Human antibody responses after dengue virus infection are highly cross-reactive to Zika virus

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Zika virus (ZIKV) is an emerging mosquito-borne flavivirus of significant public health concern. ZIKV shares a high degree of sequence and structural homology compared with other flaviviruses, including dengue virus (DENV), resulting in immunological cross-reactivity. Improving our current understanding of the extent and characteristics of this immunological cross-reactivity is important, as ZIKV is presently circulating in areas that are highly endemic for dengue. To assess the magnitude and functional quality of cross-reactive immune responses between these closely related viruses, we tested acute and convalescent sera from nine Thai patients with PCR-confirmed DENV infection against ZIKV. All of the sera tested were cross-reactive with ZIKV, both in binding and in neutralization. To deconstruct the observed serum cross-reactivity in depth, we also characterized a panel of DENV-specific plasmablast-derived monoclonal antibodies (mAbs) for activity against ZIKV. Nearly half of the 47 DENV-reactive mAbs studied bound to both whole ZIKV virion and ZIKV lysate, of which a subset also neutralized ZIKV. In addition, both sera and mAbs from the dengue-infected patients enhanced ZIKV infection of Fc gamma receptor (FcγR)-bearing cells in vitro. Taken together, these findings suggest that preexisting immunity to DENV may impact protective immune responses against ZIKV. In addition, the extensive cross-reactivity may have implications for ZIKV virulence and disease severity in DENV-experienced populations.

Zika virus | cross-reactivity | antibodies | B-cell responses

Zika virus (ZIKV) is a mosquito-borne virus belonging to the *Flaviviridae* family of single-stranded positive-sense RNA viruses. First isolated in Uganda in 1947 (1), this virus remained largely dormant for the next six decades until it reemerged as the cause of an epidemic on Yap Islands, Micronesia in 2007 (2). ZIKV has since then been linked with several outbreaks in the Pacific and Americas, along with sporadic human cases in Africa and Asia (3, 4). Until its appearance in French Polynesia in 2013 and more recently in Brazil in 2015, ZIKV infection was primarily associated with mild self-limiting illness, with symptoms similar to and often milder than dengue virus (DENV) or Chikungunya virus (CHIKV) infections (2–4). However, the more recent outbreaks have caused severe neurological complications including Guillain–Barré Syndrome in adults and an increase in congenital microcephaly and other adverse birth outcomes in Brazil (5–7). The Pan American Health Organization has reported that as of May 2016, local transmission of ZIKV had spread to over 38 countries or territories in the Americas. In addition, a recent WHO report states that 44 new countries are experiencing their first ZIKV outbreak since 2015. Despite the improving surveillance of the virus, accurate diagnosis has been challenging given the similarities in the clinical presentation of ZIKV to other arboviral infections endemic in these regions, among other factors.

During the viremic period, ZIKV can be found in patient blood, saliva, urine, and other bodily fluids early after symptom onset (8–10). During the Yap Islands epidemic in 2007, anti-ZIKV IgM ELISAs and ZIKV plaque reduction neutralization titer (PRNT) assays were performed to confirm infection in RT-PCR negative cases (2, 8). However, as these studies showed, the cross-reactivity between ZIKV and other flaviviruses makes confirmation of infection difficult, especially when patients may have had flavivirus exposures before their suspected ZIKV infection (2, 8). Given the overlapping presence of DENV and other flaviviruses in a majority of ZIKV epidemic regions (11), there are great challenges in serology-based testing of flavivirus-immune patients (12).

The DENV envelope (E) protein, considered a major imunodominant target for antibody responses in dengue patients (13–15), bears greater than 50% homology to ZIKV E protein (16). In addition to complicating the serology-based diagnosis of ZIKV infection, this raises an interesting question about the biological implications of the cross-reactivity on protection, virulence, and immunopathology of ZIKV infections. At present, the effect of preexisting immunity to DENV or other flaviviruses on immune responses induced by ZIKV infection is unknown. To this end, we were interested in determining the degree to

Significance

In this study, we address the issue of cross-reactivity between dengue virus (DENV) and Zika virus (ZIKV) by testing sera and plasmablast-derived monoclonal antibodies from dengue patients against ZIKV. We show that both acute and convalescent dengue sera potently bind and neutralize ZIKV and that this cross-reactivity is also evident at the monoclonal level. We also demonstrate in vitro antibody-dependent enhancement of ZIKV infection in the presence of dengue-induced antibodies. Our findings strongly suggest that preexisting dengue antibodies may modulate immune responses to ZIKV infection. These data are timely and highly relevant from a public health standpoint given that a majority of regions currently experiencing Zika virus epidemics are endemic for dengue.

