A Japanese Encephalitis Vaccine From India Induces Durable and Cross-protective Immunity Against Temporally and Spatially Wide-ranging Global Field Strains

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Background. Japanese encephalitis (JE) is a vaccine-preventable acute disease. We report the results of a phase 2/3 trial of JENVAC, a Vero cell–derived vaccine developed using an Indian strain of JE virus (JEV).

Methods. JENVAC was administered in 2 doses 28 days apart, and immunogenicity was compared to that from a single dose of SA-14-14-2, the only approved JE vaccine and regimen at the time in India.

Results. After both the doses, seroconversion and seroprotection were >90% for JENVAC. For SA-14-14-2, seroconversion and seroprotection were 57.69% and 77.56%, respectively, on day 28 and 39.74% and 60.26%, respectively, on day 56. The geometric mean titers at day 28 and day 56 were 145.04 and 460.53, respectively, for JENVAC and 38.56 and 25.29, respectively, for SA-14-14-2. With a single dose of JENVAC, seroprotection titers lasted at least 12 months in >80% of the subjects. Following receipt of 2 doses, 61.17% of subjects retained seroprotection titers at 24 months, and immunogenicity criteria were higher than that for SA-14-14-2 at 12, 18, and 24 months each. Sera from JENVAC subjects neutralized JEV genotypes I, II, III, and IV equally well. Adverse events were not significantly different between the 2 vaccines.

Conclusions. JENVAC elicits long-lasting, broadly protective immunity.

Clinical Trials Registration. CTRI/2011/07/001855.

Keywords. Japanese encephalitis; vaccine; indigenous strain; genotype; cross-protection; durable immunity.

Japanese encephalitis virus (JEV) is the most common agent causing epidemic encephalitis [1, 2]. Despite high infection rates, only about 0.1%–2.0% of the individuals, mostly children <15 years old, develop encephalitis [1, 2]. However, adults are equally affected in areas where childhood immunizations are practiced [1]. JE is endemic in Southeast and East Asia, where an estimated 67 900 cases occur annually [2]. The disease is fatal in 20%–30% of those affected, and 30%–50% of the survivors have long-lasting neuropsychiatric sequelae [2, 3].

The geographic distribution of JEV overlaps with the inhabitation of approximately 60% of the global population. Millions of international military personnel and travelers visiting Asia are also at risk of being infected [1]. Combined with the potential dispersal of the mosquito vector, owing to climate change, JE poses a threat to areas beyond its traditional geographical contours, as has been observed in recent years [1]. The disease therefore imposes a severe socioeconomic burden and is an important public health concern in several countries.

The maintenance of JEV in nature, especially its amplification in pigs [1, 2], the ineffectiveness of vector control programs, and the absence of specific therapy...
make vaccination the only measure to control JE. Indeed, preexposure vaccination has significantly reduced the incidence of JE and related mortality in several countries [4, 5]. Several kinds of JE vaccines have been developed since the 1950s [4]. These include inactivated vaccines produced in the mouse brain, primary cell cultures, or continuous cell lines; the live attenuated SA-14-14-2 vaccine; the yellow fever virus (YFV)-JEV chimeric vaccine; and the recombinant poxvirus vaccines.

Both JE and JEV were recognized in India in the 1950s, but epidemics were not reported until the 1970s [5]. Outbreaks, including those that occurred as recently as 2005–2006 and 2011, have since occurred in several states [5, 6]. Incidence is highest among children during the monsoon and postmonsoon periods, although sporadic cases can occur throughout the year, and nearly all adults can be seropositive in areas of prevalence [5, 7]. An estimated 2000–3000 clinical cases occur annually, with associated encephalitis cases of 0.3% and a case-fatality rate of 25% [5, 6]. Owing to the menace of JE, vaccination was initiated in 2006. In this study, we report the results of phase 2/3 clinical trials of JENVAC, a Vero cell–derived inactivated JE vaccine developed using an Indian strain and administered to 1–50-year-old healthy Indian subjects, and compare them to findings for the live attenuated SA-14-14-2 vaccine.

