Monoclonal Antibodies against Zika Virus: Therapeutics and Their Implications for Vaccine Design

Qihui Wang,^{a,b} Jinghua Yan,^{a,b,c,d,e} George Fu Gao^{b,c,d,e,f,g}

CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China^a; Shenzhen Key Laboratory of Pathogen and Immunity, Shenzhen Third People's Hospital, Shenzhen, China^b; CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China^c; Center for Influenza Research and Early-warning (CASCIRE), Chinese Academy of Sciences, Beijing, China^d; Savaid Medical School, University of Chinese Academy of Sciences, Beijing, China^e; Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Zhejiang University, Hangzhou, China^f; National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention (China CDC), Beijing, China^g

ABSTRACT Zika virus (ZIKV) has caused global concern due to its association with neurological complications in newborns and adults. Although no vaccines or antivirals against ZIKV infection have been approved to date, hundreds of monoclonal antibodies (MAbs) have been developed in a short period. Here, we first present a complete picture of the ZIKV MAbs and then focus on the neutralizing mechanisms and immune hot spots uncovered through structural studies, which provide insight for therapeutics and vaccine design.

KEYWORDS Zika virus, therapeutics, monoclonal antibodies, neutralizing epitopes, vaccine design, clinical therapeutics

Zika virus (ZIKV) infections have previously been associated with subclinical or dengue-like symptoms (1). In contrast, since the latest outbreak, mounting clinical and experimental evidence indicates that ZIKV causes more-serious manifestations, such as microcephaly in newborns and Guillain-Barré syndrome in adults (2). To date, 85 countries, territories, or subnational areas, including China, have reported evidence of vector-borne ZIKV infections (3, 4). Unfortunately, no licensed vaccines or antivirals are available for ZIKV prevention and control.

THERAPEUTIC ANTIBODIES TARGETING E PROTEINS

ZIKV is an arbovirus that is transmitted to humans by *Aedes* mosquitoes. It belongs to the genus *Flavivirus*, family *Flaviviridae*, which also includes some important human pathogens such as dengue virus (DENV), yellow fever virus (YFV), and West Nile virus (WNV); among these, ZIKV is the species most closely related to DENV (5). In flavivirus infections, humoral immunity represents an important component of the host response, and administration of polyclonal or purified monoclonal antibodies (MAbs) helps to clear the viruses (6, 7). Accordingly, within a short period, hundreds of ZIKV MAbs have been developed from either humans or mice (8–14); among these, NS1 MAbs have been applied for diagnosis (14). Although MAbs against NS1 from other flaviviruses, such as WNV, YFV, and DENV, confer protection *in vivo* (15), the efficacy of NS1 MAbs in ZIKV clearance remains to be elucidated. Thus, in this minireview, we focus on the therapeutic MAbs that bind to the envelope (E) proteins (E MAbs) (summarized in Table 1).

To date, 461 MAbs that bind to E proteins have been characterized, including 70 MAbs that display moderate to high neutralizing activities, with half-maximal inhibitory concentration (IC₅₀), 50% plaque reduction neutralization titer (PRNT₅₀), or 50% focus

Accepted manuscript posted online 2 August 2017

Citation Wang Q, Yan J, Gao GF. 2017. Monoclonal antibodies against Zika virus: therapeutics and their implications for vaccine design. *J Virol* 91:e01049-17. <https://doi.org/10.1128/JVI.01049-17>.

Editor Michaela Ulrike Gack, University of Chicago

Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Qihui Wang, wangqihui@im.ac.cn.

