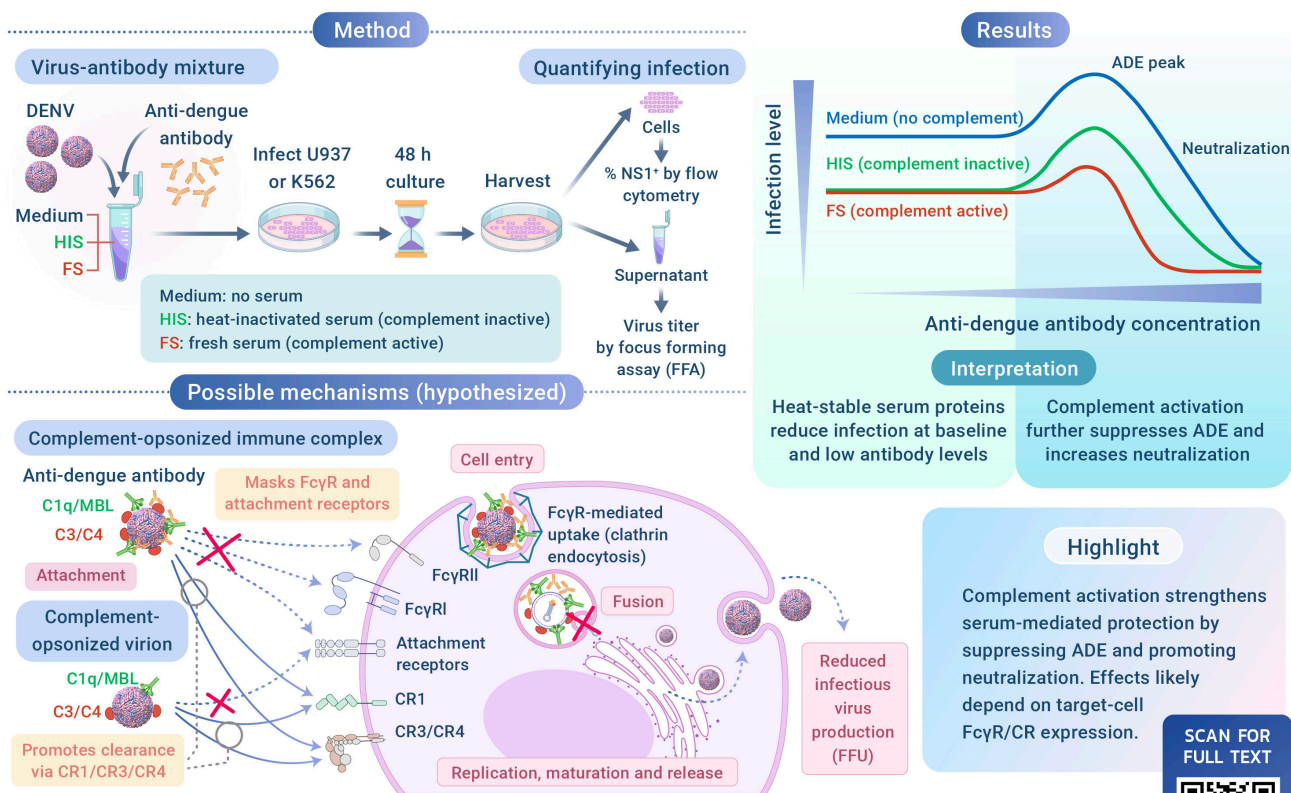


Complement in Human Serum Suppresses Antibody-Dependent Enhancement and Potentiates Neutralization of Dengue Virus

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ABSTRACT

Objective: Antibody-dependent enhancement (ADE), in which pre-existing antibodies increase viral infection, is a major concern in dengue virus (DENV) pathogenesis and vaccine development. While conventional ADE models are well-established, the role of the complement system in modulating this process remains underexplored. This study investigated how complements in human serum influence DENV infection in the presence of enhancing and neutralizing antibodies.

Materials and Methods: U937 and K562 cells were infected with DENV serotype 2 (DENV-2). The virus was pre-incubated with various concentrations of dengue antibodies, purified from pooled convalescence serum, under three conditions: culture medium, heat-inactivated human serum, or fresh human serum (as a source of active complement). After 48 hours, infectious virus production was quantified by a focus-forming assay, and infection rates were measured by flow cytometry.

Results: Both fresh and heat-inactivated human serum significantly reduced DENV infection. In the presence of antibodies, complement activation in fresh serum was significantly more effective at decreasing infection than conditions with inactive complement, particularly at antibody concentrations that mediate ADE and partial neutralization.

Conclusion: Human serum limits DENV infection, and active complement amplifies this neutralizing effect under conditions that typically promote ADE. These findings support incorporating complement into *in vitro* assessments and motivate validation in primary FcγR/complement receptor-expressing target cells.

