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A RIFT VALLEY FEVER VACCINE TRIAL

I. SIDE EFFECTS AND SEROLOGIC RESPONSE OVER A SIX-MONTH FOLLOW-UP

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Kark, J. D. (Dept. of Social Medicine, Hebrew U.—Hadassah School of Public Health and Community Medicine, Jerusalem, Israel), Y. Aynor and C. J. Peters. A Rift Valley fever vaccine trial. I. Side effects and serologic response over a six-month follow-up. *Am J Epidemiol* 1982;116:808-20.

A formalin-inactivated Rift Valley fever vaccine, originally produced in primary monkey kidney cells, has been used to protect laboratory workers. A trial of a modified vaccine, newly formulated in well-characterized diploid fetal rhesus lung cells, was conducted with 114 men aged 19–24 years. Of the 107 subjects who received up to three injections of 0.1 to 1 ml vaccine (an additional seven received a placebo) one had a local hypersensitivity-type reaction and another a generalized urticarial syndrome. Both cases had a prior history of hypersensitivity states. No pyrogenicity was detected and only insignificant systemic reactions were recorded. Mild and transient local reactions ranged from 5% at the lowest dose level to 43% at the highest. Serologic response, as assessed by plaque reduction neutralizing antibody titers, was dose dependent. Within a single vaccine lot tested at multiple dose levels, peak (day 42) geometric mean titers ranged from 48 (at 0.1 ml × 3) to 436 (at 1.0 ml × 3). Reciprocal titers of ≥40 are considered to be protective. Comparison of three lots at the 0.5 ml level indicated between lot variability, though this was not statistically significant. A sharp decline in antibody titers was observed in all vaccination groups by day 84; six months after vaccination apparently protective antibody titers were present only in groups that received 1 ml × 3 and 0.5 ml × 3 of the most antigenic lot of vaccine. These results suggest that 1) the vaccine is generally nonreactogenic, but individuals with a prior history of hypersensitivity states should be observed for allergic side effects; 2) existing vaccine supplies cannot be extended by using lower dose levels without a lower and less sustained serologic response; 3) a booster dose is necessary six months or more following the primary series; 4) although the current TSI-GSD-200 vaccine is immunogenic, a more potent vaccine is needed.

arbovirus infections; Rift Valley fever virus; serology; vaccines

Rift Valley fever is an acute febrile arboviral zoonotic disease apparently still confined to the African continent. The virus, now classified as being of the genus *Phlebovirus* of the *Bunyaviridae* family (1), was first isolated in 1931 from an epizootic in sheep in Kenya (2). Until 1977 the disease was geographically con-

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fined to sub Saharan Africa, causing periodic epizootics in many countries and was reported to cause a mild, dengue-like febrile illness in man (3). In 1975, however, the first severe clinical cases in humans were reported and first fatalities documented (4). In October 1977 the disease was identified in Egypt for the first time. A massive outbreak ensued in both animals and humans during the autumn and winter of 1977–1978, and reappeared with reduced intensity in the following years. The economic impact of this zoonosis in Egypt was extensive with severe losses of sheep and cattle (5, 6). Human infection was apparently widespread. Official reports for the three-month epidemic of 1977 showed 18,000 acute cases and 598 deaths (7), while some estimates of morbidity were in the order of 200,000 for that year alone (6). The disease outbreak in humans in Egypt was typically an acute febrile dengue-like illness with severe ocular, encephalitic or fatal hemorrhagic complications occurring not infrequently (5, 8).

Northward spread of the disease to Egypt forced international public health agencies to confront the possibility of spread of Rift Valley fever from the African continent. Israel, at risk of contiguous spread of disease, developed and instituted a coordinated prevention program (9, 10) that relied chiefly on extensive domestic animal herd vaccination as the major protective approach, both for prevention of disease in humans and for averting economic losses. The protection of personnel at high risk of infection remains a problem. A cheap, mass produced, easily administered and effective human vaccine for widescale population protection is not presently available.

A formalin-inactivated Rift Valley fever vaccine was first developed and tested by Randall et al. (11, 12) in 1964 and produced in the United States in limited quantities in 1968 (13). This vaccine, labelled NDBR-103, when used in recommended doses of 1 ml administered subcutaneously three times (days 0, 10 and 28) produced satisfactory antibody levels. Altogether several thousand people have been vaccinated with the NDBR-103 vaccine, including high risk laboratory personnel, as well as the Swedish battalions of the United Nations Expeditionary Forces in the Sinai (13, 14).

Recently, the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) modified the production process for the vaccine (13). Again, due to the biohazard safety requirements and production technique, only limited quantities of vaccine (several hundred liters in all) were processed in about 20 lots. Initial testing in human volunteers at USAMRIID showed the vaccine to be nonreactogenic and immunogenic at the recommended doses. Significant interlot differences in antigenicity were noted (13).