TABLE 1 Summary for the ZIKV MAb available to date

Origin	No. of ZIKV-specific MAbs/ total no. of MAbs	No. of ZIKV-specific MAbs with neutralization activity of <1 μ g/ml <i>in vitro</i> /total no. of MAbs with neutralization activity of <1 μ g/ml <i>in vitro</i> ^b	No. of MAbs showing efficacy <i>in vivo</i> ^c	Epitope(s) ^d	Reference(s)
Mouse	0/1	0/0	1 (2A10G6)	FL	10, 16
Human	0/9	0/5	ND		11
Human	51/113	37/41	1 (<u>ZKA64</u>)	DIII	14
Mouse	5/6	2/2	2 (<u>ZV54/ZV-67</u>)	DIII/DII	9
Human	13 (2/3) ^a	2/3	3 (<u>Z3L1/Z23/Z20</u>)	D1, DII/DIII, DI/DII	13
Human	29 (1/2) ^a	1/2	1 (<u>ZIKV-117</u>)	DII	12
Human	290 (4/19) ^a	3/17	1 (Z004)	DIII	8
Total	461 (63/153) ^a	45/70	6/9		

^aNot all isolated MAbs were subjected to cross-binding tests in the indicated stud(ies). In the values in parentheses, the first number is the number of ZIKV-specific MAbs among those tested and the second number is the total number of tested MAbs.

^bNeutralization activity is shown as IC₅₀, PRNT₅₀, or FRNT₅₀ values.

^cNames of the MAbs from the indicated stud(ies) that showed protective efficacy *in vivo* are given in parentheses; among these, the ZIKV-specific MAbs are underlined. ND, not determined.

^dEpitope(s) of the MAbs that showed protective efficacy *in vivo*.

reduction neutralization titer (FRNT₅₀) values of <1 μ g/ml. Nine MAbs further confer protection against lethal ZIKV challenge in mice. Interestingly, the proportions of ZIKV-specific MAbs in the MAb groups differ. Among the 153 tested MAbs, 63 specifically bind to ZIKV E protein (E MAbs) (8–14), while 45 of 70 MAbs that convey 50% inhibition of the virus at a concentration of <1 μ g/ml are specific to ZIKV (8–14). The proportion of ZIKV-specific MAbs is further increased in focusing on the MAbs with *in vivo* protective efficacy. Six MAbs are ZIKV specific, while three are cross-protective against other flaviviruses (8–10, 12–14). This indicates that the ZIKV-specific MAbs display higher neutralizing activities and protection efficacies against ZIKV infection than cross-binding MAbs. In addition to the protection against lethal ZIKV challenge, ZIKV-117 and convalescent-phase serum treatment markedly reduced tissue pathology, decreased vertical transmission, and prevented ZIKV-induced microcephaly in a mouse model (12, 17), emphasizing the therapeutic potential of MAbs or polyclonal antibodies in preventing ZIKV-related damage.

NEUTRALIZING MECHANISMS FOR E MAbs AND IMMUNE HOT SPOTS

Why do E MAbs exert protection against ZIKV and other flaviviruses? E protein, as a typical viral class II fusion protein, plays a pivotal role in flavivirus attachment and membrane fusion (5). The three extracellular domains (DI, DII, and DIII) of the E protein undergo major rearrangements in their relative orientations but retain most of their folded rigid-body structures in different viral life stages (Fig. 1A). On the mature virion, E proteins form dimers, but in the acidic endosome, both DII and DIII rotate clockwise with respect to DI through flexion of the interdomain linkers. Consequently, the viral envelope-anchored C terminus of DIII is close to the fusion loop (FL), which is located on top of DII and inserts into the endosomal membrane, allowing fusion to occur. ZIKV is originally assembled as immature particles, with a trimer consisting of a heterodimer of E and premembrane (prM) proteins (Fig. 1A). The prM is cleaved by furin-like protease, forming pr peptides and membranes (M), which is the hallmark for maturation. Then, peptides are released when the pH returns to neutral outside the host cells (18).

