

PHASE II SAFETY AND IMMUNOGENICITY STUDY OF LIVE CHIKUNGUNYA VIRUS VACCINE TSI-GSD-218

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Abstract. We conducted a phase II, randomized, double-blind, placebo-controlled, safety and immunogenicity study of a serially passaged, plaque-purified live chikungunya (CHIK) vaccine in 73 healthy adult volunteers. Fifty-nine volunteers were immunized one time subcutaneously with the CHIK vaccine and 14 were immunized with placebo (tissue culture fluid). Vaccinees were clinically evaluated intensively for one month, and had repeated blood draws for serological assays (50% plaque-reduction neutralization test) for one year. Except for transient arthralgia in five CHIK vaccinees, the number and severity of local and systemic reactions and abnormal laboratory tests after immunization were similar in CHIK vaccinees and placebo recipients. Fifty-seven (98%) of 58 evaluable CHIK vaccinees developed CHIK neutralizing antibody by day 28, and 85% of vaccinees remained seropositive at one year after immunization. No placebo recipients seroconverted. This promising live vaccine was safe, produced well-tolerated side effects, and was highly immunogenic.

INTRODUCTION

Chikungunya virus (CHIK) is an alphavirus borne by *Aedes* mosquitoes that produces a dengue-like illness in humans, characterized by fever, rash, painful arthralgia, and sometimes arthritis.^{1–5} The virus is widely disseminated throughout sub-Saharan Africa, Southeast Asia, India, and the Western Pacific, and numerous epidemics have been reported in these areas.^{6–11} The widespread geographic distribution, recurrent epidemics, and infection of military personnel, travelers, and laboratory staff working with CHIK have indicated the need for a safe and efficacious vaccine.^{12–15} Individual strains of CHIK are closely related antigenically,^{9,16,17} and infection with one CHIK strain leads to protection against all strains.¹⁸ Reciprocal cross-protection after infection with other alphaviruses occurs in animal models,^{19,20} although it is unclear if similar cross protection occurs in humans sequentially exposed to natural infection or live alphavirus vaccines.²¹

An isolate from a patient in Thailand, CHIK strain 15561, was used to develop a small lot of vaccine first passaged in green monkey kidney (GMK) cells and then formalin-inactivated before administration to 16 volunteers.²² The vaccine produced no untoward reactions and was highly immunogenic. The current live vaccine (Lot 1-85, TSI-GSD-218) was developed at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) and was produced at the Salk Institute, Swiftwater, PA, from a lot of the GMK-passaged strain 15561 inactivated vaccine by subsequent serial passage in MRC-5 cells.²³ The live vaccine proved to be safe and immunogenic in a phase I trial in 15 alphavirus-naïve volunteers.²¹ The current phase II, randomized, double-blind, placebo-controlled trial was designed to provide additional safety and immunogenicity data for live CHIK vaccine TSI-GSD-218.

MATERIALS AND METHODS

Vaccine. The live, attenuated, TSI-GSD-218, Lot 1-85, chikungunya vaccine is a lyophilized supernatant from human lung cell cultures (Medical Research Council-5 [MRC-5] cells, certified for vaccine use) infected with an attenuated

strain, CHIK 181/Clone 25. This attenuated vaccine seed was originally derived from a serum isolate (CHIK strain 15561) obtained from an infected patient during the 1962 outbreak of CHIK disease in Thailand. Chikungunya strain 15561 was subjected to 18 plaque-to-plaque passages in MRC-5 cultures before CHIK 181/Clone 25 was selected as the vaccine seed.^{22,23} The CHIK vaccine elicited neutralizing antibody and protected mice and monkeys against challenge.²³ The vaccine was shown to be safe and immunogenic in humans in phase I trials²¹ (Malinowski FJ, unpublished data). The placebo was MRC-5 tissue culture fluid manufactured concurrently with the CHIK vaccine, and diluted identically as the CHIK vaccine. The vaccine and placebo fluids contained less than 0.02 µg neomycin base and 0.25% human serum albumin per ml.