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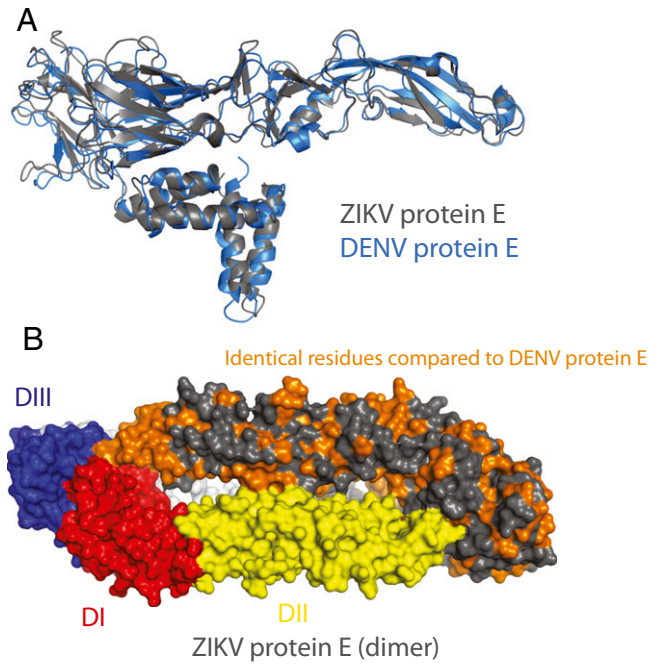

Fig. 1. The DENV2 and ZIKV E proteins share a highly similar fold and 54% sequence identity. (*A*) Overlay of DENV2 (blue) and ZIKV (gray) E protein structures (16, 34). (*B*) Structure of the ZIKV E protein dimer. The left monomer is colored by its domain structure. At right, amino acids conserved between the ZIKV PRVABC59 and DENV2 Tonga/74 E proteins are colored orange on a gray ZIKV backbone.

which dengue-induced antibodies cross-react with ZIKV in terms of binding, virus neutralization, and antibody-dependent enhancement (ADE) of ZIKV infection, both at the serum and single-cell level.

In this study, we provide an analysis of the cross-reactivity of acute and convalescent dengue-immune sera against ZIKV. The sera were collected from nine patients admitted to Siriraj Hospital in Bangkok, Thailand with confirmed DENV infection. Both acute and convalescent sera showed high binding titers to ZIKV lysate and could also neutralize ZIKV in vitro. To understand the origin and characteristics of these cross-reactive serum responses, we also analyzed a panel of plasmablast-derived DENV-reactive monoclonal antibodies (mAbs). Of the 47 mAbs tested, nearly half (22/47) bound to ZIKV lysate and an additional four to the whole virus. Seven of these mAbs also neutralized ZIKV in vitro. Five sera and a subset of the mAbs were also tested for ADE activity using the FcγR-bearing monocytic U937 cell line. All sera and ZIKV-reactive mAbs tested enhanced infection in vitro, whereas two DENV-specific but ZIKV-nonreactive mAbs did not. The data presented here have important implications for clinical diagnosis given that the current ZIKV outbreak in the Americas and the Caribbean is largely ongoing in dengue-endemic areas. Equally important, these findings set the stage for more in-depth studies that explore how preexisting flavivirus immunity may shape immune responses to ZIKV infection.

Results

Sera from DENV-Infected Patients Are Highly Cross-Reactive to ZIKV Lysate. A recently published study reported high structural similarity between the E proteins of ZIKV and other flaviviruses including DENV (16). We compared the ZIKV and DENV2 strains used in our study, ZIKV PRVABC59 and DENV2 Tonga/74, to determine the homology between their E proteins and identify potential targets for cross-reactive immune responses. The DENV2 and ZIKV E proteins share an extremely similar, superimposable structure (rmsd 1.1 Å; Fig. 1 *A* and *B*), with an overall 53.9% amino acid sequence identity (Fig. S1 *A* and *C*). E domain I (EDI) and EDII exhibit slightly higher conservation (59.1% and 56.6% identity, respectively), including the fusion loop of EDII, which is perfectly conserved between the two proteins (Fig. S1 *B* and *C*). To assess the degree of cross-reactivity of DENV-specific B-cell responses against ZIKV, mock- and ZIKV-infected Vero cell lysates were generated for use in binding assays. The lysates were tested by Western blot and probed for the presence of E protein using the mouse pan-flavivirus antibody 4G2. A band consistent with the size of ZIKV E protein was observed in ZIKV lysate and absent in the mock lysate (Fig. 2*A*). We then measured binding of both acute and convalescent dengue sera, as well as naive sera, using the ZIKV lysate by IgG ELISA (Fig. 2 *B* and *C* and Table S1).

