narra j

Original Article

The role of mediator suppressor of cytokine signaling (SOCS), toll-like receptor 3 (TLR-3) and nuclear factor kappa B (NFκB) on cytokine production during dengue virus infection

Sri Masyeni[1,2*], Kuntaman Kuntaman[3,4*], Aryati Aryati[5], Muchlis AU. Sofro[6,7], Usman Hadi[8,9], Gondo Mastutik[10], Windu Purnomo[11], Agus Santosa[1], Muhammad Iqhrammullah[12,13], Benediktus Yohan[14], Erni J. Nelwan[15,16] and R. Tedjo Sasmono[14]

[1]Departement of Internal Medicine, Faculty of Medicine and Health Sciences, Universitas Warmadewa, Bali, Indonesia; [2]Departement of Internal Medicine, Sanjiwani Hospital, Bali, Indonesia; [3]Department of Medical Microbiology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia; [4]Department of Medical Microbiology, Dr. Soetomo Hospital, Surabaya, Indonesia; [5]Department of Clinical Pathology, School of Medicine and Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia; [6]Department of Internal Medicine, Faculty of Medicine, Diponegoro University, Semarang, Indonesia; [7]Department of Internal Medicine, Dr Kariadi Hospital, Semarang, Indonesia; [8]Department of Internal Medicine, Faculty of Medicine, Airlangga University, Surabaya, Indonesia; [9]Department of Internal Medicine, Dr. Soetomo Hospital, Surabaya, Indonesia; [10]Department of Anatomical Pathologic, Faculty of Medicine, Airlangga University, Surabaya, Indonesia; [11]Department of Biostatistics, Faculty of Public Health, Universitas Airlangga Surabaya, Indonesia; [12]Faculty of Public Health, Universitas Muhammadiyah Aceh, Banda Aceh, Indonesia; [13]Innovative Sustainability Lab, PT. Biham Riset dan Edukasi, Banda Aceh, Indonesia; [14]Eijkman Research Center for Molecular Biology, Jakarta, Indonesia; [15]Division of Tropical and Infectious Disease, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Universitas Indonesia, Jakarta, Indonesia; [16]Infectious Disease and Immunology Research Center, Indonesia Medical and Education Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

*Corresponding author: masyeniputu@yahoo.com

Abstract

Inability to understand the pathogenesis of severe dengue, in particular the control mechanism of immune responses, has led to high mortality rate for patients with dengue shock syndrome (DSS). The aim of this study was to determine the control mechanism of cytokine production by mediator suppressor of cytokine signaling (SOCS), toll-like receptor 3 (TLR-3) and nuclear factor kappa B (NFκB) during DENV infection. Peripheral blood mononuclear blood cells (PBMC), isolated from healthy individuals, were infected with dengue virus (DENV)-2 strain SJN-006 Cosmopolitan genotype (isolated from Bali, Indonesia). The relative gene expression of SOCS-3, TLR-3, NFκB, and the cytokine genes (interleukin (IL)-6, IL-8, interferon inducible protein 10 (IP-10), and macrophage inflammatory protein-1 beta (MIP-1β)) were measured using qRT-PCR at 6, 12 and 24 hours post infection (hpi). Student t-test and Mann-Whitney test were used to compare the gene expressions while causal correlations were analyzed using regression test and path analyses. DENV-2 infection increased the gene expression of SOCS-3, TLR-3, and NFκB after 12 and 24 hpi. The expression of IL-6, IL-8, IP-10, and MIP-1β genes was increased and peaked at different times post-infection. NFκB and SOCS-3 genes likely have role in the upregulation of IL-8 and IL-6 gene expression, respectively. MIP-1β gene expression was significantly induced by both NFκB and SOCS-3. In conclusion, our study suggested that SOCS-3, TLR-3, and NFκB are important in regulating the production of IL-6, IL-8, IP-10, MIP-1β during early phase of DENV-2 infection. This enriches our understanding on pathogenesis pathway of DENV-associated cytokine storm.

Keywords: Dengue, SOCS-3, TLR-3, NFκB, cytokine storm

https://narraj.org/

Received: May 4, 2022 - Accepted: July 14, 2023 - Published online: July 17, 2023

Introduction

Since its first case reported in Indonesia in 1968, the incidence rate (IR) of dengue virus (DENV) infection keeps increasing in the country [1]. Globally, there are approximately 390 million dengue cases annually, of which 96 million are symptomatic [2]. DENV infection have a wide spectrum of disease, ranging from asymptomatic or a self-limiting dengue fever (DF) to life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [3]. The mortality of DENV infection is mostly contributed from patients with DSS, which is 50 times higher than those without DSS [4]. Among four DENV serotypes (DENV-1 to -4), a meta-analysis study revealed that DENV-2 serotype is a significantly associated with DSS [4].

