Dayarathna *et al.*
Journal of Biomedical Science (2024) 31:86
https://doi.org/10.1186/s12929-024-01077-8

NSTC 國家科學及技術委員會 National Science and Technology Council
The cost of publication in *Journal of Biomedical Science* is borne by the National Science and Technology Council (NSTC), Taiwan.

Journal of Biomedical Science

RESEARCH

Open Access

Dengue NS1 interaction with lipids alters its pathogenic effects on monocyte derived macrophages

Shashika Dayarathna[1], Bhagya Senadheera[1], Chandima Jeewandara[1], Madushika Dissanayake[1], Farha Bary[1], Graham S. Ogg[2] and Gathsaurie Neelika Malavige[1,2*]

Abstract

Background While dengue NS1 antigen has been shown to be associated with disease pathogenesis in some studies, it has not been linked in other studies, with the reasons remaining unclear. NS1 antigen levels in acute dengue are often associated with increased disease severity, but there has been a wide variation in results based on past dengue infection and infecting dengue virus (DENV) serotype. As NS1 engages with many host lipids, we hypothesize that the type of NS1-lipid interactions alters its pathogenicity.

Methods Primary human monocyte derived macrophages (MDMs) were co-cultured with NS1 alone or with HDL, LDL, LPS and/or platelet activating factor (PAF) from individuals with a history of past dengue fever (DF = 8) or dengue haemorrhagic fever (DHF = 8). IL-1β levels were measured in culture supernatants, and gene expression analysis carried out in MDMs. Monocyte subpopulations were assessed by flow cytometry. Hierarchical cluster analysis with Euclidean distance calculations were used to differentiate clusters. Differentially expressed variables were extracted and a classifier model was developed to differentiate between past DF and DHF.

Results Significantly higher levels of IL-1β were seen in culture supernatants when NS1 was co-cultured with LDL ($p = 0.01$, median = 45.69 pg/ml), but lower levels when NS1 was co-cultured with HDL ($p = 0.05$, median = 4.617 pg/ml). MDMs of those with past DHF produced higher levels of IL-1β when NS1 was co-cultured with PAF ($p = 0.02$). MDMs of individuals with past DHF, were significantly more likely to down-regulate *RPLP2* gene expression when macrophages were co-cultured with either PAF alone, or NS1 combined with PAF, or NS1 combined with LDL. When NS1 was co-cultured with PAF, HDL or LDL two clusters were detected based on *IL10* expression, but these did not differentiate those with past DF or DHF.

Conclusions As RPLP2 is important in DENV replication, regulating cellular stress responses and immune responses and IL-10 is associated with severe disease, it would be important to further explore how differential expression of RPLP2 and IL-10 could lead to disease pathogenesis based on NS1 and lipid interactions.

Keywords Dengue, NS1, Monocytes, Lipids, HDL, LDL, Platelet activating factor, Severe dengue, RPLP2

*Correspondence:
Gathsaurie Neelika Malavige
gathsaurie.malavige@ndm.ox.ac.uk
Full list of author information is available at the end of the article

Introduction

The dengue virus (DENV) is estimated to infect 390 million individuals annually, resulting in almost 100 million symptomatic/apparent dengue infections [9]. Although most individuals who are infected with the DENV develop inapparent or mild illness, a proportion develop severe disease manifestations due to plasma leakage, organ dysfunction and severe bleeding [30]. The reasons for occurrence of symptomatic and severe disease in some individuals remain incompletely understood. Many factors appear to be involved such as presence of host comorbidities (diabetes, cardiovascular disease and obesity), heterologous infections, the magnitude, breadth and the specificity of DENV specific antibody and T cell responses, the virulence of the infecting DENV strain, the extent viraemia and an altered dysfunctional immune responses [10, 16, 20, 43, 49].

The DENV infects many different cell types including monocytes, macrophages, hepatocytes and B cells causing disease pathogenesis [20, 30]. During infection, NS1 which is a non-structural protein, exists in intracellular form, membrane associated form and a secreted form (sNS1) [2]. In addition to the virus, experiments using recombinant sNS1 has shown to contribute to disease pathogenesis by disrupting the endothelial glycocalyx [7, 42], inducing cytokine production from immune cells [33], activating phospholipase A2 enzymes [47], by inducing MMP-9 expression and activation [37] and immune evasion by interfering with complement activation [6], based on in vitro experiments and in dengue mouse models. In vitro experiments using different endothelial cell lines have shown NS1 of different flaviviruses show varied tissue trophism. However, interestingly using in vitro experiments, both DENV and Zika NS1 were shown to equally induce endothelial glycocalyx degradation in umbilical vein endothelial cells, although Zika NS1 did not cause vascular leak in mice models [42], and does not cause vascular leak in humans. Studies in symptomatic mouse models using non-mouse adapted DENV-2 virus strains show that DENV-induced pathology was not ameliorated by passive transfer of NS1-specific antibodies or by prior vaccination with recombinant NS1 [28]. Furthermore, the secretion of NS1 varied in different DENV-2 strains and the amount of sNS1 did not associate with disease severity in mouse models [52]. Therefore, data from mouse models show that the pathogenicity of NS1 is likely to depend on the DENV strain used and varied according to the DENV serotype and strain.

It was recently shown that NS1 binds to HDL and with a lower affinity to LDL, and NS1-HDL complexes induced proinflammatory cytokine production from monocyte-derived macrophages in vitro [8]. Studies using a baculovirus expression system to express NS1 in insect cell lines showed that sNS1 circulates as a hexamer, with a lipid core, whereas the lipid free recombinant NS1 existed in both dimeric and hexameric forms in solution [1]. More recent studies showed that sNS1 derived from DENV infected Vero cells, circulates predominantly as a dimer associated with HDL [15]. Similarly, it was shown that the s NS1 of Zika virus derived from infected mammalian cells also bound to HDL [14]. During the course of the illness, the apolipoproteins that associate with NS1 have shown to change, with ApoAI and ApoB interacting with NS1 during acute illness but replaced by ApoE towards the recovery phase of illness [8]. Therefore, the lipid composition of the NS1 hexamer/tetramer or lipids that interact with NS1, could also contribute to the virulence of NS1 and potentially help explain some of the variability in previous studies [53]. Given the timing of vascular leakage that occurs in acute dengue and due to the varied data on NS1 antigen levels and disease severity in acute dengue, it is possible that the NS1 interactions with lipid could alter its pathogenicity, during the course of illness.