The nine dengue patients in this study were all confirmed for DENV infection by RT-PCR. Serum samples were collected once during the acute phase ($n = 9$) and, for five patients, a second time at convalescence ($n = 5$) (Table S1). Sera from two flavivirus-naïve donors were also included in our analyses as a comparison with dengue sera (Table S1). All 14 dengue serum samples showed high ZIKV-specific IgG endpoint dilution titers, with median values of 177,400 and 125,000 for acute and convalescent samples, respectively (Fig. 2 *B* and *C* and Table S1). All of the sera showed negligible titers against mock lysate (endpoint dilution < 250). The flavivirus-naïve samples were essentially negative against both the ZIKV-infected and the mock lysates (Fig. 2*C* and Table S1). These data illustrate that ZIKV cross-reactive antibodies can be readily detected in the serum of dengue patients living in a highly dengue-endemic country like Thailand.

Dengue-Immune Sera Exhibit High Neutralization Potency Against ZIKV. To determine whether the dengue sera could also neutralize ZIKV in vitro, we performed focus reduction neutralization tests

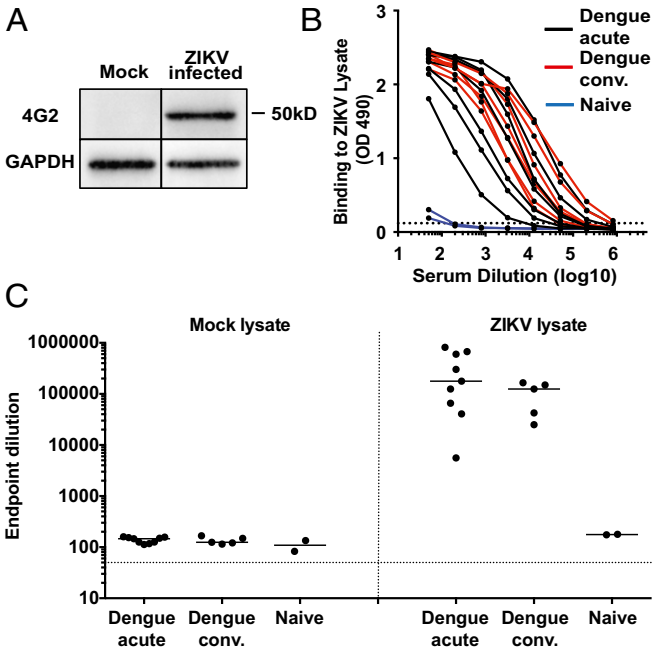

Fig. 2. Sera from patients with secondary DENV infection exhibit potent cross-reactivity against ZIKV. (*A*) Western blot of lysates from mock- or ZIKV-infected Vero cells. The pan-flavivirus–reactive mAb 4G2 was used to probe for E protein. (*B*) Binding of acute (black) and convalescent (red) dengue-immune and flavivirus-naïve (blue) sera to ZIKV lysate. Dotted line represents three times the background signal of plain blocking buffer. (*C*) Summary of binding of serum samples to lysates from mock- or ZIKV-infected Vero cells determined by ELISA. Acute ($n = 9$) and convalescent ($n = 5$) dengue and two control sera were tested. Median endpoint IgG titers for each set of sera are indicated. The dotted line represents the initial serum dilution (1/60). The binding data shown in *B* and *C* are the result of two independent experiments, and the mean value is plotted.

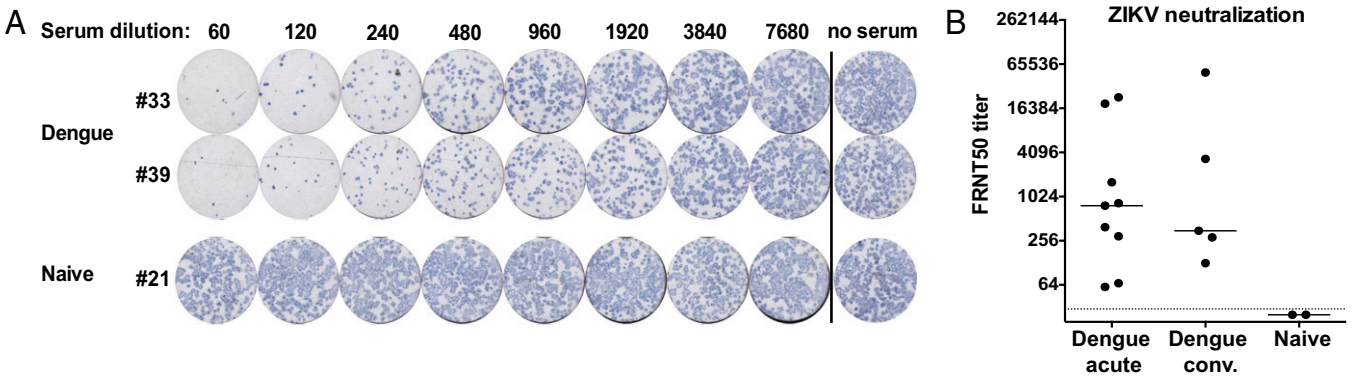

Fig. 3. Sera from acute and convalescent dengue patients can neutralize ZIKV. (*A*) Representative panel of FRNT assay showing neutralization of ZIKV by acute dengue sera (33 and 39) and one flavivirus-naïve serum sample (21). (*B*) Neutralization activity of serum samples against ZIKV. The FRNT50 titers of flavivirus-naïve ($n = 2$) sera and acute ($n = 9$) and convalescent ($n = 5$) dengue sera were determined by FRNT assay as previously described (13). The FRNT assay for each sample was repeated in two or more independent experiments. The solid line represents median FRNT50 value, and the dotted line represents the initial serum dilution (1/30).

