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ORIGINAL ARTICLE

Macrophage immune modulation: TGF-β1's influence on IL-1β dynamics in dengue virus infection

Modulación inmunitaria de los macrófagos: influencia del TGF-β1 en la dinámica de la IL-1β en la infección por el virus del dengue

Modulação imune de macrófagos: influência do TGF-β1 na dinâmica da IL-1β na infecção pelo vírus da dengue

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RESUMEN

Introduction: several pathogenic mechanisms contribute to the severity of dengue virus infection. These include viral cytotoxicity, host genetics, and comorbidities such as diabetes and dyslipidemia. Patients with severe dengue show an uncontrolled immune response with high levels of proinflammatory cytokines (TNF, IL-1β, IL8, IL-6) and chemokines, which damage the microvascular endothelium. Inflammatory cytokines (IL-4, IL-10, TGF-β1) are also increased. The role of TGF-β1in dengue remains unclear. Few studies exist, and most of them use serum data from patients. These suggest both protective and harmful effects. **Objective:** this study aimed to evaluate how TGF-1β regulates IL-1β secretion in dengue-infected macrophages *in vitro*; this was carried out in the laboratory of innate immunity, at the Autonomous University of the State of Morelos, Mexico. **Methods:** THP-1 cell line was treated with recombinant TGF-β before or after dengue virus (DENV) infection. Cells were differentiated into macrophages by PMA. Data were obtained by RT-PCR, IFA and ELISA assays. Variables analyzed included IL-1β expression and secretion. Statistical analysis included student t-tests. **Results:** RT-PCR showed that IL-1β expression was similar in pretreated and control cells. However, IL-1β secretion decreased only when cells were stimulated with TGF-β1 (SB505124) before TGF-β1 treatment and DENV infection abrogated the inhibitory effect of TGF-β1. **Conclusions:** these findings suggest that DENV might regulate TGF-β1 function in macrophages. Negative regulation of the TGF-β1 pathway is potentially a mechanism by which DENV evades the immune response. This could contribute to immunopathology.

Keywords: dengue; macrophage; IL-1β; TGF-β1; inflammation; innate immunity

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ABSTRACT

Introducción: varios mecanismos patogénicos contribuyen a la gravedad de la infección por el virus del dengue. Estos incluyen la citotoxicidad viral, la genética del huésped y comorbilidades como la diabetes y la dislipidemia. Los pacientes con dengue grave muestran una respuesta inmune descontrolada con altos niveles de citocinas proinflamatorias (TNF, IL-1β, IL-8, IL-6) y quimiocinas, que dañan el endotelio microvascular. Las citocinas antiinflamatorias (IL-4, IL-10 y TGF-β1) también se encuentran aumentadas. El papel de TGF-β1 en el dengue sigue siendo poco claro. Existen pocos estudios, y la mayoría de ellos utilizan datos séricos de pacientes. Estos sugieren efectos tanto protectores como dañinos. **Objetivo:** este estudio tuvo como objetivo evaluar cómo TGF-β1 regula la secreción de IL-1β en macrófagos infectados con dengue *in vitro*, esto se llevó a cabo en el laboratorio de inmunidad innata, en la Universidad Autónoma del Estado de Morelos, México. **Método:** la línea celular THP-1 se trató con TGF-β1 recombinante antes o después de la infección por el virus del dengue (DENV). Las células se diferenciaron en macrófagos mediante PMA. Los datos se obtuvieron mediante ensayos de RT-PCR, IFA y ELISA. Las variables analizadas incluyeron la expresión y secreción de IL-1β. El análisis estadístico incluyó pruebas t de Student. **Resultados:** la RT-PCR mostró que la expresión de IL-1β fue similar en las células pretratadas y control. Sin embargo, la secreción de IL-1β disminuyó solo cuando las células fueron estimuladas con TGF-β1 antes de la infección. El tratamiento después de la infección no tuvo efecto. El bloqueo del receptor de TGF-β1 (SB505124) antes del tratamiento con TGF-β1 y la infección por DENV anuló el efecto inhibidor de TGF-β1. **Conclusiones:** estos hallazgos sugieren que el DENV podría regular la función del TGF-β1 en macrófagos. La regulación negativa de la vía del TGF-β1 es potencialmente un mecanismo por el cual el DENV evade la respuesta inmunitaria. Esto podría contribuir a la inmunopatología.