Clinical manifestation of DENV infection may be affected by variety of either exogenous or endogenous factors [5]. Complex interactions between DENV with innate or adaptive immunity has led to investigation on differential expression of cytokines such as interleukin (IL)-6, IL-8, interferon (IFN) inducible protein 10 (IP-10), and macrophage inflammatory protein-1 beta (MIP-1β) and other soluble mediating factors such as suppressor of cytokine signaling 3 (SOCS-3), toll-like receptor 3 (TLR-3), and nuclear factor kappa B (NFκB) [6-12].

Upon its attachment to the surface of a dendritic cell or macrophage, DENV will be recognized by TLRs, mainly TLR-3 and TLR-7, followed by retinoic acid-inducible gene-I-like receptors (RIG-1) or melanoma differentiation-associated gene-5 (MDA-5). This leads the activation of transcription factor, NFκB, and regulating the production of cytokines as innate immune response. Host immune response to DENV generates a cascade of cytokine releases and it has been suggested as the primary contributor of increasing vascular permeability, followed by plasma leakage and abnormal bleeding in DENV infection [13]. In severe dengue, the levels of cytokines such as tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8, IL-10, IFN-γ, c-c motif chemokine ligand (CCL)-2, CCL-3, CXCL-8, CXCL-10, IP-10, MIP-1, and monocyte chemoattractant protein-1 (MCP-1) are increased [8, 14-16].

SOCS family proteins are attenuators of proinflammatory cytokines upon the generation of cytokine release cascade concomitant to host antiviral mechanism. Among the eight family members, SOCS-1 and SOCS-3 have been identified as potent inhibitors in Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathways [17, 18]. Previously, SOCS-1 is reported to be more potent inhibitor of JAKs compared to SOCS-3 [19]. However, a research on human immunodeficiency virus-1 (HIV-1)-infected cells suggests the JAK/STAT-free suppression of NF-κB by SOCS-3, which is central for the inflammatory stimuli [20]. SOCS-3 also has been reported to have high selectivity against JAK-1, JAK-2 and tyrosine kinase (TYK)-2 [21]. Unfortunately, amidst these conflicting findings of its importance, SOCS-3 has been underreported for its role in the pathogenesis of DHF/DSS [10, 22-25]. The data on the role of SOCS-3 on regulation of cytokine production in DENV infection is lacking. The objective of this study was to assess the role of SOCS-3, TLR-3 and NFκB in regulating the production of cytokines during the early course of DENV-2 infection in peripheral blood mononuclear cells (PBMC). Understanding on the regulation of proinflammatory cytokines through their regulators is important to have a better understanding on the pathogenesis of DHF or DSS. Therefore, the aim of this study was to determine the role of SOCS, TLR-3 and NFκB in controlling cytokine production during the early phase of DENV infection.

Methods

Study design and the virus

An experimental study with randomized post-test control group design was used. Peripheral blood mononuclear blood cells (PBMCs) were collected from seven healthy volunteers (18-25 years old) and the PBMCs from each individual were divided into two groups: (1) infected with DENV strain SJN-006 of Cosmopolitan genotype (GenBank: KY006142.1) and (2) uninfected control. The virus originated from Bali and received from Eijkman Institute for Molecular Biology (now known as Eijkman Research Center for Molecular Biology), Jakarta, Indonesia. The virus was propagated in *Aedes albopictus* cells (C6/36) as described previously [15]. The titer of the virus was determined using plaque assay using baby hamster kidney cells (BHK21) as previously

used [26]. In the present study, the independent variable was DENV2 infection; the mediating variables were the gene expression of SOCS-3, TLR-3 and NFκB; while the dependent variables were the gene expression of IL-6, IL-8, IP-10, and MIP-1β. All gene expressions were measured using quantitative real time–polymerase chain reaction (qRT-PCR).

DENV-2 infection in PBMCs

PBMCs were infected at multiplicities of infection (MOI) of 1 plaque-forming units (pfu)/cell. Samples were then incubated at 37°C and 5% CO_2 for 6, 12, and 24 hours post infection (hpi), in parallel. PBMC-infected cells and the supernatant were harvested by centrifugation at 8,000 rpm and then stored at -80°C before used for RNA extraction, enzyme linked immunosorbent assays (ELISA) and qRT-PCR.

