CONTRIBUTION OF MONOCYTE-DERIVED DENDRITIC CELLS INFECTED WITH VIRULENT- OR ATTENUATED-DENGUE VIRUS TO TNF-α REACTOGENICITY AND ASSOCIATION OF IL-1β AND IL-8 WITH ATTENUATED PHENOTYPE

Akkarat Rodpai[1, 3], Rojjanaporn Pulmanausahakul[3], Panuwat Midoeng[2], Sukathida Ubol[1] and Sutee Yoksan[3]

[1]Department of Microbiology, Faculty of Science, Mahidol University; [2]Department of Pathology, Army Institute of Pathology, Phramongkutklao Hospital, Bangkok; [3]Center for Vaccine Development, Institute of Molecular Biosciences, Mahidol University, Salaya Campus, Nakhon Pathom, Thailand

Abstract. Pathogenesis of severe dengue is partially known and involves virus and immunologic factors in orchestrating the disease severity. While serving as principal innate immune cells, dendritic cells (DCs) sub-serve as target cells for dengue virus (DENV) proliferation. Accordingly, responses of DCs toward DENV are critical for protection or disease development. We compared levels of innate cytokines secreted from primary human monocyte-derived (Mo)DCs upon infection with virulent parental strains DENV-2 16681 and DENV-3 16562, to those of MoDCs infected with their respective attenuated strains, DENV-2 PDK53 and DENV-3 PGMK30. Productions of IL-1β and IL-8 are significantly upregulated in attenuated-DENV-infected MoDCs compared to those infected with their respective parental virulent viruses. Moreover, both virulent parental viruses were potent inducers of IL-10 and TNF-α. Interestingly, although attenuated DENV-2 PDK 53 was a poor inducer of TNF-α, reactogenic-attenuated DENV-3 PGMK30 induced TNF-α production to a level similar to that induced by its virulent parental strain, suggesting a contribution of TNF-α towards the reactogenicity of DENV-3 PGMK30. These observations indicate MoDCs are able to differentiate between infection from virulent and attenuated DENV and should provide important knowledge on dengue pathogenesis useful for vaccine development.

Keywords: attenuation, DENV, IL-1β, IL-8, reactogenicity, TNF-α, virulence

Correspondence: Sukathida Ubol, Department of Microbiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.
E-mail: sukathida.ubo@mahidol.ac.th

Sutee Yoksan, Center for Vaccine Development, Institute of Molecular Biosciences, Mahidol University, Salaya Campus, Nakhon Pathom 73000, Thailand.
E-mail: sutee.yok@mahidol.ac.th

INTRODUCTION

Infection by dengue virus (DENV) is a worldwide public health problem. More than 100 countries in tropical and subtropical regions are epidemic areas of DENV, with 390 million dengue infections occurring each year, of which nearly 25% are symptomatic (Bhatt *et al*, 2013).

DENV-1 to -4 are members of the

family Flaviviridae, genus *Flavivirus*. Clinical manifestations of dengue range from relatively mild symptoms (dengue fever, DF) to severe hemorrhagic fever (dengue hemorrhagic fever and dengue shock syndrome, DHF/DSS). The pathogenic process by which infected patients develop DHF/DSS are partially known and is thought to involve two major determinants, namely, virulence of virus or viral factors and immunological factors. In the context of the immunological mediators, both pre-existing and activating mediators are associated with dengue severity (Malavige and Ogg, 2017; Srikiatkhachorn *et al*, 2017). The pathophysiological hallmarks, which differentiate DHF/DSS from DF are vascular leakage and thrombocytopenia. Vascular leakage is transient with no structural damage (Innis, 1995) and thus, soluble mediators such as cytokines have been proposed to be responsible for the alterations in vascular permeability (Martina *et al*, 2009). Circulating levels of pro- and anti-inflammatory cytokines among DHF/DSS and DF patients and healthy controls have been intensively studied, but results were inconclusive. For example, different levels of TNF-α, IFN-γ, IL-6, IL-8, IP-10, MMP-2, MMP-9 and HGP were observed when DHF is compared to DF patients (Pandey *et al*, 2015; Her *et al*, 2017; Oliveira *et al*, 2017). In addition, several studies showed higher IFN-γ level in DF than that of DHF patients (Braga *et al*, 2001; Priyadarshini *et al*, 2010), while others reported opposite results or no differences (Kurane *et al*, 1991). Moreover, similar conflicting results were also observed for IL-12, TNF-α, IL-6 and IL-8 (Hober *et al*, 1993; Libraty *et al*, 2002). It is worth noting these results were generated from patients who had secondary DENV infection.

