FLAVIVIRUSES

Zika virus infection enhances future risk of severe dengue disease

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The Zika pandemic sparked intense interest in whether immune interactions among dengue virus serotypes 1 to 4 (DENV1 to -4) extend to the closely related Zika virus (ZIKV). We investigated prospective pediatric cohorts in Nicaragua that experienced sequential DENV1 to -3 (2004 to 2015), Zika (2016 to 2017), and DENV2 (2018 to 2020) epidemics. Risk of symptomatic DENV2 infection and severe disease was elevated by one prior ZIKV infection, one prior DENV infection, or one prior DENV infection followed by one ZIKV infection, compared with being flavivirus-naïve. By contrast, multiple prior DENV infections reduced dengue risk. Further, although high preexisting anti-DENV antibody titers protected against DENV1, DENV3, and ZIKV disease, intermediate titers induced by previous ZIKV or DENV infection enhanced future risk of DENV2 disease and severity, as well as DENV3 severity. The observation that prior ZIKV infection can modulate dengue disease severity like a DENV serotype poses challenges to development of dengue and Zika vaccines.

Dengue virus serotypes 1 to 4 (DENV1 to -4) and Zika virus (ZIKV) are closely related mosquito-borne flaviviruses with high global burdens (*1*, *2*). Dengue epidemics often overwhelm health care systems as medical staff respond to life-threatening manifestations of severe dengue disease, including vascular leak syndrome and shock (*3*). ZIKV spread across the Pacific and Americas in 2013 to 2017 and caused rare but devastating clinical outcomes, including congenital microcephaly and Guillain-Barré syndrome in adults (*2*, *4*). Vaccines against both dengue and Zika are undergoing clinical evaluation (*4*, *5*). However, the only licensed dengue vaccine, Dengvaxia, increases risk of severe dengue in previously DENV-naïve individuals (*6*). Other dengue vaccine candidates are being evaluated for possible differences in safety and efficacy on the basis of DENV infection history. There remains concern that ZIKV infection or Zika vaccines could also enhance subsequent dengue disease.

A prior DENV infection is an established risk factor for future symptomatic and severe dengue during infection with a different serotype (*7*, *8*). A first DENV or ZIKV infection induces antibodies that limit disease upon reinfection with the same virus but also generates nonprotective antibodies that bind other viruses (*9*). DENV cross-reactive antibodies can facilitate heterologous DENV infection of myeloid cells by means of antibody-dependent enhancement (ADE) and can increase dengue disease severity in humans (*10*–*14*). Many cross-reactive antibodies target epitopes conserved across flaviviruses, including the envelope protein fusion loop (*15*, *16*). $CD4^+$ and $CD8^+$ T cell responses and nonstructural protein 1 (NS1) also modulate DENV and ZIKV protection and pathogenesis (*17*, *18*).

Emerging evidence suggests that prior DENV infection may not enhance noncongenital Zika disease, but whether prior ZIKV infection increases future dengue disease in humans is unknown. In vitro and mouse challenge studies have shown that antibodies raised against DENV can enhance ZIKV infection (*19*, *20*). However, prior DENV infection was not associated with ZIKV viremia or cytokine expression in experimentally challenged macaques (*21*–*23*) or in humans (*24*–*26*), nor with fetal demise or congenital Zika syndrome in pregnant women (*27*). Further, prior DENV infection was protective against uncomplicated Zika in prospective cohort studies (*28*, *29*). Prior DENV infection was also associated with stronger cytotoxic CD8 T cell responses in ZIKV-infected humans and with protection against ZIKV in mice (*30*). In contrast, anti-ZIKV antibodies increased DENV2 infection, viral output, and migration of myeloid cells in skin explants to the same degree as anti-DENV3 antibodies (*31*). In murine models, transfer of anti-ZIKV antibodies caused greater clinical severity, mortality, proinflammatory cytokine levels, and viral load after DENV2 challenge compared with untreated mice (*32*, *33*). In macaques, prior ZIKV infection produced binding but non-neutralizing antibodies to DENV2, and challenge with DENV2 resulted in elevated viral load and hematological changes associated with severe dengue, although not in all studies (*23*, *34*, *35*).

