Characteristics of Antibody Responses in West Nile Virus Seropositive Blood Donors

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ABSTRACT

Background: West Nile virus (WNV) is now endemic in the United States. Protection against infection is thought to be conferred in part by humoral immunity. Understanding of the durability and specificity of the humoral response is not well established.

Methods: We studied the magnitude and specificity of antibody responses in 370 WNV seropositive blood donors. We also recalled 18 donors infected in 2005 to compare their antibody responses at 6-months following infection vs 5 yrs post-infection.

Results: 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 seropositive in 2005 remained seropositive at 5 yrs, and maintained neutralizing antibodies. IgG levels at 5 years post-infection showed fairly minimal decreases compared with the paired level at 6 months post-infection (mean of paired differences of -0.54 S/CO units [95% CI (-0.86, -0.21)]) and only minimal decreases in PRNT titers.

Conclusion: WNV induces a significant antibody response that remains present even 5 years out from infection.

Keywords
West Nile virus; Antibody; blood donor; humoral immunity, neutralization
Introduction

West Nile virus infection is now well established in the United States with an estimated 3 million infections in the 48 contiguous states through 2010. Since the start of the epidemic in 1999 through 2012, over 15,000 persons have developed neuroinvasive disease, characterized by meningoencephalitis, or acute flaccid paralysis and over 1500 deaths have occurred (CDC Arbonet). Advanced age, male sex, and immunosuppression significantly increase the risk for neuroinvasive disease.

The production of WNV-specific IgM and IgG antibodies is important for both diagnosis and clearance of WNV infection. Persistence of IgG antibodies is thought to confer protection from subsequent re-infection. In a study of 245 viremic blood donors, IgM antibodies persisted out to a mean of 156 days, and IgG antibodies persisted at the same titer at least out to 1 year post infection. IgM antibodies persisted in up to 17% of subjects at 400 days, whereas IgG antibodies were maintained at high levels based on EIA 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 longitudinal follow-up of donors detected during acute viremia by blood donor screening for WNV RNA. We compared antibody levels in donors with recent versus
more remote infection and looked at differences by age and sex. We also assessed the specificity and neutralizing capacity of the antibody responses.

Methods

The study was approved by the institutional review boards of the participating institutions and all subjects agreed to participate and signed informed consent. Blood donors who were seropositive for WNV IgG antibodies were identified from a previously reported serosurvey of over 4500 North Dakota blood donors. In that study, 370 donors (8.2%) were IgG positive and 28 of those (7.5%) were also IgM positive.

Durability of antibody response 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 (IgM negative). Specificity and neutralizing capacity of the antibody response was assessed by assaying a subset (54 samples across the range of IgG response) of seropositive donors’ samples using a WNV plaque reduction neutralization assay to quantify plaque reduction neutralization titers (PRNT). These samples were selected by choosing every 6th sample from lowest to highest titer across the IgG response spectrum from 324 samples with adequate remaining volume for PRNT testing.

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

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 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 CDC Arbovirus Laboratory in Ft. Collins, CO as previously described.(9)(10) A titer of > 1:10 was considered positive. Each titer change represented a two-fold dilution.

