DENV-specific IgA contributes protective and non-pathologic function during antibody-dependent enhancement of DENV infection

Adam D. Wegman¹, Mitchell J. Waldran¹, Lauren E. Bahr¹, Joseph Q. Lu¹,², Kristen E. Baxter², Stephen J Thomas¹,², Adam T. Waickman¹,²

¹Department of Microbiology and Immunology, State University of New York Upstate Medical University, Syracuse, NY, USA.

²Institute for Global Health and Translational Sciences, State University of New York Upstate Medical University, Syracuse, NY, USA.

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ABSTRACT

Dengue represents a growing public health burden worldwide, accounting for approximately 100 million symptomatic cases and tens of thousands of fatalities yearly. Prior infection with one serotype of dengue virus (DENV) is the greatest known risk factor for severe disease upon secondary infection with a heterologous serotype, a risk which increases as serotypes co-circulate in endemic regions. This disease risk is thought to be mediated by IgG-isotype antibodies raised during a primary infection, which poorly neutralize heterologous DENV serotypes and instead opsonize virions for uptake by FcγR-bearing cells. This antibody-dependent enhancement (ADE) of infection leads to a larger proportion of susceptible cells infected, higher viremia and greater immunopathology. We have previously characterized the induction of a serum IgA response, along with the typical IgM and IgG responses, during dengue infection, and have shown that DENV-reactive IgA can neutralize DENV and competitively antagonize IgG-mediated ADE. Here, we evaluate the potential for IgA itself to cause ADE. We show that IgG, but not IgA, mediated ADE of infection in cells expressing both FcαR and FcγRs. IgG-mediated ADE stimulated significantly higher pro-inflammatory cytokine production by primary human macrophages, while IgA did not affect, or slightly suppressed, this production. Mechanistically, we show that DENV/IgG immune complexes bind susceptible cells significantly more efficiently than DENV/IgA complexes or virus alone. Finally, we show that over the course of primary dengue infection, the expression of FcγRI (CD64) increases during the period of acute viremia, while FcγRIIa (CD32) and FcαR (CD89) expression decreases, thereby further limiting the ability of IgA to facilitate ADE in the presence of DENV. Overall, these data illustrate the distinct protective role of IgA during ADE of dengue infection and highlight the potential therapeutic and prognostic value of DENV-specific IgA.
INTRODUCTION

Dengue virus (DENV) is a prevalent arboviral pathogen, transmitted primarily by the tropical and subtropical mosquito species Aedes aegypti and Ae. Albopictus. Dengue represents a major global disease burden: at least half of the world’s population is at risk of infection, 100 million of whom develop symptomatic disease per year, of which at least 20,000 are fatal (1, 2). There are no specific antiviral therapeutics for dengue, and the only US-FDA licensed vaccine as of March 2023 is restricted in its indication to seropositive adults (3). Clinically, ~75% of DENV infections are asymptomatic, while uncomplicated dengue present as a sudden-onset fever, often accompanied by myalgias, arthralgias, retro-orbital headache, nausea, and rash, and may include epistaxis, petechiae, and minor gingival bleeding (2, 4). However, a small percentage enter a “critical phase” of illness, characterized by increased vascular permeability and plasma leakage or bleeding. The cause of progression to severe dengue is incompletely understood, and no unifying mechanism has been described (5-9).

The DENVs comprise four immunologically and genetically distinct serotypes (10-13). Following primary infection, the antibody response to the infecting serotype is thought to durably protect against reinfection with a homologous serotype, but cross-protection against infection by heterologous serotypes wanes after 6 months to 2 years (14). Following this interval of effective cross-protection, secondary infection with a heterologous serotype represents the greatest known risk factor for developing severe disease (15). One proposed mechanistic contributor to this phenomenon is antibody-dependent enhancement (ADE) of infection, wherein a sub-neutralizing pool of IgG isotype antibodies raised against a primary infection fails to neutralize the secondary infection and instead opsonizes the virions for increased uptake by permissive FcγR-bearing cells (16, 17). Multiple lines of evidence have supported this notion: epidemiologically, with increased severe disease in DENV-experienced populations during a subsequent outbreak of a different serotype; clinically, with increased severe disease in infants born to dengue-immune mothers; and experimentally, with increased peak viremia in nonhuman primates passively immunized DENV immune sera or monoclonal antibodies (14, 18-22). Accordingly, significant effort has been put into defining the serologic profiles elicited by both infection and vaccination, with multiple putative correlates of risk and protection described.

