Aerogenic Immunization of Man with Live Tularemia Vaccine

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INTRODUCTION

Vaccines are generally administered by the subcutaneous or intramuscular route. However, the immune response produced after parenteral administration is inadequate in many instances to ensure optimal host resistance. Many infectious diseases are acquired via the respiratory tree; possibly, the immunizing antigen would be more effective in inducing high-grade host defense if the route of administration were identical to the route of acquisition of the disease. Active immunization against airborne infection by inhalation of living, attenuated microorganisms has been proved with experimental animals and, in some instances, has become routine (1–4, 11–14, 16, 20, 22). The potential for immunization of man by aerogenic vaccination with single or combined live vaccines has been recognized in the Soviet Union (1–3) and in the United States (5, 8, 9). In the Soviet Union, vaccination of man with aerosols of dried, viable tularemia vaccine, singly or in combination with living vaccines of other microorganisms, has received considerable attention. Systemic reactions were reported by Alexsandrov et al. (2) in 2 of 138 volunteers inhaling an estimated 750,000 organisms contained in an aerosol of dried tularemia vaccine. Kerostovtaev, Onikiyenky, and Khokhlov (17) noted similar complaints in three of eight persons inhaling 7,500,000 cells of a comparable product. Immunity has been measured primarily by serological procedures and by reaction to skin test preparations, but has not been proved by increase in resistance of the vaccinee to challenge

with fully virulent organisms. Tigertt (23) has reviewed selected Soviet articles on viable tularemia vaccines, and Lebidinsky (18) has reviewed the published American literature on this subject.

In the United States, live tularemia vaccine prepared from *Francisella tularensis* strain LVS (live vaccine strain) (7) and administered percutaneously has been proved immunogenic and superior to killed vaccines in studies with volunteers by Saslaw et al. (21) and by McCrumb (19). Studies by the latter investigator revealed that, although immunized volunteers were protected against challenge by the respiratory route with 200 to 2,000 virulent organisms, resistance could be overcome in about 50% of men when the challenge dose was increased approximately 10-fold. (The genus *Francisella*, honoring the late Edward Francis of the U.S. Public Health Service and providing better taxonomy, will appear in the next edition of *Bergey's Manual.*)

In an effort to enhance the immunity provided by LVS, aerogenic vaccination was studied by Eigelsbach et al. (10, 11) and White et al. (24). It was demonstrated that this route of vaccination was not associated with untoward reactions, and only a mild, nongranulomatous response was observed in the respiratory bronchioles of monkeys that received aerosolized liquid vaccine. Animals so vaccinated evidenced excellent protection when challenged with virulent organisms.

In a recent unpublished study (H. T. Eigelsbach and J. J. Tulis) designed to determine the effect of aerosolized vaccine dose on reactivity and

TABLE 1. *Effect of dose and route of inoculation on the immunogenicity of live tularemia vaccine for the monkey*

Vaccination	Vaccine dose	No. of animals	Survival at 120 days after aerogenic challenge[a]
			%
Respiratory....	10^7	16	94
Respiratory....	10^5	15	60
Respiratory....	10^2	17	47
Dermal........	Acupuncture	16	81
Control........	None	12	0

[a] With 10^4 cells of strain SCHU S4.

immunogenicity for monkey, groups of 15 to 17 *Macaca mulatta* inhaled 10^2, 10^5, or 10^7 cells of live tularemia vaccine strain LVS. Another group of 16 animals received LVS percutaneously by acupuncture; in this case, the actual number of cells introduced is unknown, because a substantial portion of the inoculum remains on the surface of the skin. Vaccination by either procedure proved innocuous, and resulted in comparable peak mean titers except in the aerosol group receiving the lowest dilution of organisms. Of the 17 animals that inhaled 10^2 organisms, 9 failed to develop agglutinins. The mean titer of the intradermal vaccinees rose earlier and faster than did titers of the aerogenic vaccinees. At 60 days after vaccination, these animals, as well as nonvaccinated controls, were challenged aerogenically with 10^4 cells of strain SCHU S4 (Table 1). All controls died within 30 days: 120 days after challenge, the per cent survival in the 10^7, 10^5, and 10^2 groups vaccinated aerogenically and in the group vaccinated dermally was 94, 60, 47, and 81, respectively. Because monkeys are less resistant to tularemia than is man, their benign response to aerosolized liquid LVS tularemia vaccine indicated that this vaccine might also be safe for man when administered aerogenically. Initial studies in volunteers indicated (9) that respiratory doses ranging from 200 to 30,000 organisms were innocuous and that approximately 1,500 inhaled cells were required to induce serological conversion consistently. These studies were expanded at the University of Maryland Research Ward at Jessup, Maryland House of Correction, and are the subject of the present report.