The present trial was conceived with the following objectives: 1) to evaluate the reactogenicity of this vaccine on a larger scale; 2) to find the minimal effective vaccine dose, permitting present stocks to be extended; 3) to examine the pattern of antibody response over a prolonged period of follow-up; 4) to compare the immunogenicity of the first three lots of TSI-GSD-200 vaccine produced.

This report deals with the issue of vaccine safety and presents serologic data for six months of follow-up.

Methods

The vaccine

Production of the formalin-inactivated cell culture-propagated TSI-GSD-200 Rift Valley fever vaccine was modified from the procedure of Randall et al. (11, 12) and was done in 1978–1979 under contract to the Salk Institute, Government Services Division, Swiftwater, Pennsylvania. The virus employed was the Entebbe strain of Rift Valley fever originally recovered from mosquitoes in Uganda in 1944. A mouse serum master seed (184th

passage) was inoculated into primary African green monkey kidney cell cultures to prepare the original Randall vaccine. The current vaccine was prepared using a similar technique except that a new master seed was made by passage of the mouse serum seed into diploid fetal rhesus monkey cells. This material was shown to be free of microbial contaminants and was used as inoculum for preparation of vaccine in diploid fetal rhesus monkey cells (DBS-103).

The cell cultures used for master seed virus, production seed virus and vaccine were grown in minimum essential medium in Earle's balanced salt solution with 50 μg/ml neomycin, 50 μg/ml streptomycin and 10 per cent fetal calf serum. The cells were extensively washed prior to virus inoculation to remove the calf serum. The maintenance medium used in the cultures following inoculation of the production-seed virus was basal medium Eagle's with 0.5 per cent human serum albumin, 50 μg/ml neomycin and 50 μg/ml streptomycin. Less than 0.01 per cent residual formalin was present in the vaccine prior to lyophilization. For a detailed report of the vaccine preparation see Eddy et al. (13). The resulting investigational vaccine has similar characteristics as the original NDBR-103 product in animal and initial human testing at USAMRIID (13). Testing of this investigational vaccine according to the protocol of the present study was authorized by the Israel Ministry of Health Human Research Committee in accordance with the Helsinki declaration.

Sample size

Sample size calculations were based on the responses to differing doses in the preliminary USAMRIID trial, and it was decided that groups of about 20 individuals should provide adequate power for detection of twofold differences in antibody titer. This group size was used for examining dose response. Smaller and consequently less powerful samples were used to test the question of interlot variation.

Study population

The study sample consisted of healthy 19–24-year-old male students of two different Yeshivas (religious colleges). Participants were given oral and written explanations regarding the trial and the possible risks involved. Following signed consent to participate, study subjects underwent a standardized medical examination. Sixty-five males in Yeshiva A and 53 in Yeshiva B volunteered. Three dropped out before receiving their first injection. One volunteer was excluded from the trial following detection of severe anemia. Therefore, 114 individuals participated in the study.

Allocation

Of the 114 participants, 99 were initially randomized into six vaccination groups who were to be injected on days 0, 10 and 28 of the study (table 1). Group 1 was to receive 1 ml × 3 of lot 1; group 2, 0.5 ml × 3 of lot 1; group 3, 0.3 ml × 3 of lot 1; group 4, 0.1 ml × 3 of lot 1; group 5, 0.5 ml × 3 of lot 2 (Yeshiva A only); and group 6, 0.5 ml × 3 of lot 3 (Yeshiva B only). Subjects were unaware of their vaccination group.

A placebo group was not randomized due to the relative paucity of study subjects. It was decided that subjects excluded by the investigators from receiving Rift Valley fever vaccine for whatever reason, would receive a normal saline injection. Therefore, seven subjects were allocated to the placebo group (group 7) following identification of psoriasis, active asthma, active hay fever, elevated blood pressure and first trimester pregnancy of a spouse.

After inception of the trial, an additional group (group 8) was formed, consisting of eight subjects who were not available on the day of the first vaccina-

TABLE 1

Distribution of Rift Valley fever vaccine (TSI-GSD-200) trial participants according to vaccination groups and schedule of vaccinations administered

Actual immunization schedule (days)	Vaccination group*								
	1	2	3	4	5	6	7	8	Total
0, 10, 28	17	19	20	20	8	9	6		99
0						1			1
0, 10	1†	1							2
10, 28							1	8	9
0, 28	2†				1				3
Total vaccinated	20	20	20	20	9	10	7	8	114

* Group 1: 1 ml × 3 of lot 1; group 2: 0.5 ml × 3 of lot 1; group 3: 0.3 ml × 3 of lot 1; group 4: 0.1 ml × 3 of lot 1; group 5: 0.5 ml × 3 of lot 2; group 6: 0.5 ml × 3 of lot 3; group 7: a saline placebo; group 8: 1 ml × 2 of lot 1 (these eight subjects were allocated to this group after they missed their first scheduled injection).

