MAJOR ARTICLE

Chimeric Live, Attenuated Vaccine against Japanese Encephalitis (ChimeriVax-JE): Phase 2 Clinical Trials for Safety and Immunogenicity, Effect of Vaccine Dose and Schedule, and Memory Response to Challenge with Inactivated Japanese Encephalitis Antigen

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ChimeriVax-JE is a live, attenuated vaccine against Japanese encephalitis, using yellow fever (YF) 17D vaccine as a vector. In a double-blind phase 2 trial, 99 adults received vaccine, placebo, or YF 17D vaccine (YF-VAX). ChimeriVax-JE was well tolerated, with no differences in adverse events between treatment groups. Viremias resulting from administration of ChimeriVax-JE and YF-VAX were of short duration and low titer; 82 (94%) of 87 subjects administered graded doses (1.8–5.8 $\log_{10}$) of ChimeriVax-JE developed neutralizing antibodies. A second dose, administered 30 days later, had no booster effect. Previous inoculation with YF did not interfere with ChimeriVax-JE, but there was a suggestion (not statistically significant) that ChimeriVax-JE interfered with YF-VAX administered 30 days later. A separate study explored immunological memory both in subjects who had received ChimeriVax-JE 9 months before and in ChimeriVax-JE–naive subjects challenged with inactivated mouse-brain vaccine (JE-VAX). Anamnestic responses were observed in preimmune individuals. ChimeriVax-JE appears to be a safe vaccine that provides protective levels of neutralizing antibody after a single dose.

Japanese encephalitis (JE) virus is a mosquito-borne member of the family *Flaviviridae*, a group of ~70 single-strand, positive-sense RNA viruses, many of which are important human and veterinary pathogens. JE is a leading cause of severe central nervous system infection in Asia and Australia, where ~35,000 cases are reported annually [1]. The disease predominantly strikes children, kills up to 35% of affected individuals, and frequently causes long-term neurologic impairment. JE also poses a risk to travelers and to military personnel deployed overseas. It is a vaccine-preventable disease, but existing products (formalin-inactivated, whole-virion vaccines produced in mouse-brain tissue) have many disadvantages, including high cost, reactogenicity, and the requirement for multiple doses for primary and booster immunization. For travelers, a cumbersome 3-dose primary regimen must be accomplished at least 1 month before embarkation. For these reasons, a high priority has been placed on development of improved vaccines [2], in particular vaccines that elicit rapid onset of immunity after a single dose. A live, attenuated vaccine (SA14-14-2) is used widely in China and has recently been approved for use in South

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Korea. Clinical trials indicate that SA14-14-2 is safe and effective, but at least 2 doses may be required for protective immunization [3, 4].

We have developed a live, attenuated vaccine against JE, using yellow fever (YF) 17D vaccine as a vector. YF 17D has a long history of safe and effective use in hundreds of millions of persons, and it rapidly induces life-long immunity after a single dose [5]. The chimeric YF-JE virus (trade name, ChimeriVax-JE; Acambis) has an improved safety profile (lower neurovirulence), compared with commercial YF 17D vaccine, in mice [6] and monkeys [7] and is expected to be less likely to cause YF vaccine–associated neurotropic adverse events (AEs) in humans. After a single dose, ChimeriVax-JE is highly immunogenic in monkeys and protects against lethal JE virus challenge [7]. A proof-of-principle clinical trial demonstrated that ChimeriVax-JE both was well tolerated and elicited high titers of specific JE neutralizing antibodies, in all YF-naive and YF-immune subjects after a single dose [8].

In the present study, we report a double-blind, placebo-controlled phase 2 clinical trial of ChimeriVax-JE in healthy adults who received graded doses of the vaccine (study 1). The response to a second, booster inoculation and interactions with YF 17D vaccine were also investigated. A separate open-label phase 2 clinical study (study 2) explored immunological memory responses in subjects who had previously received ChimeriVax-JE and who were challenged with a single dose of inactivated mouse-brain vaccine (JE-VAX; Aventis Pasteur), which served as a surrogate for natural exposure to wild-type virus. The results of the 2 studies indicate that ChimeriVax-JE is well tolerated and is highly immunogenic, with a similar clinical profile to parental YF 17D vaccine.

MATERIALS AND METHODS

Vaccines. Two vaccines were used in study 1: the experimental ChimeriVax-JE vaccine and commercial YF 17D vaccine (YF-VAX; Aventis Pasteur); and 2 vaccines were used in study 2: ChimeriVax-JE vaccine and commercial formalin-inactivated mouse-brain JE vaccine (JE-VAX; Aventis-Pasteur). JE-VAX is a monovalent vaccine against the Nakayama strain of JE virus.

ChimeriVax-JE was constructed from a full-length cDNA clone of YF 17D, as described elsewhere [6, 9]. In brief, genes encoding the premembrane (prM) and envelope (E) proteins of YF 17D vaccine virus were replaced with the corresponding genes of JE (vaccine strain SA14-14-2) virus. The chimeric cDNA was then transcribed to RNA, which was used to transfect African green monkey kidney (Vero) cells, by electroporation. The progeny virus particles contain the prM-E gene sequences derived from JE virus, encoding JE virus–specific epitopes for neutralizing antibodies as well as epitopes for cytotoxic T lymphocytes [10]. The nucleocapsid (C) protein, the 7 nonstructural proteins, and the nontranslated 3′- and 5′-termini responsible for viral replication are the original YF 17D virus sequence. The chimeric virus induces a subclinical infection in the host, an infection resembling that induced by YF 17D vaccine, but elicits specific immunity against the heterologous JE virus envelope antigens [6–9].

Vero cells were obtained from Aventis-Pasteur and were from a cell bank used by Aventis-Pasteur for manufacturing inactivated poliomyelitis vaccine. ChimeriVax-JE vaccine was produced and tested in accordance with current good manufacturing practices (GMP). Master seed, production seed, and vaccine virus stocks were produced at passages 3, 4, and 5 in Vero cells, respectively. The vaccine virus was recovered from supernatant fluid of Vero cell cultures, was treated with nuclease (benzonase) to digest host cell nucleic acids, was purified by tangential-flow microfiltration (by use of a 500-kDa cut-off membrane) and diafiltration, was stabilized with lactose and human serum albumin, and was stored frozen (less than −60°C). In the final product, host cell DNA was <0.1 ng/0.5 mL dose. The Vero cells, seed, and vaccine viruses were extensively tested for freedom from adventitious agents. Toxicological evaluation included formal monkey-neurovirulence testing. The chimeric virus was less neurovirulent than commercial YF 17D vaccine [7].

The clinical trials were performed under an investigational new-drug application approved by the US Food and Drug Administration (FDA). The protocol, amendments, informed-consent form, and certain other documents related to the study (e.g., advertisements used to recruit subjects) were approved by an institutional review board and complied with the regulatory requirements of the FDA. The studies were conducted and voluntary informed consent was obtained in accordance with the ethical principles in the Declaration of Helsinki and the applicable guidelines for good clinical practices.

Study 1. Study 1 was a randomized, double-blind, placebo-controlled out-patient study of 99 subjects: 11 subjects each were assigned to 1 of 9 groups on a random basis (table 1). The study was conducted in healthy men and women, 18–59 years old, at a single center in the United States (Pharmaceutical Research Associates). Subjects who had the following significant medical conditions were excluded: contraindications to YF 17D vaccine (pregnancy, immunosuppression, or egg hypersensitivity); positive tests for hepatitis B surface antigen (HBsAg), hepatitis C virus, or human immunodeficiency virus (HIV) antibody; pregnancy or lactation; or a history of previous vaccination against YF or JE.

The objectives of this study were the following:

1. To assess the safety of and tolerability to ChimeriVax-JE, compared with that of and that to YF-VAX and placebo, by use of laboratory tests (including quantitative viremia mea-

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Table 1. Study 1 design: day 0 and day 30 treatments for subjects in the 9 study groups.

Group (n = 11/group)[a]	Treatment for day 0 (log_{10} pfu)	Treatment for day 30 (log_{10} pfu)
1	CV-JE (5.8)	CV-JE (5.8)
2	CV-JE (4.8)	CV-JE (4.8)
3	CV-JE (3.8)	CV-JE (3.8)
4	CV-JE (2.8)	CV-JE (2.8)
5	CV-JE (1.8)	CV-JE (1.8)
6	CV-JE (4.8)	YF-VAX
7	YF-VAX	CV-JE (4.8)
8	Placebo	CV-JE (4.8)
9	CV-JE (4.8)	Placebo

NOTE. CV-JE, ChimeriVax-JE; YF-VAX, yellow fever 17D vaccine.
[a] Subjects were assigned to groups on a random basis.

surements), body-temperature measurements, and the incidence of AEs; and

2. To characterize the antibody response after administration of ChimeriVax-JE vaccine by

a. Determining the proportion of subjects seroconverting (by neutralization test) after administration of 10-fold graded doses of vaccine between 1.8 and 5.8 log_{10} pfu;

b. Comparing the immune response to that of a single dose of ChimeriVax-JE, with the immune response to 2 doses separated by 30 days; and

c. Determining the effect on antibody levels of sequential inoculation with ChimeriVax-JE followed by YF-VAX, and YF-VAX followed by ChimeriVax-JE, administered at a 30-day interval.

After screening and baseline assessments, each subject received 2 inoculations with double-blind medication, with a 30-day interval between inoculations (table 1). On day 0, subjects were inoculated by the subcutaneous (SC) route, in the upper arm, with 0.5 mL of double-blind-study medication. Subjects returned to the clinic for blood sampling and assessment of safety variables on days 1–8 (inclusive), on day 14, and on day 21. A second vaccine inoculation was administered on day 30, followed by an interim visit on day 44. Completion of the double-blind period occurred on day 60. After entry of all data, the study blind was broken, and analyses were completed. Safety assessments include incidence of AEs, body-temperature measurements, physical examinations, clinical laboratory tests, and quantitative viremia measurements.

To determine the durability of the antibody response, follow-up visits were conducted at 6 months and 11–12 months after inoculation. These data will be reported separately.