MATERIALS AND METHODS

Virus Strains
The JEV strain Kolar-821564XY, isolated [8] and characterized by the National Institute of Virology, was adapted to Vero cells at Bharat Biotech International Limited. Supplementary Table 1 shows details of this and other virus strains used in this study.

Vaccines
The live attenuated SA-14-14-2 vaccine (≥10³⁴ plaque-forming units/dose, lyophilized, batch number 201004C045-2; Chengdu Institute of Biological Products, China) was obtained commercially. The inactivated vaccine JENVAC was produced in Vero cells, chromatographically purified, and formulated with aluminum hydroxide (0.2%). The formulation was characterized for sterility, identity, and purity, as well as safety in laboratory animals (acute and repeat-dose toxicity assays were performed in rats and rabbits) by methods similar to those described elsewhere [9].

Study Subjects and Trial Design
Healthy volunteers aged 1–50 years were enrolled after they provided informed consent. Subjects with a prior history of JE, fever for >3 days in the preceding month, malaise, headache, anorexia at the time of screening or during the administration of JENVAC, past history of life-threatening illness, immunodeficiency, or hypersensitivity to vaccines were excluded from the study.

The sample size was calculated by assuming a power of 90%, a noninferiority margin of 15%, a 1-sided α, and a 97.5% confidence interval (CI). Subjects were randomized 3:1 to receive JENVAC or SA-14-14-2, using a computer-generated plan with age stratification (Figure 1). The group assignment was single blinded and was not revealed to the participants or the testing laboratory personnel.

The phase 2/3 trial was conducted between June and November of 2011 at 9 sites—3 each in coastal Andhra Pradesh and Hyderabad and 1 each in Jaipur, Kolkata, and Mysore—after obtaining approvals from the National Regulatory Authority and the respective ethics committees. The trial was conducted in compliance with the Guidelines for Clinical Trials on Pharmaceutical Products in India–Good Clinical Practices, Schedule Y, Central Drugs Standard Control Organization, Ministry of Health and Family Welfare, Government of India, and the Indian Council of Medical Research Guidelines. The study is registered with Clinical Trial Registry–India (CTRI/2011/07/001855).

Table 1. Demographic Characteristics of the Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>1 to ≤6 y</th>
<th>6 to 18 y</th>
<th>18 to ≤50 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y; Mean, SD</td>
<td>3.15 (1.57)</td>
<td>3.33 (1.59)</td>
<td>10.29 (3.24)</td>
</tr>
<tr>
<td>Female sex; n (%)</td>
<td>75 (42.13)</td>
<td>20 (37.73)</td>
<td>62 (43.05)</td>
</tr>
<tr>
<td>Weight, kg; Mean, SD</td>
<td>11.94 (2.95)</td>
<td>11.81 (2.85)</td>
<td>27.76 (11.03)</td>
</tr>
<tr>
<td>Height, cm; Mean, SD</td>
<td>90.99 (13.32)</td>
<td>91.36 (15.71)</td>
<td>122.50 (23.20)</td>
</tr>
<tr>
<td>BMI, kg/m²; Mean, SD</td>
<td>14.47 (2.02)</td>
<td>14.40 (2.94)</td>
<td>19.89 (13.98)</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.
**Intervention and Safety**

JENVAC (5 µg/0.5 mL/dose) was administered intramuscularly on day 0 and day 28±2. SA-14-14-2 was injected subcutaneously as a single dose on day 0, with a placebo administered on day 28±2, since a 2-dose regimen of this vaccine had not been approved in India at the time of initiation of the study.

Besides vital parameters, solicited adverse events (AEs) were recorded during visits on day 0, day 28±2, and day 56±2. Solicited AEs were monitored for 7 days after vaccination, using diary cards, and unsolicited AEs were followed up for the entire study duration.