The Zika epidemic was followed by several years of low DENV transmission, but in 2019, countries across Latin America reported a major resurgence of dengue cases. Since 2004, we have followed an active cohort of ~3800 children 2 to 16 years old living in Managua, Nicaragua, for DENV infection and disease (*36*, *37*). As chikungunya virus (CHIKV) and ZIKV were introduced into Nicaragua in 2014 and 2016, respectively, the cohort was extended to capture cases and infections with these emerging arboviruses (*28*). In 2019 to 2020, Nicaragua experienced the largest dengue epidemic in recorded history (Fig. 1A). An unprecedented number of cohort participants (n = 375) experienced a symptomatic DENV infection. All virologically confirmed dengue cases (n = 293) were caused by DENV2.

The epidemiology and longevity of the Nicaraguan cohort allowed us to test whether ZIKV infection modified subsequent risk of dengue disease. All children presenting with suspected dengue, undifferentiated febrile illness and, after 2016, afebrile rash were tested for DENV, ZIKV, and CHIKV infection by real-time reverse transcription–polymerase chain reaction (RT-PCR) of acute-phase samples and by serological assays run on paired acute and convalescent samples. Since 2004, cohort participants have provided healthy annual serum and/or plasma samples that have been tested for DENV-specific antibodies (DENV-Abs) using the DENV inhibition enzyme-linked immunosorbent assay (iELISA) (n = 8399 children, 57,963 samples tested) (Fig. 1B and table S1) (*12*, *37*). Paired annual samples are tested side by side for seroconversion or a ≥4-fold rise in the DENV iELISA titer (*12*). Since 2016, samples are screened for ZIKV infection and ZIKV-Abs using the ZIKV iELISA (Fig. 1C) and ZIKV NS1 blockade-of-binding assays (n = 14,159 and 14,247 measurements, respectively) (*37*, *38*). In each assay, a titer or percent inhibition is derived by measuring the serum antibody concentration required to compete for binding to the antigen of interest with an antigen-specific, labeled antibody. This detailed information on infections and cases is used to identify complete DENV and ZIKV infection histories for cohort participants (*37*) (Fig. 1D).

We estimated the probability of symptomatic DENV2 infection in the cohort during the 2019 to 2020 epidemic on the basis of ZIKV and DENV infection histories by using log-binomial

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generalized linear models (GLMs) adjusted for age and sex. A total of 8.8% of all cohort participants experienced symptomatic DENV infections in 2019 to 2020 (n = 302 cases meeting dengue case definition, of n = 3434 cohort participants with full infection histories). Unexpectedly, children with one prior ZIKV infection had a 12.1% probability of having a symptomatic DENV2 infection in 2019 to 2020 [confidence interval (CI) 9.9 to 14.5%], compared with only 3.5% (CI 2.4 to 4.6%) of flavivirus-naïve children (Fig. 2A and table S2), and similar to the 9.2% (CI 4.6 to 14.5%) of children with one prior DENV infection. The increased risk of dengue disease with one prior ZIKV infection remained when CIs were estimated using alternative modeling approaches; after adjustment for years since previous infection, for neighborhood-level risk of flavivirus infection, and for prior symptomatic ZIKV infection; and when afebrile dengue cases were included in analyses (figs. S1 to S5 and table S3).

After sequential DENV infections, individuals are thought to be at reduced risk of future dengue disease (*9*). Consistent with previous findings, children with two prior DENV infections had only a 2.5% (CI 0.0 to 9.0%) probability of symptomatic DENV2 infection. In contrast, children with one DENV infection followed by ZIKV infection remained at significantly elevated risk, with a 9.5% (CI 6.7 to 13.0%) probability of symptomatic DENV2 infection (Fig. 2A). For children with at least two prior DENV infections, the probability of dengue disease was low for those with (2.9%, CI 0.7 to 6.2%) or without (0.0%, CI 0.0 to 0.0%) a subsequent ZIKV infection.