Keywords: Human serum; complement; antibody-dependent enhancement; neutralization; U937 cells; K562 cells (Siriraj Med J 2026;78(2):96-106)

INTRODUCTION

Dengue remains a major global public health challenge, with >100 endemic countries and approximately half of the world's population at risk.¹⁻³ Dengue virus (DENV), a member of the *Flaviviridae* family, comprises four antigenically distinct serotypes (DENV-1 to DENV-4). Infection with any serotype can cause illness ranging from mild dengue fever to severe dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS).⁴ Because clinical manifestations are often nonspecific, laboratory confirmation is essential for accurate diagnosis. Current approaches include nucleic acid amplification tests (NAAT/RT-PCR) for viral genome detection, NS1 antigen assays, and serological tests for IgM and IgG antibodies, performed either individually or in combination. Numerous commercial rapid diagnostic tests (RDTs) combining NS1 antigen and antibody detection have been evaluated in multiple settings, showing high specificity but variable sensitivity influenced by the assay type, timing of sample collection, infecting serotype, and host immune status.⁵⁻¹⁰ Despite sustained research efforts, no specific antiviral therapy exists, and current vaccines provide context-dependent rather than universal protection.

Secondary infection with a heterologous serotype is the strongest epidemiologic risk factor for severe disease and is explained in part by antibody-dependent enhancement (ADE).^{11,12} At sub-neutralizing antibody concentrations, cross-reactive antibodies from prior

infection form immune complexes that facilitate virus uptake through Fc-gamma receptors (FcγR) on myeloid cells, increasing viral burden and risk of severe illness.^{13,14} The magnitude of ADE depends on antibody concentration, affinity, epitope specificity, and immunoglobulin subclass (primarily IgG1).¹⁵⁻²⁰

The complement system is a major effector arm of innate immunity that opsonizes pathogens, generates anaphylatoxins (C3a, C5a), and can lyse susceptible targets.²¹ During ADE, immune complexes can trigger classical pathway activation, suggesting that complement could enhance neutralization or, conversely, amplify infection and inflammation. Clinical studies report correlations between complement activation and severe dengue with vascular leakage,²²⁻²⁴ whereas in the related West Nile virus, complement is protective *in vivo*—underscoring context-dependent effects.²⁵⁻²⁸ How complement influences ADE of DENV infection therefore remains unresolved.

Prior *in vitro* findings are mixed. Complement-competent serum—or purified components such as C1q or C3—can attenuate ADE and reduce DENV infection in FcγR-expressing cell lines (U937, K562), particularly near the ADE peak^{29,30} yet dengue-immune sera enriched for strongly enhancing antibodies do not always confer protection.³¹ Discrepancies likely reflect differences in antibody source (monoclonal vs polyclonal), concentration, epitope specificity, subclass distribution, and complement source.

In natural DENV infection, polyclonal antibodies of diverse specificities and subclasses act in the presence of circulating complement proteins. A systematic analysis is therefore needed to define how physiologic serum factors shift antibody function across the spectrum from enhancement to neutralization. We hypothesized that complement in human serum suppresses ADE and enhances neutralization. To test this, we used U937 and K562 cells—standard ADE/neutralization models—and purified IgG from pooled convalescent sera (PCS) to approximate the human polyclonal response. We then compared fresh (complement-competent) and heat-inactivated human serum, quantifying viral output and infected-cell frequency to clarify complement's contribution to the enhancement-neutralization balance.

MATERIALS AND METHODS

Cell lines and dengue virus

Human monocytic U937 and erythroleukemia K562 cell lines were cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS; Gibco) and 100 U/mL Penicillin-Streptomycin (Pen-Strep; Gibco) at 37 °C in a 5% CO₂ incubator and passaged every 3 days. Routine PCR-based mycoplasma testing confirmed the absence of contamination. Cell-surface complement receptor (CR) expression was assessed by flow cytometry using phycoerythrin (PE)-conjugated anti-human CR1 (CD35, clone E11; BD Biosciences), fluorescein isothiocyanate (FITC)-conjugated anti-human CR3 (CD11b/MAC-1, clone ICRF44; BD Biosciences), and Brilliant Violet 650 (BV650)-conjugated anti-human CR4 (CD11c, clone B-ly6; BD Biosciences). *Aedes albopictus* C6/36 cells were maintained at 28 °C in Leibovitz's L-15 medium (Gibco) with 10% HI-FBS, 10% tryptose phosphate broth (TPB; Sigma), 100 U/mL Pen-Strep, and 2 mM L-glutamine (Gibco). African green monkey kidney (Vero) cells were grown in Minimum Essential Medium (MEM; Gibco) with 10% HI-FBS and 100 U/mL Pen-Strep at 37 °C in 5% CO₂.

DENV serotype 2 (strain 16681) was propagated in C6/36 cells (ATCC® CRL-1660™) at a multiplicity of infection (MOI) of 0.01. Infected cells were cultured in L-15 medium containing 1.5% HI-FBS for 5 days; clarified supernatants were harvested and titrated by focus-forming assay (FFA) on Vero cells.