Detection of non-structural 1 protein (NS1) antigen using ELISA

To ensure the success of infection, the production of NS1 antigen in the culture supernatants were assayed using Panbio Dengue Early ELISA kit (Standard Diagnostics, Yongin, Korea), according to the manufacturer's protocol. In brief, after the supernatant from each well was incubated at 37°C for 1 h, the plate was washed and the horseradish peroxidase (HRP)-conjugated anti-NS1 monoclonal antibody (MAb) (100 µL) was added. The measurement of absorbance was conducted at 450 nm wavelength using a microplate photometer (Thermo Scientific, Waltham, USA).

Measurement of gene expression using qRT-PCR

The gene expression of SOCS-3, TLR-3, NFκB and the cytokines (IL-6, IL-8, IP-10, and MIP-1β) were measure using qRT-PCR. Briefly, 100 ng RNA was converted into a reverse transcription template – cDNA using GoScript Reverse Transcriptase, primer Oligo(dT), and random primer (Promega, Madison, USA). The reaction condition was maintained at 25°C for 5 min, 42°C for 60 min, and 70°C for 15 min. The amplification of the cDNA was carried out using 2x GoTaq qPCR master mix (Promega, Madison, USA) and 10 µM forward and reverse primers (Macrogen, Singapore). The endogenous gene, beta actin (β-actin), was used for normalization of gene expression. The detailed primer sequences are presented in **Table 1**.

Table 1. Primers used to determine the gene expression of mediator suppressor of cytokine signaling (SOCS), toll-like receptor 3 (TLR-3) and nuclear factor kappa B (NFκB) and cytokines using qRT-PCR

Gene	Sequence
SOCS-3-F	5'-CCTGCGCCTCAAGACCTT-3'
SOCS-3-R	5'-GTCACTGCGCTCCAGTAGAA-3'
TLR-3-F	5'-GATCTGTCTCATAATGGCTTGT-3'
TLR-3-R	5'-GGCAAAGATATCCAGTTCTTCA-3'
NFκB-F	5'-CCTGGATGACTCTTGGGAAA-3'
NFκB-R	5'-TCAGCCAGCTGTTTCATGTC-3'
IL-6-F	5'-ATGAACTCCTTCTCCACAAGC-3'
IL-6-R	5'-CTACATTTGCCGAAGAGCCCTCAGGCTGGACTG-3'
IL-8-F	5'-TGCCAAGGAGTGCTAAAG-3'
IL-8-R	5'-CTCCACAACCCTCTGCAC-3'
IP-10-F	5'-TTCAAGGAGTACCTCTCTCTAG-3'
IP-10-R	5'-CTGGATTCAGACATCTCTTCTC-3'
MIP-1β-F	5'-CTGTGCTGATCCCAGTGAATC-3'
MIP-1β-R	5'-TCAGTTCAGTTCCAGGTCATAGA-3'
β-actin-F	5'-GCTCGTCGTCGACAACGGCTC-3'
β-actin-R	5'-CAAACATGATCTGGGTCATCTTCTC-3'

Statistical analysis

Distribution of the data was tested with Kolmogorov-Smirnov normality test. Comparative analysis of gene expressions was conducted for each hpi observation using Student t-test and Mann-Whitney for normal and non-normal distribution, respectively. Correlation test, regression test, and path analysis were conducted to investigate the causal relationship between variables. Positive coefficient means direct correlation; while negative coefficient means inverse correlation. The correlation was classified as very weak (coefficient <0.1), weak (coefficient 0.10–0.49), moderate (coefficient 0.50–0.74), strong (coefficient 0.75–0.90), and very strong (coefficient ≥0.90).

Results

Effect of infection time on expression of SOCS-3, TLR-3, NFκB and cytokine genes

In this study, PBMCs from seven healthy volunteers were included and were divided into two infected and uninfected control groups. The gene expression of SOCS-3, TLR-3 and NFκB as well as the cytokines for both DENV-2 infected and control PBMC are presented in **Table 2**. The expression of SOCS-3, TLR-3, and NFκB were significantly higher in DENV-2-infected PBMCs at 6, 12 and 24 hpi compared to non-infected PBMCs at 6, 12 and 24 hpi.

The gene expression of IL-8, IP-10, and MIP-1β were significantly higher in DENV-2-infected group than non-infected PBMCs at 6, 12 and 24 hpi. IL-6 expression was only statistically significant at 12 hpi, where it was 1.86 times higher in infected PBMCs compared to uninfected cells (**Table 2**). The presence of DENV-2 in the culture was confirmed in all samples collected at different time points by the positive NS1 ELISA result (index value >1.1 or Panbio Unit >11).

