Augmented Zika and Dengue Neutralizing Antibodies Are Associated With Guillain-Barré Syndrome

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The role of neutralizing antibodies in Zika-induced Guillain-Barré syndrome (GBS) has not yet been investigated. We conducted a case-control study using sera from the 2016 Zika epidemic in Colombia to determine the neutralizing antibody activity against Zika virus (ZIKV) and dengue virus serotype 2 (DENV2). We observed increased neutralizing antibody titers against DENV2 in ZIKV-infected individuals compared with uninfected controls and higher titers to both ZIKV and DENV2 in ZIKV-infected patients diagnosed with GBS compared with non-GBS ZIKV-infected controls. These data suggest that high neutralizing antibody titers to DENV and to ZIKV are associated with GBS during ZIKV infection.

Keywords. Guillain-Barré syndrome; neutralizing antibody; flavivirus; Zika; dengue.

Zika virus (ZIKV) is a flavivirus spread mainly by the Aedes aegypti mosquito. In 2015–2016, an epidemic of Zika in the Americas was accompanied by severe neurologic complications including microcephaly in babies born to mothers infected with ZIKV during pregnancy and Guillain-Barré syndrome (GBS) in adults [1]. GBS is a disorder of the peripheral nervous system often triggered by a preceding viral or bacterial infection or vaccination [2]. Although the exact cause of most GBS cases remains unknown, several studies have demonstrated that for some pathogens, such as Campylobacter jejuni, an infection-induced antibody cross-reacts with the ganglioside surface components of peripheral nerves [2]. Although the mechanism whereby Zika is associated with GBS has not been clearly elucidated, it is likely that there is a similar pathogenesis. As many vaccines currently under development for Zika are designed to elicit protective titers of neutralizing antibodies, it is critical to define the role of ZIKV antibodies in the development of GBS.

During the 2015–2016 Zika epidemic in Colombia, there was a simultaneous increase in the number of neuroinflammatory disorders reported [3]. Specifically, there was an increase in GBS cases in individuals found to be ZIKV positive (ZIKV+) by reverse-transcription polymerase chain reaction, lending support to the role of ZIKV infection in GBS pathogenesis [3]. To investigate the relationship between ZIKV infection and GBS, anti-ZIKV neutralizing antibodies were assayed in plasma samples obtained from ZIKV-infected patients and controls collected during the 2016 outbreak in Barranquilla, Colombia.

METHODS

Ethics Statement

This study was approved by the ethics committee of the Universidad El Bosque, and a nonhuman subjects determination was made by the George Washington University Institutional Review Board for analysis of de-identified data. All participants received written informed consent.

Participants and Setting

Adult patients with a clinical diagnosis of Zika and Zika-related GBS were referred to this study from the Atlántico Department and Bolivar Department, Colombia, while asymptomatic participants from Bogotá, Cundinamarca Department, a mountainous region without endemic ZIKV transmission, were enrolled as Zika-negative controls.

Case and Control Definitions

Zika-Related Guillain-Barré Syndrome Case

A Zika-related GBS (ZGBS) case was defined as a participant with clinically diagnosed ZIKV infection, confirmed by serologic analysis as described below, and GBS, as diagnosed and reported by a local neurologist. The Brighton criteria for the level of GBS diagnosis certainty was determined if documentation was available [4].

Zika-Positive Control

Participants with clinical symptoms of Zika and Zika-related GBS were referred to this study from the Atlántico Department and Bolivar Department, Colombia, while asymptomatic participants from Bogotá, Cundinamarca Department, a mountainous region without endemic ZIKV transmission, were enrolled as Zika-negative controls.
Zika-Negative Control
A Zika-negative control (ZIKV–) was defined as an asymptomatic participant confirmed to be negative for ZIKV infection by serology.

Design
Each ZGBS case was age- and sex-matched to 2 ZIKV+ controls and 1 ZIKV– control by simple random sampling within age and sex strata. All patients with a clinical diagnosis of ZIKV or ZGBS completed a brief symptom questionnaire prior to blood sample collection. A retrospective chart review was performed for cases of ZGBS cases where the medical records were available.