AEROGENIC VACCINATION OF VOLUNTEERS

Materials and Methods

F. tularensis LVS and highly virulent challenge strain SCHU S4 (6) were cultivated in a modified casein partial-hydrolysate liquid medium (R. C. Mills et al., Bacteriol. Proc., p. 37, 1949). Cultures, harvested after 16 hr of incubation with continuous shaking at 37 C, contained 35 $\times$ 10^9 to 40 $\times$ 10^9 viable organisms per milliliter. For aerogenic immunization with strain LVS or challenge with strain SCHU S4, aerosols were generated with a nebulizer that produced particles primarily in the range of 1 to 5 μ diameter. Methodology was comparable to that previously described by Griffith (15).

Prior to aerosolization of LVS for use as a vaccine in man, all available information pertaining to its safety was evaluated. Extensive experience gained in volunteers and laboratory workers at Fort Detrick by the acupuncture route (8) attested to the attenuation of this strain. Serious reactions, such as secondary pneumonitis or bubo formation, were not seen. Immunogenicity was evident from the excellent protection noted in vaccinated volunteers exposed to aerosol or intracutaneous challenge. The aforementioned thorough animal evaluations suggested that no untoward reactions were likely to occur in man after inhalation of LVS.

Clinical Reactions

Five groups totaling 253 volunteers free from tularemia agglutinins were exposed to aerosolized LVS. The dose ranged from 10^4 to 10^8 organisms. Reaction rates correlated directly with size of inoculum. After inhaling a dose of 10^4 LVS cells, about 30% of 42 volunteers had minor systemic complaints. The majority noted minimal upper respiratory symptoms, such as sore throat or slight cough. Practically all had pea-sized cervical nodes after exposure to aerogenic LVS. None had fever or roentgen evidence of pneumonic infiltration. The signs and symptoms were quite insignificant and would have been overlooked with casual examination or questioning.

A more severe reaction was associated with inhalation of a 10^8 inoculum. As a result of this massive dose, 90% of the volunteers were symptomatic with headache, coryza, chest pain, and malaise. Actually, they had mild typhoidal tularemia. In 80%, there were temperature elevations of >100 F which occurred on the average at 3 days and lasted an average of 2.5 days. Of 42 men receiving this huge dose, 3 were treated with streptomycin, and several others were put to bed for periods of 2 to 3 days. Chest X rays revealed transient infiltrations in a few of the vaccinees. In general, the reaction just described can be likened to a "flu-like" syndrome. This condition did not incapacitate the majority of volunteers; they were able to con-

tinue their prison routine. Similar, but milder reactions, were seen in 79% of volunteers inhaling 10⁶ cells of LVS.

Serological Response

Results of serological studies have shown the correlation between size of inhaled dose (antigenic mass) and acquisition of serum agglutinins. Volunteers receiving the largest number of organisms developed demonstrable serum antibodies in a surprisingly short time. By the second week postvaccination, 92% of the volunteers had agglutinins, and at 3 weeks a 97% incidence was recorded. This rapid acquisition of serum antibodies following the large inhaled antigenic mass was more rapid than the rate following LVS administered by acupuncture (65% at 2 and 82% at 3 weeks). The preliminary studies with smaller aerogenic inocula revealed a delayed response when compared with the intracutaneous route of vaccination. The results of these series of investigations suggested that large groups of nonimmune people can be immunized more rapidly by the respiratory than the intracutaneous route; however, a high incidence of systemic reactions would result from exposure to large-dose vaccine aerosols. Although there is more rapid seroconversion noted with the latter method (large-dose aerosol), the geometric mean titers were no different after 8 weeks whether vaccination was accomplished by acupuncture or with smaller-dose aerosols.

