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ORIGINAL RESEARCH

Contribution of Immune Responses to *Aedes aegypti* Saliva in Dengue Severity Among Patients with Atopic Dermatitis

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Introduction: Dengue severity is influenced by viral load and the host's immune response. *Aedes aegypti* saliva increases cellularity at the bite site, which may promote viral replication. Atopic dermatitis (AD) is associated with hypersensitivity to Aedes saliva and Th2-skewed immune response, potentially increasing viral target cells in the skin and impairing the immune response to dengue. This study investigated the association between exposure to Aedes saliva, T-cell function, and dengue severity among dengue patients with AD, particularly in children, in whom the incidence of AD and the severity of dengue are higher.

Methods: This observational cross-sectional study was conducted on 62 secondary dengue patients aged 1–12 years. The ISAAC questionnaire was used to assess the history of AD. T-cell functions were evaluated by measuring the levels of IFNγ, IL10, IL13, and CCL2 cytokines in the PHA-stimulated whole blood cultures exposed to *Aedes aegypti* salivary gland extract (SGE) using the ELISA.

Results: The incidence of dengue hemorrhagic fever (DHF) was higher among dengue patients with AD than in those without AD (p=0.010). In the PHA-stimulated whole blood cultures exposed to SGE, higher CCL2 levels were observed in DHF patients than in dengue fever (DF) patients (p=0.044), and higher IL13 levels were found in dengue patients with AD compared to those without AD (p=0.026). In PHA-stimulated whole blood cultures without SGE, lower IFNγ levels were found in DHF patients than in DF patients (p=0.035).

Conclusion: AD may be associated with a higher incidence of DHF and increased T-cell IL-13 production in response to SGE. DHF may be associated with increased T-cell CCL2 production in response to SGE, which may reflect greater cell infiltration at the bite site, and reduced T-cell IFN-γ production in response to dengue infection.

Keywords: Aedes saliva, AD, dengue, T-cell functions

Introduction

Dengue is a mosquito-borne disease caused by the Dengue virus (DENV), which belongs to the Flavivirus genus within the Flaviviridae family, with *Aedes aegypti* as its primary vector. Dengue cases are reported worldwide,[1,2] and the majority occur in children.[2–4] The clinical manifestations of Dengue infection vary widely in humans, ranging from asymptomatic, fever, to severe hemorrhage and plasma leakage that may lead to death.[2,5–7] Dengue infection is classified into Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF). Both are characterized by fever, hemorrhagic manifestations, and thrombocytopenia. However, DHF is distinguished by the presence of plasma leakage that can cause shock and death, which is absent in DF.[2]

The severity of dengue infection is associated with the host's immune response to the virus. An effective immune response can control viral replication and result in mild manifestations, whereas an ineffective response leads to

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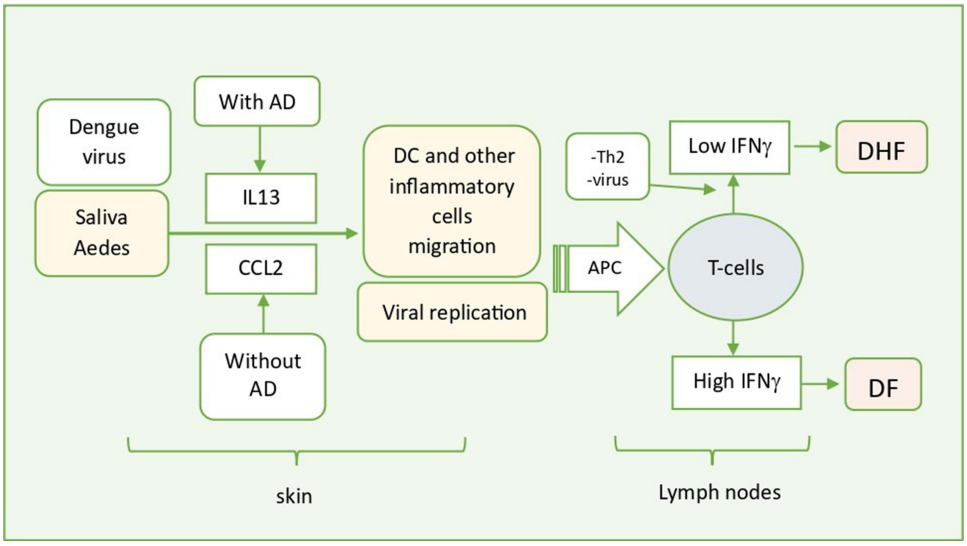
Graphical Abstract



uncontrolled viral replication and excessive inflammation and resulting in severe Dengue.[8–11] Upon viral entry through the mosquito bite, innate immune cells such as mast cells, dendritic cells, Langerhans cells, macrophages, and monocytes are activated to counter the virus.[12] These cells produce type I interferons that trigger signaling cascades to induce Interferon-Stimulated Genes (ISGs) and establish an antiviral state. Other inflammatory cytokines secreted by these cells increase endothelial permeability and promote the migration of inflammatory cells from the intravascular to the extravascular for enhancing antiviral defense.[13,14] One of the cytokines that mediates inflammatory cell migration is CCL2.[15,16] However, dendritic cells, Langerhans cells, and macrophages also serve as target cells for DENV replication,[6,17,18] and the virus can inhibit ISG induction to sustain its replication cycle.[13,19]