Table 2. Gene expression of mediator suppressor of cytokine signaling (SOCS), toll-like receptor 3 (TLR-3), nuclear factor kappa B (NFκB) and cytokines after 6, 12, and 24 hours post infection (hpi) with DENV-2

Gene	Gene expression								
	6 hours post infection			12 hours post infection			24 hours post infection		
	Infected (± SD)	Control (± SD)	*p*-value	Infected (± SD)	Control (± SD)	*p*-value	Infected (± SD)	Control (± SD)	*p*-value
SOCS-3	1.529 ± 1.60	1.020 ± 0.28	0.613[b]	2.421 ± 2.55	1.007 ± 0.43	0.006[a]	2.196 ± 2.21	1.016 ± 0.48	0.041[a]
TLR-3	0.906 ± 0.39	0.801 ± 0.29	0.215[b]	1.453 ± 0.72	0.927 ± 0.31	0.026[a]	2.629 ± 3.79	0.876 ± 0.32	0.007[a]
NFκB	1.178 ± 0.62	0.899 ± 0.42	0.180[b]	1.954 ± 1.06	0.986 ± 0.25	0.003[b]	12.32 ± 23.05	1.018 ± 0.30	0.00[a]
IL-6	0.976 ± 0.58	1.035 ± 0.30	0.505[a]	1.650 ± 1.43	0.889 ± 0.28	0.013[a]	1.816 ± 1.74	0.982 ± 0.48	0.395[a]
IL-8	1.407 ± 0.55	0.855 ± 0.51	0.011[b]	2.984 ± 1.84	0.906 ± 0.47	0.000[a]	8.993 ± 10.02	1.091 ± 0.63	0.00[a]
IP-10	7.776 ± 12.56	0.940 ± 0.33	0.009[a]	37.316 ± 52.97	0.968 ± 0.45	<0.001[a]	301.16 ± 333.60	1.018 ± 0.42	<0.001[a]
MIP-1β	1.504 ± 0.52	0.951 ± 0.20	0.001[b]	3.176 ± 2.98	1.034 ± 0.20	<0.001[a]	7.761 ± 7.17	0.899 ± 0.46	0.001[b]

[a] Analyzed using Mann-Whitney test
[b] Analyzed using Student t-test

Correlation between DENV-2 infection and expression of SOC-3, TLR-3, NFκB and cytokine genes

Correlations between independent variable (DENV-2 infection), mediating variables (SOCS-3, TLR-3, NFκB) and dependent variables (IL-6, IL-8, IP-10, and MIP-1β) are presented in **Table 3**. Significant correlations at 6 hpi were observed between infection → IL-8 (moderate), infection → IP-10 (weak), and infection → MIP-1β (moderate), SOCS-3 → IL-6 (weak), SOCS-3 → MIP-1β (weak), NFκB → IL-8 (moderate), and NFκB → MIP-1β (weak) (**Figure 1**).

After 24 h post infection, there were significant correlations between infection → SOCS-3 (weak), infection → TLR-3 (weak), infection → NFκB (weak), infection → IL-8 (moderate), infection → IP-10 (moderate), infection → MIP-1β (moderate), SOCS-3 → IL-6 (strong), SOCS-3 → MIP-1β (strong) (**Figure 1**).

Discussion

Our data suggest that the expression of SOCS-3, TLR-3, NFκB, IL-8, IL-6, IP-10, and MIP-1β were higher in the DENV-2 infected cells than in control. It was similar to the previous studies [6, 8-11]. DENV infection induced the expression or secretion of IL-8 through at least three mechanisms: non-structural protein (NS) 5 hyperphosphorylation of DENV-2 [7], recognition of DENV RNA by TLR-3 [27], and interaction between activator protein-1 (AP-1) and NFκB or c/EBP [28, 29]. Overexpression of IL-8 and IL-6 might be associated with the manifestation of DENV infection, where their consistent increment upon infection time is linked to the severity of

the disease [6, 7]. SOCS-3, TLR-3, NFκB, IP-10, and MIP-1β also had similar trend and have been suggested as biomarker for the severity [8-11] and acute stage of DENV infection [12].

