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Anti-DENV IgE correlates with dengue severity and triggers FcεRI-dependent basophil activation inhibited by Omalizumab

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Severe dengue, marked by plasma leakage, is often linked to secondary heterotypic dengue virus (DENV) infection. While mast cells and basophils contribute to dengue pathogenesis, the role of anti-DENV IgE remains unclear. Here, we investigated whether anti-DENV IgE promotes FcεRI-dependent basophil activation, potentially contributing to disease severity. Plasma from dengue fever (DF, n = 42) and dengue hemorrhagic fever (DHF, n = 56) patients, collected at febrile, defervescence, and convalescent phases, were analyzed using an in-house IgE-capture ELISA developed to detect antibodies against all four DENV serotypes. Functional assays employed RS-ATL8, a human FcεRI-expressing basophil reporter cell line, to assess IgE-mediated activation following DENV antigen cross-linking. The anti-IgE monoclonal antibody Omalizumab was used to evaluate FcεRI dependence. Anti-DENV IgE was detected in both DF and DHF, peaking at defervescence and significantly higher in DHF. Total IgE was elevated but did not differ between groups. About one-third of anti-DENV-IgE-positive plasma samples induced RS-ATL8 activation upon DENV challenge, an effect abolished by omalizumab. These findings indicate a potential pathogenic role of anti-DENV IgE and provide a rationale for further investigation of IgE-targeted interventions.

Keywords IgE, Dengue, Basophil, Mast cell, Omalizumab

Dengue viral infection is a major public health problem worldwide, particularly in subtropical and tropical countries[1]. There are four virus serotypes, which are DENV-1, DENV-2, DENV-3 and DENV-4[2]. Dengue infection results in a wide spectrum of disease outcomes from asymptomatic, undifferentiated febrile illness, dengue fever (DF), dengue hemorrhagic fever (DHF), and the most severe form, dengue shock syndrome (DSS)[2,3]. DHF and DSS are characterized by increased vascular permeability and plasma leakage, which can progress to shock and death[2,3]. Plasma leakage in DHF almost always occurs after fever subsides, when viral load is undetectable and cytokines in serum are at their highest level. In addition, epidemiologic findings repeatedly showed that DHF occurs more commonly in secondary heterotypic dengue infection, suggesting a detrimental adaptive immune response in secondary infections[4,5]. While pathogenic immune responses from IgG-mediated antibody-dependent enhancement (ADE) have been shown to play a crucial role in the severity of dengue disease[6–8]. The role of other immunoglobulin isotypes, particularly IgE, which is known to promote vascular leakage via activation of mast cells and basophils through FcεRI in allergic anaphylaxis context[9], remains poorly understood in dengue pathogenesis.

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Anti-DENV IgE antibodies have been reported in dengue patients, particularly in secondary infections[10–12], and their levels tend to correlate with disease severity[13,14]. One study associated dengue severity with the ratio of DENV-specific to total IgE (S/T ratio) and plasma chymase, although absolute IgE levels did not differ between severity groups[15]. IgE-capture assays have also been proposed for early diagnosis[11,12,16]. However, the functional role of IgE in mast cell or basophil activation remains unclear, as no human studies have examined FcεRI-mediated effects or IgE-targeted interventions. Evidence is limited to a mouse model, where IgE from DENV-2-immunized mice sensitized mast cells to release histamine[17], but its relevance to human disease is uncertain[18]. Collectively, these findings raise the possibility that anti-DENV IgE may contribute to pathogenesis by promoting mast cell degranulation and plasma leakage, thereby influencing disease severity.

The role of mast cells in DENV infection became more apparent in the past decade[19,20]. Mast cells have been found to be infected with DENV in secondary infection with pre-existing antibodies[19,21,22]. Mast cells contribute to immune surveillance and response to DENV by activating host anti-viral response and recruitment of natural killer (NK) and natural killer T (NKT) cell to the site of infection[20,23]. In contrast, several recent studies demonstrated the pathogenic role of mast cells in DENV infection by activation of endothelial cells, promote excessive coagulation and inflammation and inducing plasma leakage[20,24,25]. Level of several mast cell proteases and inflammatory mediators such as leukotriene, tryptase, chymase, vascular endothelial growth factor (VEGF) and platelet activating factor (PAF) have been implicated in dengue disease severity[15,24–30]. Mast cells mediators induced endothelial cells dysfunction and vascular leakage was found to be elevated in serum of patients with DHF and DSS, when compared to DF[24,25,30]. In addition, an increase of urinary histamine excretion was found in patients with DHF when compared to healthy controls[31]. Recent study showed that anti-DENV IgG can enhance mast cell degranulation through FcγR[21]. *In vitro*, the anti-E antibody 4G2 increased DENV2-induced degranulation while, *in vivo*, pre-sensitized mice exhibited elevated hematocrit[21]. The effect was lost in FcγRIII-deficient mast cells, confirming a key role for FcγRIII in IgG-mediated enhancement of DENV-induced MC degranulation[21]. Furthermore, recent studies demonstrated the use of mast cell stabilizers can block DENV-induced mast cells degranulation *in vitro* and reduce severity of dengue disease *in vivo*[28,32,33]. Importantly, unlike basophils, mast cells are long-lived, tissue-resident effector cells strategically located near blood vessels and mucosal surfaces, where they respond rapidly to infection and injury by releasing preformed mediators. Their tissue localization, however, makes them difficult to access and study directly in humans, particularly during acute dengue infection.

While mast cell activation has been implicated in the severity of DENV infection, the contribution of basophils to dengue immunopathogenesis remains largely unexplored. Basophils, although less abundant, are circulating IgE-bearing granulocytes that share the FcεRI-dependent activation pathway with mast cells. They release histamine and cytokines that promote Th2 polarization and can amplify inflammatory responses[34]. Given that basophils are uniquely positioned as circulating IgE-responsive innate effectors, their potential role in dengue deserves closer investigation[34].

Here, we hypothesize that anti-DENV IgE generated during natural human dengue infection can mediate basophil activation and degranulation via an FcεRI-dependent pathway, potentially contributing to disease severity. To test this, we quantified anti-DENV IgE and total IgE levels in well-characterized DF and DHF patient cohorts, assessed their temporal dynamics, and used a human FcεRI-expressing basophil reporter cell line (RS-ATL8) to examine functional activation by patient plasma. Finally, we evaluated whether omalizumab (an FDA-approved anti-IgE monoclonal antibody) can inhibit anti-DENV IgE–mediated basophil degranulation. This study provides novel insights into Anti-DENV IgE;FcεRI-dependent basophil activation potentially contributes to dengue immunopathogenesis. These findings complement previous research on mast cell-mediated mechanisms in dengue and suggest that basophils may also contribute to the complex immunopathology of the disease. Importantly, we also highlight IgE as a potential therapeutic target for modulating severe dengue outcomes.

