Cite as: S. V. Bardina *et al.*, *Science* 10.1126/science.aal4365 (2017).

Enhancement of Zika virus pathogenesis by preexisting antiflavivirus immunity

Susana V. Bardina,[1]* Paul Bunduc,[1]* Shashank Tripathi,[1,2]* James Duehr,[1]* Justin J. Frere,[1] Julia A. Brown,[1] Raffael Nachbagauer,[1] Gregory A. Foster,[3] David Krysztof, [3] Domenico Tortorella,[1] Susan L. Stramer,[3] Adolfo García-Sastre,[1,2,4]† Florian Krammer,[1]† Jean K. Lim[1]†

[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [2]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [3]American Red Cross, Gaithersburg, MD, USA. [4]Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

*These authors contributed equally to this work.

†Corresponding author. Email: adolfo.garcia-sastre@mssm.edu (A.G.-S.); florian.krammer@mssm.edu (F.K.); jean.lim@mssm.edu (J.K.L.)

Zika virus (ZIKV) is spreading rapidly into regions around the world where other flaviviruses, such as dengue (DENV) and West Nile virus (WNV) are endemic. Antibody-dependent enhancement (ADE) has been implicated in more severe forms of flavivirus disease, but whether this also applies to ZIKV infection is unclear. Using convalescent plasma from DENV and WNV infected individuals, we found substantial enhancement of ZIKV infection in vitro that was mediated through IgG engagement of FcγR receptors. Administration of DENV or WNV convalescent plasma into ZIKV-susceptible mice resulted in increased morbidity and mortality, including fever, viremia, and viral loads in spinal cord and testes. ADE may explain the severe disease manifestations associated with recent ZIKV outbreaks and highlights the need to exert great caution when designing flavivirus vaccines.

Zika virus (ZIKV) is a mosquito-transmitted flavivirus endemic to parts of Africa and Asia. Since its discovery in 1947, the virus has remained relatively obscure until 2015, when a large outbreak occurred in Brazil and rapidly spread outwards to other South and Central American countries (*1*). Today, ZIKV is endemic to several US Territories, mainly Puerto Rico, and active ZIKV transmission has been reported in Florida and Texas as of February, 2017 (www.cdc.gov). The widespread outbreaks, the virus' association with microcephaly and other neurological disorders, and its long-term persistence in human tissues leading to sexually-mediated transmission and potentially infertility, have taken the medical community by surprise and raises significant public health concerns. While many questions remain unanswered about the unusual biology and disease spectrum of this flavivirus, there is urgency in determining whether antibodies against other flaviviruses could enhance ZIKV replication and disease pathogenesis. Antibody-dependent enhancement (ADE) of different DENV serotypes has been shown to correlate with increased viremia and disease severity (*2–4*). ZIKV is phylogenetically related to other flaviviruses, such as dengue virus (DENV) and West Nile virus (WNV), which co-circulate with ZIKV in many regions across the globe. In the USA and the US territories, WNV and DENV are endemic, causing annual outbreaks of infection. South and Central America, as well as South East Asia, have experienced many outbreaks associated with all four DENV serotypes, which have resulted in hundreds of millions of DENV-seroconverted individuals in these geographical areas (*5*). Accordingly, as ZIKV continues to spread, understanding how preexisting flavivirus immunity impacts ZIKV pathogenesis is a high priority.

For all flaviviruses, the envelope (E) protein represents a primary target of neutralizing antibody responses (*6*, *7*), and recent studies show that ZIKV E protein is highly structurally similar to that of DENV and WNV (*8*, *9*). Indeed, several studies have evaluated DENV-specific monoclonal antibodies and/or a limited number of immune plasma samples for activity against ZIKV (*10–14*). Collectively, these studies showed that some DENV antibodies are cross-reactive to ZIKV and can enhance ZIKV infection at specific concentrations using in vitro systems. However, enhancement of ZIKV infection by DENV antibodies in vivo was not observed (*12*, *13*). Another important question is whether enhancement can also be driven by immunity to other related flaviviruses, such as WNV. This is particularly pertinent given that >3,000,000 US residents alone possess preexisting antibodies to WNV (*15*), a virus that is also endemic throughout parts of Europe, Africa, the Middle East, and Australia (*16*).

To begin understanding the cross-reactivity of related flaviviruses to ZIKV and its implication for enhanced infection, we evaluated convalescent plasma from 141 IgG-positive DENV-infected blood donors and 146 IgG-positive WNV-infected blood donors identified through routine

screening of blood donations in Puerto Rico and the USA, respectively (table S1) (*17*, *18*). As compared to plasma of random blood donor controls where no binding to ZIKV E protein was observed, a wide variability of binding activity against ZIKV E protein was detected for both DENV and WNV immune plasma (Fig. 1A), with DENV-elicited antibodies showing >350 fold greater binding as compared to control samples (P < 0.0001). WNV immune plasma also showed cross-reactivity to ZIKV E protein, with >35 fold higher binding compared to controls (P <0.0001). To evaluate the biological properties of these cross-reactive antibodies, we evaluated enhancement of ZIKV infection in vitro for each individual donor sample using Fcγ receptor (FcγR)-bearing human-derived K562 cells. We observed a high level of overall ADE activity among the flavivirus-exposed individuals compared to controls (P < 0.0001; Fig. 1B), with significantly higher enhancement effects observed in DENV-exposed individuals (P < 0.0001). To further understand the relationship between ZIKV-reactive antibodies in human plasma and enhancement of infection, we plotted binding as a function of enhancement for all DENV- and WNV-immune plasma. We found a strong positive correlation between levels of reactivity to ZIKV E protein and enhancement of infection for both the DENV- (Fig. 1C) and WNV-immune plasma (Fig. 1D). Due to the large number of study participants, ZIKV cross-reactivity and ADE among the DENV and WNV seropositive donors were evaluated for correlations to gender, age, severity of disease (fig. S1), and DENV-serotype (fig. S2). Interestingly for both DENV- and WNV-infected donors, a weak positive correlation for age was observed. DENV-1 positive samples had a trend toward higher ELISA titers and ADE than DENV-4 positive samples but the difference was not statistically significant (fig. S2). Evaluation of ZIKV neutralization potency of a subset of DENV- and WNV-immune plasma revealed that only the highly cross-reactive DENV immune plasma samples were capable of neutralization (fig. S3). These data indicate that preexisting immunity to DENV and WNV can enhance ZIKV infection in vitro.