Palabras clave: dengue, macrófago, IL-1β, TGF-β1, inflamación, inmunidad innata

RESUMO

Introdução: vários mecanismos patogênicos contribuem para a gravidade da infecção pelo vírus da dengue. Isso inclui citotoxicidade viral, genética do hospedeiro e comorbidades como diabetes e dislipidemia. Pacientes com dengue grave apresentam resposta imune descontrolada com altos níveis de citocinas pró-inflamatórias (TNF, IL-1β, IL-8, IL-6) e quimiocinas, que danificam o endotélio microvascular. As citocinas antiinflamatórias (IL-4, IL-10 e TGF-β1) também estão aumentadas. O papel do TGF-β1 na dengue permanece obscuro. Existem poucos estudos e a maioria deles utiliza dados séricos de pacientes. Estes sugerem efeitos protetores e prejudiciais. **Objetivo:** este estudo teve como objetivo avaliar como o TGF-β1 regula a secreção de IL-1β em macrófagos infectados com dengue *in vitro*, isto foi realizado no laboratório de imunidade inata, na Universidade Autônoma del Estado de Morelos, México. **Método:** a linhagem celular THP-1 foi tratada com TGF-β1 recombinante antes ou após a infecção pelo vírus da dengue (DENV). As células foram diferenciadas em macrófagos por PMA. Os dados foram obtidos por meio de ensaios RT-PCR, IFA e ELISA. As variáveis analisadas incluíram a expressão e secreção de IL-1β. A análise estatística incluiu testes t de Student. **Resultados:** a RT-PCR mostrou que a expressão de IL-1β foi semelhante nas células pré-tratadas e controle. No entanto, a secreção de IL-1β diminuiu apenas quando as células foram estimuladas com TGF-β1 antes da infecção. O tratamento após a infecção não teve efeito. O bloqueio do receptor TGF-β1 (SB505124) antes do tratamento com TGF-β1 e da infecção por DENV anulou o efeito inibitório do TGF-β1. **Conclusões:** esses achados sugerem que o DENV poderia regular a função do TGF-β1 em macrófagos. A regulação negativa da via do TGF-β1 é potencialmente um mecanismo pelo qual o DENV evita a resposta imune. Isso poderia contribuir para a imunopatologia.

Palavras-chave: dengue, macrófago, IL-1β, TGF-β1, inflamação, imunidade inata

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INTRODUCTION

Dengue mortality and disease burden have increased despite prevention efforts and vaccines. Dengue cases in Latin America showed significant regional variations between 2023 and 2024. Increases surpassed 60% in some areas.[1] From 1998 to 2017, years of life lost (YLLs) rose by 65.5%, and disease burden increased by 32%.[2] Dengue virus (DENV) infection symptoms vary. Most people do not develop severe conditions. About 20-25% have symptomatic infections. A few develop serious complications requiring hospitalization. These include capillary leakage and organ damage.[3,4]

Dengue patients often have increased cytokine levels. At least 25 serum molecules, including TNF, IFN-γ, IP-10, RANTES, chemokines, IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, and TGF-β1, are linked to disease severity.[5,6,7,8,9] Dengue is thought to be mainly immune-mediated disease. Cytokine dysregulation, like TNF and IL-6, causes vascular problems, plasma leakage, thrombocytopenia, and organ damage.[10]

IL-1β is a prototypical inflammatory cytokine. It is linked to various diseases with other cytokines like TNF or IL-6. The role of IL-1β in dengue is not fully understood. Human studies show conflicting results. Some link high IL-1β levels to bleeding or severe disease. Others find no association.[11,12] The efficacy of Anakinra (an IL-1 inhibitor) in dengue patients is under investigation. Clinical study results are not yet available.[13]

The role of TGF-β1 in dengue is also unclear. Some studies suggest a link between high TGF-β1 levels and severe disease. Levels were lower in controls or dengue fever patients than in Dengue hemorrhagic fever patients, especially before fever diminished.[6,14,15] However, genetic studies in Dengue patients linked high TGF-β1 production to controls.[16,17] Tillu, *et al.*[18] found more platelets in patients with higher regulatory T cells (Tregs) and TGF-β1, suggesting TGF-β1 may protect.

In vitro studies show a link between TGF-β and IL-1β. Smad6/7 proteins, made in response to TGF-β signaling, negatively regulate IL1R/TLR signaling.[19] TGF-β can also block IL-1β production by downregulating CD14 in response to lipopolysaccharide.[20] The interaction between these cytokines in dengue infection is unclear. This study investigated how TGF-β1 regulates IL-1β production by dengue-infected macrophages.

METHOD

This study was an in vitro experimental investigation conducted in the laboratory of Innate Immunity at the Universidad Autónoma del Estado de Morelos, México.

Virus, cell culture, cell differentiation, and infection of THP-1 cells

Dengue virus serotype 2 New Guinea C was provided by Dr. Humberto Lanz Mendoza (Laboratorio de Infección e Inmunidad, Centro de Investigaciones sobre Enfermedades Infecciosas del Instituto Nacional de Salud Pública, Cuernavaca, Morelos. México). 20 µL aliquots were stored at -70 °C and diluted in Dulbecco's PBS (Sigma-Aldrich) before infection. THP-1 cells (ATCC Tib 202) were grown in Advance-RPMI 1640 with 3 % Fetal Bovine Serum (complete medium; Thermo Scientific) at 37 °C and 5 % CO_2.

To become macrophages, cells were treated with 10 nM PMA (phorbol myristate acetate, Sigma-Aldrich) for 72 hours before infection. Differentiated cells were cultured in 24-well plates (Corning) at $2x10^5$ cells/well with glass coverslips for microscopy or ELISA. For RT-PCR, cells were cultured in 25 cm^2 flasks (Corning) at $4x10^6$ cells/mL.

For infection, cells were washed with PBS. Dengue virus serotype 2 was added at a multiplicity of infection of 1 (MOI 1) in Dulbecco's PBS with $CaCl_2$ and antibiotics (Sigma-Aldrich). After 2 hours, cells were washed and incubated for 24 hours in fresh medium. Infection was confirmed by fluorescent microscopy using anti-dengue antibody diluted 1:20 (ab155042, Abcam), anti-mouse Alexa 488 1:200 (Jackson Immunology), or by RT-PCR for the prM/M-C region.

Effect of TGF-β1 before DENV infection on IL-1β

To test TGF-β1's effects, differentiated cells were washed and fasted for 2 hours in serum-free RPMI-1640. Recombinant TGF-β1 (20 ng/mL, PeproTech) was added and incubated for 3 hours. Cells were washed, and infection proceeded.

