylation, which is associated with enhanced DENV pathogenesis (Leela et al., 2016). The reduction in ERK1/2 phosphorylation, in turn, upregulates the expression of antiviral genes such as OAS1, OAS3 and IFN-α. Thus,

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minocycline may be a new anti-DENV drug. Prochlorperazine, a dopamine D2 receptor antagonist that has been approved to treat nausea, vomiting, and headache in humans is able to inhibit DENV entry (Simanjuntak et al., 2015). Surprisingly, no obvious beneficial clinical effect has been noted for any of these drugs so far.

Another group of potential antiviral drugs comprises peptides. Peptides were once neglected for drug development due to their instability and propensity to be readily degraded by various human proteases (Fosgerau and Hoffmann, 2015). However, with recent technological advancements, interest in peptides has been revived. The greatest successful antiviral peptide is Fuzeon™ (enfuvirtide), which has been commercialized as an anti-HIV drug (Ding et al., 2017). This success has opened another dimension of antiviral research. Currently, more than 100 peptide therapeutics are in clinical trials (Lau and Dunn, 2018).

In the present work, we investigated the anti-DENV activity of the mixture of insect-derived peptides called viprolaxikine. Viprolaxikine is produced by mosquito cells persistently infected with DENV-2 (NGC). By LC-MS/MS analysis, viprolaxikine is a mixture of at least 4 types of anionic septapeptides that have the common sequence motif D-D/E-X-X-X-Q-D (Laosutthipong et al., 2013). Here, we characterized the anti-DENV activity of these peptides using primary cultures of human monocytes, one of the main target cells of DENVs. We found that the treatment of DENV-infected human monocytes with these insect cell-derived septapeptides resulted in the downregulation of DENV multiplication. In addition, we found that the treatment of human monocytes with these peptides can alter innate antiviral responses, including the upregulation of the expression of antiviral-associated miRNAs.

2. Materials and Methods

2.1. Cells and viruses

Vero cells (African green monkey kidney cells) were cultured at 37 °C in minimum essential medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine in a 5% CO_2 atmosphere.

C6/36 cells (*Aedes albopictus*) were grown at 28 °C in minimum essential medium.

Dengue viruses (DENV-1 16007, DENV-2 16681, DENV-3 16562, and DENV-4 1036) were propagated in C6/36 cells. The stock viruses were aliquoted and stored at −80 °C. The titers of the stock viruses were determined using plaque assays with Vero cell monolayers. These viruses were a gift from Dr. Sutee Yoksan, Mahidol University, Bangkok, Thailand.

2.2. Primary human monocyte cultures

Primary human monocyte cultures were prepared as previously described (Nantachit et al., 2017). In brief, peripheral blood mononuclear cells (PBMCs) were isolated from the buffy coat using a Ficoll-Hypaque density gradient centrifugation. The purified PBMCs were then subjected to monocyte isolation using magnetic beads against CD_{14} (Miltenyi Biotec, Germany). The purified CD_{14}^{+}-monocytes were cultured at 37 °C in RPMI 1640 medium supplemented with 10% heat-inactivated human AB serum, 2 mM L-glutamine and 1.2% antibiotics (penicillin G and streptomycin) in a 5% CO_2 atmosphere.

2.3. Synthetic peptides

Four peptides (DDHELQD, DETELQD, DEVMLQD and DEVLMQD) were synthesized, purified and provided at a purity greater than 97% by PEPTIDE 2.0, USA. Each preparation was accompanied by a mass spectrum to validate the peptide mass. Each peptide stock was dissolved in deionized distilled water at a concentration of 1 mg/ml, aliquoted and stored at −20 °C.

2.4. Detection of antiviral activity using primary human monocytes

A total of 3×10^6 purified monocytes were seeded into each well of a 12-well culture plate and cultured for 18 h at 37 °C in a 5% CO_2 incubator. The loosely adhered monolayer of purified monocytes was washed once with PBS. The monocyte cultures were treated with 100 µg (25 µg per peptide) or 10 µg (2.5 µg per peptide) of a mixture of the 4 synthetic peptides. Peptide treatment was carried out at 37 °C for 3 h. The treated cultures were washed twice with PBS and then inoculated with DENV at an MOI of 1 PFU/cell at 37 °C for 1.5 h. At the end of the inoculation period, the cultures were washed twice with PBS, and 1.5 ml of growth medium was added to each well. Aliquots of supernatant were harvested every 24 h for 4 constitutive days. The harvested samples were subjected to a plaque assay by using Vero cells. In this experiment, peptide diluent-treated monocytes infected with DENV were used as an infection control.