Anti-DENV antibodies

Pooled convalescent dengue sera (PCS) containing polyclonal antibodies against all four DENV serotypes (titers > 1:10,240) were obtained from AFRIM. IgG was purified by Protein G affinity chromatography

(Pharmacia); aggregates were reduced by centrifugation (14,000 rpm, 10 min, 4 °C). By Western blot, purified PCS IgG bound multiple DENV proteins, (envelope, capsid, pre-membrane, and NS1) (data not shown). Dengue-specific monoclonal antibodies (mAbs) included anti-NS1 (clones 2G6, DENV-2 specific) and pan-flavivirus anti-E (clone 4G2), kindly provided by Dr. Chunya Puttikhunt, (BIOTEC, NSTDA). Alexa Fluor 647-conjugated goat anti-mouse IgG (Invitrogen) was used as secondary antibody.

Normal human serum (complement source)

Normal human serum (NHS) was collected from an anonymous healthy adult with ethically institutional approval (Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand; Protocol 632/2559). The donor was seronegative for all DENV serotypes by dengue IgG/IgM-capture ELISA and PRNT (data not shown).^{32,33} NHS was used either as fresh serum (FS; complement-competent) or heat-inactivated serum (HIS), prepared by incubating at 56 °C for 30 min to inactivate heat-labile complement. Loss of complement activity was confirmed by absence of C3d deposition on yeast (flow cytometry; data not shown).

DENV infection assay

U937 and K562 cells were resuspended in RPMI with 10% HI-FBS. DENV-2 (MOI = 1) was pre-incubated at 37 °C for 30 min with serial dilutions of PCS-derived IgG under three conditions: (i) Medium (RPMI + 10% HI-FBS), (ii) 10% HIS, or (iii) 10% FS. Virus-antibody mixtures were added to cells for 2 hours (h) at 37 °C, followed by two washes with plain RPMI and one wash with RPMI + 10% HI-FBS. Cells were cultured 48 h in 96-well plates. Supernatants were collected (500 x g, 5 min, 4 °C) for FFA, and cells were processed for intracellular NS1 staining and flow cytometry.

Detection of DENV-infected cells by flow cytometry

At 48 h post-infection, cells were fixed with 2% formaldehyde (FA) in PBS for 30 min at room temperature (RT), washed with PBS + 2% HI-FBS, and permeabilized with 0.1% Triton X-100 (Fluka) in PBS for 10 min at RT. After washing, intracellular NS1 was labeled with anti-DENV NS1 mAb 2G6 (hybridoma supernatant; 50 µL/well, 1 h, RT), followed by Alexa Fluor 647-conjugated goat anti-mouse IgG (1:500, 30 min, RT, dark). Mock-infected cells (Medium only) were processed in parallel to set NS1⁺ gates (isotype controls where indicated). Samples were acquired by flow cytometry and analyzed using standard gating (cells by FSC/SSC, singlets by FSC-H vs

FSC-A). The primary readout was % NS1-positive cells (% NS1⁺).

Background subtraction and limit of detection (LOD) handling. For each experiment, %NS1⁺ in mock-infected controls was subtracted from all conditions. Negative values were floored to LOD/2, LOD was the mean %NS1⁺ in mock wells for that experiment.

Fold-change calculation (baseline normalization). To evaluate changes relative to baseline infection, fold change was calculated per experiment as the ratio of each condition (Medium, HIS, or FS) at a given antibody dose to the Medium condition at 0 µg/mL PCS. Background-corrected %NS1⁺ values were used:

$$\text{Fold change (\%NS1}^+) = \frac{\text{(Percentage of NS1 positive cells in Medium, HIS, or FS at each PCS)}}{\text{(Percentage of NS1 positive cells in Medium at PCS 0 µg/mL)}}$$

If subtraction yielded a value ≤ 0 , it was set to LOD/2 before computing the ratio. Fold-change data are summarized as mean $\pm$ SEM across independent experiments.

Measurement of infectious virus production by focus-forming assay (FFA)

Viral titers in culture supernatants were quantified by FFA on Vero monolayers. Supernatants were 10-fold serially diluted and added in duplicate to 96-well plates seeded with Vero cells (~90% confluence). After 2 h adsorption at 37 °C (5% CO₂), inocula were replaced with an overlay of 1.2% gum tragacanth (Sigma) in MEM supplemented with 2% HI-FBS, 100 U/mL penicillin–streptomycin, and 2 mM L-glutamine. Plates were incubated for 72 h at 37 °C.

Cells were fixed with 3.7% formaldehyde (FA) in PBS (15 min, RT), permeabilized with 2% Triton X-100 in PBS (10 min, RT), and stained with anti-E mAb 4G2 (1 h, 37 °C), followed by HRP-conjugated goat anti-mouse IgG (Dako; 1:1,000; 1 h, 37 °C, protected from light). After washing, foci were visualized with 3,3'-diaminobenzidine (DAB; Sigma) (typically 0.8 mg/mL containing 0.16% [w/v] NiCl₂ and 0.014% [v/v] H₂O₂) for 10–15 min at RT. Foci were counted and titers calculated as focus-forming units per mL (FFU/mL) from dilutions yielding countable foci when multiple dilutions were available, estimates were averaged.

