1. REPORT DATE: 11 MAR 2009
2. REPORT TYPE: N/A
3. DATES COVERED: -

4. TITLE AND SUBTITLE
Immune interference after sequential alphavirus vaccine vaccination.
Vaccine 27:4679-4882

5a. CONTRACT NUMBER: -
5b. GRANT NUMBER: -
5c. PROGRAM ELEMENT NUMBER: -
5d. PROJECT NUMBER: -
5e. TASK NUMBER: -
5f. WORK UNIT NUMBER: -

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8. PERFORMING ORGANIZATION REPORT NUMBER
TR-08-086

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) -

10. SPONSOR/MONITOR’S ACRONYM(S) -

11. SPONSOR/MONITOR’S REPORT NUMBER(S) -

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
The original document contains color images.

14. ABSTRACT
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15. SUBJECT TERMS
Venezuelan equine encephalitis virus, VEE, vaccines, alphavirus, antibody responses, human studies

16. SECURITY CLASSIFICATION OF:
a. REPORT: unclassified
b. ABSTRACT: unclassified
c. THIS PAGE: unclassified

17. LIMITATION OF ABSTRACT: SAR
18. NUMBER OF PAGES: 5
19a. NAME OF RESPONSIBLE PERSON -

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18
Short communication

Immune interference after sequential alphavirus vaccine vaccinations

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A R T I C L E   I N F O

Article history:
Received 21 November 2008
Received in revised form 23 February 2009
Accepted 24 February 2009
Available online 11 March 2009

Keywords:
Alphaivirus
Eastern equine encephalitis
Western equine encephalitis
Venezuelan equine encephalitis

A B S T R A C T

We compared the effect of order of administration of investigational alphavirus vaccines on neutralizing antibody response. Volunteers who received the inactivated eastern and western equine encephalitides (EEE and WEE) vaccines before live attenuated Venezuelan (VEE) vaccine had significantly lower rates of antibody response than those receiving VEE vaccine before EEE and WEE vaccines (66.7% vs. 80.6%; \( p = 0.026 \)). The odds of having a VEE antibody non-response among those initially receiving EEE and WEE vaccines, adjusted for gender, were significant (odds ratio [OR] = 2.20; 95% CI = 1.2–4.1 [\( p = 0.0145 \)]) as were the odds of non-response among females adjusted for group (OR = 1.81; 95% CI = 1.2–2.7 [\( p = 0.0037 \)]). Antibody interference and gender effect have major implications for vaccine strategy among those receiving multiple alphavirus vaccines and those developing next generation vaccines for these threats.

1. Introduction

Venezuelan equine encephalitis (VEE), eastern equine encephalitis (EEE), and western equine encephalitis (WEE) viruses are important causes of morbidity and mortality among humans and equids in the Western hemisphere [1]. All are caused by RNA viruses of the Alphavirus genus within the family Togaviridae [2,3]. Although the three diseases are acquired in nature after the bite of infected mosquitoes, the efficiency with which they can be transmitted via the aerosol route also makes them attractive candidates for use as biological weapons by adversary governments and/or terrorists [4–9].

For veterinary use, there are live, attenuated and inactivated VEE vaccines as well as inactivated vaccines for EEE and WEE [10]. However, there are no licensed EEE, WEE, or VEE vaccines marketed for use in humans. For more than 25 years, investigational vaccines against these agents have been administered at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) to laboratory workers and others at occupational risk for acquiring these infections. In 1973, Calisher et al. [11] demonstrated the phenomenon of vaccine interference in Texas horses receiving alphavirus vaccinations. McClain et al. [12] discovered immunologic suppression when live attenuated VEE and chikungunya (CHIK) virus vaccines were administered sequentially. In this report, we show that suppression of neutralizing antibody response to the live attenuated VEE vaccine can occur among volunteers who were previously vaccinated with eastern equine encephalitis and western equine encephalitis (EW) vaccines.

2. Methods

2.1. Volunteers and experimental design

Sources and preparation procedures of VEE, WEE, and EEE vaccines were described previously [13–16]. The VEE vaccine TC-83, attenuated, NDBR-12, Lot 4, was prepared at the National Drug Company in 1971. The virus was propagated in primary fetal guinea pig heart tissue culture maintained under Eagle’s basal medium (BME) containing 50 \( \mu \)g mL\(^{-1} \) each of neomycin and streptomycin and supplemented with 0.5% human serum albumin, U.S.P. The lyophilized vaccine is the filtered supernatant fluid harvested from cultures ca 30 h after infection and diluted to ca \( 10^4 \) p.f.u. dose\(^{-1} \). The vaccine is stored at ca 20 °C. The lyophilized vaccine is reconstituted with sterile water for injection, U.S.P. and is administered by inoculating 0.5 mL of the vaccine subcutaneously.