2.5. Detection of innate cytokines

Monolayers of purified monocytes were washed once with PBS before being treated with either 10 µg or 100 µg of peptide mixture for 3 h at 37 °C. At the end of treatment, 1.5 ml of growth medium was added to each well. The cultures were then incubated at 37 °C in a humidified CO_2 incubator. Aliquots of supernatant were harvested at 3, 24, 48 and 72 h of incubation. The harvested supernatants were analyzed for the levels of proinflammatory cytokines, including Th 1- and Th 2- related cytokines, chemokines, and growth factors, by using a Bio-Plex human cytokine assay kit (Bio-Rad). Antiviral cytokines (IFN-α and IFN-β) were quantitated using an ELISA kit (R&D Systems). The harvested cells were subjected to qRT-PCR analysis to measure the levels of miRNA expression.

2.6. Migration assay

A cell migration assay was performed as previously described (Phuklia et al., 2013). The assay was carried out using 96-well microchemotaxis plates with 8.0 µm pore-diameter polycarbonate filters (Corning, Corning NY). The purified human monocytes were added to the upper compartment of the chamber, while culture supernatants from peptide-treated monocytes were added to the lower compartment. Cell migration was scored by counting the number of monocytes that reached the bottom chamber after 2 h of incubation at 37 °C. The number of migrating cells was expressed as the chemoattractant index (CI), which was calculated as the number of cells migrating to the test medium divided by the number of cells migrating to the control medium. In this experiment, the control medium was medium harvested from monocyte cultures treated with only peptide diluent.

2.7. Detection of miRNA expression

RNA isolation, reverse transcription, and qPCR amplification were performed as follows. Peptide-treated monocytes were subjected to RNA isolation using High Pure miRNA Isolation kits (Roche, Mannheim, Germany) according to the manufacturer's instructions. For reverse transcription, a total of 10 ng of purified RNA was subjected to cDNA synthesis using specific primers and a TaqMan® MicroRNA Reverse Transcription kit (Applied Biosystems) in a volume of 15 µl. The reaction contained 10 ng of RNA template, 7 µl of RT Master Mix and 3 µl of specific primer. The RT reaction program was 80 min at 16 °C, followed by 30 min at 42 °C and 5 min at 85 °C. qPCR amplification was performed in a 20 µl reaction volume using a TaqMan® Small RNA assay kit containing 1 µl of TaqMan® Universal PCR Master Mix II and nuclease-free water. PCR amplification was carried out by an initial incubation at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and annealing/extension at 60 °C for 1 min with an ABI PRISM 73000 Real-Time PCR system (Applied Biosystems, USA).

The data obtained were analyzed using the comparative C_T ($\Delta\Delta C_T$) method for calculating the relative quantitation of gene expression. The relative expression of each miRNA was calculated by the $2^{-(\Delta\Delta CT)}$ formula in which $\Delta CT = CT_{miRNA} - CT_{RNU-48}$, and $\Delta\Delta CT = \Delta CT_{peptide\text{-}treated} - \Delta CT_{mock\text{-}treated}$. Subsequently, the relative quantitation value underwent log 2 transformation to compare the expression levels of candidate miRNAs.

2.8. Statistical analysis

All data are reported as the mean ± SD from three independent experiments. Statistical analysis was performed using Student's *t*-test, and differences with $P \leq 0.05$ were considered statistically significant.

3. Results

3.1. Treatment with insect-derived peptides suppresses DENV production

Monolayer cultures of purified human monocytes were treated with the peptide mixture before being inoculated with DENVs as described in the Materials and Methods. The production of infectious viruses was quantitated using plaque assays. As revealed in Fig. 1, primary human monocytes infected with DENV-1 16007, DENV-2 16681, DENV-3 16562 or DENV-4 1036 yielded infectious virions with titers of 1.2×10^3, 9.2×10^4, 8×10^2 and 1.1×10^3 PFU/ml, respectively. Interestingly, treatment with the peptide mixture at a dose of 100 μg but not at a dose of 10 μg (data not shown) significantly suppressed DENV multiplication. At a dose of 100 μg of peptide mixture, DENV-1 16007 multiplication was downregulated by approximately 60% and 100% by days 1 and 2 post exposure, respectively. Surprisingly, the inhibitory activity of the peptide mixture against DENV-2 16681 was not detected.

