Characteristics of Antibody Responses in West Nile Virus-Seropositive Blood Donors

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West Nile virus (WNV) is now endemic in the United States. Protection against infection is thought to be conferred in part by humoral immunity. An understanding of the durability and specificity of the humoral response is not well established. We studied the magnitude and specificity of antibody responses in 370 WNV-seropositive blood donors. We also recalled 18 donors who were infected in 2005 to compare their antibody responses at 6 months following infection versus at 5 years postinfection. There were no significant differences in IgG antibody levels based on age, sex, or recent infection (as evidenced by IgM positivity). Specific antibody responses by viral plaque reduction neutralization testing (PRNT) were seen in 51/54 subjects evaluated. All donors who were seropositive in 2005 remained seropositive at 5 years and maintained neutralizing antibodies. IgG levels at 5 years postinfection showed fairly minimal decreases compared with the paired levels at 6 months postinfection (mean of paired differences, $-0.54$ signal-to-cutoff ratio (S/CO) units [95% confidence interval (CI), $-0.86$ to $-0.21$ S/CO units]) and only minimal decreases in PRNT titers. WNV induces a significant antibody response that remains present even 5 years after infection.

West Nile virus (WNV) infection is now well established in the United States, with an estimated 3 million infections in the 48 contiguous states through 2010 (1). Since the start of the epidemic in 1999 through 2012, >15,000 persons have developed neuroinvasive disease, characterized by meningoencephalitis or acute flaccid paralysis, and >1,500 deaths have occurred (CDC ArboNET). Advanced age, male sex, and immunosuppression significantly increase the risk for neuroinvasive disease (2, 3).

The production of WNV-specific IgM and IgG antibodies is important for both the diagnosis and the clearance of WNV infection (4). The persistence of IgG antibodies is thought to confer protection from subsequent reinfection (4–6). In a study of 245 viremic blood donors, IgM antibodies persisted for a mean of 156 days, and IgG antibodies persisted at the same titer for at least 1 year postinfection (7). IgM antibodies persisted in up to 17% of subjects at 400 days postinfection, whereas IgG antibodies were maintained at high levels based on enzyme immunoassay (ELISA) signal-to-cutoff levels among all subjects. It is unclear if antibodies persist beyond that time, if those antibodies are specific for and neutralize WNV, and if antibody responses and persistence vary depending on age or sex.

We studied the characteristics of WNV antibody responses in two different groups of blood donors, one identified by a cross-sectional serosurvey and the second by a longitudinal follow-up of donors detected during acute viremia by blood donor screening for WNV RNA. We compared the antibody levels in donors with recent versus more remote infections and looked at differences according to age and sex. We also assessed the specificity and neutralizing capacities of the antibody responses.

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MATERIALS AND METHODS

This study was approved by the institutional review boards of the participating institutions, and all subjects agreed to participate and signed informed consents. Blood donors who were seropositive for WNV IgG antibodies were identified from a previously reported serosurvey of >4,500 North Dakota blood donors (2). In that study, 370 donors (8.2%) were IgG positive, and 28 of those (7.5%) were also IgM positive. The durability of the antibody responses was assessed by comparing IgG antibody levels among recently infected donors (those who were IgM seropositive) versus donors presumed to be infected >1 year prior (i.e., were IgM negative). The specificity and neutralizing capacity of the antibody response were assessed by assaying a subset (54 samples across the range of IgG response) of samples from seropositive donors using a WNV plaque reduction neutralization assay to quantify plaque reduction neutralization titers. These samples were selected by choosing every 6th sample from the lowest to highest titers across the IgG response spectrum from 324 samples with adequate volume remaining for plaque reduction neutralization testing (PRNT).

We also studied a group of 18 donors who were originally identified with acute WNV infection by screening for blood plasma RNA with nucleic acid amplification technology (NAT) in 2005 and who were enrolled in a 1-year longitudinal follow-up study (7). Their WNV IgG, IgM, and PRNT levels were assessed at 6 months and 5 years postinfection. These samples were tested in parallel and under code to minimize interrun variability and biases in assay performance and interpretation.