In order to avoid other factors that might have arisen as a consequence of secondary infection, Cruz-Hermandez *et al* (2016) investigated the association of pro-inflammatory cytokines with dengue severity during primary infection, and reported the association between high levels of pro-inflammatory cytokines (IL-12p70, IFN-γ, TNF-α, and IL-6) and dengue disease severity does not always occur. These conflicting results may be due to a lack of appropriate animal models and an inability to perform direct study comparisons.

The development of live-attenuated dengue vaccine candidates using the classical approach of serial adaptation of virulent DENV in cells of non-natural hosts has led to successful attenuation all four DENV serotypes with various degrees of attenuation (Bhamarapravati and Yoksan, 2000). Two sets of virulent viruses and their attenuated variants, namely, DENV-2 16681 and its attenuated variant DENV-2 PDK53, and DENV-3 16562 and its attenuated but reactogenic counterpart DENV-3 PGMK30 are interesting. DENV-2 PDK53, derived by sub-culturing its parental strain in primary dog kidney (PDK) cells for 53 passages is clinically safe, stimulates protective response in humans and is used as a backbone in the construction of chimeric vaccines for other DENV serotypes (Osorio *et al*, 2016). DENV-3 PGMK30, generated by serial sub-culturing of its parental strain in primary green monkey kidney (PGMK) cells for 30 passages and further subculturing in Vero cells, exerts an attenuating phenotype, but clinical trials reveal reactogenic activity of this DENV strain that causes dengue-liked syndrome in recipients (Kanesa-thasan *et al*, 2001; Kitchener *et al*, 2006). Thus, DENV-3 PGMK30 is considered as an under-attenuated strain.

Using these two sets of virulent and attenuated or under-attenuated DENVs for direct comparisons, we hypothesized that dendritic cells, the first line of im-

mune cells to encounter DENVs, should be able to differentiate between virulent and attenuated virus. The resulting information should provide a better understanding of DENV virulence and attenuation in dengue pathogenesis.

MATERIALS AND METHODS

Viruses

The two sets of DENVs, DENV-2 16681 and its attenuated counterpart DENV-2 PDK53 and DENV-3 16562 and its attenuated but reactogenic counterpart DENV-3 PGMK30, were obtained from the Center for Vaccine Development, Mahidol University. DENVs were propagated in Vero cells and their titers were quantified using a plaque assay (Poole-Smith *et al*, 2015).

Human monocyte-derived dendritic cells (MoDCs)

MoDCs were prepared as previously described (Hunsawong *et al*, 2015). In brief, $CD14^+$ cells were purified from peripheral blood mononuclear cells before being differentiated into MoDCs using a differentiating medium (RPMI 1640; Gibco, Thermo Fisher Scientific, Waltham, MA) supplemented with 10% inactivated human AB serum, 2 mM L-glutamine, 1,000 U/ml GM-CSF (R&D Systems, Minneapolis, MN), 500 U/ml IL-4 (R&D Systems), 50 U/ml penicillin and 50 U/ml streptomycin). MoDCs phenotype were confirmed on day 7 by staining using anti-DC-SIGN antibodies (R&D Systems). The stained cells were analyzed using a flow cytometer (CytoFlex; Beckman Coulter, Indianapolis, IN). MoDCs obtained from the same six healthy donors were used throughout the study to minimize variations from lot to lot of host cells.

Infection of MoDCs

MoDcs ($5x10^6$ cells) were cultured as described above, then were washed twice with 1 × phosphate-buffered saline pH 7.4 (PBS) before being infected with virulent viruses or their attenuated counterparts at an MOI of 1.0 PFU/cell or 3.0 PFU/cell, respectively. Infected cultures were cultivated in RPMI 1640 medium supplemented with 10% heat-inactivated human serum, 50 U/ml penicillin and 50 U/ml streptomycin. Aliquots of culture medium were harvested every 24 hours for four constitutive days and stored at -80°C until used. Infected cells were harvested on day 2 post-inoculation and subjected to anti-DENV antibody staining. In brief, harvested cells were stained with anti-DENV antibody (HB114, ATCC, Manassas, VA) for 1 hour at 37°C, and washed twice with 1 × PBS. Cells that interact with anti-DENV antibodies were detected by fluorescent-labelled secondary antibody (Invitrogen, Carlsbad, CA) before being analyzed by flow cytometry as described above.