Viral antigens are subsequently presented by dendritic cells, which act as antigen-presenting cells (APCs), to the adaptive immune system to initiate a more specific and potent immune response.[12] The adaptive immune response against viral infections involves T-helper 1 (Th1) cells and $CD8^+$ cytotoxic T-cells, which produce interferon-gamma (IFN-γ).[8,12,20] Low IFNγ and high IL-10 production by T-cells are associated with severe dengue. IL-10 suppresses T-cell response to the virus by inhibiting IFN-γ production.[21–23]

Aedes aegypti saliva has also been reported to have a role in Dengue infection. *Aedes aegypti* saliva promotes the migration of inflammatory cells to the bite site, which facilitates viral replication.[24] Previous studies have demonstrated an increased number of dendritic cells, Langerhans cells, and macrophages at mosquito bite sites.[25] Mosquito saliva induces CCL2 production and promotes inflammatory cell migration.[25,26] This recruitment is mediated by the salivary protein NeSt1,[25] and sialokinin.[26]

Beyond acting as an immunogen that stimulates inflammation, Aedes saliva also acts as an allergen that can trigger hypersensitivity reactions. Hypersensitivity to *Aedes aegypti* saliva is more frequent among individuals with Atopic Dermatitis (AD).[27] AD is a chronic inflammatory skin disease that usually appears in childhood and is associated with Th2-polarized immune responses.[28–30] The prevalence of AD in children is estimated to be around 20–30%.[28] AD is characterized by erythematous, pruritic papules and scaling, often occurring on extensor surfaces in children. This disease is commonly associated with a family history of atopy, such as AD, allergic rhinitis, or asthma.[28,29]

The skin serves as the portal of entry for the Dengue virus via mosquito bites. The Th2-skewed immune response observed both locally in the skin and systemically in patients with AD may impair antiviral Th1 responses.[28,31–38] Impaired Th1 responses have been associated with severe dengue.[39] Based on this, we hypothesize that AD is associated with dengue severity. Previous studies have reported associations between allergic diseases and asthma with dengue

severity.[40–42] However, studies on the relationship between AD, the role of Aedes saliva, and dengue severity in humans remain limited. This study aims to investigate the relationship between AD and dengue severity, and the contribution of immune responses to *Aedes aegypti* saliva exposure in dengue severity (DF vs DHF), particularly among children with AD, in whom the incidence of AD and the severity of dengue are higher.[4,29,30] The immune parameters assessed in this study include T-cell function in producing IFN-γ, IL-13, IL-10, and CCL2 in PHA-stimulated whole blood cultures from dengue patients with AD, which were exposed to *Aedes aegypti* salivary gland extract (SGE), for understanding the adaptive immune responses to mosquito saliva present within the lymphatic system, blood, and the skin, which possesses immunological memory against antigens and may enhance local innate immune responses in the skin. The findings are expected to enhance understanding of dengue pathogenesis by integrating immune responses to *Aedes aegypti* saliva, especially among individuals with AD, and may contribute to future dengue management strategies and the development of Aedes saliva-based vaccines for mosquito-borne disease prevention.

Materials and Methods

Study Design, Participants, and Procedure

A cross-sectional observational study was conducted from September 2024 to July 2025. Sixty-two samples of peripheral blood were collected from children aged 1–12 years diagnosed with secondary dengue infection, based on clinical manifestations and positive anti-dengue IgM and IgG serology, consecutively, after obtaining informed consent from the parents at Welas Asih Hospital and Al Islam Hospitals in Bandung. Dengue patients with comorbid with other infections, malnutrition, diabetes mellitus, systemic corticosteroid use, or a history of allergies, asthma, or allergic rhinitis without AD were excluded. Blood samples were obtained on days four to six after the onset of fever, concurrently with routine serial hematological testing. The classification of dengue severity into DF and DHF followed the WHO 2011 guidelines.[2] AD history was obtained from parents using the ISAAC questionnaire through structured interviews.[43,44]

All of the blood sampling procedures were conducted according to the policy of the Faculty of Medicine, Universitas Padjadjaran, Welas Asih Hospital, and Al Islam Hospital. Ethical approval was obtained from the Research Ethics Committee of Universitas Padjadjaran (approval number 24/UN6.KEP/EC/2024) and the Ethics Committee of Al Islam Hospital, Bandung (approval number 035/KEPK-RSAI/6/2024). The study adhered to the Declaration of Helsinki. Written informed consent was obtained from the parents of all participants.