For DENV-3 16562 and DENV-4 1036, treatment with the peptides at the 100 μg dose significantly suppressed virus production. By day 2, DENV-3 16562 production was completely inhibited. DENV-4 1036 production was also significantly inhibited by day 2, and this inhibition continued until the end of the experiment. The results indicated that the insect-derived septapeptides strongly suppressed the multiplication of DENV-1 16007, DENV-3 16562 and DENV-4 1036 but not that of DENV-2 16681.

3.2. Insect-derived septapeptides stimulate the production of antiviral cytokines and proinflammatory cytokines

To investigate the mechanism underlying the inhibitory effect of the peptides on DENV, antiviral cytokine/chemokine profiles were monitored in monocytes treated with 10 μg or 100 μg of peptide mixture in

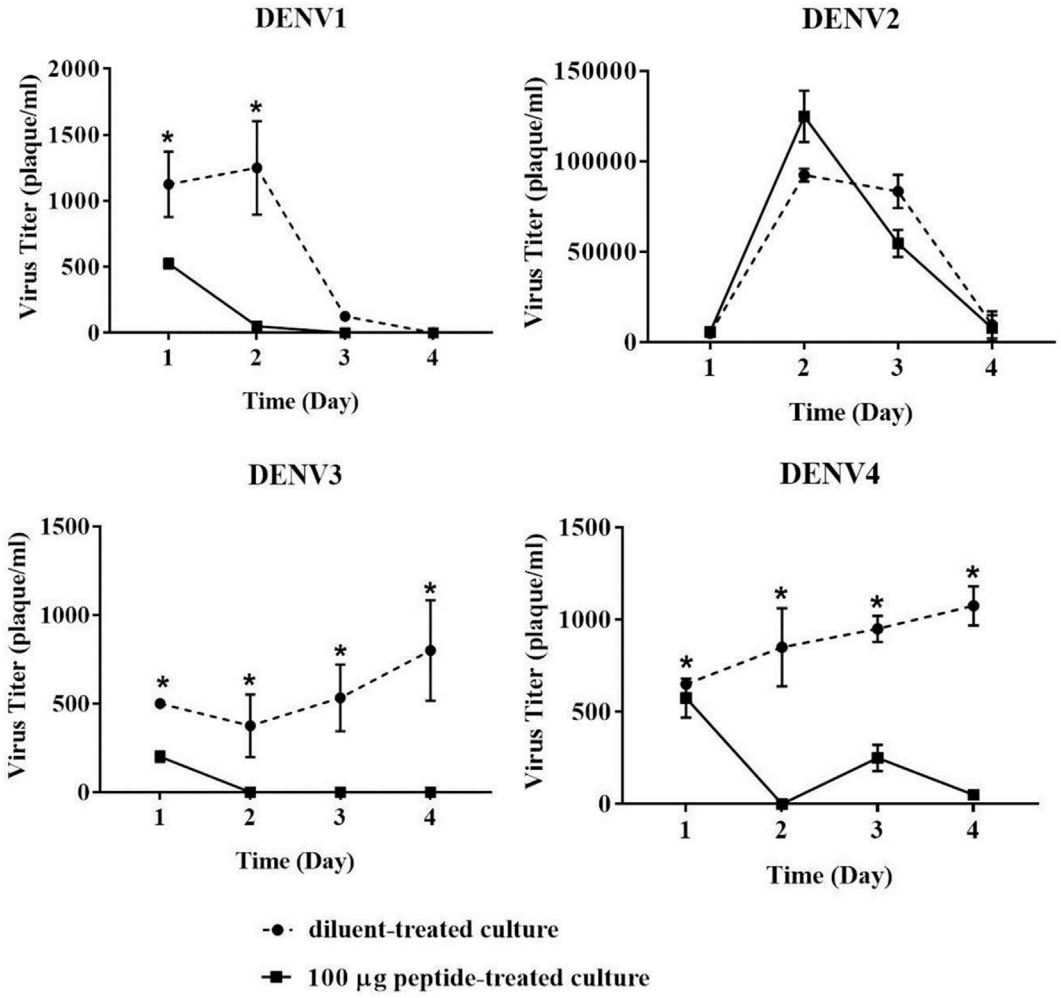

Fig. 1. Viral titers from *ex vivo* monocyte cultures challenged with 4 DENV serotypes after pre-exposure to septapeptides: Purified, cultured human $CD14^+$ cells were pre-exposed to either 10 or 100 μg of septapeptide mixture before being challenged with a DENV at an MOI of 1.0. Aliquots of culture medium were harvested every 24 h post challenge for 3 constitutive days. Viral titers were determined using plaque assays. Monocyte cultures treated with peptide diluent before being infected with DENV were used as the infected-culture control. The symbol (*) indicates a significant difference ($P \leq 0.05$) between the test and control, as determined by Student's *t*-test.