ADE is primarily mediated through the engagement of IgG antibodies to cell surface Fcγ receptors (FcγR) (*19*, *20*). We therefore tested whether the ADE induced by DENV- and WNV-immune plasma was IgG-mediated. To do this, we tested the ADE activity of plasma pooled from 15 control, DENV- and WNV-infected blood donors as compared to purified IgG from these same samples. Blood donors used for pooling were individually tested for ADE activity (figs. S4 to S6). Of note, these sera were tested for the presence of viral NS1 and were found to be negative (DENV samples only; fig. S7). ZIKV was pre-incubated with serially-diluted pooled plasma or purified IgG, and these mixtures were then used to infect K562 cells. Overall enhancement activity was maintained for both DENV- and WNV-IgG, suggesting that IgG is the primary plasma component involved (Fig. 2A). Furthermore, IgG-depleted plasma did not enhance infection, indicating that ADE in K562 cells was solely attributable to the IgG fraction (Fig. 2B). Given the role of FcγR in mediating ADE, we also investigated the dependency of DENV- and WNV-specific ADE on IgG-FcγR engagement. To do this, K562 cells were pre-incubated in the presence or absence of purified FcγR binding inhibitor (BI; biosciences) prior to infection with ZIKV in the presence of serially-diluted DENV-, WNV- or control-IgG. ZIKV ADE was ablated in the presence of the FcγR BI, suggesting that enhancement is indeed occurring through FcγRs (Fig. 2C). Since K562 cells mediate ADE through FcγRIIA (*21–23*), we next pre-incubated these cells with either an anti-CD32 antibody, which blocks IgG binding to FcγRII, or a control anti-CD16 antibody, which blocks IgG binding to FcγRIII, which is not expressed on K562 cells. The addition of anti-CD32 completely inhibited the ADE induced by DENV- and WNV-IgG (Fig. 2D), while ADE was still robustly observed in the presence of anti-CD16 (Fig. 2E). This suggests that IgG-FcγRIIA interactions are specifically responsible for the enhanced infection in K562 cells. We further verified this by treatment of purified IgG from control, DENV-, or WNV-positive donors with PNGase F. PNGase F removes N-linked glycans from the Fc domain of IgG, which are necessary for FcγR engagement (*24*). This treatment specifically ablated the ability of DENV- and WNV-IgG to induce ADE (Fig. 2F), while the ability of purified IgG to bind ZIKV by ELISA was identical irrespective of PNGase F treatment (fig. S8). Together, our data demonstrate that IgG elicited by infection with DENV and WNV is capable of mediating ADE of ZIKV through FcγR engagement in vitro.

Given the binding and enhancement effects observed with DENV- and WNV- convalescent plasma in vitro, we next evaluated whether ADE could occur during ZIKV infection in vivo. Several studies with numerous flaviviruses have shown that viral proteins antagonize the host innate immune response through targeting STAT2 (*25–29*). In pilot studies, we also evaluated the replication of ZIKV in different mouse strains and discovered that *Stat2*$^{-/-}$ C57BL/6 mice displayed considerable morbidity and mortality in response to infection (*30*). Therefore, *Stat2*$^{-/-}$ mice were given pooled immune plasma from control, DENV, or WNV positive donors. Two hours post transfer, all mice were infected with ZIKV strain PRVABC59 and monitored daily for survival, weight loss, and clinical symptom development.

In response to ZIKV infection, mice that received control plasma exhibited a 93.3% survival rate (Fig. 3A). In contrast, the vast majority of mice that received DENV-positive donor plasma succumbed to infection by day 8 (21.43% survival rate; P < 0.05 compared to mice receiving control plasma).

These mice also exhibited significant weight loss (Fig. 3B) and an enhanced clinical symptom score (Fig. 3C), marked by the development of severe neurological symptoms including paralysis of several limbs and, in some cases, total body paralysis. We also observed a decreased survival rate among mice receiving WNV immune plasma (60% survival; Fig. 3A), although this difference was not statistically distinct. Symptoms were less severe in mice receiving control plasma or PBS. These results correlated directly with the difference in measured ZIKV cross reactivity (Fig. 1).

To further assess clinical symptom development, core body temperatures were measured daily prior to and for several days following infection. Prior to infection, all mice showed an average body temperature between 36°C-37°C. Mice receiving control plasma showed a small but insignificant increase in body temperature on day 3, rising to an average of 37.6°C. However, mice that received DENV-positive plasma developed fever on day 3 post infection, averaging 38.3°C. Mice receiving WNV-positive plasma also developed fever (38.0°C), although not as high as the DENV-positive plasma group (Fig. 3, D to G). To our knowledge, this is the first animal model showing a direct correlation between ADE and fever. Fever was the only symptom observed in the sentinel monkey in 1947 that resulted in the isolation and identification of ZIKV (*31*) and is the hallmark clinical feature in individuals with dengue hemorrhagic fever, West Nile fever, and ZIKV infections (*1*, *4*, *32–34*).

In humans, ADE caused by secondary infection with a heterotypic DENV strain can result in increased viremia, enhanced clinical symptoms, and mortality. To characterize ZIKV replication in the context of preexisting antibodies against DENV or WNV in vivo, we measured viral loads in the blood and several organs known to be associated with ZIKV infection and sexual transmission. In the blood of mice receiving control plasma or PBS, ZIKV RNA levels were elevated on day 3 and then decreased on day 6 post infection (Fig. 4A). Mice receiving DENV-positive donor plasma had a significantly elevated viremia, with >10 fold higher levels on day 3 compared to mice receiving control plasma or PBS. Mice that received WNV immune plasma also had significantly higher viremia at this time point, with ~2 fold increase compared to control mice or mice receiving control plasma or PBS. These differences in viremia were no longer detectable by day 6. Furthermore, ZIKV was detected in the spinal cord and testes in all groups of mice (Fig. 4, B and C) but titers were significantly elevated in mice receiving plasma from DENV- or WNV-seropositive donors. Immunofluorescent staining of ZIKV NS3 in the spinal cord and testes confirmed these observations, showing elevated ZIKV staining in mice receiving DENV- and WNV-infected donor plasma in comparison to the control mice or mice injected with PBS (Fig. 4, D and E). Interestingly, ZIKV staining in the testes was primarily located in the region containing spermatid. We also tested ZIKV levels in several additional organs, including brain, ovaries, and eyes, but found no differences in viral titers (Fig. 4, F to H). Together, our data suggest that ADE induced by cross-reactive DENV and WNV antibodies exacerbates in vivo viremia that may lead to more efficient viral spread to the spinal cord and testes, two organs associated with human ZIKV disease and sexual transmission.