† Three subjects received only two of their planned three 1 ml injections, one on days 0, 10, and two on days 0, 28. For serologic analyses these were included in group 8 (n = 11).

tion. These were subsequently allocated to be injected on days 10 and 28 with 1 ml of lot 1. Three additional individuals who received the 1 ml dose twice only (on days 0 and 10 or on days 0 and 28), because they missed a scheduled vaccination, are included in group 8 for some analyses.

Groups 2, 5 and 6 were designed to permit a comparison of the immunogenicity of the different vaccine lots.

Group 8 was used for limited evaluation of a different schedule (i.e., injections 18 days apart using the 1 ml dose).

All vaccinations were administered subcutaneously into the upper arm. All participants were interviewed by a physician prior to each vaccination. Subjects with an oral temperature ≥37.5 C or feeling unwell were vaccinated one or two days later or received no injection.

Side effects

A standard form designed to assess side effects was completed by a physician prior to each vaccination and 24 hours post-vaccination for each individual. Oral temperature, pulse rate, systemic symptoms (headache, malaise, weakness, myalgia, interference with daily activities, nasal discharge, sore throat, cough, nausea or anorexia, vomiting, and diarrhea), and signs and symptoms at the injection site (pain, tenderness, erythema, swelling, induration) were recorded. Each symptom was individually scored on a 0–3 scale as absent, mild, moderate, or severe. The diameter of the injection site signs was measured.

Blood samples

Venous blood was drawn on days 0, 10, 28, 42, 84 and 180 into plain vacuum-containers (for serum samples) and into tubes containing disodium ethylenediaminetetraacetate (EDTA) (for plasma samples). The blood samples were immediately refrigerated, transported to the laboratory and separated within 24 hours. Aliquots were stored at −20 C until examination. Samples were airfreighted on dry ice to the USAMRIID laboratories at Frederick, Maryland, for serologic testing.

Immune response

Antibodies to Rift Valley fever virus were measured by the plaque reduction neutralization test. The laboratory received sequentially numbered tubes with no identification as to the vaccine group. Twofold dilutions of sera were mixed with 50 to 150 plaque-forming units of the ZH501 strain of Rift Valley fever virus and incubated for one hour at 37 C. Both serum and virus were diluted in Hanks

balanced salt solution buffered to pH 7.3 with Hepes and containing 2 per cent heat-inactivated calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Residual virus was assayed by inoculating duplicate 35 mm diameter monolayers of VERO cells with 0.2 ml. After one hour at 37 C in 5 per cent CO_2, an agarose overlay of Basal Medium Eagle (Earle's salts and Hepes buffer) with 4 per cent heat inactivated calf serum and antibiotics was applied and the plates were incubated for an additional three days before adding an identical overlay containing 1:9000 neutral red. On day 4, plaques were enumerated and the endpoint was determined as the lowest final dilution of serum reducing the inoculum by 80 per cent or more. The ZH501 strain was isolated from the serum of a fatal hemorrhagic fever case in Egypt by Dr. J. Meegan of the US Naval Medical Research Unit (NAMRU-3) in Egypt. We reisolated virus in fetal rhesus lung cells and passed the virus a second time before use. It is serologically indistinguishable from the Entebbe vaccine seed (15).

Statistical analysis

For calculation of geometric mean titers, a reciprocal titer less than 10 was defined as 5. Statistical testing was performed on the log transformed titers using analysis of variance and *t*-tests for comparing vaccine group means, and linear regression for testing dose response and for testing the independent effects of early antibody response and vaccine dose on the persistence of antibody over six months.

Results

Of the 107 individuals allocated to the seven Rift Valley fever vaccine groups, six (or 5.6 per cent) did not complete their intended schedule due either to concurrent illness on the scheduled day (two subjects) or side effects following the prior vaccination (three subjects), while one person could not be located upon the day of injection. Three of these six individuals received two 1 ml injections and were included in some analyses (in group 8). The number of individuals actually receiving the various schedules is shown in table 1.

Side effects

Eighty five per cent of the subjects were examined by a physician 24 hours after vaccination. The remainder were contacted; none reported untoward reactions to the vaccine.

Local reactions (i.e., either erythema, swelling, tenderness or pain at the site of the injection) were checked in 216 of the 306 injections given (71 per cent). These seemed to be strongly dose dependent, ranging from 43 per cent at the 1 ml dose level down to 5 per cent at the 0.1 ml dose (table 2).