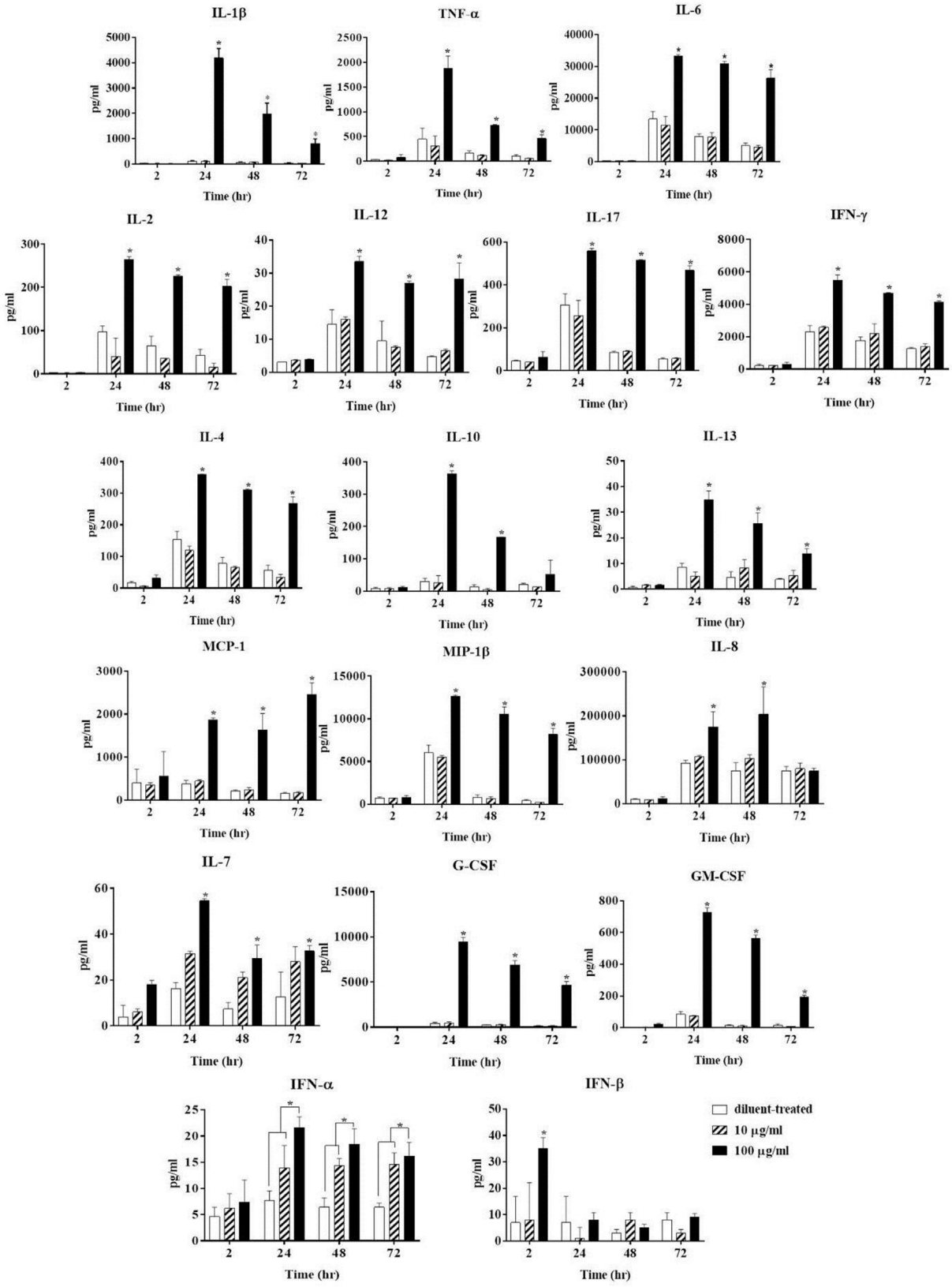

Fig. 2. Levels of antiviral cytokines/chemokines produced by *ex vivo* monocyte cultures after exposure to septapeptides: Cultures of purified human $CD14^+$ cells were treated with either 10 or 100 μg of septapeptide mixture. Aliquots of culture media were harvested every 24 h for 3 constitutive days and subjected to cytokine/chemokine quantification by ELISA. The symbol (*) indicates a significant difference ($P \leq 0.05$) between the test and control, as determined by Student's *t*-test.