Cytokines detection

The harvested supernatants from infected MoDCs cultures were subjected to cytokine (IL-1β, IL-8, IL-10, TNF-α, and IFN-α) quantification using ELISA (Quantikine™; R&D System, Minneapolis, MN).

Statistical analysis

Student's *t*-test was used to compare differences in cytokine production among test and control groups. A p-value <0.05 is considered statistically significant.

Ethical considerations

The study protocols were approved by the Institutional Review Board, Mahidol University (MU-IRB 2012/005.2806).

RESULTS

Response of MoDCs to DENV-2 16681 and attenuated DENV-2 PDK53 infection

MoDC cultures were infected with

either DENV-2 16681 or DENV-2 PDK53 at MOI of 1.0 or 3.0 PFU/cell, respectively. Both DENV-2 16681 and PDK53 replicated at the same efficiency and yielded similar number of infected cells (Fig 1).

Aliquots of infected-MoDC culture media were harvested on days 1, 2 and 3, and the amounts of IL-1β, IL-8, IL-10, TNF-α, and IFN-α in the harvested media were quantified using ELISA. DENV-2 16681 was a strong inducer of IL-10 and TNF-α production but a weaker stimulator for that of IL-1β and IL-8, whereas attenuated DENV-2 PDK53 was a potent stimulator of IL-1β, and IL-8 production but a weaker inducer of IL-10 and TNF-α (Fig 2). Both virulent and attenuated DENV-2 strains stimulated high levels of type-I interferon.

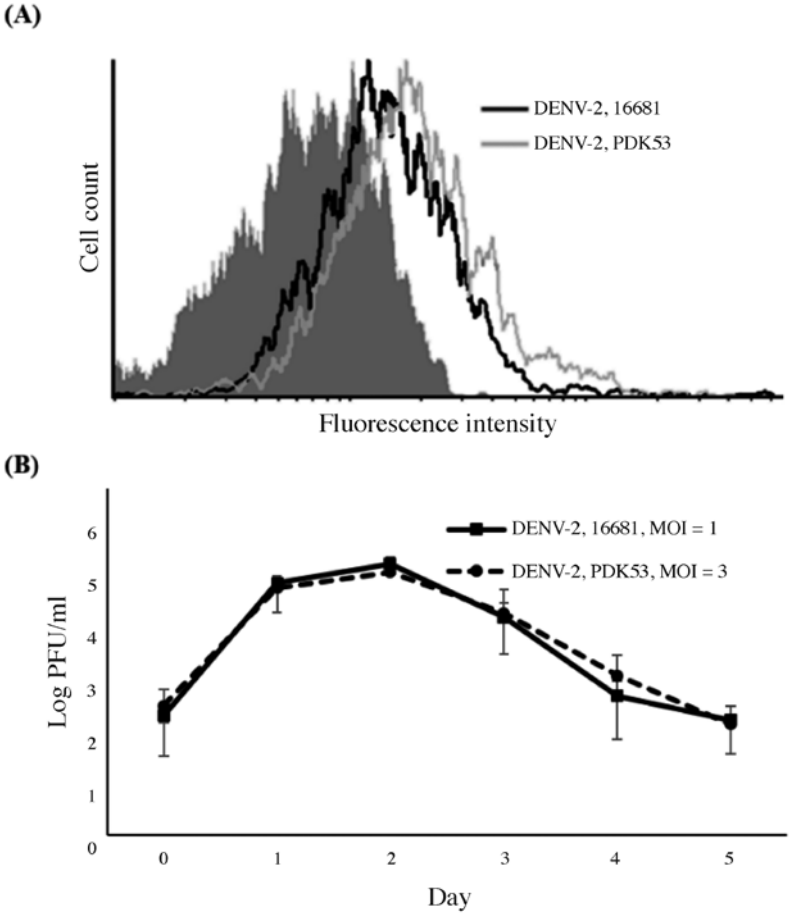

Fig 1-Replication efficiency of DENV-2 16681 and DENV-2 PDK53. Human monocyte derived dendritic cells (MoDCs) were infected with DENV-2 16681 or DENV-2 PDK53 at MOI of 1 or 3, respectively. Cell were harvested on day 2 of infection, fixed, permeabilized, and stained with anti-DENV-2 antibody. Numbers of DENV antigen-positive cells were quantified using flow cytometry. Aliquots of supernatant fluid were harvested every 24 hours for five consecutive days and subjected to virus quantification by plaque assay. A. Numbers of infected MoDCs on day 2 of infection. B. Replication kinetics of DENV-2 16681 and DENV-2 PDK53.