Effect of TGF-β1 after DENV infection on IL-1β

For the post-infection test, fasted cells were infected with DENV in PBS for 2 hours. Complete medium with TGF-β1 (20 ng/mL, PeproTech) was added. Cells were incubated for 24 hours.

Effect of SB505124 on IL-1β secretion

Differentiated THP-1 cells were washed and incubated for 2 hours with 1 mM SB505124 (TOCRIS Biosciences) in serum-free RPMI-1640. TGF-β1 (20 ng/mL) was added and incubated for 3 hours before infection.

Stimulation of THP-1 with LPS

To induce an inflammatory response, PMA-differentiated THP-1 cells were treated with10 ng/mL of LPS from *E. coli* O11:B4 (Sigma-Aldrich).[21]

Quantification of secreted IL-1β

24 hours after infection, supernatant was collected and centrifuged at 575 *g* for 10 minutes (Spectrafuge, Labnet). Supernatant was aliquoted and stored at -20 °C. IL-1β was measured using the Human IL-1β ELISA MAX kit (Biolegend).

Total RNA extraction and RT-PCR

Total RNA was isolated from THP-1 cells using Tri-Reagent (Sigma-Aldrich). RNA concentration and quality were assessed using a spectrophotometer (Bioteck), and agarose gel electrophoresis. cDNA was synthesized from 1 µg of RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). PCR was performed using PCR Master Mix (Thermo Scientific). Reaction conditions were 35 cycles of denaturation at 95 °C for 30 seconds, annealing (57-60 °C) for 60 seconds, and extension at 72 °C for 30 seconds, in a 3PRIME Thermal Cycler (TECHNE, Bibby Scientific).

PCR products were analyzed by agarose gel electrophoresis, using ChemiDoc XRS-Quantity One Image Analyzer (BioRad). Primers sequences: IL-1β: forward 5'-GTCATTCGCTCCCACATTCT-3', reverse 5'-ACTTCTTGCCCCCTTTGAAT-3' (Bozza, Cruz *et al*. 2008). β-actin: forward 5'-AGGATCTTCATGAGGTAGT-3', reverse 5'-GCTCCGGCATGTGCAA-3'. TGF-β1: forward 5'-GGTACCTGAACCCGTGTTGCT-3', reverse 5'-TGTTGCTGTATTTCTGGTACAGCTC-3'.

DENV RNA detection by PCR

PCR was used to detect DENV RNA. Total RNA was extracted, and cDNA was synthesized. cDNA reactions were adjusted to 500 ng/µL and used for PCR in Master Mix 2x (Thermo Scientific). Reaction conditions were 35 cycles of denaturation at 95 °C for 20 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 60 seconds. Primers sequences: DENV ALL RV 5'-CCCCATCTATTCAGAATCCCTGC-3' and DENV ALL F 5'-CAATATGCTGAAACGAGAGAGAA-3'. Nested PCR was performed with the amplicon from the first PCR product, diluted 1:100, using the same reaction conditions for cycling, with the second pair of primers. DENV ALL F 5'-CAATATGCTGAAACGAGAGAGAA-3' and DENV2RV 5'-TGCTGTTGGTGGGATTGTTA-3'.

Statistical analysis

Descriptive statistics were used (mean and SD). Student's t-tests were used to compare conditions. $P < 0.05$ was considered significant. GraphPad Prism v.8.1.0 software was used for graphs and statistics.

RESULTS

TGF-β1 reduces IL-1β secretion only before infection

40% of cells were infected at 24 hours post-infection (MOI of 1, Figure 1). DENV infection induced IL-1β secretion ($p < 0,001$), less than LPS. THP-1 cells were treated with TGF-β1 (20 ng/mL) for 3 hours before infection. This pretreatment inhibited IL-1β secretion ($p < 0,03$) in infected cells, but not in LPS-treated cells. This effect did not occur if cells were infected first, then treated with TGF-β1 two hours later ($p = 0,46$) (Figure 2).

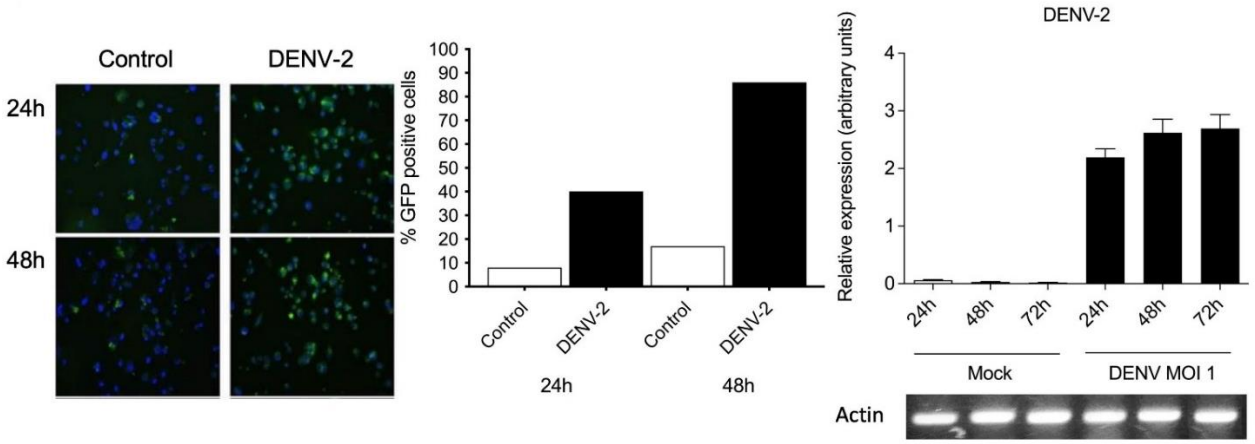

Fig. 1. Left: Immunofluorescence assay showed infected cells at 24- and 48-hours post-infection. Center: Semi-automatic cell counting was performed; fluorescent-background cells were counted in the control group. Right: Endpoint RT-PCR at three time points indicated viral absence in control and increasing relative expression in infected cells with MOI 1.