Blood plasma specimens were tested for WNV IgM and IgG by using Food and Drug Administration–approved enzyme-linked immunosorbent assay (ELISA) kits manufactured by Focus Diagnostics (8). In accordance with the kit inserts, an IgG signal-to-cutoff ratio (S/CO) of 1.5 and an IgM S/CO of 1.1 were considered positive. PRNT was performed at the CDC Arbovirus Diagnostic Laboratory in Fort Collins, CO, as pre-
viously described (9, 10). A titer of $\geq 1:10$ was considered positive. Each titer change represented a 2-fold dilution.

For the statistical analysis, a Pearson’s correlation coefficient (95% confidence interval) was used to measure the linear association between WNV IgG antibodies with log$_2$ PRNT/10. For those patients who were identified by NAT screening in 2005 as being infected with WNV, to evaluate whether the average measured WNV IgG antibody level dropped at 5 years postinfection from 6 months, a one-sided paired Student’s $t$ test and associated one-sided confidence interval (CI) were used. After transforming the PRNT values by dividing them by 10 and taking log$_2$, the mean-transformed PRNT levels were similarly analyzed. Finally, the IgG ELISA S/CO values were characterized as a function of age (in years) and sex using a linear model, allowing for an age-by-sex interaction to test whether any linear age association depended on the patient’s sex. Sex-specific estimates and CIs of IgG level as a function of age were computed adjusting for multiplicity using Bonferroni’s adjustment. The homogeneity of variance between the sexes was evaluated by fitting models with common and different variances by sex and comparing the fits using a likelihood ratio test. Standard residual diagnostics were employed to evaluate deviations from the linear model assumptions.

RESULTS

In the serosurvey donor group, there was no significant difference in the level of IgG production based on the presumption of more recent infection (those who were IgM positive) versus those with more remote infection (i.e., who were IgM negative); the mean ± standard deviation IgG levels were 4.72 ± 1.38 and 4.55 ± 1.46 S/CO units, respectively ($P = 0.54$). In the 18 recalled donors, IgG levels trended down somewhat over the 5-year time span, with a mean of paired differences (5 years to 6 months) of $-0.54$ S/CO units (95% CI, $-0.86$ to $-0.21$ S/CO units). Two subjects had a slight rise in their IgG levels and had a corresponding lack of change in their PRNT titers. The remaining subjects who had small decreases in their IgG levels over time showed no change in their PRNT titers in 4/16 subjects, a 1-fold decrease in 7/16 subjects, and a 2-fold decrease in the titer in 4/16 subjects. One subject had a 1-fold dilution increase in titer. Note that they all remained seropositive, and the majority of them had levels that were remarkably similar to their baseline levels (Fig. 1A). Twelve out of 18 (67%) subjects were IgM positive at 6 months, whereas none (0%) of the subjects were positive at 5 years (exact McNemar’s test, $P = 0.0004883$; p2-p1 [exact 95% CI], $-0.67$ [-0.87 to $-0.37$]).

PRNT of the WNV IgG-reactive donor samples from the serosurvey showed that 51 out of 54 subjects had neutralizing antibodies, with titers ranging from 1:2.5 to 1:2,560 (Fig. 2). Titers of $<1:10$ were not considered significant. There was a strong correlation between IgG level and the transformed PRNT titer (Pearson’s correlation, 0.81 [95% CI, 0.69 to 0.89]). All of the 18 recalled donors still had significant PRNT titers out to 5 years (Fig. 1B). Six of the 18 had the same titer as that at baseline, 1 subject had an increase in titer, and 7 subjects’ titers fell by only one dilution.

Using a linear model, there was a statistically significant difference in IgG level as a function of age between males and females ($F_{1,366} = 5.73; P = 0.017$) (see Fig. 3). Though not a strong effect, the decrease in IgG level for each 10-year age group for females was statistically significant (0.31 S/CO units; simultaneous 95% CI, 0.14 to 0.48 S/CO units), while for males, it was statistically nonsignificant (0.08 S/CO units; simultaneous 95% CI, $-0.05$ to 0.22 S/CO units). There was no indication that variances differed by sex ($P = 0.38$, likelihood ratio test), and all the residual analyses indicated no significant departures from the model assumptions.

