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Immunogenicity and safety of an inactivated Rift Valley fever vaccine in a 19-year study☆

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ABSTRACT

An investigational, formalin-inactivated Rift Valley fever (RVF) vaccine, known as The Salk Institute-Government Services Division (TSI-GSD) 200 vaccine, was administered to 1860 at-risk subjects (5954 doses) between 1986 and 2004 as a three-dose primary series (days 0, 7, and 28) followed by booster doses as needed for declining titers. An initial positive serological response ($PRNT_{80} \geq 1{:}40$) to the primary series was observed in 90% of subjects. Estimate of the $PRNT_{80}$ response half-life in initial responders to the primary series by Kaplan–Meier plot was 315 days after the primary series dose 3. Differences in a serological response were observed at 2 weeks after dose 3 of the primary series between vaccine lots and for gender (women > men); a trend was observed for age (<40 years). When response to the primary series was measured by $PRNT_{50}$ titer $\geq 1{:}40$, nearly all subjects (99.1%) responded. In individuals not initially responding to the primary series ($PRNT_{80} < 1{:}40$), a response was observed in most subjects after receiving only one booster dose. Immune response (all subjects) to subsequent booster doses for a declining titer ($PRNT_{80} < 1{:}40$) was 98.4%. The vaccine was well-tolerated; vaccine-related adverse reactions were generally mild and self-limited. Differences in adverse events were observed with vaccine lot and sex. The data support the safety and immunogenicity of the inactivated RVF vaccine, and may serve as a standard of comparison for immunogenicity and safety for future RVF vaccines.

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1. Introduction

Rift Valley fever virus was initially recognized in the Rift Valley of Kenya in 1931 and is endemic to many areas of sub-Saharan Africa [1–4]. After initial introduction to Egypt in 1977 that resulted in an extensive epidemic in humans and domestic animals, the virus has since emerged in Madagascar, Yemen, and Saudi Arabia [4,5]. While illness from RVF virus most commonly presents as an undifferentiated febrile illness, severe disease in humans may cause retinitis (that may result in permanent loss of vision), a hemorrhagic syndrome associated with gastrointestinal hemorrhage and hepatitis, or meningio-encephalitis [5–9]. Infection in humans is generally acquired from close contact with the blood of infected livestock or the bite of an infected mosquito. As the *Aedes* mosquito vector resides in many areas of the world, introduction of virus to these areas could have a significant public health and agricultural impact during epizootics [10].

There is currently no Food and Drug Administration (FDA)-approved vaccine for RVF infection. The Salk Institute-Government Services Division (TSI-GSD) 200 vaccine is an investigational, formalin-inactivated vaccine that was manufactured in 1979 from a plaque-cloned version of the seed virus and cellular substrate of diploid fetal rhesus lung cells [11]. The vaccine development was based on an earlier investigational formalin-inactivated RVF vaccine developed from monkey kidney cells infected with a pantropic strain of the virus, known as the National Drug Biological Research Company (NDBR) 103 vaccine. The NDBR 103 vaccine had demonstrated (1) immunogenicity and efficacy in animal models and (2) safety and immunogenicity in over 2000 at-risk individuals [12–16].

Although reports of the TSI-GSD 200 RVF vaccine were previously published, the earlier reports involved smaller cohorts with inadequate numbers to address demographical differences (age

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and sex) in immune response and adverse events as observed in this study report [11,17–19]. The experience of the RVF TSI-GSD 200 vaccine administered to at-risk individuals (mainly laboratory workers) from 1984 to 2004 was reviewed to better define the safety and immunogenicity of the vaccine. In addition to differences in immunogenicity related to gender, age, and lot (only interlot differences in immunogenicity previously reported with this vaccine), this study noted differences in adverse events related to gender, age, and vaccine lot [11,18]. Also, earlier publications reported data only by 80% plaque-reduction neutralization ($PRNT_{80}$) assay response and not by $PRNT_{50}$ results, which has since become a standard in vaccine development. With the emergence within the past year of several RVF vaccine candidates demonstrating immunogenicity in animal models, the information on the inactivated TSI-GSD 200 RVF vaccine may provide insight into immunogenicity and safety issues for clinical trials with future recombinant vaccine candidates [20].

2. Methods

2.1. Vaccine

The formalin-inactivated, TSI-GSD-200 RVF vaccine was developed in 1979, using a master seed made from passage of the mouse serum seed into diploid fetal rhesus monkey lung cells, DBS 103. Details of the vaccine have been described in previous publications [11,17]. The lyophilized vaccine product (lots 1–16, and lot 18) was stored at $-20^{\circ} \pm 10\,^{\circ}C$ and reconstituted with 5 ml of sterile water before injection. A total of 31 lots and runs of the vaccine were administered during the study.