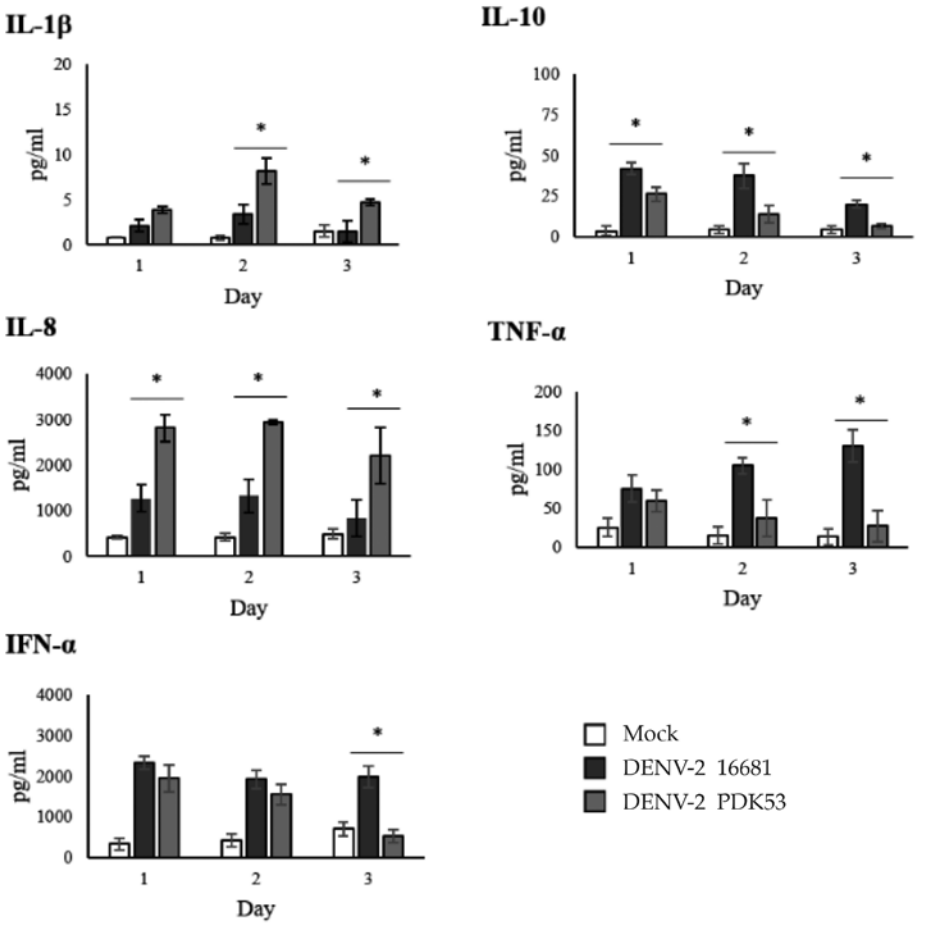

Fig 2-Levels of innate cytokines in response to DENV-2 16681 or DENV-2 PDK53 infection. Human monocyte-derived dendritic cells (MoDCs) were infected with DENV-2 16681 or DENV-2 PDK53 at MOI of 1.0 or 3.0, respectively. Supernatants were harvested every 24 hours post-infection for three consecutive days and subjected to ELISA assay for cytokine quantification. *Significant difference at p-value <0.05.

Response of MoDCs to DENV-3 16562 and attenuated but reactogenic DENV-3 PGMK30

A similar study was performed using DENV-3 16562 and attenuated but reactogenic DENV-3 PGMK30, with the same lots of MoDCs and MOIs infected with that yielded the same percent infected cells (31.6% and 32.5%, respectively). Replication of both DENV-3 strains DENV-3 peaked on day 2 post-infection, yielding virus titer of $3.5x10^4$ and $6.0x10^4$ PFU/ml for DENV-3 16562 and DENV-3 PGMK30, respectively.