**Immunogenicity**

Sera were obtained from all the subjects on day 0, day 28±2, and day 56±2 and from available subjects at 12, 18, and 24 months, and subjected to the determination of 50% plaque reduction.
neutralization titer (PRNT<sub>50</sub>). Briefly, heat-inactivated sera were serially diluted 4-fold starting at a dilution of 1:10, incubated with JEV 821564XY, and inoculated onto Vero cells in 6-well plates. The monolayers were overlaid with 0.84% methylcellulose and incubated for 5 days before staining with 0.1% crystal violet and counting plaques. The PRNT<sub>50</sub> was computed by using the formula \[ X - 50/47.7662 + Y \], where \( X \) is the percentage of plaques reduced in the next dilution above 50%, and \( Y \) is the next log dilution above 50%, as described previously [10]. The PRNT<sub>50</sub> values were transformed to seroconversion, seroprotection, and geometric mean titer (GMT) data. A titer of >10 was considered to be seropositive and seroprotective [11]. Seroconversion was defined as a titer of >10 if the baseline titer was <10 or as a 4-fold increase if the baseline titer was >10. GMTs were calculated as the anti-log of the mean of the log transformation of titers for all the subjects within a group.

**Statistical Analyses**

Comparison of seroconversion rates and AEs between groups was performed using the 2-proportions test and that of GMTs was performed using the 2-sample paired \( t \) test.

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**RESULTS**

The strain 821564XY was selected on the basis of its superiority over several other Indian strains in protecting mice against challenge with Beijing-1, Nakayama, and SA-14-14-2 strains (National Institute of Virology, unpublished data). Preclinical toxicity studies revealed no treatment-related effects of JENVAC in rats and rabbits (data not shown). Immunogenicity studies in mice showed that there was no significant difference in seroconversion between JENVAC and JenceVac (killed mouse brain-derived vaccine [MBDV]; Korean Green Cross Vaccine) or JE-Vax (killed MBDV; Government Pharmaceutical Organization, Thailand), whereas GMTs were similar between JENVAC and JE-Vax, while the GMT for JenceVac was lower than that for both (Bharat Biotech International Limited, unpublished data). In the phase 1 randomized, placebo-controlled study, it was found that both the 2-dose and 3-dose regimens produced >90% seroconversion and seroprotection, with GMTs of 78.11 and 92.54 at 12 and 18 months, respectively, after 2 doses and 149.79 and 122.92 at 12 and 18 months, respectively, after 3 doses of JENVAC (Supplementary Figure 1). Since there was...
no significant difference between the 2-dose and 3-dose regimen, the phase 2/3 studies were conducted with 2 doses. Of the 644 subjects enrolled in the phase 2/3 study, 212, 201, and 231 belonged to the 18–50-year, 6–18-year, and 1–6-year age groups, respectively; 478 and 166 were randomly assigned to the JENVAC and SA-14-14-2 groups, respectively; and 34 were lost to follow-up or migration from the study area (24 from the JENVAC group and 10 from the SA-14-14-2 group; Figure 1). The various characteristics of subjects, including sex ratio, were similar between the JENVAC and SA-14-14-2 recipients in all the age groups, although the proportion of males ranged from 70% to 71%, 53% to 57%, and 58% to 62% in the 18–50-year, 6–18-year, and 1–6-year age groups, respectively (Table 1). Baseline seroprevalence was 14.18% (n = 275), 11.11% (n = 221), 15.38% (n = 65), 15.38% (n = 13), and 8.82% (n = 34) in coastal Andhra Pradesh, Hyderabad, Jaipur, Kolkata, and Mysore, respectively (data not shown).