2.2. Serology

Immunological response of volunteers was assessed using an 80% plaque reduction neutralization ($PRNT_{80}$) assay, as described in previous publications [18,19]. RVF virus was diluted to approximately 100 plaque-forming units (PFU)/0.2 ml and mixed with sera in serial twofold dilutions. After incubation overnight at 4 °C, the mixtures were placed into 23-mm wells containing confluent monolayers of VERO cells (0.1 ml/well). After incubation at 37 °C for 1 h with 5% CO_2, the inoculated cells were overlaid with nutrient medium containing 1% agar, 5% fetal bovine serum, 200 U of penicillin/ml, and 200 mg of streptomycin/ml, and reincubated again at 37 °C with 5% CO_2. The cells were then overlaid again with the nutrient medium to which a 1:7500 dilution of neutral red solution had been added. The highest dilution of serum that inhibited 80% or more of the plaques (compared to virus control titration) was defined as the $PRNT_{80}$ titer. $PRNT_{50}$ titers were calculated but were not used for clinical decisions in the protocol.

2.3. Vaccination

Subjects were randomized to receive a specific vaccine lot until 1993. After 1993, only the vaccine lot(s) currently in use at the time was administered to all subjects (same lot always used to complete the primary series). The primary series of the vaccine consisted of three 1.0-ml subcutaneous injections in the triceps region of the arm (given at day 0, day 14, and day 28). If the subject's $PRNT_{80}$ titer was ≥1:40 after the primary series (referred to as an initial responder to the primary series), $PRNT_{80}$ titers were subsequently obtained at month 2, 5, 8, and 11 after dose 3 of the primary series, and then at 6-month intervals. If the subject's $PRNT_{80}$ was <1:40 after the primary series (referred to as an initial nonresponder), the subject was given a booster dose (maximum of four booster doses in a 1 year) until a $PRNT_{80}$ titer ≥1:40 was achieved. In 2001, the protocol was amended to extend the windows of time for vaccine doses to day 0, days 7–14, and days 28–42, and to only obtain titers at days 21–35 after vaccine doses and then annually in responders with a $PRNT_{80}$ titer ≥1:40.

2.4. Study recruitment

From 1984 to 2000, at-risk individuals for exposure to RVF virus were recruited and vaccinated under informed consent both in the Special Immunizations Program (SIP) at USAMRIID and 59 external sites (39 domestic and 21 nondomestic sites). Beginning in May 2000, all vaccinations were performed only at USAMRIID. Study volunteers were evaluated with a baseline history and physical examination, complete blood count (CBC), serum chemistries, urinalysis, hepatitis panel, human immunodeficiency virus (HIV), enzyme-linked immunosorbent assay (ELISA), electrocardiogram (EKG), and chest X-ray. Enrollment criteria required individuals to be at-risk for exposure to RVF virus, and to be ≥18 years of age and in general good health. Women of childbearing potential were required to have a negative beta subunit human chorionic gonadotropin (BhCG) pregnancy test. Individuals were excluded for a history of an allergy to a vaccine component (formaldehyde, neomycin sulfate, and streptomycin), a previous severe reaction to the vaccine, or evidence of immunodeficiency.

2.5. Adverse events

Adverse events were collected by passive reporting until May 29, 2000, when the study was amended to actively collect adverse events on day 1 postvaccination and then weekly through day 28 after a vaccine dose. Serious adverse events were collected for the duration of the study.

2.6. Statistical analysis

2.6.1. Immunogenicity

Serological analysis assessed the (1) percentage of initial responders to the primary series ($PRNT_{80}$ titer ≥1:40 after dose 3 of the primary series) and (2) the number of booster doses in initial nonresponders required to achieve a $PRNT_{80} \geq 1{:}40$. Persistence of immunogenicity in initial responders to the primary series was assessed by both the percentage of subjects with a $PRNT_{80}$ titer ≥1:40 and by the geometric mean titer (GMT) at time points from 2 weeks to 11 months after dose 3 of the primary series, and by the estimated numbers of days until the $PRNT_{80}$ titer fell below 1:40 using a Kaplan–Meier plot. The serological response of subjects was also determined for $PRNT_{50}$ titers. For the most frequently used vaccine lots, immunogenicity was compared at time points from 2 weeks to 11 months after dose 3 of the primary series by logistic regression for lot, sex, age, and race.

2.6.2. Adverse events

Analysis of adverse events data was performed primarily on vaccine doses given May 29, 2000 through 2004, when adverse event data were actively collected (the absence of adverse event data for a significant number of vaccine doses given before May 29, 2000 prohibited meaningful analysis of these adverse events other than descriptive analysis). Only adverse events assessed to be definitely, probably, or possibly related to the vaccine were included in the analysis. The percentage of subjects with related adverse reactions was compared for vaccine lot, shot series (primary versus booster doses), sex, age, and race (Caucasian versus non-Caucasian) by multiple logistic regression analysis.