comparison to diluent-treated control cells. The amounts of cytokines in the supernatants were quantified by ELISA. As shown in Fig. 2, IL-1β, IL-6 and TNF-α production were strongly stimulated in response to 100 μg but not 10 μg of peptides. A similar result was found for the production of Th1-related cytokines (IL-2, IL-12, and IL-17), Th 2-related cytokines (IL-4, IL-10 and IL-13) and chemokines (MCP-1, MIP-1, and IL-8) at 100 μg but not 10 μg of peptides. For growth factors, the 100 μg peptide treatment increased the production of IL-7, G-CSF and GM-CSF, while treatment with 10 μg of peptides stimulated the production of only IL-7 (Fig. 2). Notably, the production of these cytokines and growth factors lasted until the end of our experiment (day 3).

Regarding the production of type I IFNs, at both the 10 μg and 100 μg doses, these insect-derived peptides strongly stimulated the production of IFN-α. The upregulation of IFN-α expression was sustained until the end of the experiment. In contrast, the peptides only transiently activated IFN-β production. As shown in Fig. 2, the upregulation of IFN-β expression occurred within 2 h following treatment, and then IFN-β expression decreased to an undetectable level within 24 h.

3.3. Treatment with insect-derived septapeptides induces monocyte/macrophage migration

As shown in Fig. 2, treating monocytes with the insect-derived peptides significantly increased the production of chemokines (IL-8, MCP-1 and MIP-1). To determine whether supernatants from peptide-treated monocytes could induce monocyte/macrophage migration, an assay using purified human CD_{14}^{+} cells was carried out. As shown in Fig. 3, the migration of monocytes in chambers containing supernatants from peptide-treated cells was significantly greater than the background migration using supernatants from diluent-treated cells. The results suggested that treatment with the peptides could facilitate inflammatory cell migration to sites of infection.

3.4. Treatment with insect-derived peptides alters miRNA expression

MicroRNAs (miRNAs) are classified as a component of innate immune responses to viral infection. These small, noncoding RNAs serve as posttranscriptional regulators of cellular homeostasis. For infectious diseases, miRNAs can target the viral genome to regulate viral replication (Bruscella et al., 2017). Thus, we investigated the possibility that the insect-derived peptides could affect the expression of immune response-related and virus-related miRNAs. Monocyte cultures were treated with 100 μg of peptide mixture or peptide diluent. At 3, 6 and 21 h following treatment, the monocytes were harvested for expression analysis of 7 selected miRNAs (hsa-miR-146a, hsa-miR-147, hsa-miR-155, hsa-miR-30e*, hsa-miR-133a, hsa-miR 223 and hsa-miR-122) using the level of RNU-48 as an internal control.

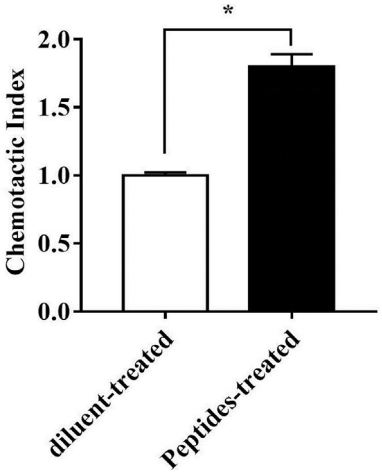

Fig. 3. Migration of human monocytes in response to cytokines/chemokines produced by monocyte cultures after exposure to septapeptides: Culture supernatants from monocyte cultures treated with 100 μg of peptide mixture or diluent control were added into the lower wells of 96-well microchemotaxis plates, while purified human monocytes were plated in the upper wells. The migrated cells were counted, and the results are expressed as a chemoattractant index (CI). The data were obtained from three independent experiments. The symbol (*) indicates a significant difference ($P \leq 0.05$) between the test and control, as determined by Student's *t*-test.