The AEs observed are shown in Figure 2. Overall, AEs were recorded in 138 subjects (28.87%) and 23 subjects (5.09%) after the first and the second dose of JENVAC, respectively; these findings were similar to those for the SA-14-14-2 group, in which the proportions were 29.52% (n = 49) and 5.77% (n = 9), respectively. No hypersensitivity reactions were observed. Pain at the injection site was the most common local AE, being reported in 9.8% and 13.2% of JENVAC and SA-14-14-2 vaccinees, respectively, after the second dose. Fever was the most common systemic AE, being reported in 19.5% and 19.3% of the JENVAC and SA-14-14-2 recipients, respectively, after the first dose. The other systemic AEs were headache (1.9% after the first dose and 0.4% after the second dose), vomiting, diarrhea (only after the first dose, at 0.6% for each group), and cold (only after the first dose, at 0.4%). There was no significant difference between the JENVAC and SA-14-14-2 groups in either local or the systemic AEs (P > .05). When the AEs were analyzed according to age groups, although some individual AEs were different between the treatment groups (Supplementary Figure 2), there was no significant difference in the overall AEs between JENVAC and SA-14-14-2 recipients in any of the age groups (P > .05 in all cases). The AEs were treated symptomatically and resolved within 48 hours.

Immunogenicity at baseline, day 28, and day 56 was analyzed using the PRNT50 values against the 821564 strain. Baseline seroprotection in subjects administered JENVAC and SA-14-14-2 was 9.46%–13.67% and 13.72%–25.49%, respectively. The levels were slightly higher in both the 6–18-year and 18–50-year age groups in the JENVAC group and were considerably higher in the 18–50-year age group among SA-14-14-2 recipients (Table 2). The seroconversion and seroprotection rates were ≥90% for JENVAC after both primary and booster immunizations in all the age groups. Comparatively, the seroconversion rate was 55%–59% at day 28 and 31%–45% at day 56, and the

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Parameter, GMT (95% CI)</th>
<th>Analysis Interval</th>
<th>JENVAC</th>
<th>SA-14-14-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ≤ 6 months</td>
<td>Day 0</td>
<td>5.70 (5.05–6.39)</td>
<td>6.04 (5.35–6.75)</td>
<td></td>
</tr>
<tr>
<td>6 ≤ 23 months</td>
<td>Day 0</td>
<td>5.05 (4.39–5.87)</td>
<td>4.61 (3.64–5.85)</td>
<td></td>
</tr>
<tr>
<td>26 ≤ 59 months</td>
<td>Day 0</td>
<td>5.05 (4.39–5.87)</td>
<td>4.61 (3.64–5.85)</td>
<td></td>
</tr>
<tr>
<td>60 ≤ 79 months</td>
<td>Day 0</td>
<td>5.05 (4.39–5.87)</td>
<td>4.61 (3.64–5.85)</td>
<td></td>
</tr>
<tr>
<td>80+ months</td>
<td>Day 0</td>
<td>5.05 (4.39–5.87)</td>
<td>4.61 (3.64–5.85)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: GMT, geometric mean titer. Parameter values were deduced from 50% plaque reduction neutralization titers obtained against the 821564 strain.
The seroprotection rate was 74%–80% at day 28 and 56%–69% at day 56 in the SA-14-14-2 group. GMTs in JENV AC vaccinees rose from 6.13 at baseline to 145.04 after the first dose and 460.53 after the second dose. Comparatively, GMTs in the SA-14-14-2 group rose from 6.52 at baseline to 38.56 on day 28 and 25.29 on day 56 (Table 2). With JENV AC, 1.33%, 12.83%, 39.16%, 34.29%, and 12.39% of the subjects elicited antibodies with PRNT50 of <10, 10–40, 40–160, 160–640, and >640, respectively, at day 28; the proportions were 0.22%, 5.31%, 11.95%, 42.04%, and 40.49%, respectively, at day 56. Comparatively, the respective 821564-reactive PRNT50 values were 22.44%, 35.26%, 20.50%, 10.26%, and 11.54% at day 28 and 39.74%, 23.72%, 14.74%, 15.39%, and 6.49% at day 56, respectively, for the SA-14-14-2 vaccinees. By day 28, 98.5% and 50.98% of the baseline seronegative and seropositive subjects, respectively, had seroconverted.