Data from patients with acute dengue, suggests the relative contribution of sNS1 in causing disease pathogenesis and vascular leak is not clear. Studies done by Libraty et al. and others have shown while high NS1 antigen levels associate with severe dengue, not all individuals with high levels develop severe disease [29, 35]. Furthermore, lower NS1 levels are reported in acute DENV-2 infection, which is associated with increased risk of severe disease [17]. In addition, circulating NS1 levels are higher in primary dengue, which is usually associated with a lower risk of severe dengue, whereas NS1 is cleared early in secondary dengue, as it is bound to NS1 specific antibodies [17, 50]. Plasma leakage in patients with acute illness usually occurs between day 3 to 6 of illness when the NS1 levels decline [30]. As the rise of NS1 antibodies coincides with development of plasma leakage in patients with severe dengue, it is possible that NS1-antibody complexes also contribute to disease pathogenesis [22].

We have shown that various inflammatory lipid mediators such as platelet activating factor (PAF), leukotrienes, phospholipase A2 enzymes and prostaglandin metabolites are elevated in patients with acute dengue [19, 23, 24, 46]. In mouse models, DENV infection was shown to induce intestinal inflammation, leading to gut barrier dysfunction [40] also observed in patients with acute dengue [13, 45, 51]. HDL is known to bind to LPS, and dysfunctional HDL (HDL with altered surface proteins and lipid cargo) was associated with severe disease outcomes in sepsis [34, 48]. [25]. Given the timing of the vascular leak in relation to circulating NS1 and the variability of data on NS1 and disease pathogenesis, we

hypothesize that the pathogenicity of NS1 varies based on NS1 interactions with lipids. Therefore, to investigate if the pathogenicity of NS1 is altered when it interacts with different lipid mediators, we studied their effects on primary monocyte derived macrophages in individuals with a past history of mild or asymptomatic dengue and DHF.

Methodology

Culture of monocytes with different lipid mediators

Primary human monocytes were isolated from peripheral blood mononucleocytes (PBMCs) from 16 healthy individuals with past inapparent dengue/DF ($n=8$) or DHF ($n=8$). Those with a past history of DHF were who were hospitalized with an acute dengue infection and who were classified as having DHF based on the WHO 2011 guidelines [54]. Of those who had past DF/inapparent dengue, 3 were hospitalized due to a dengue infection, but had no evidence of DHF, one was treated as an outpatient and 4 had an inapparent dengue infection (Additional file 2: Table S1). The four individuals who had an inapparent dengue infection, were found to have neutralizing antibodies to several DENV serotypes by the FRNT assay, suggestive of past multi-typic dengue infections.

Monocyte isolation was carried out using CD14 magnetic beads (Miltenyi Biotech, Germany) and cultured in 96 well cell culture plates (0.125×10^6 cells in 250 µl media per well), for 7 days with 5% CO_2 at 37 °C to convert into macrophages. The resting media contained RPMI-1640 (Thermofisher Scientific, USA), 2 mM L-glutamine (Thermofisher Scientific, USA), 1% penicillin-streptomycin (Thermofisher Scientific, USA), 10 mM Na Pyruvate (Thermofisher Scientific, USA), 10 mM HEPES (Thermofisher Scientific, USA), 1% MEM vitamins (Thermofisher Scientific, USA), 1% NEAA (Thermofisher Scientific, USA), 50 µM beta-mercaptoethanol (Thermofisher Scientific, USA), and 15% AB-ve human serum (Sigma-Aldrich, USA). After 7 days, resting media was removed, and the monocyte derived macrophages co-cultured with DENV-1-NS1 antigen (HEK293) (NativeAntigen, USA) (2 µg/ml), LPS (100ng/ml, Invivogen, USA, Cat: tlrl-eblps), PAF (500ng/ml, Sigma Aldrich, USA, Cat: 511075), HDL (2.5 µg/ml, Sigma Aldrich, USA, Cat: L1567) and LDL (20 µg/ml, Sigma Aldrich, USA, Cat: L8292) alone or with NS1 in combination with the different lipid mediators. For all experiments investigating the effect of NS1 and different lipids on monocyte derived macrophages, FBS was used instead of human serum similar to experiments done by Benfrid et al. [8], as human serum contains varying levels of HDL. Macrophages co-cultured with media alone (5% FBS instead) was considered as the controls. The plates were rocked at every 15 min for 90 min for homogenous distribution of media and rested for 24 h at 37 °C with 5% CO_2 after which the culture supernatants and cells were harvested and RNA extracted. IL1-β levels were measured in culture supernatants using a quantitative ELISA (R&D systems, USA, Cat no: DY201) according to manufacturer's instructions.

RNA extraction and quantitative real time PCR

RNA was extracted using Geneaid rSYNC RNA Isolation kit (Geneaid Biotech Ltd, Taiwan, Cat No: RDHF100) and converted into cDNA using high-capacity cDNA Reverse Transcription kit with RNase inhibitor (Thermofisher Scientific, USA). Quantitative real-time PCR was carried out in triplicate for the relative expression of GAPDH, IL-10, NLRP3, IFN-β1, RIGI, RPLP2 and SMPD1 (Thermofisher Scientific, USA) using Invitrogen Superscript III Platinum master mix kit reagents (Thermofisher Scientific, USA) in ABI 7500 (Applied Biosystems, USA). ΔCt values were calculated for each gene for each test condition. ΔΔCt values for each gene was calculated separately by subtracting the endogenous GAPDH ΔCt values of each test condition followed by Fold change (FC) calculation for each gene for different test conditions. Due to the limited availability of sample volume, Gene expression experiments were performed for 12/16 individuals (DF = 6, DHF = 6).

The six genes were selected on the basis of being associated with severe clinical disease and also because of their requirement in DENV replication. Several studies have shown that IL-10 was associated with severe disease [16, 38], while some others have shown that NLPR3 was associated with disease pathogenesis [27, 44]. RIGI activation and IFNB1 are crucial for activation of innate antiviral immune pathways in DENV [30]. RPLP2 is required for DENV replication [12]. High sphingomyelin levels along with low SMPD1 expression have been observed during WNV infection in-vivo, and the role of this enzyme has not been studied in dengue [32].

Phenotyping of monocytes

To characterize the monocyte populations, flowcytometry was carried out in whole blood of the 12 individuals in whom we performed gene expression analysis (DHF = 6, DF = 6). Briefly, red blood cell (RBC) lysis was carried out using RBC lysis buffer (BD Biosciences, USA) according to manufacturer's protocol. Cells were stained with Live/Dead Fixable aqua (Invitrogen, USA), CD14-FITC (BD Biosciences, USA), CD16-Pacific blue (BD Biosciences, USA) and acquired through FACSAria III (BD Biosciences, USA). The results were analyzed in FlowJo version 10.8.1 (BD Biosciences, USA) and the gating strategy for different populations of monocytes is shown in Additional file 1: Fig. S1.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 10 and (Dotmatics, USA), and R software. Mann–Whitney *U* test was performed when comparing two unpaired samples, and the Wilcoxon test was used to compare paired samples. Hierarchical cluster analysis with Euclidean distance calculations were used to differentiate clusters under different culture conditions of NS1 with different lipid mediators. Clustering and visualization of heatmaps for gene expression under different culture conditions were performed with R V4.3.2. Differentially expressed variables were extracted based on two parameters (> 0.1 FC and < 0.1 p-value) and a volcano plot was constructed. The conditions which satisfy both the above parameters were identified as potential features in differentiating DF from DHF and the results were visualized in a scatter plot. Based on the extracted features, a classifier model was developed to differentiate the biological groups with Random Forest decision tree (10-fold cross validation) [11]. Accuracy of the model was validated internally and externally (Confusion Matrix and Cohen Kappa).