To further understand the relationship between DENV and WNV antibody titers and the potential for enhanced ZIKV disease in vivo, we evaluated a range of doses for control, DENV, and WNV immune plasma and its subsequent effect on ZIKV infection. High concentrations of DENV immune plasma (200μl per mouse) resulted in protection against ZIKV infection, with 100% survival, no weight loss, and decreased symptoms. Lower concentrations (20μl and 2μl per mouse) showed nearly identical survival, weight loss and symptom development (fig. S9). In mice injected with WNV immune plasma, we observed a dose dependent response, with a trend of higher survival and better symptom outcome with high concentrations of plasma. Interestingly, the highest dose of control plasma (200μl) resulted in a decrease in survival and greater symptom development, suggesting that a non-specific response, e.g., from polyreactive antibodies that may occur at high plasma concentrations. However, comparison of control plasma injected mice versus mice receiving DENV and WNV plasma at high concentrations highlights the protective effects of cross-reactive flavivirus antibodies when present at sufficient concentrations. These data suggest that in vivo enhancement may occur optimally at low concentrations of ZIKV-reactive IgG, while high levels may be protective.

In order to evaluate which DENV neutralizing titers correlate with ADE in vivo, we assessed the neutralizing activity of mouse plasma two hours post transfer of 200μl, 20μl, or 2 μl of the pooled human plasma from controls or DENV-exposed individuals. We detected no neutralization activity in mice injected with 20μl or 2 μl of DENV immune plasma, and only residual neutralization of DENV in mice injected with 200μl of DENV immune plasma (fig. S10). This resembles titers of humans with waning immune responses after natural exposure to DENV (*35*, *36*).

In addition to DENV and WNV, there are several clinically relevant flaviviruses and flavivirus vaccines that may also induce ADE. Particularly relevant in South America is Yellow Fever virus (YFV), for which a vaccine has been commercially available since the 1930's, and has significantly reduced the disease burden (*37*). To test the extent to which preexisting immunity against YFV could promote ADE of ZIKV infection, serum samples from macaques vaccinated against YFV using the 17D strain were evaluated for binding

to ZIKV E protein and enhancement of ZIKV infection in vitro (fig. S11). Compared to pre-bleed samples, sera obtained from all three macaques 30 days after 17D vaccination (early immune) showed negligible reactivity to ZIKV E protein by ELISA and no ADE activity on K562 cells, while weak reactivity and enhancement was observed for one of three macaques during the 'late immune' phase (between 6-12 months post vaccination). For comparison, we also tested serum samples from DENV-infected macaques (n = 4), where cross-reactivity to ZIKV E and in vitro ADE was robust at 30 days post infection (early immune) and was even stronger at the later immune time point. The response observed for DENV in the macaques fell well within the range observe for DENV-infected humans (Fig. 1). Though the numbers of animals used in this experiment are small, these data suggest that flaviviruses that are phylogenetically and serologically more distant from ZIKV may have progressively less cross-reactivity (*38*, *39*). Another possibility is that attenuated flavivirus vaccines induce lower IgG titers that lead to less cross-reactivity and therefore, less enhancement. In any case, our data suggest that YFV vaccination, particularly within 30 days post vaccination, poses little risk of enhanced disease outcome if followed by ZIKV infection.

This study reports the first large-scale analysis of ZIKV binding and enhancement by human immune plasma obtained from individuals infected with DENV and WNV. We show that the naturally occurring polyclonal antibody responses against WNV in humans are cross-reactive to ZIKV and can enhance ZIKV in vitro and in vivo. WNV-elicited antibodies appear to show less cross-reactivity and enhancement compared with DENV in vitro. Likewise, the same amount of WNV-positive immune plasma in mice results in a less aggressive ADE phenotype compared to mice receiving DENV immune plasma. We also describe a mouse model of ZIKV infection that recapitulates ADE in vivo, resulting in clinically relevant phenotypes that mimic human disease. Furthermore, we show, for the first time that fever in mice directly correlates with enhanced viral replication exacerbated by the presence of preexisting antibodies against other flaviviruses. Other studies evaluating the effect of DENV monoclonal antibodies on the pathogenesis of ZIKV in vivo found no enhancement of ZIKV disease (*11*, *12*). Possible reasons for these differences may involve the mouse model used, or the concentration of antibodies tested. Further, it is unclear whether strains of ZIKV from distinct lineages or geographic locations induce ADE to different extents. Preliminary studies using ZIKV Nigeria 1968 and Cambodia 2010 strains revealed distinct ADE curves in vitro (fig. S12). Furthermore, our study used polyclonal plasma for in vivo studies, rather than monoclonal antibodies, mimicking naturally occurring immunity. This may have also contributed to differences in outcome. Of note, it has been shown that antibodies against ZIKV can enhance DENV infection in vivo (*11*).

Currently, the breadth of flavivirus-induced ADE of ZIKV infection is unclear, but includes at least DENV and WNV. Other clinically relevant viruses, for which natural infection and large-scale vaccination campaigns have resulted in large numbers of seropositive individuals around the world, may be included, such as YFV, Japanese encephalitis virus, and tick-borne encephalitis virus. Given the high prevalence of DENV antibodies in the geographical areas most affected by the ZIKV, our results suggest that preexisting immunity to DENV may have contributed to the rapid spread of ZIKV in the Americas, possibly associated with increased viremia and clinical symptoms, including microcephaly. In addition, the high prevalence of WNV antibodies in the US raises concerns if ZIKV continues to spread into North America. Our results also have broad implications for vaccine efforts against DENV, WNV, and other flaviviruses. Cross-reactive antibodies induced by these vaccines might lead to enhanced infection when individuals are subsequently exposed to ZIKV. Our results highlight the urgent need for epidemiological studies in humans to understand the impact of preexisting flavivirus antibodies for ZIKV induced disease and sequelae, while exercising great caution on the design and use of flavivirus vaccines in ZIKV affected areas.

