Therapeutic and protective efficacy of a dengue antibody against Zika infection in rhesus monkeys

Peter Abbink, Rafael A. Larocca, Wanwisa Dejnirattisai, Rebecca Peterson, Joseph P. Nkolola, Erica N. Borducchi, Piyada Supasa, Juthapith Mongkolapaya, Gavin R. Screaton and Dan H. Barouch

Strategies to treat Zika virus (ZIKV) infection in dengue virus (DENV)-endemic areas are urgently needed. Here we show that a DENV-specific antibody against the E-dimer epitope (EDE) potently cross-neutralizes ZIKV and provides robust therapeutic efficacy as well as prophylactic efficacy against ZIKV in rhesus monkeys. Viral escape was not detected, suggesting a relatively high bar to escape. These data demonstrate the potential for antibody-based therapy and prevention of ZIKV.

Zika virus (ZIKV) has been associated with fetal microcephaly and other congenital abnormalities as well as Guillain–Barre syndrome. Our laboratory and others have shown that ZIKV-specific neutralizing antibodies correlate with vaccine protection in both mice and monkeys as well as with rapid control of viremia following infection in monkeys. Several groups have also demonstrated therapeutic efficacy of ZIKV-specific monoclonal antibodies in immunosuppressed mice, and a cocktail of three ZIKV-specific monoclonal antibodies that targeted domain III was shown to prevent ZIKV infection in nonhuman primates. In the present study, we assessed the therapeutic and prophylactic efficacy of a potent ZIKV-specific antibody in rhesus monkeys.

Substantial humoral cross-reactivity exists between DENV and ZIKV, and DENV-specific antibodies have been associated with antibody-dependent enhancement of ZIKV infection in vitro and in certain mouse models. We previously reported that DENV E-dimer epitope (EDE)-specific monoclonal antibodies bind a quaternary epitope formed at the interface of head-to-tail E-dimers and efficiently cross-neutralize ZIKV. EDE-specific monoclonal antibodies poorly bind to monomeric E-proteins but efficiently bind to stable E-dimers. These antibodies can be subdivided into two groups, EDE1 and EDE2, according to their insensitivity or sensitivity, respectively, to removal of N-linked glycan at position 153. EDE1 monoclonal antibodies typically exhibit greater potency. Moreover, the EDE1-specific monoclonal antibody B10 has been shown to prevent and treat ZIKV infection in mice. We evaluated 33 EDE1-specific antibodies isolated from patients infected with DENV and found that B10 was the most potent in neutralizing a French Polynesian ZIKV strain (ZIKV-PF13; Fig. 1a). B10 neutralized ZIKV-PF13 (50% neutralization titer (NT50) of 0.016 ± 0.001 nM; NT90 of 0.100 ± 0.009 nM (mean ± s.e.m.)) even more potently than DENV-1, DENV-2, and DENV-3 but showed poor neutralization of DENV4 (Fig. 1b).

To confirm the antiviral activity of B10 against ZIKV in vivo, we performed a titration study in immunocompetent Balb/c mice. Groups of Balb/c mice (n = 5 mice per group) received a single infusion of 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.097, 0.048, or 0 μg B10 and were subsequently challenged with 10^6 viral particles (VP) (10^5 plaque-forming units (PFU)) of ZIKV-BR via the intravenous route (Supplementary Fig. 1). In naive mice, ZIKV-BR infection led to peak viral loads of 5.24–6.18 log RNA copies per ml, similar to previous findings with this challenge stock. B10 doses as low as 3.12 μg, corresponding to serum levels of 0.5–0.9 μg/ml (3–6 nM), resulted in complete protection against ZIKV-BR challenge in mice (Supplementary Fig. 1). Subprotective B10 doses of 0.19–1.56 μg provided partial protection of a subset of mice and attenuation of viral loads in infected animals. These data confirm B10 potency against ZIKV challenge in mice.