Table 3. Results of causality test of DENV-2 infection on gene expression of mediator suppressor of cytokine signaling (SOCS), toll-like receptor 3 (TLR-3), nuclear factor kappa B (NFκB) and cytokines

Variable	6 hours post infection		12 hours post infection		24 hours post infection	
	b/η	*p*-value	b/η	*p*-value	b/η	*p*-value
Infection → SOCS-3	NA	NA	0.390	0.006*	0.377	0.041*
Infection → TLR-3	0.155	0.430	0.461	0.026*	0.322	0.007*
Infection → NFκB	NA	NA	0.627	0.003*	0.440	<0.001*
Infection → IL-6	NA	NA	0.364	0.013*	NA	NA
Infection → IL-8	0.554	0.011*	0.666	<0.001*	0.509	0.004*
Infection → IP-10	0.422	0.009*	0.460	<0.001*	0.560	<0.001*
Infection → MIP-1β	0.661	0.001*	0.486	<0.001*	0.577	0.001*
SOCS-3 → IL-6	0.402	0.028*	1.000	<0.001*	0.754	<0.001*
SOCS-3 → IL-8	0.190	0.189	0.017	0.880	0.032	0.850
SOCS-3 → IP-10	-0.169	0.389	0.191	0.329	0.185	0.345
SOCS-3 → MIP-1β	0.367	0.037*	0.814	<0.001*	0.798	<0.001*
NFκB → IL-6	0.229	0.194	0.032	0.612	0.156	0.234
NFκB → IL-8	0.529	0.001*	0.499	0.001	0.219	0.295
NFκB → IP-10	0.300	0.121	0.264	0.174	0.103	0.604
NFκB → MIP-1β	0.349	0.026*	0.133a	0.261	0.049	0.664
TLR-3 → NFκB	0.031	0.875	0.224	0.252	-0.065	0.743

The arrow indicates the causal relationship between variables.
*Statistically significant at p=0.05.

We suspect TLR-3 to be suppressed at the early stage of infection (6 hpi) because the significant difference was observed only afterward. The suppression of TLRs (including TLR-3) is also exhibited by hepatitis C virus in HepG-2 cells [30]. Similarly, DENV infection downregulates TLRs expression during the entry of DENV into monocytes via Fc receptor [31]. Significant correlation between DENV infection and TLR-3 was observed at 12 and 24 hpi, which corresponds to the significant increase of its expression.

Direct induction of MIP-1β by DENV-2 in PBMC has been previously reported; deduced from the absence of correlation between MIP-1β and other possible mediating factors [32]. Nonetheless, our data suggest the correlation between MIP-1β induction with NFκB and SOCS-3. Correlation between NFκB and MIP-1β that was identified in our study, in line with the previous study in hepatocytes, in which NFκB silencing yields to downregulation of MIP-1β mRNA [33]. Furthermore, SOCS-3-induced NFκB expression has been reported by a study using influenza A virus-infected A549 cells [34]. Hence, it contributes to the strong correlation between SOCS-3 and MIP-1β.

Upon infection, IP-10 is induced by IFN-γ and produced by multiple cell types (endothelial cells, monocytes, keratinocytes, and dendritic cells) as innate immune response [35, 36]. Along with MIP-1β, IP-10 plays a key role in the recruitment of leukocytes at the DENV-infected tissues or organs [32, 37-39]; explaining their significant correlations with the infection. Additionally, it was further found that IP-10 limits the viral replication through competitive binding to heparan sulphate on the cell surface [39].

In line with the results of our study, upregulation of SOCS-3 expression is a common viral resistance mechanism against host immune response that inhibits JAK/STAT pathway [40]. A study using DENV-antibody-dependent enhancement (ADE)–infected macrophages revealed the role of IL-6 in SOCS-3 induction [22]. Correlation between infection and IL-6 can be explained through the upregulation of IL-6 by DENV NS1 through TLR-2 and TLR-6 expressions [41]. The correlation was recorded at 12 hpi, when the IL-6 expression was significantly higher than control. At the same time, SOCS-3 was at the peak of expression; further confirming IL-6-mediated SOCS-3 induction.