REFERENCES AND NOTES

1. D. Musso, D. J. Gubler, Zika Virus. *Clin. Microbiol. Rev.* **29**, 487–524 (2016). doi:10.1128/CMR.00072-15 Medline
2. S. B. Halstead, Pathogenesis of dengue: Challenges to molecular biology. *Science* **239**, 476–481 (1988). doi:10.1126/science.3277268 Medline
3. S. C. Kliks, S. Nimmanitya, A. Nisalak, D. S. Burke, Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am. J. Trop. Med. Hyg.* **38**, 411–419 (1988). Medline
4. D. W. Vaughn, S. Green, S. Kalayanarooj, B. L. Innis, S. Nimmannitya, S. Suntayakorn, T. P. Endy, B. Raengsakulrach, A. L. Rothman, F. A. Ennis, A. Nisalak, Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J. Infect. Dis.* **181**, 2–9 (2000). doi:10.1086/315215 Medline
5. C. P. Simmons, J. J. Farrar, V. Nguyen, B. Wills, Dengue. *N. Engl. J. Med.* **366**, 1423–1432 (2012). doi:10.1056/NEJMra1110265 Medline
6. L. Dai, Q. Wang, J. Qi, Y. Shi, J. Yan, G. F. Gao, Molecular basis of antibody-mediated neutralization and protection against flavivirus. *IUBMB Life* **68**, 783–791 (2016). doi:10.1002/iub.1556 Medline
7. J. T. Roehrig, Antigenic structure of flavivirus proteins. *Adv. Virus Res.* **59**, 141–175 (2003). doi:10.1016/S0065-3527(03)59005-4 Medline
8. L. Dai, J. Song, X. Lu, Y.-Q. Deng, A. M. Musyoki, H. Cheng, Y. Zhang, Y. Yuan, H. Song, J. Haywood, H. Xiao, J. Yan, Y. Shi, C.-F. Qin, J. Qi, G. F. Gao, Structures of the Zika virus envelope protein and its complex with a flavivirus broadly protective antibody. *Cell Host Microbe* **19**, 696–704 (2016). doi:10.1016/j.chom.2016.04.013 Medline
9. D. Sirohi, Z. Chen, L. Sun, T. Klose, T. C. Pierson, M. G. Rossmann, R. J. Kuhn, The 3.8 Å resolution cryo-EM structure of Zika virus. *Science* **352**, 467–470 (2016). doi:10.1126/science.aaf5316 Medline
10. L. Priyamvada, K. M. Quicke, W. H. Hudson, N. Onlamoon, J. Sewatanon, S. Edupuganti, K. Pattanapanyasat, K. Chokephaibulkit, M. J. Mulligan, P. C. Wilson, R. Ahmed, M. S. Suthar, J. Wrammert, Human antibody responses after dengue virus infection are highly cross-reactive to Zika virus. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 7852–7857 (2016). doi:10.1073/pnas.1607931113 Medline