The placebo group experienced no local response. Erythema was the most common reaction and was noted in about 25 per cent of all injections at the 1 ml dose, but was totally absent at the 0.1 ml dose. There was no difference in reaction at the first, second, or third injection (i.e., no "sensitizing" to the material responsible for the local response). Most instances of erythema were less than 4 cm diameter (36/39) while 2/39 were between 4 and 6 cm and 1/39 was over 7 cm. Swelling and induration were less marked. One subject complained of pain in the axilla of the injected arm, but lymphadenopathy was not detected.

Systemic responses were generally few and mild. There were no febrile reactions whatsoever. The mean systemic score was less than 1 for the various dose levels (i.e., there was less than one mild systemic reaction recorded per vaccinee in each group). There was no apparent dose effect.

Four unusual cases are described in somewhat more detail.

Case A had a history of asthma and penicillin sensitivity. Four to six hours after receiving a first dose of vaccine

TABLE 2

Proportions of all local reactions, and erythema alone 24 hours post-vaccination at varying dose levels of Rift Valley fever vaccine (TSI-GSD-200)

Dose	Any local reaction		Erythema	
	No.*	%	No.*	%
Lot 1: 1 ml	23/53	43.3	15/53	24.5
Lots 1, 2, 3: 0.5 ml	30/81	37.0	21/81	25.9
Lot 1: 0.5 ml	19/45	42.2	11/45	24.4
Lot 2: 0.5 ml	4/14	28.5	3/14	21.4
Lot 3: 0.5 ml	7/22	31.8	7/22	31.8
Lot 1: 0.3 ml	6/42	14.3	3/42	7.1
Lot 1: 0.1 ml	2/40	5.0	0/40	0.0
Saline: 1 ml	0/13	0.0	0/13	0.0
Total†	61/216	28.2	39/216	18.1

* Number with reactions/number checked. The denominator is the number of injections given and checked 24 hours later (not the number of individuals vaccinated).

† Of 306 injections given to the 107 subjects who received the Rift Valley fever vaccine, 216 were examined for local reactions. Placebo controls are not included in the total.

(0.5 cc lot 3) he complained of headache, malaise, fatigue and a sore throat. When examined 24 hours after injection, the pharynx was mildly inflamed, and there was mild tenderness and erythema at the site of the injection. At 48 hours the injection site was swollen, inflamed and tender, with ragged well-defined borders extending more than 7 cm in diameter. There was no axillary pain, tenderness or adenopathy. Temperature was normal. The white blood count at 48 hours was 14,800, with 66 segmented polymorphonuclears, 2 band cells, 3 eosinophiles, 6 mononuclears and 23 lymphocytes. Only symptomatic treatment was given. All signs and symptoms disappeared by 96 hours. This individual's immune response was the strongest of all vaccinees at all dose levels upon day 10 with a reciprocal titer of 80 following a single dose of 0.5 ml. A titer of 40 was maintained at six months. This subject was excluded from further immunization.

Case B had a history of urticarial reactions attributed to penicillin and, he believed, also to certain foodstuffs (eggplants). Three hours after receiving a second dose of 1 ml from vaccine lot 1, he complained of malaise, frontal headache, fatigue, and weakness. Multiple generalized urticarial wheals appeared several hours later. At the injection site there was mild erythema of 1–2 cm. Temperature, breathing, and chest examination were all normal and there were no other findings of interest. The wheals receded 24 hours later, reappearing elsewhere and finally disappearing three days following vaccination. The third vaccination was withheld. This subject's antibody titer at day 10 was highest of all those receiving the 1 ml dose.

Case C, who had a history of penicillin sensitivity, received 2 doses of 0.5 ml of lot 1; 48 hours after the second vaccination, the subject reported sudden brief, severe pain in the left upper abdomen. This recurred several times in the epigastrium during the next two days and appeared to be exacerbated by eating. A somewhat similar event had occurred a year earlier. Temperature, physical examination and routine laboratory studies were normal, except for a questionable palpable spleen which was not felt upon re-examination several days later. This reaction was most probably not vaccine-related. However, the subject was not further immunized.

Case D. Two months after receiving his third vaccination (0.3 cc lot 1) this subject, not residing in the Yeshiva at the time, was referred to a hospital emergency room with fever, weakness, severe headache and a suspected diagnosis of meningitis. The patient refused lumbar puncture. Several other individuals from the same area were diagnosed as having viral meningitis during the same period. A University hospital neurologist summarized the case as most probably aseptic meningitis. Recovery was rapid and uneventful. There was no evidence of a Guillain-Barré type syndrome. This sub-

TABLE 3

Proportion of participants in the Rift Valley fever vaccine (TSI-GSD-200) trial with reciprocal titers of ≥10 and ≥40 at each follow-up, by vaccine dose groups