Further analysis of the PRNT50 titers showed that JENVAC produced similar seroconversion rates, seroprotection rates, and GMTs against 821564 (homologous) and SA14-14-2 (heterologous) viruses on both day 28 and day 56 (92% seroconversion, 96% seroconversion, and a GMT of approximately 160 on day 28 and 96%–98% seroprotection, 100% seroprotection, and GMTs of 488 or 581 on day 56; Table 3). On the other hand, SA-14-14-2 produced seroconversion rates (76.92% vs 61.54% on day 28 and 61.54% vs 38.46% on day 56), seroprotection rates (84.62% vs 69.23% on day 28 and 69.23% vs 61.54% on day 56), and GMTs (86.85 vs 52.82 on day 28 and 32.10 vs 20.32 on day 56) that were slightly better against SA-14-14-2 than against 821564. Comparatively, seroconversion rates, seroprotection rates, and GMTs were all better for JENV AC homologous sera-virus pairs than for SA-14-14-2 (Table 3). The seroconversion rates were 92.31% versus 76.92%, the seroprotection rates were 96.15% versus 84.62%, and the GMTs were 154.78 versus 86.85 on day 28 for JENV AC recipients versus SA-14-14-2 recipients; the seroconversion rates were 96.15% versus 61.54%, the seroprotection rates were 100% versus 69.23%, and the GMTs were 487.64 versus 32.10 on day 56, respectively (Table 3).

A proportion of the immunized subjects (41.15%–51.10% for the JENVAC group and 24%–38% for the SA-14-14-2 group) were available for follow-up at 12, 18, or 24 months. At 12 months, 79.57% of the JENV AC subjects showed 821564-reactive seroprotection levels, and titers persisted for as long as 24 months in >60% of the individuals. The GMTs fell >8-fold from 2 to 12 months and then tapered gradually. On the other hand, the overall 821564-reactive GMTs for SA-14-14-2 recipients were ≤25 at all the evaluated time points (Figure 3A). Data for the different age-groups have been presented in Figures 3B–3D.

Table 3. Reciprocal Immunogenicity End-points for JENV AC and SA-14-14-2 Immune Sera, by Time After Vaccination

<table>
<thead>
<tr>
<th>Parameter, Challenge Virus</th>
<th>Anti-JENV AC Sera (n = 52)</th>
<th>Anti-SA-14-14-2 Sera (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 28</td>
</tr>
<tr>
<td>Seroconversion, % (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>821564XY</td>
<td>NA</td>
<td>92.31 (85.07–99.55)</td>
</tr>
<tr>
<td>SA14-14-2</td>
<td>NA</td>
<td>92.31 (85.07–99.55)</td>
</tr>
<tr>
<td>Seroprotection, % (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>821564XY</td>
<td>15.38 (5.58–25.19)</td>
<td>96.15 (90.93–100)</td>
</tr>
<tr>
<td>SA14-14-2</td>
<td>13.46 (4.18–22.74)</td>
<td>96.15 (90.93–100)</td>
</tr>
<tr>
<td>GMT (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>821564XY</td>
<td>8.31 (5.98–11.54)</td>
<td>154.78 (11.54–200.00)</td>
</tr>
<tr>
<td>SA14-14-2</td>
<td>5.77 (5.21–6.40)</td>
<td>163.04 (111.42–238.58)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; GMT, geometric mean titer; JEV, Japanese encephalitis virus; NA, not applicable.

* JEV strain from which JENVAC was developed.
SA14-14-2–immunized subjects, at 60.25 (95% CI, 43.30–83.85), 35.57 (95% CI, 25.19–50.23), and 75.06 (95% CI, 49.95–112.79). The P values for differences between the 2 groups at 12, 18, and 24 months were .0444, .1152, and .1160, respectively. Assessment of immunogenicity in some subjects who withdrew after the first dose showed that the seroprotection rates were 81.82% and 44.44% for JENVAC (n = 22) and SA14-14-2 (n = 9) recipients, respectively, whereas the 821564-reactive GMTs were 40.90 and 29.44, respectively, after 12 months (Figure 3E).