11. K. Stettler, M. Beltramello, D. A. Espinosa, V. Graham, A. Cassotta, S. Bianchi, F. Vanzetta, A. Minola, S. Jaconi, F. Mele, M. Foglierini, M. Pedotti, L. Simonelli, S. Dowall, B. Atkinson, E. Percivalle, C. P. Simmons, L. Varani, J. Blum, F. Baldanti, E. Cameroni, R. Hewson, E. Harris, A. Lanzavecchia, F. Sallusto, D. Corti, Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. *Science* **353**, 823–826 (2016). doi:10.1126/science.aaf8505 Medline
12. J. A. Swanstrom, J. A. Plante, K. S. Plante, E. F. Young, E. McGowan, E. N. Gallichotte, D. G. Widman, M. T. Heise, A. M. de Silva, R. S. Baric, Dengue virus envelope dimer epitope monoclonal antibodies isolated from dengue patients are protective against Zika virus. *MBio* **7**, e01123-16 (2016). doi:10.1128/mBio.01123-16 Medline
13. W. Dejnirattisai, P. Supasa, W. Wongwiwat, A. Rouvinski, G. Barba-Spaeth, T. Duangchinda, A. Sakuntabhai, V.-M. Cao-Lormeau, P. Malasit, F. A. Rey, J. Mongkolsapaya, G. R. Screaton, Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. *Nat. Immunol.* **17**, 1102–1108 (2016). doi:10.1038/ni.3515 Medline
14. L. M. Paul, E. R. Carlin, M. M. Jenkins, A. L. Tan, C. M. Barcellona, C. O. Nicholson, S. F. Michael, S. Isern, Dengue virus antibodies enhance Zika virus infection. *Clin. Transl. Immunology* **5**, e117 (2016). doi:10.1038/cti.2016.72 Medline
15. L. R. Petersen, P. J. Carson, B. J. Biggerstaff, B. Custer, S. M. Borchardt, M. P. Busch, Estimated cumulative incidence of West Nile virus infection in US adults, 1999–2010. *Epidemiol. Infect.* **141**, 591–595 (2013). doi:10.1017/S0950268812001070 Medline
16. C. Chancey, A. Grinev, E. Volkova, M. Rios, The global ecology and epidemiology of West Nile virus. *BioMed Res. Int.* **2015**, 376230 (2015). doi:10.1155/2015/376230 Medline
17. S. L. Stramer, C. T. Fang, G. A. Foster, A. G. Wagner, J. P. Brodsky, R. Y. Dodd, West Nile virus among blood donors in the United States, 2003 and 2004. *N. Engl. J. Med.* **353**, 451–459 (2005). doi:10.1056/NEJMoa044333 Medline
18. S. L. Orton, S. L. Stramer, R. Y. Dodd, Self-reported symptoms associated with West Nile virus infection in RNA-positive blood donors. *Transfusion* **46**, 272–277 (2006). doi:10.1111/j.1537-2995.2006.00710.x Medline
19. S. J. Balsitis, K. L. Williams, R. Lachica, D. Flores, J. L. Kyle, E. Mehlhop, S. Johnson, M. S. Diamond, P. R. Beatty, E. Harris, Lethal antibody enhancement of dengue disease in mice is prevented by Fc modification. *PLOS Pathog.* **6**, e1000790 (2010). doi:10.1371/journal.ppat.1000790 Medline
20. S. Kliks, Antibody-enhanced infection of monocytes as the pathogenetic mechanism for severe dengue illness. *AIDS Res. Hum. Retroviruses* **6**, 993–998 (1990). Medline
21. B. J. Mady, I. Kurane, D. V. Erbe, M. W. Fanger, F. A. Ennis, Neuraminidase augments Fc_{γ} receptor II-mediated antibody-dependent enhancement of dengue virus infection. *J. Gen. Virol.* **74**, 839–844 (1993). doi:10.1099/0022-1317-74-5-839 Medline
22. B. Guy, P. Chanthavanich, S. Gimenez, C. Sirivichayakul, A. Sabchareon, S. Begue, S. Yoksan, C. Luxemburger, J. Lang, Evaluation by flow cytometry of antibody-dependent enhancement (ADE) of dengue infection by sera from Thai children immunized with a live-attenuated tetravalent dengue vaccine. *Vaccine* **22**, 3563–3574 (2004). doi:10.1016/j.vaccine.2004.03.042 Medline
23. R. Jefferis, J. Lund, Interaction sites on human IgG-Fc for $Fc_{\gamma}R$: Current models. *Immunol. Lett.* **82**, 57–65 (2002). doi:10.1016/S0165-2478(02)00019-6 Medline
24. D. Kao, H. Danzer, M. Collin, A. Groß, J. Eichler, J. Stambuk, G. Lauc, A. Lux, F. Nimmerjahn, A monosaccharide residue is sufficient to maintain mouse and human IgG subclass activity and directs IgG effector functions to cellular Fc receptors. *Cell Rep.* **13**, 2376–2385 (2015). doi:10.1016/j.celrep.2015.11.027 Medline
25. W. J. Liu, X. J. Wang, V. V. Mokhonov, P.-Y. Shi, R. Randall, A. A. Khromykh, Inhibition of interferon signaling by the New York 99 strain and Kunjin subtype of West Nile virus involves blockage of STAT1 and STAT2 activation by nonstructural proteins. *J. Virol.* **79**, 1934–1942 (2005). doi:10.1128/JVI.79.3.1934-1942.2005 Medline
26. A. Grant, S. S. Ponia, S. Tripathi, V. Balasubramaniam, L. Miorin, M. Sourisseau, M. C. Schwarz, M. P. Sánchez-Seco, M. J. Evans, S. M. Best, A. García-Sastre, Zika virus targets human STAT2 to inhibit type I interferon signaling. *Cell Host Microbe* **19**, 882–890 (2016). doi:10.1016/j.chom.2016.05.009 Medline
27. J. Ashour, M. Laurent-Rolle, P. Y. Shi, A. García-Sastre, NS5 of dengue virus mediates STAT2 binding and degradation. *J. Virol.* **83**, 5408–5418 (2009). doi:10.1128/JVI.02188-08 Medline
28. M. Mazzon, M. Jones, A. Davidson, B. Chain, M. Jacobs, Dengue virus NS5 inhibits interferon-α signaling by blocking signal transducer and activator of transcription 2 phosphorylation. *J. Infect. Dis.* **200**, 1261–1270 (2009). doi:10.1086/605847 Medline
29. J. Ashour, J. Morrison, M. Laurent-Rolle, A. Belicha-Villanueva, C. R. Plumlee, D. Bernal-Rubio, K. L. Williams, E. Harris, A. Fernandez-Sesma, C. Schindler, A. García-Sastre, Mouse STAT2 restricts early dengue virus replication. *Cell Host Microbe* **8**, 410–421 (2010). doi:10.1016/j.chom.2010.10.007 Medline
30. S. Tripathi, V. R. M. T. Balasubramaniam, J. A. Brown, I. Mena, A. Grant, S. V. Bardina, K. Maringer, M. C. Schwarz, A. M. Maestre, M. Sourisseau, R. A. Albrecht, F. Krammer, M. J. Evans, A. Fernandez-Sesma, J. K. Lim, A. García-Sastre, A novel Zika virus mouse model reveals strain specific differences in virus pathogenesis and host inflammatory immune responses. *PLOS Pathog.* **13**, e1006258 (2017). doi:10.1371/journal.ppat.1006258 Medline
31. G. W. Dick, S. F. Kitchen, A. J. Haddow, Zika virus. I. Isolations and serological specificity. *Trans. R. Soc. Trop. Med. Hyg.* **46**, 509–520 (1952). doi:10.1016/0035-9203(52)90042-4 Medline
32. D. H. Libraty, T. P. Endy, H.-S. H. Houng, S. Green, S. Kalayanarooj, S. Suntayakorn, W. Chansiriwongs, D. W. Vaughn, A. Nisalak, F. A. Ennis, A. L. Rothman, Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *J. Infect. Dis.* **185**, 1213–1221 (2002). doi:10.1086/340365 Medline
33. W. K. Wang, D.-Y. Chao, C.-L. Kao, H.-C. Wu, Y.-C. Liu, C.-M. Li, S.-C. Lin, S.-T. Ho, J.-H. Huang, C.-C. King, High levels of plasma dengue viral load during defervescence in patients with dengue hemorrhagic fever: Implications for pathogenesis. *Virology* **305**, 330–338 (2003). doi:10.1006/viro.2002.1704 Medline
34. E. B. Hayes, D. J. Gubler, West Nile virus: Epidemiology and clinical features of an emerging epidemic in the United States. *Annu. Rev. Med.* **57**, 181–194 (2006). doi:10.1146/annurev.med.57.121304.131418 Medline
35. M. Taketa-Graham, J. L. Powell Pereira, E. Baylis, C. Cossen, L. Oceguera, P. Patiris, R. Chiles, C. V. Hanson, B. Forghani, High throughput quantitative colorimetric microneutralization assay for the confirmation and differentiation of West Nile Virus and St. Louis encephalitis virus. *Am. J. Trop. Med. Hyg.* **82**, 501–504 (2010). doi:10.4269/ajtmh.2010.09-0297 Medline
36. V. Vorndam, M. Beltran, Enzyme-linked immunosorbent assay-format microneutralization test for dengue viruses. *Am. J. Trop. Med. Hyg.* **66**, 208–212 (2002). Medline
37. T. Ishikawa, A. Yamanaka, E. Konishi, A review of successful flavivirus vaccines and the problems with those flaviviruses for which vaccines are not yet available. *Vaccine* **32**, 1326–1337 (2014). doi:10.1016/j.vaccine.2014.01.040 Medline
38. M. W. Gaunt, A. A. Sall, X. de Lamballerie, A. K. Falconar, T. I. Dzhivanian, E. A. Gould, Phylogenetic relationships of flaviviruses correlate with their epidemiology, disease association and biogeography. *J. Gen. Virol.* **82**, 1867–1876 (2001). doi:10.1099/0022-1317-82-8-1867 Medline
39. S. S. Baba, A. H. Fagbami, O. D. Olaleye, Antigenic relatedness of selected flaviviruses: Study with homologous and heterologous immune mouse ascitic fluids. *Rev. Inst. Med. Trop. Sao Paulo* **40**, 343–349 (1998). doi:10.1590/S0036-46651998000600002 Medline
40. S. L. Orton, S. L. Stramer, R. Y. Dodd, Self-reported symptoms associated with West Nile virus infection in RNA-positive blood donors. *Transfusion* **46**, 272–277 (2006). doi:10.1111/j.1537-2995.2006.00710.x Medline
41. R. S. Lanciotti, O. L. Kosoy, J. J. Laven, J. O. Velez, A. J. Lambert, A. J. Johnson, S. M. Stanfield, M. R. Duffy, Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg. Infect. Dis.* **14**, 1232–1239 (2008). doi:10.3201/eid1408.080287 Medline
42. S. V. Bardina, D. Michlmayr, K. W. Hoffman, C. J. Obara, J. Sum, I. F. Charo, W. Lu, A. G. Pletnev, J. K. Lim, Differential roles of chemokines CCL2 and CCL7 in monocytosis and leukocyte migration during West Nile Virus infection. *J. Immunol.* **195**, 4306–4318 (2015). doi:10.4049/jimmunol.1500352 Medline
43. D. Michlmayr, S. V. Bardina, C. A. Rodriguez, A. G. Pletnev, J. K. Lim, Dual function of Ccr5 during Langat virus encephalitis: Reduction in neutrophil-mediated central nervous system inflammation and increase in T cell–mediated