Results

IL-1β levels in culture supernatants with NS1 and different lipid mediators

Significantly higher levels of IL-1β were seen in primary human monocyte derived macrophages (MDMs) culture supernatants when NS1 was co-cultured with LDL ($p=0.01$), while lower IL-1β were detected when NS1 was co-cultured with HDL ($p=0.05$) and PAF ($p=0.82$) than with NS1 alone which appeared to have a suppressive effect (Fig. 1a). The median IL-1β levels of the culture supernatants when MDMs were co-cultured with NS1 alone (8.219 pg/ml) was higher ($p=0.05$) compared when MDMs were co-cultured with NS1 + HDL (4.617 pg/ml). In contrast, when MDMs were co-cultured with NS1 + LDL, the IL-1β levels (45.69 pg/ml) were significantly higher ($p=0.01$) than in MDMs co-cultured with NS1 alone (8.219 pg/ml). The IL-1β levels in media alone, with NS1, LPS and NS1 and LPS were similar to the levels observed by Benfrid et al. [8]. Interestingly, when monocyte derived macrophages were co-cultured with PAF and NS1 they produced a significantly higher IL-1β level than when co-cultured with PAF alone ($p<0.0001$). Similarly, the macrophages produced significantly higher levels of IL-1β ($p<0.0001$) when NS1 was co-cultured with LDL than with LDL alone. In contrast, IL-1β levels were significantly lowered when NS1 was co-cultured with HDL compared to HDL ($p=0.03$) (Fig. 1a).

Previously we found that monocyte-derived macrophages of those with a past history DHF produced higher levels of cytokines and higher viral loads when infected with different DENV serotypes than in those with past DF [26]. Therefore, we sought to investigate if similar changes were seen when monocyte derived macrophages from individuals with past DF and DHF produced different levels of IL-1β under different conditions. We found that although those with past DHF produced a trend towards more IL-1β when NS1 was co-cultured with LPS and LDL, but differences were only significant when NS1 was co-cultured with PAF ($p=0.02$) (Fig. 1b).

Differential gene expression of monocyte-derived macrophages with NS1 co-cultured with different lipid mediators

We carried out hierarchical cluster analysis to identify differential expression of *IL-10*, *NLRP3*, *IFN-β1*, *RIGI*, *RPLP2* and *SMPD1* when NS1 was co-cultured with different lipid mediators. Accordingly, we only identified two main clusters based on the expression of *IL-10* (Fig. 2). In cluster 1, high *IL-10* expression was seen when monocyte derived macrophages were co-cultured with PAF alone, NS1 + PAF, NS1, LDL, HDL and NS1 + HDL. Based on the overall expression of other genes, no significant differences were found. The two main clusters identified did not distinguish between those with past DF or DHF.

As different monocyte subsets have shown to produce different cytokines in response to the DENV and other stimuli, we sought to investigate if those who had higher levels of *IL-10* expression compared to those with lower expression levels had a different composition of monocytes. To identify different monocyte subsets, PBMCs in those gene expression analysis was carried out were gated as follows: $CD14^{high}$ $CD16^{low}$, $CD14^{high}$ $CD16^{high}$, $CD14^{dim}$ $CD16^{low}$ and $CD14^{dim}$ $CD16^{high}$. The predominant monocyte subpopulation was the classical monocytes ($CD14^{+}CD16^{-}$), followed by non-classical monocytes ($CD14^{dim}CD16^{+}$), and intermediate monocytes ($CD14^{+}CD16^{+}$). We did not observe any significant differences in the proportion of different monocyte subpopulations in those who had high expression of *IL-10* (cluster 1) compared to those with lower *IL-10* expression (cluster 2).

Differential gene expression in those with past DF and DHF when NS1 was co-cultured with different lipid mediators

We then proceeded to differential gene expression in those with past DF and DHF and constructed a volcano plot to identify significant differences between these two groups (Fig. 3a). We identified significant differences in expression of *RPLP2*, when monocyte-derived macrophages of those with past DF or DHF were co-cultured with PAF alone, PAF + NS1 or NS1 + LDL.