Serologic ZIKV Infection Determination
Participants were considered to be positive for a ZIKV infection if they fulfilled the following diagnostic criteria: ZIKV nonstructural protein 1 (NS1) antibody positive by the previously described Zika NS1 blockade-of-binding assay [5] or a reciprocal 50% neutralizing titer (NT50) against ZIKV strain H/PF/2013 that was at least 2-fold greater than the NT50 titer against dengue virus serotype 2 (DENV2) 16681.

Reporter Virus Particle Neutralization Assays
Neutralization of ZIKV H/PF/2013 and DENV2 16681 by plasma samples was measured using a reporter virus particle assay as described previously [6]. In brief, heat-inactivated plasma was serially diluted 5-fold from 1:50 and incubated with 100 μL of virus for 1 hour at 37°C, after which 50 μL of target Vero cells (400,000 cells/mL) was added. Input virus dilution was calculated from titration experiments to ensure sufficient luciferase output within the linear portion of the titration curve. Cell-only and virus-only controls were included on each plate, and all serum samples (and virus only) were run in triplicate. After a 48-hour incubation, luciferase activity was measured, and neutralization curves were calculated by averaging luciferase units from triplicates, subtracting cell-only control background and calculating the percentage difference in serum samples to virus-only controls. Data was fit by nonlinear regression using the asymmetric 5-parameter logistic function in GraphPad Prism. The 50%, 80%, and 90% neutralizing titers (NT50, NT80, and NT90, respectively) were defined as the reciprocal serum dilution resulting in a 50%, 80%, or 90% reduction in infectivity.

Statistical Analysis
Nonparametric Mann–Whitney U tests were performed to determine if there were differences in reciprocal dilutions between ZGBS and ZIKV+ groups for neutralization of DENV and ZIKV, respectively. Samples with no neutralization at a dilution of 1:50 were assigned a titer of 49 for statistical analysis. The differences between the mean reciprocal dilution vectors for neutralization of DENV2 and ZIKV in these groups (ZGBS and ZIKV+) were further assessed with Hotelling T2 test and graphically with 95% probability confidence ellipses. Spearman rank correlation was used to determine the association between neutralization of DENV and ZIKV for each group. Statistical analyses were conducted using SAS software, version 9.4 (SAS Institute, Cary, North Carolina), and tests were considered statistically significant with a P value ≤.05.

RESULTS
The clinical and serological factors associated with ZGBS were studied in 23 patients with a clinical diagnosis of Zika and GBS in Barranquilla, Colombia, from December 2015 through May 2016. Six participants were excluded from further analysis because their clinical Zika diagnosis was not serologically confirmed. Seventeen ZGBS cases, 34 age- and sex-matched ZIKV+ controls, and 17 age- and sex-matched ZIKV– controls were included in the current analysis. The ZGBS cases were adults with median age of 49 years, and 47% were male (Supplementary Table 1). Two patients reported a history of a previous suspected DENV infection, and 2 patients of a suspected prior chikungunya virus infection. All patients reported viral symptoms during ZIKV infection including arthralgias (94%), fever (88%), and myalgias (88%). The median time from onset of ZIKV symptoms to neurologic symptoms was 10 days (interquartile range [IQR], 7–19; Supplementary Table 1). Access to medical records allowed Brighton criteria GBS classification in 8 of the 17 patients, demonstrating certainty of diagnosis level 1 (based on both nerve conduction studies and cerebrospinal fluid [CSF] analysis) in 18% of cases, level 2 in 18% of cases based on either nerve conduction studies or CSF analysis, and level 3 (based on clinical features) in 12% of cases [4]. One patient was diagnosed with Miller–Fisher syndrome (Supplementary Table 1). Two patients demonstrated demyelination and axonal involvement based on nerve conduction studies.

The most common neurologic symptoms were lower extremity weakness (100%), inability to walk (88%), and paresthesias (100%). The great majority of patients were cared for in the intensive care unit (88%). Half the patients had difficulty breathing, and 38% had respiratory failure requiring intubation. Most patients were treated with intravenous immunoglobulin (63%) or plasmapheresis (25%), and none were treated with steroids. The median duration of hospitalization was 11 days (IQR, 7–24 days), with a median of 9 days (IQR, 5–13 days) in the intensive care unit. One patient died, one-fourth had a full recovery, and 63% reported chronic morbidities including upper and lower extremity weakness, facial tremors, and sensory alterations.