DENV-3 PGMK30 was a stronger inducer of IL-1β and IL-8 production compared to DENV-3 16562 (Fig 3). In contrast to DENV-3 16562, a potent stimulator of IL-10 production, DENV-3 PGMK30 was much less able to upregulate IL-10 production compared to DENV-3 16562, but still significantly higher than that of mock-

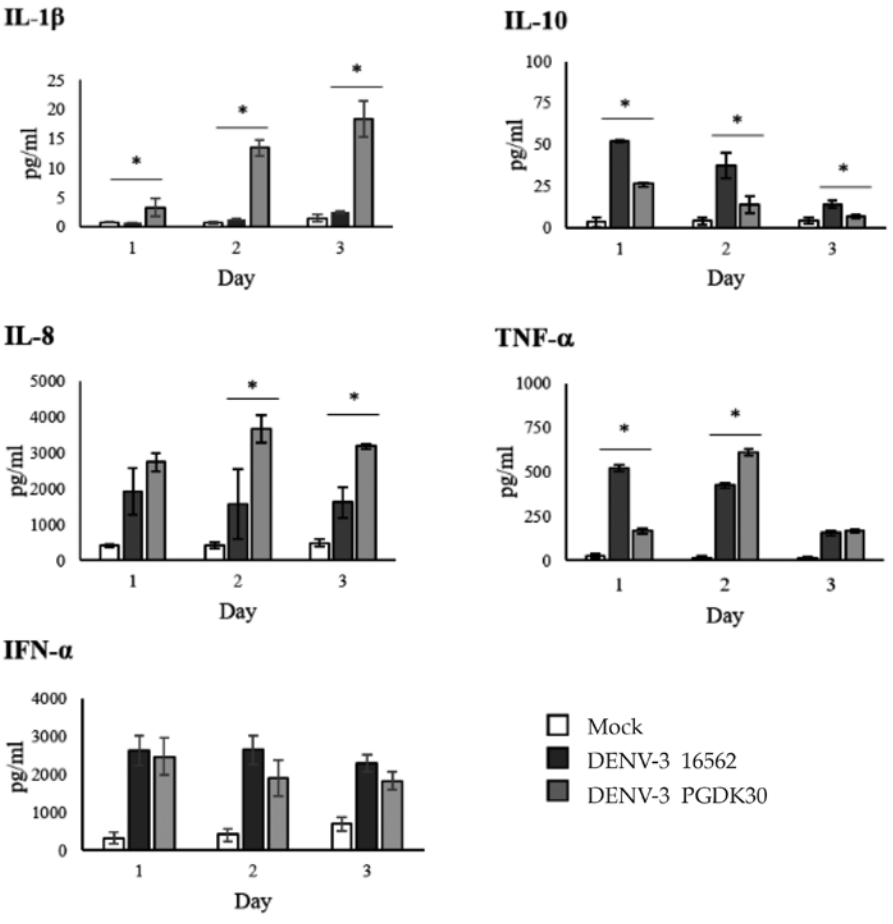

Fig 3-Levels of innate cytokines in response to DENV-3 16562 or DENV-3 PGMK30 infection. Human monocyte-derived dendritic cells (MoDCs) were infected with DENV-3 16562 or DENV-3 PGMK30 at MOI of 1.0 or 3.0, respectively. Supernatants were harvested every 24 hours post-infection for three consecutive days and subjected to ELISA assay for cytokine quantification. *Significant difference at p-value <0.05.

infected cultures. Interestingly, DENV-3 16562 and DENV-3 PGMK30 were strong inducers of TNF-α production. While DENV-3 16562 rapidly activated TNF-α production to the highest level (522 $\pm$ 16 pg/ml,) within 24 hours of infection, DENV-3 PGMK30-infected cultures produced TNF-α within 24 hours of infection and peaked (613 $\pm$ 17 pg/ml) on day 2. These data showed that, although DENV-3 PGMK30 is known to be the attenuated counterpart of DENV-3 16562, this variant still inherited the ability to induce TNF-α production to a similar level stimulated by the parental virulent virus.

DISCUSSION

In the present study, we reveal that DENV-infected MoDCs were able to differentiate between the attenuated variants from their parental virulent viruses

through, in part, the profile of innate cytokine responses. Upregulation of IL-8 and IL-1β production was strongly associated with infection by the attenuated DENV phenotypes while stimulation of IL-10 and TNF-α production with infection by the virulent parental viruses. Interestingly, the attenuated but reactogenic DENV-3 PGMK30 retains its capability to induce TNF-α production.