(FRNTs) on all 14 dengue sera against ZIKV. A representative example of the ZIKV neutralization assay with two acute dengue sera (#33 and #39) and one flavivirus-naïve serum sample (#21) is shown in Fig. 3*A*. The ZIKV FRNT50 titers of the acute dengue samples ranged from 60 (#60) to 23,109 (#79), with a median value of 770. The convalescent dengue sera ranged in FRNT50 titers from 126 (#60R) to 50,346 (#79R), with a median titer of 350. Although neutralization titers increased between the acute and convalescent bleeds for three patients, convalescent titers for patients 55 and 67 were lower than their acute titers (Fig. 3*B* and Table S1). Of note, the convalescent samples for these two donors were obtained at a much later time point after fever onset (61–100 d) than the other three convalescent sera (Table S1). These data show that dengue-immune sera can neutralize ZIKV in vitro. The impact of these neutralizing titers on either protective immunity or disease severity after ZIKV infection remains to be defined.

mAbs Derived from Dengue-Induced Plasmablasts Are Highly Cross-Reactive to ZIKV. Although analysis of polyclonal sera from the dengue patients clearly illustrates ample cross-reactivity of dengue-immune sera against ZIKV, serum analyses alone cannot determine the origin of these cross-reactive antibodies. In other words, whether the serum cross-reactivity was caused by two individual pools of antibodies, one DENV-specific and the other ZIKV-specific, or by antibodies that recognize both viruses can only be conclusively determined by analyzing functional cross-reactivity at the monoclonal level. To dissect the cross-reactivity between DENV infection-induced antibodies and ZIKV, we characterized the binding and neutralization activity of a panel of plasmablast-derived mAbs against ZIKV. These mAbs were generated from in vivo-activated, single cell-sorted plasmablasts isolated during ongoing infection from four DENV2 patients and were previously shown to be DENV-reactive either in binding or in both binding and neutralization (13).

Of the 47 mAbs tested, 22 bound with high affinity to ZIKV lysate (Fig. 4*A*). An additional four ZIKV cross-reactive mAbs were identified using a whole-virus capture ELISA (Fig. 4*B* and Table S2). A majority of the ZIKV-specific mAbs (20/26) came from the plasmablasts of donors 31 and 39. Only a handful of mAbs from donors 32 and 33 cross-reacted with ZIKV, with several of these recognizing only whole ZIKV. Although nearly half of all DENV-reactive mAbs bound ZIKV lysate or whole virus, only seven of the mAbs neutralized ZIKV in vitro (Fig. 4 *C* and *D* and Table S2). Six of these seven mAbs exhibited moderate neutralizing activity against ZIKV, with FRNT50 titers ranging between 5 μg/mL and 1 μg/mL. In contrast, mAb 33.3A06 was highly potent in ZIKV neutralization with a ZIKV FRNT50 titer of 0.03 μg/mL. Interestingly, despite the overall lower frequency of ZIKV-binding mAbs isolated from 32 and 33, half of all ZIKV-neutralizing mAbs in the panel, including the three most potently neutralizing mAbs, were derived from these two patients. Repertoire analysis of the cross-reactive mAbs showed broad immunoglobulin variable gene use and junctional diversity. The cross-reactive cells were also highly mutated, illustrating that these responses were likely the result of multiple previous DENV exposures (Table S2).

Dengue-Induced Antibodies Can Enhance ZIKV Infection of an FcγR-Bearing Monocytic Cell Line in Vitro. We tested the ability of five dengue sera and 11 plasmablast-derived mAbs to enhance ZIKV infection using a human FcγR-bearing monocytic cell line, U937. The U937 cell line is widely used to study ADE of DENV infection, and it is not typically permissive to high levels of DENV infection in the absence of enhancing antibodies (17). The five dengue sera tested were all acute samples from DENV2-infected patients, including patients 31, 32, 33, and 39 from whose plasmablasts the mAbs in this study were derived. The mAbs tested included seven ZIKV-neutralizing mAbs, of which six were intermediate in neutralization ($ELISA^{+}/Neut^{int}$) and one potent ($ELISA^{+}/Neut^{++}$), two ZIKV-reactive but nonneutralizing mAbs ($ELISA^{+}/Neut^{neg}$), and two mAbs that bound DENV but did not cross-react with ZIKV ($ELISA^{-}/Neut^{neg}$). In addition to the dengue sera and mAbs, one flavivirus-naïve serum sample (#21) and two irrelevant mAbs (cholera and influenza-specific) were also tested for ZIKV ADE activity. A representative example of the flow cytometry-based assay showing ADE activity of mAb 31.3F01 is provided in Fig. 5*A*. Each of the five dengue sera tested was able to enhance ZIKV infection of U937 cells, with peak percent infection between 27% (#31) to 66% (#55). The bell-shaped ADE curves observed with this assay generally seemed to shift to lower dilutions as the neutralizing potency of the serum sample increased (Fig. 5*B* and Table S1), presumably due to complete neutralization of the virus at higher concentrations. The flavivirus-naïve serum sample did not enhance ZIKV infection of U937 cells (Fig. 5*B*).