The literature on dengue serology has focused almost exclusively on IgM and IgG isotype antibodies, for several reasons. One of the first papers describing ADE as a phenomenon compared the enhancement potential of IgM and IgG, but not IgA isotype antibodies, and concluded that “the infection-enhancing factor was a noncytophilic antibody of the IgG class” (16). Later, the IgM/IgG ratio became a routine measurement used to differentiate primary from secondary infections (23), further contributing to the focus on these two isotypes. However, in addition to IgM and IgG, there is a serum IgA component to the antibody response. There is a small body of literature examining this, including initial attempts to correlate isotypes and subclasses with clinical outcome (24), as well as evaluation as a potential diagnostic tool (25). Our lab and colleagues have characterized the IgA response at the serological and single-cell (plasmablast) levels during dengue infection (26, 27). We have previously shown that DENV-reactive monoclonal IgA can antagonize IgG-mediated ADE in a dose-dependent manner in the FcγR-bearing K562 cell line (28). Since ADE occurs via phagocytosis (29), and the FcαR is capable of mediating phagocytosis of IgA-opsonized targets (30-33), the capacity of IgA to contribute to ADE of DENV infection remains unclear.
Here, we demonstrate that IgG, but not IgA, mediates ADE of DENV infection in cell lines and primary human cells which express of FcγR and FcαR. IgG-mediated ADE – but not IgA - increases production of viral particles after infection as well as the secretion of pro-inflammatory cytokines by primary human macrophages. Mechanistically, we show that DENV/IgG immune complexes bind more effectively to FcγR/FcαR expressing cells than DENV/IgA immune complexes, indicating that the inability of IgA to mediate ADE is largely due to the low affinity of IgA to its cognate Fc receptor. Finally, we highlight the dynamic nature of FcγRs and FcαRs expressing during acute primary DENV infection, wherein FcγR expression is dramatically increased and FcαR expressing is suppressed. In their totality these results illustrate the distinct protective role of IgA during ADE of dengue infection and highlight the potential therapeutic and prognostic value of DENV-specific IgA during acute dengue.

RESULTS

IgG, but not IgA, mediates ADE in FcγR/FcαR expressing cells. Our group has previously demonstrated that DENV-specific IgA antagonizes IgG-mediated antibody-dependent enhancement of DENV infection in the FcγR-bearing K562 cell line (28). However, while the K562 line is the gold standard for DENV ADE assays, it does not express the human myeloid-restricted FcαR (CD89) (34). CD89 is broadly expressed by monocytes, macrophages and dendritic cells and can facilitate antibody-dependent phagocytosis of immune complexes (30, 31). Therefore, our previous work left unaddressed the potential contribution of DENV-specific IgA to ADE via FcαR. To fill this knowledge gap, we utilized the U937 pro-monocytic cell line, which is routinely used in studies of DENV ADE and has been described to express high levels of FcαR (18, 35, 36). We first confirmed the expression of the Fc-receptors and observed that the U937 cell line expresses robust levels of both FcγRIIa (CD32), FcγRI (CD64), and FcαR (CD89), but little or no surface DC-SIGN (CD209) (Figure 1A). To assess the ability of DENV-specific IgG and IgA to enhance DENV infection in U937 cells we utilized a previously described DENV-specific monoclonal antibody (VDB33) which we synthesized with either an IgG or IgA Fc domain (26, 28). The conversion of this parentally IgG1 isotype antibody to an IgA1 format did not impact the binding or neutralization activity of the antibody (Supplemental Figure 1B, Figure 1C, Supplemental Figure 2).

However, despite expressing FcαR, no enhancement of DENV infection was observed in U937 cells cultured with IgA/DENV immune complexes despite the abundant expression of FcαR on the cells.