IL-8 is associated with pleural effusion and alteration of cytoskeleton and tight junction of microvascular endothelium (Pace *et al*, 1999; Talavera *et al*, 2004). Several reports showed high levels of circulating IL-8 in severe dengue and proposed IL-8 as a possible predictor of severe DENV infection (Pandey *et al*, 2015; Mehta *et al*, 2017). On the other hand, the protective role of IL-8 in infection is well documented. IL-8 induces migration of neutrophils into infected sites (Reeves *et al*, 2015) where they eliminate invading pathogens via various mechanisms, such as oxygen-dependent (α-defensins) and oxygen-independent (burst oxidation) pathways and neutrophil-extracellular traps (Vorobjeva and Pinegin, 2014). In addition, recruited neutrophils interact and crosstalk with various immune cells, such as monocytes/macrophages and NK cells (Scapini and Cassatella, 2014). As the attenuated DENV variants are stronger inducers of IL-8 production than their virulent parental viruses, this suggests that IL-8 produced from infected dendritic cells at the sites of mosquitoes bite may act differently from the circulating IL-8. In other words, at the site of infection, IL-8 from DENV-infected DCs may serve as protective rather than pathogenic chemokine.

IL-1β is another mediator that may determine severity of dengue as severe dengue patients have higher levels of circulating IL-1β than mild dengue patients (Bozza *et al*, 2008). High level of circulating IL-1β may contribute to vascular leakage through disruption of vascular junction by dissociation of VE-cad/β catenin complex resulting in decreased expression of adhesion molecules at the junction (Haidari *et al*, 2012). While acting as a pathogenic factor, IL-1β may well sub-serve in protection as it facilitates expansion and proliferation of antigen-specific $CD4^+$ and $CD8^+$ T cells, resulting in the migration of these effector cells to the periphery and maintenance of memory (Ben-Sasson *et al*, 2013a). In addition, IL-1β is reported to enhance vaccine potency (Ben-Sasson *et al*, 2013b). Here, we revealed that the attenuated DENV variants, but not the virulent parental viruses, are strong inducers of IL-1β production, and thus, it is possible that 1L-1β produced from DENV-infected DCs may be one of the protective markers during DENV infection.

Our present study shows an association of high levels of TNF-α and IL-10 with MoDC infection by virulent DENV phenotypes. TNF-α is the master regulator of vascular leakage in the liver and liver damage in mouse lacking type-I and -II interferon receptors (Phanthanawiboon *et al*, 2016). This may partly be mediated by the ability of TNF-α to induce endothelial barrier instability through reorganization of PECAM-1 and downregulation of a tight junction protein, occludin (Inyoo *et al*, 2017). IL-10 is another circulating mediator that is positively correlated with severe dengue, being elevated in fatal dengue and associated with plasma leakage and hepatic dysfunction in children in Brazil with dengue (Chen *et al*, 2006; Ferreira *et al*, 2015). IL-10 may also exert its pathogenic roles through immune suppression, such as by downregulating

expression of co-stimulatory molecules, MHC and other cell surface molecules, resulting in depressed antigen presentation and suppressed immune responses (Ng *et al*, 2013; Mittal and Roche, 2015). In addition, IL-10 induces antigen-specific anergy in $CD4^+$ T cells in a mouse model and has counter regulatory properties to a number of proinflammatory cytokines (Maris *et al*, 2007; Lee *et al*, 2016). These roles of IL-10 and TNF-α suggest the ability to induce IL-10 and TNF-α may be one of the virulent phenotypes of DENV-2 16681 and DENV-3 16562.

Significantly, we found that the attenuated but reactogenic DENV-3 PGMK30 retains its TNF-α-inducing capacity while clearly reducing its IL-10-stimulating characteristic. Three hypotheses for the reactogenicity of DENV-3 PGMK30 have been proposed. Firstly, DENV-3 PGMK30-based vaccine induces insufficient response to inhibit virus infection, which in turn contributes to reactogenicity (Balas *et al*, 2011). Secondly, adaptive immunity plays a role in DENV-3 PGMK30 reactogenicity (Sanchez *et al*, 2006). Thirdly, DENV serotype 3 is a particularly aggressive serotype (Halstead and Marchette, 2003). Thus, the vaccine derived from DENV-3 PGMK30 may have retained some virulence properties, such as the ability to activate mediators involved in dengue pathogenesis. The capability to stimulate TNF-α could be one of the virulent characteristics retained by DENV-3 PGMK30.

In summary, we present data demonstrating MoDCs served as a relevant *in vitro* human model for the study of virulence and attenuating markers produced during DENV infection. No appropriate animal model for dengue is yet available, and conflicting results on vaccine human trials and pathogenesis of dengue have been reported. The *in vitro* model presented in our study should provide a useful tool for further research on dengue vaccine development and disease pathogenesis.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Institute of Science and Technology Agency, Thailand (P-11-00016 to SY) and partly by the National Vaccine Institute, Thailand (2559.1/11 to SU). The authors thank the volunteers for donating the primary monocytes used in the study.