Results

Study design and study population

To investigate the levels and kinetics of anti-DENV IgE in individuals with dengue fever (DF) and dengue hemorrhagic fever (DHF), samples from 42 DF patients and 56 DHF patients were examined during the febrile, defervescence, and convalescent phases (Fig. 1a and supplementary table 1). In addition, total IgE levels, mast cell mediators, and in vitro mast cell activation were assessed on the day of defervescence (Fig. 1a). The demographic characteristics of patients, including age, gender, current dengue virus serotype, and DENV infection status (primary or secondary infection), were comparable between the DF and DHF groups (Table 1 and Fig. 1b-e). Most patients in both groups experienced secondary dengue infections, 34 of 42 cases (80.9%) in DF and 53 of 56 cases (94.6%) in DHF (Table 1 and Fig. 1e). The average age was also comparable between the two groups, 25.31 years in DF and 25.20 years in DHF (Table 1 and Fig. 1b). In the DF group, 26 of 42 patients (61.9%) were male and 16 (38.1%) were female, whereas in the DHF group, 23 of 56 patients (41.1%) were male and 33 (58.9%) were female (Table 1 and Fig. 1c). Notably, DENV-3 was the most prevalent serotype in both DF (10 of 42 cases, 47.6%) and DHF (19 of 56 cases, 33.9%), while DENV-2 was the least common (5 of 42 cases, 11.9% in DF; and 10 of 56 cases, 17.9% in DHF) (Table 1 and Fig. 1d). To investigate the contribution of IgE in DENV infection, we first compared total IgE level between DF and DHF at D0. Total IgE was elevated above the normal range in most patients in both groups (59.5% of DF and 64.3% of DHF patients had total IgE > 120 IU/mL) (Fig. 1f). However, there was no significant difference in total IgE concentrations between the two groups (Fig. 1g). Antigen-specific IgE level would provide a better evidence, however, a commercially assay is not available.

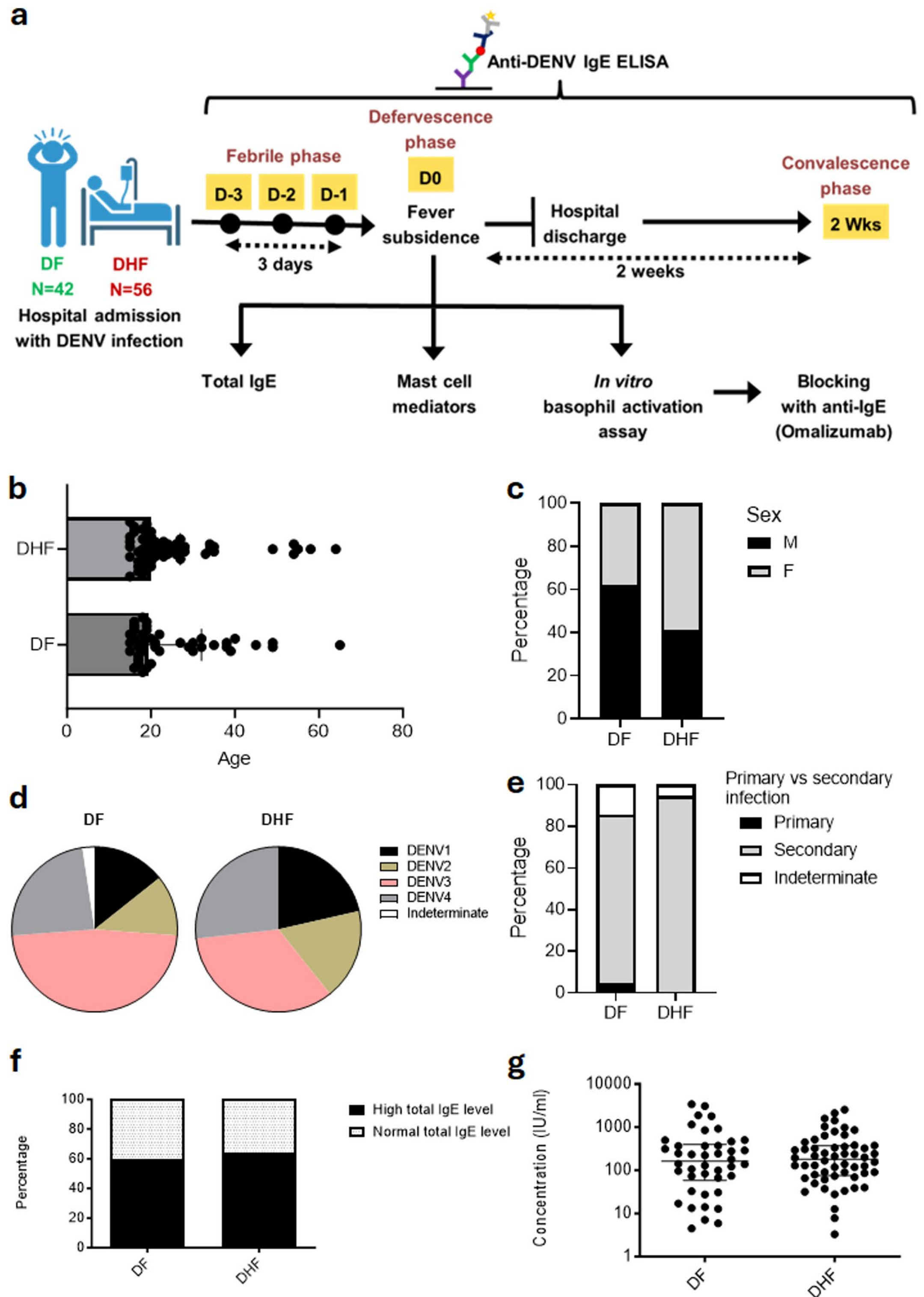

Fig. 1. Experimental design and validation of sample matching. (**a**) Experimental design and blood sample collection over the course of dengue viral infection in DENFREE cohort. (**b**–**e**) Demographic characteristics of patients, including age (**b**), gender (**c**), current dengue virus serotype (**d**), and DENV infection status (primary or secondary infection) (**e**) in DF and DHF groups. (**f**) Percentage of DF and DHF patients that showed high level and normal level of total IgE (reference range = 0–120 IU/ml). (**g**) Total IgE level of DF, and DHF patients at the defervescence (D0) phase. Each dot represents the total IgE level of each patient, and the line represents the median and interquartile range of each group. Each dot represents an individual patient, and the line indicates the median and interquartile range for each group. The Mann–Whitney U test (b, g) was used for statistical analysis, with $p \leq 0.05$ considered statistically significant.