IgG, but not IgA, mediates ADE in primary human macrophages. While the U937 cell line is a widely used and tractable system for ADE studies it is not a fully representative target cell for DENV. As a myeloleukemic cell line, it is replicatively immortal, whereas DENV infects non-replicating cells of the myeloid lineage (37, 38). Moreover, U937 cells are immature pro-monocytes, and Fc-receptor expression levels are known to be modulated during the normal lifespan of monocytes as well as their differentiation into macrophages (39).
To address this limitation, we next utilized primary human monocyte-derived macrophages as a target of DENV ADE (40, 41). Consistent with previous reports and *ex vivo* phenotyping (40), our M-CSF differentiated monocytes express high levels of CD14 and CD163 and retained expression of FcγRIIa, FcγRI, and FcαR (Figure 2A, Figure 2B, Supplemental Figure 3). Importantly, our monocyte differentiation protocol did not induce expression of the dendritic cell-specific CD209, further confirming the differentiated cells as bona fide macrophages (Figure 2B). Consistent with the literature, the rate of infection with DENV alone was low in our primary monocyte derived macrophages (40). However, significant infection enhancement was observed with DENV complexed with IgG, but not IgA (Figure 2C, Figure 2D, Supplemental Figure 4). The production of progeny DENV virions from infected macrophages was also enhanced by the addition of IgG proportionally to the frequency of DENV infected cells, while no such enhancement was observed in cultures treated with DENV/IgA immune complexes (Figure 2E). Thus, as was observed in the U937 cell line, DENV-reactive IgA exhibited no DENV infection-enhancing ability despite expression of FcαR on primary human monocyte-derived macrophages.

**DENV-specific IgG, but not IgA, enhances pro-inflammatory cytokine production by primary human macrophages.** Although the exact immunopathologic mechanisms responsible for the development of severe dengue have not been determined, it is clear that serum levels of pro-inflammatory cytokines and chemokines correlate with disease severity (8). In light of the differential ability of DENV-specific IgG and IgA to enhance DENV infection in primary monocyte-derived macrophages, we hypothesized that DENV-specific IgG and IgA may also impact the cellular immunopathogenesis of DENV infection. To this end, we tested the supernatants of infected macrophage cultures with a multiplex cytokine assay to determine the levels of various cytokines associated with dengue disease (42).

The abundance of IFN-α2a (Figure 3A) IFN-β (Figure 3B), IL-6 (Figure 3C), MIP-1α (Figure 3D) and TNFα (Figure 3E) in supernatants collected from human macrophage cultures infected with DENV alone or with DENV/IgG or DENV/IgA immune complexes was assessed using a multiplex cytokine array. These cytokines are well-established soluble markers of inflammation and several are known pyrogens, which is consistent with a pathologic contribution of IgG-mediated ADE to severe dengue. As we observed with the overall burden of DENV infected cells, DENV-specific IgG significantly enhanced the production of all these cytokines/chemokines above what was observed with DENV infection alone in a fashion directly proportional to the frequency of DENV infected cells in the culture. However, no such enhancement was observed upon the addition of DENV-specific IgA to the infection cultures. These results suggest that DENV-reactive IgA is capable of reducing both the DENV-infected cell burden relative to idiotype-matched IgG, but also limits infection-elicited cytokine production.

**IgA exhibits lower affinity for its cognate Fc receptor than IgG and does not facilitate low-valency binding to DENV-permissive cells.** Having established the ability of DENV-reactive IgG, but not IgA, to mediate antibody-dependent enhancement of infection and consequent inflammation, we endeavored to determine the mechanism responsible. We have verified that the mAbs used in our experiments bind the DENV virion with equal affinity (Supplemental Figure 1) (28), indicating that this effect is not due to differential ability of DENV-specific IgG or IgA to bind DENV. We therefore tested the next possible source of difference, namely the binding of the IgG and IgA Fc domains to their cognate Fc receptors at a cellular and molecular level.
To the end, we first incubated U937 cells and primary monocyte-derived macrophages in the presence of human serum-derived polyclonal IgG or IgA followed by staining with the appropriate secondary antibody, and analyzed the total antibody-binding by flow cytometry. Strikingly, both U937 cells (Figure 4A) and primary human monocyte-derived macrophages (Figure 4B) bound more polyclonal IgG than polyclonal IgA at multiple antibody concentrations. To extend these observations to a more physiologically relevant setting, we performed a virus binding assay to assess the ability of DENV, DENV/IgG immune complexes, or DENV/IgA immune complexes to bind either U937 cells or primary human monocyte-derived macrophages. This assay was performed by incubating DENV, DENV/IgG, or DENV/IgA immune complexes with cells on ice to prevent virion internalization. After extensive washing, the abundance of cell-associated virions was assessed by RT-qPCR. Consistent with the relative receptor abundance and receptor/antibody affinity, DENV/IgG immune complexes bound both U937 cells and primary human monocyte-derived macrophages at a significantly higher rate than DENV alone (Figure 4E, Figure 4F). However, IgA/DENV immune complexes exhibited no increased binding to these cells over that observed with DENV alone. These data are consistent with the patterns of infection burden described above, and support the conclusion that the differential affinity of IgG and IgA for their cognate Fc receptors is responsible for the variance in ability to mediate ADE.