LOD transformations. Samples with no detectable foci (across duplicates) were assigned LOD/2 before analysis; for this assay LOD = 10 FFU/mL, thus LOD/2 = 5 FFU/mL. FFU/mL values were log₁₀-transformed for visualization where indicated.

Fold-change calculation (baseline normalization). Similarly, virus output for each condition was expressed relative to the Medium condition at 0 µg/mL PCS:

$$\text{Fold change (FFU)} = \frac{\text{(Log10 in Medium, HIS, or FS at each PCS)}}{\text{(Log10 in Medium at PCS 0 µg/mL)}}$$

For samples below detection limit, LOD/2 (5 FFU/mL) was substituted before computation. Fold-change data are summarized as mean $\pm$ SEM across independent experiments.

Statistical analysis

All analyses were performed in GraphPad Prism (GraphPad Software). Data are presented as mean $\pm$ SEM. Normality of distributions was assessed using the Kolmogorov–Smirnov test. For normally distributed data, comparisons between two groups were made using unpaired two-tailed Student's t-test. Comparisons among three conditions (Medium, HIS, FS) were performed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple-comparison test for pairwise differences. A *p*-value < 0.05 was considered statistically significant.

Because data in Fig 2 were expressed as fold change relative to the Medium baseline (0 µg/mL PCS), a value of 1 represents no change from baseline. Each data point in the figures represents an independent biological replicate, and all analyses were based on results from at least three independent experiments to ensure reproducibility.

RESULTS

Within-condition antibody titrations establish ADE and neutralization (Fig 1)

To validate ADE, U937 and K562 cells were infected with DENV-2 in the presence of titrated pooled dengue convalescent serum IgG (PCS). In Medium, infection peaked at 1 µg/mL PCS in both cell types: NS1⁺ cells increased to 5.4% in U937 (from 1.5%; NS, *p* = 0.1324) and to 53.2% in K562 (from 1.5%; *p* = 0.0011). Virus production rose from 4 × 10³ to 2 × 10⁵ FFU/mL in U937 (*p* = 0.0053) and from 2 × 10⁵ to 2 × 10⁶ FFU/mL in K562 (*p* = 0.0085). At higher antibody doses, infection declined: complete neutralization occurred at 100 µg/mL in U937 (*p* < 0.0001) and partial neutralization at 50 µg/mL in K562 (*p* = 0.0237), defining the canonical bell-shaped ADE curve followed by neutralization.

We then repeated the within-condition titrations with serum present. With heat-inactivated serum (HIS), U937 showed suppression of the ADE peak (1 µg/mL: 1.6% NS1⁺; 4 × 10² FFU/mL) relative to its own 0 µg/mL