	DF	DHF
Gender Male Female	26 (61.9%) 16 (38.1%)	23 (41.1%) 33 (58.9%)
Average age	25.31	25.20
DENV serotype DENV-1 DENV-2 DENV-3 DENV-4 Indetermined	6 (14.3%) 5 (11.9%) 20 (47.6%) 10 (23.8%) 1 (2.4%)	12 (21.4%) 10 (17.9%) 19 (33.9%) 15 (26.8%) 0 (0%)
DENV infection status Primary Secondary Indetermined	2 (4.8%) 34 (80.9%) 6 (14.3%)	0 (0%) 53 (94.6%) 3 (5.4%)

Table 1. Demographic characteristics of DF and DHF patients.

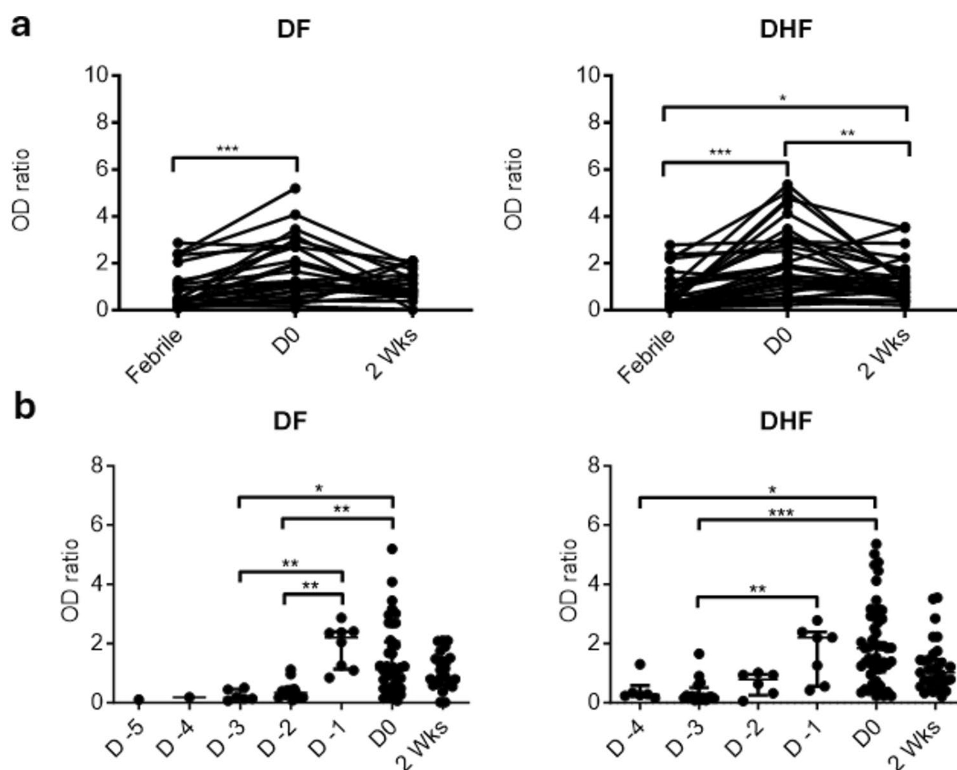

Fig. 2. Kinetics of anti-DENV IgE over the course of infection. (**a**) The anti-DENV IgE OD ratio over the course of dengue virus infection including febrile, defervescence (D0), and convalescent (2 Wks) phases. The left panel shows DF patients, and the right panel shows DHF patients, with each line connecting data from individual patients across time points. (**b**) The OD ratio of anti-DENV IgE over the course of dengue virus infection, designated as days -5, -4, -3, -2, -1, 0, and F1, where day 0 represents the date of defervescence. Each dot represents an individual patient's anti-DENV IgE OD ratio, and the line indicates the median and interquartile range for each group. The Wilcoxon signed-rank test (**a**) and Kruskal–Wallis test with Dunn's multiple-comparisons post hoc test (**b**) were used for statistical analysis, with $p \leq 0.05$ considered statistically significant (*$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$).

Kinetics of anti-DENV IgE over the course of infection

To investigate the levels of anti-DENV IgE antibodies, we developed an IgE captured ELISA assay specific to DENV of all 4 serotypes. The levels of anti-DENV IgE antibodies over the course of dengue infection, as quantified by both the OD ratio (Fig. 2a, b) and the titer of anti-DENV IgE antibodies (Supplementary Fig. 1a, b), were evaluated in 42 DF patients and 56 DHF patients at different stages of infection including febrile, defervescence, and convalescent phases using heparinized plasma.

The kinetics study revealed that the OD ratio of anti-DENV IgE antibodies in both DF and DHF patients was highest during the defervescence phase (DF: median: 1.120, IQR = 0.5527–2.057; DHF: median: 1.841, IQR: 1.082–2.952), showing statistically significant differences compared to the febrile (DF: median: 0.4297, IQR:

0.1803–1.109, $p < 0.0001$; DHF: median: 0.3254, IQR: 0.1897–0.9429, $p < 0.0001$) and convalescent phases (DF: median: 0.9000, IQR: 0.6188–1.634, $p = 0.0946$; DHF: median: 1.078, IQR: 0.6194–1.597, $p = 0.0013$) (Fig. 2a). When tracking the daily progression of each patient throughout the course of infection designated as days -5, -4, -3, -2, -1, 0, and 2 weeks, with day 0 representing the date of defervescence, the data demonstrated a clear trend of increasing anti-DENV IgE levels, peaking at day 0, followed by a decline during the convalescent phase in both DF and DHF patients (Fig. 2b).

Similarly, analysis of anti-DENV IgE titers at these time points showed that titers also peak during the defervescence phase, with statistically significant differences compared to the febrile and convalescent phases (Supplementary Fig. 1a). Tracking the daily progression in the same time-course manner reinforced the pattern of an ascending trend in anti-DENV IgE titers, peaking at day 0 and subsequently declining during convalescence in both DF and DHF patients (Supplementary Fig. 1b). A subgroup analysis in only secondary dengue infection also exhibited a similar temporal pattern (Supplementary Fig. 2a, c).

Collectively, these findings confirm the presence of anti-DENV IgE antibodies in dengue-infected individuals, with levels peaking during the defervescence phase, the timepoint co-incide with the beginning of critical period, and declining thereafter, suggesting a potential association between anti-DENV IgE levels and the clinical course of dengue infection.

Anti-DENV IgE levels show an association with disease severity

To investigate the potential association between anti-DENV IgE antibody levels and disease severity, the OD ratio (Fig. 3a, b) and antibody titers (Fig. 3c, d) were measured in 42 patients with dengue fever (DF) and 56 patients with dengue hemorrhagic fever (DHF), day 0 and the results were compared between the two groups.