Volunteers. Seventy-three adult volunteers (47 men, 26 women) were recruited by poster, published advertisement, and by word-of-mouth at the University of Maryland, Baltimore, MD and at the University of Maryland, College Park, MD. Healthy volunteers, 18–40 years of age, were screened and included if they had no clinically significant history of medical or psychiatric disease (including Lyme disease and personal and family history of arthritis). Additional screening consisted of a standard but brief physical examination and the usual serum chemistries, urinalysis, and complete blood count. Negative results of serum HIV, hepatitis virus B and C, and alpha virus serology (PRNT₅₀ <1:10 for CHIK, Venezuelan equine encephalitis virus [VEE], western equine encephalitis virus [WEE], and eastern equine encephalitis virus [EEE]) were required. In addition, we documented that female volunteers were not pregnant within 48 hours of vaccine administration.

The study was approved by the Institutional Review Boards of the University of Maryland at Baltimore and the University of Maryland at College Park, and by the Human Subjects Research and Review Board, Office of the Surgeon General, United States Army. Informed consent was obtained from all volunteers.

Study design. Volunteers were randomized in blocks of 5 (4 vaccine and 1 placebo recipient) using a computer random-number generator. For logistic reasons, volunteers were

TABLE 1
Number of volunteers developing clinical reactions during the first 28 days after immunization with the chikungunya (CHIK) vaccine

Reaction	CHIK vaccinees* n = 59 No. showing reaction (%)	Placebo recipients n = 14 No. reaction (%)
Local symptoms and signs†	12 (20)	3 (21)
Any systemic symptoms and signs‡	34 (58)	9 (64)
Systemic symptoms possibly associated with immunization§	19 (32)	4 (29)
Arthralgia in isolation	5 (8)	0
Flu-like symptom(s)	13 (22)	4 (29)
Urticaria	1 (2)	0

* All differences between CHIK and placebo groups were NS ($P > 0.58$, Fisher's exact test). Clinical reactions were graded and associated with immunization in a blinded fashion before vaccine code was broken.

† Pain, tenderness, erythema, induration, and restricted arm motion.

‡ Malaise, fever, chills, headache, photophobia, myalgia, arthralgia, anorexia, nausea, vomiting, pruritis, and rash.

§ Based on temporal association with immunization 28 days before onset of symptom, and by the absence of another identifiable condition that may have accounted for symptom or symptoms.

divided into five cohorts. In total, 59 volunteers (35 men, 24 women) received the CHIK vaccine, and 14 volunteers (12 men, 2 women) received the placebo preparation. Sixty (82%) of the 73 volunteers were Caucasian, and 13 were either African-American (5 persons), Asian (5 persons), or Hispanic (3 persons).

The vaccine or placebo was administered as a 0.5 ml dose of either the reconstituted vaccine ($\sim 10^5$ plaque-forming units [pfu]/dose) or placebo by injection subcutaneously over the deltoid muscle. Neither the volunteer nor the investigators knew whether virus or placebo fluid was administered until after the completion of the study. After inoculation, all volunteers remained in the vicinity of the clinic for 30 minutes for observation of immediate reactions. Volunteers subsequently returned for clinical checks on study Days 1–4, 10, 14, and 28. Parameters examined and recorded at these times included local pain, tenderness, warmth, erythema, and induration at the inoculation site, and limited range of arm movement. Systemic signs and symptoms noted included temperature, subjective fever or chills, malaise, arthralgia, arthritis, myalgia, rash, pruritus, sore throat, photophobia, headache, anorexia, nausea, and vomiting. Reactions were quantitatively scored on the basis of severity of symptoms: (0 = none, 1 = mild, symptom can be ignored; 2 = moderate, symptom affects activity; and 3 = severe, symptom requires analgesics). Volunteers were given a disposable thermometer and instructed to report to the research nurse or physician whenever they suspected a reaction to the vaccine. A physician investigator was available on a 24-hour basis throughout the study to screen any subject who reported suspected vaccine side effects.

Blood for the safety screen included complete blood counts with differential, platelet count, chemistry panel (electrolytes, glucose, blood urea nitrogen, creatinine, glucose, calcium, phosphorus, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and alkaline phosphatase) and urinalysis obtained on Days 0, 14, and 28 after inoculation. Clinical laboratory tests were performed by licensed, commercial pathology laboratories approved by the American College of Pathology. Blood for CHIK serology was obtained on Days 0, 14, 28, 42, 180, and 365 after vaccination.