DISCUSSION

Humoral immunity is a critical aspect of protection against flavivirus infection. It is necessary for the initial containment and clearance of infection, as well as for subsequent protection against reinfection (6, 11, 12). Eliciting a protective antibody response is a primary goal in the development of safe and effective vaccines (12). Our study suggests that infection with WNV elicits a strong and durable antibody response, with evidence of stable high-titer neutralizing antibodies even as far out as 5 years. All 18 recalled donors had significant levels of WNV-specific IgG antibodies as evidenced by ELA and PRNT at the 5-year assessment. Although there was a downward trend in some subjects by both assays for WNV antibodies compared with their 6-month postseroconversion values, the declines were remarkably small. None had evidence of persistent IgM antibodies at 5 years follow-up, which

In neither case did there appear to be what would likely be clinically significant decreases in antibody levels with advancing age.

FIG 1 IgG levels (A) and plaque reduction neutralizing antibody levels (B) in WNV-infected blood donors recalled at 6 months and 5 years postinfection.

FIG 2 Antibody response (WNV IgG ELISA level) and specificity (plaque reduction neutralization titer) in WNV-seropositive blood donors.
would be expected in the absence of reinfection. The durability of the antibody response was also indirectly suggested by the fact that in the seropositive donors identified in the cross-sectional serosurvey, there were no differences in IgG levels in the subjects who were IgM positive (presumptively because of more recent infection) and in those who were IgM negative.

There are several possible reasons for the persistence of antibody production. The most likely explanation is that WNV induces a strong humoral response that endures in the absence of ongoing viral infection or persistent antigenic stimulation. Other studies have shown a similar durability in antibody responses following flavivirus infection. Exposure to dengue virus in military personnel during World War II and in volunteers exposed experimentally to the virus led to detectable antibody levels in the majority of subjects from 35 to 60 years after infection (13, 14). Similarly, up to 97% of military personnel vaccinated with yellow fever vaccine during World War II still had neutralizing antibody titers up to 35 years after vaccination (15). However, the persistence of humoral responses may be due to reexposure to and/or reinfection with the West Nile virus or a closely related flavivirus. The lack of IgM in any of the recalled donors would go somewhat against the hypothesis of reexposure or reinfection to West Nile virus, although it is unclear if IgM is induced upon reexposure to virus in someone previously infected, and it is not known how long this would persist. Furthermore, the cumulative seroprevalence of WNV in a highly endemic state was estimated to be only 8.2% of the population over the entire epidemic (2). It is highly unlikely that the majority of the recalled donors faced repeat exposures or infections in the years following their initial infection. Another possible explanation for antibody persistence is persistent viral infection. This has been suggested by viral RNA detection in human whole blood out to 90 days postinfection, nucleic acids in tissues in a mouse model out to 6 months, and RNA detection in urine from previously infected humans out to 6 years (16–18). However, others have not been able to verify the latter finding (19).

Antibody response was not significantly associated with age or sex in our group of subjects. This is somewhat surprising in that both advancing age and male sex have been identified as significant risk factors for the development of neuroinvasive disease (2, 3). There are likely many other factors that are important in determining the immunologic and pathophysiologic risk factors for central nervous system invasion other than the formation or characteristics of IgG antibodies. Our study does not address the early immune response that is critical for the initial containment of the virus. It does, however, suggest that once infected, almost all persons will eventually develop significant antibody levels that are quite durable and potentially protective.

Our study is limited in that the serosurvey donor group was selected by having a positive antibody titer, so we are unable to assess if there were substantial numbers of donors who had been infected but never seroconverted. However, this is unlikely to be the case. A prior study of 245 blood donors with WNV viremia showed that all subsequently seroconverted with both IgM and IgG antibodies (7). Finally, our study was limited to blood donors and hence did not include subjects who developed clinical severe WNV disease manifestations. Further studies comparing humoral immune responses in persons with asymptomatic versus symptomatic infections are warranted.

Conclusions. WNV infection induces a significant humoral response that is stable over time. This suggests that persons who have been infected with the virus may well have long-lasting protection from reinfection. Furthermore, the development of vaccines that mimic natural infection may hopefully be expected to provide similar enduring immunity.

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The authors declare no conflicts of interest.

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