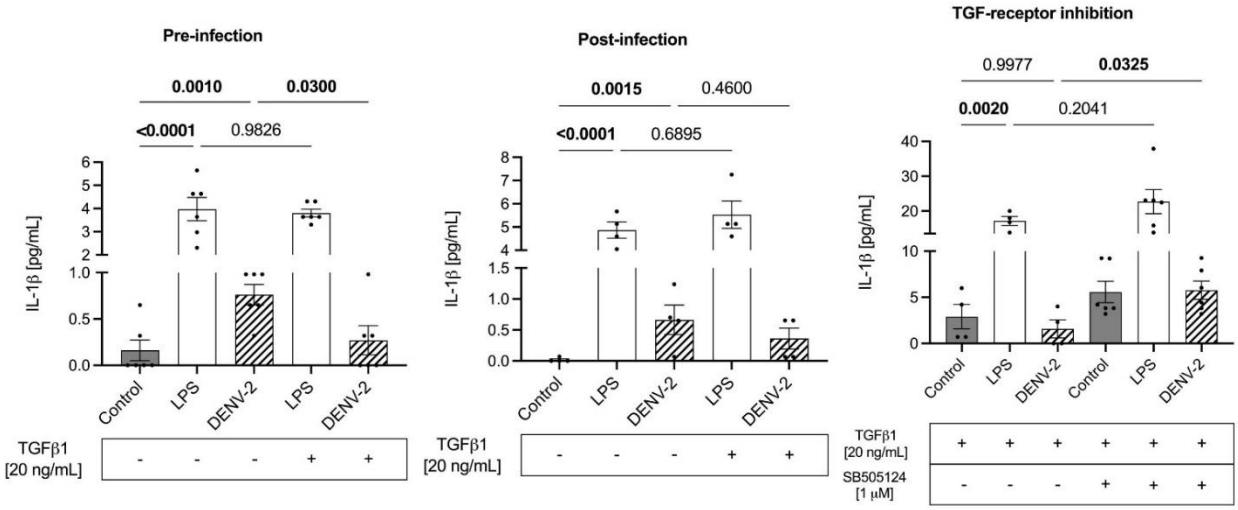

Fig. 2. Left: Differentiated THP-1 cells were pre-treated with 20 ng/mL TGF-β1 or PBS for 3 h, then infected with DENV-2 MOI 1 or treated with 10 ng/mL LPS for 24 hours. Control cells were incubated with PBS or LPS. Center: Cells were first infected with DENV-2 and then treated with TGF-β1 2 hours later. Control cells were incubated with PBS or LPS. Right: Cells were pre-incubated with 1 mM SB505124 for 2 hours, followed by TGF-β1 treatment for 3 hours before DENV-2 infection. In all panels, supernatant IL-1β was quantified by ELISA after 24 hours post-infection. Differences between treatments were evaluated by Student's t-test, with $p<0.05$*, $p<0.01$**, and $p<0.005$*** indicating significant differences.

Inhibiting the TGF-β1 receptor reverses TGF-β1's effect

THP-1 cells were pretreated with SB505124, a TGF-βRI inhibitor, before TGF-β1 (20 ng/mL). Infection with DENV followed. This nullified TGF-β1's inhibitory effect. IL-1β secretion increased more with DENV infection ($p < 0,04$) compared to non-inhibited cells. Uninfected and LPS-treated cells also increased IL-1β production (Figure 2).

TGF-β1's effect on IL-1β secretion does not affect IL-1β expression

IL-1β is synthesized as a 31 kDa precursor that needs processing for secretion. To test if TGF-β1 repressed IL-1β expression was measured it by RT-PCR. Infection and LPS increased IL-1β mRNA. However, there was no change between infected cells with or without TGF-β1. This suggests TGF-β1 mainly affects secretion (Figure 3).