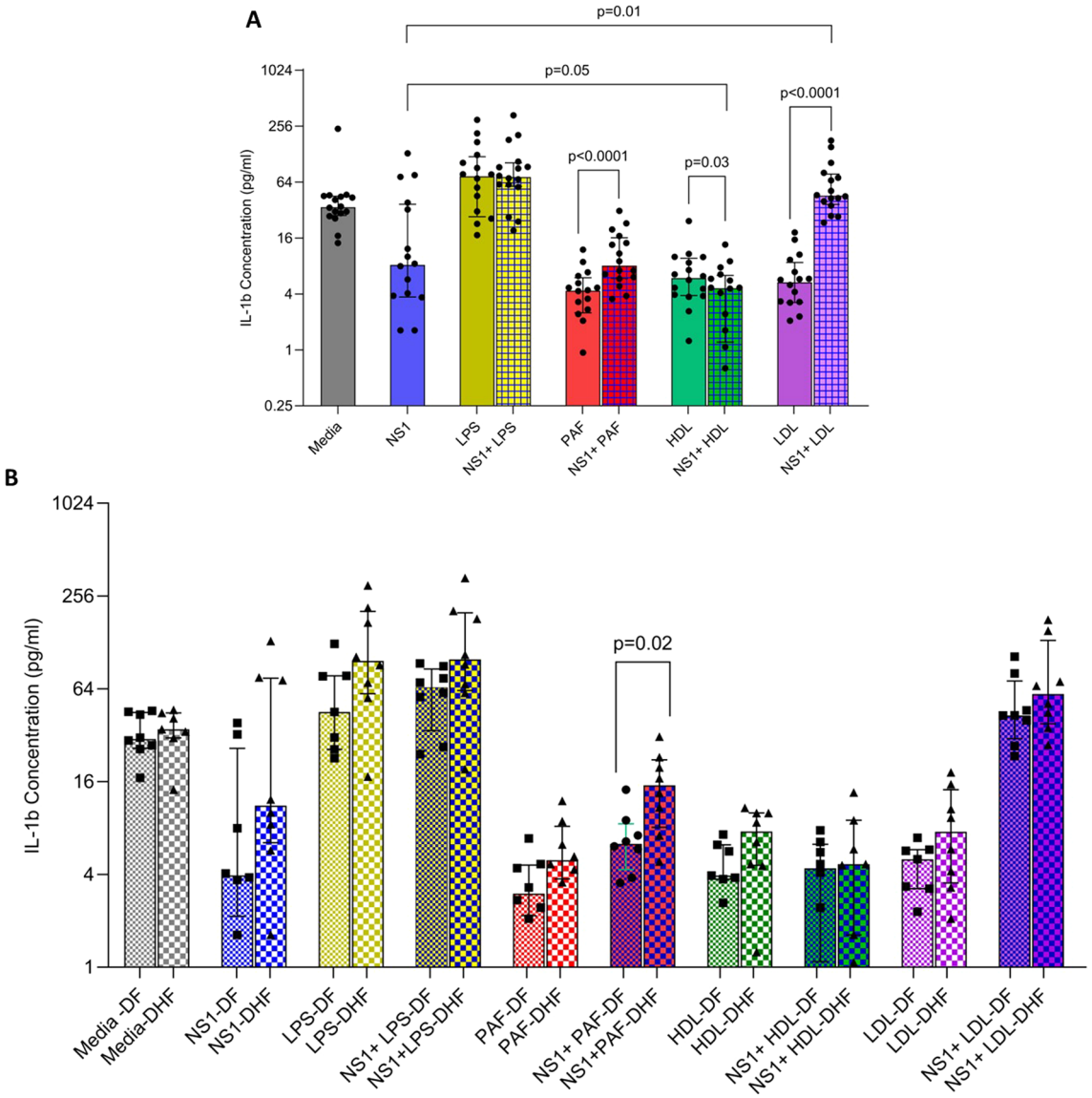

Fig. 1 IL-1β levels in culture supernatant of primary human monocyte derived macrophages (MDMs) co-cultured with NS1 and different lipid mediators. MDMs from individuals with a history of DF ($n=8$) and DHF ($n=8$) were co-cultured with NS1 alone, different lipid mediators alone or with NS1 and different lipid mediators and the IL-1β levels in the supernatants were measured by ELISA after 24 h. The Wilcoxon matched pairs signed rank test was used for comparison of IL-1 β levels in culture supernatants in the same individual under different conditions (**a**). IL-1β levels in culture supernatant in those with past DF vs. DHF, under different culture conditions were compared using Mann-Whitney U test for unpaired groups (**b**). The lines indicate the median and IQR

The differences in expression levels of *RPLP2* under these three culture conditions were then used to generate a 3D scatter plot. Furthermore, with the three features, the classifier model was created using 50% of the dataset and was found to predict the differences in the two biological groups (past DF and DHF) with 100% accuracy using our dataset. To avoid the bias by using our internal dataset, we then used the remaining 50% of the dataset as the external validation dataset, which gave the accuracy of the model as 68%. The generated 3D scatter plot and heatmaps using this model clearly distinguished those with past DF and DHF, except for one individual with past DHF who was also classified under the past DF group (Fig. 3b, c).

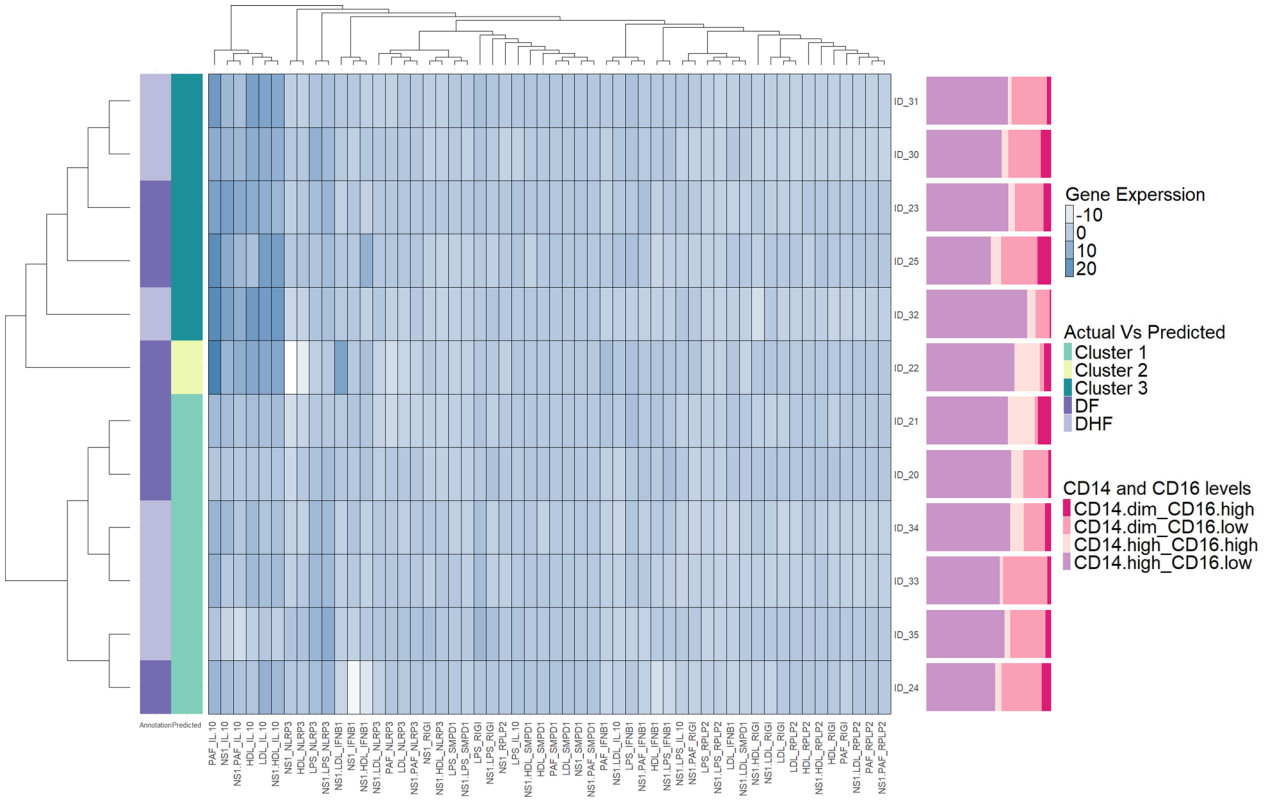

Fig. 2 Patterns of expression of different genes in monocyte derived macrophages (MDMs) when co-cultured with NS1 and different lipid mediators. A heat-map was generated for expression of *RIGI*, *IL-10*, *NLRP3*, *IFN-β1*, *SMPD1*, *RPLP2*, when MDMs were co-cultured from individuals with a history of DF ($n=6$) and DHF ($n=6$) with NS1 alone, different lipid mediators alone or with NS1 and different lipid mediators. Hierarchical cluster analysis with Euclidean distance calculations were used to differentiate clusters under different culture conditions of NS1 with different lipid mediators. The color intensity corresponds to the Z-score that indicates the number of standard deviations by which the normalized raw value was below or above. The differences of monocyte subpopulations identified of each individual is indicated in the Y axis