While the observations described above might indicate that IgA/FcaR interaction are biologically extraneous in the setting of many infectious diseases, it must be noted that IgA is a potent opsonin which can facilitate the phagocytic uptake of bacteria, parasites, and even whole-cells in an FcξR dependent fashion (30, 43). Indeed, we observed that the same DENV-reactive IgA mAb that exhibited no ADE activity in the assays described above was able to facilitate the phagocytic uptake of DENV-E protein coated polystyrene beads at levels only slightly lower than what was observed with IgG opsonized beads (Supplemental Figure 5). This leads us to suggest that FcξR expression and the affinity of IgA/FcξR interactions are tuned to selectively facilitate the uptake of high-valency antigens, while the higher expression and higher affinity of FcγR allows for the effective uptake of even small antigens such as IgG-opsonized virions.

**Fc receptor expression is dynamic during acute DENV infection.** The data presented thus far suggests that IgA mediated ADE is unlikely to occur due to the low affinity of IgA for its cognate Fc receptor and the relatively low expression of FcξR on permissive cells. However, the expression of FcR on monocytes, macrophages, and other cells permissive to DENV infection is highly dynamic and can be influenced by pro-inflammatory cytokines and other soluble inflammatory mediators (44, 45). Indeed, a recent comparison of FcγR gene transcription on circulating monocytes between dengue patients and healthy controls showed alterations in dengue patients (46), but did not assess FcξR expression. While the cells chosen for our *in vitro* assays closely mirror the natural cellular reservoir of DENV in susceptible human hosts, our analysis leaves unaddressed the possibility that acute viral infection may modulate the expression of FcRs in such a fashion that would increase the possibility of IgA-mediated ADE.

Considering this, we sought to characterize the changes in FcR surface expression over the course of a dengue infection to refine our understanding of the potential contribution of DENV-specific IgA to dengue pathogenesis. We selected pre-infection, acute illness, and convalescent PBMC samples collected from four previously flavivirus naive patients infected with an attenuated DENV-3 challenge virus and assessed the expression of Fc-receptors FcγRIa (CD32), FcγRI (CD64), and FcξR (CD89) on PBMC monocytes by flow cytometry (Supplemental Figure 6). Consistent with previously published
reports, CD14⁺CD16⁺ monocytes were the dominate subtype of observed at all time points, (Figure 5A, Supplemental Figure 7), but the frequency of CD14⁺CD16⁺ intermediate monocytes increased during the peak of DENV infection and resolved by 28 days post infection (Figure 5A, Supplemental Figure 7). Within the CD14⁺CD16⁺ classical monocyte compartment, expression of FcγRI increased dramatically during the acute phase of DENV infection, while the expression of FcγRIIa remained unchanged, and the expression FcαR was suppressed (Figure 5A, Figure 5C). Accordingly, we posit that DENV-elicited inflammation further reduces the likelihood of IgA-mediated ADE – but increase the likelihood of IgG-mediated ADE - due to the suppressed expression of FcαR and increased expression of FcγRs relative to monocytes at homeostasis.

DISCUSSION

In this study we have shown that DENV/IgG immune complexes, but not DENV/IgA complexes, can mediate enhanced infection of DENV in both a monocytic cell line and in monocyte-derived macrophages. IgG-mediated ADE significantly increases production of pro-inflammatory cytokines including type I interferon, IL-6, MIP-1α, and TNF, which are associated with dengue symptoms and development of pathogenesis. Mechanistically, we have shown that this differential effect is largely attributable to lower avidity of IgA-opsonized DENV for susceptible cells; and both lower Fc – FcαR interaction affinity as well as lower expression of FcαR contribute to this effect.