Laboratory Procedures

Mosquito Salivary Gland Extract Preparation

Aedes aegypti salivary glands were obtained from five-day-old adult female mosquitoes that were reared from eggs at room temperature (20–27°C) with 80–90% humidity. The salivary glands were dissected from the mosquitoes and pooled, with every ten pairs stored in 100µL of PBS in microcentrifuge tubes.[45] The samples were kept at –80°C until protein extraction. Protein extraction was performed using a Hwanshin Power Sonic 420 bath sonicator for 45 minutes, followed by centrifugation at 10,000g for 10 minutes at 4°C. The supernatant was sterilized through a nitrocellulose membrane filter with a 0.02µm pore size.[46] Protein concentration was quantified using a Nanodrop spectrophotometer at 280nm.[47] Approximately 3µg of protein was extracted from each pair of glands. The SGE solution was stored at –80°C until use.[46]

Whole Blood Culture and Cytokine Assay

A total of 200 µL of heparinized whole blood sample was diluted in 600µL of Roswell Park Memorial Institute (RPMI) medium supplemented with 1% L-glutamine and 1% penicillin/streptomycin,[48] 100µL of phytohemagglutinin (PHA, 5 µg/100µL) was added,[49] and with or without the addition of 100µL of *Aedes aegypti* SGE (2 µg/100µL).[50] The cultures were incubated at 37°C with 95% humidity and 5% CO_2 for 24 hours.[49] Levels of cytokines IFN-γ, IL-13, IL-10, and CCL2 in the supernatant of unstimulated culture, PHA-stimulated culture, and PHA-stimulated culture exposed to SGE were measured using ELISA,[51] following the manufacturer's instructions (Bio-Techne R&D Systems Human DuoSet ELISA kits: IFN-γ, Cat. No. DY285B-05; IL-13, Cat. No. DY213-05; IL-10, Cat. No. DY217B-05; and CCL2, Cat. No. DY279-05).

Assessment of T-Cell Function Level

Since PHA is a mitogen that proliferates T and B cells,[49,52] and unstimulated whole blood cultures cytokine levels (without PHA and SGE), representing serum cytokine levels produced by various cells in the blood compartment, were used to assess baseline cytokine levels. The assessment of T-cell function level in response to dengue was performed by measuring the difference between supernatant cytokine levels in PHA-stimulated whole blood cultures and those in unstimulated cultures. The assessment of T-cell function level in response to dengue and SGE, as dengue patients inevitably mount an immune response to dengue, was performed by measuring the difference between supernatant cytokine levels in PHA-stimulated whole blood cultures exposed to SGE and those in unstimulated cultures.[51]

Statistical Analysis

Non-normally distributed data are presented as median, and the non-parametric tests: Wilcoxon, Chi-Square, Mann–Whitney, and Kruskal–Wallis are used for data analysis. All analyses were performed using SPSS version 29.0. Statistical significance was set at $P<0.05$.

Results

Impaired T-Cell Function in IL-13 Production Among Dengue Patients

First, we analyzed the differences in cytokine levels in supernatant PHA-stimulated whole blood cultures and unstimulated whole blood cultures in 62 pediatric dengue patients to assess T-cell function in response to Dengue in dengue patients. Table 1 shows that the median of cytokine levels of IFN-γ, IL-10, and CCL2 in the supernatants of PHA-stimulated whole blood cultures were significantly higher than those in unstimulated cultures ($p < 0.001$). This increase indicates that T-cells of dengue patients were actively producing IFN-γ, IL-10, and CCL2 in response to Dengue. However, as shown in Table 1, there was no significant difference in IL-13 levels between PHA-stimulated and unstimulated whole blood cultures ($p = 0.359$). This suggests an impairment in IL-13 production by T cells in patients with dengue.

Similarly, T cells of dengue patients also exhibited impaired IL-13 production upon exposure to *Aedes aegypti* SGE. Table 2 shows that there is no difference in T-cell function level between the response to dengue alone and the response to dengue and SGE exposure in dengue patients ($p = 0.53$). In contrast, SGE exposure significantly enhanced the T-cell function level in IFN-γ and IL-10 production ($p < 0.001$), while reducing T-cell function levels in CCL2 production ($p < 0.001$) in dengue patients. These findings indicate an impairment of Th2-immune responses among dengue patients.

Atopic Dermatitis May Increase the Incidence of DHF in Dengue Patients

Theoretically, AD may serve as a risk factor for severe dengue due to immune system dysregulation occurring in AD.[38] However, studies on the correlation between AD and dengue severity remain limited. This study employed a cross-sectional design as a preliminary investigation to examine the correlation between the incidence of AD and DHF, with a minimum sample for basic

Table 1 Differences in Cytokine Levels in Supernatant PHA-Stimulated Whole Blood Cultures and Unstimulated Whole Blood Culture Indicate T-Cell Function in Dengue Patients in Response to Dengue

Cytokine	Median of Cytokine Level (pg/mL)		p
	Unstimulated Culture (n=62)	PHA-Stimulated Culture (n=62)	
IFNγ	109.39	519.65	**<0.001**
IL10	9.70	78.41	**<0.001**
IL13	304.51	255.96	0.359
CCL2	404.50	1752.11	**<0.001**

Notes: Test: Wilcoxon. Bolded values indicate statistical significance. ELISA methods were performed for the supernatant of the 24 h whole blood culture cytokine assay. T-cell function level in response to dengue: the difference between supernatant cytokine levels in PHA-stimulated whole blood cultures and those in unstimulated cultures.