For the statistical analysis, Pearson’s correlation coefficient (95% confidence interval) was used to measure the (linear) association between WNV IgG antibodies with base-2 logarithm-transformed (log2) PRNT / 10. For those patients who were identified as WNV-infected using NAT screening in 2005, to evaluate whether the average measured WNV IgG antibody level dropped at 5 years post-infection from 6 months, the 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 log2, mean transformed PRNT levels were similarly analyzed. Finally, IgG ELISA S/CO values were characterized as a function of age (years) and sex using a linear model, allowing for an age-by-sex interaction to test whether any linear age association depended on sex. Sex-specific estimates and CIs of IgG level as a function of age were computed adjusting for multiplicity using the Bonferroni adjustment. 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) vs those with more remote infection (IgM negative); mean +/- standard
deviation IgG level was 4.72 +/- 1.38 vs 4.55 +/-1.46 S/CO units, 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 – 6 months) of -0.54 S/CO units [95% CI (-0.86, -
0.21)]. Two subjects had a slight rise in their IgG level, and had a corresponding no
change in their PRNT titer. The remaining subjects who had small decreases in their
IgG levels over time showed no change in PRNT titer in 4/16 subjects, a 1-fold
decrease in 7/16 subjects, and a 2-fold decrease in titer in 4/16 subjects. One subject
had a 1-fold dilution increase in titer. Note that all remained seropositive, and the
majority were remarkably similar to their baseline levels (figure 1, panel A). Twelve out
of 18 (67%) were IgM positive at 6 mos where none (0%) were positive at 5 years.
[exact McNemar’s test, p = 0.0004883; p2-p1 (exact 95% CI) = -0.67 (-0.87, -0.37)].

PRNT testing 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 1:2560 (figure 2). Titers less than 1:10 were not considered significant. There
was a strong correlation between the IgG level and the transformed PRNT titer
[Pearson’s correlation 0.81 (95% CI 0.69, 0.89)]. All of the 18 recalled donors still had
significant PRNT titers out to 5 years (figure 1, panel B). Six of the 18 had the same
titer as at baseline, 1 subject had an increase in titer, and 7 subjects 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 Figure 3. Though not a strong effect, the decrease in IgG level for each 10 year age strata for females was statistically significant [0.31 S/CO units, simultaneous 95% CI (0.14, 0.48)], while for males it was statistically non-significant [0.08 S/CO units, simultaneous 95% CI (-0.05, 0.22)]. There was no indication that variances differed by sex (likelihood ratio test p = 0.38), and all residual analyses indicated no significant departure from model assumptions. In neither case, therefore, did there appear to be what would likely be clinically significant decreases in antibody levels with advancing age.

Discussion

Humoral immunity is a critical aspect of protection against flavivirus infection. It is necessary for initial containment and clearance of infection, as well as subsequent protection against re-infection.\(^{(6)}\)(\(^{(11)}\)\(^{(12)}\)) 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 out to 5 years. All 18 recalled donors had significant levels of WNV-specific IgG antibodies as evidenced by EIA 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 post-seroconversion values, the declines were remarkably small. None had evidence of persistent IgM antibodies at
five years of follow-up which would be expected in the absence of re-infection.

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 more recent infection) compared to those who were IgM negative.

There are several possible reasons for persistence of antibody production. The most likely explanation is that WNV induces a strong humoral response that is enduring in the absence of ongoing viral infection or persistent antigenic stimulation. Other studies have shown similar durability of 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 35-60 years after infection. 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. However persistence of humoral responses may be due to re-exposure to and/or re-infection 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 re-exposure or re-infection to West Nile virus, although it is unclear if IgM is induced upon re-exposure to virus in someone previously infected, and 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. It is highly unlikely that the majority of the recalled donors faced repeat exposures or infections in the years following their initial infection. Another possibility to explain antibody persistence is persistent viral infection. This has been
suggested by viral RNA detection in human whole blood out to 90 days post-infection, 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. However, others have not been able to verify the latter finding.

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. There are likely many other factors that are important in determining the immunologic and pathophysiologic risk factors for central nervous system invasion other than 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. 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 vs 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 re-infection. Furthermore, the development of vaccines that mimic natural infection may hopefully be expected to provide similar enduring immunity.

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FIGURE LEGENDS

Figure 1. IgG levels (Panel A) and plaque reduction neutralizing antibody levels (Panel B) in WNV infected blood donors recalled at 6 mos and 5 years post-infection.

Figure 2. Antibody response (WNV IgG ELISA level) and specificity (plaque reduction neutralization titer) in WNV seropositive blood donors.

Figure 3. WNV antibody levels by age and sex. Females – Panel A, Males – Panel B.


**FOOTNOTES:**

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