Serologic procedures. Sera were assayed for 50% plaque-reduction neutralization test (PRNT₅₀) antibody,²⁴ modified for use with the vaccine strain of CHIK.²¹ The as-

says were performed by the Diagnostic Systems Division at USAMRIID. A positive antibody titer was considered to be $\geq 1:20$ by the PRNT₅₀ assay. The end-point titer was expressed as a reciprocal of the highest initial dilution that demonstrated $\geq 50\%$ reduction in the number of plaques for the average viral dose. Less than 50% neutralization by the initial serum test dilution of 1:10 was reported as 5.

Statistical methods. Each volunteer's clinical reactions and serological results were entered into the computer before the study code was broken. Both the clinical and the clinical laboratory responses to vaccine versus placebo were compared using Fisher's exact tests, evaluated at two-tailed ($P = 0.05$).

RESULTS

Volunteer retention. One volunteer whose serum interfered non-specifically with the antibody assay was not included in the serological analysis. Thus, 72 volunteers (58 of 59 CHIK vaccinees and all 14 placebo vaccinees) were evaluated serologically and all 73 volunteers were evaluated for side effects. Only three of 365 scheduled blood specimens were obtained off schedule, each from a different volunteer. One Day 180 serum was donated on Day 270, one Day 365 serum was donated on Day 385, and one Day 365 serum was not obtained.

Local reactions. The number of volunteers who experienced one or more local symptoms or signs was 12 (20%) of 59 CHIK vaccinees, and 3 (21%) of the 14 placebo controls (Table 1). The few reactions noted at the immunization site were invariably mild, except one CHIK volunteer who developed moderate intermittent pain in her inoculated arm between 12 and 48 hours after immunization that was associated with a mildly pruritic eczema-like rash at the vaccination site.

Systemic reactions. The CHIK vaccine was well tolerated. Except for transient arthralgia, systemic symptoms were no more frequent or severe in CHIK vaccinees than in placebo recipients (Table 1). The only severe systemic symptoms reported after CHIK vaccination occurred in two individuals who treated their headaches with analgesics for one day. Nineteen (32%) of CHIK vaccinees had symptoms judged to be possibly associated with immunization, compared to 4 (29%) of placebo recipients. Of these immunization-associated reactions, no CHIK vaccinee developed a

TABLE 2
Arthralgia in volunteers immunized with the chikungunya vaccine

Volunteer no.	Description of arthralgia
11	Mild intermittent pain in the left elbow beginning Day 4 (lasted 24 hours)
19	Moderate pain in the left wrist on Day 1 (lasted 10 minutes)
22	Mild arthralgia in the left hand on Day 13 (lasted 3 hours)
32	2-3 bouts of episodic pain lasting 15 minutes in left elbow and wrist on Days 1, 2, and 4 that limited typing
43	Mild left knee and thigh pain on Day 1 (lasted 6 hours)

temperature elevation > 100.0°F, and afebrile, flu-like symptoms occurred with similar frequency in vaccinated and placebo recipients (Table 1). Five (8%) of CHIK vaccinees (3 men, 2 women) had transient arthralgia without arthritic signs or associated flu-like symptoms (Table 2). The most severely affected volunteer (No. 32, Table 2) suffered pain in her left elbow and wrist which required her to stop typing episodically over three days. No placebo recipient reported arthralgia at any time, and no CHIK vaccinee reported joint symptoms between Day 14 and Day 360, the period of passive surveillance.

Clinical laboratory results. No clinically significant abnormal laboratory tests were noted in any volunteer. No significant difference existed in the percentage of CHIK and placebo-group volunteers who had abnormal laboratory tests at baseline or at post-vaccination (data not shown).

Serological results. Fifty-seven (98.3%) of 58 Chik volunteers seroconverted ($\geq 1:20$ by PRNT₅₀) by Day 28, and although antibody levels declined somewhat over time, 85% of CHIK vaccinees were still seropositive at one year (Figure 1A). The highest geometric mean titer (GMT) (1:582) was reached at Day 28 and declined slowly to 1:105 on Day 360 (Figure 1B). Maximum titers reached 1:10,240 on Days 28 and 42, and declined to 1:1,280 on Days 180 and 360. No placebo recipient seroconverted.