We next evaluated the therapeutic and prophylactic efficacy of B10 in rhesus monkeys (Macaca mulatta). Sixteen monkeys received the following antibodies by intravenous infusion either before or after challenge with the Brazil/ZKV2015 strain (ZIKV-BR) (n = 4 monkeys per group): (i) 10 mg per kg body weight (mg/kg) B10 on day –1, (ii) 10 mg/kg isotype matched control antibody (PGT121), (iii) 10 mg/kg isotype matched control antibody (PGT121) on day –1, (iii) 10 mg/kg B10 on day +2, or (iv) 10 mg/kg isotype matched control antibody (PGT121) on day +2. We selected this antibody dose on the basis of our previous experience with therapeutic HIV-1-specific antibody studies in SHIV-infected rhesus monkeys. Antibody pharmacokinetics was monitored using ELISA, and peak B10 levels were 78–306 μg/ml (0.5–2 μM) on the day after infusion (Fig. 1c).

On day 0, all monkeys were challenged via the subcutaneous route with 10^6 VP (10^4 PFU) of ZIKV-BR, and viral loads were quantitated using RT-PCR. Animals that received the isotype matched sham control antibody either before or after ZIKV-BR challenge exhibited approximately 7 log of viremia with median peak viral loads of 6.40 (range, 5.31–6.60) log RNA copies per ml on day 3–5 following challenge (Fig. 2a), consistent with our previous studies using this ZIKV-BR challenge stock in rhesus monkeys. Administration of B10 on day –1 before challenge resulted in complete protection, as evidenced by no detectable plasma viremia at any time point (P = 0.02 comparing infection of B10 group versus controls; Fisher’s exact test). Administration of B10 on day +2 after challenge, which was during the exponential rise of plasma viremia, resulted in an abrupt termination of viral replication and rapid clearance of virus.
from peripheral blood by day 3 (Fig. 2a; P = 0.02 comparing viremia on days 3–7 of B10 group versus controls; Fisher’s exact test).

We observed prolonged ZIKV-BR shedding in cerebrospinal fluid (CSF), lymph nodes (LN), and colorectal (CR) biopsies from sham-treated controls (Fig. 2b,c and Supplementary Fig. 2), consistent with our previous observations. Monkeys that received B10 on day –1 before challenge had no detectable viral load in these tissues, in accordance with complete protection against infection. Moreover, these animals had no detectable cellular immune responses following ZIKV-BR challenge, as measured by interferon-γ (IFN-γ) ELISPOT assays to ZIKV envelope protein (Env), nonstructural protein 1 (NS1), capsid protein (Cap), and premembrane (prM) peptide pools (Supplementary Fig. 3). Monkeys that received B10 on day +2 after challenge also showed a substantial reduction of viral load in tissues. However, ZIKV-BR was still detected in CSF of 2 of 4 monkeys on day 7 and in CSF of 1 of 4 monkeys on day 14. In this animal (12-083), the peak B10 level in CSF was 1 μg/ml (0.5% of plasma levels). The prM–Env sequence from the virus in CSF on day 14 was identical to that of the ZIKV-BR challenge stock (Supplementary Fig. 4), suggesting that the virus did not specifically escape from B10. These data demonstrate that therapeutic B10 administration in monkeys acutely infected with ZIKV-BR rapidly controlled virus replication in the periphery within 24 h but incompletely cleared virus from immunoprivileged sites, likely as a result of reduced antibody penetration into these anatomic compartments.

To further evaluate the capacity of ZIKV to escape EDE1-specific monoclonal antibodies, we incubated ZIKV with escalating concentrations of the B10 or C8 antibodies in vitro at 0.002, 0.015, and 0.070 μg/ml (corresponding to 50% focus reduction neutralization test (FRNT) and 90% and 99% FRNT) for two, three, and five passages, respectively. After ten passages, parental and passaged ZIKV stocks were used in the infection experiments. We observed that B10 inactivated ZIKV stocks more efficiently than C8 as measured by FRNT assays (Supplementary Fig. 5), suggesting a relatively high bar to resistance. These findings are consistent with the observed therapeutic and prophylactic efficacy with B10 in rhesus monkeys even when delivered as monotherapy (Fig. 2). In contrast, a cocktail of three domain III–specific monoclonal antibodies was required to prevent ZIKV infection in nonhuman primates.