Dose	Titers ≥10, by day			Titers ≥40, by day					
	0	10	18	10	18	28	42	84	180
Lot 1: 1 ml × 3	0/17	8/17		1/17		16/17	17/17	16/17	15/17
Lot 1: 1 ml × 2	0/11	1/1	6/8	1/1	5/8	10/11	3/3	10/11	4/11
Lot 1: 0.5 ml × 3	0/19	5/19		1/19		19/19	19/19	16/19	12/19
Lot 2: 0.5 ml × 3	0/8	3/7		0/7		8/8	8/8	8/8	3/7
Lot 3: 0.5 ml × 3	0/9	2/9		0/9		6/9	8/9	6/9	4/9
Lot 1: 0.3 ml × 3	0/20	2/20		0/20		14/20	19/20	13/20	5/18
Lot 1: 0.1 ml × 3	1/20	1/20		0/20		7/20	12/19	4/20	3/19

ject showed no serologic response (titer <1:10) at any point during follow-up, including the acute and convalescent phases of his illness.

Nineteen study subjects reported hypersensitivity or allergic type conditions in their medical history taken prior to participation. These conditions were as follows: sensitivity to penicillin in eight persons, to sulfa in two, to streptomycin in one, dust allergy in three, hay fever, allergic rhinitis or allergic conjunctivitis in four, asthma or allergic bronchitis in four, and allergy to certain food stuffs in one subject. The two hypersensitivity-type side effects noted in this study (cases A and B) were among the three subjects who reported more than one allergy. It is of interest that cases A, B and C all had history of penicillin sensitivity. The one participant who reported sensitivity to streptomycin (used in preparation of the vaccine) evinced no unusual response.

Serology

In this free-living study population it was impossible to adhere strictly to the initial blood-drawing schedule for all participants. Thus, day 10 sera were drawn between 8–10 days (median 9), day 28 sera between days 26–34 (median 28), day 42 sera between days 39–48 (median 42), day 84 sera between days 81–92 (median 84), and day 180 sera between days 152–182 (median 175).

There was no statistically significant difference in serologic responses between the two Yeshivas. Thus, the data were pooled for analysis. All participants (including cases A–D) were negative at day zero except one subject who had a titer of 20 at day zero but less than 10 on day 10. Antibody response was prompt and was clearly dose dependent (table 3). Three subjects did not show any response to vaccination. They received 0.3 ml × 3 (one case) and 0.1 ml × 3 (two cases).

Peak response was noted on day 42 with 95 per cent or more developing a ≥40 titer in all vaccine groups except the group receiving 0.1 ml × 3 in whom 63 per cent responded. By three months a decline had occurred, more overtly at the ≥40 cut-point in the lower vaccine dose groups (0.3 ml and 0.1 ml × 3). Using the geometric mean titer (GMT) as the unit of measurement (table 4 and figure 1), the steep rise in antibody production between days 10 and 42 and the similarly steep decline by day 84 are striking. The smallest dose level (0.1 ml × 3) clearly gave the lowest GMT, but was not devoid of antigenicity.

Figure 2 shows the dose response at each of days 28, 42, 84, and 180 of follow-up. Overall, at each follow-up point, the impression is one of a generally linear response, especially evident upon day 42. The proportion of the total variance (R^2) of log serologic response attributable to differing dose levels (0.1 ml × 3, 0.3 ml × 3, 0.5 ml × 3, and 1.0 ml × 3) of lot 1 vaccine varied slightly from 35 to 46 per cent at the different follow-up days. A linear regression term best described the

TABLE 4

Serologic response to varying dose levels of Rift Valley fever vaccine (TSI-GSD-200) at each follow-up day

Dose		Follow-up day						
		0	10	18	28	42	84	180
Lot 1: 1 ml × 3	GMT*	5	8		186	437	94	80
	(*n*)†	(17)	(17)		(17)	(17)	(17)	(17)
	log ± SD	0.69 ± 0.0	0.89 ± 0.28		2.27 ± 0.40	2.64 ± 0.35	1.97 ± 0.34	1.90 ± 0.36
Lot 1: 1 ml × 2	GMT	5		37	320		43	17
	(*n*)	(11)		(8)	(11)		(11)	(11)
	log ± SD	0.69 ± 0.0		1.56 ± 0.59	2.50 ± 0.35		1.63 ± 0.28	1.22 ± 0.40
Lot 1: 0.5 ml × 3	GMT	5	6		170	234	55	48
	(*n*)	(19)	(19)		(19)	(19)	(19)	(19)
	log ± SD	0.69 ± 0.0	0.81 ± 0.25		2.23 ± 0.37	2.37 ± 0.38	1.74 ± 0.35	1.68 ± 0.55
Lot 2: 0.5 ml × 3	GMT	5	6		174	320	62	30
	(*n*)	(8)	(8)		(8)	(8)	(8)	(7)
	log ± SD	0.69 ± 0.0	0.81 ± 0.15		2.24 ± 0.25	2.50 ± 0.39	1.79 ± 0.32	1.47 ± 0.34
Lot 3: 0.5 ml × 3	GMT	5	6		86	148	34	21
	(*n*)	(9)	(9)		(9)	(9)	(9)	(9)
	log ± SD	0.69 ± 0.0	0.79 ± 0.21		1.94 ± 0.76	2.17 ± 0.59	1.53 ± 0.39	1.33 ± 0.53
Lot 1: 0.3 ml × 3	GMT	5	5		48	145	31	15
	(*n*)	(20)	(20)		(20)	(20)	(20)	(18)
	log ± SD	0.69 ± 0.0	0.73 ± 0.09		1.67 ± 0.60	2.16 ± 0.45	1.49 ± 0.3	1.18 ± 0.38
Lot 1: 0.1 ml × 3	GMT	5	5		19	48	13	10
	(*n*)	(20)	(20)		(20)	(19)	(20)	(19)
	log ± SD	0.69 ± 0.0	0.71 ± 0.06		1.28 ± 0.66	1.68 ± 0.59	1.12 ± 0.38	0.99 ± 0.41