Table 2 T-Cell Function Level of Dengue Patients in Response to *Aedes aegypti* SGE

Cytokine	Median of T-Cell function Level (pg/mL)		p
	In Response to Dengue (n=62)	In Response to Dengue and SGE (n=62)	
IFNγ	379.08	518.87	**<0.001**
IL10	31.89	80.94	**<0.001**
IL13	2.77	−3.98	0.537
CCL2	1112.55	334.42	**<0.001**

Notes: Test: Wilcoxon. Bolded values indicate statistical significance. T-cell function level in response to dengue: the difference between supernatant cytokine levels in PHA-stimulated whole blood cultures and those in unstimulated cultures. T-cell function level in response to dengue and SGE: the difference between supernatant cytokine levels in PHA-stimulated whole blood cultures exposed to SGE and those in unstimulated cultures.

analysis without an a priori power calculation, as no previous studies have investigated the association between dermatitis and dengue severity. The findings indicate a possible correlation between the incidence of AD and DHF (p = 0.010). The incidence of DHF was three times higher in dengue patients with AD compared to those without AD (Table 3).

AD was observed in 14 of 62 patients (22.6%) included in this study. Information regarding AD was obtained through interviews with parents or guardians using the ISAAC questionnaire.[43,44] None of the dengue patients with AD reported persistent itching lasting more than six months within the past year, but all had experienced pruritic episodes before the age of two.

Dengue Patients with AD Exhibit Hypersensitivity to Aedes SGE and Impaired T-Cell Function in IFNγ Production in Response to Dengue

As shown in Table 4, dengue patients with AD exhibited a significantly higher median T-cell function level in IL-13 production in response to SGE exposure compared with patients without AD (p = 0.026). This suggests that AD patients

Table 3 Association Between AD and Dengue Severity

	Dengue Severity		Total	p	Prevalence Ratio
	DHF	DF			
With AD	7 (50%)	7 (50%)	14	**0.010**	3.0
Without AD	8 (16.7%)	40 (83.3%)	48		

Notes: Test: Chi-square. Bolded values indicate statistical significance.

Table 4 Serum Cytokine and T-Cell Function Level in Whole Blood Culture Supernatants of Dengue Patients with and without AD

	IFNγ		IL10		IL13		CCL2	
	Median (pg/mL)	p	Median (pg/mL)	p	Median (pg/mL)	p	Median (pg/mL)	p
Serum cytokine level								
With AD	141.77	0.312	13.67	0.698	437.98	0.372	436.67	0.775
Without AD	102.00		9.7		246.72		388.98	
T-cell function level in response to dengue								
With AD	325.12	0.126	31.56	0.674	5.96	0.590	781.53	0.625
Without AD	393.43		36.53		2.24		1158.76	
T-cell function level in response to dengue and SGE								
With AD	84.35	0.134	85.52	0.827	−17.28	**0.026**	524.82	0.775
Without AD	217.38		80.11		−7.94		327.19	

Notes: Test: Mann–Whitney U. Bolded values indicate statistical significance. Serum cytokine level: the level of unstimulated 24 h whole blood cultures cytokine measured by ELISA.

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may be associated with hypersensitivity to Aedes SGE, which is mediated through a Th2 immune response. Furthermore, both the median serum IL-13 levels and the T-cell function level in IL-13 production in response to Dengue alone tended to be higher among dengue patients with AD than in those without AD, although these differences did not reach statistical significance. Conversely, dengue patients with AD tended to have lower median T-cell function level in IFN-γ production than those without AD, both in response to Dengue alone and in response to Dengue and SGE exposure. This finding suggests a Th2-skewed immune response in dengue patients with AD.

DHF May Be Associated with Low T-Cell Function Levels in IFNγ Production in Response to Dengue Alone and High T-Cell Function Levels in CCL2 Production in Response to Dengue and SGE Exposure

To elucidate the immunopathogenesis underlying dengue severity and the potential role of *Aedes aegypti* SGE, we analyzed T-cell function in relation to dengue severity, that classified as DF and DHF in pediatric patients. Among the 62 dengue patients included in this study, 15 (24.19%) developed DHF. As shown in Table 5, the median by T-cell function level in IFNγ production in response to Dengue in DHF patients was significantly lower than that observed in DF patients ($p = 0.035$). This finding suggests that impaired T-cell function in IFNγ production in response to Dengue may be associated with the development of DHF.

Conversely, the median T-cell function level in CCL2 production in response to Dengue and SGE exposure was significantly higher in DHF patients than in DF patients ($p = 0.044$). This indicates that enhanced T-cell function in CCL2 production upon exposure to *Aedes aegypty* SGE components may contribute to disease severity in dengue patients.

Although not statistically significant, serum IFNγ levels tended to be higher in DHF patients compared to DF patients, suggesting possible differences in whole blood cytokine profile that may not be reflected at the T-cell functional level.