viral clearance. *J. Immunol.* **196**, 4622–4631 (2016). doi:10.4049/jimmunol.1502452 Medline
44. M. C. Schwarz, M. Sourisseau, M. M. Espino, E. S. Gray, M. T. Chambers, D. Tortorella, M. J. Evans, Rescue of the 1947 Zika virus prototype strain with a cytomegalovirus promoter-driven cDNA clone. *mSphere* **1**, e00246-16 (2016). doi:10.1128/mSphere.00246-16 Medline

ACKNOWLEDGMENTS

We thank Richard Cadagan, Osman Lizardo, and Fatima Amanat for excellent technical assistance. We also thank Dr. Matthew Evans' laboratory for providing the RNA standards for determination of ZIKV load by Taqman PCR assay, Christian Schindler for providing the original *Stat2*$^{-/-}$ mouse strain, and Ana Fernandez-Sesma for ZIKV and DENV strains. We are grateful to Dr. Dusan Bogunovic for invaluable evaluation of our manuscript. We thank the microscopy core at Icahn School of Medicine for their support and technical assistance. We also thank Gorky Estrella and Jeffery Bethea at the Center for Comparative Medicine at the Icahn School of Medicine at Mount Sinai for their efforts to rapidly accommodate ZIKV studies in our vivarium. The following reagent was obtained through BEI Resources, NIAID, NIH: Pre-Immune Dengue Virus Type 1 Sera NR-41782; Pre-Immune Dengue Virus Type 2 Sera NR-41783; Pre-Immune Dengue Virus Type 3 Sera NR-41784; Pre-Immune Dengue Virus Type 4 Sera NR-41785; Early-Immune Dengue Virus Type 1 Antiserum, NR-30247; Early-Immune Dengue Virus Type 2 Antiserum, NR-29321; Early-Immune Dengue Virus Type 3 Antiserum, NR-29323; Early-Immune Dengue Virus Type 4 Antiserum, NR-29327; Late-Immune Dengue Virus Type 1 Antisera NR-41786; Late-Immune Dengue Virus Type 2 Antisera NR-41787; Late-Immune Dengue Virus Type 3 Antisera NR-41788; Late-Immune Dengue Virus Type 4 Antisera NR-41789; Pre-Immune Yellow Fever Virus Serum, NR-42556, NR-42564, and NR-42565; Early-Immune Yellow Fever Virus Antiserum, NR-29335, NR-29337, and NR-29338; Late-Immune Yellow Fever Virus Antiserum, NR-42567, NR-42575, and NR-42576. This work was partially supported by a supplement to NIAID grant U19AI118610 and R21AI130299. Human sample IRB protocols include Nucleic Acid Testing (NAT) for West Nile Virus (WNV) Protocol # 2003-011 and Dengue Virus (Gen-Probe Procleix) Clinical Protocol #2012-016. All the data supporting our conclusions are contained in this manuscript.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/cgi/content/full/science.aal4365/DC1
Materials and Methods
Figs. S1 to S12
Table S1
References (*40*–*44*)

18 November 2016; accepted 17 March 2017
Published online 30 March 2017
10.1126/science.aal4365

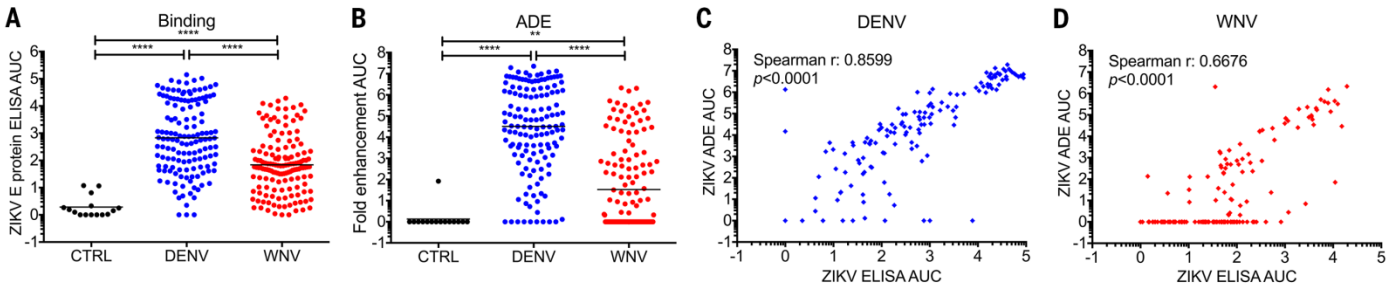

Fig. 1. ZIKV binding and enhancement of infection by DENV- and WNV-immune plasma. Plasma from seropositive DENV- (n = 141), WNV-infected (n = 146), or sero-negative control (n = 15) donors were evaluated for reactivity to ZIKV E protein by ELISA (**A**) or enhancement of ZIKV infection of K562 cells (**B**). Area under the curve (AUC) calculations based on serially-diluted plasma measurements are shown. (**C** and **D**) Scatter plot showing the relationship between ZIKV binding and enhancement of ZIKV infection for DENV (C) and WNV (D) immune plasma. Each point represents one donor. Significance was analyzed by nonparametric unpaired Mann-Whitney U test for (A) and (B) or by non-parametric Spearman's rank correlation for (C) and (D). $^{**}P < 0.01$; $^{****}P < 0.0001$.