A history of ZIKV infection was also a significant risk factor for severe dengue disease. The probability of experiencing dengue with warning signs or severe dengue (DwWS/SD) [2009 World Health Organization (WHO) criteria; n = 144] was significantly elevated for individuals with one DENV infection (5.4%, CI 2.0 to 9.6%), one ZIKV infection (5.9%, CI 4.3 to 7.7%), or one DENV and one ZIKV infection (4.8%, CI 2.9 to 7.0%) compared with flavivirus-naïve children (0.7%, CI 0.3 to 1.2%) (Fig. 2B and table S4). The risk of dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS) (1997 WHO criteria; n = 15) was also unusually high (*39*) and was significantly greater for children with one prior ZIKV infection (probability, 1.1%; CI 0.3 to 1.8%) and one DENV and one ZIKV infection (0.9%, CI 0 to 3.3%) compared with flavivirus-naïve children (0%) (Fig. 2C and table S5). These relationships held for individual manifestations of severe dengue disease (fig. S6). In contrast, multiple prior DENV infections did not enhance dengue disease severity.

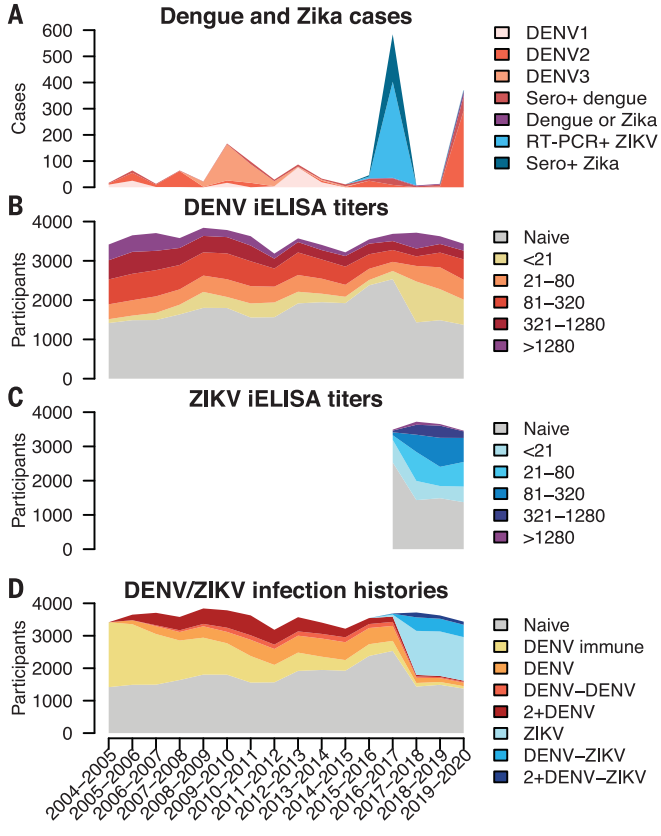

Fig. 1. Dengue and Zika cases, DENV-Ab and ZIKV-Ab titers, and infection histories in the PDCS (2004 to 2020). (**A**) Confirmed dengue and Zika cases by epidemic season and infecting virus. (**B** to **D**) DENV iELISA titers (B), ZIKV iELISA titers (C), and DENV and ZIKV infection histories (D) for cohort participants, measured at the beginning of each epidemic season. Ab titers were grouped by reciprocal serum dilution, ranging from <21 to >1280. Infection histories were grouped as follows: flavivirus-naïve (naive), entered cohort DENV-immune without subsequent infections (DENV immune), entered flavivirus-naïve with one DENV (DENV) or ZIKV (ZIKV) infection, one prior DENV infection followed by a DENV (DENV-DENV) or ZIKV (DENV-ZIKV) infection, ≥2 prior DENV infections without (2+DENV) or with (2+DENV-ZIKV) a subsequent ZIKV infection.