REFERENCES

Balas C, Kennel A, Deauvieau F, *et al*. Different innate signatures induced in human monocyte-derived dendritic cells by wild-type dengue 3 virus, attenuated but reactogenic dengue 3 vaccine virus, or attenuated nonreactogenic dengue 1-4 vaccine virus strains. *J Infect Dis* 2011;203:103-8.

Ben-Sasson SZ, Hogg A, Hu-Li J, *et al*. IL-1 enhances expansion, effector function, tissue localization, and memory response of antigen-specific CD8 T cells. *J Exp Med* 2013a;210:491-502.

Ben-Sasson SZ, Wang K, Cohen J, *et al*. IL-1beta strikingly enhances antigen-driven CD4 and CD8 T-cell responses. Cold Spring Harbor Symposia on Quantitative Biology. 2013b;78:117-24.

Bhamarapravati N, Yoksan S. Live attenuated tetravalent dengue vaccine. *Vaccine*. 2000;18:44-7.

Bhatt S, Gething PW, Brady OJ, *et al*. The global distribution and burden of dengue. *Nature* 2013;496:504-7.

Bozza FA, Cruz OG, Zagne SM, *et al*. Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity. *BMC Infect Dis* 2008;8:86-96.

Braga EL, Moura P, Pinto LM, *et al*. Detection

of circulant tumor necrosis factor-alpha, soluble tumor necrosis factor p75 and interferon-gamma in Brazilian patients with dengue fever and dengue hemorrhagic fever. *Mem Inst Oswaldo Cruz* 2001;96:229-32.

Chen LC, Lei HY, Liu CC, *et al*. Correlation of serum levels of macrophage migration inhibitory factor with disease severity and clinical outcome in dengue patients. *Am J Trop Med Hyg* 2006;74:142-7.

Cruz Hernández SI, Puerta-Guardo HN, Flores Aguilar H, *et al*. Primary dengue virus infections induce differential cytokine production in Mexican patients. *Mem Inst Oswaldo Cruz* 2016;111:161-7.

Ferreira RA, de Oliveira SA, Gandini M, *et al*. Circulating cytokines and chemokines associated with plasma leakage and hepatic dysfunction in Brazilian children with dengue fever. *Acta Trop* 2015;149:138-47.

Haidari M, Zhang W, Chen Z, *et al*. Atorvastatin preserves the integrity of endothelial adherens junctions by inhibiting vascular endothelial cadherin tyrosine phosphorylation. *Exp Cell Res* 2012;318:1673-84.

Halstead SB, Marchette NJ. Biologic properties of dengue viruses following serial passage in primary dog kidney cells: studies at the University of Hawaii. *Am J Trop Med Hyg* 2003;69(6 Suppl):5-11.

Her Z, Kam YW, Gan VC, *et al*. Severity of plasma leakage is associated with high levels of interferon γ-inducible protein 10, hepatocyte growth factor, matrix metalloproteinase 2 (MMP-2), and MMP-9 during dengue virus infection. *J Infect Dis* 2017;215:42-51.

Hober D, Poli L, Roblin B, *et al*. Serum levels of tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 beta) in dengue-infected patients. *Am J Trop Med Hyg* 1993;48:324-31.

Hunsawong T, Sunintaboon P, Warit S, *et al*. Immunogenic properties of a BCG adjuvanted chitosan nanoparticle-based dengue vaccine in human dendritic cells. *PLOS Negl Trop Dis* 2015;9:e0003958.

Innis BL. Dengue and dengue hemorrhagic fever. In: Porterfield JS, ed. Exotic viral infections. London: Chapman & Hall, 1995:103-46.

Inyoo S, Suttitheptumrong A, Pattanakitsakul SN. Synergistic effect of TNF-α and dengue virus infection on adhesion molecule reorganization in human endothelial cells. *Jpn J Infect Dis* 2017;70:186-91.

Kanesa-thasan N, Sun W, Kim-Ahn G, *et al*. Safety and immunogenicity of attenuated dengue virus vaccines (Aventis Pasteur) in human volunteers. *Vaccine* 2001;19:3179-88.

Kitchener S, Nissen M, Nasveld P, *et al*. Immunogenicity and safety of two live-attenuated tetravalent dengue vaccine formulations in healthy Australian adults. *Vaccine* 2006;24:1238-41.

Kurane I, Innis BL, Nimmannitya S, *et al*. Activation of T lymphocytes in dengue virus infections. High levels of soluble interleukin 2 receptor, soluble CD4, soluble CD8, interleukin 2, and interferon-gamma in sera of children with dengue. *J Clin Invest* 1991;88:1473-80.