The six $ELISA^{+}/Neut^{int}$ mAbs enhanced ZIKV infection at the maximum concentration tested (10 μg/mL), whereas the potent neutralizer 33.3A06 exhibited minimal ADE above 2 μg/mL, again potentially due to complete viral neutralization. At lower concentrations, however, the mAb facilitated the infection of U937 cells, reaching a maximal percent infection of 81% (Fig. 5*C*). The two ZIKV $ELISA^{+}/Neut^{neg}$ mAbs also enhanced ZIKV infection, similar to the neutralizing mAbs. Two mAbs that were previously shown to be DENV1-specific (13) and were ZIKV-nonreactive (Fig. 4) did not enhance ZIKV infection (Fig. 5*C*). These data demonstrate that ZIKV-reactive antibodies can potentiate

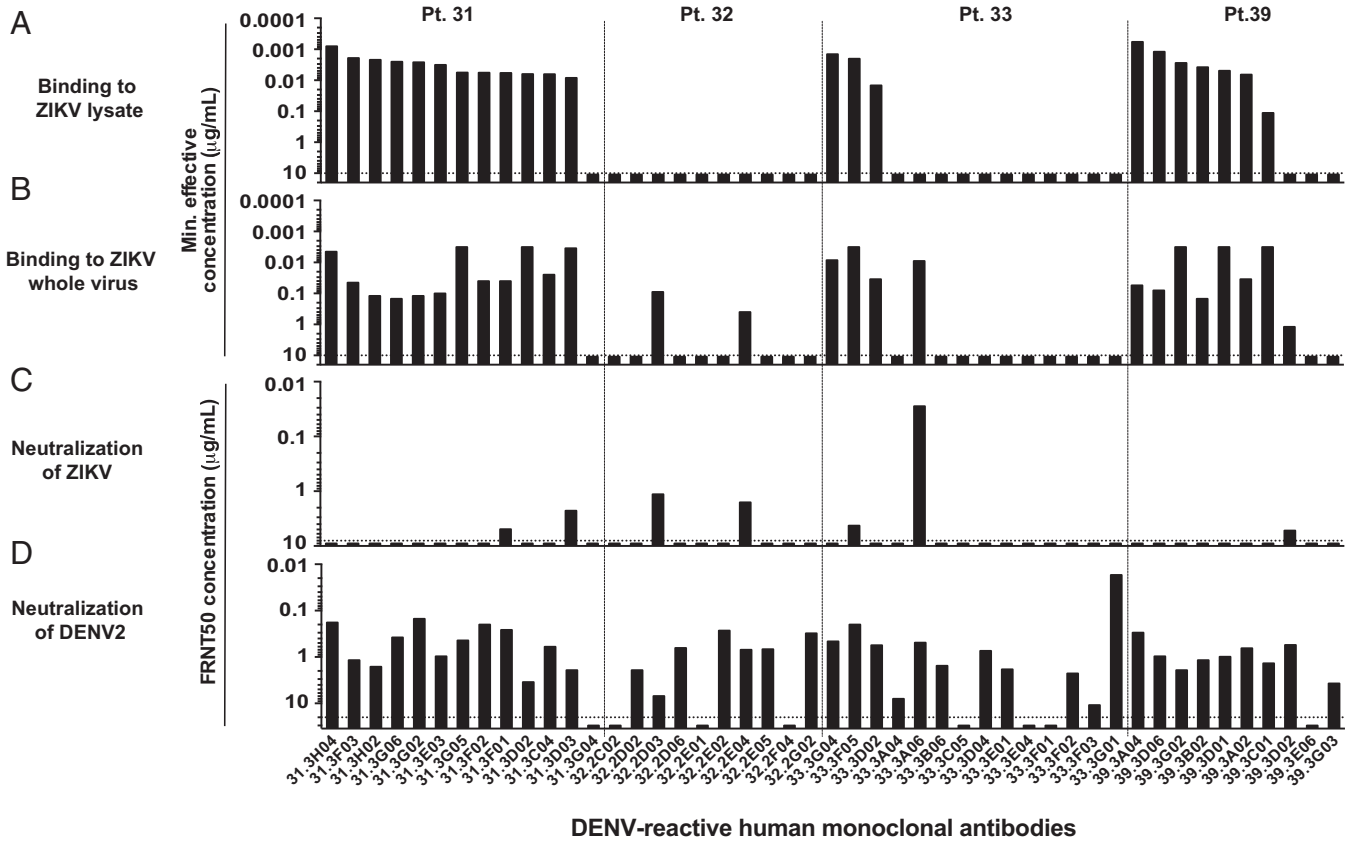

Fig. 4. A subset of DENV-specific plasmablast-derived mAbs cross-react to ZIKV by both binding and neutralization. Binding of DENV-reactive mAbs ($n = 47$) to (*A*) ZIKV lysate or (*B*) whole ZIKV. The mAbs are grouped by patient (Pt.). Values plotted represent the minimum concentration required for three times the background signal from an irrelevant mAb. Dotted line represents the maximum concentration of mAb tested in ELISA: 10 μg/mL. FRNT50 of DENV-reactive mAbs against ZIKV (*C*) and DENV2 (*D*). Dotted line represents the maximum concentration of mAb tested: 8 μg/mL (ZIKV FRNT) and 20 μg/mL (DENV FRNT). The DENV2 neutralization data in *D* have been adapted from previously published data (13). All experimental data shown is the result of two or more independent experiments.

infection of FcγR-bearing human monocytic cells in vitro and that both maximal infection and the effective concentration range of individual antibodies vary significantly.

Discussion

The emerging ZIKV shares a high degree of sequence and structural homology compared with other flaviviruses, such as DENV (16). For the current outbreak in the Americas and the Caribbean, this is of major public health concern. It is not clear how preexisting antibody titers to other flaviviruses might affect the quality of immune responses generated to ZIKV infection and, equally important, whether such cross-reactive antibodies provide protective immunity or impact disease severity in infected adults (18). In the study presented here, we have determined the degree by which dengue-induced antibodies cross-react with ZIKV, both at a serum level as well as at a single-cell level.