We further tested whether history of ZIKV infection increased risk of dengue disease in a separate study based at the Nicaraguan National Pediatric Reference Hospital. Since 2005, the study has followed 5832 children 6 months to 14 years of age during and after presentation to the hospital for suspected dengue, Zika, or chikungunya. In 2018 and 2019, 388 children were enrolled in the hospital study, and all virologically confirmed cases were caused by DENV2 (n = 277). For a random subset of the hospital study's DENV2 cases (n = 88), we analyzed acute- and convalescent-phase samples (days 1 to 5 and 12 to 26 post–symptom onset, respectively) by the ZIKV and DENV E-domain III ELISA and DENV iELISA (*40*). In combination, these assays had 96% sensitivity and 96% specificity for detecting prior ZIKV infection in a separate set of children from the Pediatric Dengue Cohort Study (PDCS) with known infection histories (n = 53) (*37*). Compared with the full PDCS cohort (53%), significantly more cohort (76%) and hospital study (86%) dengue cases had a prior ZIKV infection, with or without a prior DENV infection (Kruskal-Wallis rank sum test, $P < 2.2 \times 10^{-16}$). By contrast, for those with prior DENV infection only, there were no differences between groups (P = 0.25), suggesting that ZIKV infection history helped explain the higher rate of disease (Fig. 2D). Further, among DENV2 cases in the cohort only or cohort and hospital studies (Fig. 2, E and F; and table S6; logistic regression adjusted for age and sex), the probabilities of experiencing DwWS/SD or DHF/DSS were significantly greater for children with histories of ZIKV with or without DENV (DHF/DSS: 11.6%, CI 6.2 to 17.2%), compared with naïve children (0.0%) and similar to the rate for those with only prior DENV infection (14.0%, CI 2.5 to 31.4%).

We previously showed that the level of preexisting DENV-Abs correlates with subsequent symptomatic and severe dengue disease (*12*, *14*). Here, we found that children with the same number of prior DENV or DENV and ZIKV infections had similar levels of preexisting DENV-Ab titers (Fig. 2G), whereas preexisting ZIKV-Ab titers were higher for children with prior ZIKV infection, with or without prior DENV infection (Fig. 2H), consistent with previous studies (*41*–*43*). We used log-binomial GLMs to test whether preexisting DENV-Abs, including cross-reactive antibodies induced by prior ZIKV infection, and preexisting ZIKV-Abs were associated with dengue disease risk in 2019 to 2020. Children with any level of preexisting cross-reactive DENV-Abs were at significantly greater risk of symptomatic DENV2 than flavivirus-naïve children (Fig. 2I). Those with a range of intermediate preexisting DENV-Abs were at the greatest risk of symptomatic DENV2 disease (titers of 21 to 80: 13.7%, CI 10.3 to 17.4; naïve: 3.5%, CI 2.4 to 4.6) (Fig. 2I), DwWS/SD (titers of 21 to