Solving the structures of neutralizing MAbs can help to identify neutralizing hot spots for vaccine development. The MAbs that interfere with E protein function at any stage (e.g., blocking E-mediated viral attachment, hindering E protein rearrangement in the endosome, or blocking FL insertion into the endosomal membrane) might prevent the flavivirus infection. Structural studies have shed light on the neutralizing mechanisms of MAbs. Taking MAb C10 as an example (19), this MAb covers the region of FL on DII of one protomer of the virion and makes contact with DIII, DI, and DII on the

FIG 1 Domain rearrangements in the ZIKV E protomer and the neutralizing epitopes on the E protein. In this figure, the three extracellular domains (DI, DII, and DIII) are marked in red, yellow, and blue, respectively, while the fusion loops (FLs) are colored in green. (A) The three structures displayed as cartoons represent the E protomer at the immature (PDB: 5u4w), mature (PDB: 5iz7), and postfusion stages. The ZIKV E protein in the postfusion state was generated by docking structure 5iz7 on the DENV postfusion E structure (PDB: 1ok8). (B to F) Structure-defined neutralizing epitopes are displayed with a cutoff of 4.5 Å, except for Z23. Due to the low resolution (9.6 Å) of ZIKV complexed with Z23, as determined by cryo-electron microscopy (cryo-EM), the detailed epitope could not be distinguished and is highlighted with an ellipse. Carbohydrates are indicated as spheres. In each panel, the epitope of the MAb marked in black is colored white on the surface structure. The circles in panels B, C, and E represent the epitopes of the MAbs in the same color. The PDB identifiers for the epitope analysis are as follows: 5h37 (C10), 5lcv (A11), 5lbs (C8), 5gzo (Z20), 5uhy (ZIKV-117), 5gzn (Z3L1), 5gzs (Z23), 5vig (Z006), 5kvg (ZV-67), and 5jhl (2A10G6).

other protomer (Fig. 1B). This MAb also binds to the interdimer interface. Thus, E dimers are locked by MAb C10, which inhibits domain reorganization. Consistently, the E protein layer of the ZIKV-C10 complex at pH 6.5 remains at a radius similar to that seen with the uncomplexed ZIKV at pH 8.0, unlike uncomplexed ZIKV at pH 6.5 (19). Similar intradimer epitopes are also observed for MAb C8 and MAb A11 (11) (Fig. 1B). Interestingly, all three MAbs were initially isolated from a DENV infection patient and then their cross-neutralization activities against ZIKV were confirmed, indicating that the epitope is a cross-neutralization hot spot (11, 20). E dimers might be also locked by MAbs interacting across DII of protomers, as exemplified by Z20 and ZIKV-117 (13, 21) (Fig. 1C). Although isolated from different patients, the two MAbs bind to similar regions, indicating that this epitope might be another neutralization hot spot. Of note, with a few varied residues in the epitope, ZIKV-117 displays ZIKV specificity, while Z20 exerts weak cross-neutralization against DENV. MAbs such as Z3L1, which was isolated along with Z20 and is specific for ZIKV, can also inhibit domain rearrangement through fixation of the interdomain linker (Fig. 1D) (13).

DIII-binding MAbs might prevent ZIKV infection by either impeding E reorganization or blocking viral attachment to host cells because DIII is hypothesized to be responsible for receptor binding (22, 23). The human MAb Z23 mainly binds to the lateral regions (LRs) on DIII and inserts into the cleft between two adjacent dimers (Fig. 1E) (13). ZV67, derived from mice, interacts with the LR similarly to Z23 (Fig. 1E) (9). Both MAbs are specific to ZIKV, suggesting the LR is a probable ZIKV-specific hot spot. Z006, isolated from another patient, also bound to the LR and is a strong neutralizer of both ZIKV and DENV serotype 1 virus (DENV-1) (Fig. 1E) (8).

During membrane fusion, FL-targeting MAbs might cause steric hindrance and inhibit E insertion into the endosomal membrane. The murine MAb 2A10G6, which interacts with the conserved FL (Fig. 1F) and displays broad protection against flavivi-

ruses, is hypothesized to function this way (10, 16). Notably, the neutralizing activity of 2A10G6 against ZIKV is weaker than that seen with other flaviviruses, such as WNV and DENV (13). A majority of human and murine FL MAbs also show much weaker neutralization activities against ZIKV infection (10, 13, 14). This difference might result from the unique structural features of ZIKV. ZIKV displays higher thermal stability than other flaviviruses (24), leading to less “breathing” of the E protein and a FL that is less accessible to solvent. Instead, these MAbs might enhance the virus infection, a phenomenon called antibody-dependent enhancement (ADE). Therefore, ADE epitopes should be avoided in vaccine development.