Among these patients, 26.19% of DF cases and 37.5% of DHF cases tested positive for anti-DENV IgE antibodies, defined by an OD ratio greater than 2 (Fig. 3a). Importantly, during the defervescence phase, DHF patients exhibited a significantly higher OD ratio of anti-DENV IgE antibodies (median: 1.840, IQR: 1.038–2.919) compared to DF patients (median: 1.120, IQR: 0.5527–2.057, $p = 0.0449$) (Fig. 3b). By comparison, during the febrile phase, anti-DENV IgE levels did not differ significantly between DF and DHF (Supplementary Fig. 3).

A similar trend was observed when analyzing antibody titer. Detectable levels of anti-DENV IgE were found in 30.90% of DF patients and 48.20% of DHF patients (Fig. 3c). At day 0, DHF patients tended to associate with higher median antibody titers (median: 4.522, IQR: 1–160.1) compared with DF patients (median: 0, IQR: 1–19.32), although the difference was only marginally significant ($p = 0.0525$) (Fig. 3d). Subgroup analysis of secondary infection did not reach statistical significance, likely due to small sample size (Supplementary Fig. 2b, d).

In addition, there was no significant correlation between anti-DENV IgE and total IgE levels in both DF ($p = 0.645$, $r = -0.073$) and DHF ($p = 0.195$, $r = 0.176$) patients (Fig. 3e). This suggests that the elevated anti-DENV IgE in DHF is not merely due to an overall atopic tendency or polyclonal IgE rise, but rather a specific anti-DENV-IgE response.

Because IgE is known to activate mast cells and basophil degranulation, we next assessed plasma mast cell and basophil mediators (tryptase and chymase) at day 0. Surprisingly, unlike previous reports which found correlation of these mediators with disease severity[15,23–25,30], no significant differences in median tryptase and chymase levels between DF and DHF patients were observed in our cohort (Fig. 3f). The discrepancy might be due to the difference in timing of sample collection as these mediators are known to have short half-life and rapid decline in plasma level[35].

Anti-DENV IgE mediated FcεRI dependent basophil activation

To investigate the potential role of anti-DENV IgE in activating basophils via FcεRI in a more direct and controlled fashion in vitro, we used RS-ATL8 cells, a rat basophil cell line engineered to express the human FcεRI α/β/γ subunits and a luciferase reporter gene. We first compared RS-ATL8 activation priming with anti-DENV IgE-positive and -negative plasma samples, then cross-linking with DENV. As expected, priming with anti-DENV IgE positive plasma showed significantly higher luciferase activity (median: 1.364, IQR: 0.8731–2.051) compared to those with anti-DENV IgE negative plasma in DF (median: 0.5559, IQR: 0.4482–1.197, $p = 0.046$) in DF (Fig. 4a). A similar trend was observed in DHF, with higher luciferase expression when priming with anti-DENV IgE positive plasma (median: 1.527, IQR: 0.9738–2.803) than those with anti-DENV IgE negative plasma (median: 0.8657, IQR: 0.7513–1.171, $p = 0.030$) (Fig. 4a). Together, these results suggested that some of the samples with anti-DENV IgE-positive are able to activate basophils upon DENV crosslinking.

Among the anti-DENV IgE-positive plasma, only a proportion were able to activate basophils. Of those anti-DENV IgE-positive, a slightly higher proportion of DHF (34.62%) as compared to DF (30.77%) could induce robust basophil activation when challenged with DENV (as defined by the increased in luciferase activity more than twice from background without plasma priming (Supplementary Fig. 4b-4)) (Fig. 4b).

Omalizumab blocks dengue plasma induced basophils activation

To investigate if the activation of the RS-ATL8 basophil cell line by anti-DENV IgE and DENV is mediated through the IgE-FcεRI pathway, a blocking experiment was conducted using anti-human IgE (Omalizumab) which binds to Cε3 portion of IgE, preventing its binding to FcεRI.

In anti-DENV IgE-positive samples capable of inducing RS-ATL8 cell activation, Omalizumab treatment markedly suppressed RS-ATL8 activation, reducing luciferase fold induction from 3.173 (IQR: 2.514–4.786) to 0.8563 (IQR: 0.6631–0.9244; $p = 0.0001$). In DHF patients, a comparable reduction was observed, with activation decreasing from 3.173 (IQR: 2.726–4.793) to 0.7999 (IQR: 0.6050–0.9244, $p = 0.0020$). Similarly, but to a lesser extent in DF samples, luciferase fold induction declined from 2.642 (IQR: 2.333–4.484) to 0.8369 (IQR: 0.7884–1.039, $p = 0.0625$) following Omalizumab treatment (Fig. 4c). Within all DF and DHF samples, (Supplementary