Given that our infection studies with DENV virions revealed no evidence of ADE, we sought to confirm that our mAbs were capable of opsonizing targets for uptake by the FcαR. Adapting a bead-based phagocytosis assay (32, 47), we showed that primary human monocytes were capable of uptaking both VDB33-IgG and -IgA opsonized polystyrene beads more efficiently than un-opsonized beads. These aligned with our other observations showing a clear efficiency advantage of IgG over IgA in opsonizing targets. These observations are consistent with the hypothesis that FcαR-dependent uptake is a function of the total avidity of the receptor-ligand interactions, wherein a larger quantity of IgA on the surface of a target could overcome the otherwise prohibitively low affinity of the individual interactions. This is consistent with the known ability of IgA to opsonize relatively large targets (such as bacteria and human cells) for phagocytic uptake by FcαR expressing cells, while at the same time exhibiting poor binding to the same receptors in monomeric or low-valency aggregates (30, 43). Indeed, previously reports have suggested that binding of IgA to FcαR only appears to occur when 5 or more IgA antibodies are multimerized in the same complex, with little-or-no binding of monomeric IgA (48). This would also explain a similar observation by Tay and colleagues (47), who reported equal phagocytosis efficiency of IgA and IgG opsonization of HIV env-conjugated beads, but greater efficiency of IgG opsonization of the HIV virion itself.

One implication of these data concerns the many attempts to define a correlate of protection for dengue (49), along with other studies of seroprevalence, such as those used as endpoint measures in vaccine trials. The data shown here, as well as our previously published work, present an often-unexamined factor that could additionally influence the probability of neutralization versus enhancement: the presence and quantity of DENV-reactive IgA in the serum. This is relevant because some measures of dengue seropositivity, such as HAI, could vary between samples as a function of DENV-reactive serum IgA titer, even if the IgM and IgG titers were otherwise identical by ELISA. If IgA were identified as a distinct factor conferring protective immunity, that in turn would suggest selective IgA deficiency, the most common primary immunodeficiency, as a potential risk factor for severe disease. Given its unique
induction and decay kinetics compared to IgM and IgG, the IgA titer during dengue could also be potentially used to infer the timing of a prior infection serologically (21).

Another translational application of these data is the prophylactic and therapeutic potential of IgA mAbs. Given the risk of ADE, human monoclonal IgG antibodies proposed for dengue have included the LALA mutation to attenuate Fc region binding activity (50, 51). Another proposed neutralizing mAb initially characterized as a mouse mAb and suggested as a candidate for humanization (52) would presumably need a similar modification. However, several of the commonly used Fc domain modifications do not fully abrogate binding to FcRs or to C1q (53), while others show reduced FcRn binding, possibly leading to a reduced half-life in vivo (54). By contrast, an IgA isotype therapeutic mAb would not require humanization or other modification to counter the risk of ADE. By the same reasoning, an IgA mAb could also be suitable as a prophylactic immunotherapy, especially for individuals who have already experienced a primary infection and whose window of potential re-exposure is known in advance (e.g., travelers, military personnel).

There are several limitations to our study, which merit further investigation. While we used exclusively purified monoclonal IgA1 in our experiments, there is a small proportion (~10%) of dimeric IgA circulating in plasma (55), which according to some reports has a higher affinity for monocytes than monomeric IgA (56, 57). The IgA in our enhancement assays was all DENV-reactive monoclonal IgA, which would form immune complexes with DENV more readily than when diluted by non-DENV-reactive IgA. It has been shown that free IgA binding to the FcαR results in generally anti-inflammatory signaling, while immune complexed IgA binds more avidly and results in an activated, pro-inflammatory state (58-60). This effect has been shown on a clinical scale, with infusion of pooled human serum IgA reducing inflammation in synovial infiltrates in rheumatoid arthritis as well as reducing symptoms of arthritis in human FcαR transgenic mice (61). Given this, the anti-inflammatory contribution of free non-DENV-reactive IgA in vivo would further weigh against any inappropriate inflammation arising from IgA/DENV immune complexes. With the observation that IgA is incapable of mediating ADE in the systems described herein, we suggest that DENV-reactive IgA may possess the ability to not only limit DENV infection, but also DENV-associated immunopathogenesis.