The relationship between antibody responses to ZIKV infection and a clinical diagnosis of GBS was assessed by comparing neutralizing antibody titers between the ZGBS cases and the ZIKV+ and ZIKV– controls. Because DENV2 recently circulated in Colombia, plasma neutralizing antibody titers against both
**Figure 1.** Neutralizing antibody titers to Zika virus (ZIKV) and dengue virus serotype 2 (DENV2) in cases and controls. Age and sex are listed for each case-control participant as well as the time from reported Zika symptoms to date of sampling in days. Reciprocal 50%, 80%, and 90% neutralizing antibody titers to ZIKV strain H/PF/2013 and DENV2 strain 16681 are reported and shaded according to potency as indicated in the key. Abbreviations: DENV2, dengue virus serotype 2; GBS, Guillain-Barré syndrome; NT50, 50% neutralizing titer; NT80, 80% neutralizing titer; NT90, 90% neutralizing titer; ZIKV, Zika virus.
NT80: ZGBS cases to ZIKV+ controls, we found significantly elevated post–Zika infection in the ZGBS group. When comparing We observed a trend toward lower NT80 with increasing days interval between onset of disease and sampling (79–137 days).

DENV neutralizing antibody titers for each patient, along with Table 5). These data are summarized by graphing ZIKV vs neutralizing antibodies in ZIKV infection was recently reported after ZIKV infection [13]. Confirmation of the role of DENV neutralizing antibodies in ZIKV infection was recently reported in a study of longitudinal B-cell responses to ZIKV after previous DENV infection. It was observed that both ZIKV and DENV neutralizing antibodies are boosted following ZIKV infection but that they derive from distinct B-cell populations and that the anamnestic dengue response occurs first, followed by a de novo ZIKV response [14]. It is possible that the development of GBS results from indirect effects such as molecular mimicry, where virus specific antibodies (either ZIKV or DENV) cross-react with nerve cells, but it is also possible this association between newt day. The time course between the onset of Zika-like symptoms and the development of neurologic symptoms is sufficient for antibody production and would be compatible with the role of an adaptive immune response in this process; however, this observation does not prove that adaptive immunity causes GBS. The onset of neurologic symptoms occurred in median of 10 days after the initial onset of viral symptoms, comparable with other GBS cohorts from Colombia [3, 9, 11].

Higher titers of anti-DENV2 antibodies in ZIKV-infected participants compared to uninfected participants provides more evidence for the observation that DENV B cells are activated after ZIKV infection. This is in agreement with a previous reports such as an analysis of samples from Brazil where neutralizing antibody titers to ZIKV and DENV1 were boosted after ZIKV infection [13].

DISCUSSION

The clinical and demographic characteristics of ZGBS cases from Barranquilla, Colombia, are in accordance with other studies [3, 7–12]. GBS may occur with rapid onset of both motor and sensory neurologic symptoms following symptomatic ZIKV infection. These cases reported here tended to be severe, with most patients (88%) admitted to intensive care units and more than one-third requiring mechanical ventilation.

The higher neutralizing antibody titers found in the ZGBS cases compared to ZIKV+ controls provide evidence of a correlation between these titers and the development of GBS in these patients, though not causation. This finding could represent an indirect effect that results from high virus load. The time course between the onset of Zika-like symptoms and the development of neurologic symptoms is sufficient for antibody production and would be compatible with the role of an adaptive immune response in this process; however, this observation does not prove that adaptive immunity causes GBS. The onset of neurologic symptoms occurred in median of 10 days after the initial onset of viral symptoms, comparable with other GBS cohorts from Colombia [3, 9, 11].

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as high virus load or high immune activation. This cross-sectional analysis cannot fully resolve that issue. Further research is needed to characterize the specific antibody populations responsible for ZGBS, and this information will be critical for Zika vaccine development.

Supplementary Data
Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copy-edited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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