DISCUSSION

This phase II, double-blind study was designed to examine the safety and immunogenicity of the live CHIK vaccine TSI-GSD-218 in healthy adult volunteers. Side effects experienced by vaccinee and placebo volunteers who were followed actively and passively for one year were compared. The neutralizing antibody response was also studied for one year. This study confirms the encouraging clinical reaction profile and antibody response noted in a phase I study of 15 alphavirus-naive volunteers immunized with this vaccine,²¹ and in several phase I trials conducted at USAMRIID (Malinoski FJ, unpublished data). In the current blinded phase II study, the number and severity of adverse clinical signs and symptoms were similar in the vaccine-immunized and placebo-inoculated volunteers. No CHIK volunteer developed clinically important reactions to the vaccine. Clinical laboratory abnormalities were also comparable in both volunteer groups in the current study, although minor differences in hematologic values were noted in the previous phase I trial.²¹

Because virulent chikungunya virus can cause acute ar-

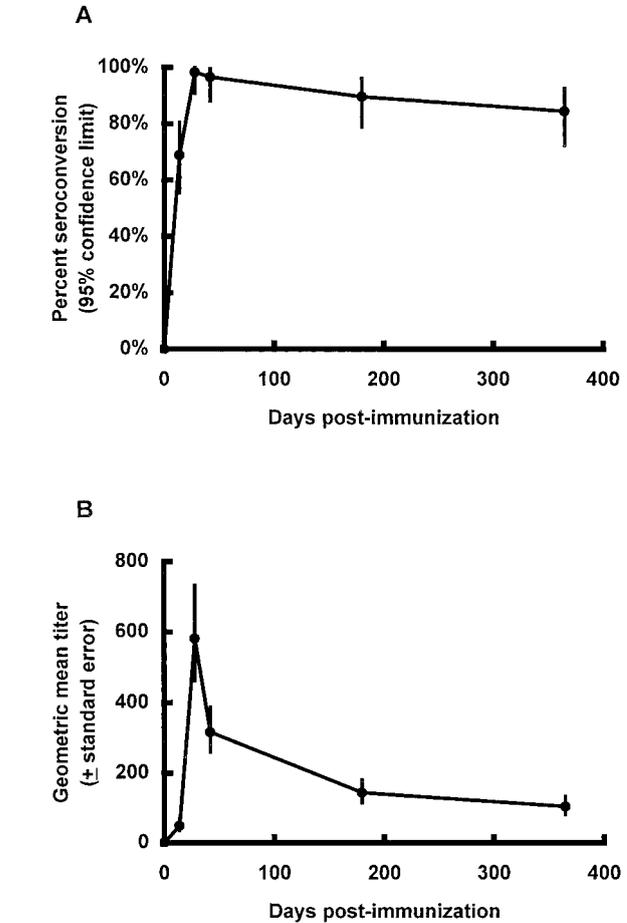


FIGURE 1. Chikungunya virus (CHIK) 50% plaque reduction neutralization test (PRNT₅₀) antibody titers in the sera of 58 alphavirus-naive volunteers after immunization with attenuated CHIK vaccine. Serum was obtained on Days 0, 14, 28, 42, 180, and 360. (A) Percent seroconversion ($\geq 1:20$ PRNT₅₀), 95% confidence intervals. (B) Geometric mean titer \pm standard error. No placebo recipients seroconverted (data not shown).

thralgia and arthritis,^{1,3-5,25,26} which on occasion may persist for months or years, we were particularly interested in enumerating joint symptoms. Five of 59 CHIK vaccinees developed transient arthralgia without other known cause (Table 2), while none of 14 controls developed arthralgia ($P = 0.33$). The incidence of arthralgia in vaccine recipients compared to controls may have reached statistical significance had we conducted a larger study. Three of 5 volunteers noted arthralgia one day after immunization, which is earlier than predicted based on the fact that CHIK vaccine viremia occurred in previous trials on Day 2-4 as reported by McClain and others²¹ and noted by FJ Malinoski, (unpublished data). Nevertheless, it is plausible medically that some of the arthralgia episodes were caused by the attenuated CHIK virus. No volunteer reported symptoms of arthralgia or arthritis during passive surveillance conducted between 1 and 12 months after immunization. One Army volunteer complained of mild, intermittent small-joint pains without evidence of arthritis or abnormal laboratory studies after long-term follow-up (Malinoski FJ, unpublished data). The sum of the safety data in the current phase II trial and in the phase I

Army trials summarized above strongly suggest that the live CHIK vaccine is safe and well-tolerated and that it produces no more severe or more frequent symptoms than found in placebo recipients.