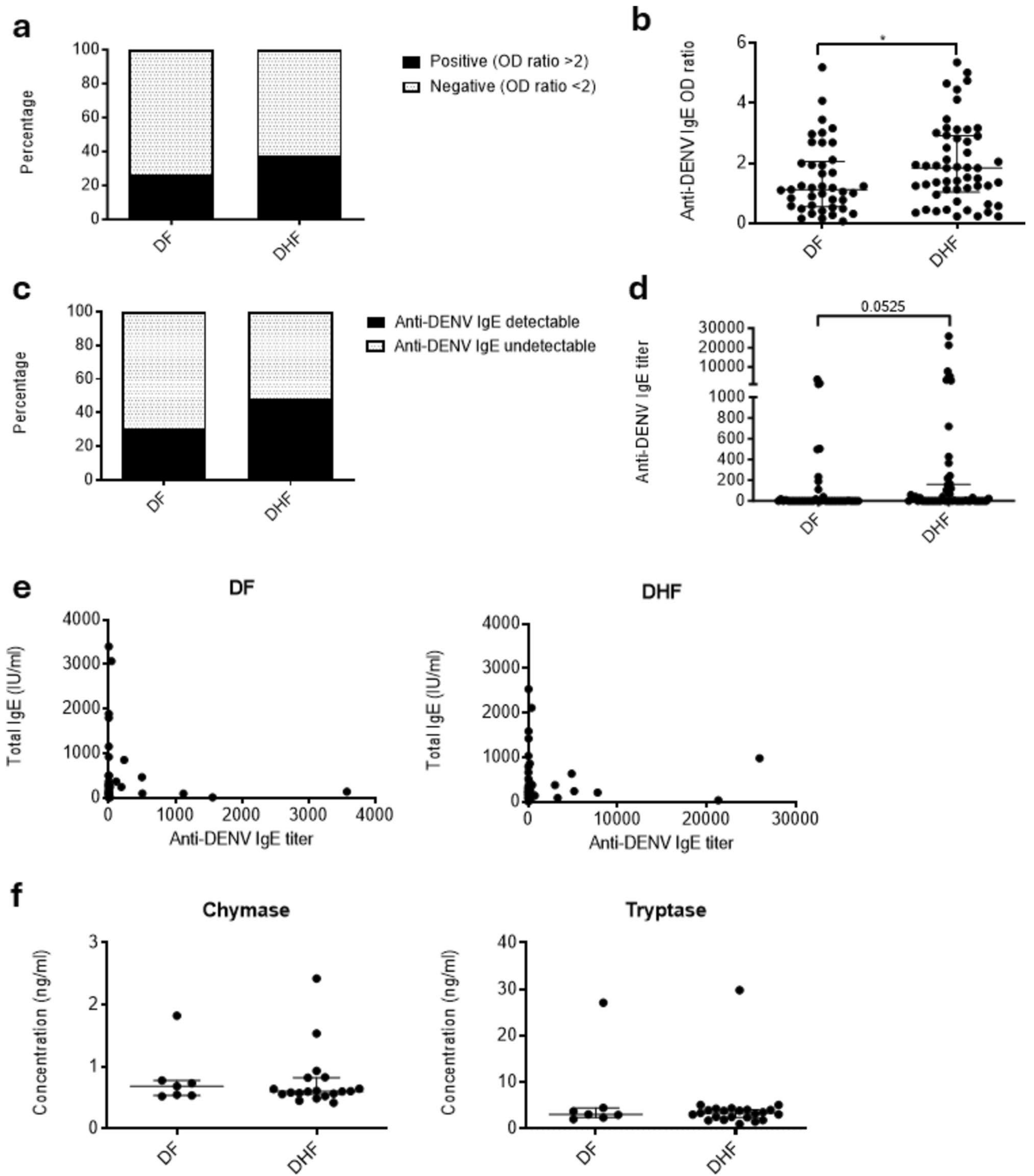

Fig. 3. Anti-DENV IgE levels show an association with disease severity. The percentages of DF and DHF patients with measurable anti-DENV IgE levels based on OD ratio (**a**) and antibody titer (**c**). (**b**) The OD ratio and (**d**) titer of anti-DENV IgE in DF and DHF patients during the defervescence (D0) phase. (**e**) Correlation between anti-DENV IgE titer and total IgE level in DF and DHF patients. (**f**) Level of mast cell mediators including chymase and tryptase in DF and DHF patients. Each dot represents an individual patient's anti-DENV IgE OD ratio (**b**) or titer (**d**), or mast cell mediators (**f**) and the line indicates the median and interquartile range for each group. The Mann–Whitney U test (**b–d**, and **f**) and Spearman's rank correlation test (**e**) were used for statistical analysis, with $p \leq 0.05$ considered statistically significant (* $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$).

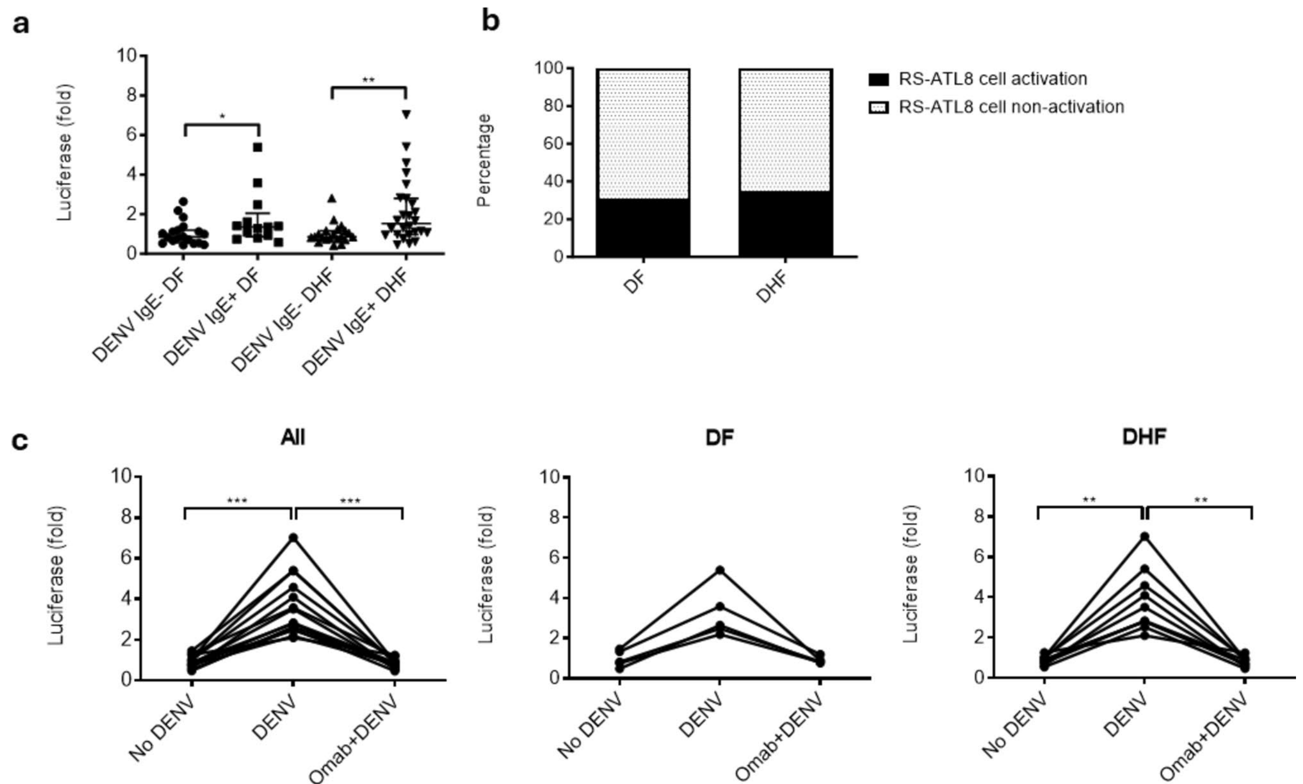

Fig. 4. Anti-DENV IgE mediated FcεRI dependent basophil activation. (**a**) The level of luciferase activity in RS-ATL8 cells that were sensitized with plasma from DF and DHF patients who tested negative or positive for anti-DENV IgE, followed by cross-linking with DENV. Each dot represents the level of luciferase expression of each patient, and the line indicates the median and interquartile range of each group. (**b**) Percentage of DF and DHF that mediated RS-ATL8 cell activation upon sensitization with anti-DENV positive heparinized plasma and challenge with DENV. (**c**) Luciferase activity of RS-ATL8 cells sensitized with plasma from all patients, and from DF and DHF subgroups, in the presence or absence of anti-human IgE or Omalizumab. Each line connects data obtained from the same patient under different experimental conditions. The Mann–Whitney U test (**b**) and Wilcoxon signed-rank test (**c**) were used for statistical comparisons, with $p \leq 0.05$ considered statistically significant (*$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$).