**METHODS**

**Viruses:** DENV-3 (strain CH53489) stocks were prepared by propagating a low-passage inoculum in Vero cells. Supernatants were harvested, centrifuged at 3000g at 4°C to pellet suspended cells and debris, aliquoted, and stored at -80°C. Infectious titer (PFU) was determined by plaque assay on Vero cells.

**Cell lines:** The U937 cell line was kindly supplied by Dr. Scott Blystone. Cells were maintained in RPMI media (Corning, #10-040-CV) supplemented to 10% (v/v) FBS, 1% penicillin/streptomycin, and 1% L-glutamine. Culture flasks were incubated in a humidified incubator at 37°C, 5% CO₂.

**Monocyte-derived macrophage differentiation:** Monocytes from cryopreserved PBMC obtained from healthy normal donors were differentiated into macrophages largely following Boonak et al. (40). In short, monocytes were isolated by negative selection according to the manufacturer’s protocol (Biolegend, #480059) and were resuspended in RPMI supplemented to with 100ng/mL M-CSF
(Peprotech, #300-25), 10% (v/v) FBS (Gibco), 1% penicillin/streptomycin, 1% L-glutamine. Cell density was adjusted to 1.25-1.5x10^5 cells per mL and 2mL/well plated in tissue culture-treated 24 well plates (Corning, #3524). Plates were centrifuged for 2 minutes at 500 g to adhere monocytes to the bottom of the well and incubated at 37°C/5% CO2. The day of isolation and plating was defined as day 1. The culture media was repleted on day 5 by adding 1mL of differentiation media per well.

**Monocyte-derived macrophage DENV infection:** Inocula were prepared by mixing DENV with antibody dilutions (ADE conditions) or media (virus-only condition) and incubating at 37°C/5% CO2 for 60 minutes to allow for immune complex formation. Supernatants from 6-day monocyte-derived macrophage cultures were aspirated and 200 µL of inoculum added to each well. Plates were rocked to ensure even coverage by the inoculum and incubated for 2 hours at 37°C/5% CO2. After incubation, the inoculum was aspirated, wells were washed twice with 2mL RPMI medium, 500 µL differentiation media added, and plates incubated for 48 hours at 37°C/5% CO2. After 48 hours, the supernatant was removed, aliquoted, and stored at -80C for cytokine expression and viral burden analyses. Macrophages were detached from the plates by adding 600 µL of Accutase (Stemcell technologies, 07920) per well and incubated at 37°C for 20 minutes, dissociated by pipetting, transferred to round-bottom polypropylene 96-well plates, and analyzed as described for each respective assay. Cytokine production from DENV infection macrophages was quantified using a MSD QuickPlex SQ120 instrument and a human U-PLEX cytokine panel (Meso Scale Diagnostics).

**Quantification of DENV infected cells:** DENV-infected cell lines or macrophages were transferred to round-bottom polypropylene 96-well plates, washed, and fixed with IC Fixation Buffer (Invitrogen, 00-82222-49). Fixed cells were washed twice and permeabilized with 1X IC Permeabilization Buffer (Invitrogen, 00-8333-56). Cells were stained with the DENV PrM-reactive monoclonal 2H2 primary antibody (Millipore Sigma, MAB8705) at 1µg/mL, and with PE-goat anti-mouse IgG secondary antibody (#550589, BD Biosciences) at 0.4 µg/mL. The frequency of DENV infected cells was quantified on a BD LSRII flow cytometer (BD Biosciences). Data were analyzed with FlowJo version 10 (Becton Dickinson).

**Flow cytometry.** Surface staining for flow cytometry analysis was performed in PBS supplemented with 2% FBS and TruStain FcX (Biolegend, 422301) at room temperature. Aqua Live/Dead (ThermoFisher, L34957) was used to exclude dead cells in all PBMC phenotyping experiments. Antibodies and dilutions used for flow cytometry analysis are listed in Supplemental Table 1. Data collection was performed on a BD LSRII or Fortessa flow cytometer and analyzed using FlowJo v10.2 software (Becton Dickinson).

**Staining for surface-bound antibody:** Macrophages or U937 cells were harvested, washed, resuspended in serum-purified human polyclonal IgG or IgA (12511-10MG and 14036-1MG, Sigma Aldrich), and incubated at 4°C for 30 minutes. Cells were washed and stained with AF647-conjugated goat anti-human IgG or IgA as appropriate (2050-31 and 2040-31, Southern Biotech) and analyzed by flow cytometry as described above.