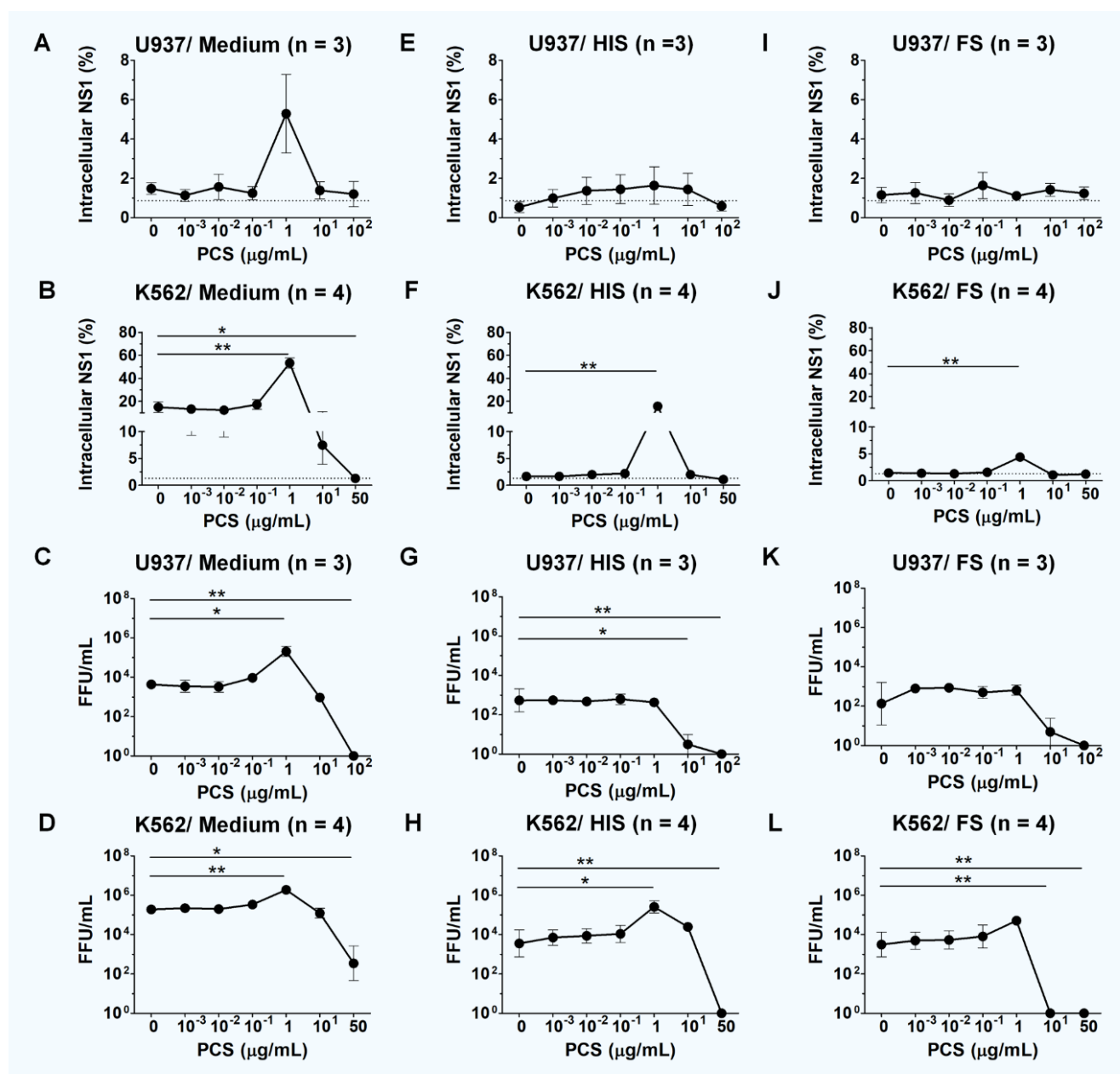


Fig 1. Antibody titrations within each condition reveal ADE and subsequent neutralization in Medium, HIS, and FS. U937 and K562 were infected with DENV-2 (MOI = 1) in the presence of PCS at 0, 10^{-3} , 10^{-2} , 10^{-1} , 1, and 10 $\mu\text{g/mL}$ (plus 50 $\mu\text{g/mL}$ for K562; 100 $\mu\text{g/mL}$ for U937). Conditions were Medium (A–D), 10% HIS (E–H), or 10% FS (I–L). At 48 hpi, intracellular NS1 was quantified by flow cytometry (A, B, E, F, I, J), and infectious virus production in supernatants by focus-forming assay (FFA) (C, D, G, H, K, L). Data represent mean $\pm$ SEM from independent experiments (n as indicated in panels). Statistical comparisons were made using unpaired two-tailed t-tests vs the 0 $\mu\text{g/mL}$ PCS baseline within each condition; significance: * $p < 0.05$, ** $p < 0.01$; NS, not significant.

PCS baseline (0.5%, 5×10^2 FFU/mL), whereas K562 still exhibited ADE (15.7%, 3×10^5 FFU/mL vs 1.6%, 4×10^3 FFU/mL; $p = 0.0487$). At 10 $\mu\text{g/mL}$ PCS, U937 virus production fell markedly (3 FFU/mL; $p = 0.0440$), while K562 showed no significant change. Infectious virus was eliminated by HIS at 100 $\mu\text{g/mL}$ in U937 and at 50 $\mu\text{g/mL}$ in K562 ($p = 0.0097$ and 0.0021, respectively). With fresh serum (FS; complement-competent), infection was broadly reduced across titrations in both cell types; K562 still showed an ADE peak in NS1⁺ at 1 $\mu\text{g/mL}$

($p = 0.0013$), but FS yielded complete neutralization of infectious virus by 10 $\mu\text{g/mL}$ PCS. Fig 1 therefore defines the ADE to neutralization curves in Medium, HIS, and FS for each cell line.

Serum effects at matched antibody doses reveal complement-dependent restriction (Fig 2)

For Fig 2, fold-change values at each antibody dose were calculated relative to the Medium baseline (0 $\mu\text{g/mL}$ PCS) within each experiment. Thus, 1 denotes no