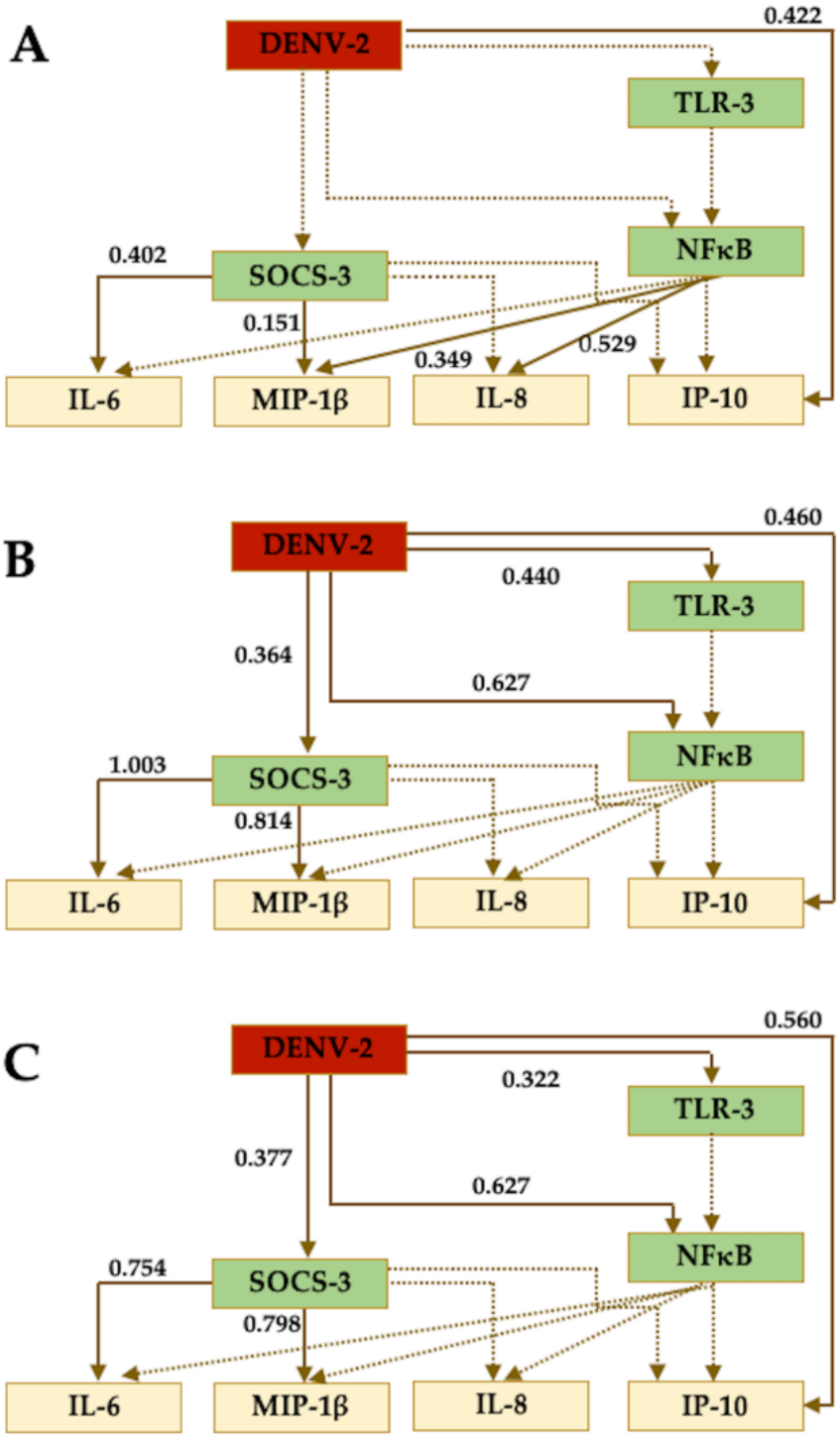

Figure 1. Proposed regulatory pathways of SOCS-3, TLR-3 and NFκB in regulation of cytokines during DENV-2 infection in 6 (A), 12 (B) and 24 hours (C) post-infection. The numbers are the co-efficient of the significant correlations. Continuous lines indicate significant correlation at $p<0.05$ while dashed-lines indicate non-significant correlation. Coefficient <0.1 (very weak), 0.10–0.49 (weak), 0.50–0.74 (moderate), 0.75–0.90 (strong), and ≥0.90 (very strong).

There are some limitations of this study. The gene expressions of SOCS-3, TLR-3, NFκB and the cytokines were measured using qRT-PCR. Since there is post-transcriptional attenuation phenomenon in which not all the mRNA will be translated into protein, a study to measure both RNA and the protein concentration will provide better evidence. In this present study, only one serotype was used, DENV-2. The effects of other DENV serotypes might also worth to be assessed.

Conclusion

DENV-2 infection induces the overexpression of SOCS-3, TLR-3, NFκB, IL-6, IL-8, IP-10, and MIP-1β. SOCS-3 involved in the dysregulation of IL-6 and MIP-1β; while NFκB involved in induction of IL-8 and MIP-1β. Further studies are required to confirm the proposed and reveal other feasible mechanisms.

Ethics approval

The protocol of this study was approved by Ethical Review Boards of Universitas Udayana (No. 2072/UN.14.2/KEP/2017). All PBMC donors were voluntarily required to sign informed consent prior to the study.