Many reports indicate that the primary infection with DENV generates an antibody response that protects against homologous serotypes, but it might cause the ADE effect during subsequent heterologous infections and exacerbate disease (7, 25, 26). Due to the similarity between DENV and ZIKV, the MAbs stimulated by ZIKV infection also enhance DENV infection (14). The cross-reactive MAbs, especially the FL MAbs, greatly contributed to the ADE effect (27). Thus, during ZIKV vaccine design, FL MAbs should be avoided. Thus far, many platforms have been developed for ZIKV vaccines (28), among which the mRNA vaccine candidate incorporates T76R, Q77E, W101R, and L107R mutations to destroy the FL. The modified mRNA vaccine can prevent ZIKV disease and be adapted to reduce the risk of ADE during subsequent exposure to DENV, indicating the most potential for future clinical application (29).

IMPLICATIONS FOR THERAPEUTICS AND VACCINE DESIGN

Since the latest outbreak of ZIKV infection, hundreds of E MAbs have been isolated from patients or mice. Six human MAbs, including four ZIKV-specific ones, have proven *in vivo* protection efficacies in mice against lethal ZIKV challenge. Moreover, ZIKV-117 treatment also protects against mother-to-fetus transmission, infection, and disease, suggesting its therapeutic potential for clinical applications. However, one obstacle for future MAb treatment is that viruses, especially those with RNA genomes, display high mutation rates and readily generate escape mutations. Combinations consisting of different MAbs targeting different epitopes and, therefore, functioning through different mechanisms might suppress escape mutations. Here, a structural analysis provides detailed epitope information and is fundamental for therapeutic MAb “cocktail” design. Moreover, more human-protective MAbs are expected to be discovered, enhancing our understanding of ZIKV immune responses and protective therapeutics.

Another antigen that is important during flavivirus infections is the NS1 protein. Previously, NS1 polyclonal antibodies or MAbs had been shown to demonstrate protection in mice challenged with YFV and WNV (30, 31). Studies of ZIKV indicate that increasing secretion of NS1 is associated with high transmission between mosquitos and humans. In support of this idea, administration of antiserum against ZIKV NS1 protein decreases transmission between the two hosts. Thus, ZIKV NS1-specific MAbs may provide additive protection in combination with E-specific MAbs, though further investigations should be conducted to support this hypothesis.

Through the dissection of MAbs targeting E protein, three neutralizing hot spots were uncovered. More E MAb complex structures are desired for identification of additional hot spots. Further, it is also proposed that FL antibodies readily stimulate the ADE effect. Although FL mutations decrease the antigenicity of this epitope (29), they reciprocally evoke lower neutralizing serum titers, possibly by destabilizing the interaction between the mutated DII on one protomer and the DIII on the other protomer. Many strategies, including but not limited to the introduction of substitution mutations on the counterpart of DIII with hydrophilic amino acids to enhance the electrostatic interactions between the two domains and the introduction of disulfide bonds between the two protomers to stabilize the dimer conformation, are worth trying to solve the problem. Recently, a hypothesis was proposed on the basis of the study of influenza vaccine that preexisting human antibody levels negatively correlate with boosting responses to the same epitope (32, 33). Whether the administration of ZIKV vaccines along with FL antibodies could

suppress responses to the cognate antigenic sites remains to be elucidated, and the results will aid in ZIKV vaccine development.

ACKNOWLEDGMENTS

We apologize to all those colleagues whose excellent work could not be discussed due to space limitations. We appreciate the participation of Yi Shi, Jianxun Qi, Hao Song, Lianpan Dai, and Shuguang Tan in discussions in the preparation of the manuscript.