* GMT, geometric mean titers. A titer of <1:10 was defined as 5.
† Number vaccinated.

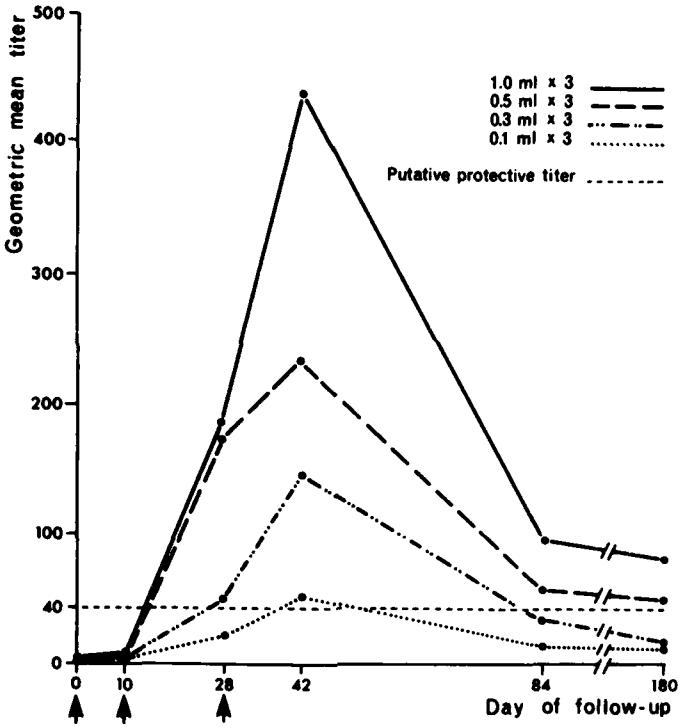

FIGURE 1. Geometric mean titer antibody response to varying doses of lot 1 Rift Valley fever vaccine over a six-month follow-up. Vaccine administered on days 0, 10 and 28 (see arrows).

dose response at each day of follow-up ($p < 0.0001$). Addition of higher order terms made no significant contribution.

Comparing the 1 ml × 2 and the 0.5 ml × 3 lot 1 groups in whom a somewhat comparable total antigenic mass was given (table 4), the enhanced early antibody response of the 1 ml × 2 group at four weeks compared with the 0.5 ml × 3 group is in contrast with the lower titers of the 2-dose group at three and six months. Three doses resulted in a more persistent antibody response than two doses, even though total vaccine volume was lower.

Day 28, 42, and 84 antibody log titers were strongly correlated with antibody levels measured on subsequent days of follow-up. Day 28 titers, in subjects receiving lot 1 three times, predicted the day 42, 84, and 180 titers equally well (Pearson's $r = 0.70$ to 0.75). This provides evidence for considerable "tracking" of Rift Valley fever antibody levels in individuals over a period of six months. Persistence of antibody levels is strongly dependent on initial (28 day) titers. A single measure at day 28 provides fairly good prediction of subsequent levels for six months of follow-up at least.

The independent contribution of achieved antibody titers on days 28, 42, and 84 follow-up and the dose of antigen to prediction of six-month antibody levels was examined by regression (table 5). Knowledge of the dose received and the titer reached on day 28 explain 62 per cent of the variance of the day 180 log titers. When both variables are in the regression, dose level remains a significant independent predictor of day 180 levels, but is weaker than titer, particularly at day 84, as seen from the standardized regression coefficients. Terms for squared dose and a dose-titer interaction did not significantly improve prediction when forced