We characterized the ZIKV binding and neutralization potential of sera obtained from PCR-confirmed dengue patients sampled during acute disease and at convalescence. Both acute and convalescent sera had high IgG binding titers to ZIKV and potently neutralized the virus in vitro (Figs. 2*C* and 3*B* and Table S1). Although no obvious correlation was observed between DENV2- and ZIKV-specific neutralization titers in the same patients (Table S1), it is evident that a significant proportion of serum antibodies present after DENV infection cross-react with ZIKV. Although a majority of the dengue sera tested neutralized DENV2 more potently than ZIKV, sera from patients #55 and #79 had higher FRNT50 titers to ZIKV compared with DENV2 (Table S1). For patient #79, the lower dengue titers could simply be attributed to the mismatch between the serotype of infection (DENV1) and the virus tested (DENV2). For patient #55, this could have been caused by genetic differences between the laboratory-adapted DENV2 strain used in our study and the infecting DENV2 strain. An alternative possibility is that these patients were previously exposed to ZIKV and thus had ZIKV-reactive antibodies in their sera as a result. In fact, in the past few years, isolated cases of ZIKV transmission in Thailand have been reported (10, 19, 20). Although there is no evidence of previous ZIKV epidemics in Thailand, the possibility that the patients in our study are ZIKV-immune and that the extensive cross-reactivity of their sera against ZIKV is due to preexisting ZIKV-induced antibodies cannot be formally ruled out. To definitively conclude that antibodies induced by DENV infection cross-react with ZIKV, it is important to demonstrate this cross-reactivity at the monoclonal level as well. In addition, from the serum data it is unclear whether the observed cross-reactivity is caused by a small number of highly potent, cross-reactive antibodies or if this is the result of a broader, low-level cross-reactivity.

To deconstruct the cross-reactivity observed at the serum level, we analyzed the ZIKV binding and neutralization activities of plasmablast-derived mAbs generated from four acutely infected DENV2 patients. We found that over half of the DENV-reactive mAbs bound with high affinity to ZIKV (Fig. 4 *A* and *B*). At least 23 of the 26 ZIKV cross-reactive mAbs were E protein-specific, as they were previously shown to bind recombinant DENV E protein (13). Although cross-reactive binding was abundant and all 26 ZIKV-reactive mAbs neutralized DENV2,