Acknowledgments

We thank Dengue Unit of the Eijkman Institute for Molecular Biology, Jakarta by providing DENV-2 strain SJN-006 of Cosmopolitan genotype.

Conflicting of interest

All the authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

All data underlying the results can be requested from the corresponding author (SM).

How to cite

Masyeni S, Kuntaman K, Aryati A, *et al.* The role of mediator suppressor of cytokine signaling (SOCS), toll-like receptor 3 (TLR-3) and nuclear factor kappa B (NFκB) on cytokine production during dengue virus infection. Narra J 2023; 3 (2): e167 - http://doi.org/10.52225/narra.v3i2.167.

References

1. Harapan H, Michie A, Mudatsir M, *et al.* Epidemiology of dengue hemorrhagic fever in Indonesia: analysis of five decades data from the National Disease Surveillance. BMC Res Notes 2019; 12(1):350.
2. Bhatt S, Gething PW, Brady OJ, *et al.* The global distribution and burden of dengue. Nature 2013; 496(7446):504-507.
3. WHO. Handbook for Clinical Management of Dengue. Geneva, Switzerland: WHO Press; 2012
4. Huy NT, Van Giang T, Thuy DH, *et al.* Factors associated with dengue shock syndrome: a systematic review and meta-analysis. PLoS Negl Trop Dis 2013; 7(9):e2412.
5. Halstead SB. Dengue. Lancet 2007; 370(9599):1644-1652.
6. Butthep P, Chunhakan S, Yoksan S, *et al.* Alteration of cytokines and chemokines during febrile episodes associated with endothelial cell damage and plasma leakage in dengue hemorrhagic fever. Pediatr Infect Dis J 2012; 31(12):e232-238.
7. Medin CL, Fitzgerald KA, Rothman AL. Dengue virus nonstructural protein NS5 induces interleukin-8 transcription and secretion. J Virol 2005; 79(17):11053-11061.
8. Patro ARK, Mohanty S, Prusty BK, *et al.* Cytokine Signature Associated with Disease Severity in Dengue. Viruses 2019; 11(1).
9. 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.
10. Flores-Mendoza LK, Estrada-Jimenez T, Sedeno-Monge V, *et al.* IL-10 and socs3 Are Predictive Biomarkers of Dengue Hemorrhagic Fever. Mediators Inflamm 2017; 2017:5197592.
11. Balakrishna Pillai A, Cherupanakkal C, Immanuel J, *et al.* Expression Pattern of Selected Toll-like Receptors (TLR's) in the PBMC's of Severe and Non-severe Dengue Cases. Immunol Invest 2020; 49(4):443-452.