Study 2. To characterize the immunological response after challenge with JE-VAX, a separate study was performed on subjects previously inoculated with ChimeriVax-JE. This open-label study was conducted on an out-patient basis on 20 healthy subjects, at a single study center in the United States. Ten subjects who had received ChimeriVax-JE at least 6 months before enrollment in the study and who had not previously received YF vaccine were inoculated with JE-VAX. A control group, consisting of the same number of age- and sex-matched subjects who were naive to JE and YF vaccines, was also administered JE-VAX. Subjects who had the following significant medical conditions were excluded: contraindications to JE-VAX vaccine (hypersensitivity to proteins of rodent origin, to neural tissue, or to thimerosal); positive test results for HBsAg, hepatitis C virus, or HIV antibody; pregnancy or lactation; previous travel to or residence in Asia, Africa, or South America; or military service with overseas deployment.

The objectives of this study were the following:

1. To characterize the safety of administration of JE-VAX in subjects who were inoculated at least 6 months before with ChimeriVax-JE, by monitoring incidence of AEs;
2. To characterize the magnitude and kinetics of the recall (anamnestic) response to challenge with JE-VAX vaccine in subjects who were inoculated at least 6 months before with ChimeriVax-JE. The anamnestic response was characterized by measuring the neutralizing antibodies at intervals of up to 30 days after the administration of JE-VAX; and
3. To compare the cross-reactive neutralizing antibody response to multiple JE virus genotypes in subjects with previous inoculations of ChimeriVax-JE, before and after receiving a booster inoculation of JE-VAX.

After screening assessments were completed, subjects were administered a single 1.0-mL SC dose of JE-VAX on day 0. For measurement of neutralizing antibodies and for recording AEs, clinic visits occurred on days 3, 7, 14, and 30. Comparisons were made between the 2 treatment groups by use of safety parameters, the magnitude and the kinetics of the neutralizing antibody response to JE-VAX, and the cross-reactive neutralizing antibody response to wild-type JE virus strains.

Viremia (study 1). Serum samples collected daily through day 8, for viremia determinations, were frozen at less than −60° C within 3 h of collection. Viremia titers were determined by direct plaquing of 0.1 mL of a 1:2 and 1:20 dilution of serum in duplicate wells of Vero cell cultures grown in 12-well plates.

Serological tests. The humoral immune response was measured (T cell studies were not performed in these trials). Two different neutralization tests were performed. The primary outcome measure was a constant-serum, varying-virus plaque-reduction neutralization test (PRNT) against homologous viruses (ChimeriVax-JE and YF 17D) performed in Vero cells. Stock virus pools for use in neutralization tests were prepared from ChimeriVax-JE (GMP production virus seed; passage level

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4) and commercial YF-VAX vaccine, by a single passage in Vero cells. The homologous test system is routinely used for determination of the response to many live viral vaccines, such as YF 17D, vaccinia, and measles. The JE or YF antibody titer was defined as the $\log_{10}$ neutralization index (LNI; $\log_{10}$ difference between the virus titer in serum-virus mixture for the test sample and that in a negative control serum) [8, 11]. For JE and YF neutralization tests, seroconversion was defined as an increase in LNI of $\geqslant$0.7, representing the cut-off for protective immunity in monkeys against YF [11, 12], a virus that is significantly more lethal than JE.

The secondary test was a serum dilution, constant-virus $PRNT_{50}$ performed in LLC-MK2 cells, as described elsewhere [13]. Paired serum samples from all subjects were tested for antibodies against wild-type strains of JE virus and ChimeriVax-JE. Wild-type strains included Beijing-1 (isolated in China in 1948) and Nakayama (isolated in Japan in 1935). These strains are used to produce killed vaccines in Japan. A third wild-type virus, 902/97 (isolated from a child in Vietnam in 1997), was used at low passage (1 C6/36 *Aedes albopictus* cells, 1 suckling mouse brain, and 3 LLC-MK_2 cells). All wild-type strains belong to JE virus genotype III [14, 15] (S.Y., unpublished data for strain 902/97), which is both the same genotype from which the donor genes were obtained for construction of ChimeriVax-JE and the most prevalent genotype causing human disease in Asia. The end point for neutralization was the highest dilution of serum reducing plaques by 50%, compared with a negative serum control, determined by probit analysis. YF-neutralization tests were not performed by use of the $PRNT_{50}$ method.

STATISTICAL METHODS

Study 1

Sample size and power calculations. Sample size was based on the responses observed in a previous clinical trial of ChimeriVax-JE [8]. A sample size of 10 subjects/group provided 90% power to establish a dose response among groups 1–5 (table 1) in a logistic-regression model for the proportion seroconverting at day 30, at significance level 0.05. Since groups 4, 6, and 9 all received the same initial dose of 4.8 $\log_{10}$ pfu of ChimeriVax-JE (table 1), the SE at this dose level was ~42% that of the other starting doses at day 30. Eleven subjects were enrolled per group, to allow for an estimated dropout rate of 10%.

Baseline demographics. Tests for baseline comparability, between randomized treatment groups, used analysis of variance (ANOVA) techniques, for the age variable, and Fisher's exact test, for the sex and race variables.

AEs. AEs were determined for the safety population, which was defined as subjects who received at least 1 dose of study medication (active or placebo). AEs were coded in accordance with the MedDRA dictionary. Treatment-emergent AEs were defined as those events starting or worsening after dosing with study medication. The overall incidence of subjects with treatment-emergent AEs was compared between treatment groups at day 30 and at day 60, by use of Fisher's exact test.

Viremia. In calculating statistics for viremia, absence of detectable virus in serum is designated as the value "0." In fact, the lowest detectable value, on the basis of the test method used, is 10 pfu (1.0 $\log_{10}$/mL). An ANOVA model was used to compare viremia (peak, duration, and area under the curve [AUC], calculated by use of the linear trapezoidal method) after the first dose, for groups 1–6 and for group 9 versus group 8. A residual analysis showed that assumptions of normality and equality of variance were met within the model. Differences between treatment groups were investigated by use of the Kruskal-Wallis test.

Antibody response. Antibody responses were evaluated for the per-protocol population, which was defined as subjects who received at least 1 inoculation with double-blind study medication, who provided serum samples to at least day 30, and who had no significant protocol deviations identified before unblinding. A logistic-regression model was used to examine the seroconversion rate as a function of dose, on day 30 after inoculation, using groups 1–6, 8, and 9 (the LNI for groups 1–6 and 9 were measured on day 30 and that for group 8 on day 60). The dose calculated to produce seroconversion in 50% of individuals (ED_{50}) and in 90% of individuals (ED_{90}) was estimated from the fitted curve. To determine the effect on antibody levels after sequential inoculation with ChimeriVax-JE and YF-VAX, Fisher's exact test was used to compare seroconversion rates in group 6 at day 30 with those in group 7 at day 60, for JE, and to compare seroconversion rates in group 6 at day 60 to those in group 7 at day 30, for YF (see table 1 for treatment group assignments).

Study 2

AEs. The COSTART dictionary was used to code the investigator's AE terms to body system and preferred term. The number and percentage of subjects experiencing at least 1 treatment-emergent AE overall, as well as those for each body system and preferred term, were summarized by immune status.

Antibody responses. The LNI was summarized by use of descriptive statistics at each time point after inoculation (days 3, 7, 14, and 30). The anamnestic response was assessed by comparing the previously exposed and ChimeriVax-JE–naive subjects, by use of a 2-sample *t* test at each time point.

RESULTS

Study 1. Ninety-eight of 99 subjects in the per-protocol population completed the study; 1 subject in group 1 withdrew on

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day 3. No subjects withdrew because of either AEs or noncompliance with the protocol.

Demographics. Study groups were similar with respect to age (mean, 34.2 years [range, 18–58 years]; $P = .8705$), sex (male, 52%; $P = .6139$), height (mean, 171.2 cm; $P = .9421$), weight (mean, 81.0 kg; $P = .7123$), and body mass index (mean, 27.58 kg/m^2; $P = .7748$). The racial composition differed between the groups, with groups 7 and 8 having higher proportions of nonwhite subjects ($P = .0416$).

Safety. There were no serious AEs related to study medication. One subject in group 2 was hospitalized with cholecystitis ~3 weeks after the second dose of study medication, but the event was considered to be unrelated to the vaccine.

Oral body temperature was recorded by subjects on diary cards (on days 1–60) and was also measured by the investigator during clinic visits (days 0 [before and after inoculation], 1–8, 14, 21, 30, and 60). The symptom pyrexia was reported as an AE for 13 subjects (after the first inoculation in 6 subjects and after the second inoculation in 7 subjects). Objective elevation in body temperature during study visits was recorded as an AE in 8 subjects (4 after the first inoculation and 4 after the second inoculation). The symptom pyrexia was considered to be possibly, probably, or definitely related to ChimeriVax-JE vaccine in 5 subjects (1 each in groups 1, 3, 4, 5, and 9) and at least possibly related to YF-VAX in 2 subjects. The highest temperature elevation considered to be at least possibly related to study medication was 38.6°C (1 subject in group 5, 9 days after inoculation), and the longest temperature elevation was 2 consecutive days.

The majority of subjects in all groups reported at least 1 treatment-emergent AE (table 2). AEs were more frequent after the first dose than after the second dose (table 2; figure 1). There were no differences in the incidence of AEs between treatment groups, after either the first dose ($P = .7874$, Fisher's exact test) or the second dose ($P = .7626$). The most frequently reported AEs were injection-site erythema, injection-site pain, fatigue, myalgia, headache, and diarrhea. Injection-site reactions occurred at the highest frequency in subjects receiving the highest doses (5.8 $\log_{10}$ pfu) of ChimeriVax-JE (group 1) and in those receiving YF-VAX (groups 6 and 7) (figure 1). Local reactions were either absent or infrequent in subjects receiving placebo or doses of ChimeriVax-JE <5.8 $\log_{10}$ pfu. Since the placebo groups received active vaccine (4.8 $\log_{10}$ pfu of ChimeriVax-JE) in a cross-over design (table 1), it is necessary to examine reactogenicity for the first and second dose between the placebo group and the active-treatment group (figure 1). This analysis revealed that, whereas injection-site reactions were attributable to the study vaccines, systemic AEs were less clearly associated.