This work was supported by the State Key Research Development Program of China (grant no. 2016YFC1200300) and the National Natural Science Foundation of China (NSFC; grant no. 31502078). Q.W. is supported by the Young Elite Scientist Sponsorship Program of the China Association for Science and Technology (CAST; grant no. YESS20150040). G.F.G. is a leading principal investigator in the NSFC Innovative Research Group (grant no. 81621091).

REFERENCES

1. Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, Kolenc M, Resman Rus K, Vesnaver Vipotnik T, Fabjan Vodusek V, Vizjak A, Pizem M, Petrovec M, Avšič Županc T. 2016. Zika virus associated with microcephaly. *N Engl J Med* 374:951–958. <https://doi.org/10.1056/NEJMoa1600651>.
2. Paixão ES, Barreto F, Teixeira Mda G, Costa MDC, Rodrigues LC. 2016. History, epidemiology, and clinical manifestations of Zika: a systematic review. *Am J Public Health* 106:606–612. <https://doi.org/10.2105/AJPH.2016.303112>.
3. WHO. 2017. Zika virus disease. WHO, Geneva, Switzerland. <http://www.who.int/csr/disease/zika/en/>.
4. Zhang Y, Chen W, Wong G, Bi Y, Yan J, Sun Y, Chen E, Yan H, Lou X, Mao H, Xia S, Gao GF, Shi W, Chen Z. 2016. Highly diversified Zika viruses imported to China, 2016. *Protein Cell* 7:461–464. <https://doi.org/10.1007/s13238-016-0274-5>.
5. Knipe DM, Howley PM, Cohen JL, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B (ed). 2013. *Fields virology*, 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
6. Dai L, Wang Q, Qi J, Shi Y, Yan J, Gao GF. 2016. Molecular basis of antibody-mediated neutralization and protection against flavivirus. *IUBMB Life* 68:783–791. <https://doi.org/10.1002/iub.1556>.
7. Dowd KA, Pierson TC. 2011. Antibody-mediated neutralization of flaviviruses: a reductionist view. *Virology* 411:306–315. <https://doi.org/10.1016/j.virol.2010.12.020>.
8. Robbiani DF, Bozzacco L, Keffe JR, Khouri R, Olsen PC, Gazumyan A, Schaefer-Babajew D, Avila-Rios S, Nogueira L, Patel R, Azzopardi SA, Uhl LFK, Saeed M, Sevilla-Reyes EE, Agudelo M, Yao KH, Golijanin J, Gristick HB, Lee YE, Hurley A, Caskey M, Pai J, Oliveira T, Wunder EA, Jr, Sacramento G, Nery N, Jr, Orge C, Costa F, Reis MG, Thomas NM, Eisenreich T, Weinberger DM, de Almeida ARP, West AP, Jr, Rice CM, Bjorkman PJ, Reyes-Teran G, Ko A, MacDonald MR, Nussenzweig MC. 2017. Recurrent potent human neutralizing antibodies to Zika virus in Brazil and Mexico. *Cell* 169:597–609.e511. <https://doi.org/10.1016/j.cell.2017.04.024>.
9. Zhao H, Fernandez E, Dowd KA, Speer SD, Platt DJ, Gorman MJ, Govero J, Nelson CA, Pierson TC, Diamond MS, Fremont DH. 2016. Structural basis of Zika virus-specific antibody protection. *Cell* 166:1016–1027. <https://doi.org/10.1016/j.cell.2016.07.020>.
10. Dai L, Song J, Lu X, Deng YQ, Musyoki AM, Cheng H, Zhang Y, Yuan Y, Song H, Haywood J, Xiao H, Yan J, Shi Y, Qin CF, Qi J, Gao GF. 2016. Structures of the Zika virus envelope protein and its complex with a flavivirus broadly protective antibody. *Cell Host Microbe* 19:696–704. <https://doi.org/10.1016/j.chom.2016.04.013>.
11. Barba-Spaeth G, Dejnirattisai W, Rouvinski A, Vaney MC, Medits I, Sharma A, Simon-Loriere E, Sakuntabhai A, Cao-Lormeau VM, Haouz A, England P, Stiasny K, Mongkolsapaya J, Heinz FX, Screaton GR, Rey FA. 2016. Structural basis of potent Zika-dengue virus antibody cross-neutralization. *Nature* 536:48–53. <https://doi.org/10.1038/nature18938>.