**Virus/antibody immune complex binding:** DENV/antibody immune complexes were generated by mixing DENV-3 with VDB33-IgG or VDB33-IgA and incubating at 37C/5% CO2 for 60 minutes and were then chilled to 4C. U937 cells or macrophages were harvested as described, counted, plated in a round-bottom polypropylene 96-well plates, centrifuged and supernatant decanted. Cells were
resuspended in the chilled immune complexes, mixed gently by pipetting, and incubated at 4C for 90 minutes. After incubation, cells were washed 4 times in cold FACS buffer. After the last wash, cells were centrifuged, decanted, resuspended in 200mL RLT-plus buffer (Qiagen) with 10mL/mL 2-mercaptoethanol, and stored at -80C for analysis.

**Experimental DENV-3 human infection model.** PBMC for flow cytometric analysis were obtained from a phase 1, open-label study (ClinicalTrials.gov identifier: NCT04298138) that was conducted between August 2020 and July 2021 at the State University of New York, Upstate Medical University (SUNY-UMU) in Syracuse, New York. Participants received a single subcutaneous inoculation of 0.7 x 10^3 PFU (0.5ml of a 0.7 x 10^3 PFU solution) of the CH53489 DENV-3 infection strain virus manufactured at the WRAIR Pilot Bioproduction Facility, Silver Spring, MD (US FDA Investigational New Drug 19321). All participants were pre-screened to ensure an absence of preexisting flavivirus using the Euroimmun dengue, West Nile, and Zika IgG ELISA kits (Lübeck, Germany). Subjects were monitored in an outpatient setting unless the hospitalization criteria were met. The Dengue Human Infection Model and associated analysis was approved by the State University of New York Upstate Medical University (SUNY-UMU) and the Department of Defense’s Human Research Protection Office.

**Statistical analysis:** All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, La Jolla, CA) with a p-value < 0.05 considered significant.

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**Competing interests:** Authors ADW and ATW are co-inventors on the provisional patent “IgA monoclonal antibodies as a prophylactic and therapeutic treatment for acute flavivirus infection”. ATW and SJT are co-founders of Azimuth Biologics, Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

**Data availability.** The authors declare that all data supporting the findings of this study are available within this article or from the corresponding author upon reasonable request.
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Figure 1: DENV-specific IgG, but not IgA, mediates ADE in U937 cells. A) Quantification of FcγRIIa (CD32), FcγRI (CD64), FcαR (CD89), and DC-SIGN (CD208) expression on U937 cells by flow cytometry. Histogram labels are isotype-subtracted geometric mean fluorescence intensity (MFI) values. Dashed line indicates isotype control staining. B) Assessment of DENV infection-enhancing activity of DENV-specific IgG and IgA in U937 cells. Data shown as fold change in U937 cell infection frequency relative to DENV alone at the indicated antibody concentration. Dashed line indicates infection rate observed with virus alone, set to a value of 1 for each biological replicate. C) The area under the ADE curves for each of 6 independent biological replicates of IgG and IgA infection experiments relative to infection achieved with DENV alone. * p < 0.05, Wilcoxon matched-pairs test.

Figure 2: DENV-specific IgG, but not IgA, mediates ADE in primary human monocyte-derived macrophages A) Assessment of CD14 and CD163 expression on primary human monocyte-derived macrophages by flow cytometry. Dashed line indicates isotype control. B) Quantification of FcγRIIa (CD32), FcγRI (CD64), FcαR (CD89), and DC-SIGN (CD208) on primary human monocyte-derived macrophages by flow cytometry. Histogram labels are isotype-subtracted geometric mean fluorescence intensity (MFI) values. Dashed line indicates isotype control staining. C) Assessment of DENV infection-enhancing activity of DENV-specific IgG and IgA in primary human monocyte-derived macrophages. Data shown as fold change in primary human monocyte-derived macrophages cell infection frequency relative to DENV alone at the indicated antibody concentration. Dashed line indicates infection rate observed with virus alone (set to 1). D) The area under the ADE curves for each of 6 independent biological replicates of IgG and IgA infection experiments. * p < 0.05, Wilcoxon matched-pairs test.