Conversion rates were reduced and geometric mean agglutinin titers were delayed as the inhaled dose was lowered. After the 10⁴ log dose of LVS, geometric mean titers did not begin to rise significantly until the 3rd week postvaccination, and antibody levels comparable to those associated with acupuncture vaccination occurred between the 4th and 5th weeks (Fig. 1). Both geometric mean titers lagged behind those of the two largest aerogenic vaccine doses. Similarly,
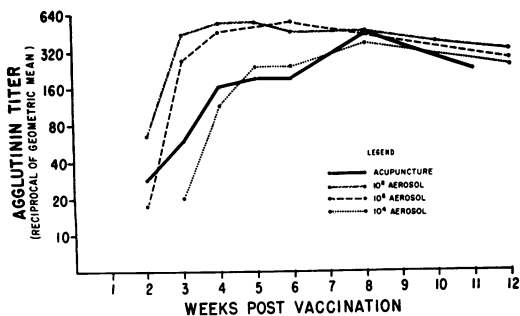

FIG. 1. *Agglutinin response to LVS vaccine administered in varying doses by the aerogenic route compared with response after intradermal inoculation.*

TABLE 2. *Response of volunteers to large-dose respiratory challenge after aerogenic vaccination*

Interval between vaccination and challenge	No. challenged[a]	No. with fever >100 F	No. requiring therapy[b]	Per cent protection[c]
months				
2	22	15	6	73
4	30	18	0	100
6	16	10	0	100
14	32	26	16	50
18	2	2	1	50
Summation, 2–18	102	71 (70%)	23 (23%)	77
Controls	47	44 (94%)	42 (89%)	

[a] With 2.5 × 10⁴ organisms, strain SCHU S4.

[b] Criterion for treatment was 103 F or greater for over 24 hr.

[c] Uncorrected with respect to control data.

seroconversion rates peaked at the 90% level 5 weeks postvaccination, compared with 3 weeks. Nevertheless, geometric mean titers eventually reached antibody levels achieved with larger aerosol doses.

CHALLENGE OF AEROGENIC VACCINEES WITH VIRULENT *F. TULARENSIS*

Aerogenic Challenge

The presence of circulating tularemia agglutinins is not tantamount to resistance to the disease. It remained, therefore, to evaluate the degree of protection of the volunteers to challenge with virulent *F. tularensis*. Table 2 outlines the results of aerogenic challenge with 2.5 × 10⁴ organisms. This challenge represents approximately 2,500 times the minimum infective dose for man, which has been estimated to be 10 to 50 organisms (21). This was a severe challenge and probably far exceeds the number encountered during natural exposures. In this experiment, the interval between vaccination and time of challenge did not appear to be a determining factor in the extent of protection. At 2 months 73% of 22 and at 14 months 50% of 32 volunteers exposed developed disease and were treated with antibiotics. (The difference was not significant by the chi square test.) These two groups received the same dose of aerosolized vaccine. Those men challenged at 4 and 6 months received the two highest doses of LVS (10⁶ and 10⁸), and the subsequent mild vaccine infection may have contributed to the excellent overall resistance of the groups.

Table 3 illustrates the significance of the method of vaccination of these volunteers in relation to

TABLE 3. *Relationship of route and dose of LVS vaccine to resistance to tularemia aerosol challenge*

Dose	Route	No. challenged	No. (%) with fever >100 F[a]	Per cent requiring treatment	Percent protection[b]
10^8	Respiratory	30	18 (60)	0	100
10^6	Respiratory	16	10 (62)	0	100
10^4	Respiratory	56	43 (77)	41	59
—[c]	Dermal	46	29 (63)	46	54
	None	47	44 (94)	89	

[a] Mean incubation period: volunteers vaccinated aerogenically or by acupuncture, approximately 4 days; controls, approximately 3 days.

[b] Uncorrected with respect to control data.

[c] Acupuncture technique.

resistance to respiratory challenge. High-grade protection was acquired by the men inhaling 10^8 or 10^6 doses of LVS. A somewhat lower grade protection was observed in man immunized aerogenically with 10^4 LVS or by acupuncture; similar protection resulted in both groups. The incidence of *infection* in all four groups of vaccinees was equivalent (60 to 77% had fever of 100 F or greater), but the incidence of the disease was quite different. The 28 infected men in the two groups who had received large doses of LVS by the aerogenic route reacted to the initial infectious process developing from the severe respiratory challenge, but the acquired resistance prevented progression to overt disease requiring specific treatment.