Our data demonstrate that a DENV EDE1–specific monoclonal antibody has potent cross-reactive neutralizing activity against ZIKV and provides robust therapeutic as well as prophylactic efficacy against ZIKV infection in rhesus monkeys. On the basis of the rapid clearance of plasma virus by 24 h after B10 infusion, we speculate that this antibody functions therapeutically by opsonization of virus followed by clearance. Previous studies have evaluated ZIKV-specific monoclonal antibodies in therapeutic studies in...
immunosuppressed mice models\textsuperscript{6–11}. Our data extend these prior studies by demonstrating the therapeutic and prophylactic efficacy of a ZIKV-specific antibody in nonhuman primates. These findings encourage clinical development of ZIKV-specific monoclonal antibodies for both therapy and prevention.

The potency of B10 and the apparent relatively high bar to escape raise the possibility of antibody monotherapy, which would be logistically far simpler than the development of antibody cocktails\textsuperscript{12} or bispecific antibodies\textsuperscript{9}. The structure of B10 remains to be determined, but the related cross-reactive DENV–ZIKV EDE1-specific monoclonal antibody C8 binds a conserved quaternary site at the interface between the two Env subunits in the dimer at the interaction site of prM\textsuperscript{16}, which may explain its high bar to escape.

A potential challenge for any antibody-based ZIKV therapeutic strategy will likely involve persistent virus in immunoprivileged sites, as the virus may be quickly seeded in these sites within the first few days of infection. Such sites include the central nervous system, LN, and placental and fetal tissues. We previously reported that ZIKV persists in CSF, LN, and CR mucosa in monkeys for substantial periods of time after viremia resolves, and viral persistence at these sites correlates with activation of mTOR and proinflammatory signaling pathways\textsuperscript{7}. We show here that B10 poorly penetrates into the CSF and thus may not fully clear CSF virus that was seeded before antibody administration.

A unique aspect of B10 is that it was derived from a DENV-infected individual before the ZIKV epidemic. Certain DENV-specific antibodies have been shown to enhance ZIKV replication in vitro and in mice\textsuperscript{13–15}, although the relevance of these observations for humans remains to be determined. In our experiments, subneutralizing doses of B10 did not result in enhanced ZIKV replication in mice (Supplementary Fig. 1). Nevertheless, the possibility of antibody-dependent enhancement with a cross-reactive DENV–ZIKV-specific antibody requires further investigation, and if necessary, mutations inactivating the fragment crystallizable interface between the two Env subunits may explain its high bar to escape.

We thank V. Cao-Lormeau (Unit of Emerging Infectious Diseases, Institut Louis Malardé; provided Zika virus strain PF-13), E. Moseley, K. McMahan, M. Boyd, M. Kirilova, O. Nanayakkara, Z. Li, N. Mercado, A. Badamchi-Zadeh, M. Iampietro, C. Bricault, P. Gandhi, S. Khatiwada, S. Mojta, B. Alimonti, A. Chandrashekar, A. Brinkman, M. Ferguson, and W. Rinaldi for generous advice, assistance, and reagents. We acknowledge support from the US National Institutes of Health (AI124377, AI126603, AI128751, AI129797) (D.H.B.), the National Institute for Health Research Biomedical Research Centre funding scheme UK (G.R.S), MRC-Newton UK (J.M.), and the Ragon Institute of MGH, MIT, and Harvard (D.H.B.). G.R.S is a Wellcome Trust Senior Investigator.