Figure 3: IgG mediated antibody dependent enhancement increases pro-inflammatory cytokine production. Quantification of A) IFN-α2a, B) IFN-β, C) IL-6, D) MIP-1α, and E) TNFα in supernatants collected from human macrophage cultures infected with DENV alone or with DENV/IgG or DENV/IgA immune complexes. Data shown as fold change in cytokine production relative to DENV alone. Dashed line indicates cytokine production observed with virus alone (set to 1). Right panel indicates relationship between cytokine production enhancement (fold over DENV alone) and infection enhancement achieved at equimolar concentrations of IgG or IgA. * p < 0.05, Wilcoxon matched-pairs test.

Figure 4: IgG more efficiently binds its cognate Fc receptor than IgA. A) Assessment of U937 cell ability to bind purified human serum-derived polyclonal IgG or IgA at the indicated concentration. Results are representative of 3 independent experiments. B) Assessment of primary human monocyte-derived macrophages to bind purified human serum-derived polyclonal IgG or IgA at the indicated concentration. Results are representative of 3 independent experiments. C) DENV/IgG immune complexes bind FcγR and FcαR expressing cells more avidly than DENV/IgA immune complexes. DENV or DENV pre-complexed with VDB33-IgG or VDB33-IgA were added to U937 cells for 90 min at 4°C and extensively washed. Cell-bound virus quantified by qRT-PCR. Results are shown as background-subtracted GE/ml of cell lysate. D) DENV or DENV pre-complexed with VDB33-IgG or VDB33-IgA were added to monocyte-derived macrophages cells for 90 min at 4°C and extensively washed. Cell-bound virus quantified by qRT-PCR. Results are shown as background-subtracted GE/ml of cell lysate. * p < 0.05, ** p < 0.01, one-way ANOVA with Tukey post-hoc test.
**Figure 5: Evolution of monocyte FcR expression over the course of a primary DENV-3 infection.**

Cryopreserved PBMC from a DENV-3 infected patient at the indicated timepoints were analyzed by flow cytometry. **A)** Frequencies of CD14^+^CD16^−^ (classical), CD14^+^CD16^+^ (intermediate), and CD14^−^CD16^+^ (non-classical) monocytes gated on viable CD3^−^CD56^−^CD19^−^ leukocytes (left). **B)** Aggregated fold-change of geometric mean fluorescence intensity of FcγRIIa (CD32), FcγRI (CD64), FcαR (CD89) and DC-SIGN (CD209) on CD14^+^CD16^−^ monocytes. Stained samples were isotype control-subtracted prior to fold-change analysis. Red highlight indicates window of viremia following DENV-3 challenge.
Figure 1

A) U937 cell line

B) Infection enhancement (fold over virus alone)

C) AUC

- CD32 PE (FcγRII)
- CD64 PE (FcγRI)
- CD89 PE (FcaR)
- CD209 PE (DC-SIGN)

VDB33-IgG
VDB33-IgA

Infection enhancement

[Ab], ug/mL

0.001 0.01 0.1 1 10 100

IgG
IgA

AUC

VDB33

*
Figure 2

A) Monocyte-derived macrophages

B) Monocyte-derived macrophages

C) Infection enhancement (fold over virus alone)

D) AUC

E) Virus production enhancement (fold over virus alone)

Infection frequency enhancement (fold over virus alone)
Figure 3

Fold-change vs. DENV-3 alone

Cytokine production enhancement

Cytokine enhancement

Infection enhancement

A) IFN-α2a

B) IFN-β

C) IL-6

D) MIP-1α

E) TNF-α
**Figure 4**

A) **U937 cell line**

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B) **Monocyte-derived macrophages**

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C) **U937 cell line**

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D) **Primary macrophages**

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- IgG AF647
- IgA AF647

- 1.0 µg/mL
- 0.1 µg/mL
- 2° only

- DENV GEq

- ** ns**
- ** ns**
- ** ns**
- ** ns**

- DENV-3
- VDB33-IgG
- VDB33-IgA

- -
- +
- +
- +
- -
- +
- +
- -
- +
Figure 5

A) % of CD3-19-56- PBMC

B) Fold-change gMFI from day 0

CD14+CD16- (classical) monocytes