The Difference in the Pattern of High T-Cell Function Level in CCL2 Production in Response to Dengue and SGE Exposure, and Low T-Cell Function Level in IFNγ Production in Response to Dengue Alone, Among DHF Patients with and without AD

A distinct pattern of high T-cell function in producing CCL2 in response to Dengue and SGE among patients with DHF was observed when comparing DHF patients with and without AD, although the results did not reach statistical significance (Table 6). High T-cell function level in CCL2 production in response to SGE and dengue in DHF patients with AD was parallel to high IL-13 production; in patients with DHF without AD, it was parallel to IFN-γ production. Furthermore, Table 6 also demonstrates the different patterns of low T-cell function level in IFN-γ production in response to Dengue alone between DHF patients with and without AD. In DHF patients with AD, low T-cell function level in IFNγ production in response to Dengue was accompanied by high T-cell function level in IL-13 production. This may

Table 5 Cytokine Serum and T-Cell Function Level in Whole Blood Culture Supernatants DHF and DF Patients

Dengue Severity	IFNγ		IL10		IL13		CCL2	
	Median (pg/mL)	p	Median (pg/mL)	p	Median (pg/mL)	p	Median (pg/mL)	p
Serum cytokine level								
DHF	159.09	0.086	38.13	0.143	567.82	0.194	326.99	0.495
DF	104.19		6.02		233.00		413.58	
T-cell function level in response to dengue								
DHF	309.92	**0.035**	32.71	0.663	−15.91	0.297	1749.55	0.128
DF	408.27		31.08		3.68		823.49	
T-cell function level in response to dengue and SGE								
DHF	484.68	0.075	77.30	0.267	5.32	0.274	1190.74	**0.044**
DF	539.15		84.58		−7.51		309.68	

Notes: Test: Mann–Whitney U. Bolded values indicate statistical significance.

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Table 6 Cytokine Serum and T-Cell Function Level in Whole Blood Culture Supernatants DF and DHF Patients with and without AD

	IFNγ		IL10		IL13		CCL2	
	Median (pg/mL)	P	Median (pg/mL)	p	Median (pg/mL)	p	Median (pg/mL)	p
Serum cytokine level								
DHF with AD	159.09	0.364	25.93	0.307	536.53	0.199	459.77	0.856
DHF without AD	181.08		42.77		567.82		288.74	
DF with AD	124.46		3.60		437.98		413.58	
DF without AD	93.29		6.87		189.60		408.34	
T-cell function level in response to dengue								
DHF with AD	316.91	0.146	32.71	0.830	10.06	0.218	1673.01	0.300
DHF without AD	304.05		42.08		−32.25		2856.34	
DF with AD	452.92		10.39		3.68		458.00	
DF without AD	403.26		36.53		3.73		950.42	
T-cell function level in response to dengue and SGE								
DHF with AD	357.13	0.240	77.30	0.737	29.47	0.158	617.59	0.150
DHF without AD	737.60		81.53		−6.52		2497.18	
DF with AD	581.48		131.81		15.91		435.05	
DF without AD	537.53		80.11		−7.87		271.31	

Note: Test: Kruskal–Wallis.

reflect a Th2-skewed immune profile in DHF patients with AD. Conversely, in DHF patients without AD, low T-cell function level in IFNγ production in response to Dengue was accompanied by equally low T-cell function level in IL-13 production. This suggests a reduction of both Th1 and Th2 cytokine responses.

Discussion

T-Cell Function in Dengue Patients

The profile of T-cell function in response to Dengue in dengue patients in this study showed that T-cells were actively producing IFN-γ, IL-10, and CCL2. IFN-γ is a cytokine produced by $CD4^+$ T-cells, specifically those that differentiate into Th1 cells, as part of the adaptive immune response against viral infections. IFN-γ enhances the activity of $CD8^+$ T cells, which also produce IFN-γ, to establish an antiviral state. Furthermore, IFN-γ secreted by Th1 cells also promotes the maturation of M1 macrophages and NK cells to enhance viral elimination.[53]

Production of IFN-γ during the antiviral immune response is counterbalanced by the secretion of IL-10 from regulatory T-cells (Tregs) or even Th1 cells themselves.[54,55] In addition to being a Th2-associated cytokine, IL-10 serves as an anti-inflammatory cytokine that plays a key immunoregulatory role in preventing tissue damage due to excessive inflammation.[54] Binding of IL-10 to its receptor activates the JAK1–TYK2–STAT3 signaling pathway, leading to the expression of immunomodulatory genes. IL-10 suppresses T-cell proliferation and IFN-γ production by Th1 cells through the inhibition of APC function.[55] It reduces the expression of MHC class II and B7 molecules on monocytes and macrophages, thereby limiting antigen presentation to T-cells and inhibiting CD28-mediated co-stimulation, ultimately downregulating Th1 and Th2 activation.[20,56,57]

T-cells also produce CCL2,[15,16] which production correlates with increased levels of IFN-γ and TNF.[16,58] This chemokine promotes increased vascular permeability, as well as the migration and infiltration of monocytes and T-cells into infected tissues through several pathways, including tyrosine kinase, cAMP, MAPK (p38), PIP3, JNK, and calcium influx signaling.[15,16] The migration of monocytes to sites of infection is to carry out their function in promoting inflammation or facilitating tissue homeostasis.[59].