Discussion

In this study we show that primary monocyte-derived macrophages produced significantly higher levels of IL-1β when NS1 was co-cultured with LDL, but not HDL. In fact, IL-1β levels in culture supernatants were lower when the macrophages were co-cultured with HDL and NS1, rather than HDL alone. These observations are contrary to those made by Benfrid et al., where they saw significantly higher levels of proinflammatory cytokines when macrophages were co-cultured with NS1 and HDL [8]. These differences could be attributed to the differences in NS1 concentrations (NS1 concertation being 10 μg/ml vs. our concentrations of 2 μg/ml) used and the differences in the sources of HDL and LDL used in our study. For instance, Libraty et al. reported NS1 antigen levels of ≥600 ng/ml (0.6 μg/ml) were associated with progression to DHF [29], Allonso et al. reported a median NS1 antigen concentrations varying from 22.6 to 36.8 ng/ml based on day of illness [4], Nunes et al. reported mean levels of 4.72 and 4.92 ng/ml in those with primary and secondary dengue and Alcon-LePoder et al. reported plasma levels between 10 and 100 ng/ml [3, 36]. Although the NS1 concentrations used here was also higher than levels found in patients with acute dengue, we wished to use values that are closer to those reported in those with severe dengue [29]. Furthermore, we used NS1 of DENV1 derived from mammalian HEK293 cells, while Benfrid et al. used NS1 of DENV2, expressed recombinant in Drosophila S2 cells [50]. DENV NS1 antigen levels have shown to vary in different DENV serotypes in patients with acute dengue. Therefore, the differences between NS1 used and the concentrations used in the experiments could account for the differences in results.

Previously we showed that primary human monocyte responses to all four serotypes of the DENV varied based on past dengue infection, with those with past DHF producing higher viraemia and proinflammatory cytokines in culture supernatants [26]. In this study, we observed that macrophages in individuals with past DHF produced significantly higher levels of IL-β, when they were co-cultured with NS1 and PAF. Furthermore, using volcano plots when we evaluated differential gene expression of

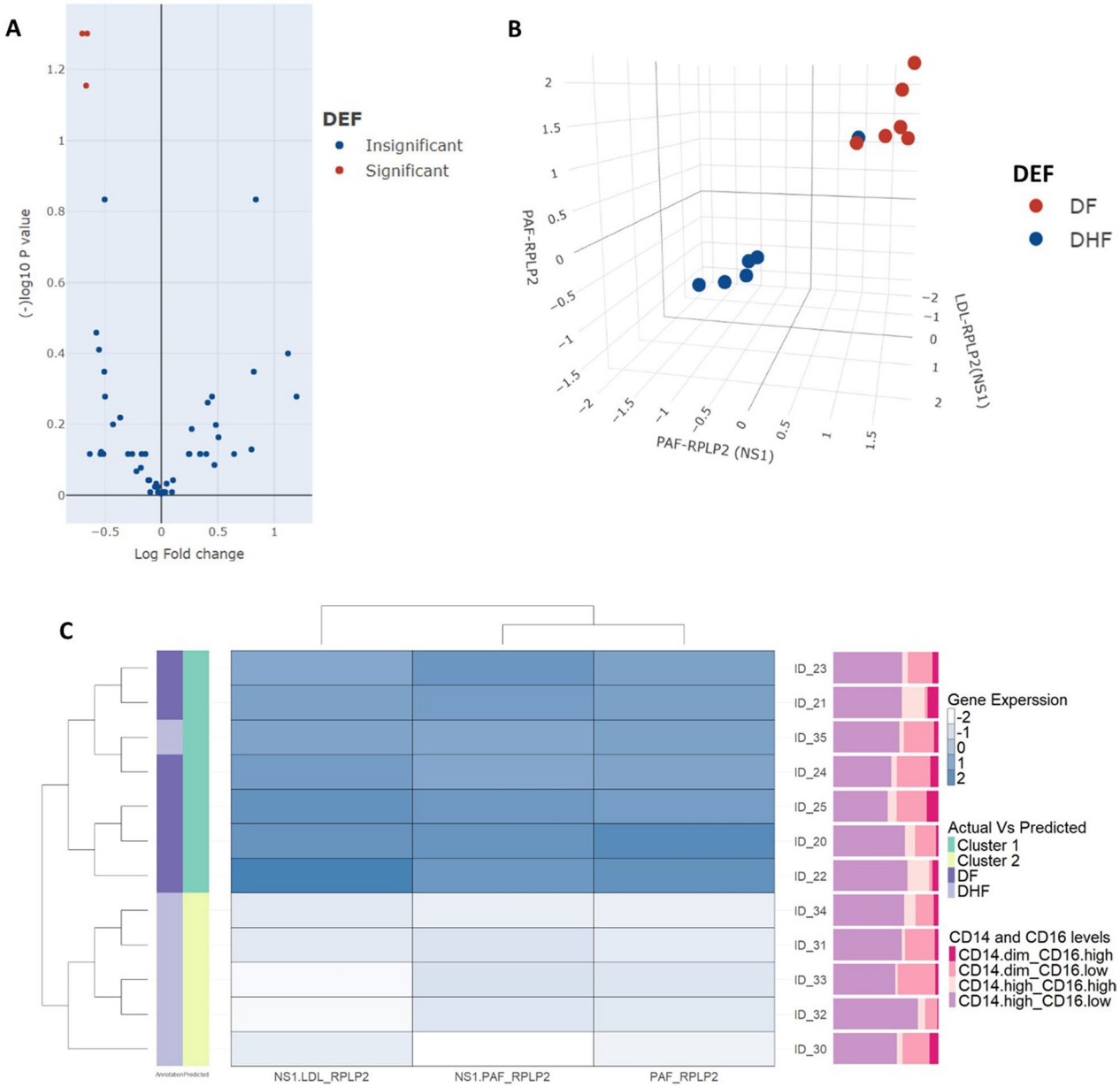

Fig. 3 Differences in gene expression patterns in monocyte derived macrophages (MDMs) when co-cultured with NS1 and different lipid mediators in those with past DF and DHF. A volcano plot was generated to identify significant differences in gene expression in MDMs in those with past DF ($n=6$) and past DHF ($n=6$), with NS1 alone, different lipid mediators alone or with NS1 and different lipid mediators (**a**). Significant differences in *RPLP2* gene expression were observed when MDM2s of those with past DHF were co-cultured with PAF, NS1 and PAF and NS1 and LDL, which are also displayed in a 3D plot view with individuals with DHF (blue) and DF (red) (**b**). A heatmap was generated for differential expression of *RPLP2* identified by the volcano plot in those with past DF and DHF. The differences in monocyte subpopulations identified of each individual is indicated in the Y axis (**c**)