Acknowledgements
We thank V. Cao-Lormeau (Unit of Emerging Infectious Diseases, Institut Louis Malardé; provided Zika virus strain PF-13), E. Moseley, K. McMahan, M. Boyd, M. Kirilova, O. Nanayakkara, Z. Li, N. Mercado, A. Badamchi-Zadeh, M. Iampietro, C. Bricault, P. Gandhi, S. Khatiwada, S. Mojta, B. Alimonti, A. Chandrashekar, A. Brinkman, M. Ferguson, and W. Rinaldi for generous advice, assistance, and reagents. We acknowledge support from the US National Institutes of Health (AI124377, AI126603, AI128751, AI129797) (D.H.B.), the National Institute for Health Research Biomedical Research Centre funding scheme UK (G.R.S), MRC-Newton UK (J.M.), and the Ragon Institute of MGH, MIT, and Harvard (D.H.B.). G.R.S is a Wellcome Trust Senior Investigator.

Author contributions
D.H.B. and G.R.S designed the studies. W.D., P.S, and J.M. produced and characterized the B10 antibody. R.A.L. conducted the mouse studies. P.A. and R.P conducted the virologic assays. J.P.N. and E.N.B. conducted the monkey study and immunologic assays. D.H.B. wrote the paper with all coauthors.

Competing interests
The B10 antibody is the subject of patents held by Imperial College and Institute Pasteur on which G.R.S, W.D, and J.M. are inventors.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41591-018-0056-0.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to G.R.S or D.H.B.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Methods

Animals, vaccines, and challenges. Female 6- to 8-week-old Balb/c mice were housed at Beth Israel Deaconess Medical Center. 16 outbred, Indian-origin male and female rhesus monkeys (Macaca mulatta) were housed at AlphaGenesis, Yemassee, SC, USA. Animals received in-house-manufactured B10 or isotype matched control antibody (PGT121) infusions via the intravenous route either before or after challenge. Antibodies were negative for endotoxin by Pierce LAL Chromogenic Endotoxin Quantitation kit (Thermo Scientific). Balb/c mice were challenged with 10^5 viral particles (VP) (10^2 plaque-forming units (PFU)) ZIKV-BR (Brazil ZIKV2015). Rhesus monkeys were challenged by the subcutaneous route with 10^6 VP (10^3 PFU) ZIKV-BR. Animals were randomly allocated to groups. Immunologic and virologic assays were performed blinded. Animal studies were approved by the Institutional Animal Care and Use Committees (IACUCs) at AlphaGenesis and Beth Israel Deaconess Medical Center as well as the Central Animal Welfare Ethical Review Board at Imperial College London.

Focus reduction neutralization assay. Virus was incubated with serial dilutions of antibodies at a 1:1 ratio for 1 h at 37 °C. The monoclonal antibody–virus mixtures were then inoculated onto Vero cells (a gift from AFRIMS). After 1 h of incubation, the cell monolayers were overlaid with 1.5% (wt/vol) carboxymethyl cellulose (C4888; Sigma) and incubated for 2 d (for ZIKV) or 3 d (for DENV). The viral foci were visualized by staining with monoclonal antibody 4G2 supernatant (mouse anti-DENV fusion loop that cross-reacts to ZIKV; a gift from AFRIMS) followed by peroxidase-conjugated goat anti–mouse immunoglobulin at a 1:1,000 dilution (P0047, Sigma). The foci (infected cells) were visualized by staining with monoclonal antibody–virus mixtures as described.3,4 RNA was extracted from plasma or other samples with a QIAcube HT (Qiagen). The wild-type ZIKV BeH815744 cap gene was used as a standard. RNA was purified (Zymo Research), and RNA quality and concentration were assessed by the BIDMC Molecular Core Facility. Log dilutions of the RNA standard were reverse transcribed and included with each RT-PCR assay. Viral loads were calculated as virus particles (VP) per ml or per 1×10^6 cells and were confirmed by PFU assays. Assay sensitivity was 100 copies per ml or 1×10^6 cells.

