Short Report: Failure of Japanese Encephalitis Vaccine and Infection in Inducing Neutralizing Antibodies against West Nile Virus, People’s Republic of China

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Abstract. We examined whether live attenuated Japanese encephalitis (JE) vaccine is effective in preventing West Nile virus (WNV) infection in the People’s Republic of China. Three groups were recruited into the study: patients with Japanese encephalitis (JE), healthy controls vaccinated with live attenuated 2 SA14-14 vaccine against JE virus (JEV), and unvaccinated healthy controls. Serum samples were collected and screened for IgG antibodies against JEV by an indirect immunofluorescence assay. Positive samples were then analyzed for levels of antibodies against JEV and neutralizing antibodies against West Nile virus (WNV) by a plaque-reduction neutralization test (PRNT). Although most persons had medium to high levels of JEV-reactive IgG and neutralizing antibodies, only 2 of the 82 unvaccinated control samples were positive for the WNV-reactive antibodies. These findings suggest that previous JEV infection or vaccination did not induce adequate levels of WNV-reactive antibodies in the population studied. However, how these persons would respond to a secondary flavivirus infection and whether their prior experience with wild-type or attenuated JE vaccine will provide some cross-protection against WNV disease still warrants further investigation.

The appearance of West Nile virus (WNV) in North America, which resulted in human and equine cases of encephalitis, brought worldwide attention to this serious public health issue. Strategies to control the vectors or reduce disease in vertebrate hosts are difficult to implement, and vaccination against WNV remains a desired goal. West Nile virus and Japanese encephalitis virus (JEV) are both included in the JEV serocomplex of the genus Flavivirus, which belongs to the family Flaviviridae. Cross-protection between WNV and JEV had been demonstrated in multiple animal models. Thus, it was suggested that JE vaccines might protect humans from WNV infection, especially in countries where inactivated and live-attenuated JE vaccine was widely used. However, only limited studies have addressed whether immune responses to JE vaccines in humans are cross-reactive with WNV.

Several studies have been published in attempts to address this issue. One study tested whether immune responses to JE vaccines in humans are cross-reactive with WNV. The conclusions of this study indicated that protective levels of neutralizing antibodies to WNV were not detected after vaccination against JEV. In the absence of challenge experiments, additional studies of immune responses to commercially available flavivirus vaccines in humans are needed to clarify the issue of cross-reactivity. In contrast, another study found low but consistently detectable levels of neutralizing antibodies to WNV in sera of all immunized donors and those given booster immunizations. It was suggested in these two studies that such vaccination might limit disease severity and progression. In a study that involved serosurvey analysis after a WNV outbreak, investigators concluded that previous flaviviral vaccination does not markedly affect the development of WNV fever and that the IgM antibody response in patients without neuroinvasive WNV disease is transient. Discrepancies in the results of these studies might be caused by different types of vaccines used (killed versus live attenuated), differences in plaque-reduction neutralization test (PRNT) methods, or timing of serum collections after vaccination. Because all of these studies were fairly small, differences in individual responses to flavivirus or to prior exposures to flaviviruses might contribute to the different results. A study involving a larger and more diversified cohort might provide a better answer to this issue.

Although it is still not clear whether there is circulating WNV in China, JE has spread throughout the country and vaccine for this disease has been administered widely. We were interested in knowing whether immunity to JEV would induce protective titers of WNV-reactive neutralizing antibodies.