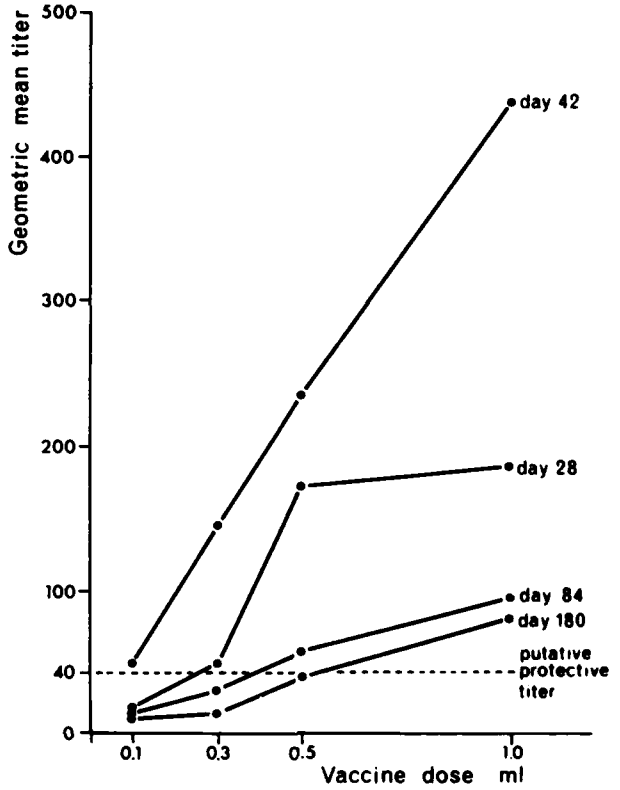

FIGURE 2. Dose response curves for geometric mean titer antibody to Rift Valley fever vaccine on days 28, 42, 84 and 180 of follow-up (vaccine lot 1 administered on days 0, 10 and 28).

TABLE 5

Prediction of Rift Valley fever antibody titer at day 180 by previous titers at each of days 28, 42 and 84 of follow-up and by dose received. A regression analysis*

	Standardized regression coefficient	*p* value	Multiple correlation	R^2	Simple correlation
Day 28 titer	0.593	<0.001	0.75	0.56	0.75
Dose level	0.286	<0.005	0.78	0.62	0.60
Day 42 titer	0.538	<0.001	0.71	0.50	0.71
Dose level	0.296	<0.005	0.75	0.56	0.60
Day 84 titer	0.740	<0.001	0.82	0.68	0.82
Dose level	0.133	>0.10	0.83	0.69	0.60

* Log transformation of reciprocal antibody titers.

into the regression after titer and dose were already in the equation.

Two different schedules for administering the first two injections of 1 ml of lot 1 vaccine were compared. Eight subjects who received two vaccinations 18 days apart had a GMT of 349 when sampled on day 32. The 17 individuals in the 1.0 ml × 3 group were bled on day 28 after having received their first two injections 10 days apart; they developed a GMT of only 186 on day 28. This difference is not signifi-

cant at the 0.05 level ($0.10 < p_{(2\ tailed)} < 0.20$). Although it is possible that the slight difference in day of examination (day 32 versus day 28) may have affected this comparison, the longer interval between injections may have produced an enhanced immune response.

In order to examine interlot differences in vaccine potency we tested the first three lots of vaccine produced, at the 0.5 ml dose level (tables 3 and 4). Using the GMT, lots 1 and 2 appear to be more immunogenic than lot 3 with about twofold differences between the most antigenic and least antigenic lots. These differences were not statistically significant at any follow-up day tested. The sample sizes were small, however, and only substantial differences could be detected with these numbers.

Discussion

The formalin-inactivated Rift Valley fever vaccine tested here (TSI-GSD-200) produces rather frequent but mild and transient local reactions. The reactogenicity is clearly dose dependent and is not the result of increasing sensitization with subsequent doses of vaccine. The vaccine is not pyrogenic. Of the 107 vaccinees who received a total of 306 injections, there were two hypersensitivity-type-reactions—one response perhaps of the Arthus type and one a generalized urticarial syndrome. It is interesting that both cases had a prior history of hypersensitivity states. Although both reported sensitivity to penicillin, this antibiotic was not used in preparation of the vaccine. The cells used to make the vaccine were grown in fetal bovine serum, but they were extensively rinsed prior to vaccine inoculation. Nevertheless, one cannot exclude the possibility of selective adsorption of components to cells and this could result in reactions in presensitized individuals. Use of the vaccine in persons with histories of allergic conditions (especially to more than one allergen) may carry an increased risk and such individuals should be specially supervised if vaccinated. The apparently enhanced early immune response of these two cases is intriguing, but was not sustained, presumably because their subsequent scheduled vaccinations were withheld when side-effects materialized.