macrophages in those with past DF and DHF, we found that macrophages of individuals with past DHF, were significantly more likely to down-regulate *RPLP2* expression when co-cultured with PAF, NS1 and PAF and NS1 and LDL. The *RPLP2* gene encodes for 60 S subunit of the ribosomes and is responsible for the elongation step of protein biosynthesis. RPLP2 has numerous functions including regulating glycolysis, cell proliferation, regulation of the MAPK1/ERK2 signaling pathway, regulation of generation of reactive oxygen species (ROS), endoplasmic reticulum stress and also binds to TLR4 [5, 21, 55]. Furthermore, RPLP2 was shown to be required for many

flaviviruses such as dengue, yellow fever and zika virus [12]. DENV replication results in accumulation of ROS and proinflammatory responses [18]. The unfolded protein response, which is induced as a result of endoplasmic reticulum stress in altered in DENV infection, resulting in altered autophagy and cellular immune responses [39]. High levels of expression of RPLP2 reduced accumulation of ROS and reduced ER stress and regulates the unfolded protein response [5, 21]. Therefore, it is interesting to note that *RPLP2* gene expression was significantly downregulated in macrophages of individuals with past DHF, when co-cultured with PAF, NS1 and PAF or NS1 and LDL. These differences observed in response to NS1 and different lipids in those with varying severity of past dengue, could be due to certain genetic or epigenetic changes in monocytes/macrophages of such individuals, which predispose them to have an altered response to NS1 and the DENV.

Although elevated IL-10 is associated with severe disease in many viral infections including dengue [31, 41], compared to illnesses such as severe COVID-19, the IL-10 levels in those who have DHF are several folds higher [16]. PAF, NS1 and PAF, HDL and NS1 and LDL and NS1 combinations led to a significant upregulation of *IL-10* gene expression in one cluster of individuals, while the other cluster showed no upregulation or down regulation. These two clusters, which differed on *IL-10* expression levels did not associate with past dengue disease severity. As different monocyte subsets produce different cytokine profiles to inflammatory stimuli, we investigated the different monocyte subpopulations in those who had high *IL-10* expression vs. normal expression levels and compared monocyte subpopulations in those with past DF and DHF. We did not find any difference in the monocyte subpopulation proportions in those who had high *IL-10* expression compared to no change in *IL-10* expression when co-cultured with NS1 and other lipids. Therefore, rather than differences in monocyte subpopulations, other mechanisms that lead to increased *IL-10* gene transcription could be responsible for these differences observed between the two clusters.

Conclusions

In summary, to delineate if NS1 and lipid interactions alter the pathogenicity of NS1, we investigated their effects on primary human monocyte derived macrophages. We found that NS1 and LDL led to increased production of IL-1β. However, significantly lower expression of *RPLP2* was observed in the macrophages of those with past DHF when co-cultured with PAF, PAF and NS1 and NS1 and LDL. As RPLP2 is important in DENV replication and in regulating cellular stress responses and immune responses, it is important to further explore how differential expression of *RPLP2* could lead to disease pathogenesis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12929-024-01077-8.**Acknowledgements**
None.

Author contributions
Conceptualization: GNM, GSO. Experiments and investigations: SD, MD, FB. Data analysis: BS, SD, MD. Project administration and supervision: GNM, CJ. Funding acquisition: GNM, CJ, GSO. Writing the manuscript: GNM, SD. Reviewing the manuscript: GNM, GSO, CJ, BS.

Funding
We are grateful to the NIH, USA (Grant No. 5U01AI151788-02), Accelerating Higher Education Expansion and Development (AHEAD) Operation of the Ministry of Higher Education funded by the World Bank, and the UK Medical Research Council.

Availability of data and materials
All data is available within the manuscript and the supporting files.

Declarations

Ethics approval and consent to participate
Ethics approval was obtained from the Ethics Review Committee of the University of Sri Jayewardenepura. All participants gave informed written consent.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Allergy Immunology and Cell Biology Unit, Department of Immunology and Molecular Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka. [2]MRC Translational Immune Discovery Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK.

Received: 27 May 2024 Accepted: 21 August 2024
Published online: 04 September 2024

References

1. Akey DL, Brown WC, Dutta S, Konwerski J, Jose J, Jurkiw TJ, DelProposto J, Ogata CM, Skiniotis G, Kuhn RJ, Smith JL. Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system. Science. 2014;343(6173):881–5.
2. Alcon-LePoder S, Drouet MT, Roux P, Frenkiel MP, Arborio M, Durand-Schneider AM, Maurice M, Le Blanc I, Gruenberg J, Flamand M. The secreted form of dengue virus nonstructural protein NS1 is endocytosed by hepatocytes and accumulates in late endosomes: implications for viral infectivity. J Virol. 2005;79(17):11403–11.
3. Alcon-LePoder S, Sivard P, Drouet MT, Talarmin A, Rice C, Flamand M. Secretion of flaviviral non-structural protein NS1: from diagnosis to pathogenesis. Novartis Found Symp. 2006;277:233–47 discussion 247–253.
4. Allonso D, Meneses MD, Fernandes CA, Ferreira DF, Mohana-Borges R. Assessing positivity and circulating levels of NS1 in samples from a 2012 dengue outbreak in Rio De Janeiro, Brazil. PLoS ONE. 2014;9(11):e113634.
5. Artero-Castro A, Perez-Alea M, Feliciano A, Leal JA, Genestar M, Castellvi J, Peg V, Ramon YCS, Lleonart ME. Disruption of the ribosomal P complex leads to stress-induced autophagy. Autophagy. 2015;11(9):1499–519.