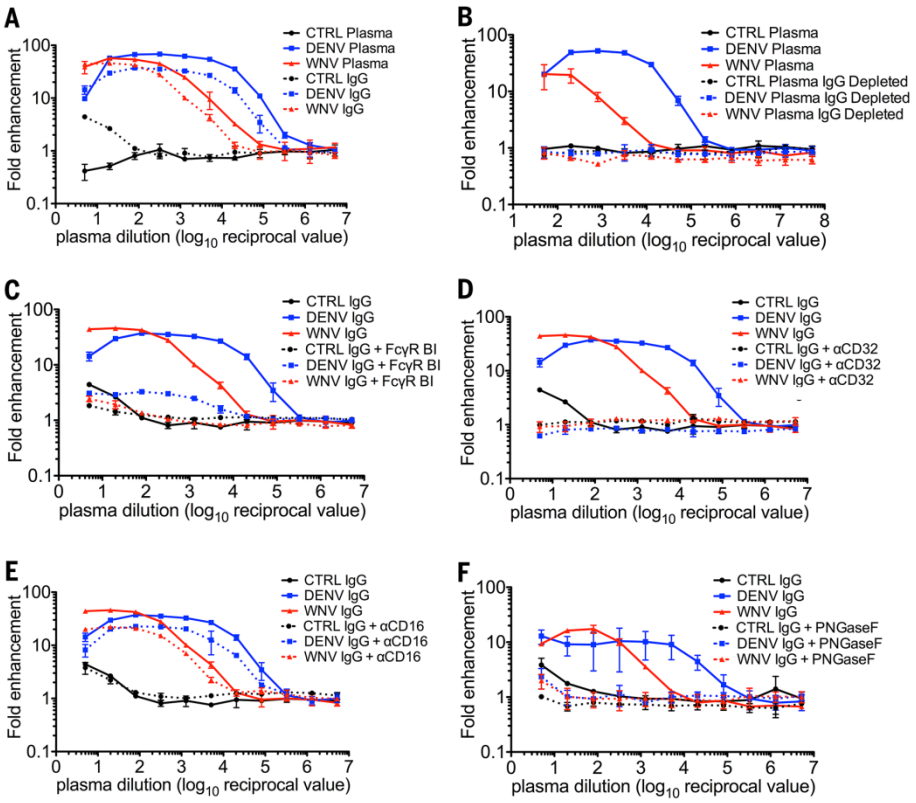

Fig. 2. Enhancement of ZIKV infection via IgG engagement of FcγR receptors. (**A**) IgG purified from plasma pooled (*n* = 15 per group) from control blood donors, DENV-, or WNV-infected donors was evaluated for enhancement of ZIKV infection on K562 cells. (**B**) Pooled plasma samples from control donors, DENV-, or WNV-seropositive donors were evaluated for enhancement of ZIKV infection on K562 cells before and after IgG purification. (**C** to **E**) Purified IgG from control, DENV-, or WNV-seropositive donors was tested for enhancement of ZIKV infection on K562 cells in the presence or absence of FcγR binding inhibitor (C), anti-CD32 antibody (D), or anti-CD16 antibody (E). (**F**) Plasma IgG from control, DENV-, or WNV-seropositive donors was incubated in the presence or absence of PNGase F prior to evaluation of ZIKV enhancement on K562 cells.