Some of the sera from JENVAC group were also tested against another Indian genotype III (G-III) virus. The baseline (day 0) seroprotection rates and GMTs were higher against heterologous virus, and the GMTs were higher but the seroconversion...
rates were equal or lower against heterologous virus on day 28 and day 56 (data not shown). Furthermore, when sera selected randomly from JENVAC subjects (n = 5–10) at different times were tested against JEV strains belonging to G-I, G-II, G-III, and G-IV, the GMTs were found to be highest against G-III, followed by similar GMTs against the other genotypes at the 2-month time point (Table 4). A similar trend of higher GMTs to the homologous genotype was observed at 12 and 18 months, and the reactivity to heterologous strains was variable (Table 4). Persistently high levels of antibodies were observed against 2 recent G-I strains (Table 4).

**DISCUSSION**

Several types of JE vaccines have been developed since the 1950s, and 3 have been widely used for immunizations. The inactivated MBDV needs to be administered in multiple doses, can cause serious hypersensitivity in some vaccinees, and can potentially cause neurological side effects [11]. Moreover, its manufacturing process is less stringent than that for cell culture–derived vaccines. The live attenuated SA-14-14-2 vaccine [4, 12] is efficacious but produced in primary cells, which are less preferred than continuous cell lines for vaccine production. The YFV-JEV chimera is also safe and efficacious with a single dose [13]. Among the available inactivated JE vaccines, only Ixiaro (also Jespect; Intercell/Valneva) is derived using in a continuous cell line. JENVAC is the only cell culture–based inactivated vaccine developed and commercialized starting from the strain in India. Our phase 2/3 studies revealed that JENVAC elicits long-term, broadly cross-protective responses that are noninferior to and may be slightly better than those of SA-14-14-2 in 1–50-year-old Indian subjects.

The most meaningful comparison of our studies would be against other cell culture–derived inactivated vaccines. The first such study used the Beijing-1 strain propagated in Vero cells, in which seroconversion rates of 93%–97% and GMTs of 2.09–2.19 were achieved after a single immunization, with not much increase in GMTs following 1 or 2 more boosters [14]. Later studies showed that a Vero cell–derived SA-14-14-2 killed vaccine was equivalent to an MBDV [15, 16]. Seroconversion rates were 77.3%–95.6%, depending on the dose, after a single immunization and >95% following a booster, whereas seroconversion after 3 doses of MBDV was 84%. The GMTs were 61.2–328.3 after 1 dose and 186.1–516.3 after 2 doses, whereas those for MBDV were 128–132 after 3 doses [15]. Long-term studies have shown that (1) GMTs decreased from 219.3 at 2 months to 46.6 at 6 months, 18 at 18 months, and 16.2 at 24 months and (2) a booster at 11 or 23 months could produce seroconversion in 100% of the subjects, with GMTs of 676.2 and 2496.1, respectively [17]. Another study showed that 95% and 83% of the subjects were still seroconverted, with GMTs of 84 and 41, after 6 and 12 months, respectively, following a single dose [18]. A booster at 15 months could produce seroconversion rates of 98.5%–100% and GMTs of 900, 487, and 361 on day 28, at 6 months, and at 12 months, respectively [19]. Our results show that JENVAC was immunogenic, with 79.57%, 66.67%, and 61.17% of the subjects still seroprotected at 12, 18, and 24 months, respectively, following the receipt of 2 doses. Although the above comparisons are not head-to-head, our results demonstrate that JENVAC is noninferior to other commercially available vaccines.