Most AEs were mild or moderate in degree. There were 16 events that were scored severe (2 cholecystitis, 2 bruising at venipuncture site, 1 myalgia, 1 contact dermatitis, 1 urticaria, and 9 erythema) at the injection site. Injection-site erythema was arbitrarily assigned a very conservative severity score on the basis of diameter (>1 inch).

Clinical laboratory tests. Clinical laboratory tests (complete blood counts, blood chemistry [glucose, creatinine, aspartate aminotransferase /AST/, and alanine aminotransferase /ALT/], and urinalysis) were performed 1 week after the first (day 8) and second (day 44) inoculations. There were no apparent changes in hematological test parameters associated with treatment. Mean values for blood-chemistry variables were similar in each treatment group at baseline. There were a few subjects with shifts (from within the normal range to above the normal range) in blood-chemistry variables from screening to day 8 and from screening to day 44, but there was no pattern with respect to treatment group. Five subjects had increased levels of AST at day 8; 4 of these subjects had received 4.8 $\log_{10}$ pfu of ChimeriVax-JE (2 in group 2 and 2 in group 6), and 1 subject had received placebo. Increases in levels of AST were minimal, with the highest value being 45 IU/L (reference range, 10–40 IU/L). Two subjects had increased levels of ALT at day 8 (1 each in the groups receiving 3.8 and 5.8 $\log_{10}$ pfu of ChimeriVax-JE). Increases in levels of AST were also minimal, with the highest value being 59 IU/L (reference range, 3–50 IU/L). All enzyme abnormalities resolved by day 44. There were few subjects with abnormal urinalysis findings, and there was no obvious pattern between the treatment groups.

Viremia. Overall, viremia in subjects who received ChimeriVax-JE vaccine was characterized by short duration and low titer. Fifty percent to 100% of subjects receiving ChimeriVax-JE and 64% of subjects receiving YF-VAX developed detectable viremia on at least 1 day after inoculation (table 3, figure 2; $P = .0999$, 2-tailed Fisher's exact test). An inverse relationship between dose and the proportion of viremic subjects was found in the groups receiving ChimeriVax-JE ($\rho = .0180$ was considered to be statistically significant, by 2-sided Cochran-Armitage Trend test). In addition, the magnitude of viremia tended to be higher in the groups receiving lower doses of ChimeriVax-JE, although the differences in mean peak viremia and AUC did not quite reach statistical significance. These data were skewed by an individual in the group receiving 2.8 $\log_{10}$ pfu who developed a relatively high viremia, 110 and 220 pfu/mL on days 4 and 5, respectively. However, viremias were also of longer duration at lower doses of ChimeriVax-JE vaccine, and the difference between treatment groups was nearly significant ($\rho = .0547$). The viremia after inoculation with YF-VAX occurred quite uniformly between days 4 and 6, whereas the viremia after inoculation with ChimeriVax-JE occurred more variably, with some subjects having early, late, or intermittent viremias (figure 2). We determined the relationship between viremia level and duration and peak neutralizing-

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Table 2. Study 1: no. (%) of subjects with adverse events (AE), reported by at least 2 subjects in any treatment group (subjects counted only once for each body system or preferred term).

Body system/preferred term	Treatment group (n = 11/group)									Total (n = 99)	P[j]
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	8[h]	9[i]		
Total AEs	44	54	49	36	52	78	72	43	49	477	
Subjects with any AE	10 (91)	9 (82)	10 (91)	9 (82)	10 (91)	11 (100)	9 (82)	10 (91)	9 (82)	87 (88)	
After first and before second inoculation	8 (73)	9 (82)	10 (91)	9 (82)	10 (91)	10 (91)	9 (82)	10 (91)	7 (64)	82 (83)	.7874
After second inoculation	6 (55)	4 (36)	4 (36)	5 (45)	6 (55)	8 (73)	7 (64)	6 (55)	7 (64)	53 (54)	.7626
Gastrointestinal	4 (36)	4 (36)	3 (27)	2 (18)	4 (36)	4 (36)	3 (27)	3 (27)	6 (55)	33 (33)	
Diarrhea	3 (27)	1 (9)	1 (9)	0 (0)	2 (18)	2 (18)	3 (27)	1 (9)	4 (36)	17 (17)	
Nausea	0 (0)	2 (18)	1 (9)	0 (0)	0 (0)	3 (27)	1 (9)	2 (18)	2 (18)	11 (11)	
Pharyngolaryngeal pain	0 (0)	1 (9)	0 (0)	1 (9)	2 (18)	1 (9)	1 (9)	1 (9)	2 (18)	9 (9)	
General disorders and administration site conditions	8 (73)	7 (64)	4 (36)	3 (27)	4 (36)	8 (73)	8 (73)	6 (55)	5 (45)	53 (54)	
Fatigue	0 (0)	4 (36)	2 (18)	1 (9)	2 (18)	1 (9)	3 (27)	2 (18)	4 (36)	19 (19)	
Injection-site bruising	0 (0)	2 (18)	0 (0)	1 (9)	0 (0)	0 (0)	0 (0)	2 (18)	1 (9)	6 (6)	
Injection-site erythema	7 (64)	1 (9)	1 (9)	0 (0)	1 (9)	7 (64)	7 (64)	2 (18)	1 (9)	27 (27)	
Injection-site pain	3 (27)	1 (9)	1 (9)	0 (0)	2 (18)	7 (64)	5 (45)	2 (18)	0 (0)	21 (21)	
Injection-site edema	2 (18)	0 (0)	0 (0)	0 (0)	0 (0)	2 (18)	0 (0)	0 (0)	0 (0)	4 (4)	
Malaise	1 (9)	3 (27)	1 (9)	0 (0)	0 (0)	2 (18)	1 (9)	1 (9)	0 (0)	9 (9)	
Pyrexia	1 (9)	1 (9)	2 (18)	2 (18)	2 (18)	3 (27)	1 (9)	0 (0)	1 (9)	13 (13)	
Rigors	1 (9)	2 (18)	0 (0)	0 (0)	0 (0)	0 (0)	1 (9)	0 (0)	0 (0)	4 (4)	
Infections and infestations	0 (0)	1 (9)	2 (18)	0 (0)	0 (0)	1 (9)	0 (0)	1 (9)	0 (0)	5 (5)	
Injury, poisoning, procedural complications	0 (0)	2 (18)	0 (0)	3 (27)	3 (27)	2 (18)	0 (0)	1 (9)	0 (0)	11 (11)	
Investigations	4 (36)	2 (18)	2 (18)	1 (9)	2 (18)	1 (9)	1 (9)	0 (0)	2 (18)	15 (15)	
Body temperature increased	2 (18)	2 (18)	1 (9)	0 (0)	2 (18)	1 (9)	0 (0)	0 (0)	0 (0)	8 (8)	
Musculoskeletal and connective tissue disorders	3 (27)	4 (36)	6 (55)	5 (45)	7 (64)	8 (73)	3 (27)	4 (36)	6 (55)	46 (46)	
Back pain	0 (0)	0 (0)	0 (0)	0 (0)	2 (18)	2 (18)	0 (0)	0 (0)	3 (27)	7 (7)	
Myalgia	2 (18)	3 (27)	5 (45)	2 (18)	3 (27)	4 (36)	3 (27)	3 (27)	2 (18)	27 (27)	
Nervous system disorders	3 (27)	6 (55)	7 (64)	5 (45)	7 (64)	8 (73)	6 (55)	5 (45)	7 (64)	54 (55)	
Headache NOS	3 (27)	6 (55)	7 (64)	5 (45)	7 (64)	8 (73)	6 (55)	5 (45)	7 (64)	54 (55)	
Reproductive system and breast disorders	1 (9)	1 (9)	1 (9)	0 (0)	0 (0)	2 (18)	0 (0)	0 (0)	1 (9)	6 (6)	
Respiratory, thoracic and mediastinal disorders	1 (9)	2 (18)	1 (9)	2 (18)	1 (9)	1 (9)	2 (18)	1 (9)	1 (9)	12 (12)	
Sinus congestion	0 (0)	2 (18)	0 (0)	1 (9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3)	
Skin and subcutaneous tissue	2 (18)	1 (9)	1 (9)	1 (9)	1 (9)	5 (45)	4 (36)	2 (18)	0 (0)	17 (17)	
Pruritus NOS	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (9)	2 (18)	0 (0)	0 (0)	3 (3)	

NOTE. CV-JE , ChimeriVax-JE; NOS, not otherwise specified; YF-VAX, yellow fever 17D vaccine.

[a] 5.8 $\log_{10}$ pfu of CV-JE (day 0) and 5.8 $\log_{10}$ pfu of of CV-JE (day 30).
[b] 4.8 $\log_{10}$ pfu of CV-JE (day 0) and 4.8 $\log_{10}$ pfu of CV-JE (day 30).
[c] 3.8 $\log_{10}$pfu of CV-JE (day 0) and 3.8 $\log_{10}$ pfu of CV-JE (day 30).
[d] 2.8 $\log_{10}$ pfu of CV-JE (day 0) and 2.8 $\log_{10}$ pfu of CV-JE (day 30).
[e] 1.8 $\log_{10}$ pfu of CV-JE (day 0) and 1.8 $\log_{10}$ pfu of CV-JE (day 30).
[f] 4.8 $\log_{10}$ pfu of CV-JE) (day 0) and YF-VAX (day 30).
[g] YF-VAX (day 0) and 4.8 $\log_{10}$ pfu of CV-JE (day 30).
[h] Placebo and 4.8 $\log_{10}$ pfu of CV-JE (day 30).
[i] 4.8 $\log_{10}$ pfu of CV-JE (day 0) and placebo (day 30).
[j] Fisher's exact test.