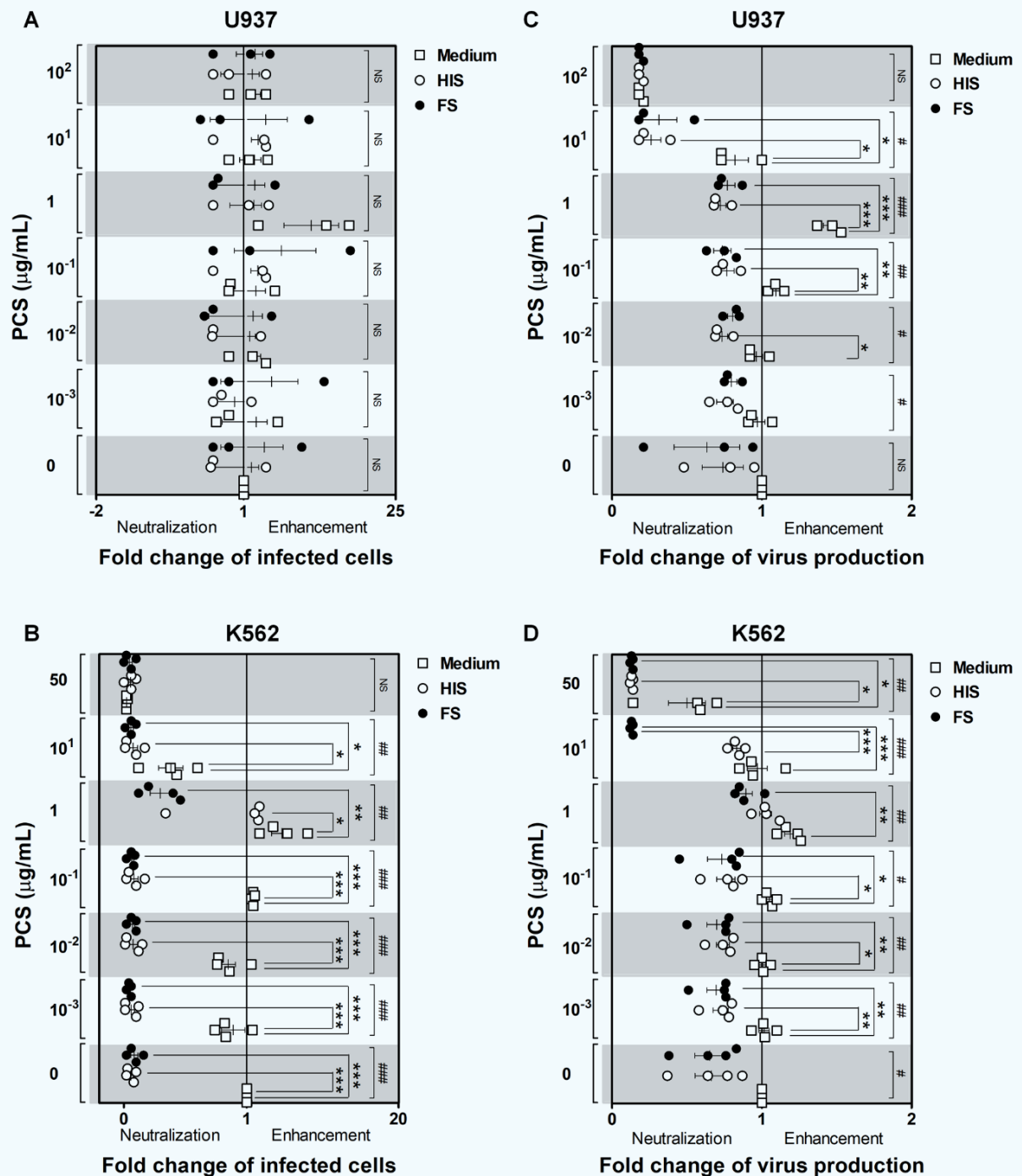


Fig 2. Serum effects at matched antibody doses shown as fold change relative to medium.

For each PCS dose and experiment, the effect of HIS (open circles) and FS (filled circles) vs Medium was computed as fold change (condition/Medium) for % NS1⁺ cells (A, B) and FFU/mL (C, D) in U937 (A, C) and K562 (B, D). Points show individual experiments; bars show mean $\pm$ SEM. The reference line at 1 indicates no difference from Medium. Statistical comparisons show fold change against the reference line and, where indicated, FS vs HIS at the same dose (multiple-comparison adjusted where applicable); exact *p*-values are annotated in the panels. For FFU values at the detection limit, the assay's LOD handling is described in Methods. (Underlying raw titration curves are shown in Fig 1. At each PCS dose, a one-way ANOVA tested differences among Medium, HIS, and FS (sharp symbols: #, ##, ###). Bonferroni-adjusted pairwise comparisons are shown with asterisks (*, **, ***). Symbol thresholds: * or # $p \leq 0.05$; ** or ## $p \leq 0.01$; *** or ### $p \leq 0.001$; NS, not significant. Because data are plotted as fold change, the Medium group equals 1 (reference line).

change from baseline; because ADE/neutralization shifts infection at non-zero doses, Medium can be > 1 (ADE) or < 1 (neutralization).

U937. Across PCS doses, NS1⁺ fold-changes for HIS and FS clustered near 1 (little consistent difference vs Medium), whereas FFU fold-changes were significantly < 1 at 0.1, 1, and 10 $\mu\text{g/mL}$ PCS for both HIS and FS. Mean FFU fold-changes ($n=3$ experiments) at 0.1, 1, and 10 $\mu\text{g/mL}$ were 0.77, 0.72, 0.26 (HIS), and 0.74, 0.77, 0.31 (FS); corresponding Medium were 1.09, 1.46, 0.82, reflecting ADE then neutralization. Significant neutralization of the enhancing antibody effects was observed at 0.1 and 1 $\mu\text{g/mL}$, with near-complete neutralization at 10 $\mu\text{g/mL}$. Pairwise (Bonferroni-adjusted) mean differences vs Medium were: for HIS, 0.33 (95% CI: 0.1070–0.5464, $p \leq 0.01$), 0.73 (95% CI: 0.5222–0.9445, $p \leq 0.001$), and 0.56 (95% CI: 0.1232–0.9968, $p \leq 0.05$) at 0.1, 1, and 10 $\mu\text{g/mL}$, respectively; for FS, 0.36 (95% CI: 0.1370–0.5764, $p \leq 0.01$), 0.69 (95% CI: 0.4755–0.8978, $p \leq 0.001$), and 0.51 (95% CI: 0.0699–0.9434, $p \leq 0.05$). Thus, in U937, serum—particularly with intact complement—reduces infectious output (FFU) even when the percentage of infected cells changes little.