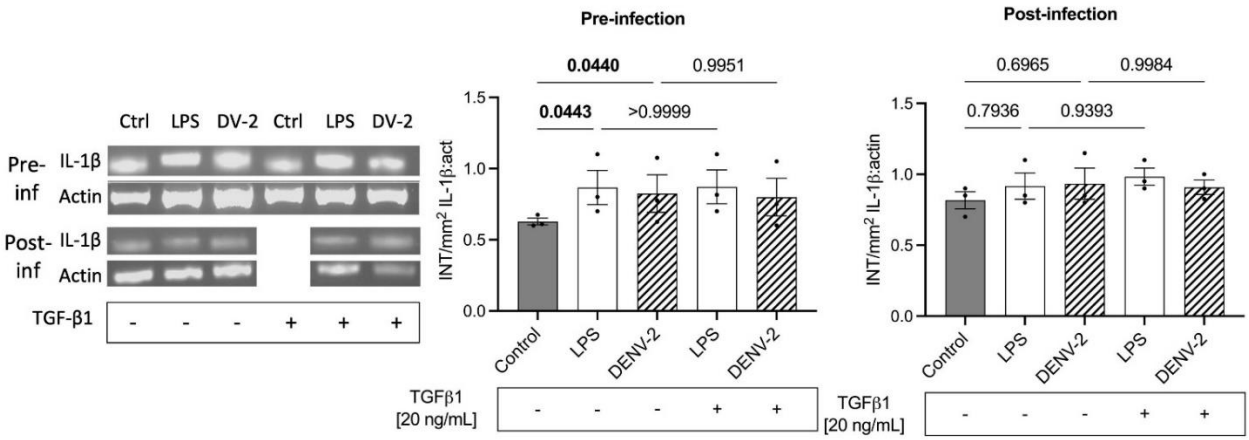

Fig. 3. Left: Endpoint RT-PCR of IL-1β in THP-1 differentiated cells treated with 20 ng/mL TGF-β1 or PBS 3 hours before DENV infection. Center: Pixel densitometry analysis of IL-1β bands normalized to β-actin bands for pre-treatment with TGF-β1. Right: Pixel densitometry analysis of IL-1β bands normalized to β-actin bands for post-treatment with TGF-β1. No significant differences were observed in any treatment.

DISCUSSION

DENV infection is complex and affected by virus genotype and serotype, mosquito proteins, prior infections, host genetics, and conditions like obesity, cardiovascular disease, or asthma.[10] The host's immune response is key in dengue's pathogenesis, increasing both inflammatory and anti-inflammatory cytokines.[1,10,12,22]

Cytokine imbalance may worsen disease. One hypothesis suggests that cytokines like TNF, IL-6, or IL-8 had a direct effect on blood vessels. This disrupts the endothelial barrier, increasing vascular permeability and causing pleural effusion or ascites.[8] Finding biomarkers to predict disease severity early is ongoing.

The 24-48 hours before body temperature drops are particularly important. Vascular changes, plasma leakage, increased hematocrit, decreased platelets, and organ damage occur 2-3 days after defervescence.[23] Identifying biomarkers is difficult due to patient differences and varying methods.[24]

In the late 1990s, some studies linked high TGF-β1 levels to severe disease. Levels were lower in controls or dengue fever patients than in dengue hemorrhagic fever patients. This was more noticeable before the temperature drop and lasted 9 days after fever onset.[6,14,25] However, polymorphism studies linked high TGF-β1 producers to controls, suggesting TGF-β1 protection role.

Djamiatun *et al*. found TGF-β1 plasma levels were lower than in healthy children, even if there was an increase in patients with hemorrhagic manifestations.[26] *In vitro* studies show cells from a DENV serotype donor produce higher TGF-β1 when re-challenged with the same serotype but not with heterologous serotypes. As severe dengue has been associated with reinfection, this study could also suggest a protective role for TGF-β1.[16,27]

TGF-β1 is a multifunctional cytokine with varying immunomodulatory effects based on concentration, location, and context. In T cells, it suppresses Th1, Th2, and CD8 activation and promotes FoxP3/Tregs generation.[28,29] Tillu, *et al.*[18] found more Tregs in mild dengue, suggesting a protective role, and noted a link between TGF-β1 levels and more platelets in these patients. TGF-β1's effects on macrophages can be stimulating or inhibitory, depending on other cytokines and maturation.[25,30]

To study the link between TGF-β1 and macrophage response to DENV infection using THP-1 cells differentiated into macrophages. 40% of cells were DENV-positive at 24 hours post-infection. A significant increase in IL-1β secretion was observed. Other studies also report that DENV-infected macrophages secrete IL-1β up to 6 hours post-infection.[31]

A total reduction in IL-1β secretion in infected cells treated with TGF-β1 3 hours before infection but not in cells stimulated with LPS. The concentration of IL-1β released by macrophages stimulated by LPS was almost five times higher than those infected by DENV. This may be because LPS and DENV bind to different TLRs.

In the assay of pre-treated THP-1 cells with SB505124, a TGF-β1 receptor inhibitor, TGF-β1's inhibitory effect on IL-1β secretion was annulled. Also, TGF-β1 no longer inhibited IL-1β secretion once infection started. There was no change in IL-1β mRNA. This suggests TGF-β1's mechanism relates to events after IL-1β expression. However, further confirmation with qPCR is necessary.

Kwon, *et al.*[32] showed that knocking-down Smad7, a TGF-β signaling inhibitor, reduces infection. They also showed that infected U937-DC-SIGN cells increased Smad7 expression in response to DENV. Thus, DENV might manipulate TGF-β1 response by modigying cellular pathways. It is important to evaluate changes in Smad 2/3 and Smad7 expression and activation.

IL-1β is produced as a 34 kDa precursor that needs activation by proteases like Caspase-1 before secretion. IL-1β is a cytosolic protein without a signal sequence, and its release mechanism is unclear. It requires a non-canonical pathway independent of the Golgi. Release mechanisms may involve pores associated with phosphatidylserine, autophagy vesicles, or pyroptosis.[33,34,35]

There was no evidence that TGF-β1 inhibits IL-1β secretion by activating caspase-1 and inducing pyroptosis. Some studies show DENV-infected monocytes or macrophages die by pyroptosis after caspase-1 activation.[35,36] Another study shows caspase-4 role in IL-1β activation and maturation in DENV-infected cells.[37] IL-1β secretion was seen almost 90 hours before caspase-1 activation or cell death. This suggests IL-1β could be processed by other proteases and not necessarily due to its secretion leading to cell death.[38] In this experimental data, was found elevated IL-1β secretion at 24 hours post-infection, as others reported.[31]

Autophagy regulates IL-1β by both maturation and release, and by degradation before secretion. The autophagy vesicle for IL-1β maturation and release seems to be non-classical. IL-1β binds TRIM16 and traffics to an LC3-II-positive membrane. A vesicle forms and binds SEC22b. This vesicle undergoes SNARE-modified fusion to reach the plasma membrane.[34] However, autophagy inhibitors or defective autophagy cells increase IL-1β secretion but not TNF or IL-6 in LPS-stimulated cells.