From August through September 2006, we conducted a seroprevalence study in Shanxi Province, an area in China endemic for JEV infection that recently had a new outbreak of JE in July 2006. We recruited three groups into the study. The first group was composed of 11 JE patients (mean ± SD age = 62.36 ± 16.64 years) who were newly diagnosed in hospitals in Shanxi Province during the July JE outbreak. The interval from disease onset to sample collection in these patients ranged from 5 to 30 days, and all patients had been previously confirmed as infected with JE by detection of JEV-reactive IgM by an indirect immunofluorescence assay (IFA). None of these patients had ever received JE vaccine prior to this infection. The second group was composed of 92 vaccinated healthy controls (mean ± SD age = 34.37 ± 14.85 years) who were vaccinated with 0.5 mL of SA14-2-2 JE vaccine (Chengdu Institute of Biological Products, Chengdu, Peoples’ Republic of China) by subcutaneous injection three weeks before the investigation. The third group was composed of 287 unvaccinated controls (mean ± SD age = 35.54 ± 16.47 years) from the same hospitals who had never been vaccinated against JEV or any other flaviviruses. Demographic data and health history information were obtained from all participants through self-administered questionnaires and hospital admission records. In Shanxi Province, no other flavivirus infections had been reported other than JEV. Therefore, the participants were asked only to recall any symptoms of infection with JEV.
Detection of antibodies against Japanese encephalitis virus (JEV) and West Nile virus (WNV) in patients with Japanese encephalitis, vaccinated controls, and unvaccinated controls, People’s Republic of China*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>JE patients (n = 11)</th>
<th>Vaccinated controls (n = 92)</th>
<th>Unvaccinated controls (n = 287)</th>
<th>Negative controls (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JEV-reactive IgG No. positive/no. tested (%)</td>
<td>10/11 (90.91)</td>
<td>85/92 (92.39)</td>
<td>82/287 (28.57)</td>
<td>ND</td>
</tr>
<tr>
<td>GMT of positive samples</td>
<td>1:65.63</td>
<td>1:29.68</td>
<td>1:26.09</td>
<td>ND</td>
</tr>
<tr>
<td>Titer range of positive samples</td>
<td>1:20–1:320</td>
<td>1:20–1:320</td>
<td>1:20–1:1280</td>
<td>ND</td>
</tr>
<tr>
<td>JEV-reactive antibodies No. positive/no. tested (%)</td>
<td>10/10 (100)</td>
<td>85/85 (100)</td>
<td>82/82 (100)</td>
<td>0/100 (0)</td>
</tr>
<tr>
<td>GMT of positive samples</td>
<td>1:65.00</td>
<td>1:59.51</td>
<td>1:57.33</td>
<td>&lt;1:10</td>
</tr>
<tr>
<td>Titer range</td>
<td>1:15–1:320</td>
<td>1:14–1:320</td>
<td>1:10–1:360</td>
<td>NA</td>
</tr>
<tr>
<td>WNV-reactive antibodies No. positive/no. tested (%)</td>
<td>0/10 (0)</td>
<td>0/85 (0)</td>
<td>2/82 (2.44)</td>
<td>0/100 (0)</td>
</tr>
<tr>
<td>GMT of positive samples</td>
<td>&lt;1:10</td>
<td>&lt;1:10</td>
<td>1:61.71</td>
<td>&lt;1:10</td>
</tr>
<tr>
<td>Titer range</td>
<td>NA</td>
<td>NA</td>
<td>1:34–1:112</td>
<td>NA</td>
</tr>
</tbody>
</table>

* GMT = geometric mean titer; ND = not detectable; NA = not applicable.

After informed consent was obtained, serum samples were obtained from each participant for detection of JEV-reactive IgG and JEV/WNV-reactive neutralizing antibodies. Briefly, serial two-fold dilutions (1:10–1:1,280) of samples were subjected to an IFA and a PRNT. Briefly, air-dried, acetone-fixed slides coated with JEV (Beijing-1 strain)–infected C6/36 cells were used as sources of antigens in the IFA. Non-infected C6/36 cells were used as controls. Serially diluted samples were placed on the coated wells and incubated in a moist chamber for 40 minutes at 37°C. Slides were washed in phosphate-buffered saline (PBS) for 10 minutes and rinsed with deionized water. A fluorescein isothiocyanate–conjugated rabbit anti-human IgG antibody was then applied to the slides and incubated for 30 minutes. The slides were washed in PBS for 10 minutes. Results were determined on the basis of cytoplasmic fluorescence observed for each sample. A positive control of an in-house human reference serum was included in every slide. Sera were considered positive for JEV-reactive antibodies when specific green fluorescence was located in the cytoplasm or on the plasma membrane in approximately 25% of the cells, and if no fluorescence staining was observed in uninfected cells. The IgG titer was defined as the highest serum dilution that showed positive results.

Levels of neutralizing antibodies to JEV and WNV were measured by PRNT. Briefly, 0.1 mL of diluted virus stock, which contained 100 plaque-forming units of WNV (Chin-01 strain, GenBank accession no. AY490240) or JEV (Beijing-1 strain), was added to each serum dilution. The serum–virus mixture was placed on assay plates containing confluent layers of Vero cells and plates were incubated at 37°C for 90 minutes in an atmosphere of 5% CO₂. Overlay medium containing 1% methylcellulose was added and the plates were incubated at 35°C for 6 days in an atmosphere of 5% CO₂. Neutralization titer was defined as the highest serum dilution that reduced numbers of plaques by 50%.