K562. NS1⁺ and FFU fold-changes were < 1 for HIS and FS across 0–1 $\mu\text{g/mL}$ PCS, with significant effects at each dose. At 1 $\mu\text{g/mL}$, mean NS1⁺ fold-changes ($n=4$ experiments) were 0.29 (FS), 1.43 (HIS), and 4.95 (Medium). Bonferroni-adjusted mean differences were FS vs Medium: 4.655 (95% CI 1.347–7.963, $p \leq 0.01$), HIS vs Medium: 3.520 (95% CI 0.2122–6.828, $p \leq 0.05$). At 10 $\mu\text{g/mL}$, mean NS1⁺ fold-changes were 0.0575 (FS), 0.0750 (HIS), and 0.3825 (Medium), with FS vs Medium: 0.3250 (95% CI 0.06681–0.5832, $p \leq 0.05$) and HIS vs Medium: 0.3075 (95% CI 0.04931–0.5657, $p \leq 0.05$). For FFU at 10 $\mu\text{g/mL}$, mean fold-changes were 0.1325 (FS), 0.8325 (HIS), and 0.9700 (Medium); FS vs Medium: 0.8375 (95% CI 0.6668–1.008, $p \leq 0.001$) and FS vs HIS: 0.7000 (95% CI 0.5293–0.8707, $p \leq 0.001$). Under FS at 10 $\mu\text{g/mL}$, FFU fell below the detection limit (neutralization). These data confirm a complement-dependent amplification of neutralization, most evident in K562 near the ADE peak and at sub-neutralizing antibody levels.

DISCUSSION

Antibody-dependent enhancement (ADE) is a recognized risk factor for severe dengue.¹⁵ *In vitro* models remain essential for dissecting mechanisms and guiding evaluation of vaccine and therapeutics. U937 and K562 are widely used because ADE depends on Fc γ -receptor (Fc γ R)–mediated uptake.^{34,35} Given that severe dengue is frequently linked to DENV-2^{36,37} and that DENV-2

has posed a substantial burden in Thailand in the past decade³⁸, we used DENV-2 infection of U937 and K562 as a relevant model. Consistent with prior studies, both lines supported ADE when DENV-2 was incubated with titrated dengue-immune IgG (PCS). Despite this shared susceptibility, their behaviors diverged, reflecting differences in intrinsic permissivity and Fc γ R profiles (U937: Fc γ RI and Fc γ RII; K562: Fc γ RII only).^{39–43} To separate antibody-driven enhancement from direct infection, we referenced each line's no-PCS baseline. On this “effective enhancement” scale, the ADE peak at 1 $\mu\text{g/mL}$ PCS was larger in U937 than in K562 (Fig 1), indicating that U937 is more sensitive for detecting enhancing activity.

We then examined how human serum shapes this enhancement–neutralization balance. Two points emerged. First, heat-inactivated serum (HIS) reduced infection across a wide antibody range—including in the absence of dengue antibody—implicating heat-stable recognition molecules such as C1q and MBL that can bind virions or immune complexes and interfere with attachment/uptake.^{29,30,44–46} Yet, the ADE peak persisted in K562 under HIS (Fig 1), indicating that recognition alone did not fully counter enhancement in this line. Second, fresh serum (FS)—which retains complement activity—produced greater restriction than HIS, most clearly in K562 near the ADE peak and at sub-neutralizing PCS doses. In Fig 2, where fold change values are normalized to the Medium baseline (0 $\mu\text{g/mL}$ PCS), this appears as values < 1 for HIS and FS, with FS $<$ HIS at 1 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$, and complete neutralization (below LOD) in FS at 10 $\mu\text{g/mL}$.

These patterns fit a model in which immune-complex size and opsonization control complement potency. Complement activation is favored at higher epitope density and at equivalence or antibody excess.⁴⁷ At ADE/sub-neutralizing doses, immune complexes are abundant and can recruit C1q/MBL, trigger C4/C3 deposition, and restrict infection by (i) masking Fc γ R and other entry receptors and/or (ii) reducing the fraction of infectious virions. Notably, in U937 we often observed large decreases in FFU with little change in %NS1⁺, suggesting that serum—especially with complement—diminishes infectious yield more than initial entry in this line.