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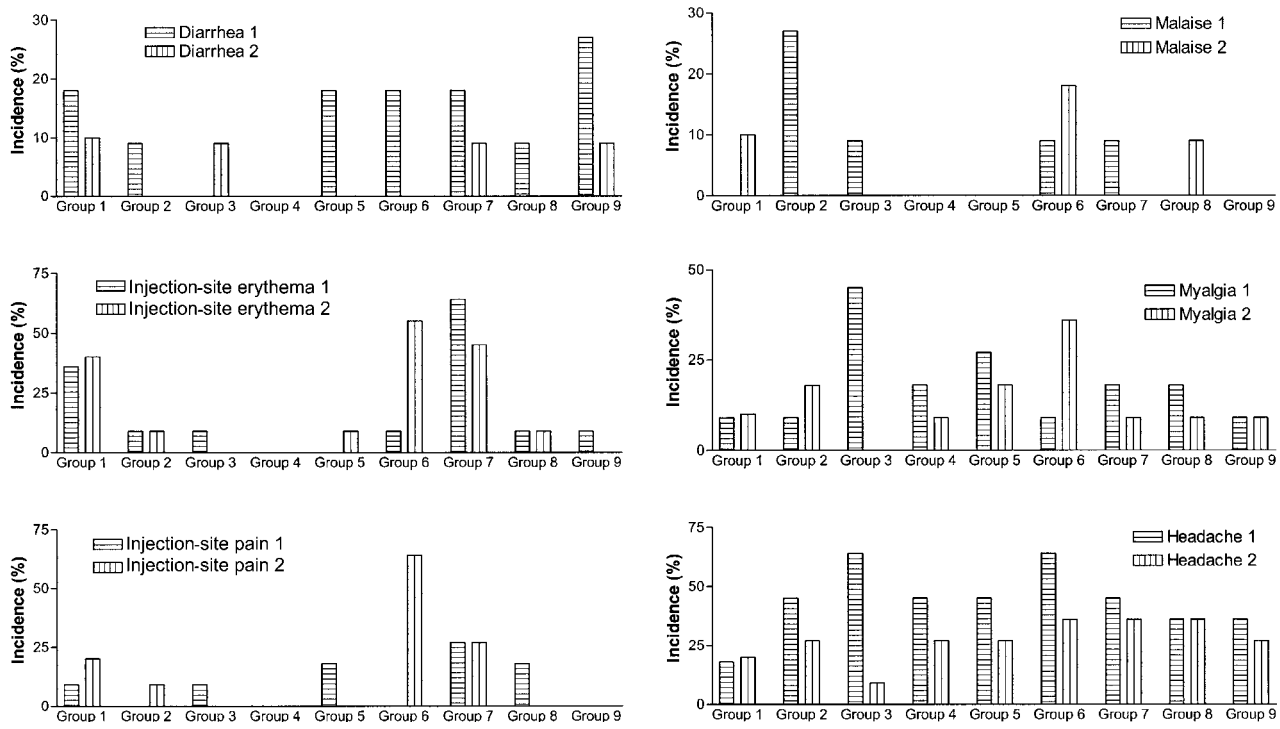

Figure 1. Study 1: incidence of the most common treatment-emergent adverse events, after the first and second inoculations

antibody response to ChimeriVax-JE and found no correlations (data not shown).

Neutralizing antibody response to ChimeriVax-JE vaccine. The primary end point of the study was the seroconversion rate to JE in subjects who were seronegative at baseline. The homologous JE virus strain (ChimeriVax-JE) was used as the challenge virus in LNI tests for measuring the primary end point.

None of the subjects in the trial had detectable neutralizing antibodies to JE virus at baseline. Eighty-two (94.3%) of 87 subjects who underwent primary immunization with a single inoculation of ChimeriVax-JE, at all dose levels (groups 1–6, 8, and 9), seroconverted to JE within 30 days, as determined by neutralization test (table 4). Seroconversion rates varied between 82% and 100%, between a range of doses of 1.8–5.8 $\log_{10}$ pfu, without relationship to dose (table 4). By day 14, 48 (55%) of 87 subjects in all dose groups had seroconverted, and, by day 21, 76 (87%) of 87 subjects were seropositive. Seroconversion possibly occurred earlier in the groups receiving higher doses of ChimeriVax-JE than in those receiving lower doses (figure 3). On day 14, 18% and 27% of subjects receiving 2.8 and 1.8 $\log_{10}$ pfu were seropositive, compared with 45%–75% in higher dose groups. ChimeriVax-JE rarely elicited a cross-reactive neutralizing antibody response (table 4).

Because seroconversion rates exceeded 90% in all dose groups (table 4), it was difficult to accurately calculate the ED_{50} or ED_{90} values or the confidence intervals for these values. Since the proportion of subjects who seroconverted to JE was so high, the dose-response logistic model did not fit very well. From the regression analysis, it was estimated that the ED_{90} value could be as low as 17.3 pfu and the ED_{50} value as low as 0.12 pfu, but these estimates should be considered to be unreliable.

A second inoculation of ChimeriVax-JE, at the same dose level administered 30 days after primary inoculation, had a modest effect on seroconversion rate. One subject in group 2 (4.8 $\log_{10}$ pfu) seroconverted after primary inoculation, seroreverted by day 30, and became seropositive again after receiving a booster inoculation. A second subject in this group did not seroconvert at the time of receiving either primary or booster inoculation. The only subject in group 3 (3.8 $\log_{10}$ pfu) who did not seroconvert at the time of primary inoculation developed a transient seroconversion 14 days after receiving a booster inoculation (day 44) but became seronegative again by day 60. Of the 2 subjects in group 4 (2.8 $\log_{10}$ pfu) who did not seroconvert after primary inoculation, 1 seroconverted after receiving a booster inoculation.

The magnitude of the neutralizing antibody response to ChimeriVax-JE was similar between dose groups (table 4). By 30 days after primary inoculation, mean LNI values ranged from 1.38 to 2.02. No statistical differences in mean antibody titers were found between dose groups ($\rho = .7070$, ANOVA). Antibody titers increased rapidly 2–3 weeks after primary inoculation, appeared to peak around day 30, did not increase after booster inoculation, and, in fact, tended to decrease slightly by day 60 (figure 4).

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Table 3. Proportion of subjects developing viremia, mean peak, duration and area under the curve (AUC), by treatment group (study 1).

Parameter, measure	ChimeriVax-JE dose, $\log_{10}$ pfu					YF-VAX (5.0)[b]	P[c]	P[d]
	5.8	4.8[a]	3.8	2.8	1.8			
No. of subjects	10	33	11	11	11	11		
Viremic on ⩾1 d, no. viremic/total (%)	5/10 (50)	22/33 (67)	9/11 (82)	11/11 (100)	9/11 (82)	7/11 (64)	.0999[e]	.0180[f]
Peak viremia, pfu/mL								
Mean	7.0	13.0	16.4	40.9	18.2	21.8	.1293[g]	.0646
SD	8.2	13.3	14.3	62.8	14.0	28.2		
Range	0–20	0–40	0–50	0–220	0–50	0–80		
Duration of viremia, d								
Mean	0.9	1.6	1.4	2.7	2.2	1.2	.0652	.0547
SD	1.3	1.5	1.1	1.7	1.7	1.2		
Range	0–4	0–5	0–3	1–6	0–5	0–3		
AUC								
Mean	10.0	21.7	21.4	75.0	34.1	38.2	.1367	.0717
SD	14.1	24.1	18.2	119.2	33.6	60.0		
Range	0–40	0–100	0–50	5–410	0–110	0–180		

[a] Eleven subjects from groups 2, 6, and 9 (table 1).
[b] Nominal dose indicated by manufacturer.
[c] Difference between treatment groups.
[d] Difference between ChimeriVax-JE treatments.
[e] Two-tailed Fisher's exact test.
[f] Two-sided Cochran-Armitage Trend test.
[g] Kruskall-Wallis test (applied to all tests for differences in mean peak, duration, and AUC).

Neutralizing antibodies to wild-type JE virus strains. PRNTs were performed against ChimeriVax-JE and 3 wild-type JE virus strains (table 5). All subjects were seronegative (titer <10) at baseline, except for 1 subject in group 8 (placebo) who had a titer of 12 against Beijing-1 virus. A high proportion of subjects in all groups receiving ChimeriVax-JE developed neutralizing antibodies to all JE virus strains by day 30. There was no dose effect for the primary end point, and 91%–100% of subjects inoculated with the lowest dose (1.8 $\log_{10}$ pfu) seroconverted to all virus strains.

In all dose groups, geometric mean neutralizing antibody titers were 2–10-fold higher against the homologous virus (ChimeriVax-JE) than against the wild-type viruses, and the differences were statistically significant (table 5). In individual subjects, antibody levels varied greatly, with some subjects having low and others having very high antibody titers, often broadly reactive with all 4 viruses. The latter observation raised the question of whether subjects with very high levels of antibody responses could have been primed by previous exposure to a heterologous flavivirus endemic in the United States, particularly St. Louis encephalitis (West Nile virus was not present in the region represented by the study population at the time of the trial). To examine this possibility, we compared the kinetics of the immune response in subjects with and without very high and cross-reactive neutralizing antibody responses to JE; this was done by examining the ChimeriVax-JE LNI on day 14, the earliest sample tested after inoculation. In only 1 case (1 subject in group 3) was the response on day 14 suggestive of an anamnestic response in a primed individual (data not shown).

Interactions between YF-VAX and ChimeriVax-JE. Seven subjects had YF-neutralizing antibodies at baseline (3 in group 1 [5.8 $\log_{10}$ pfu of ChimeriVax-JE], 2 in group 5 [1.8 $\log_{10}$ pfu of ChimeriVax-JE], 1 in group 7 [YF-VAX], and 1 in group 8 [placebo]) (table 4), presumably due to inoculation during military service, although no history of inoculation was elicited during screening. Inoculation in the YF-immune subjects occurred at a remote time before randomization in the trial, but the interval was not defined. The viremia and anti-JE antibody responses of YF-immune subjects to inoculation with ChimeriVax-JE were compared and did not differ from those of YF-naive subjects (data not shown).