12. Huang J, Liang W, Chen S, *et al*. Serum Cytokine Profiles in Patients with Dengue Fever at the Acute Infection Phase. Dis Markers 2018; 2018:8403937.
13. Srikiatkhachorn A, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. Semin Immunopathol 2017; 39(5):563-574.
14. Costa VV, Fagundes CT, Souza DG, *et al*. Inflammatory and innate immune responses in dengue infection: protection versus disease induction. Am J Pathol 2013; 182(6):1950-1961.
15. Yohan B, Kendarsari RI, Mutia K, *et al*. Growth characteristics and cytokine/chemokine induction profiles of dengue viruses in various cell lines. Acta Virol 2014; 58(1):20-27.
16. Harapan H, Michie A, Sasmono RT, *et al*. Dengue: A Minireview. Viruses 2020; 12(8).
17. Dimitriou ID, Clemenza L, Scotter AJ, *et al*. Putting out the fire: coordinated suppression of the innate and adaptive immune systems by SOCS1 and SOCS3 proteins. Immunol Rev 2008; 224:265-283.
18. Baker BJ, Akhtar LN, Benveniste EN. SOCS1 and SOCS3 in the control of CNS immunity. Trends Immunol 2009; 30(8):392-400.
19. Liau NPD, Laktyushin A, Lucet IS, *et al*. The molecular basis of JAK/STAT inhibition by SOCS1. Nat Commun 2018; 9(1):1558.
20. Sood V, Lata S, Ramachandran VG, *et al*. Suppressor of Cytokine Signaling 3 (SOCS3) Degrades p65 and Regulate HIV-1 Replication. Front Microbiol 2019; 10:114.
21. Babon JJ, Kershaw NJ, Murphy JM, *et al*. Suppression of cytokine signaling by SOCS3: characterization of the mode of inhibition and the basis of its specificity. Immunity 2012; 36(2):239-250.
22. Rolph MS, Zaid A, Rulli NE, *et al*. Downregulation of interferon-beta in antibody-dependent enhancement of dengue viral infections of human macrophages is dependent on interleukin-6. J Infect Dis 2011; 204(3):489-491.
23. Masyeni S, Hadi U, Kuntaman, *et al*. Detection of micro RNA hsa-let-7e in peripheral blood mononuclear cells infected with dengue virus serotype-2: preliminary study. IOP Conference Series: Earth and Environmental Science 2018; 125.
24. Estrada-Jimenez T, Millan-Perez Pena L, Flores-Mendoza L, *et al*. Upregulation of the Suppressors of Cytokine Signaling 1 and 3 Is Associated with Arrest of Phosphorylated-STAT1 Nuclear Importation and Reduced Innate Response in Denguevirus-Infected Macrophages. Viral Immunol 2016; 29(2):95-104.
25. Ubol S, Phuklia W, Kalayanarooj S, *et al*. Mechanisms of immune evasion induced by a complex of dengue virus and preexisting enhancing antibodies. J Infect Dis 2010; 201(6):923-935.
26. Warnasih S. Induksi Ekspresi Gen Sitokin/Kemokin pada Sel Makrofag Manusia yang Dipapar Virus Dengue Isolat Indonesia. Current Biochemistry 2014; 1(3):146-157.
27. Tsai YT, Chang SY, Lee CN, *et al*. Human TLR3 recognizes dengue virus and modulates viral replication in vitro. Cell Microbiol 2009; 11(4):604-615.
28. Medin CL, Rothman AL. Cell type-specific mechanisms of interleukin-8 induction by dengue virus and differential response to drug treatment. J Infect Dis 2006; 193(8):1070-1077.
29. Hoffmann E, Thiefes A, Buhrow D, *et al*. MEK1-dependent delayed expression of Fos-related antigen-1 counteracts c-Fos and p65 NF-kappaB-mediated interleukin-8 transcription in response to cytokines or growth factors. J Biol Chem 2005; 280(10):9706-9718.
30. Chang S, Kodys K, Szabo G. Impaired expression and function of toll-like receptor 7 in hepatitis C virus infection in human hepatoma cells. Hepatology 2010; 51(1):35-42.
31. Modhiran N, Kalayanarooj S, Ubol S. Subversion of innate defenses by the interplay between DENV and pre-existing enhancing antibodies: TLRs signaling collapse. PLoS Negl Trop Dis 2010; 4(12):e924.
32. Spain-Santana TA, Marglin S, Ennis FA, *et al*. MIP-1 alpha and MIP-1 beta induction by dengue virus. J Med Virol 2001; 65(2):324-330.
33. Zhang T, Guo CJ, Li Y, *et al*. Interleukin-1beta induces macrophage inflammatory protein-1beta expression in human hepatocytes. Cell Immunol 2003; 226(1):45-53.
34. Pauli EK, Schmolke M, Wolff T, *et al*. Influenza A virus inhibits type I IFN signaling via NF-kappaB-dependent induction of SOCS-3 expression. PLoS Pathog 2008; 4(11):e1000196.
35. Satish L, Yager D, Wells A. Glu-Leu-Arg-negative CXC chemokine interferon gamma inducible protein-9 as a mediator of epidermal-dermal communication during wound repair. J Invest Dermatol 2003; 120(6):1110-1117.
36. Vazirinejad R, Ahmadi Z, Kazemi Arababadi M, *et al*. The biological functions, structure and sources of CXCL10 and its outstanding part in the pathophysiology of multiple sclerosis. Neuroimmunomodulation 2014; 21(6):322-330.

37. Becquart P, Wauquier N, Nkoghe D, *et al*. Acute dengue virus 2 infection in Gabonese patients is associated with an early innate immune response, including strong interferon alpha production. BMC Infect Dis 2010; 10:356.
38. Biron CA, Nguyen KB, Pien GC, *et al*. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 1999; 17:189-220.
39. Chen JP, Lu HL, Lai SL, *et al*. Dengue virus induces expression of CXC chemokine ligand 10/IFN-gamma-inducible protein 10, which competitively inhibits viral binding to cell surface heparan sulfate. J Immunol 2006; 177(5):3185-3192.
40. Akhtar LN, Benveniste EN. Viral exploitation of host SOCS protein functions. J Virol 2011; 85(5):1912-1921.
41. Chen J, Ng MM, Chu JJ. Activation of TLR2 and TLR6 by dengue NS1 protein and its implications in the immunopathogenesis of dengue virus infection. PLoS Pathog 2015; 11(7):e1005053.