To exclude false-positive results in the IFA and PRNT, we also tested 100 serum samples from healthy residents in a non-endemic region (Xinjiang Autonomous Region) who were never vaccinated against JE. The validity of the assay was confirmed after no positive results were obtained with samples from these donors (Table 1).

Positive JEV-reactive IgG antibody responses were detected in 10 (90.91%) of 11 patients with JE, 85 (92.39%) of 92 vaccinated controls, and 82 (28.57%) of 287 unvaccinated controls. Geometric mean titers (GMTs) were calculated for participants with positive results in each of the three groups. The GMTs for JEV-reactive IgG antibodies were 1:65.63 for JE patients, which was significantly higher than titers in vaccinated controls (1:29.68) and unvaccinated controls (1:26.09).

The IgG-positive samples were then subjected to a JEV-reactive PRNT, which showed that all donors had JEV-neutralizing antibodies with GMTs of 1:65.00, 1:59.51, and 1:57.33 in patients, vaccinated controls, and unvaccinated controls, respectively. Neutralizing antibody titers in the two control groups were higher than IgG antibody titers. Conversely, positive neutralizing antibody titers (>1:10) to WNV were detected only in two of the unvaccinated controls (titers = 1:34 and 1:112, respectively). These two donors also showed much higher JEV-reactive neutralizing antibody titers (1:360 and 1:224, respectively). However, on the basis of information obtained from the questionnaire, we could not deduce previous JE in these two persons. Because WNV infection has not been detected in Shanxi Province, we suspect that the positive neutralizing antibody results may have been caused by cross-protection from other flavivirus infections, rather than WNV infection. However, the likelihood that these persons were previously exposed to flaviviruses other than WNV and JEV (e.g., dengue) is unclear because no infections with flaviviruses other than JEV have been reported in Shanxi Province.

There were no statistically significant differences between the two control groups with respect to GMTs of JEV-reactive IgG and neutralizing antibodies, which indicates the effectiveness of sub-clinical infection of JEV in inducing protection against the disease, in comparison with the vaccination group. These results are not surprising because Shanxi Province is one of areas in China endemic for JE, and a high proportion of the population is naturally immune to JEV or other flavivirus infections. The negative neutralizing response in the three tested populations in our study suggests that effective JEV vaccination or clinical and sub-clinical infections did not induce neutralizing antibody responses against WNV. However, it is not known how the persons in our study would respond to a secondary flavivirus infection, and whether their prior experience with wild-type or attenuated JEV would provide cross-protection against WNV clinical disease.

Limited protection by heterologous immunity had been demonstrated in previous studies. Vaccination against JE can induce cross-protective immunity against dengue virus infection, but this cross-protective immunity is short-lived and lasts no longer than six months after vaccination with a killed vac-
cine. Heterologous neutralizing antibody against dengue is not protective but may modify disease severity after infections with other dengue virus serotypes. One study showed no WNV neutralizing antibodies after vaccination against JEV. However, in the absence of challenge experiments, our study could not rule out the possibility that pre-existing immunity to heterologous flaviviruses might protect against severe disease.

It is noteworthy that in the vaccinated control group the JEV-reactive IgG-positive rate of 92.39% and the IgG GMT of 1:29.68 after vaccination were lower than expected. We believe that the difference might be caused by population heterogeneity, vaccine application method, or timing of serum collection after vaccination. Other possibilities, such as muted response in persons with a high pre-vaccination serologic response because natural immunity to JE virus, should also be considered. Levels of JEV-reactive neutralizing antibodies in these patients were low and similar to those in the previously vaccinated cohort. This finding might be caused by the relative older age of these patients than those in the two control groups. Only one patient was IgG negative, which resulted in a lower positive rate of 90.91% in the patient group. This patient had a serum sample collected five days after JE infection, which is a reasonable time point for antibody detection because most patients would have detectable IgG for 7 to 20 days. However, we could not rule out the possibility that this interval was not long enough to detect IgG antibody in this patient.

In summary, our study failed to detect any protective neutralizing antibodies to WNV after vaccination or infection with JEV. Additional studies in animal models are required to demonstrate whether immunization with heterologous vaccines or pre-infection with heterologous flaviviruses have protective efficacy against WNV.

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