Fig. 4d) and within anti-DENV IgE positive DF and DHF samples (Supplementary Fig. 4e), Omalizumab-treated plasma also significantly reduced RS-ATL8 activation. In contrast, no significant effect was detected in anti-DENV IgE–negative samples (Supplementary Fig. 4f.).

Together, these findings suggest that the activation of RS-ATL8 cells by anti-DENV IgE-containing plasma and DENV is mediated through the IgE-FcεRI pathway in both DF and DHF patients and can be blocked with omalizumab.

Discussion

Our study identifies a potential role for anti-DENV IgE in dengue pathogenesis through FcεRI-dependent basophil activation. Anti-DENV IgE levels peaked at defervescence, coinciding with the onset of the critical phase, and showed an association with disease severity. Plasma containing anti-DENV IgE activated the FcεRI-expressing basophil reporter line RS-ATL8 upon DENV exposure, and this activation was effectively inhibited by Omalizumab. These findings suggest that anti-DENV IgE can engage the FcεRI pathway and may contribute to dengue immunopathogenesis, broadening current understanding of antibody isotype diversity in dengue immunopathogenesis[10–15].

Previous studies have associated elevated mast cell mediators including chymase, tryptase, leukotrienes, VEGF, and PAF with endothelial dysfunction, vascular leakage, and disease severity[15,24–30]. Although increased chymase and tryptase levels have been linked to acute and severe dengue[15,24–27,30], our data revealed no such association in adults sampled at defervescence. This discrepancy may stem from differences in cohort composition and sampling time, as prior studies largely examined pediatric patients and earlier febrile phases[15,24,30]. Notably, chymase elevations appear more pronounced in children[26], and mediator measurements in earlier studies were typically performed during days 3–7 of illness[24,30]. Considering that mast cell proteases such as tryptase peak within 1–2 h of activation[35,36], the absence of elevated mediators in our cohort likely reflects temporal rather than mechanistic differences.

To date, only one study has addressed IgE-mediated mast cell activation in dengue, showing in a mouse model that IgE from DENV-2-immunized mice sensitizes peritoneal mast cells, triggering histamine release upon antigen challenge[18]. Extending these findings to humans, we demonstrate that plasma containing anti-DENV IgE can sensitize RS-ATL8 basophils, which are activated upon DENV cross-linking in both DF and DHF patients.

Notably, not all anti-DENV IgE-positive plasma induced activation, suggesting that the IgE quantity alone could not completely predict effector cell activation or disease severity. Qualitative features of anti-DENV IgE, such as antigenic affinity, epitope specificity, and interactions with other antibody isotypes, might influence its functional capacity and warrant further investigation. Since natural infection elicits IgE against structural (E, prM, C) and non-structural proteins (NS1, NS3, NS5) with distinct effector functions[37], future studies employing epitope mapping are warranted to clarify these mechanistic relationships. Our blocking experiments demonstrated that Omalizumab efficiently inhibited RS-ATL8 activation, confirming that anti-DENV IgE mediates basophil activation via the IgE-FcεRI pathway in both DF and DHF patients.

FcγRIII-dependent enhancement of mast cell degranulation by anti-DENV IgG was demonstrated in a mouse model, a mechanism distinct yet potentially complementary to FcεRI-mediated IgE activation[21]. Together, these data suggest that both anti-DENV IgG and IgE may drive mast cell and basophil activation in dengue. Future studies should simultaneously assess IgG and IgE levels, evaluate degranulation in human effector cell models, and employ *in vivo* systems lacking FcγR and FcεRI. Our findings suggest that human anti-DENV IgE can mediate basophil activation via the FcεRI pathway *in vitro*, which can be inhibited by the anti-IgE drug Omalizumab, indicating a potential role for IgE-targeted approaches in modulating dengue severity. While mast cell stabilizers such as Ketotifen[32] or the tryptase inhibitor Nafamostat[24] have reduced dengue severity in mouse models, broad inhibition may also affect protective antiviral functions[23,34,38]. Omalizumab, as a specific anti-IgE therapy approved for allergic asthma[39,40], could offer a more targeted intervention. Considering that anti-DENV IgE levels peak around defervescence, timely modulation of IgE-mediated activation might influence disease progression, and it may also interact with antiviral pathways via FcεRI-TLR7-mediated Type I interferon responses[41–43]. As Omalizumab inhibited anti-DENV IgE–mediated basophil activation *in vitro* and anti-DENV IgE levels peaked during the defervescence phase, we hypothesize that Omalizumab may have potential as an adjunctive, personalized therapy in a subset of patients with severe dengue and high anti-DENV IgE levels at defervescence, alongside standard supportive care. As these observations are based on an in vitro RS-ATL8 reporter system, the findings remain exploratory and further functional validation using primary cells with actual mediator release, subsequent biological effects, *in vivo* model and clinical trials are required to establish the biological and clinical relevance of anti-DENV IgE in treating severe dengue.

These findings, though consistent with prior reports, should be interpreted with caution. Although we showed association of higher anti-DENV IgE OD ratio in DHF compared to DF, the correlation in endpoint titer is only marginally significant, confirmation of severity correlation in a larger cohort is warranted. As most participants had secondary infections and no DSS cases, our study could not assess IgE dynamics across the full disease spectrum. Reported variations in anti-DENV IgE peak timing likely reflect differences in cohort composition and phase definitions[13,15,16]. Defining time relative to defervescence (D0) may improve temporal resolution, but validation in larger, more diverse cohorts is needed. Additionally, to accommodate heterotypic secondary infections and enable broad detection, our ELISA used a mixture of DENV-1–4 antigens to quantify dengue-reactive IgE. We also employed the 4G2 antibody, which recognizes epitopes on the envelope (E) protein. This design limits both serotype-specific and antigen-specific interpretation. Although DENV culture supernatants contain virions and NS1, replacing 4G2 with the NS1-specific antibody 1F11 yielded no detectable anti-NS1 IgE (data not shown), which may reflect limited assay sensitivity and/or low NS1-specific IgE responses in this cohort. Future studies using validated serotype- and antigen-specific IgE assays, and including DSS and primary infections, will be important to further define the pathogenic relevance of anti-DENV IgE.

Based on our findings, we propose a model in which anti-DENV IgE contributes to the pathogenesis of natural dengue infection (Fig. 5). Patients lacking detectable anti-DENV IgE tend to develop milder diseases, whereas those with anti-DENV IgE may be at higher risk of severe manifestations. *In vitro*, blocking anti-DENV IgE with Omalizumab reduced basophil activation, suggesting a potential avenue for modulating IgE-mediated effects. This model highlights anti-DENV IgE as a possible marker associated with disease severity and a target for further investigation. While these results provide a mechanistic link between anti-DENV IgE and FcεRI-dependent activation, further in vivo and clinical studies are needed to evaluate its predictive and therapeutic potential in dengue.