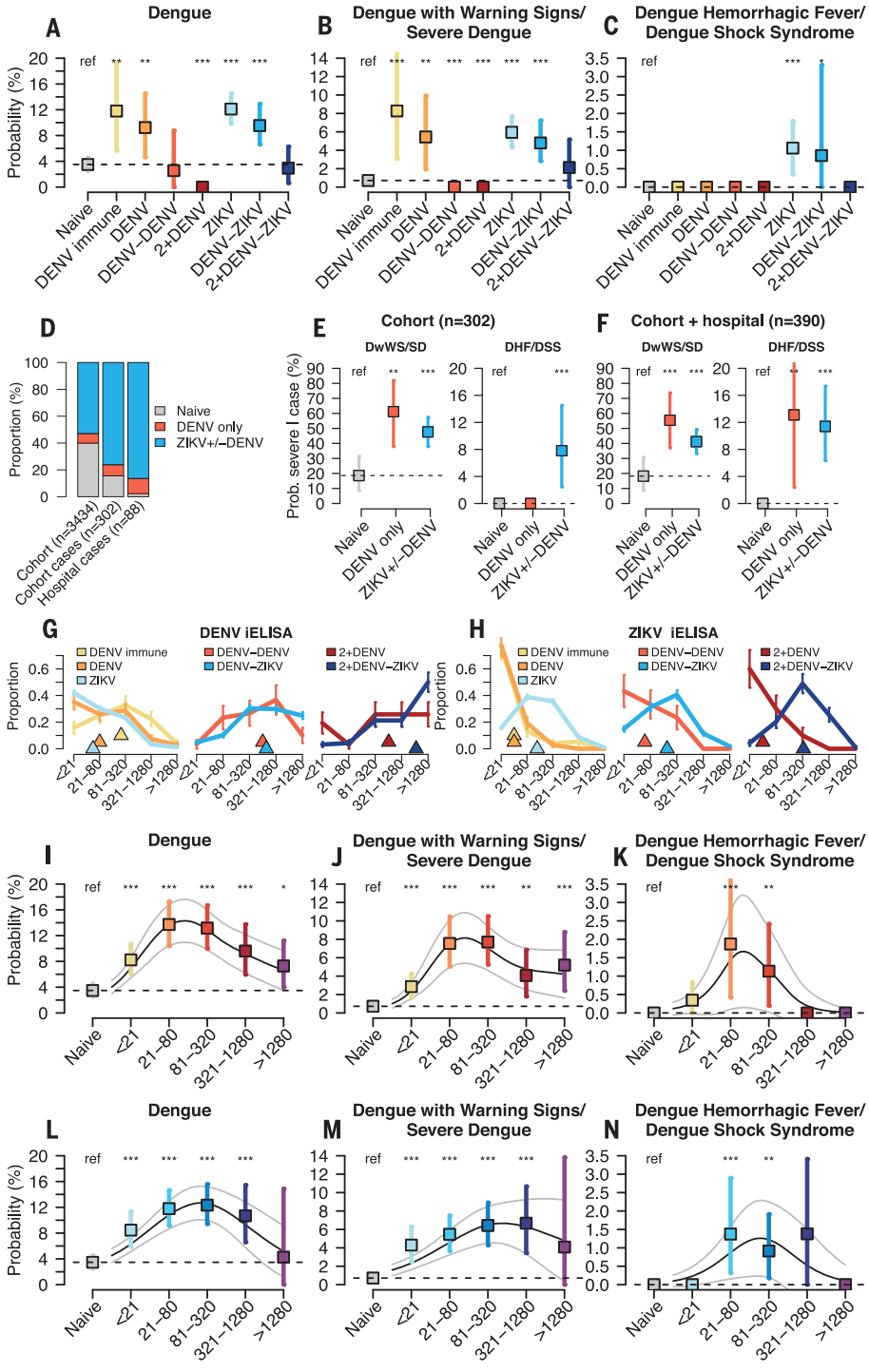

Fig. 2. Probability of symptomatic and severe DENV2 infection by prior DENV and ZIKV infection history and preexisting antibody titer, 2019 to 2020. (**A** to **C**) Log-binomial GLMs were used to estimate the probability of dengue disease in 2019 to 2020 in the cohort study on the basis of DENV and ZIKV infection history with bootstrap resampling (n = 10,000) to construct 95% CIs and calculate P values. (**D**) Infection histories for all children in the cohort, for cohort dengue cases, and for hospital study dengue cases. Differences in the proportion with histories of prior ZIKV and/or DENV or DENV only were tested with a Kruskal-Wallis rank sum test. (**E** and **F**) Probability of severe dengue disease among confirmed dengue cases in cohort (E) or cohort and hospital studies (F) by infection history, estimated using logistic regression. Bootstrap resampling (n = 10,000) was used to construct 95% CIs and calculate P values. (**G** and **H**) DENV iELISA (G) and ZIKV iELISA (H) titer distributions (grouped by reciprocal serum dilution, from <21 to >1280) for cohort participants in 2019 to 2020 by DENV and ZIKV infection history (triangles show median values, vertical bars show ±1 standard deviation). (**I** to **K**) Log-binomial GLMs were used to estimate the probability of dengue disease in 2019 to 2020 in the cohort study on the basis of preexisting DENV iELISA titers. (**L** to **N**) Log-binomial GLMs were used to estimate the probability of dengue disease in 2019 to 2020 in the cohort study on the basis of preexisting ZIKV iELISA titers. Continuous relationships between titers and disease were modeled with log-binomial generalized additive models (GAMs; black lines show probabilities, gray lines show 95% CIs). All models were adjusted for age and sex, and probabilities are shown for an average study participant (male, age 8). *P < 0.05; **P < 0.01; ***P < 0.001. P values indicate significantly different probability estimates from the naïve group.