The average incubation period for the control subjects was less by 1 day than that of the vaccinees. The shorter incubation period in the controls plus equal incubation time for all vaccinees, acupuncture as well as aerogenic, suggests that respiratory exposure to LVS did not sensitize the lung parenchyma. If a hypersensitivity reaction had occurred in men vaccinated aerogenically, immediate febrile or systemic reactions might have been expected. No evidence of such reaction was observed.

Table 4 presents data accumulated from additional experiments wherein volunteers, immunized by the acupuncture technique, were challenged aerogenically at varying intervals postvaccination. Although the numbers of subjects were small, results were similar to those observed after small-dose aerosol LVS. Immunity waned at about 1 year to the same extent.

Unvaccinated volunteers without demonstrable tularemia agglutinins served as controls in these aerosol challenges. Five of 47 men failed to

TABLE 4. *Response of volunteers to large-dose respiratory challenge after acupuncture vaccination*

Interval between vaccination and challenge	No. challenged[a]	No. with fever >100 F	No. requiring therapy[b]	Per cent protection[c]
months				
2	5	1	0	100
4	13	10	4	69
11	8	6	6	25
14	19	11	10	47
36	1	1	1	
Summation 2–36	46	29 (65%)	21	54
Controls	47	44 (94%)	42 (89%)	

[a] With 2.5×10^4 organisms, strain SCHU S4.

[b] Criterion for treatment, 103 F per os or greater for over 24 hr.

[c] Uncorrected with respect to control data.

develop disease. Actually, four men represent these five failures; two were rechallenged and developed pneumonic tularemia, a third was reexposed on two additional occasions before disease was induced, and the fourth has not been rechallenged. Each appeared to be a complete "miss" at time of exposure without subsequent subclinical infection, because antibodies were not demonstrable. Mechanical difficulties, i.e., loose-fitting masks, were implicated as the significant reasons for failure to produce disease and not natural host resistance, because of susceptibility to rechallenge. Similar incidents may also have occurred in the exposed vaccinees. The low frequency of "misses" and presumed equal distribution would not invalidate the per cent protection observed in the challenged vaccinees.

Intradermal Challenge

Small numbers of volunteers receiving vaccine by the respiratory route have been challenged by the intradermal inoculation of 1,000 to 10,000 infectious doses per man of SCHU S4 strain (Table 5). Protection was excellent. Not only was there no evidence of lessened immunity after 6 months, but also resistance to massive challenges was uniform. The disease rates were comparable to those following challenge of volunteers vaccinated by acupuncture. The clinical appearance of inoculation sites was strikingly different from the lesions in controls. The skin lesion resembled a delayed hypersensitivity reaction in the immune individual; control

TABLE 5. *Response of vaccinated volunteers to intradermal challenge*

Type of vacci-nation	Challenge dose (organisms)	Interval be-tween vacci-nation and challenge	No. chal-lenged	No. re-quiring therapy[a]	Number protected/ total	Controls requir-ing therapy/ total
		months				
Acupunture	3×10^3	6	10	1	9/10	10/10
	8×10^3	6	8	1	7/8	1/1
	1×10^4	2	3	1	2/3	2/2
	1×10^5	2	3	1	2/3	—
Total	3×10^3–1×10^5	2–6	24	4	20/24 (83%)	
Aerogenic	1×10^4	2	7	0	7/7	2/2
	1×10^4	6	4	0	4/4	4/4
	1×10^5	2	3	2	1/3	—
Total	1×10^4–1×10^5	2–6	14	2	12/14 (86%)	

[a] Criterion for treatment, development of a typical ulceroglandular infection similar to that in controls.

subjects showed progressively developing ulcers. Based on this small experience, it appears that aerosolized LVS produces effective immunity to ulceroglandular tularemia.

Relationship of Antibody Titers to Immunity

Analysis of agglutinating antibody titers in the vaccinees suggests that higher levels were associated with less severe illness, and groups of inmates requiring treatment had baseline geometric mean titers one-half the value of those groups not treated. On the other hand, absence of disease in the respiratory challenge group which received the massive dose of aerogenic LVS cannot be explained solely on the basis of elevated titers. Geometric mean titers in this group were equivalent to those of the other aerogenically vaccinated groups. Challenge results were quite different; 6 of 22 men had disease when exposed 2 months after small-dose aerosol vaccination, but none of 30 men had disease following challenge at 4 months after large-dose vaccine aerosol. Thus, although absolute level of agglutinins cannot be correlated with immunity, presence of these antibodies in the sera of volunteers exposed to virulent challenge suggests that members of the group will resist infection to a greater degree than unvaccinated controls.

