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Short communication

Persistence of antibodies to West Nile virus nonstructural protein 5

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Abstract

Background: Because IgM antibody against West Nile virus (WNV) pre-membrane/envelope (preM/E) recombinant protein may persist for more than 1 year, an assay distinguishing recent from past WNV infection would be useful. Published findings for a single patient suggest that the presence of antibody against WNV nonstructural protein 5 (NS5) indicates recent infection.
Objectives: To compare the persistence of WNV NS5 antibodies and preM/E IgM using plasma samples from blood donors who were viremic at the time of donation.
Study design: Follow-up plasma samples from 35 viremic donors were tested for WNV NS5 antibodies using a microsphere immunoassay, and compared to WNV preM/E IgM antibodies determined on the same samples using an enzyme-linked immunosorbent assay (ELISA).
Results: At 90 ± 14 days of follow-up, 20/26 donors (77%) were positive for NS5 antibodies; 6/25 (24%) were positive at 180 ± 27 days, and 3/23 (13%) were positive at 365 ± 55 days. The comparable values for preM/E IgM antibodies were 77%, 32% and 17%, respectively.
Conclusion: Persistence of WNV NS5 antibody in plasma is similar to that of preM/E IgM antibody. WNV NS5 antibody cannot be used to distinguish recent from past WNV infection.

Keywords: West Nile virus; PreM/E IgM; NS5 antibodies; Persistence

1. Introduction

The major laboratory tool used in the diagnosis of recent West Nile virus (WNV) infection is measurement of WNV-specific IgM (Petersen and Marfin, 2002; Sampathkumar, 2003). Most WNV IgM assays utilize recombinant pre-membrane/envelope (preM/E) protein (Davis et al., 2001). These assays exhibit excellent sensitivity for identifying individuals recently exposed to WNV (Hogrefe et al., 2004; Muerhoff et al., 2004; Holmes et al., 2005; Tilley et al., 2005; Rawlins et al., 2007).

A limitation to the diagnostic utility of WNV preM/E IgM antibody for recent WNV exposure is its persistence, albeit usually at low levels, for over a year in some individuals (Roehrig et al., 2003; Prince et al., 2007). These findings have led to a search for a serologic assay to distinguish recent (current season) from past (prior season) WNV infection. One potential assay is measurement of total antibodies to WNV nonstructural protein 5 (NS5), a replication phase protein with enzymatic activity (Azzi and Lin, 2007). Wong et al. (2003) utilized a microsphere immunoassay to show that NS5 antibodies were absent from two sera collected >77 days after symptom onset from one WNV-infected patient.

In an effort to confirm the findings of Wong et al. (2003), we developed an NS5 antibody fluorescent microsphere immunoassay similar to that of Wong et al. (2003) to measure NS5 antibodies in follow-up plasma specimens from blood donors who had WNV viremia (by PCR) (Prince et

Abbreviations: WNV, West Nile virus; NS5, nonstructural protein 5; preM/E, pre-membrane/envelope protein; ELISA, enzyme-linked immunosorbent assay; MFI, median fluorescent intensity; Ig, immunoglobulin; NAT, nucleic acid amplification test.

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al., 2007). We then compared the persistence of NS5 antibodies to the persistence of preM/E IgM antibodies in these specimens.

2. Materials and methods

2.1. Specimens

Twenty WNV preM/E IgM-negative sera from healthy employees of a California laboratory (control serum panel) were used to establish WNV NS5 antibody assay conditions and define the parameters for expressing results. To define the upper threshold for a negative value using plasma, 72 preM/E IgM-negative plasma samples collected in 2004 from Los Angeles-area blood donors were tested for WNV NS5 antibodies.