80: 7.5%, CI 5.0 to 10.4%; naïve: 0.7%, CI 0.3 to 1.2%) (Fig. 2J), and DHF/DSS (titers of 21 to 80: 1.9%, CI 0.4 to 3.9%; naïve: 0%) (Fig. 2K). Intermediate preexisting ZIKV-Ab titers were also associated with enhancement of symptomatic and severe dengue (Fig. 2, L to N).

Epidemiological evidence suggests that prior DENV immunity differentially affects disease caused by each DENV serotype. For instance, DENV2 and DENV4 more commonly manifest as symptomatic or severe disease in secondary infections than do DENV1 and, in some studies, DENV3 (*7*, *8*, *44*, *45*). Immune correlate analyses in natural infection and vaccine studies have shown that high preexisting neutralizing antibody titers protect against DENV1 and DENV3 but not necessarily DENV2 (*46*–*48*). Consistent with these observations, we found that in the pre-Zika era (2004 to 2015), children with intermediate preexisting DENV-Ab titers also had increased probability of symptomatic (0.8%, CI 0.5 to 1.1; vs. naïve, 0.3%, CI 0.2 to 0.4) or severe dengue disease caused by DENV2 (DwWS/SD: 0.3%, CI 0.1 to 0.6% vs. naïve: 0.02%, CI 0.01 to 0.06; DHF/DSS: 0.1%, CI 0.03 to 0.3%, vs. naive, 0%) (Fig. 3, A to C; and table S7). The magnitude of the enhancing effect was greater in the post-Zika era, due in part to the higher incidence of dengue cases in 2019 to 2020 (Fig. 2, I to K; vs. Fig. 3, A to C). In the pre-Zika era, low and