The impaired IL-13 production observed in T-cells of dengue patients in this study may be related to the Th1-polarized immune response that inhibits Th2 cytokine synthesis during Dengue infection.[60,61] IL-13 is a Th2 cytokine produced in

response to IL-4 stimulation. IL-4 interferes with Th1 activation by increasing the expression of the transcription factor GATA3, which suppresses IFN-γ production and TBX21 gene expression, thereby reducing Th1 activity. Conversely, IL-12, which promotes Th1 responses by upregulating the transcription factor T-bet and IFN-γ, suppresses GATA3 expression and reduces IL-4 production, thereby attenuating Th2 activation.[61]

This study also found that *Aedes aegypti* SGE stimulated T-cells to produce IFN-γ and IL-10. Although mosquito saliva primarily functions as a vasodilator, anticoagulant, and anti-inflammatory agent facilitating blood feeding,[62] it is also an immunogen capable of inducing inflammatory responses. Aedes saliva has been shown to enhance IFN-γ and IL-10 production at the skin site of mosquito bites.[25] Supporting this finding, previous studies on macrophage cultures stimulated with saliva and infected with Dengue virus also reported increased levels of IL-10 and IFN-β.[63] Several Aedes salivary proteins known to induce inflammation include AgBR-1, which promotes inflammatory cytokine secretion and immune cell recruitment,[64] and aegyptin (Aed-a3), which stimulates the release of granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-5, and IL-6.[65] However, these results contrast with studies reporting that *Aedes aegypti* saliva suppresses proinflammatory cytokine IFN-γ production following LPS stimulation in skin biopsy specimens from mosquito bite sites.[25] This discrepancy may be explained by differences in the type of sample and stimulant used between studies.

Exposure to *Aedes aegypti* saliva in PHA-stimulated whole blood culture in this study resulted in a significant decrease in CCL2 levels in the culture supernatant. This finding differs from previous reports suggesting that mosquito saliva increases CCL2 levels, leading to the recruitment of dendritic cells, Langerhans cells, and M2 macrophages at the bite site.[24,25] Previous studies have also shown that saliva increases the number of macrophages expressing the C–C chemokine receptor type 2 (CCR2) at the site of mosquito bites.[66] CCR2, which is the receptor for CCL2, is also expressed on T-cells in addition to macrophages.[67,68] Based on this, we hypothesize that saliva not only stimulates CCL2 production but also upregulates CCR2 expression on T-cells. The decrease in CCL2 levels observed in the supernatant of PHA-stimulated whole blood cultures exposed to Aedes saliva may result from greater CCL2 binding to CCR2 compared to the amount produced. Further study is required to confirm this hypothesis.

Exposure to *Aedes aegypti* SGE did not increase IL-13 levels in PHA-stimulated whole blood cultures from dengue patients in this study. In fact, there was a tendency toward decreased IL-13 levels in the supernatant. This finding contrasts with previous studies showing elevated IL-4 and IL-13 levels, as well as increased Th2 cell responses, at mosquito bite sites.[25] Prior study has demonstrated that the Aedes salivary protein SAAG-4 induces a Th2 immune response.[69] The inconsistency between these findings and ours may be due to our samples were derived from dengue patients, whose T-cells exhibit impaired IL-13 production due to a Th1 immune response to Dengue. We speculate that the observed reduction in IL-13 levels following Aedes SGE exposure in PHA-stimulated whole blood cultures is caused by the induction of IL-13 binding to its receptor on B-cells in the context of impaired IL-13 production by T-cells. IL-13 is known to stimulate IgE production by B-cells.[20,70] B-cell proliferation may occur in PHA-stimulated blood culture by T cell stimulation.[52,71] Further studies are needed to confirm IL13 production by T-cells in dengue patients and the binding of IL13 to B-cells in Aedes SGE exposure.

In this study, the assessment of T-cell function did not employ cell-specific methods for measuring cytokine production, such as flow cytometry. Instead, a more economical alternative approach was used to estimate T-cell cytokine responses by calculating differences in cytokine levels between PHA-stimulated cultures, which promote T-cell and B-cell proliferation, and unstimulated cultures without PHA. While this approach does not allow precise attribution of T-cell cytokine production, the contribution from other immune cells cannot be entirely excluded.

T-Cell Function in Atopic Dermatitis

In this study, 62 dengue patients were recruited as participants, of whom 14 children (22.6%) had a history of AD. The proportion of dengue patients with AD in this study falls within the reported prevalence range of childhood AD in Asia, which is 0.96% to 22.6%.[30] The proportion of DHF among the participants was 24.19%, which is consistent with previous dengue studies reporting that approximately 30% of hospitalized dengue patients develop DHF.[72] DHF represents a severe manifestation of dengue infection characterized by plasma leakage, and plasma leakage is one of the warning signs that may lead to shock and death.[2]

AD has been suggested as one of the risk factors of severe dengue, as patients with AD exhibit dysregulated immune responses that support Dengue virus replication and trigger subsequent inflammation.[38] The results of this study demonstrate a possible association between the incidence of AD and dengue severity. However, the small sample size may lead to selection bias. We have minimized potential confounding factors, such as age and timing of sample collection, by analyzing group differences. No significant differences were observed between age groups (12 to 59 months and 5–12 years) or between sampling at the end of the febrile and critical phases (data not shown). Nutritional status was pre-controlled by recruiting only participants with normal nutritional status. A larger sample study, multiple study sites, and case-control or cohort study method would be needed to more reliably assess any relationship between atopic dermatitis and the severity of dengue.