The WEE vaccine, TSI-GSD-210 (lot 1–81), is a lyophilized product originating from the supernatant harvested from primary chicken fibroblast cell cultures. The vaccine was prepared from specific pathogen-free eggs infected with the attenuated CM4884 strain of WEE virus. The supernatant was harvested and filtered, and the virus was inactivated with formalin. The residual formalin was neutralized by sodium bisulfite and the supernatant was...
The EEE vaccine, TSI-GSD-104 (lot 2-1-89), is a lyophilized product originating from the supernatant harvested from primary chicken embryo cell cultures. The vaccine was prepared from specific pathogen-free eggs infected with the attenuated PE-6 strain of EEE virus. The supernatant was harvested and filtered, and the virus was inactivated with formalin. The residual formalin was neutralized by sodium bisulfite and the supernatant was lyophilized. The final product was manufactured at the Salk Institute, Government Services Division, Swiftwater, PA. The lyophilized vaccine was stored at −20 °C, reconstituted with 5 mL of sterile water for subcutaneous injection and used within 2 h of reconstitution. The primary vaccination series consists of 3 subcutaneous injections (0.5 mL) on days 0, 7, and 28.

The study population consisted of 766 human volunteers in the Special Immunizations Program (SIP) at USAMRIID who received a single primary vaccination of an investigational live attenuated VEE vaccine (TC-83) [17] and priming doses of both inactivated investigational EEE and WEE vaccines in different sequences between 1 January 1976 and 30 April 1997. Written informed consent was obtained from each volunteer before receipt of any investigational vaccine.

For each vaccinee, dates of VEE, EEE, and WEE vaccinations were recorded in the SIP database. The chronological relationships between the EEE and WEE vaccination dates, and the VEE primary vaccination date, were used as criteria to allocate individuals into two groups. In group A, VEE vaccine was administered first, followed by EEE and WEE vaccines. In group B, EEE and WEE vaccines were administered first, followed by VEE vaccination.

2.2. Serology

The neutralizing antibody titers to VEE vaccine were determined for both groups between 14 and 98 days after receipt of VEE vaccine, before any subsequent EEE and WEE vaccines in group A. Antibodies were measured against Trinidad strain VEE virus, using a constant-virus, serum dilution technique as previously described [17]. A plaque reduction neutralization titer 80% (PRNT_{80}) ≥ 1:20 was considered positive for seroconversion.
Table 1
Overall VEE neutralizing antibody response rates of 766 volunteers who were administered VEE vaccine only (Group A) or VEE vaccine after EEE and WEE vaccines (Group B).

<table>
<thead>
<tr>
<th>Group</th>
<th>Total vaccines (N)</th>
<th>Non-responders</th>
<th>Responders</th>
<th>GMT of responders (95% CL)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N) (% )</td>
<td>Non-response (n) (% )</td>
<td>Response (n) (%)</td>
<td>Non-response (n) (% )</td>
</tr>
<tr>
<td>A⁴</td>
<td>718</td>
<td>139 (19.4)</td>
<td>579 (80.6)</td>
<td>157 (141–174)</td>
</tr>
<tr>
<td>B⁵</td>
<td>48</td>
<td>16 (33.3)</td>
<td>32 (66.7)</td>
<td>150 (104–215)</td>
</tr>
<tr>
<td>p-Value</td>
<td>(p = 0.026)</td>
<td></td>
<td></td>
<td>(p = 0.845)</td>
</tr>
</tbody>
</table>

² CL = Confidence limits.

4. Discussion
These data indicate that vaccinating humans against EEE and WEE with inactivated vaccines may interfere with subsequent neutralizing antibody response to the live attenuated VEE TC-83 vaccine. This interference was manifested by the diminished ability to elicit an adequate antibody response of at least 1:20.

The phenomenon of alphavirus vaccine-induced interference was first observed in horses as a result of previous inoculations of EEE and WEE vaccines [11]. Similar immune suppression occurred in humans when two live alphavirus vaccines were administered sequentially in a study by McClain et al. [12]. In that study, a live attenuated CHIK vaccine was administered after inoculation of live attenuated VEE vaccine, TC-83, in humans or vice versa. Significant interference with the ability to elicit a neutralizing antibody response to the second vaccine was observed regardless of which vaccine was administrated first. The present study appears to be the first report of alphavirus vaccine interference, involving VEE, EEE, and WEE vaccines in humans. The exact mechanisms responsible for this interference are unknown. When we analyzed individuals who received VEE before EEE or WEE inactivated vaccines, we did not observe interference (data not shown). Several animal stud-

Table 2
VEE neutralizing antibody response rates by group, stratified by gender (N = 766).