The autophagy vesicle formed without an inflammasome-inducing signal is TRIM16-independent and co-localizes with LAMP-1 and STX17, leading to IL-1β transport and degradation by lysosomes.[39,40] Viral infection manipulates pathways like interferon (IFN) response and autophagy. Studies show DENV activates autophagy for its replication.[41] Inhibiting ER stress signaling limits DENV's ability to induce autophagy and decrease mature virion formation.[41] Viral replication complex proteins do not co-localize with autophagosome membranes, suggesting they may have another role, such as immune evasion.[42] TGF-β1 increases autophagy via Smad and JKN pathways.

It was hypothesized that, without DENV infection, TGF-β1 causes IL-1β and inflammasome proteins to accumulate in autophagic vesicles with STX17 and SNARE, directing the complex to lysosomes. However, when dengue virus infects macrophages, mediated by TLR 3, 4, and 7, the signal pathways lead to the accumulation of IL-1β in vesicles associated with TRIM16 and SEC22b, eventually resulting in the secretion of IL-1β. Evidently, this proposal must be confirmed by experimental data. DENV-infected macrophages may not respond to TGF-β1 due to downstream signaling pathways.

CONCLUSIONS

A limitation in the study was the absence of antibodies. Macrophage activation differs if the virus binds directly or via immunocomplexes and FcRγ. Dengue-Antibody complexes can increase IL-1β secretion only with homologous sera. TGF-β1 reduces FcRγ expression on cells. In conclusion, TGF-β1 may limit dengue development and severity, but only in individuals with TGF-β1 present before infection.

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REFERENCES

1. de Almeida MT, Merighi DGS, Visnardi AB, Boneto Gonçalves CA, Amorim VMF, Ferrari ASA, de Souza AS, Guzzo CR. Latin America's Dengue Outbreak Poses a Global Health Threat. Viruses [Internet]. 2025 Jan [cited 8 Feb 2025]; 17(1):57. DOI: https://doi.org/10.3390/v17010057
2. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet [Internet]. 2018 Nov [cited 14 May 2024]; 392(10159):1736–88. DOI: https://doi.org/10.1016/s0140-6736(18)32203-7
3. Martinez JD, Garza JAC de la, Cuellar-Barboza A. Going Viral 2019: Zika, Chikungunya, and Dengue. Dermatol Clin

[Internet]. 2019 Jan [cited 14 May 2024]; 37(1):95–105. DOI: https://doi.org/10.1016/j.det.2018.07.008

4. Wilder-Smith A, Ooi EE, Horstick O, Wills B. Dengue. Lancet Lond Engl [Internet]. 2019 Jan 26 [cited 14 May 2024]; 393(10169):350–63. DOI: https://doi.org/10.1016/s0140-6736(18)32560-1
5. Pandey N, Jain A, Garg RK, Kumar R, Agrawal OP, Lakshmana Rao PV. Serum levels of IL-8, IFNγ, IL-10, and TGF β and their gene expression levels in severe and non-severe cases of dengue virus infection. Arch Virol [Internet]. 2015 Jun [cited 14 May 2024]; 160(6):1463–75. DOI: https://doi.org/10.1007/s00705-015-2410-6
6. Patra G, Mallik S, Saha B, Mukhopadhyay S. Assessment of chemokine and cytokine signatures in patients with dengue infection: A hospital-based study in Kolkata, India. Acta Trop [Internet]. 2019 Feb [cited 14 May 2024]; 190:73–9. DOI: https://doi.org/10.1016/j.actatropica.2018.10.017
7. Soo KM, Khalid B, Ching SM, Tham CL, Basir R, Chee HY. Meta-analysis of biomarkers for severe dengue infections. PeerJ [Internet]. 2017 Sep [cited 14 May 2024]; 5:e3589. DOI: https://doi.org/10.7717/peerj.3589
8. Srikiatkhachorn A, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. Semin Immunopathol [Internet]. 2017 Jul [cited 14 May 2024]; 39(5):563–74. DOI: https://doi.org/10.1007/s00281-017-0625-1
9. Palmal S, Kundu S, Ganguly S, Dey JB, Sandhukhan S, Pattanayak AK. Immunologic Crosstalk and Host-Specific Immune Signature Associated with Dengue. ACS Omega [Internet]. 2024 Aug [cited 30 Dic 2024]; 9(36):37418–29. DOI: https://doi.org/10.1021/acsomega.4c02506
10. St. John AL, Rathore APS. Adaptive immune responses to primary and secondary dengue virus infections. Nat Rev Immunol [Internet]. 2019 Apr [cited 14 May 2024]; 19(4):218–30. DOI: https://doi.org/10.1038/s41577-019-0123-x
11. Bozza FA, Cruz OG, Zagne SM, Azeredo EL, Nogueira RM, Assis EF, Bozza PT, Kubelka CF. Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity. BMC Infect Dis [Internet]. 2008 Jun [cited 14 May 2024]; 8:86. DOI: https://doi.org/10.1186/1471-2334-8-86
12. Chaturvedi UC, Nagar R, Shrivastava R. Macrophage and dengue virus: friend or foe? Indian J Med Res [Internet]. 2006 Jul [cited 14 May 2024]; 124(1):23–40. Disponible en: https://pubmed.ncbi.nlm.nih.gov/16926454/
13. Huyen TB, Trieu HT, Vuong NL, Minh Nguyet N, Tam DTH, McBride A, *et al*. Anakinra for dengue patients with hyperinflammation: protocol for a randomized double-blind placebo-controlled trial. Wellcome Open Res [Internet]. 2024 [cited 30 Dic 2024]; 9:689. DOI: https://doi.org/10.12688/wellcomeopenres.21017.1
14. Agarwal R, Elbishbishi EA, Chaturvedi UC, Nagar R, Mustafa AS. Profile of transforming growth factor-beta 1 in patients with dengue haemorrhagic fever. Int J Exp Pathol [Internet]. 1999 Jun [cited 14 May 2024]; 80(3):143–9. DOI: https://doi.org/10.1046/j.1365-2613.1999.00107.x
15. Laur F, Murgue B, Deparis X, Roche C, Cassar O, Chungue E. Plasma levels of tumour necrosis factor a and transforming growth factor β-1 in children with dengue 2 virus infection in French Polynesia. Trans R Soc Trop Med Hyg [Internet]. 1998 [cited 14 May