Similarly, the neutralizing antibody response to ChimeriVax-JE was not influenced by intentional inoculation against YF performed 30 days before (table 6). All 11 YF-naive subjects in group 6 and 10 (91%) of 11 YF-immune subjects in group 7 developed JE antibodies after inoculation with ChimeriVax-JE ($P = 1.000$, Fisher's exact test). The mean ± SD LNI to Chi-

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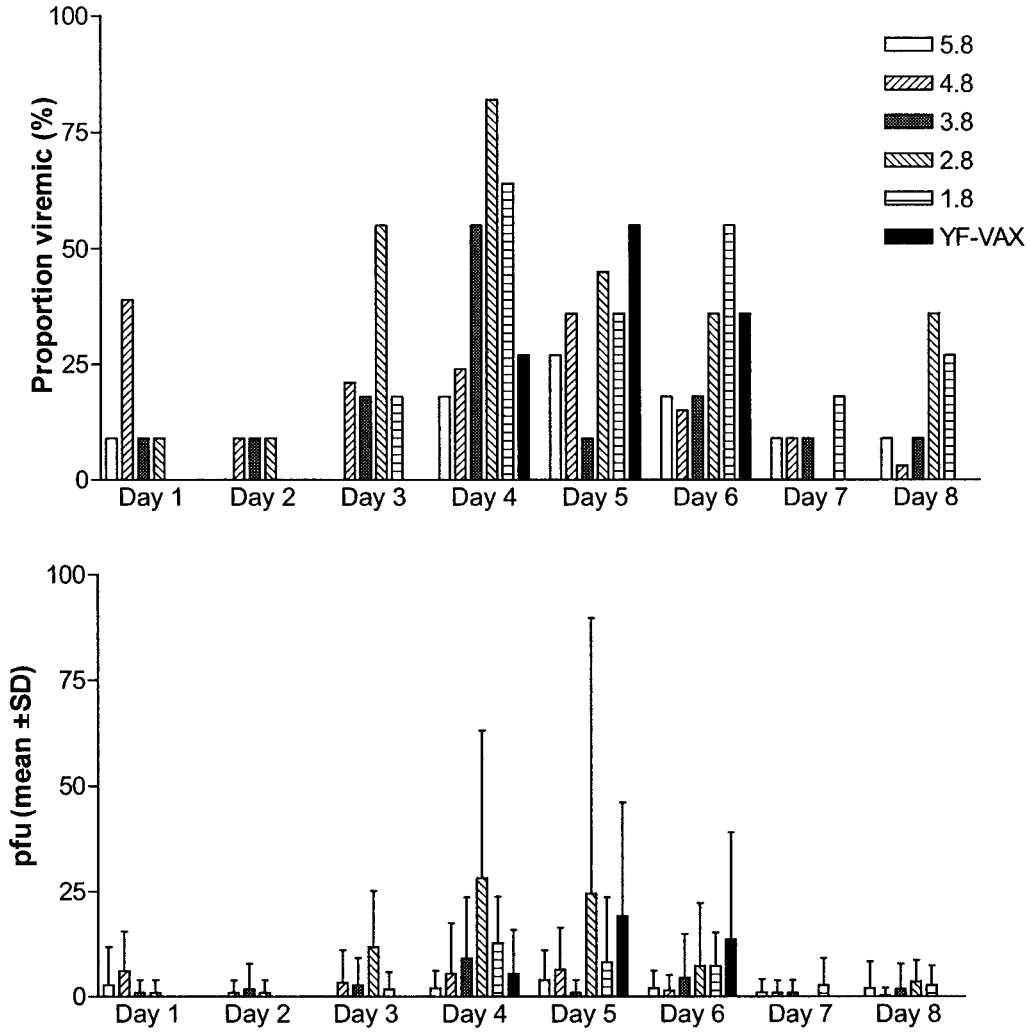

Figure 2. Study 1: proportion of subjects with detectable viremia *(top)* and mean viremia (pfu/mL) *(bottom),* by study day and treatment group. YF-VAX, yellow fever 17D vaccine.

meriVax-JE 30 days after inoculation in YF-naive subjects (1.92 ± 1.06) was not significantly different from that in YF-immune subjects (1.42 ± 1.11; $\rho = .0935$, *t* test).

Subjects in group 6 received ChimeriVax-JE, followed by YF-VAX 30 days later (table 6). There was a suggestion that previous inoculation of ChimeriVax-JE diminished the serological response to YF 17D. Sixty-four percent of ChimeriVax-JE–immune subjects (group 6) seroconverted to YF, compared with 91% of ChimeriVax-JE–naive subjects (group 7); the difference was, however, not statistically significant ($\rho = .3108$, Fisher's exact test). The mean ± SD LNI to YF 30 days after inoculation was lower in ChimeriVax-JE–immune subjects (group 6; 1.59 ± 1.47) than in ChimeriVax-JE–naive subjects (group 7; 2.29 ± 1.03), but, again, the difference was not statistically significant ($\rho = .2256$, *t* test).

Study 2. This open-label study was performed to assess the memory-immune response of previously inoculated subjects to challenge with JE antigen (JE-VAX). A total of 20 healthy adults were enrolled, 10 of whom had been inoculated with ChimeriVax-JE (4.0 or 5.0 $\log_{10}$ pfu) ~9 months before [8] and 10 of whom were naive to ChimeriVax-JE. None of the subjects withdrew prematurely from the study. Seventy percent of subjects in both groups were women. Treatment groups were well matched with respect to age, race, height, weight, medical history abnormalities, baseline clinical laboratory tests, and vital signs.

Safety. Four (40%) previously exposed and 4 (40%) ChimeriVax-JE–naive subjects experienced at least 1 treatment-emergent AE during the study. Among previously exposed subjects, the only AE reported by 2 subjects was injection-site pain. All other AEs reported were experienced by 1 subject only and included single incidences of chills, headache, injection-site re-

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Table 4. Baseline seropositive rate, seroconversion rate, and log neutralization indices (LNI) to JE (homologous strain) and YF, 30 days after 1 dose of ChimeriVax-JE, yellow fever (YF) 17D vaccine (YF-VAX), or placebo.

	ChimeriVax-JE dose (log_{10} pfu)						
Virus used in neutralizing antibody test, measure	5.8	4.8	3.8	2.8	1.8	YF-VAX	Placebo
ChimeriVax-JE							
No. of subjects	10	44[a]	11	11	11	11	11
Seropositive on day 0, no. of subjects (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Seroconversion on day 30, no. of subjects (%)	10 (100)	42 (95)	10 (91)	9 (82)	11 (100)	0 (0)	0 (0)
LNI on day 30, mean	1.64	1.76	1.69	1.38	2.02	−0.08	−0.15
SD	0.90	1.08	1.30	0.94	0.86	0.22	0.14
Minimum	0.7	0.3	0.6	0.5	0.7	−0.3	−0.3
Maximum	3.1	5.2	4.9	3.6	3.5	0.4	0.0
YF 17D							
No. of subjects	10	44	11	11	11	11	11
Seropositive on day 0, no. of subjects (%)	3 (30)	0 (0)	0 (0)	0 (0)	2 (18)	1 (9)	1 (9)
Seroconversion on day 30, no. of subjects (%)[b]	0 (0)	1 (2)	0 (0)	0 (0)	1 (9)	10 (91)[c]	0 (0)
LNI on day 30, mean	0.75	−0.02	−0.19	−0.14	0.45	2.29	0.02
SD	1.53	0.68	0.08	0.10	1.48	1.13	0.93
Minimum	−0.3	−0.5	−0.3	−0.3	−0.4	0.0	0.0
Maximum	3.7	3.1	−0.1	0.0	4.3	4.1	2.8

[a] Includes groups 2, 6, and 9 (day 30) and group 8 (day 60) (table 1).
[b] Proportion of subjects who are seropositive or seronegative on day 0 and demonstrate an increase in YF LNI of ⩾0.7 by day 30.
[c] The subject seropositive on day 0 seroconverted; 1 subject, who was seronegative on day 0, did not seroconvert.

action, vasodilation, nausea, vomiting, agitation, and skin disorder. Among naive subjects, the only AE reported by 2 subjects was injection-site reaction. All other AEs reported were experienced by 1 subject only and included single incidences of headache, pain, rhinitis, and dysmenorrhea. All AEs reported during the study were mild or moderate in intensity; no severe events were reported. No deaths were reported during the study.

Immune response. Subjects with previous exposure to ChimeriVax-JE had enhanced neutralizing antibody responses to challenge with a single dose of JE-VAX, compared with those of ChimeriVax-JE–naive subjects (figure 5). Anamnestic response was assessed by comparing seroconversion rates and mean neutralizing antibody titers, for multiple time points after inoculation, of the previously exposed subjects with those of the ChimeriVax-JE–naive subjects. Responses of preimmune subjects to challenge were significantly different from those of ChimeriVax-JE–naive subjects (figure 5).

Neutralizing antibody titers to ChimeriVax-JE and to wild-type viruses were also measured by $PRNT_{50}$, at baseline and at 30 days after administration of JE-VAX. The analysis revealed that subjects who had received primary inoculation of ChimeriVax-JE and booster inoculations with JE-VAX (Nakayama strain) responded with higher titers against ChimeriVax-JE than against either Nakayama or the other wild-type strains, illustrating the phenomenon of "original antigenic sin." The ChimeriVax-JE–naive subjects receiving JE-VAX also developed the highest levels of neutralizing antibodies against the homologous virus (Nakayama) (figure 6), consistent with other studies [16].

DISCUSSION

ChimeriVax-JE was well tolerated at all dose levels. There were no serious AEs related to study medication. Since there were no active infection–indicating differences between dose groups, in virological or serological results, the safety results for these subjects may be combined to estimate the probability of observing an uncommon AE. With the sample size of 99 subjects, an upper bound of 3% was established for the 95% confidence interval for the incidence of an AE that was not observed in this trial.

Most subjects experienced at least 1 treatment-emergent AE (table 2). Between the treatment groups, there was no statistically significant difference in the number of subjects with treatment-emergent AEs after either the first or the second inoculation. Indeed, the incidence of any AE after the first inoculation in the placebo group (group 9) was similar to that in the active-treatment groups. Both between treatment groups and overall, the incidence of AEs was higher after the first inoculation than it was after the second inoculation. This finding suggests that many AEs were causally related, since immunity following the first inoculation would have prevented AEs associated with active virus replication. However, it is also

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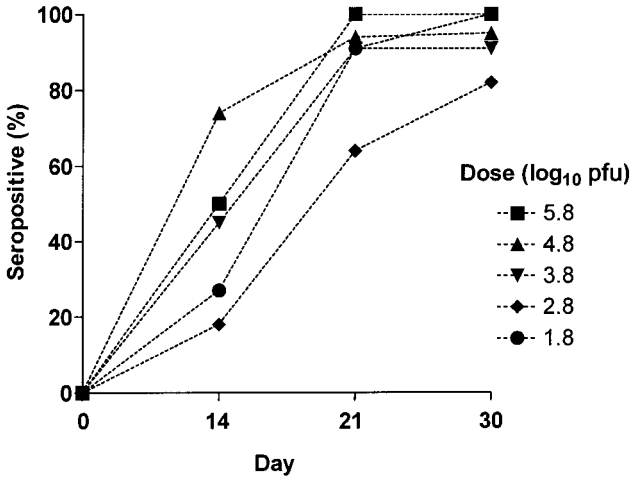

Figure 3. Study 1: seroconversion rate, by day after primary inoculation with graded doses of ChimeriVax-JE. The data on the 4.8 log_{10}-pfu dose combine groups 2, 6, and 9, after the first inoculation, and group 9, after the second inoculation (see table 1).

likely that subjects were more inclined to report AEs during the initial phase of the clinical study.