Methods

Ethics statement

The study complied with the Declaration of Helsinki and was approved by the IRBs of Vajira Hospital (No.015/12), the Faculty of Tropical Medicine, Mahidol University (TMEC 13–041), and Ramathibodi Hospital, Mahidol University (MURA2016/219, MURA2019/603). Written informed consent was obtained from all participants or their legal guardians.

Study population

Dengue patients and their household members were enrolled through the DENFREE Thailand cohort at Vajira Hospital[44]. Inclusion criteria for dengue infected cases were: (1) detection of DENV RNA by reverse transcription real-time PCR (RT-PCR), NS1 antigen, or anti-DENV IgM by rapid test (SD, Korea); (2) fever ≥ 38 °C; and (3) at least two clinical manifestations of dengue illness, such as severe headache, retro-orbital pain, myalgia, arthralgia, rash, or bleeding symptoms. Disease severity was categorized as dengue fever (DF) or dengue hemorrhagic fever (DHF) following the WHO 1997 guidelines[2]. To distinguish primary from secondary dengue infections, paired

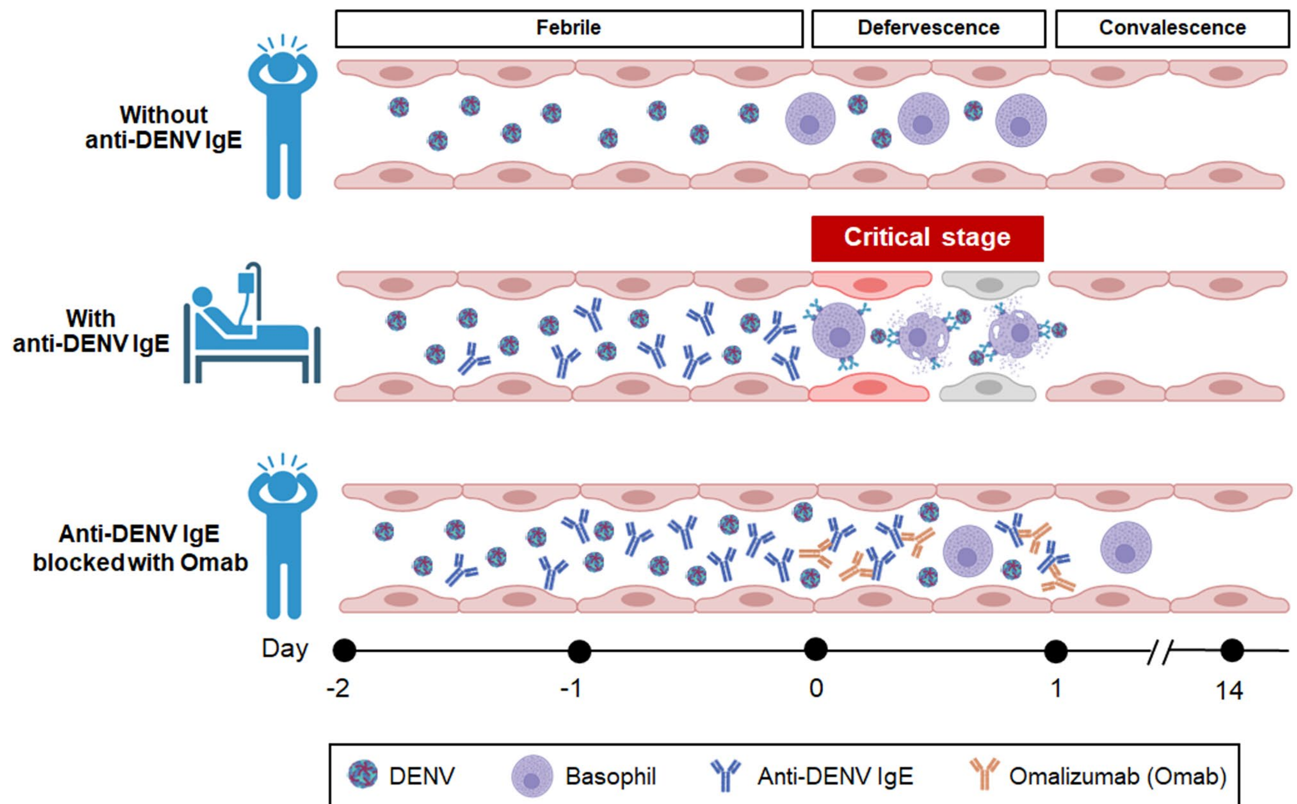

Fig. 5. Proposed hypothetical model of the role of anti-DENV IgE and potential benefit of blocking with Omalizumab in severe dengue infection. Our findings suggest a model for the potential role of anti-DENV IgE in natural dengue infection. Subset of patients with severe dengue who have high anti-DENV IgE might potentially benefit from blocking anti-DENV IgE with Omalizumab (Omab) as a personalized adjunctive treatment.

acute and convalescent plasma samples were analyzed using hemagglutination inhibition (HI), focus reduction neutralization test (FRNT), and the IgM/IgG ratio determined by ELISA, in accordance with WHO 1997 criteria.

Patients with confirmed dengue infection were enrolled into the DENFREE Thailand cohort at the time of hospital admission. Heparinized blood samples were obtained at the first day of hospital admission during the febrile phase, and the day of fever subsidence (defervescence), which was consistently defined as Day 0 (D0). To systematically account for time-course variations during the febrile phase, samples were retrospectively labeled as one day before defervescence (D-1), two days before defervescence (D-2), and progressively earlier time points. Blood samples were also collected from dengue-infected patients during the convalescent phase (2 Wks) (Fig. 1a). Heparinized plasma was separated from blood cells and stored at – 80 °C until further analysis.

Dengue virus propagation

Prototype strains of all four DENV serotypes (DENV1 Hawaii, DENV2 16681, DENV3 H87, DENV4 H241) were propagated in C6/36 cells and used as antigens. Cells were cultured to 80–90% confluency, infected at an MOI of 0.01 in 2% FBS L-15 media for 4 h at room temperature, then supplemented with media and incubated at 28 °C. Supernatants were collected on days 5 and 7, clarified by centrifugation, aliquoted, and stored at – 80 °C for subsequent experiments.

Dengue virus antigen titration

DENV antigen in culture supernatants was quantified by ELISA (Supplementary Fig. 5a). Serial twofold dilutions (1:25–1:800) were coated on 96-well plates, blocked with 3% BSA, and incubated with anti-flavivirus E protein (4G2, 1:16) followed by HRP-conjugated anti-mouse IgG. After TMB development and stopping with 1N HCl, OD450 was measured. A titration curve was plotted to determine the optimal dilution corresponding to OD = 1 (Supplementary Fig. 5b).