WNV RNA-positive blood donors ($N = 35$) were identified by nucleic acid amplification test (NAT) screening of donations made June–November 2005 (Busch et al., 2005). Approximately one-third of donors were from the West/Southwest (CA, NV, AZ, NM), one-third were from the Upper Midwest (ND, SD, MN), and one-third were from South Central states (AR, MS, TX). Follow-up plasma specimens ($N = 474$) were collected 5–404 days after the viremic (index) donation; 7–16 specimens were collected per donor. Informed consent was obtained from all donors at the local blood donation site; protocols for NAT screening and follow-up were approved by the University of California, San Francisco's Committee on Human Research, and the Food and Drug Administration (FDA).

2.2. PreM/E ELISA

Viremic donor follow-up plasmas were tested for WNV preM/E IgM antibody using an ELISA kit (Focus Diagnostics, Cypress, CA); a sample-to-cutoff ratio (SCR) > 1.1 was considered positive (Prince et al., 2007). This kit is FDA cleared for serum only; in-house studies verified plasma as an acceptable specimen (Prince and Hogrefe, 2005).

2.3. NS5 antibody assay

WNV NS5 total antibodies were measured using a fluorescent microsphere immunoassay patterned after the assay described by Wong et al. (2003) Recombinant NS5 (New York State Department of Health, Albany, NY) was covalently linked to 6.25×10^6 carboxylated microspheres (Luminex Corporation, Austin, TX) using a two-step carbodiimide reaction. Filter-bottom microplates (Millipore, Bedford, MA) were pre-wet with phosphate-buffered saline containing 0.05% Tween 20 (PBST). Following vacuum filtration, NS5-coupled microspheres (2500 microspheres in 50 μL phosphate-buffered saline containing 1% BSA and 0.1% Proclin 950 [PBP buffer]) were added to wells, followed by 50 μL of serum or plasma diluted 1:50 in PBP buffer. The microplate was incubated 20 min on a shaker at room temperature (RT), followed by three washes with PBST. Wells then received 50 μL of polyvalent phycoerythrin-conjugated goat anti-human immunoglobulins (IgG, IgA, and IgM) (Biosource International, Camarillo, CA) diluted 1:400 in PBST. After incubation for 20 min at RT on a plate shaker and three washes with PBST, microspheres were suspended in 125 μL PBST and analyzed using a Luminex L100 instrument (Luminex Corporation). The median fluorescent intensity (MFI) of 100 microspheres was quantified for each well. Each assay run included the control serum panel, and the cutoff MFI for a given run was defined as the control serum panel average MFI plus three standard deviations. The cutoff MFI was then used to calculate the SCR for each specimen using the formula: SCR = specimen MFI/cutoff MFI. All assay runs included quality control (QC) samples designed to yield low-negative (QC1), high-negative (QC2), or positive (QC3) NS5 antibody results.

2.4. Follow-up time windows

Five follow-up (days post-index) time windows were selected to assess the proportions of viremic blood donors positive for NS5 and preM/E IgM antibodies over time; these windows represent a targeted number ±15% of follow-up days. The five windows were thus 30 ± 4, 60 ± 9, 90 ± 14, 180 ± 27, and 365 ± 55 days. To avoid bias due to multiple specimens from some donors, results from only one specimen per donor (if available) were included in each time window; if multiple results were available for a given donor within a given time window, the result for the sample collected closest to the window target was used.

3. Results

3.1. NS5 antibody assay development

As outlined by Wong et al. (2003) the average MFI value plus three standard deviations for a control serum panel was used as the basis for interpreting NS5 antibody results. Fig. 1 presents the SCR results for 20 control sera and 72 plasma specimens from Los Angeles blood donors without WNV infection. The distribution of SCR values in the Los Angeles blood donor plasma panel was similar to that of the control serum panel; 71 of 72 (99%) plasma specimens exhibited SCR values <1.1. A plasma SCR value <1.1 was thus defined as negative for NS5 antibodies and ≥1.1 was defined as positive.

Performance characteristics for the NS5 antibody assay are shown in Table 1. Intra-assay and inter-assay variation for QC2 and QC3 were <10%; the larger variations observed for QC1 reflected the very low SCR values obtained for this specimen.