Injection-site erythema and injection-site pain were the most commonly reported local AEs, reported by 27 (27%) of 99 and 21 (21%) of 99 subjects, respectively. The incidence of both AEs was highest in group 1 (subjects who received the highest dose of ChimeriVax-JE) and groups 6 and 7 (subjects who received YF-VAX as the second or first dose, respectively) (figure 1). All reports of injection-site erythema and injection-site pain were considered to be at least possibly related to the study vaccine. Reactions were generally mild to moderate, except in 6 subjects who received YF-VAX, all of whom had erythema >1 inch in diameter (designated as "severe" in this study). Local reactions were very infrequent in subjects receiving primary inoculation of $\leq$4.8 log_{10} pfu of ChimeriVax-JE, and there was no evidence of an increase in reactogenicity or for allergic phenomena at the time of reinoculation. In a recent, large clinical study of YF vaccines, injection-site inflammation and injection-site pain were noted in 29% and 39% of subjects, respectively, receiving YF-VAX [11], a finding consistent with the present study. These reactions were less frequent in subjects with preexisting YF immunity [11], a finding suggesting that they were related to active virus replication rather than to impurities in the vaccine. However, in the present study, a decrease in injection-site erythema was not observed in the ChimeriVax-JE group receiving a second dose of 5.8 log_{10} pfu (figure 1), a finding suggesting that components other than infectious virus may have elicited the response in the high-dose group, although these were diluted in the lower-dose groups. Injection-site reactions also appeared to be more frequent in subjects who were inoculated with ChimeriVax-JE 30 days after receiving YF-VAX (group 7; figure 1). The latter finding will require further study in future vaccine-interaction trials but may represent recruitment of inflammatory cells due to antigenic cross-reactivity or possibly to enhanced local virus replication (immune enhancement).

Headache and myalgia were the most commonly reported systemic AEs; these AEs are those that are the most often associated with YF vaccines [11] and are likely due to the release of cytokines, such as interferon-α and tumor necrosis factor–α [17, 18]. However, the incidence of headache and myalgia was similar in many active-treatment groups and in the placebo groups (groups 8 and 9), a finding suggesting that many events were unrelated to study medication. Headache and myalgia were mild to moderate in nearly all subjects. Severe myalgia, which was considered by the investigator to be probably related to the study vaccine, was reported for only 1 subject, in group 5 (1.8 log_{10} pfu of ChimeriVax-JE).

Viremia was an objective measure of vaccine safety in this study, in particular the comparison of viremia in subjects inoculated with ChimeriVax-JE and that in subjects inoculated with YF-VAX. Viremia reflects the level of replication in extraneural tissues and is regarded as a measure of viscerotropism of YF vaccine virus [19]. However, since virus levels in blood reflect both the countervailing forces of virus release from infected cells and rapid virus clearance by the reticuloendothelial system [20], the daily measurements made in this study provide only a "snapshot" of the dynamics of virus replication in the host. The cells and tissues involved in replication of YF 17D virus and ChimeriVax-JE, in the host, are unknown and may differ, since tropism is determined by the viral envelope.

Viremia levels were low in all treatment groups, with the highest viremia being 2.3 log_{10} pfu/mL. When all treatment groups were considered, there was no statistical evidence of a difference between treatment groups, for any of the viremia summary measures (table 3). The proportion viremic, peak viremia, duration, and AUC for ChimeriVax-JE–treated subjects were similar to those for YF-VAX–treated subjects. However, the temporal pattern of viremia differed between groups receiving YF-VAX and those receiving ChimeriVax-JE. YF-VAX induced a consistent pattern of viremia on days 3–5, consistent with data published elsewhere [8, 17, 21], whereas ChimeriVax-JE tended to induce intermittent viremia, with some subjects positive as early as day 1 and as late as day 8. A recent study using a sensitive reverse-transcriptase polymerase chain reaction demonstrated YF 17D virus in blood for longer periods than were detectable by infectivity (plaque) assays [17]. It is likely that the use of a similar method for detection of ChimeriVax-JE would fill in the gaps between intermittent viremic determinations and would reveal a more-accurate temporal picture of active virus replication.

When only the ChimeriVax-JE doses were considered, the

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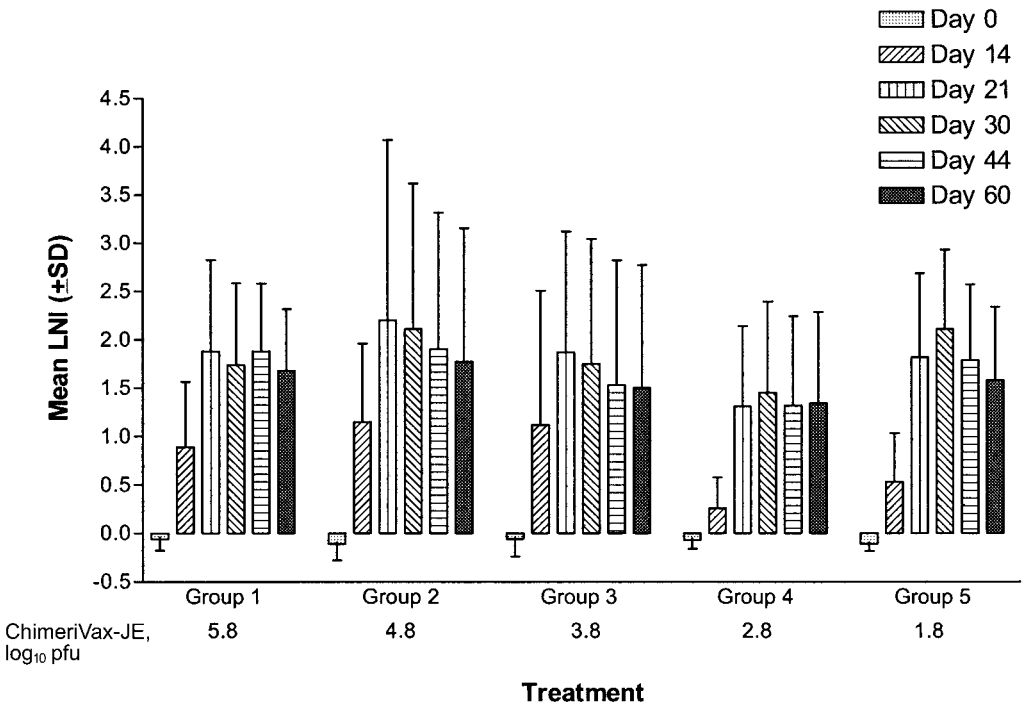

Figure 4. Study 1: mean $\log_{10}$ neutralizing antibody titers, by day after inoculation of ChimeriVax-JE (groups 1–5), showing kinetics of the immune response after the first dose (day 0) and lack of a booster effect after the second dose, on day 30. To show the effect of booster inoculations, data for subjects receiving 4.8 $\log_{10}$ pfu in this figure are limited to group 2. LNI, $\log_{10}$ neutralization index.

differences between the dose groups were either significant or approached statistical significance (table 3). The proportion viremic, peak titer, and duration tended to be greater in subjects inoculated with the lower doses of ChimeriVax-JE, particularly 2.8 $\log_{10}$ pfu. The inverse relationship between dose and virus replication (as reflected by viremia) is not unexpected, on the basis of observations with both YF 17D and ChimeriVax-JE vaccines [8]. Smith et al. [22] reported significantly higher antibody responses in humans administered very low doses of YF 17D than in those administered high doses. In rhesus monkeys, viremia was inconsistent and was lower in magnitude and briefer in duration after high doses of YF 17D than after inoculation of diluted virus [23]. This dose prozone effect is also clearly evident in mice [6]. It is possible that high doses induce stronger innate immune responses, which limit virus replication.

Viremia was not measured after the second dose, and, thus, the effect of previous YF inoculation on virus replication, estimated by viremia after inoculation with ChimeriVax-JE, was not defined. However, 5 subjects in groups 1 (5.8 $\log_{10}$ pfu of ChimeriVax-JE) and 5 (1.8 $\log_{10}$ pfu of ChimeriVax-JE) were YF-immune at baseline. Viremic responses in these subjects were similar to those in YF-naive subjects. There was no evidence that previous YF immunity enhanced or inhibited the ChimeriVax-JE infection in YF-immune subjects.

The immunogenicity of ChimeriVax-JE was similar to that of the parental YF 17D vaccine used to construct the chimeras. Overall, 82 (94%) of 87 subjects in all groups (groups 1–6, 8, and 9) receiving primary inoculations of ChimeriVax-JE developed antibodies to the homologous virus (ChimeriVax-JE) (table 4). When the $PRNT_{50}$ assay was used, 86 (99%) of 87 subjects seroconverted (table 5). In the group receiving YF-VAX, 10 (91%) of 11 subjects seroconverted, determined by the LNI test, to the homologous virus (YF 17D). Thus, the immune response to ChimeriVax-JE closely approximates that to YF 17D vaccine.

There was no statistical evidence of a linear dose response to ChimeriVax-JE. From a regression analysis, it was estimated that the ED_{90} value was ~17 pfu. Previous studies of YF 17D vaccines in human subjects indicate that, when dose response is considered, YF vaccine may actually be slightly less immunogenic than ChimeriVax-JE. Fox et al. [24] found that 70% of subjects seroconverted at a dose of 14 adult-mouse intracerebral LD_{50} (estimated to be ~700 pfu). Other published studies [25, 26] indicate that doses of 100–200 pfu result in seroconversion of >90% of vaccinated individuals. A recent trial of YF 17D vaccine manufactured in the United Kingdom showed that 93% of subjects who were administered 200 pfu seroconverted (Acambis and Evans Vaccines; unpublished data, 1999).