In-house anti-DENV IgE ELISA development

To measure the concentration of anti-DENV IgE, we developed a quantitative captured ELISA (Supplementary Fig. 6a). Optimization was performed by testing different concentrations of anti-human IgE during the coating step, various coating solutions, control serum dilutions, conjugated antibody concentrations, and different substrates with varying incubation times (Supplementary Fig. 6b-f). In the final protocol, a 96-well ELISA plate was coated with goat anti-human IgE diluted 1:200 in carbonate buffer (pH 9.6) and incubated overnight at 4 °C.

The plate was then washed three times with 0.05% PBST and blocked with 3% BSA in PBS at room temperature for 1 h. Heparinized plasma from DENV-infected patients was serially twofold diluted in 3% BSA, ranging from 1:2.5 to 1:5120, and added to each well. The plate was incubated at 37 °C for 2 h, followed by five washes with 0.05% PBST. Next, a mixture of the four DENV serotypes at the optimal dilution of each serotype corresponding to OD = 1 or mock culture supernatant (used as background control) diluted in 3% BSA was added, and the plate was incubated overnight at 4 °C. After another five washes with 0.05% PBST, anti-flavivirus E protein antibody (4G2), diluted 1:16 in 3% BSA, was added and incubated at 37 °C for 1 h. The washing step was repeated as before. Subsequently, anti-mouse IgG conjugated with alkaline phosphatase (AP), diluted 1:1000 in 3% BSA, was added to the plate. After five additional washes, p-nitrophenyl phosphate (PNPP) substrate was added and incubated for 1 h. The reaction was detected by measuring the optical density (OD) at 405 nm using an ELISA plate reader. We used dengue-positive serum (PCS) as the positive control, while the negative controls included dengue-negative serum (PNS) and patient plasma with mock supernatant (patient OD background). This setup yielded clear anti-DENV IgE signals compared with PNS and patient OD background.

The anti-DENV IgE OD ratio for each sample at a 1:2.5 dilution was calculated using the formula: OD ratio = (OD sample—OD background) / (OD background × 2). An OD ratio ≥ 2 was considered positive for anti-DENV IgE. To enhance quantitation, anti-DENV IgE endpoint titer was also determined. A titration curve was plotted, and the endpoint titer was determined as the dilution yielding an OD value twice that of the average background (Supplementary Fig. 6 g). Inter-experimental controls were included on each plate to ensure assay consistency and standardization.

Unlike prior studies that used negative control serum for background, our method incorporates patient-specific mock controls and serial plasma dilutions to generate titration curves and end-point titers[10,11,13–15]. This approach increases sensitivity, overcomes the prozone effect, and enables more accurate detection and quantification of anti-DENV IgE.

Total IgE ELISA

The concentration of total IgE in DENV-infected heparinized plasma was determined at D0 using the sandwich ELISA method with a commercialized ELISA kit (ImmunoCAP® Phadia, Sweden).

Tryptase and Chymase ELISA

Heparinized plasma samples obtained from DENV-infected patients at the defervescence phase (D0) were subjected to analysis for the levels of basophil/mast cell mediators, including tryptase (Phadia, Sweden) and chymase (USCN Life Science, China). This analysis was performed using commercially available ELISA kits, following the manufacturer's instructions.

RS-ATL8 cell line culture

The human FcεRI-expressing rat basophilic leukemia reporter cell line (RS-ATL8), which has been genetically modified to stably integrate the NF-AT-responsive luciferase reporter gene into human FcεRI-expressing RBL-SX38 cells, was utilized in this study. The RS-ATL8 cell line was cultured in minimum essential media (MEM) supplemented with 10% FBS, 100 U/ml penicillin, 0.5 mg/ml geneticin, 0.2 mg/ml hygromycin B, and GlutaMAX-I at 37 °C with 5% CO2.

RS-ATL8 cells stimulation with DENV-IgE containing plasma and DENV

RS-ATL8 cells, were harvested and incubated on a 96-well plate cells were sensitized overnight with heparinized plasma diluted 1:20 in complete MEM. The sensitized RS-ATL8 cells were stained with anti-FcεRI APC and anti-IgE PEcy7. Flow cytometry confirmed FceRI expression on on sensitized RS-ATL8 (Supplementary Fig. 4a). This indicates that we successfully sensitized RS-ATL8 with anti-DENV-containing plasma.

Subsequently, a mixture of DENV serotypes 1–4, combined in equal amounts, was added to the cells to induce cross-linking. As a positive control, RS-ATL8 cells sensitized with IgE-containing plasma and cross-linked with anti-human IgE were used.

Luciferase activity, using the ONE-Glo™Luciferase Assay System (Promega Corporation), was measured after sensitizing the RS-ATL8 cells with heparinized plasma from DF and DHF patients, with or without anti-DENV IgE, followed by cross-linking with a mixture of DENV serotypes (Supplementary Fig. 4b-3). As a positive control, RS-ATL8 cells sensitized with IgE-containing plasma and cross-linked with anti-human IgE were employed (Supplementary Fig. 4b-1). Negative controls included: patient plasma with mock supernatant (Supplementary Fig. 4b-2), mixed DENV without plasma (Supplementary Fig. 4b-4), and mock supernatant without plasma (Supplementary Fig. 4b-5). RS-ATL8 activation was considered positive when luciferase activity exceeded twice the value of the negative control (mixed DENV without plasma).

Blocking experiment with anti-IgE drug (Omalizumab)

To determine whether RS-ATL8 cell activation by anti-DENV IgE-containing plasma and DENV is mediated through the IgE-FcεRI pathway, a blocking experiment was performed using the anti-human IgE monoclonal antibody Omalizumab (Xolair, Genetech, Novartis, USA). Prior to sensitization, the plasma was pre-incubated with 0.5 mg/ml of Omalizumab for 30 min. Subsequently, a mixture of DENV serotypes 1–4 was added (Supplementary Fig. 4b-6). Luciferase activity was then measured as previously described.

This setup yielded clear signals for anti-DENV IgE-positive plasma (Supplementary Fig. 3c), whereas luciferase activity in anti-DENV IgE-negative plasma, serum-free controls (no plasma), Omalizumab (Omab) blocking, and mock virus (no DENV) conditions remained consistently low (Supplementary Fig. 4c).

Statistical analysis
The Mann–Whitney U test was used for comparing unpaired data, while the Wilcoxon signed-rank test was used for analyzing paired data. The Kruskal–Wallis test with Dunn's multiple-comparisons post hoc test was used for multiple-group comparisons. Spearman's rank correlation test was used for assessing correlation between variables. A significance level of $p \leq 0.05$ was considered statistically significant.

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

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Author contributions
Conceptualized and designed the study: P.M., W.C. Clinical samples: DENFREE Thailand, W.C., W.T., P.M. Methodology: W.C. Data analysis and interpretation: W.C., P.M., V.V., T.D., J.M. Wrote manuscript: W.C., P.M., V.V. All authors have reviewed and approved the submitted manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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