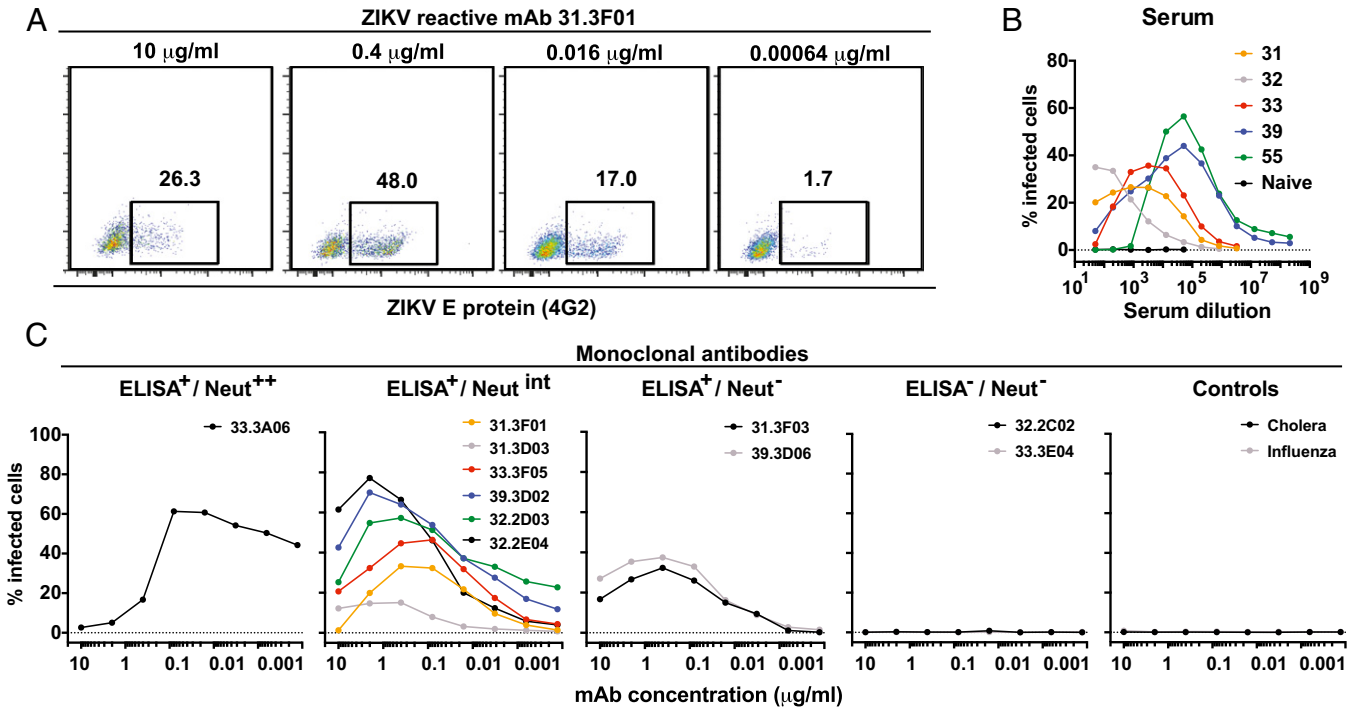

Fig. 5. Sera and mAbs from DENV-infected patients can enhance ZIKV infection of U937 cells. (*A*) Representative flow cytometry panel of mAb 31.3F01 showing percent infection at a range of mAb concentrations. (*B*) ADE activity of five dengue sera and one flavivirus-naïve serum sample. (*C*) ADE activity of dengue patient-derived ($n = 11$) and control ($n = 2$) mAbs. The antibodies are grouped by ZIKV cross-reactivity phenotype. ELISA$^{+/-}$ refers to binding activity to ZIKV by capture virus ELISA, whereas Neut$^{++/+/neg}$ refers to ZIKV neutralization activity. Infected cells were identified by 4G2 staining. The dotted line in *B* and *C* represents percent infection in the absence of antibody (virus only). Data shown are representative of two or more independently performed experiments.

less than a third neutralized ZIKV in vitro. Furthermore, of the seven ZIKV-neutralizing mAbs, only one displayed potent neutralization activity (Fig. 4*C*). Therefore, even though a large number of dengue patient mAbs were able to bind viral epitopes, the capacity to cross-neutralize ZIKV was restricted to a select few. Additionally, a majority of these DENV-reactive mAbs were previously shown to neutralize more than one DENV serotype (Table S2) (13). Hence, for a large proportion of our mAb panel, the ability to cross-neutralize the virus did not extend beyond the DENV species to ZIKV. Lastly, no obvious patterns in terms of VH gene use or dominant clones were observed for the ZIKV-reactive mAbs (Table S2) (13). Thus, the cross-reactivity observed at the serum level, at least for these four patients, appears to be caused by a diverse repertoire of B cells.

The ZIKV E protein shares a high degree of homology with the E protein of other flaviviruses including DENV (16). We compared the E proteins of the ZIKV and DENV2 strains used in our study and found an overall sequence identity of 54% (Fig. S1 *A* and *C*). EDI and EDII were relatively more conserved than EDIII, which had a lower sequence identity of 44.6% (Fig. S1*C*). Notably, the fusion loop is 100% conserved between the two viruses and also compared with other flaviviruses including yellow fever virus, West Nile virus, and Japanese encephalitis virus (Fig. S1*B*). The fusion loop has been described as a target for broadly cross-reactive antibodies against DENV (21, 22) as well as other flaviviruses (23–25) and could be one of the epitopes targeted by the cross-reactive antibodies described in our study. In addition, despite the amino acid differences between the DENV2 and ZIKV E proteins compared, the two proteins share nearly identical structures (Fig. 1*A*). This could have important implications for antibodies against conformationally sensitive epitopes, which depend on the quaternary structure of the E protein for recognition and binding (26–28). In fact, four out of the seven ZIKV-neutralizing antibodies characterized in this study bound to whole virus but failed to bind ZIKV lysate, suggesting that they recognize a conformational epitope. Efforts to map some of the antibodies described earlier are ongoing, focused especially on the potent ZIKV neutralizer 33.3A06. Identifying potential targets for broadly cross-neutralizing antibody responses could inform the design of vaccines or antibody-based therapies in the future.