The dose-response study also provided further evidence that responses of nonhuman primates predicted the clinical behavior of chimeric vaccines and YF 17D virus in humans. The

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Table 5. Seroconversion rate, geometric mean titer (GMT), 95% confidence intervals (CI), and minimum and maximum neutralizing antibody titers to JE (homologous and wild-type strains), 30 days after 1 dose of ChimeriVax-JE, yellow fever (YF) 17D vaccine (YF-VAX), or placebo (by 50% plaque-reduction neutralization method).

Virus used in neutralizing antibody test, measure	ChimeriVax-JE dose (log_{10} pfu)					YF-VAX (n = 11)	Placebo (n = 11)	P[b]
	5.8 (n = 10)	4.8 (n = 44)[a]	3.8 (n = 11)	2.8 (n = 11)	1.8 (n = 11)			
ChimeriVax-JE								
Seroconversion, %	100	100	100	91	100	9	0	
GMT	262[c]	299	210	103	285	7[c]	5[c]	.3277
95% CI	99–694	197–453	65–681	29–364	111–734	3–17	5–5	
Minimum, maximum	16, 2100	23, 10000	23, 24000	<10, 2500	35, 2200	<10, 330	<10, <10	
Beijing-1								
Seroconversion, %	70	82	100	73	100	9	9[e]	
GMT	21	31	35	19	74	6	6	.0881
95% CI	9–51	21–45	12–99	9–39	39–140	4–11	5–8	
Minimum, maximum	<10, 120	<10, 310	10, 3000	<10, 130	12, 330	<10, 82	<10, 12[f]	
Nakayama								
Seroconversion, %	80	86	91	73	91	0	0	
GMT	38	61	83	40	132	5	5	.4212
95% CI	16–90	39–97	17–415	11–140	34–516	5–5	5–5	
Minimum, maximum	<10, 140	<10, 1300	<10, 40000	<10, 2200	<10, 3000	<10, <10	<10, <10	
902/97								
Seroconversion, %	100	89	82	91	100	18	0	
GMT	59	85	133	71	160	6	5	.6490
95% CI	29–119	50–142	22–792	22–223	52–488	5–8	5–5	
Minimum, maximum	10, 150	<10, 2600	<10, 70000	<10, 3600	13, 2300	<10, 16	<10, <10	
P[d]	<.0001	<.0001	<.0001	<.0001	.0098			

[a] Includes groups 2, 6, and 9 (day 30) and group 8 (day 60) (table 1).
[b] Differences between treatments, ChimeriVax-JE–treatment groups only (analysis of variance [ANOVA]).
[c] Values <10 were assigned the number 5, for calculating GMT.
[d] Differences between antibody titers, by treatment group (ANOVA).
[e] One subject in the placebo group had no detectable antibody at day 0 and a titer of 10 on day 30.
[f] Seropositive on day 0.

viremia and antibody responses to graded doses of ChimeriVax-JE in rhesus monkeys [7] were similar to those in humans. Low doses ($\leq$2 log_{10} pfu) of vaccine induced antibodies in humans and monkeys and were associated with a longer time to peak response than were high doses of vaccine. The availability of an animal model that both responds to vaccine in a way that is similar to the way humans respond and is susceptible to disease after challenge with wild-type virus [7, 27] is especially important. Regulatory approval of novel chimeric vaccines against JE and West Nile viruses may be based on efficacy in animal models, as a surrogate for field trials, as proposed by Markoff [28].

Administration of a second dose of ChimeriVax-JE, 30 days after primary inoculation, did not result in a boost in antibody titers, measured by LNI or $PRNT_{50}$ (figure 4). It appeared that the initial dose provided sterilizing immunity. There are no previous reports of reinoculation with a live flavivirus vaccine at such a short interval. In the case of YF 17D, reinoculation at longer intervals (generally years) is associated with a blunted response, compared with that of primary inoculation, the occurrence of a booster-inoculation response being more likely in individuals with low neutralizing antibody titers. In one study [22], only 1 of 8 subjects who were inoculated 1–14 years before and who had LNIs between 2.1 and 3.6 developed an increase in levels of neutralizing antibodies after reinoculation. In a second study [29], 7 (64%) of 11 subjects reinoculated 14 months after primary inoculation had developed an increase in antibody titer, but, in most subjects, the response was lower than that to primary inoculation. We previously reported that only 3 of 6 subjects reinoculated with YF-VAX after a 9-month interval developed increases in levels of neutralizing antibodies [8]. In both the present and the earlier studies, neutralization of the live virus inoculum by preformed antibody is the likely mechanism limiting the response to reinoculation. The kinetics of JE virus neutralization in the presence of serum complement are extremely rapid [30]. At 30 days after primary inoculation,

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Table 6. Effect of previous inoculation with yellow fever (YF) 17D vaccine (YF-VAX) on the neutralizing antibody response to ChimeriVax-JE and the reciprocal interaction (see text for statistical analyses).

First vaccine/second vaccine, group, measure	First vaccine (day 30)		Second vaccine (day 60)	
	CV-JE[a]	YF 17D	CV-JE[a]	YF 17D
4.8 $\log_{10}$ pfu of ChimeriVax-JE/YF-VAX, group 6 (n = 11)				
Seropositive, %[b]	100	0	91	64
LNI, mean	1.92	−0.15	1.61	1.59
SD	1.06	0.16	1.22	1.47
Minimum, maximum	0.9, 4.5	−0.5, 0.1	0.6, 4.8	−0.2, 3.6
YF-VAX/4.8 $\log_{10}$ pfu of ChimeriVax-JE, group 7 (n = 11)				
Seropositive, %[c]	0	91	91	91
LNI, mean	−0.08	2.29	1.42	2.27
SD	0.22	1.13	1.11	1.14
Minimum, maximum	−0.1, 0.4	0.0, 4.1	0.9, 4.4	−0.1, 3.7

[a] CV-JE, ChimeriVax-JE virus.
[b] All subjects were seronegative on day 0.
[c] One subject who was YF seropositive on day 0 and had a significant increase in LNI after receiving a booster inoculation of YF-VAX is included in the analysis.

neutralizing antibody levels and cytotoxic T lymphocyte responses would have peaked and would be able to sterilize the booster inoculum. Longer intervals would be expected to allow sufficient virus replication for boosting immunity. It is also possible that the 30-day interval between primary and booster inoculations, in the present study, may not have been sufficient to optimize memory T and B cell responses, since memory cell populations appear late. Lee and Scherer [31] investigated the booster responses to JE virus in immune hosts exposed to the bite of infected mosquitoes. A boost in immunity did not depend on replication of live virus, and even a low dose of viral antigen induced an increased antibody response. However, memory B cells depend on the expression of high-affinity receptors activated by low levels of antigen (e.g., in this case, the inoculum of ChimeriVax-JE neutralized by antibody), and the 30-day interval between primary and booster inoculations was likely insufficient for selection of high affinity B cell clones.

We used both the homologous virus (ChimeriVax-JE) and several wild-type JE virus strains to assess the neutralizing antibody response to inoculation. Antibody responses and seroconversion rates, to the homologous virus, were higher than those to the 3 wild-type strains used in the present study (table 5). The wild-type viruses belonged to the same genotype (III) as did SA14-14-2, the prM-E gene donor strain in ChimeriVax-JE. However, it is well known that antigenic variation occurs among strains of JE virus belonging to the same JE virus genotype [32]. In neutralization tests, inactivated mouse-brain JE vaccine produced from Nakayama or Beijing-1 strains (both genotype III) elicited stronger immune responses against the homologous strain than it did against the heterologous strain [33]. Antigenic differences tend to be greater between JE viral genotypes; neutralizing antibodies induced by Beijing-1 vaccine were significantly higher against Nakayama or Beijing-1 virus than against Thai strains, which presumably belonged to genotype II [4]. These observations have raised concerns about the ability of a JE vaccine prepared from 1 virus strain/genotype to protect against all strains/genotypes circulating in nature. However, there is no evidence that antigenic strain variation limits the protective efficacy of JE vaccines. In a large field trial, inactivated JE vaccine prepared from monovalent Nakayama or bivalent Nakayama-Beijing-1 virus was effective against Thai strains [34]. The protective activity of JE vaccines probably depends on the memory-immune response after exposure to the bite of an infected mosquito. Studies of vaccinated mice have demonstrated that protection is dependent on the anamnestic postchallenge neutralizing antibody response [35]. Studies of monkeys inoculated with ChimeriVax-JE and challenged with virulent JE virus, by the intracerebral route, also suggest that the postchallenge recruitment of immunologically specific cells into the brain, during the incubation period, is associated with protection [7]. The secondary immune response after challenge with JE virus is characterized by high-affinity antibodies and a broadening of the cross-reactivity between JE virus strains. This phenomenon undoubtedly underlies the dictum that immunity to 1 (vaccine) strain protects against disease caused by all other strains of JE virus [36].