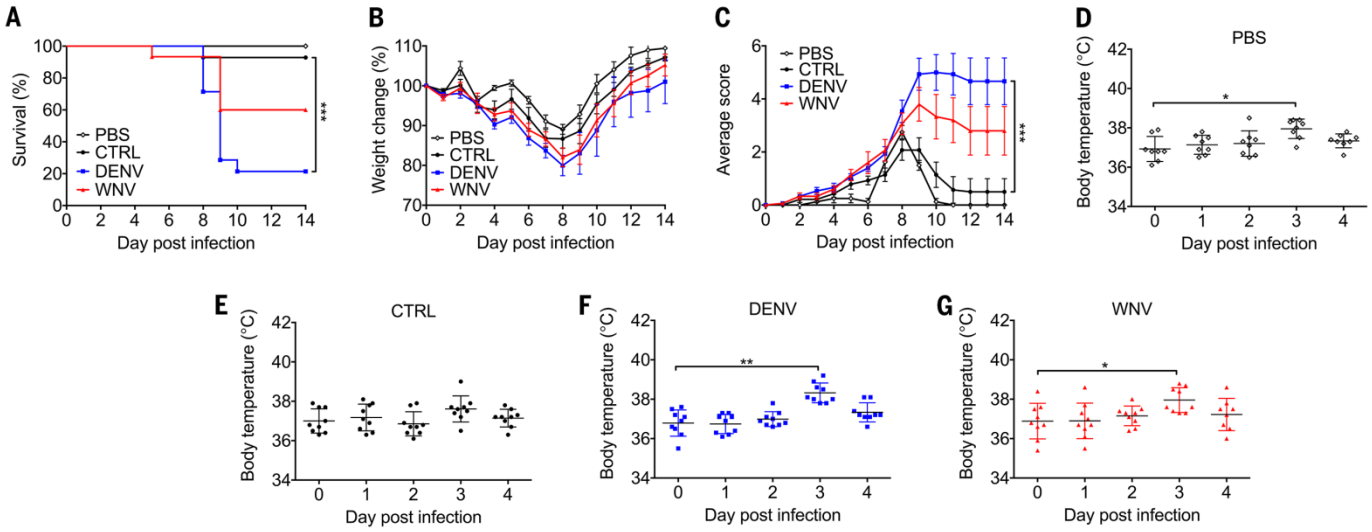

Fig. 3. In vivo enhancement of ZIKV infection by convalescent DENV and WNV plasma. (**A**) 20μl of convalescent plasma from control, DENV, and WNV donors was administered intraperitoneally to *Stat2*$^{-/-}$ mice 2 hours prior to intradermal inoculation with 1×10^5 PFU of ZIKV strain PRVABC59. Kaplan-Meier survival curve is shown; significance was determined by using the Mantel-Cox log-rank test and adjusted for multiple comparisons using the Bonferroni correction. Mice were monitored for (**B**) weight loss and (**C**) clinical score using a 6-point system ($n = 5$ per group) with a score of 7 awarded to deceased animals. Significance was determined by using the student's t test, and then adjusted for multiple comparisons using the Bonferroni correction. In symptom score comparisons, the day with the highest average score per group was used. Daily rectal body temperature measurements were taken for PBS (**D**), control (**E**), DENV (**F**), or WNV (**G**) immune plasma. Statistically significant differences were calculated by comparing day 3 (the day of the highest total average temperature) and day 0 for each group. *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$.

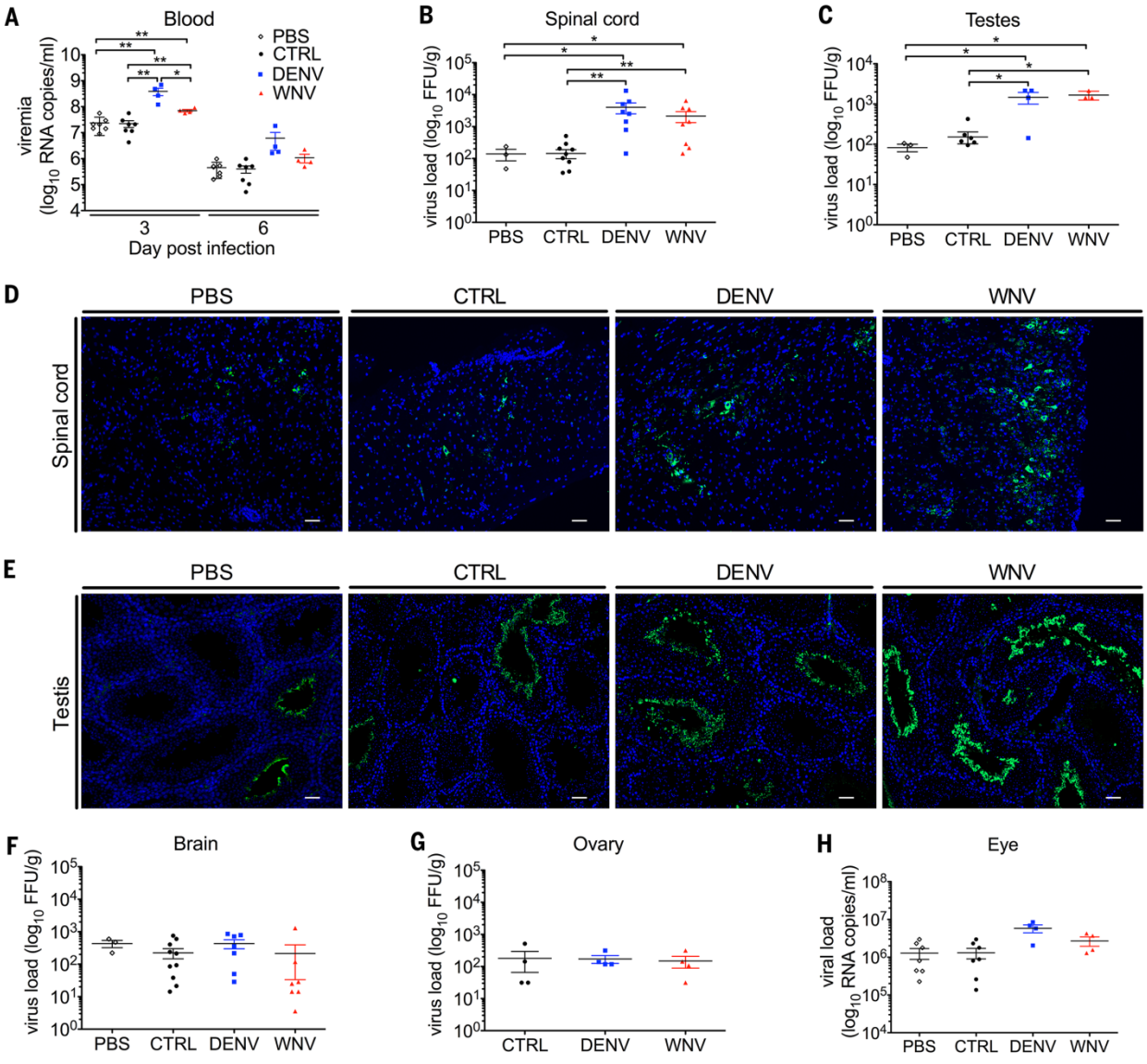

Fig. 4. In vivo ADE correlates with amplified ZIKV replication. (**A**) Blood viral titers of mice treated with control, DENV, or WNV-positive donor plasma were assessed by real-time PCR on day 3 and 6 post infection. Viral titers were quantified by a plaque assay on spinal cords (**B**) and testes (**C**) isolated on day 6 post infection from ZIKV infected *Stat2*$^{-/-}$ mice treated with a low dose of either control, DENV-, or WNV-positive donor plasma. Paraffin embedded spinal cords (**D**) and testes (**E**) from ZIKV-infected *Stat2*$^{-/-}$ mice on day 6 post infection treated with a low dose of either control, DENV-, or WNV-positive donor plasma sections were stained for ZIKV (green) and nuclear DAPI (blue). Representative images are shown at 10× magnification and 50 μm scale bars. Brains (**F**), ovaries (**G**), and eyes (**H**) were also evaluated for viral loads on day 6. *$P < 0.05$; **$P < 0.01$.

Enhancement of Zika virus pathogenesis by preexisting antiflavivirus immunity

Susana V. Bardina, Paul Bunduc, Shashank Tripathi, James Duehr, Justin J. Frere, Julia A. Brown, Raffael Nachbagauer, Gregory A. Foster, David Krysztof, Domenico Tortorella, Susan L. Stramer, Adolfo García-Sastre, Florian Krammer and Jean K. Lim (March 30, 2017)
published online March 30, 2017

Editor's Summary

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.