The question of rare and severe side effects such as the Guillain-Barré syndrome associated with the swine-influenza vaccination program (16) cannot be answered in a study of this scale. With rates of approximately 1/100,000 in swine influenza vaccinees, it is clear that only very large-scale vaccine usage can identify a problem of this sort. We do not consider the case of meningitis in our study population to be vaccine related. In the Swedish vaccination program (14), one of the approximately 1000 soldiers in the United Nations Expeditionary Forces immunized with the NDBR-103 vaccine developed a neurologic syndrome diagnosed as Guillain-Barré. Though this outcome cannot at present be causally linked to the vaccine, there is some cause for concern. No neurologic reactions were reported among the 3000–4000 additional individuals who have received the NDBR-103 vaccine.

Deaths and severe complications from Rift Valley fever infection were not uncommon in the Egyptian epidemic. Thus, widescale use of Rift Valley fever vaccine, were it available, would appear justifiable in the face of a threat of widespread Rift Valley fever infection and morbidity. However, known world vaccine stocks are limited and in spite of the apparent safety of the vaccine, our experience with it is still relatively small and has been limited largely to healthy young males. Since the vaccine is still in an investigational phase, its use should at present be limited to those potentially at high risk of infection after careful consideration and informed consent.

Immunogenicity of the vaccine has been assessed by its ability to induce spe-

cific antibody detectable in the plaque reduction neutralization test. Minimal protective levels in humans are not established, but titers as low as 10 and 40 appear to have prevented disease in laboratory workers and similar or even lower titers are protective against parenteral challenge of experimental animals (15). Antibody appeared promptly after vaccination. Most subjects were negative on day 10 but the small group sampled on day 18 showed active antibody production. Using the ≥40 cutpoint, the lowest dose (0.1 ml × 3) would have protected 63 per cent of the vaccinees on day 42, and all higher doses, 98 per cent of vaccinees (table 3). By three and six months, 100 per cent and 88 per cent, respectively, who received the 1 ml dose would still be protected; however, only 20 per cent and 16 per cent of those receiving 0.1 ml had levels ≥40. Peak titers were measured on day 42 but had declined considerably by day 84 and were further reduced by day 180. For example, the most potent dose (1 ml lot 1 × 3) produced a GMT of 437 on day 42, which fell to 94 on day 84 but was sustained at 80 on day 180. This vaccine given in three doses thus appears to be characterized by short-lived antibody response with a steep drop-off even at the highest dose level used. Clearly the 1 ml dose level of this vaccine cannot be substituted by smaller doses if the intent of the immunization is prolonged protection. The pattern of response in all vaccine groups indicates that a booster is mandatory to maintain or achieve continued protection.

Although published studies of the older NDBP-103 vaccine (13) do not permit quantitative comparisons with the present trial data, both vaccines would appear to have immunogenicity and reactogenicity of the same order of magnitude.

Thirty-five to 46 per cent of the variance in serologic response at days 28, 42, 84, and 180 was explained by differing dose levels. This suggests that a substantial amount of the variability in immune response to Rift Valley fever vaccine would appear to be due to host factors. The fact that both antibody level at days 28 and 42 and dose levels were independent predictors of day 180 serologic responses suggests, on the one hand, that host factors play a considerable role in determining antibody levels (at a given vaccine dose), while on the other hand, that antigenic mass is a determinant of antibody persistence independently of the early antibody response.

Use of a two rather than a three-dose schedule at the 1 ml level produced adequate titers at day 42 but of very short duration. By six months the antibody level equalled that of the 0.3 ml × 3 dose level although three times the total amount of antigen was used. The three-dose series is clearly preferable Although not statistically significant, the twofold higher titer obtained shortly after giving the first two vaccinations 18 days apart, rather than 10 days apart, suggests that further manipulation of dose schedules might enhance immunogenicity.

There were twofold differences in titer between the three vaccine lots tested at the 0.5 ml dose level. The differences were not statistically significant; however, sample size was small. Contrasting additional lots in the less extensive USAMRIID studies showed significant interlot differences (13). This may necessitate future use of varying dose levels for the various vaccine lots.

Since the amount of Rift Valley fever vaccine currently in stock and available for emergency use is very limited, these trial results could suggest different vaccination policies depending on priorities. For protection of a maximum number of at risk individuals in case of an epidemic, one might use smaller dose levels aiming for short-term protection. Solid and prolonged protection of a defined group of recipients such as veterinarians or laboratory workers would require a full dose with boosting.

The current status of human Rift Valley fever vaccination is clearly far from ideal. There are no rigorous data in humans to support the 1:40 titer inferred to be protective from animal experiments. Nor is there information on the relative contributions of circulating antibody, primed B cells, or other immune effector mechanisms to protection.

Furthermore, our data strongly suggest the need for a more antigenic and more easily administered vaccine that would require less than a three injection primary immunization schedule and a booster. However, the available TSI-GSD-200 vaccine appears to be useful in protecting high-risk persons, but requires additional testing. We are presently evaluating the response to boostering at 18 months using different doses and routes of administration.

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