DENV can engage all three complement pathways, which have dual roles in protection and pathogenesis. The classical pathway can inhibit infection during ADE (e.g., C1q binding to immune complexes or E protein³⁰), while the lectin pathway can neutralize via MBL.^{45,46} Activation of these pathways promotes viral clearance through

C4/C3 deposition and opsonophagocytosis.^{48,49} By contrast, excessive alternative pathway activity correlates with disease severity and inflammatory injury.⁵⁰ Our functional data—suppressed ADE and enhanced neutralization in FS versus HIS—are consistent with a predominant contribution of classical/lectin mechanisms under our conditions, though we did not map pathways directly.

Complement can also, under certain conditions, enhance infection through complement-receptor (CR) pathways. Yamanaka *et al.* reported complement-mediated enhancement in U937 at high serum fractions (50–75%), whereas such effects were absent in K562 conditions under their conditions.²⁹ Complement-facilitated dissemination has also been described for HIV, HSV, and West Nile virus.^{51–53} In our study, using 10% serum, restriction predominated in both lines. We detected CR1 on U937 but little/no CR3/CR4, and no CR1/CR3/CR4 on K562 (Supplementary Fig S1). Because primary monocytes express higher CR1 than U937^{54,55} and are important DENV targets^{56,57}, the *in vivo* balance between complement-mediated neutralization and enhancement likely depends on serum fraction/opsonin density, antibody dose, immune-complex size, and the FcγR/CR context of the target cell.

While our experiments in U937 and K562 demonstrate that serum proteins and complement activation suppress ADE—and can lower DENV-2 infection even in the absence of anti-DENV antibody—validation in primary human target cells (monocytes, macrophages, and monocyte-derived dendritic cells) is warranted to confirm physiological relevance. Because complement–antibody interactions can vary by DENV serotype, parallel evaluation across DENV-1–4 should be prioritized.^{58–60} Our results provide functional evidence that complement from seronegative human serum inhibits ADE mediated by pooled convalescent IgG. However, the specific complement pathways and IgG subclasses responsible remain to be defined; complement engagement depends on subclass and epitope specificity.^{58,59} Accordingly, pathway-resolved studies—e.g., C1q- or C3-depleted sera with add-back, MgEGTA/MBL blockade to parse classical vs lectin/alternative pathways, and virion-associated C4/C3 deposition assays using subclass-defined anti-DENV monoclonal antibodies—will be essential to delineate the antibody–complement mechanisms that modulate ADE.

Beyond neutralization, the protective efficacy of anti-DENV antibodies in people is strongly linked to complement engagement. Complement-fixing antibodies are associated with protection from symptomatic disease

and reduced progression to severe disease (DHF/DSS)⁶¹, with murine studies showing that C1q binding antibodies accelerate viremia clearance.⁶² In humans, C1q-fixing antibody levels induced by the TAK-003 correlated with neutralizing antibody titers across all four DENV serotypes.⁶³ Mechanistically, efficient classical pathway activation occurs when C1q binds IgG1/IgG3 arranged in hexamers on antigen surfaces.^{64,65} These data argue that evaluating both neutralization potency and complement-activating capacity should improve how we assess vaccine and therapeutic antibody candidates.

Finally, our study has boundaries: we used one DENV-2 strain (16681), two cell lines, and 10% human serum from defined donors; we did not quantify CH50/AH50, C3a/C5a, or virion-associated C4b/C3b/iC3b, nor did we profile IgG subclasses or block FcγR/CR directly. Pathway-specific depletion/reconstitution, opsonin-deposition assays, and studies in primary FcγR/CR-expressing cells will be important next steps.

CONCLUSIONS

Human serum reduces DENV infection during direct infection as well as across low, enhancing, and sub-neutralizing antibody concentrations. Heat-stable recognition (e.g., C1q/MBL) is sufficient to lower infection, but complement activation in fresh serum provides greater restriction, most evident near the ADE peak and at sub-neutralizing antibody levels—particularly in K562. In U937, serum (with or without complement) markedly lowers infectious output (FFU) even when %NS1⁺ changes little. The magnitude of protection depends on antibody dose and target cell type, consistent with effects of immune-complex size, opsonization, and receptor context. These findings refine how serum and complement shape the ADE–neutralization balance and motivate mechanistic studies in primary FcγR/CR-expressing cells to define pathway-specific roles in dengue infection and immunity.

Data availability

Data and material requests should be addressed to the corresponding author (panisadee.avi@mahidol.ac.th).

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Conflict of Interest

All authors declare no conflicts of interest.

Registration Number of Clinical Trial

None.

Author Contributions

Conceptualization and methodology, H.P. and P.A. ;
Investigation, H.P., N.P. ; Formal analysis, H.P. and
S.T. ; Visualization and writing – original draft, H.P.,
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manuscript.

Use of Artificial Intelligence

ChatGPT (OpenAI) was used for language editing of
the Introduction, Results, selected Methods subsections,
Discussion, Conclusions, and Data Availability section.
The authors verified and edited all AI-assisted text. No
AI was used for data analysis, figure generation, or image
editing; no identifiable data were shared with AI tools.

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