ELISA. Mice and monkey ZIKV Env ELISA kits (Alpha Diagnostic International) were used to assess B10 levels. 96-well plates coated with ZIKV Env protein were first equilibrated at room temperature with 300 µl of kit working wash buffer for 5 min. 6 µl of serum was added to the top row, and threefold serial dilutions were tested in the remaining rows. Samples were incubated at room temperature for 1 h, and plates were washed four times. 100 µl of anti-mouse or anti-human IgG HRP-conjugate working solution was then added to each well and incubated for 30 min at room temperature. Plates were washed five times, developed for 15 min at room temperature with 100 µl of 3,3',5,5'-tetramethylbenzidine (TMB) substrate, and stopped by the addition of 100 µl of stop solution. Plates were analyzed at 450 nm and 550 nm on a VersaMax microplate reader using Softmax Pro 6.0 software (Molecular Devices). B10 levels were assessed against a standard curve.

In vitro selection with B10 and C8. To try to select ZIKV mutants resistant to neutralization by B10 or C8 (manufactured in-house), ZIKV was incubated with monoclonal antibody for 1 h at 37 °C. Viruses were then inoculated onto Vero cells and incubated for 2 d. In parallel, mock-neutralized virus was used as wild-type virus control. Viral titers were determined, and virus-containing cell suspension was harvested for the next passage. This process was repeated through ten passages, with 0.002, 0.015, and 0.070 µg/ml of antibody (FRNT50, FRNT90, and FRNT100) for two, three, and five passages, respectively. After 10 passages, parental and passaged viruses were analyzed for resistance to B10 or C8 neutralization by FRNT assays.

Viral sequencing. Viral RNA was extracted by QIAamp Viral RNA Mini Kit or QIAxcel Viral RNA Mini Kit or QIAxcel Viral RNA Mini Kit or QIAxcel Viral RNA Mini Kit and sequenced. Analysis of virologic and immunologic data was performed using GraphPad Prism v6.03 (GraphPad Software). Comparisons of groups were performed using Fischer's exact tests and Wilcoxon rank-sum tests.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All data supporting this study are available from the corresponding author upon reasonable request.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- **n/a**  Confirmed
- **✓**  The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- **✓**  An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- **✓**  The statistical test(s) used AND whether they are one- or two-sided
  
  *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- **✓**  A description of all covariates tested
- **✓**  A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- **✓**  A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- **✓**  For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  
  *Give P values as exact values whenever suitable.*
- **✓**  For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- **✓**  For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- **✓**  Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- **✓**  Clearly defined error bars
  
  *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

- **Data collection**  Softmax Pro 6.0 (Molecular Devices)
- **Data analysis**  GraphPad Prism v6.03 (GraphPad Software)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All raw data is provided in the figures and supplementary figures
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences  
- Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | For the NHP study, sample sizes of N=4/group were selected as standard for NHP studies to detect large differences between treatment and control arms. For the mouse studies, sample sizes of N=5/group were selected based on our prior experience of variability for this model system. |
| Data exclusions | No data was excluded. |
| Replication | Mouse experiments were repeated twice. All attempts at replication were successful. Viral loads in the NHP study were done by qualified assays with multiple measures per animal. |
| Randomization | Animals were randomly allocated to groups. |
| Blinding | Virologic and immunologic data was generated blinded. |

Materials & experimental systems

Policy information about availability of materials

- n/a

Involved in the study

- Unique materials
- Antibodies
- Eukaryotic cell lines
- Research animals
- Human research participants

Unique materials

- Obtaining unique materials
  The B10 antibody is available by MTA

Antibodies

- Antibodies used
  B10 (ZIKV-specific), PGT121 (HIV-specific)

Validation

The B10 antibody was validated against known standards by functional neutralization activity against DENV and ZIKV, as depicted in Figure 1.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

- Animals/animal-derived materials
  Rhesus monkeys, mixed male and female, age 3-8; Balb/c mice, female, age 6-8 weeks; all studies were IACUC approved

Method-specific reporting

- n/a

Involved in the study

- ChIP-seq
- Flow cytometry
- Magnetic resonance imaging