The level of neutralizing antibodies required for protection against JE infection has not been defined in humans but may be inferred from observations of the inactivated mouse-brain vaccine and the Chinese SA14-14-2 live, attenuated vaccine, both of which have been proven to be efficacious in field trials [3, 34]. In preclinical tests, a single dose of the live, attenuated SA14-14-2 vaccine elicited neutralizing antibodies that fell to a very low level (1:5) after 6 months; however, animals were fully protected against challenge [37]. Moreover, protection was observed after challenge with a variety of heterologous wild-type JE strains [38]. In humans, a single dose of SA14-14-2 was effective in a field trial in Nepal [39], and the neutralizing antibody response to a single inoculum of SA14-14-2 vaccine is characterized by low geometric means of antibody titers (~1:20) [33], lower than we observed for heterologous JE virus strains after inoculation with ChimeriVax-JE in the present study. In the case of the inactivated mouse-brain vaccines that have controlled JE disease in much of Asia, the levels of preexposure antibody that appear to be protective are even lower than observed in the case of live vaccine. The 2-dose primary series of inactivated JE vaccine elicits a geometric mean antibody titer of 1:28 [16]. After 3 doses, the titer is higher (1:

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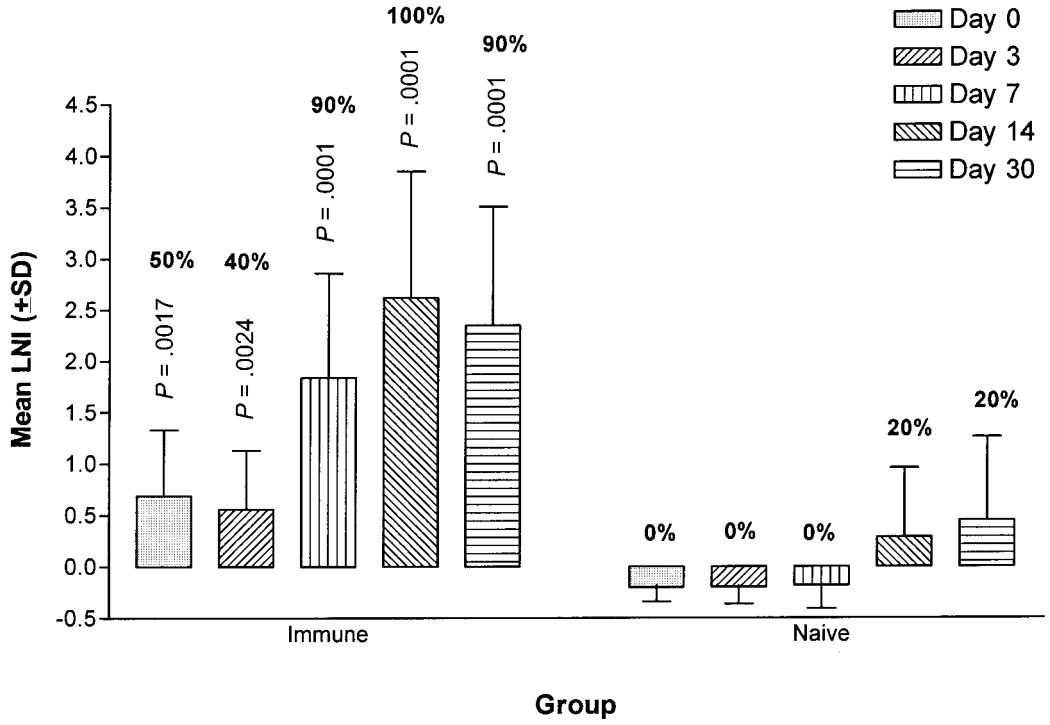

Figure 5. Study 2: mean neutralizing antibody titers (vs. ChimeriVax-JE), by day after inoculation of JE Nakayama vaccine, in subjects with and without previous inoculation with ChimeriVax-JE. The proportion (%) of seropositive subjects is shown above each bar. *P* values are shown for 2-sample *t* tests comparing preimmune subjects and naive subjects, at each time point. LNI, $\log_{10}$ neutralization index; $PRNT_{50}$, 50% plaque-reduction neutralization test.

141), but titers wane rapidly, falling below 1:8 after 6–12 months [16]. Also, as mentioned above, the inactivated product elicits significantly lower levels of antibody responses to heterologous JE virus strains than to the antigen (Nakayama) used to produce the vaccine. Finally, in a study of rhesus monkeys immunized with ChimeriVax-JE, levels of neutralizing antibody responses to wild-type JE virus strains were lower than those

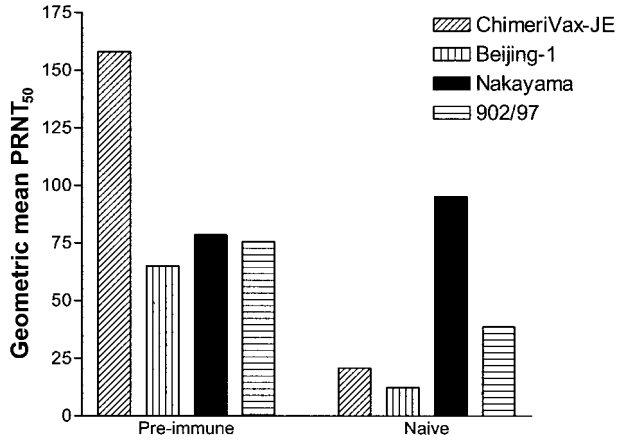

Figure 6. Study 2: geometric mean 50% plaque-reduction neutralization test ($PRNT_{50}$) titers to ChimeriVax and wild-type JE virus strains, after challenge with inactivated JE Nakayama vaccine (JE-VAX).

to the homologous virus (ChimeriVax-JE) and were in the same range as those seen in our clinical trial [7]. Nevertheless, monkeys with neutralizing antibody titers to the wild-type challenge strain as low as 1:20 were solidly protected against a severe (intracerebral) challenge [7]. Thus, ChimeriVax-JE appears to elicit a neutralizing antibody response in humans, a response that would be expected to be as protective or more protective than that elicited by existing vaccine products.

There was considerable individual variation in the neutralizing antibody response to ChimeriVax-JE, with some individuals developing very high and others developing low levels of antibody responses (see minimum and maximum data in tables 4 and 5). Genes controlling the 2′5′oligoA synthetase system that are responsible for cellular resistance to flaviviruses in the JE antigenic complex have been described in animals [40, 41]. However, we have found no correlation between antibody titer and the level or duration of viremia, reflecting the level of virus replication in the host, and thus our data suggest that genetic factors controlling susceptibility to flavivirus infection were not responsible for the variation in immune response. However, the adaptive immune response to flavivirus infection appears to be under genetic control in humans [42], and genetic factors underlie T and B cell responses to many viral infections and vaccines [43]. Thus, it is likely that the marked individual var-

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iability in neutralizing antibody responses to ChimeriVax-JE was also due to genetic determinants. In only 1 subject, who had an accelerated antibody response, was there evidence that priming with a heterologous flavivirus, probably St. Louis encephalitis (a member of the JE antigenic complex), might have enhanced the immune response to the vaccine. However, immunity to the more distantly related YF virus did not have a significant effect on anti-JE responses.

Because ChimeriVax-JE contains genes encoding YF antigens that may induce antivector immunity, we studied the interactions of ChimeriVax-JE and YF-VAX, in both directions. We showed elsewhere that YF vaccination 9 months before inoculation of ChimeriVax-JE did not interfere with viremia or antibody response [8]. In the present study, some subjects had preexisting YF antibodies, but, with respect to viremia and neutralizing antibodies against JE, their response to ChimeriVax-JE vaccine was identical to that of YF-naive subjects. We also intentionally preimmunized subjects with YF-VAX 30 days before a second inoculation of ChimeriVax-JE. This interval was selected because it is generally recommended that live viral vaccines be administered either simultaneously or separated by 30 days. Previous administration of YF-VAX did not interfere with either seroconversion or antibody titer to ChimeriVax-JE. However, when subjects were first administered ChimeriVax-JE, followed by YF-VAX 30 days later, the seroconversion rate and the antibody titer to YF were somewhat reduced. Although the differences were not statistically significant, it is possible that a larger sample size would reveal a significant interference effect. Since the basis for interference is likely to be immune responses against antigens encoded by the YF virus backbone (antibodies against NS1 or cytotoxic T lymphocytes against NS3), it is not clear why the interference operated in only 1 direction. It is possible that ChimeriVax-JE induces a more active antivector response than does YF-VAX, since ChimeriVax-JE appeared more immunogenic than YF-VAX, on the basis of dose-response considerations (see discussion of ED_{90} above). T cell responses, which were not measured in this study, might shed light on the basis of the one-way interference effect. It will be interesting to define the anti-NS1 antibody and anti-NS3 T cell responses to YF in future trials. On the basis of previous studies, it is unlikely that the JE-specific anti-E protein immune responses elicited by ChimeriVax-JE cross-protected against YF 17D vaccine. Subjects with monotypic neutralizing antibodies against JE had equivalent neutralizing antibody responses after inoculation with YF 17D as did JE-naive subjects [44]. Moreover, viremia levels after inoculation with YF 17D were not different between subjects with JE immunity and those without JE immunity, a finding indicating that replication of YF 17D virus was not restricted [45]. Further studies of vaccine interactions will be necessary to elucidate vaccination schedules for chimeric viruses and YF 17D vaccine. It is likely that simultaneous inoculation or a longer interval between inoculations of ChimeriVax-JE and YF 17D will resolve any interference effects. Few individuals require both vaccines, since YF and JE viruses are endemic in distinct geographic areas.

The durability of the neutralizing antibody response to YF 17D is well known and is believed to be life-long in the majority of individuals, even without natural exposure to YF virus [5]. In study 2, we found that only 50% of subjects who had been successfully immunized 9 months before with ChimeriVax-JE had detectable neutralizing antibodies (figure 5), whereas all individuals had responded 30 days after the initial inoculation vaccination [8]. This finding, if confirmed in subsequent trials, suggests that ChimeriVax-JE induces protective immunity after a single dose, immunity sufficient for protection during travel or a single JE virus–transmission season, but that a booster inoculation may be required for longer-term protection.

In study 2, individuals who received a primary inoculation of ChimeriVax-JE exhibited immunological memory when challenged with JE Nakayama antigen (JE-VAX). As noted above, the anamnestic response to challenge infection is probably critical to protection against JE disease [35]. Thus, waning of the antibody response to primary infection does not necessarily imply that vaccinated individuals are not protected against natural infection. Similar observations have been made in the case of other live viral vaccines. For example, antibody responses to measles vaccine wane to low or undetectable levels over time, but protection against disease is life-long. As in our study, individuals without detectable measles antibodies respond to revaccination or natural infection with a typical anamnestic immune response [46].

In summary, ChimeriVax-JE appears to be well tolerated, with a safety profile and viremia pattern consistent with those of YF-VAX. In preclinical toxicology tests, the vaccine virus is significantly less neurovirulent than YF-VAX [7] and is expected to be less likely to cause vaccine-associated neurotropic accidents, a rare complication of YF-VAX. ChimeriVax-JE rapidly elicits high titers of neutralizing antibodies after a single inoculation at very low doses, an advantage over existing inactivated vaccines that require multiple doses. Additional clinical trials are planned to elucidate the safety and efficacy profile of this vaccine.

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