JEV strains have been classified into 5 genotypes, G-I through G-V, all belonging to a single serotype [20]. G-III is widely distributed in Asia, and therefore all of the vaccines developed so far have used G-III strain viruses. Immunogenicity analyses show that all vaccines induce cross-protection against all the tested genotypes, although titers are typically higher against homologous genotypes or against homologous strains within a genotype [21–24]. However, in recent times, G-I strains have been isolated at an increased rate, to the extent that G-I is now the dominant genotype [25, 26], although it may be genetically more homogeneous and has been difficult to isolate from humans than from pigs and mosquitoes [26]. Despite the fact that most JE vaccines induce similar antibody titers against most JEV strains [16, 22], there is evidence that titers, although still protective, can be low against G-I and, possibly, G-IV strains [27–31]. The 821564 strain also belongs to G-III but elicits antibodies reacting well to heterologous G-III, as well as heterotypic viruses, including 2 recent G-I strains (SM1 and 12/290), suggesting that JENVAC may be better than other vaccines. It should, however, be noted that JENVAC is yet to be tested against any strain belonging to G-V, a more divergent and reemerging genotype [32].

The only published study on clinical trials for a cell culture–derived inactivated JE vaccine in Indian subjects is that for the SA14-14-2 strain propagated in Vero cells (Ixiaro). That study used 3 and 6 µg of antigen in 1–3-year-old subjects and found that 65.2% and 71.4% of the subjects seroconverted, with GMTs

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**Table 4. Cross-reactivity of Sera With Different Genotypes of Japanese Encephalitis Virus (JEV), by Time After Vaccination**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>2 mo</th>
<th>12 mo</th>
<th>18 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991, TVP-8236</td>
<td>I</td>
<td>1404.72</td>
<td>741.96</td>
<td>583.55</td>
</tr>
<tr>
<td>B 1034/8</td>
<td>II</td>
<td>1555.21</td>
<td>354.22</td>
<td>724.45</td>
</tr>
<tr>
<td>Beijing-1</td>
<td>III</td>
<td>2276.67</td>
<td>803.88</td>
<td>809.87</td>
</tr>
<tr>
<td>JKT 9092 TVP-6265</td>
<td>IV</td>
<td>1453.55</td>
<td>524.59</td>
<td>465.31</td>
</tr>
<tr>
<td>SM1</td>
<td>I</td>
<td>233.74</td>
<td>839.99</td>
<td>492.21</td>
</tr>
<tr>
<td>12/290</td>
<td>I</td>
<td>692.03</td>
<td>1073.54</td>
<td>842.91</td>
</tr>
</tbody>
</table>

Seroprotection was 100% in all cases except for the 12-month time point for SM1 and 12/290, at which seroprotection was 88.89% in both cases. Experiments with SM1 and 12/290 were performed separately from tests involving other strains.

Abbreviation: GMT, geometric mean titer.
of 24 and 21, respectively, after 1 dose and that seroconversion rates were 95.7% and 95.2%, with GMTs of 201 and 218, respectively, after 2 doses [33]. The same vaccine produced in India as Jeev (Biologicals Evans) following transfer of technology has been reported to produce seroconversion rates of 56.28% and 92.42% after 1 and 2 doses, respectively, in 1–3-year-old subjects [34]. Analysis of our data from subjects in the same age group showed that seroconversion rates and GMTs produced by JENVAC were 97.8% and 226.74, respectively, after 1 dose and 98.89% and 1050.17, respectively, after 2 doses. Comparatively, the seroconversion rates and GMTs for SA-14-14-2 were 60.00% and 61.07, respectively, on day 28 and 43.33% and 27.59, respectively, on day 56 after a single dose. These data indicate that, in 1–3-year-old subjects, a 2-dose regimen of JENVAC is better than 2 doses of Ixiaro/Jeev or 1 dose of SA-14-14-2.