2024]; 92(6):654–6. DOI: https://doi.org/10.1016/s0035-9203(98)90800-8

16. Perez AB, Sierra B, Garcia G, Aguirre E, Babel N, Alvarez M, *et al*. Tumor necrosis factor-alpha, transforming growth factor-β1, and interleukin-10 gene polymorphisms: implication in protection or susceptibility to dengue hemorrhagic fever. Hum Immunol [Internet]. 2010 Nov [cited 14 May 2024]; 71(11):1135–40. DOI: https://doi.org/10.1016/j.humimm.2010.08.004
17. Yeo ASL, Azhar NA, Yeow W, Talbot CC, Khan MA, Shankar EM, *et al*. Lack of clinical manifestations in asymptomatic dengue infection is attributed to broad down-regulation and selective up-regulation of host defence response genes. PloS One [Internet]. 2014 [cited 14 May 2024]; 9(4):e92240. DOI: https://doi.org/10.1371/journal.pone.0092240
18. Tillu H, Tripathy AS, Reshmi PV, Cecilia D. Altered profile of regulatory T cells and associated cytokines in mild and moderate dengue. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol [Internet]. 2016 Mar [cited 14 May 2024]; 35(3):453–61. DOI: https://doi.org/10.1007/s10096-015-2561-0
19. Lee YS, Kim JH, Kim ST, Kwon JY, Hong S, Kim SJ, *et al*. Smad7 and Smad6 bind to discrete regions of Pellino-1 via their MH2 domains to mediate TGF-beta1-induced negative regulation of IL-1R/TLR signaling. Biochem Biophys Res Commun [Internet]. 2010 Mar 19 [cited 14 May 2024]; 393(4):836–43. DOI: https://doi.org/10.1016/j.bbrc.2010.02.094
20. Imai K, Takeshita A, Hanazawa S. Transforming Growth Factor-β Inhibits Lipopolysaccharide-Stimulated Expression of Inflammatory Cytokines in Mouse Macrophages through Downregulation of Activation Protein 1 and CD14 Receptor Expression. Infect Immun [Internet]. 2000 May [cited 14 May 2024]; 68(5):2418–23. DOI: https://doi.org/10.1128/iai.68.5.2418-2423.2000
21. Tsatsanis C, Androulidaki A, Dermitzaki E, Gravanis A, Margioris AN. Corticotropin releasing factor receptor 1 (CRF1) and CRF2 agonists exert an anti-inflammatory effect during the early phase of inflammation suppressing LPS-induced TNF-alpha release from macrophages via induction of COX-2 and PGE2. J Cell Physiol [Internet]. 2007 [cited 14 May 2024]; 210(3):774–83. DOI: https://doi.org/10.1002/jcp.20900
22. Pang T, Cardosa MJ, Guzman MG. Of cascades and perfect storms: the immunopathogenesis of dengue haemorrhagic fever-dengue shock syndrome (DHF/DSS). Immunol Cell Biol [Internet]. 2007 Jan [cited 14 May 2024]; 85(1):43–5. DOI: https://doi.org/10.1038/sj.icb.7100008
23. Yacoub S, Wills B. Predicting outcome from dengue. BMC Med [Internet]. 2014 Dec [cited 14 May 2024]; 12(1):1–10. DOI: https://doi.org/10.1186/s12916-014-0147-9
24. John DV, Lin YS, Perng GC. Biomarkers of severe dengue disease – a review. J Biomed Sci [Internet]. 2015 Oct [cited 14 May 2024]; 22:83. DOI: https://doi.org/10.1186/s12929-015-0191-6
25. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol [Internet]. 1998 [cited 14 May 2024]; 16:137–61. DOI: https://doi.org/10.1146/annurev.immunol.16.1.137
26. Djamiatun K, Faradz SMH, Setiati TE, Netea MG, van der Ven AJAM, Dolmans WMV. Increase of plasminogen activator inhibitor-1 and decrease of transforming growth factor-b1 in children with dengue haemorrhagic fever in Indonesia. J Trop Pediatr [Internet]. 2011 Dec [cited 14 May 2024]; 57(6):424-32. DOI: https://doi.org/10.1093/tropej/fmq122