6. Avirutnan P, Fuchs A, Hauhart RE, Somnuke P, Youn S, Diamond MS, Atkinson JP. Antagonism of the complement component C4 by flavivirus nonstructural protein NS1. J Exp Med. 2010;207(4):793–806.
7. Beatty PR, Puerta-Guardo H, Killingbeck SS, Glasner DR, Hopkins K, Harris E. Dengue virus NS1 triggers endothelial permeability and vascular leak that is prevented by NS1 vaccination. Sci Transl Med. 2015;7(304):304ra141.
8. Benfrid S, Park KH, Dellarole M, Voss JE, Tamietti C, Pehau-Arnaudet G, Raynal B, Brule S, England P, Zhang X, Mikhailova A, Hasan M, Ungeheuer MN, Petres S, Biering SB, Harris E, Sakuntabhai A, Buchy P, Duong V, Dussart P, Coulibaly F, Bontems F, Rey FA, Flamand M. Dengue virus NS1 protein conveys pro-inflammatory signals by docking onto high-density lipoproteins. EMBO Rep. 2022;23(7):e53600.
9. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SI. The global distribution and burden of dengue. Nature. 2013;496(7446):504–7.
10. Bos S, Graber AL, Cardona-Ospina JA, Duarte EM, Zambrana JV, Ruiz Salinas JA, Mercado-Hernandez R, Singh T, Katzelnick LC, de Silva A, Kuan G, Balmaseda A, Harris E. Protection against symptomatic dengue infection by neutralizing antibodies varies by infection history and infecting serotype. Nat Commun. 2024;15(1):382.
11. Breiman L. Random forests. Mach Learn. 2001;45:5–32.
12. Campos RK, Wijeratne HRS, Shah P, Garcia-Blanco MA, Bradrick SS. Ribosomal stalk proteins RPLP1 and RPLP2 promote biogenesis of flaviviral and cellular multi-pass transmembrane proteins. Nucleic Acids Res. 2020;48(17):9872–85.
13. Chancharoenthana W, Leelahavanichkul A, Ariyanon W, Vadcharavivad S, Phatcharophaswattanakul S, Kamolratanakul S, Leaungwutiwong P, Phumratanaprapin W, Wilairatana P. Leaky gut syndrome is associated with endotoxemia and serum (1–>3)-beta-D-glucan in severe dengue infection. Microorganisms. 2021;9:11.
14. Chew BLA, Ngoh AQ, Phoo WW, Weng MJG, Sheng HJ, Chan KWK, Tan EYJ, Gelbart T, Xu C, Tan GS, Vasudevan SG, Luo D. Structural basis of Zika virus NS1 multimerization and human antibody recognition. npj Viruses. 2024;2(1):14.
15. Chew BLA, Ngoh AQ, Phoo WW, Chan WKK, Ser Z, Lim SS, Weng MJG, Watanabe S, Choy MM, Low JG, Ooi EE, Sobota RM, Vasudevan SG, Luo D. Secreted dengue virus NS1 is predominantly dimeric and in complex with high-density lipoprotein. eLife. 2022;12:RP90762.
16. Dayarathna S, Jeewandara C, Gomes L, Somathilaka G, Jayathilaka D, Vimalachandran V, Wijewickrama A, Narangoda E, Idampitiya D, Ogg GS, Malavige GN. Similarities and differences between the 'cytokine storms' in acute dengue and COVID-19. Sci Rep. 2020;10(1):19839.
17. Duyen HT, Ngoc TV, Ha do T, Hang VT, Kieu NT, Young PR, Farrar JJ, Simmons CP, Wolbers M, Wills BA. Kinetics of plasma viremia and soluble nonstructural protein 1 concentrations in dengue: differential effects according to serotype and immune status. J Infect Dis. 2011;203(9):1292–300.
18. Ferrari M, Zevini A, Palermo E, Muscolini M, Alexandridi M, Etna MP, Coccia EM, Fernandez-Sesma A, Coyne C, Zhang DD, Marques ETA, Olagnier D. And Hiscott J. Dengue virus targets Nrf2 for NS2B3-mediated degradation leading to enhanced oxidative stress and viral replication. J Virol. 2020;94:24.
19. Fonseka CL, Hardman CS, Woo J, Singh R, Nahler J, Yang J, Chen YL, Kamaladasa A, Silva T, Salimi M, Gray N, Dong T, Malavige GN, Ogg GS. Dengue virus co-opts innate type 2 pathways to escape early control of viral replication. Commun Biol. 2022;5(1):735.
20. Ghita L, Yao Z, Xie Y, Duran V, Cagirici HB, Samir J, Osman I, Rebellon-Sanchez DE, Agudelo-Rojas OL, Sanz AM, Sahoo MK, Robinson ML, Gelvez-Ramirez RM, Bueno N, Luciani F, Pinsky BA, Montoya JG, Estupinan-Cardenas MI, Villar-Centeno LA, Rojas-Garrido EM, Rosso F, Quake SR, Zanini F, Einav S. Global and cell type-specific immunological hallmarks of severe dengue progression identified via a systems immunology approach. Nat Immunol. 2023;24(12):2150–63.
21. Jang GY, Kim YS, Lee SE, Lee JW, Han HD, Kang TH, Park YM. Improvement of DC-based vaccines using adjuvant TLR4-binding 60S acidic ribosomal protein P2 and immune checkpoint inhibitors. Cancer Immunol Immunotherapy: CII. 2021;70(4):1075–88.
22. Jayathilaka D, Gomes L, Jeewandara C, Jayarathna GSB, Herath D, Perera PA, Fernando S, Wijewickrama A, Hardman CS, Ogg GS, Malavige GN. Role of NS1 antibodies in the pathogenesis of acute secondary dengue infection. Nat Commun. 2018;9(1):5242.
23. Jeewandara C, Gomes L, Wickramasinghe N, Gutowska-Owsiak D, Waithe D, Paranavitane SA, Shyamali NL, Ogg GS, Malavige GN. Platelet activating factor contributes to vascular leak in acute dengue infection. PLoS Negl Trop Dis. 2015;9(2):e0003459.
24. Jeewandara C, Gomes L, Udari S, Paranavitane SA, Shyamali NL, Ogg GS, Malavige G. N. Secretory phospholipase A2 in the pathogenesis of acute dengue infection. Immun Inflamm Dis. 2017;5(1):7–15.
25. Kamaladasa A, Gomes L, Jeewandara C, Shyamali NL, Ogg GS, Malavige G. N. Lipopolysaccharide acts synergistically with the dengue virus to induce monocyte production of platelet activating factor and other inflammatory mediators. Antiviral Res. 2016;133:183–90.
26. Kamaladasa A, Gomes L, Wijesinghe A, Jeewandara C, Toh YX, Jayathilaka D, Ogg GS, Fink K. And Malavige G.N. altered monocyte response to the dengue virus in those with varying severity of past dengue infection. Antiviral Res. 2019;169:104554.
27. Khan RA, Afroz S, Minhas G, Battu S, Khan N. Dengue virus envelope protein domain III induces pro-inflammatory signature and triggers activation of inflammasome. Cytokine. 2019;123:154780.
28. Lee PX, Ting DHR, Boey CPH, Tan ETX, Chia JZH, Idris F, Oo Y, Ong LC, Chua YL, Hapuarachchi C, Ng LC, Alonso S. Relative contribution of nonstructural protein 1 in dengue pathogenesis. J Exp Med. 2020;217(9).
29. Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, Vaughn DW, Nisalak A, Ennis FA, Rothman AL. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. J Infect Dis. 2002;186(8):1165–8.
30. Malavige GN, Ogg GS. Molecular mechanisms in the pathogenesis of dengue infections. Trends Mol Med. 2024.
31. Malavige GN, Gomes L, Alles L, Chang T, Salimi M, Fernando S, Nanayakkara KD, Jayaratne S, Ogg GS. Serum IL-10 as a marker of severe dengue infection. BMC Infect Dis. 2013;13:341.
32. Martin-Acebes MA, Gabande-Rodriguez E, Garcia-Cabrero AM, Sanchez MP, Ledesma MD, Sobrino F, Saiz JC. Host sphingomyelin increases West Nile virus infection in vivo. J Lipid Res. 2016;57(3):422–32.
33. Modhiran N, Watterson D, Muller DA, Panetta AK, Sester DP, Liu L, Hume DA, Stacey KJ, Young PR. Dengue virus NS1 protein activates cells via toll-like receptor 4 and disrupts endothelial cell monolayer integrity. Sci Transl Med. 2015;7(304):304ra142.
34. Morin EE, Guo L, Schwendeman A, Li XA. HDL in sepsis - risk factor and therapeutic approach. Front Pharmacol. 2015;6:244.
35. Nainggolan L, Dewi BE, Hakiki A, Pranata AJ, Sudiro TM, Martina B, van Gorp E. Association of viral kinetics, infection history, NS1 protein with plasma leakage among Indonesian dengue infected patients. PLoS ONE. 2023;18(5):e0285087.
36. Nunes PCG, Nogueira RMR, Heringer M, Chouin-Carneiro T, Dos Santos Rodrigues D, de Filippis C, Lima AMBM, Santos DFB. NS1 Antigenemia and Viraemia load: potential markers of progression to Dengue. Fatal Outcome? Viruses. 2018;10:6.
37. Pan P, Li G, Shen M, Yu Z, Ge W, Lao Z, Fan Y, Chen K, Ding Z, Wang W, Wan P, Shereen MA, Luo Z, Chen X, Zhang Q, Lin L, Wu J. DENV NS1 and MMP-9 cooperate to induce vascular leakage by altering endothelial cell adhesion and tight junction. PLoS Pathog. 2021;17(7):e1008603.
38. Patro ARK, Mohanty S, Prusty BK, Singh DK, Gaikwad S, Saswat T, Chattopadhyay S, Das BK, Tripathy R, Ravindran B. Cytokine Signature Associated with Disease Severity in Dengue. Viruses. 2019;11(1).
39. Perera N, Miller JL, Zitzmann N. The role of the unfolded protein response in dengue virus pathogenesis. Cell Microbiol. 2017;19(5).
40. Pliego Zamora A, Kim J, Vajjhala PR, Thygesen SJ, Watterson D, Modhiran N, Bielefeldt-Ohmann H, Stacey KJ. Kinetics of severe dengue virus infection and development of gut pathology in mice. J Virol. 2023;e0125123.
41. Puc I, Ho TC, Yen KL, Vats A, Tsai JJ, Chen PL, Chien YW, Lo YC, Perng GC. Cytokine Signature of Dengue Patients at different severity of the Disease. Int J Mol Sci. 2021;22(6).
42. Puerta-Guardo H, Glasner DR, Espinosa DA, Biering SB, Patana M, Ratnasiri K, Wang C, Beatty PR, Harris E. Flavivirus NS1 triggers tissue-specific vascular endothelial dysfunction reflecting disease tropism. Cell Rep. 2019;26(6):1598–613.
43. Sangkaew S, Ming D, Boonyasiri A, Honeyford K, Kalayanarooj S, Yacoub S, Dorigatti I, Holmes A. Risk predictors of progression to severe disease