Although no previous studies have investigated the association between atopic dermatitis and dengue severity, some previous studies have reported that AD increases the risk of skin infections, systemic infections, and complications in infection.[73–75] The occurrence of severe infection, particularly in dengue, among individuals with AD is related to immune dysregulation. In the skin of patients with moderate to severe AD, there is an increase in dendritic cells, mast cells, and M2 macrophages, which are target cells for viral replication. Furthermore, AD patients exhibit altered NK cell cytotoxicity, increased type 2 innate lymphoid cells (ILC2), and Th2-skewed immune responses.[38]

The Th2-skewed immune responses in AD,[38] may interfere with Th1 responses.[39,61] Lower levels of IFN-γ have been observed in cultured blood samples from AD patients.[76] Impaired Th1 responses have been linked to dengue severity.[8–11,22,77] Although no statistically significant differences were found, this study shows a tendency toward low T-cell function level in producing IFN-γ in response to Dengue among dengue patients with AD compared to those without AD, and also among DHF patients with AD. This finding indicates that immune dysregulation in AD may contribute to the development of DHF.

In addition to immune dysregulation, dengue patients with AD also exhibited a tendency toward hypersensitivity to *Aedes aegypti* SGE. The T-cell function level in IL13 production in response to Dengue and SGE in dengue patients with AD was higher than that of dengue patients without AD. This finding suggests that AD patients may be associated with type I hypersensitivity to Aedes SGE.[27] As discussed earlier, mosquito saliva induces IL-13 production and stimulates IL-13 binding to B-cells. T cells in dengue patients with AD remain functional in producing IL-13 upon Aedes SGE exposure under conditions of Th2 response disruption due to Th1-skewed immune response in response to Dengue, compared to dengue patients without AD. Based on this, we assume that T-cells in dengue patients with AD remain active to produce IL-13 when exposed to SGE and are accompanied by IL-13 binding to B cells.

Type I hypersensitivity reactions are mediated by Th2 cytokines, particularly IL-4 and IL-13, which promote IgE production.[78] Patients with AD tend to produce IgE in response to environmental proteins even in small quantities, including mosquito saliva proteins.[27,79] Salivary allergens are presented by T-cells to B cells via CD40–CD40L interactions and together with IL-13, stimulate IgE production by B cells.[20,70] Hypersensitivity to Aedes SGE may be mediated through the Aegyptin protein, which also acts as an allergen. Aegyptin as an allergen promotes the migration of inflammatory cells that serve as Dengue virus targets, thereby increasing the risk of DHF.[80] SGE exposure also seems to induce higher CCL2 production in dengue patients with AD compared to those without AD. This finding aligns with previous studies reporting that CCL2 plays an important role in the migration of inflammatory cells in AD and can be stimulated by Aedes saliva.[81,82]

Mosquito saliva can trigger histamine release, increasing capillary permeability and inducing the migration of inflammatory cells through two mechanisms: IgE-mediated hypersensitivity reactions and non-IgE–mediated inflammatory responses. In AD patients, histamine release due to mosquito saliva exposure may occur through both pathways.[82] Individuals with IgE-mediated hypersensitivity to mosquito saliva proteins exhibit larger wheal diameters following mosquito bites.[82,83] Larger wheal diameters are associated with higher numbers of dendritic cells and M2 macrophages.[25]

Upon saliva exposure, IL-10 may act as a Th2 cytokine that supports Th2 immune activation.[84] T-cell function in producing IL-10 upon Aedes SGE exposure in dengue patients with AD seems higher than in those without AD, while T-cell function in producing IFN-γ upon saliva exposure tended to be lower in dengue patients with AD than in those without AD. This is in line with the Th2-biased immune tendency of AD patients,[85] although the results of this study did not reach significance. This result is consistent with previous studies reporting an increase in IFN-γ levels at mosquito bite sites,[25] although the Th1 response in AD patients is impaired.[31,86]

T-Cell Function in Relation to Dengue Severity

T-cell function in response to Dengue and SGE in patients with DHF showed significantly higher CCL2 levels compared to those with DF. This finding suggests a role of *Aedes aegypti* SGE in the dengue severity. The *Aedes aegypti* SGE may contribute to the development of DHF by enhancing CCL2 production and CCR2 expression, which induce the migration of inflammatory cells to the bite site. Aedes saliva has been shown to upregulate both CCL2 and CCR2 expression at mosquito bite sites.[24,66] The NeSt1 protein in Aedes saliva stimulates CCL2 production.[64,87,88] High CCL2 levels enhance vascular permeability, which is essential for macrophage migration, and also induce basophils to secrete histamine, further increasing vascular permeability.[16,89]