27. Sierra B, Perez AB, Vogt K, Garcia G, Schmolke K, Aguirre E, *et al*. Secondary heterologous dengue infection risk: Disequilibrium between immune regulation and inflammation? Cell Immunol [Internet]. 2010 [cited 14 May 2024]; 262(2):134–40. DOI: https://doi.org/10.1016/j.cellimm.2010.02.005
28. Chen W, Ten Dijke P. Immunoregulation by members of the TGFβ superfamily. Nat Rev Immunol [Internet]. 2016 Nov [cited 14 May 2024]; 16(12):723–40. DOI: https://doi.org/10.1038/nri.2016.112
29. Li MO, Flavell RA. TGF-beta, T-cell tolerance and immunotherapy of autoimmune diseases and cancer. Expert Rev Clin Immunol [Internet]. 2006 Mar [cited 14 May 2024]; 2(2):257–65. DOI: https://doi.org/10.1586/1744666x.2.2.257
30. Travis MA, Sheppard D. TGF-β activation and function in immunity. Annu Rev Immunol [Internet]. 2014 [cited 14 May 2024]; 32:51–82. DOI: https://doi.org/10.1146/annurev-immunol-032713-120257
31. Callaway JB, Smith SA, McKinnon KP, de Silva AM, Crowe JE, Ting JPY. Spleen Tyrosine Kinase (Syk) Mediates IL-1β Induction by Primary Human Monocytes during Antibody-enhanced Dengue Virus Infection. J Biol Chem [Internet]. 2015 Jul 10 [cited 14 May 2024]; 290(28):17306–20. DOI: https://doi.org/10.1074/jbc.m115.664136
32. Kwon YJ, Heo J, Wong HEE, Cruz DJM, Velumani S, da Silva CT, *et al*. Kinome siRNA screen identifies novel cell-type specific dengue host target genes. Antiviral Res [Internet]. 2014 Oct [cited 14 May 2024]; 110:20–30. DOI: https://doi.org/10.1016/j.antiviral.2014.07.006
33. Brough D, Pelegrin P, Nickel W. An emerging case for membrane pore formation as a common mechanism for the unconventional secretion of FGF2 and IL-1β. J Cell Sci [Internet]. 2017 Oct [cited 14 May 2024]; 130(19):3197–202. DOI: https://doi.org/10.1242/jcs.204206
34. New J, Thomas SM. Autophagy-dependent secretion: mechanism, factors secreted, and disease implications. Autophagy [Internet]. 2019 Oct [cited 14 May 2024]; 15(10):1682–93. DOI: https://doi.org/10.1080/15548627.2019.1596479
35. Wu MF, Chen ST, Yang AH, Lin WW, Lin YL, Chen NJ, *et al*. CLEC5A is critical for dengue virus-induced inflammasome activation in human macrophages. Blood [Internet]. 2013 Jan 3 [cited 14 May 2024]; 121(1):95–106. DOI: https://doi.org/10.1182/blood-2012-05-430090
36. Tan TY, Chu JJH. Dengue virus-infected human monocytes trigger late activation of caspase-1, which mediates pro-inflammatory IL-1β secretion and pyroptosis. J Gen Virol [Internet]. 2013 Oct [cited 14 May 2024]; 94(Pt 10):2215–20. DOI: https://doi.org/10.1099/vir.0.055277-0
37. Cheung KT, Sze DMY, Chan KH, Leung PHM. Involvement of caspase-4 in IL-1 beta production and pyroptosis in human macrophages during dengue virus infection. Immunobiology [Internet]. 2018 [cited 14 May 2024]; 223(4–5):356–64. DOI: https://doi.org/10.1016/j.imbio.2017.10.044
38. Netea MG, Veerdonk FL van de, Meer JWM van der, Dinarello CA, Joosten LAB. Inflammasome-Independent Regulation of IL-1-Family Cytokines. Annu Rev Immunol [Internet]. 2015 Mar [cited 14 May 2024]; 33:49–77.DOI: https://doi.org/10.1146/annurev-immunol-032414-112306
39. Harris J. Autophagy and cytokines. Cytokine [Internet]. 2011 Nov [cited 14 May 2024]; 56(2):140–4. DOI: https://doi.org/10.1016/j.cyto.2011.08.022

40. Harris J. Autophagy and IL-1 Family Cytokines. Front Immunol [Internet]. 2013 Apr 5 [cited 14 May 2024]; 4:83. DOI: https://doi.org/10.3389/fimmu.2013.00083
41. Datan E, Roy SG, Germain G, Zali N, McLean JE, Golshan G, *et al*. Dengue-induced autophagy, virus replication and protection from cell death require ER stress (PERK) pathway activation. Cell Death Dis [Internet]. 2016 Mar [cited 14 May 2024]; 7(3):e2127–e2127. DOI: https://doi.org/10.1038/cddis.2015.409
42. Heaton NS, Randall G. Dengue virus and autophagy. Viruses [Internet]. 2011 Aug [cited 14 May 2024]; 3(8):1332–41. DOI: https://doi.org/10.3390/v3081332

Conflict of interest:
The authors declare no conflicts of interest.

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Archivo complementario (*Open Data*):
Results obtained in the investigation titled Macrophage immune modulation, TGF-β1's influence on IL-1β dynamics in dengue virus infection