As shown in Fig. 4, treatment with the insect-derived peptides resulted in the upregulation of the expression of 5 out of the 7 investigated miRNAs (hsa-miR-146a, hsa-miR-147, hsa-miR-30e*, hsa-miR-133a, and hsa-miR-223). The induction of hsa-miR-146a expression was detected within 3 h post treatment, and then this expression decreased almost completely by 21 h after treatment. The upregulation of hsa-miR-147 expression was also detected within 3 h of treatment, while has-miR-30e* expression was not stimulated until 21 h post treatment. Different patterns of expression were revealed for has-miR-133a and hsa-miR-223. Both exhibited upregulated expression within 3 h post treatment, and the increased expression was sustained through the end of the experiment (21 h). In contrast, has-miR-155 expression was upregulated at 3 h post treatment and then significantly suppressed through 21 h post treatment.

In summary, the exposure of primary cultures of human monocytes to insect-derived septapeptides resulted in the upregulation of the expression of immune response-related miRNAs (has-miR-146a and hsa-miR-147) and antiviral-associated miRNAs (hsa-miR-30e*, hsa-miR-133a, and hsa-miR-223).

4. Discussion

Peptides have highly selective and relatively safe characteristics, making them attractive candidates for drug development. As a result, antimicrobial peptides (AMPs) with diverse antiviral activities have recently gained increasing attention (Bahar and Ren, 2013; Hsieh and Hartshorn, 2016). For example, the peptide HS-1 derived from the skin of the frog *Hypsiboas semilineatus* (order *Anura*) acts directly on DENV by breaking down the DENV envelope (Monteiro et al., 2018). Other peptides that inhibit DENV multiplication have been intensively studied and found to affect various steps of the DENV life cycle. For example, peptides that target the E and prM proteins inhibit DENV in the post-entry phase (Alhoot et al., 2013; Wang et al., 2009). Another peptide that interacts specifically with NS2B-NS-3B or NS5 serves as a translation or replication inhibitor (García et al., 2017).

Apart from their direct effects on viruses, AMPs are well accepted as important components of the innate immune response in insects (Bulet et al., 2004; Yi et al., 2014). For example, cecropins and attacins are important anti-RNA virus molecules in Drosophila (Imler and Bulet, 2005). Gloverin-4 is an AMP that protects against Bombyx mori nucleopolyhedrovirus, which infects the silkworm *Bombyx mori* (Kawaoka et al., 2008). Alloferons are cationic, nonglycosylated 12–13 amino acid peptides isolated from the hemolymph of Dipterans. Synthetic alloferon-1 stimulates natural killer cells, upregulates IFN-I production and inhibits influenza A and B viruses in a mouse model (Chernysh et al., 2002). Several AMPs, such as a cecropin-like peptide and CxVago, have been identified in mosquitoes. A cecropin-like peptide containing 59 amino acids was isolated from the *Aedes aegypti* salivary gland and found to have anti-DENV and anti-Chikungunya virus activities (Luplertlop et al., 2011). CxVago, a cysteine-rich, anionic peptide of 113 amino acids isolated from *Culex quinquefasciatus,* functions as an IFN-like antiviral cytokine (Paradkar et al., 2012).

In this study, we investigated the anti-DENV activity of anionic septapeptides, which were previously reported to suppress the multiplication of all 4 serotypes of DENV in both insect (invertebrate) cells and Vero (vertebrate) cells (Laosutthipong et al., 2013). We demonstrated here that these septapeptides strongly and rapidly inhibited the