12. Sapparapu G, Fernandez E, Kose N, Bin C, Fox JM, Bombardi RG, Zhao H, Nelson CA, Bryan AL, Barnes T, Davidson E, Mysorekar IU, Fremont DH, Doranz BJ, Diamond MS, Crowe JE. 2016. Neutralizing human antibodies prevent Zika virus replication and fetal disease in mice. *Nature* 540:443–447. <https://doi.org/10.1038/nature20564>.
13. Wang Q, Yang H, Liu X, Dai L, Ma T, Qi J, Wong G, Peng R, Liu S, Li J, Li S, Song J, Liu J, He J, Yuan H, Xiong Y, Liao Y, Li J, Yang J, Tong Z, Griffin BD, Bi Y, Liang M, Xu X, Qin C, Cheng G, Zhang X, Wang P, Qiu X, Kobinger G, Shi Y, Yan J, Gao GF. 2016. Molecular determinants of human neutralizing antibodies isolated from a patient infected with Zika virus. *Sci Transl Med* 8:369ra179. <https://doi.org/10.1126/scitranslmed.aai8336>.
14. Stettler K, Beltramello M, Espinosa DA, Graham V, Cassotta A, Bianchi S, Vanzetta F, Minola A, Jaconi S, Mele F, Foglierini M, Pedotti M, Simonelli L, Dowall S, Atkinson B, Percivalle E, Simmons CP, Varani L, Blum J, Baldanti F, Cameroni E, Hewson R, Harris E, Lanzavecchia A, Sallusto F, Corti D. 2016. Specificity, cross-reactivity and function of antibodies elicited by Zika virus infection. *Science* 353:823–826. <https://doi.org/10.1126/science.aaf8505>.
15. Watterson D, Modhiran N, Young PR. 2016. The many faces of the flavivirus NS1 protein offer a multitude of options for inhibitor design. *Antiviral Res* 130:7–18. <https://doi.org/10.1016/j.antiviral.2016.02.014>.
16. Deng YQ, Dai JX, Ji GH, Jiang T, Wang HJ, Yang HO, Tan WL, Liu R, Yu M, Ge BX, Zhu QY, Qin ED, Guo YJ, Qin CF. 2011. A broadly flavivirus cross-neutralizing monoclonal antibody that recognizes a novel epitope within the fusion loop of E protein. *PLoS One* 6:e16059. <https://doi.org/10.1371/journal.pone.0016059>.
17. Wang S, Hong S, Deng YQ, Ye Q, Zhao LZ, Zhang FC, Qin CF, Xu Z. 2017. Transfer of convalescent serum to pregnant mice prevents Zika virus infection and microcephaly in offspring. *Cell Res* 27:158–160. <https://doi.org/10.1038/cr.2016.144>.
18. Shi Y, Gao GF. 2017. Structural biology of the Zika virus. *Trends Biochem Sci* 42:443–456. <https://doi.org/10.1016/j.tibs.2017.02.009>.
19. Zhang S, Kostyuchenko VA, Ng TS, Lim XN, Ooi JS, Lambert S, Tan TY, Widman DG, Shi J, Baric RS, Lok SM. 2016. Neutralization mechanism of a highly potent antibody against Zika virus. *Nat Commun* 7:13679. <https://doi.org/10.1038/ncomms13679>.
20. Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-Spaeth G, Duangchinda T, Sakuntabhai A, Cao-Lormeau VM, Malasit P, Rey FA, Mongkolsapaya J, Screaton GR. 2016. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with Zika virus. *Nat Immunol* 17:1102–1108. <https://doi.org/10.1038/ni.3515>.
21. Hasan SS, Miller A, Sapparapu G, Fernandez E, Klose T, Long F, Fokine A, Porta JC, Jiang W, Diamond MS, Crowe JE, Jr, Kuhn RJ, Rossmann MG. 2017. A human antibody against Zika virus crosslinks the E protein to prevent infection. *Nat Commun* 8:14722. <https://doi.org/10.1038/ncomms14722>.
22. Chu JH, Rajamanonmani R, Li J, Bhuvanathan R, Lescar J, Ng ML. 2005. Inhibition of West Nile virus entry by using a recombinant domain III from the envelope glycoprotein. *J Gen Virol* 86:405–412. <https://doi.org/10.1099/vir.0.80411-0>.
23. Bhardwaj S, Holbrook M, Shope RE, Barrett ADT, Watowich SJ. 2001. Biophysical characterization and vector-specific antagonist activity of domain III of the tick-borne flavivirus envelope protein. *J Virol* 75:4002–4007. <https://doi.org/10.1128/JVI.75.8.4002-4007.2001>.
24. Kostyuchenko VA, Lim EX, Zhang S, Fibriansah G, Ng TS, Ooi JS, Shi J, Lok