Lee BR, Kwon BE, Hong EH, *et al*. Interleukin-10 attenuates tumor growth by inhibiting interleukin-6/signal transducer and activator of transcription 3 signaling in myeloid-derived suppressor cells. *Cancer Lett* 2016;381:156-64.

Libraty DH, Endy TP, Houng HS, *et al*. Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *J Infect Dis* 2002;185:1213-21.

Malavige GN, Ogg GS. Pathogenesis of vascular leak in dengue virus infection. *Immunology* 2017;151:261-9.

Maris CH, Chappell CP, Jacob J. Interleukin-10 plays an early role in generating virus-specific T cell anergy. *BMC Immunol* 2007;8:8-16.

Martina BE, Koraka P, Osterhaus AD, *et al*. Dengue virus pathogenesis: an integrated view. *Clin Microbiol Rev* 2009;22:564-81.

Mehta VK, Verma R, Garg RK, Malhotra HS, Sharma PK, Jain A. Study of interleukin-6 and interleukin-8 levels in patients with neurological manifestations of dengue. *J Postgrad Med* 2017;63:11-5.

Mittal SK, Roche PA. Suppression of antigen presentation by IL-10. *Curr Opin Immunol* 2015;34:22-7.

Ng TH, Britton GJ, Hill EV, Verhagen J, Burton BR, Wraith DC. Regulation of adaptive immunity; the role of interleukin-10. *Front Immunol* 2013;4:129-41.

Oliveira RA, Cordeiro MT, Moura PM, *et al*. Serum cytokine/chemokine profiles in patients with dengue fever (DF) and dengue hemorrhagic fever (FHD) by using protein array. *J Clin Virol* 2017;89:39-45.

Osorio JE, Wallace D, Stinchcomb DT. A recombinant, chimeric tetravalent dengue vaccine candidate based on a dengue virus serotype 2 backbone. *Expert Rev Vaccine*s 2016;15:497-508.

Pace E, Gjomarkaj M, Melis M, *et al*. Interleukin-8 induces lymphocyte chemotaxis into the pleural space. Role of pleural macrophages. *Am J Respir Crit Care Med* 1999;159(5 Pt 1):1592-99.

Pandey N1, Jain A, Garg RK, *et al*. Serum levels of IL-8, IFNγ, IL-10, and TGF β and their gene expression levels in severe and non-severe cases of dengue virus infection. *Arch Virol* 2015;160:1463-75.

Phanthanawiboon S, Limkittikul K, Sakai Y, Takakura N, Saijo M, Kurosu T. Acute systemic infection with dengue virus leads to vascular leakage and death through tumor necrosis factor-α and Tie2/angiopoietin signaling in mice lacking type I and II interferon receptors. *PLOS One* 2016;11:e0148564.

Poole-Smith BK, Hemme RR, Delorey M, *et al*. Comparison of vector competence of *Aedes mediovittatus* and *Aedes aegypti* for dengue virus: implications for dengue control in the Caribbean. *PLOS Negl Trop Dis* 2015;9:e0003462.

Priyadarshini D, Gadia RR, Tripathy A, *et al*. Clinical findings and pro-inflammatory cytokines in dengue patients in Western India: a facility-based study. *PLOS One* 2010;5:e8709.

Reeves EP, McCarthy C, McElvaney OJ, *et al*. Inhaled hypertonic saline for cystic fibrosis: reviewing the potential evidence for modulation of neutrophil signalling and function. *World J Crit Care Med* 2015;4:179-91.

Sanchez V, Gimenez S, Tomlinson B, *et al*. Innate and adaptive cellular immunity in flavivirus-naïve human recipients of a live-attenuated dengue serotype 3 vaccine produced in Vero cells (VDV3). *Vaccine* 2006;24:4914-26.

Scapini P, Cassatella MA. Social networking of human neutrophils within the immune system. *Blood* 2014;124:710-9.

Srikiatkhachorn A, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. *Semin Immunopathol* 2017;39:563-74.

Talavera D, Castillo AM, Dominguez MC, Gutierrez AE, Meza I. IL8 release, tight junction and cytoskeleton dynamic reorganization conducive to permeability increase are induced by dengue virus infection of microvascular endothelial monolayers. *J Gen Virol* 2004;85(Pt 7):1801-13.

Vorobjeva NV, Pinegin BV. Neutrophil extracellular traps: mechanisms of formation and role in health and disease. *Biochemistry* 2014;79:1286-96.