Because the current ZIKV outbreak is largely localized within dengue-endemic areas, the potential for preexisting dengue-induced antibodies to enhance ZIKV infection is of concern. ADE is hypothesized to contribute to the increased disease severity often observed in secondary DENV infections (29). ADE is thought to occur when preexisting cross-reactive antibodies form virus–antibody complexes that then facilitate the infection of FcγR-bearing cells (30). This may increase the number of infected cells and cause higher serum viral loads, which have been shown to positively correlate with higher disease severity (31, 32). To determine whether dengue antibodies can enhance ZIKV infection in vitro, we infected the FcγR-bearing U937 monocytic cell line in the presence of five acute sera and 11 dengue mAbs. All five sera and the nine ZIKV-reactive mAbs tested enhanced ZIKV infection in vitro (Fig. 5 *B* and *C*). Two DENV1-specific mAbs that did not react to ZIKV by binding or neutralization assays failed to enhance ZIKV infection in this system (Fig. 5*C*). These data clearly illustrate that ZIKV cross-reactive antibodies induced after DENV infection can enhance ZIKV infection in vitro. However, it is important to point out that the physiological relevance of this mechanism must be carefully examined in vivo to determine its importance in the context of ZIKV infection of flavivirus-immune patients.

Our findings raise important questions regarding the role of cross-reactive antibodies in protective immunity as well as their potential impact on ZIKV pathogenesis and disease severity. The data presented suggest that ZIKV infection may have the potential to reactivate cross-reactive dengue-induced memory responses in patients with prior DENV exposures. There may thus be interesting differences between the immunological responses of DENV-immune patients versus those of a flavivirus-naïve individual to ZIKV. To address these issues, ongoing comparative

studies of immune responses, disease severity, and clinical outcomes in ZIKV-infected patients in both flavivirus-endemic and nonendemic areas are required. One of the most critical aspects of the current ZIKV virus outbreak is the ability of the virus to cause congenital microcephaly (6, 7). It will be essential to determine if the preexisting cross-reactive antibodies may be involved in the context of maternal–fetal transmission of ZIKV. Equally important, studying cross-reactivity against multiple ZIKV isolates, derived from both recent and previous epidemics, might shed light on the cause for the increased disease severity observed in the current outbreak. Finally, as additional ZIKV-reactive human plasmablast and memory B-cell–derived mAbs are identified, characterizing their in vivo properties in murine and macaque models will be an important step in generating potential prophylactic/therapeutic treatments. Such studies will also improve our understanding of the immunobiology of ZIKV infection and how preexisting antibodies to DENV or other flaviviruses might modulate the ZIKV-immune response.

Materials and Methods

Patient Samples. The dengue serum samples in this study were collected at Siriraj Hospital in Bangkok, Thailand. All patients were diagnosed with DENV infection by serotype-specific RT-PCR (33), and serum samples were collected during acute infection and/or convalescence. From four of these patients, a panel of mAbs was derived from single cell-sorted plasmablasts (13). Two flavivirus-naïve sera were also included as controls. All studies were preapproved by the Faculty of Medicine at Siriraj Hospital and the Emory institutional review board (IRB) (#IRB00015730).

Viruses and Viral Antigens. The ZIKV and DENV2 strains used in this study were ZIKV PRVABC59 (KU501215.1) and DENV2 Tonga/74 (AY744147.1). For a detailed description of how the virus was propagated and ZIKV lysate was prepared, *SI Materials and Methods*.

Binding, Neutralization, and ADE Assays. Sera and mAbs were tested for binding to ZIKV lysate and whole virus by ELISA. The endpoint titer/minimum effective concentration was determined as the concentration required for three times the background signal of flavivirus-naive serum/irrelevant mAb. The ZIKV neutralization activity of samples was determined by FRNT. FRNT50 was determined as the concentration or dilution factor of the sample required for 50% neutralization of the virus. A flow cytometry-based ADE assay was performed to determine percent enhancement of ZIKV infection in the presence of dengue antibodies. U937 cells were infected with a mixture of serum/mAb and ZIKV, and infected cells were stained using 4G2 and anti-mouse A488. Detailed descriptions of the ELISA, FRNT, and ADE assays are provided in *SI Materials and Methods*.

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