- SM. 2016. Structure of the thermally stable Zika virus. *Nature* 533: 425–428.
25. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martinez E, Nathan MB, Pelegrino JL, Simmons C, Yoksan S, Peeling RW. 2010. Dengue: a continuing global threat. *Nat Rev Microbiol* 8:S7–S16. <https://doi.org/10.1038/nrmicro2460>.
 26. Flipse J, Smit JM. 2015. The complexity of a dengue vaccine: a review of the human antibody response. *PLoS Negl Trop Dis* 9:e0003749. <https://doi.org/10.1371/journal.pntd.0003749>.
 27. Dejnirattisai W, Jumnainsong A, Onsirisakul N, Fitton P, Vasanawathana S, Limpitikul W, Puttikhunt C, Edwards C, Duangchinda T, Supasa S, Chawansuntati K, Malasit P, Mongkolsapaya J, Screaton G. 2010. Cross-reacting antibodies enhance dengue virus infection in humans. *Science* 328:745–748. <https://doi.org/10.1126/science.1185181>.
 28. Barouch DH, Thomas SJ, Michael NL. 2017. Prospects for a Zika virus vaccine. *Immunity* 46:176–182. <https://doi.org/10.1016/j.immuni.2017.02.005>.
 29. Richner JM, Himansu S, Dowd KA, Butler SL, Salazar V, Fox JM, Julander JG, Tang WW, Shresta S, Pierson TC, Ciaramella G, Diamond MS. 2017. Modified mRNA vaccines protect against Zika virus infection. *Cell* 168: 1114–1125. <https://doi.org/10.1016/j.cell.2017.02.017>.
 30. Chung KM, Nybakken GE, Thompson BS, Engle MJ, Marri A, Fremont DH, Diamond MS. 2006. Antibodies against West Nile virus nonstructural protein NS1 prevent lethal infection through Fc gamma receptor-dependent and -independent mechanisms. *J Virol* 80:1340–1351. <https://doi.org/10.1128/JVI.80.3.1340-1351.2006>.
 31. Schlesinger JJ, Foltzer M, Chapman S. 1993. The Fc portion of antibody to yellow fever virus Ns1 is a determinant of protection against Yf encephalitis in mice. *Virology* 192:132–141. <https://doi.org/10.1006/viro.1993.1015>.
 32. Sasaki S, He XS, Holmes TH, Dekker CL, Kemble GW, Arvin AM, Greenberg HB. 2008. Influence of prior influenza vaccination on antibody and B-cell responses. *PLoS One* 3:e2975. <https://doi.org/10.1371/journal.pone.0002975>.
 33. Angeletti D, Gibbs JS, Angel M, Kosik I, Hickman HD, Frank GM, Das SR, Wheatley AK, Prabhakaran M, Leggat DJ, McDermott AB, Yewdell JW. 2017. Defining B cell immunodominance to viruses. *Nat Immunol* 18: 456–463. <https://doi.org/10.1038/ni.3680>.