CCL2 production is associated with increased IFN-γ levels.[16,58] In DHF patients without AD, there was a tendency for higher T-cell function in producing CCL2 in response to Dengue and Aedes SGE, accompanied by high production of IFN-γ, which is a Th1 cytokine. Aegyptin, an immunogenic component of Aedes saliva, induces the production of inflammatory cytokines such as GM-CSF, IFN-γ, IL-5, and IL-6 at the bite site and promotes the recruitment of inflammatory cells that serve as Dengue virus target cells.[65] In contrast, DHF patients with AD showed high T-cell production of CCL2, accompanied by high IL-13, which is a Th2 cytokine. Aegyptin, which can also act as an allergen, promotes the migration of inflammatory cells that serve as dengue virus targets, which can increase DHF incidence.[80] AD patients exhibit type I hypersensitivity tendencies to Aedes saliva.[27] Saliva's mosquito contributes to the enhancement of viral infection through increased vascular permeability rather than through immune responses directed against the saliva itself.[26] Both Th1 and Th2 immune responses in response to SGE may be associated with DHF.

This study also found that DHF patients exhibited impaired T-cell function in producing IFN-γ in response to Dengue. Low IFN-γ production, which indicates a weakened Th1 immune response, is associated with an ineffective adaptive immune defense against the virus and contributes to DHF development.[53] But there is a distinct pattern in low T-cell function in IFN-γ production in DHF patients without and with AD. In DHF patients without AD, low T-cell function in IFN-γ production was accompanied by low T-cell function in IL-13 production. This reduction of both Th1 and Th2 cytokine production may result from defective antigen presentation by dengue-infected dendritic cells, leading to inadequate T-cell activation.[90] On the other hand, the low T-cell function in IFN-γ production observed in DHF patients with AD may correlate with a Th2-skewed immune response. In these patients, low T-cell function in IFN-γ production in response to Dengue was accompanied by high T-cell function in IL-13 production.

Ineffective adaptive immune responses lead to further innate immune activation and aggravate dengue-associated inflammation.[8–11,22,77] Although the results were not statistically significant, serum IFN-γ levels in DHF patients seem higher than those in DF patients. The absence of a significant difference in serum cytokine levels between patients with DF and DHF in this study may be attributed to the inclusion of only DHF grade 1 and 2 cases, which are characterized by plasma leakage without hemodynamic disturbance and are therefore classified as non-severe dengue, similar to DF. No DHF grade 3 or 4 cases meeting the inclusion criteria were observed in this study. Previous studies have demonstrated differences in serum cytokine profiles between patients with severe dengue and those with non-severe dengue.[91,92]

High serum IFN-γ level reflects increased systemic inflammation and is a marker of dengue severity.[91,92] IFN-γ is a key antiviral cytokine produced not only by T-cells but also by innate immune cells such as macrophages and NK cells.[93,94] However, excessive IFN-γ activity can cause tissue damage, necrosis, and inflammation, contributing to disease pathology.[93] High IFN-γ levels during dengue infection can stimulate macrophages to secrete TNF, IL-6, CCL2, and other cytokines that increase vascular permeability. IFN-γ also recruits inflammatory cells, further enhancing vascular leakage,[93,95] and can induce endothelial damage.[96]

The higher serum IFN-γ levels observed in DHF, which are likely largely secreted by innate immune cells, may occur as a response to ineffective adaptive immunity, are accompanied by higher serum IL-10 levels. IL-10, as an anti-inflammatory cytokine, is secreted to suppress T-cell proliferation and IFN-γ production,[20,55,97,98] and prevent excessive tissue damage caused by inflammation during infection. Elevated IFN-γ stimulates IL-10 secretion by T-cells, NK cells, macrophages, and other immune cells.[54,97] Previous studies have identified IL-10 as one of the biomarkers of dengue severity.[53,91,98]

Conclusion

We found that *Aedes aegypti* SGE may contribute to the occurrence of DHF in dengue infection. *Aedes aegypti* SGE may be associated with the migration of inflammatory cells that serve as target cells for viral replication through CCL2 production, which appears to be driven by Th2 immune response in patients with DHF with AD, and by Th1 immune response in patients with DHF without AD. The dengue severity may further correlate with low function of T-cells in producing IFN-γ during the immune response to dengue virus infection. The impairment of IFNγ production may be related to virus-mediated disruption of T-cell activation and Th2-skewed immune response. An ineffective adaptive immune response may lead to uncontrolled viral replication, which triggers higher innate cellular responses and aggravates dengue inflammation. The observed association between AD and DHF provides a basis for future studies to further elucidate the relationship between atopic dermatitis and dengue severity, which can offer recommendations to clinicians in managing dengue in patients with AD. Further studies are also needed to understand the mechanisms underlying cytokine production following SGE exposure to support the development of Aedes saliva–based vaccines.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; All authors took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure Statement

The author(s) report no conflicts of interest in this work.

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