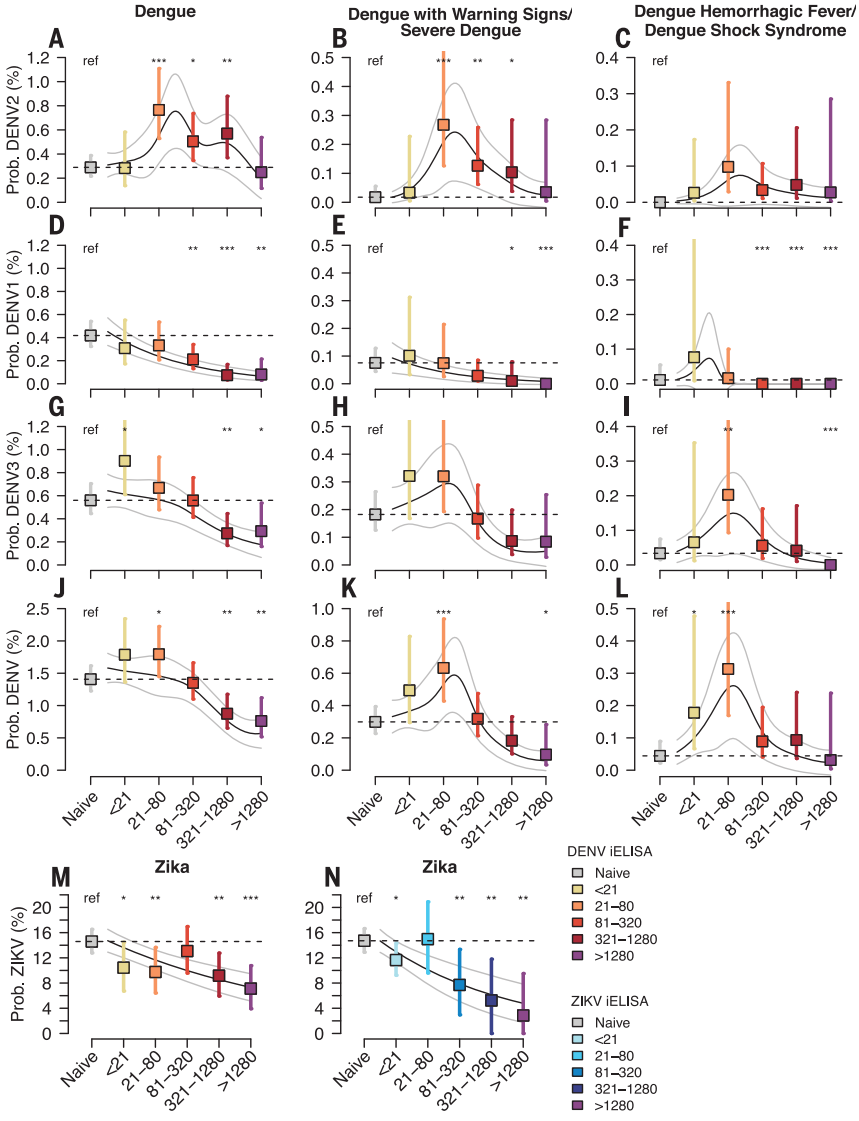

Fig. 3. Probability of disease caused by DENV2, DENV1, DENV3, all DENV, and ZIKV infection by preexisting DENV and ZIKV iELISA titers, 2004 to 2017. Probability of each disease outcome for each infecting virus was modeled as a function of preexisting antibody titer on both discrete (colored bars) and continuous (black lines) scales, shown with 95% CIs. All continuous relationships were modeled using log-binomial GAMs. (**A** to **L**) Probabilities of dengue [(A), (D), (G), and (J)], DwWS/SD [(B), (E), (H), and (K)], and DHF/DSS [(C), (F), (I), and (L)] by discrete DENV iELISA titer bins were modeled separately for [(A) to (C)] DENV2, [(D) to (F)] DENV1, [(G) to (I)] DENV3, and [(J) to (L)] all serotypes in the pre-Zika era (2004 to 2015) (J to L) using generalized estimating equation log-binomial models. Point estimates and confidence intervals correspond to probabilities, and *P* values correspond to iELISA titer bins with significantly different relative risk compared with the naive group. (**M** and **N**) Probabilities of Zika (2016) by preinfection DENV iELISA (M) and ZIKV iELISA (N) titer bins were modeled using log-binomial GLMs, using bootstrap resampling (n = 10,000 to calculate 95% CIs and *P* values.). All models were adjusted for age and sex, and model estimates are shown for an average study participant (male, age 8). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

intermediate DENV-Ab titers also had an enhancing effect on symptomatic and severe DENV3 infection (0.2%, CI 0.09 to 0.4%; vs. naïve: 0.03%, CI 0.01 to 0.08) (Fig. 3, G to I) but not on symptomatic or severe DENV1 infection (Fig. 3, D to F). By contrast, high preexisting DENV-Abs had a protective effect against symptomatic DENV1 and DENV3 infections (Fig. 3, D and G; and table S7). When all DENV cases were analyzed simultaneously, an overall protective effect was observed against symptomatic disease, whereas the enhancing effect increased as the definition of severity narrowed to DHF/DSS (Fig. 3, J to L), as we observed previously (*12*).

Similar to observations for DENV1, high preexisting DENV-Ab and ZIKV-Ab titers were associated with reduced probability of uncomplicated Zika during the 2016 Zika epidemic (Fig. 3, M and N; and table S8) (we did not observe severe Zika in the cohort). Consistent with our previous epidemiological findings (*28*), one or multiple prior DENV infections were protective against Zika (table S8). However, intermediate DENV-Ab and ZIKV-Ab titers were not significantly protective, possibly indicating more complex relationships (alternative titer bins are provided in fig. S8).

We find that prior ZIKV infection modulates future dengue disease risk to a similar degree as prior infection with a DENV serotype. A single prior ZIKV infection, like one prior DENV infection, increases the probability of symptomatic and severe dengue disease caused by DENV2. Further, one DENV followed by one ZIKV infection also increased future risk of dengue disease, unlike sequential DENV infections, which reduced future risk, suggesting an important difference between secondary flavivirus infection with ZIKV versus a DENV serotype. Our findings also show that the relationship between preexisting anti-flavivirus antibodies and disease depends on the secondary infecting virus. Intermediate preexisting cross-reactive DENV-Ab or ZIKV-Ab titers enhance risk of DENV2 and DENV3 disease severity, but not that of DENV1 or ZIKV. High titers protect against symptomatic DENV1, DENV3, and ZIKV, but not DENV2 infection. Thus, we find that asymmetry exists among DENV serotypes and between DENV and ZIKV infections.