Our results show that JENVAC is more immunogenic than SA-14-14-2 in Indian subjects. The poor immunogenicity of SA-14-14-2 in Indian subjects is perplexing. Studies from China have reported seroconversion rates of 85%–100% after a single dose [8]. Reports suggest that seroconversion rates overall and in previously naive individuals are 73.9% and 67.2%, respectively, after a single dose, with a protective effect of 62.5%; the efficacy is reported to be 43.1% overall and 35% in preimmune subjects after 1 year [35]. Other studies have reported that SA-14-14-2 has an effectiveness of 75%–80% [36, 37]. On the other hand, tolerability of JENVAC after a single dose in Indian subjects is equivalent to or slightly better than the reported tolerability of SA-14-14-2 in Chinese [38, 39] and Indian [40, 41] subjects. Further studies are needed to compare immunogenicity, efficacy, and safety between JENVAC and SA-14-14-2 in Indian subjects.

Despite the introduction of the live attenuated SA-14-14-2 vaccine in 2006, the incidence of acute encephalitis syndrome and the associated case-fatality rate have increased in India [42, 43]. Although several viruses have been found to be associated, a significant proportion of the cases are attributed to JEV [34, 44, 45]. It is not clear whether the continued increased incidence of acute encephalitis syndrome/JE is simply because of increased reporting or a result of poor vaccination coverage, ineffectiveness of the vaccine, emergence of a strain against which the vaccine is poorly protective, or, perhaps, a combination of these factors. Robust epidemiological investigations of outbreaks, as well as large-scale postvaccination serosurveillance studies, should resolve some of these issues. On the other hand, vaccines must be tested against a battery of virus strains that are geographically disparate and temporally well separated. Our data on the ability of JENVAC to elicit protective responses against strains (1) isolated from humans, pigs, and mosquitoes, (2) separated by >30 years, and (3) belonging to all the major genotypes of JEV suggest that JENVAC can be used as a universal vaccine against JE. In addition, the ability of JENVAC to elicit protective responses that are sustained, with either a single dose or 2 doses, is an added benefit in JE-endemic countries where vaccination coverage and compliance are an impediment to tackling JE.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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An externally contracted, independent statistician designed the trial. M. M., G. S., P. V., J. V. R., R. K., M. K. G., S. K., B. S., and N. B. are employed by BBIL and contributed to trial conduct. A. S. and V. K. M. contributed to trial implementation, and K. M. E. provided the overall direction. K. G., Y. K., and M. M. G. contributed to design and implementation of animal experiments and laboratory analyses. A. S. and Y. K. contributed to data analyses. V. K. M. and K. M. E. contributed to preparation of pilot lots and provided intellectual input into trial design and implementation. M. M. G. provided expert advice and technical input throughout vaccine production and clinical trials. N. R. H. compiled and prepared the data and wrote the manuscript, with input from Y. K., A. S., M. M. G., V. K. M., and K. M. E. All authors reviewed and approved the manuscript.

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**Potential conflicts of interest.** The vaccine strain was licensed from the National Institute of Virology (NIV), India to BBIL. The study sponsor had no role in the study design but was involved in data collection, analysis, and interpretation. Vaccine development and production, preclinical toxicity studies, and assessment of antibody titer were done at BBIL, with guidance from the NIV. All serum samples were initially evaluated at the in-house quality control laboratory of BBIL. Further confirmatory neutralization and cross-neutralization were performed at the NIV and Mahidol University. A. S., Y. K., K. G., V. K. M., and K. M. E. are employees of BBIL. Clinical trials, off-site laboratory analyses, and statistical studies were sponsored by BBIL. M. M., G. S., P. V., J. V. R., B. K., M. K. G., S. S. K., B. S., N. B. R., and M. M. G. received personal fees and other financial support from BBIL during the conduct of the current study. M. M., G. S., P. V., M. K. G., S. S. K., B. S., and N. B. R. also received personal fees and other financial support from BBIL during the last 36 months for the conduct of another study. The Ella Foundation, to which N. R. H. is affiliated, received funds from BBIL for scientific and technical input, data compilation, and manuscript preparation and submission. K. M. E. is also a director of the Ella Foundation.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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