DISCUSSION

Immunization of man against tularemia can be accomplished safely by employing aerosolized living attenuated vaccine. The dose necessary to ensure development of serum antibodies in at least 90% of volunteers is 10^4 organisms. Systemic subjective reactions at this dose were not significant, and close clinical observation was

necessary to reveal subtle objective findings, i.e., appearance of pea-sized cervical lymph nodes.

The inhaled dose can be increased without undue risk if more rapid induction of antibodies is desired. As many as 10^8 organisms have been delivered to volunteers as an immunizing dose. Low-grade febrile disease occurred in more than 90% of the volunteers with this dose. However, the reaction was mild and self-limiting, and did not interfere with the daily routine of most inmates. After this vaccination, a high-grade immunity was observed against a severe aerogenic challenge conducted 4 months after vaccination. Assurance is provided, thereby, that, even if unlikely dilution errors would create such concentrated aerosols, exposed healthy young adults would experience only mild discomfort. Acceptability of this aerosolized antigen is questionable in people with chronic lung disease, congestive failure, or other diseases affecting the integrity of respiratory defense mechanisms. Perhaps small doses of aerosolized LVS could be tolerated in such patients. Sufficient evidence bearing on this point is unavailable.

These studies validate the respiratory route as a means of introducing an attenuated bacterium into the human host. It remains to be determined whether this route is more advantageous than the conventional dermal site. Aerosolized vaccine does lead to an immune state. The incidence of disease after challenge of volunteers vaccinated by this method was less than that recorded in the men challenged after immunization by acupuncture. This difference occurred primarily in groups that received the larger doses of aerosolized LVS. These men had mild tularemia after vaccination, and the virulent challenge can

almost be considered a rechallenge. This type of immunization provoked more resistance to infection. Circulating antibodies alone are not sufficient to explain differences in protection; the geometric mean titers were identical for the aerogenic vaccine groups challenged at 2 and 4 months, respectively; yet, disease rate was greater in the former. Perhaps the lower disease rate results from the ability of lung tissue previously exposed to LVS to confine better the inhaled pathogens through better phagocytosis, tissue antibody effect, or other local defense mechanisms. Thus, it seems reasonable to expect that in respiratory-acquired infectious diseases prior vaccination with sufficient antigen given by the aerogenic route will produce increased host protection. Present evidence is insufficient to allow conclusions regarding the protection afforded aerogenically vaccinated individuals against the ulceroglandular form of tularemia. Following the reasoning above, the acupuncture method should be the best way to prevent this disease. The differences in distribution of vaccine by the two routes into the two organs initiates dissimilar reactions for developing local tissue defense. Therefore, analogous reasoning cannot be applied to the skin.

One disadvantage of the aerogenic vaccination technique is the lack of a "marker" indicating vaccine reaction. The scar from the acupuncture route is obvious for weeks. Nevertheless, the need for visible evidence of reaction to vaccine is lessened when over 90% of an exposed population are immunized by simple inhalation of LVS. In addition, serological proof of vaccination is easily obtained.

The elaborate exposure equipment used in these studies allowed for precision in uniformity of particle size and quantitation of the inhaled dose. The application of aerosolized vaccines on a mass basis will require simple, less complicated apparatus. Efforts to create such instruments should be encouraged. Soviet literature contains reference to mass aerogenic vaccination of troops exposed in tents (6). Vaccination by the respiratory route for tularemia is effective, and this fact should serve as an impetus for future experimental studies with viral and bacterial vaccines.

SUMMARY

Live, attenuated LVS tularemia vaccine has been administered via the respiratory route in doses ranging from 10^4 to 10^8 organisms. Mild self-limiting typhoidal tularemia was induced by doses of 10^6 to 10^8 vaccine organisms. Rapidity of induction of agglutinin titers in the human host varies directly with size of inhaled inoculum. Immunity to aerogenic virulent *F. tularensis* challenge appeared to be greater than that produced by the conventional acupuncture method of vaccine administration. Protection against ulceroglandular tularemia was also demonstrated. The pulmonary tree in man can be safely and successfully utilized for application of *F. tularensis* strain LVS and possibly for other microorganisms.

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