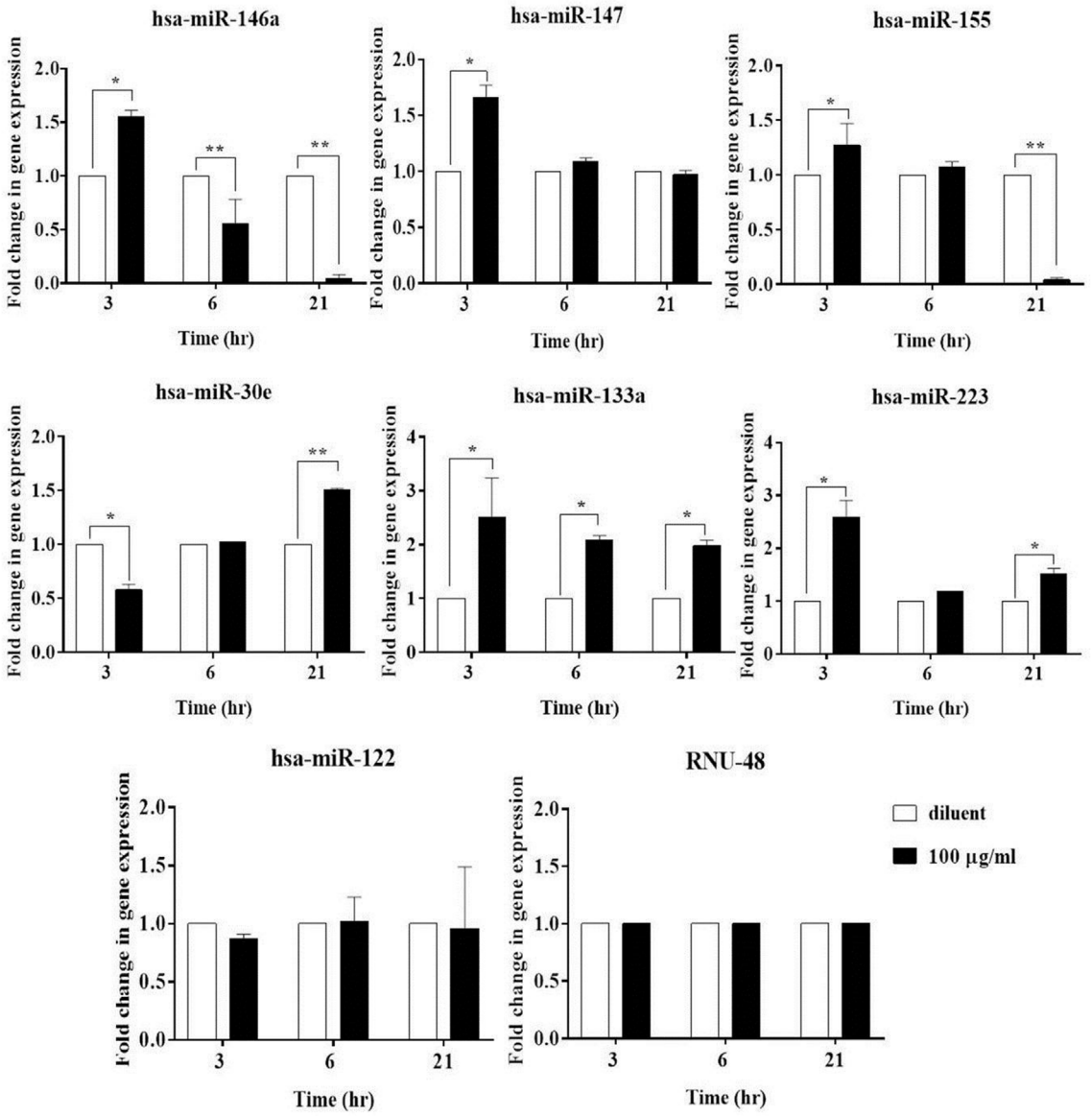

Fig. 4. Expression profiles of miRNAs in human monocytes after exposure to septapeptides: Cultures of purified human $CD14^+$ cells were treated with either 10 or 100 µg of septapeptide mixture. The treated cells were harvested at 3, 6, and 21 h after treatment, and miRNA expression was analyzed semiquantitatively. The symbol (*) indicates a significant difference ($P \leq 0.05$) between the test and control, as determined by Student's *t*-test.

multiplication of DENV-1, DENV-3 and DENV-4 but not that of DENV-2 in primary human monocytes. The contradiction between our finding and that of the report by Laosutthipong et al. on anti-DENV activity is unclear. It is worth noting that DENV-2 16681, which was used in our study, was isolated from a DSS patient, while the previous report tested the activity of these peptides against DENV-2 NGC, which was isolated from a DF patient (Guzman et al., 1995). Therefore, the contribution of strain differences to the susceptibility to antiviral activity deserves further study. In addition, we revealed that the replication efficiency of DENV-2 16681 was 10-fold higher than that of DENV-1 or DENV-4 and 100-fold higher than that of DENV-3 (Fig. 1). The antiviral mediators stimulated in our model may not be potent enough to suppress the multiplication of DENV-2 16681. However, our results and the report by Laosutthipong et al. indicated that these septapeptides are panserotype DENV inhibitors due to their ability to suppress DENV-1 (Hawii and 16007), DENV-2 (NGC), DENV-3 (H87 and 16562) and DENV-4 (H241 and 1036).