On the basis of our findings and previous literature on DENV1 to -4 (*7*, *8*, *44*–*48*), we posit that prior ZIKV infection, like prior DENV infection, is particularly capable of enhancing DENV2 disease but that enhancement of other serotypes is possible. Mechanistic studies in animal models and human skin explants have shown that prior primary ZIKV infection induces anti-DENV2 antibodies that facilitate classical ADE of infection and increase disease severity during DENV2 challenge (*23*, *31*–*34*). In humans, primary ZIKV infection induces lower heterologous neutralizing antibody titers to DENV1 to -4 than primary DENV1 to -3 infection (*41*, *42*), suggesting the potential for enhancement of multiple serotypes (fig. S9). Further, although secondary ZIKV infection induces highly specific ZIKV-Abs and boosts heterologous DENV binding antibodies, ZIKV infection does not induce the broadly cross-neutralizing antibodies observed after secondary DENV infection (fig. S9) (*41*, *42*, *49*). However, memory B cells and monoclonal antibodies with high DENV1-, DENV2-, and DENV3-ZIKV cross-neutralization activities have been isolated, suggesting cross-protection is possible (*42*, *43*, *50*). The tighter structure of the ZIKV virion may modify the types of antibodies ZIKV induces

and limit their neutralizing potency against DENV, as well as reduce the ability of cross-reactive DENV antibodies to enhance ZIKV infection (*51*, *52*). In addition, anti-ZIKV cytotoxic T cells mostly target structural protein epitopes, which may not protect against dengue, as anti-DENV $CD8^+$ T cells mostly target conserved regions of nonstructural proteins (*53*, *54*). Finally, whether anti-ZIKV immunity enhances other DENV serotypes may depend on characteristics specific to the secondary infection, including virion structure and maturation state, cell infection mechanism, and fucosylation of immunoglobulin G (*51*, *52*, *55*, *56*).

Our findings suggest that protective and pathogenic interactions between DENV1 to -4 and ZIKV could affect vaccine efficacy and safety. If monovalent Zika vaccines induce cross-reactive DENV antibodies such as those observed after natural ZIKV infection, Zika vaccines could increase risk of subsequent symptomatic and severe dengue disease. In contrast, we found that DENV-Abs induced by natural DENV infection modestly protect against uncomplicated Zika, but the effect on vertically transmitted ZIKV in humans and risk of congenital Zika syndrome requires additional study. Further, differences in efficacy in the phase 3 trials of multiple dengue vaccines may be explained not only by lack of protection but also by enhancement of certain serotypes (*6*). Our findings demonstrate why it is important to analyze separately correlates of protection and risk for dengue caused by specific serotypes. We have illustrated critical immunological interactions among ZIKV and the DENV serotypes and revealed differences in the relationship between preexisting cross-reactive antibodies and serotype-specific risk of symptomatic and severe disease. Elucidating how immunity to DENV1 to -4, ZIKV, and possibly other flaviviruses modulates future disease risk is of utmost concern for developing and deploying safe, effective flavivirus vaccines and preventing future epidemics.

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SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/369/6507/1123/suppl/DC1
Materials and Methods
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MDAR Reproducibility Checklist

View/request a protocol for this paper from *Bio-protocol*.

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Science

Zika virus infection enhances future risk of severe dengue disease

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Double whammy

Dengue and Zika virus epidemics have been lapping each other around the globe. These are closely related mosquito-borne viruses with about 40% homology within the envelope protein. We know that subsequent dengue infections bring a risk of antibody-dependent disease enhancement. Whereas emphasis has been placed on how prior dengue immunity affects Zika infection, little is known about how prior Zika immunity may affect dengue disease. Katzelnick *et al.* have been following a well-characterized and established pediatric cohort in Nicaragua who were serially exposed to both flaviviruses in recent years (see the Perspective by Clapham). This study shows not only that a previous history of just one round of dengue is a problem but also that prior Zika immunity creates an increased risk for severe dengue virus sereotype 2 infection. By contrast, multiple infections raise antibodies to protective levels.

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