<table>
<thead>
<tr>
<th>Gender</th>
<th>Group A</th>
<th>Total vaccinees (n) (%)</th>
<th>Non-response (n) (%)</th>
<th>Response (n) (%)</th>
<th>GMT of responders (95% CL)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>157 (22)</td>
<td>44 (28)</td>
<td>113 (72)</td>
<td>119 (95–150)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>561 (78)</td>
<td>95 (17)</td>
<td>466 (83)</td>
<td>168 (149–188)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>718 (100)</td>
<td>(p = 0.003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>7 (15)</td>
<td>2 (29)</td>
<td>5 (71)</td>
<td>184 (49–686)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>41 (85)</td>
<td>14 (34)</td>
<td>27 (66)</td>
<td>144 (100–209)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>48 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

² CL = Confidence limits.

3. Results

3.1. VEE neutralizing antibody response
The distribution of VEE neutralizing antibody titers expressed as the proportion of vaccinees in groups A and B is illustrated in Fig. 1a. When VEE vaccine was administered first, the antibody response rate was 80.6% (Table 1). In contrast, the antibody response rate was only 66.7% when subjects received EW vaccines before VEE (p = 0.026). There were no group differences in GMT against VEE among responders, regardless of group (p = 0.845).

3.2. Neutralizing antibody response rates: effects of age, gender, race, and vaccination sequence
Because this was an observational study and not a randomized trial, it was necessary to examine demographic factors as possible confounders with group membership. Fig. 1b and c show the distribution of VEE neutralizing antibody titers by group and gender. VEE neutralizing antibody response rates and GMTs are stratified by group and by gender in Table 2. For group A, the antibody response rate for males was 83%; females 72% (p = 0.003). Responder GMTs in group A also were lower in females than males (p = 0.01). Sample size for females (N = 7) was insufficient in group B for statistical gender comparisons. These results from group A imply that analysis of response to VEE TC-83 must account for gender before comparing groups.

By logistic regression analysis, gender and prior vaccination with EEE were found to be jointly significant predictors of antibody non-response (p = 0.0037 and 0.0145, respectively) (Table 3). There was no statistical evidence of differences in odds of non-response due to age (p = 0.75), race (p = 0.97), or interaction between gender and vaccination sequence (p = 0.27). In a model adjusting for vaccination sequence, the odds ratio of non-response to VEE by gender (female vs. male) was 1.81 (95% CI = 1.2–2.7; p = 0.0037). When adjusting for gender, receipt of EEE and WEE before VEE yielded an OR of 2.20 for non-response in comparison with the group receiving VEE initially (95% CI = 1.2–4.1; p = 0.0145).

4. Discussion
These data indicate that vaccinating humans against EEE and WEE with inactivated vaccines may interfere with subsequent neutralizing antibody response to the live attenuated VEE TC-83 vaccine. This interference was manifested by the diminished ability to elicit an adequate antibody response of at least 1:20.

The phenomenon of alphavirus vaccine-induced interference was first observed in horses as a result of previous inoculations of EEE and WEE vaccines [11]. Similar immune suppression occurred in humans when two live alphavirus vaccines were administered sequentially in a study by McClain et al. [12]. In that study, a live attenuated CHIK vaccine was administered after inoculation of live attenuated VEE vaccine, TC-83, in humans or vice versa. Significant interference with the ability to elicit a neutralizing antibody response to the second vaccine was observed regardless of which vaccine was administrated first. The present study appears to be the first report of alphavirus vaccine interference, involving VEE, EEE, and WEE vaccines in humans. The exact mechanisms responsible for this interference are unknown. When we analyzed individuals who received VEE before EEE or WEE inactivated vaccines, we did not observe interference (data not shown). Several animal stud-

Table 3
Adjusted odds ratio (OR) of non-response (NR) by gender and EW vaccine exposure history.

<table>
<thead>
<tr>
<th>Adjusted data odds ratio</th>
<th>p-Value</th>
<th>OR (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female vs. male</td>
<td>0.0037</td>
<td>1.81 (1.2, 2.7)</td>
</tr>
<tr>
<td>Prior EW vs. no EW WEE before VEE</td>
<td>0.0145</td>
<td>2.20 (1.2, 4.1)</td>
</tr>
</tbody>
</table>
ies have shown cross-protection among alphaviruses [11,20–24]. Indeed, some of these studies showed that cross-protection may occur with non-neutralizing antibodies [23,24]. In our case, existing antibodies against EEE and WEE interfered with the development of antibodies against the live, attenuated VEE vaccine in humans.

In a previous report [17], we showed that females had a lower antibody response rate than males to the live attenuated VEE vaccine. The finding was reproduced in this study. During this time, VEE TC-83 live attenuated vaccine was administered to women during menstruation. Whether hormonal levels or some other mechanism is responsible for this phenomenon is unknown.

These data are important to medical researchers developing alphavirus vaccines and strategies for vaccination with multiple antigens.

**Acknowledgements**

The authors thank Drs. Kelly McKee and Ellen Boudreau for their critiques of the manuscript. Special acknowledgement is given to Alberta Blake, RN, who administered the vaccines in the Special Immunizations Clinic, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, between 1987 and 1999.

**References**