In studying the mechanism of DENV inhibition, we found that treatment with the peptide mixture significantly upregulated the production of immune mediators such as antiviral cytokines (IFN-α and IFN-β), inflammatory cytokines/chemokines (IL-1β, IL-6, IL-8, MCP-1 and MIP-1), Th 1-related and Th 2-related cytokines and macrophage growth factors. Among these mediators, interferon type I was likely to be the mediator that directly suppressed DENV multiplication in our model. This hypothesis was supported by the results shown in Figs. 1 and 2, which indicated that the level of virus production inversely correlated with the level of IFN-α production. Other mediators, such as inflammatory cytokines/chemokines that induce the migration of inflammatory cells to sites of infection, are known to promote cellular antiviral activity. Th 1-related and Th 2-related cytokines regulate T and B cell responses. Unfortunately, the activities of these cell-mediated responses against DENV cannot be tested in our *in vitro* model. Moreover, these peptides also altered the expression of miRNAs, which are known to be effective weapons for host protection against viral infection (Baltimore et al., 2008). For example, miR-26a protects the host from influenza A virus infection (Gao et al., 2017). Several cellular miRNAs such as miR-122 and miR-30e* have been revealed to significantly downregulate DENV replication by reducing the DENV RNA

level (Lee et al., 2010; Zhu et al., 2014). In addition, miR-9, miR-124a and miR-142 determine tissue tropism and can therefore restrict DENV infection and prevent the spread of DENVs to target tissues or organs. Moreover, miR-133a inhibits DENV-2 replication through the down-regulation of DENV 3′-UTR interacting protein polypyrimidine tract binding protein (PTB) expression, while the overexpression of miR-233 suppresses DENV replication by decreasing the production of STMIN1 (a microtubule destabilizer). Some cellular miRNAs suppress DENV production through the regulation of antiviral responses. For example, miR-30e* targets the TLR signaling pathway, resulting in the upregulation of IFN-β expression (Zhu et al., 2014) and thereby blocking DENV production. In addition, miR-146a negatively regulates TRAF-6, IRAK1, IL-8 and RANTES and thus prevents an excessive inflammatory response, which suggests that miR-146a can lower dengue severity (Saba et al., 2014). This notion is supported by the evidence that DENV-1-infected patients have a lower expression level of miRNA-146a than healthy control subjects (Ouyang et al., 2016). Similarly, miR155 downregulates the production of MMP-1 and MMP-3, resulting in the suppression of tissue damage due to overreactive inflammation. Moreover, miR-24-1-5p, miR-512-5p, miR-4640-3p and miR-383 are associated with dengue severity (Tambyah et al., 2016). These findings indicate that cellular miRNAs regulate not only the multiplication of DENV but also the severity of disease. In our present study, we found that primary human monocytes treated with 100 μg of a cocktail of insect-derived septapeptides upregulated the expression of 3 tested antiviral miRNAs (has-miR-30e*, has-miR-133a, and has-miR-223) and transiently expressed proinflammatory miRNAs (has-miR-146a and has-miR-147). Our findings showed that treatment with the anionic, mosquito cell-derived septapeptides significantly stimulated the expression of known immune-related miRNAs as well as reported antiviral miRNAs. It is well accepted that one type of miRNA has various functions in the cellular microenvironment; thus, the mechanism of the stimulated miRNAs identified in our study in response to DENV infection requires further study.

In summary, we have demonstrated that a cocktail of synthesized, anionic septapeptides derived from DENV-infected mosquito cells can drive primary human monocytes into an anti-DENV state. Two potential anti-DENV mechanisms were described here. First, the peptides stimulated the expression of miRNAs known to suppress DENV replication. Second, the peptides promoted the production of antiviral cytokines, cytokines/chemokines that not only suppress viral multiplication but also recruit inflammatory cells to infected sites, and cytokines that regulate T and B cell responses.

Competing financial interests

All authors declare that they have no conflicts of interest.

Submission declaration and verification

This manuscript has not been submitted or accepted elsewhere. All authors have read and approved the manuscript for submission and have contributed significantly to this work.

Author contribution

Limthongkul J, Mapratiep N, and Apichirapokey S were responsible for performing the experiments. Midoeng P supervised the PBMC and monocyte isolations. Suksatu A was responsible for the data analysis and manuscript preparation. Ubol S coordinated and supervised the study and composed the manuscript.

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