during the febrile phase of dengue: a systematic review and meta-analysis. Lancet Infect Dis. 2021;21(7):1014–26.
44. Shrivastava G, Visoso-Carvajal G, Garcia-Cordero J, Leon-Juarez M, Chavez-Munguia B, Lopez T, Nava P, Villegas-Sepulveda N, Cedillo-Barron L. Dengue Virus Serotype 2 and its non-structural proteins 2A and 2B activate NLRP3 inflammasome. Front Immunol. 2020;11:352.
45. Shyamali NLA, Mahapatuna SD, Gomes L, Wijewickrama A, Ogg GS, Malavige GN. Risk Factors for Elevated Serum Lipopolysaccharide in Acute Dengue and Association with Clinical Disease Severity. Trop Med Infect Dis. 2020;5(4).
46. Silva T, Jeewandara C, Gomes L, Gangani C, Mahapatuna SD, Pathmanathan T, Wijewickrama A, Ogg GS, Malavige GN. Urinary leukotrienes and histamine in patients with varying severity of acute dengue. PLoS ONE. 2021;16(2):e0245926.
47. Silva T, Gomes L, Jeewandara C, Ogg GS, Malavige GN. Dengue NS1 induces phospholipase A2 enzyme activity, prostaglandins, and inflammatory cytokines in monocytes. Antiviral Res. 2022;202:105312.
48. Tanaka S, Diallo D, Delbosc S, Geneve C, Zappella N, Yong-Sang J, Patche J, Harrois A, Hamada S, Denamur E, Montravers P, Duranteau J, Meilhac O. High-density lipoprotein (HDL) particle size and concentration changes in septic shock patients. Ann Intensive Care. 2019;9(1):68.
49. Tissera HA, Jayamanne BDW, Raut R, Janaki SMD, Tozan Y, Samaraweera PC, Liyanage P, Ghouse A, Rodrigo C, de Silva AM, Fernando SD. Severe dengue epidemic, Sri Lanka, 2017. Emerg Infect Dis. 2020;26(4):682–91.
50. Tricou V, Minh NN, Farrar J, Tran HT, Simmons CP. Kinetics of viremia and NS1 antigenemia are shaped by immune status and virus serotype in adults with dengue. PLoS Negl Trop Dis. 2011;5(9):e1309.
51. van de Weg CA, Pannuti CS, de Araujo ES, van den Ham HJ, Andeweg AC, Boas LS, Felix AC, Carvalho KI, de Matos AM, Levi JE, Romano CM, Centrone CC, de Lima Rodrigues CL, Luna E, van Gorp EC, Osterhaus AD, Martina BE, Kallas EG. Microbial translocation is associated with extensive immune activation in dengue virus infected patients with severe disease. PLoS Negl Trop Dis. 2013;7(5):e2236.
52. Watanabe S, Tan KH, Rathore AP, Rozen-Gagnon K, Shuai W, Ruedl C, Vasudevan SG. The magnitude of dengue virus NS1 protein secretion is strain dependent and does not correlate with severe pathologies in the mouse infection model. J Virol. 2012;86(10):5508–14.
53. Watterson D, Modhiran N, Muller DA, Stacey KJ, Young PR. Plugging the Leak in Dengue Shock. Adv Exp Med Biol. 2018;1062:89–106.
54. WHO, editor. Comprehensive guidelines for prevention and control of dengue fever and dengue haemorrhagic fever. India: World Health Organization, SEARO, New Delhi; 2011.
55. Yang Q, Meng X, Chen J, Li X, Huang Y, Xiao X, Li R, Wu X. RPLP2 activates TLR4 in an autocrine manner and promotes HIF-1alpha-induced metabolic reprogramming in hepatocellular carcinoma. Cell Death Discov. 2023;9(1):440.

Publisher's note