Mild hemorrhage limited to petechia, epistaxis, and bleeding gums, or severe hemorrhage characterized by melena, hematemesis, hemoptysis, or hematuria, have occurred in up to 10% of patients during outbreaks of CHIK infection, depending of the locale.^{2,11,27,28} Some of the more severe cases of hemorrhage may have been caused by dengue virus infection.²⁷ Rarely, death in children and the aged has been associated with outbreaks of CHIK;²⁸ CHIK was isolated from the blood of one Ceylonese child who died.²⁹ Nevertheless, because of the rarity of CHIK isolations and because the live CHIK vaccine is highly attenuated for man, hemorrhagic disease and death are not considered to be vaccine risks.

Although chikungunya viral disease is not known to adversely affect the pregnant woman or fetus and no reports of teratogenicity or increased abortion have been associated with massive epidemics of CHIK, there is a theoretical potential that any replicating vaccine virus will adversely affect the developing fetus. This vaccine has not been tested for teratogenicity or abortogenicity in animal models.

The theoretical risk of transmission of the attenuated CHIK vaccine virus to mosquitoes is considered to be remote. Because of the transient low level of viremia produced in inoculated volunteers, it is unlikely that mosquitoes would become infected by feeding on a person inoculated with the CHIK vaccine.³⁰ Moreover, although the vaccine was successfully transmitted by mosquitoes after intrathoracic inoculation, there was no evidence of reversion to a virulent phenotype.³⁰ Studies to detect possible revertant isolates from vaccine recipients indicated that most isolates retain the biological characteristics of the vaccine virus. One isolate from a volunteer in an Army study had suckling mouse lethality intermediate between the vaccine (0%) and parent (100%) viruses and induced viremia in monkeys that was higher than that produced by the parent vaccine virus. However, the viremia in the volunteer was probably too low to infect a feeding mosquito (Malinowski FJ, unpublished data).

The CHIK vaccine was highly immunogenic. Forty vaccinees (69%) seroconverted by Day 14, and 57 vaccinees (98%) seroconverted by Day 28 (Figure 1A). Neutralizing antibody was still detected in 85% of volunteers after 12 months. Long-term follow-up studies would be needed to determine the durability of the immune response. The GMT of the vaccinees was high (Figure 1B). All 15 alphavirus-naive individuals seroconverted in the single published phase I trial.²¹ By contrast, seroconversion in laboratory workers who had previously been immunized with the VEE TC-83 vaccine was only 36%,²¹ suggesting that the VEE TC-83 vaccine interferes immunologically with the live CHIK vaccine. These and other investigations suggest that sequential attenuated alphavirus immunization is detrimental to the development of neutralizing antibody and may impair the development of protective responses to subsequent CHIK immunization. It is unknown if previous attenuated alphavirus vaccines can interfere with subsequent infection and illness caused by virulent CHIK.²¹

In summary, the live CHIK vaccine was safe, produced well-tolerated side effects such as transient arthralgia in 5 (8%) of 59 volunteers, and was highly immunogenic. It is, therefore, a promising vaccine for use in alphavirus naive individuals.

Acknowledgments: The authors thank the participation of the study volunteers in Baltimore and College Park, Maryland. Skilled research nursing was provided by Kathleen Palmer, Helen Secrest, and JoAnna Becker. Dr. Alvin Sills provided clinical support, and Julie Hile and Dennise Danner assisted in performing PRNT₅₀ assays. We thank Dr. David J. McClain, Dr. Phillip R. Pittman, and Dr. Frank J. Malinoski for reviewing the paper and sharing unpublished data.

Financial support: The study was supported by Contract No. DAMD17-92-C-2058 (Task Order 94-008) to the University of Maryland by the United States Army Medical Research Acquisition Activity, Fort Detrick, Frederick, MD.

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