Aerogenic Immunization of the Monkey and Guinea Pig with Live Tularemia Vaccine. (27049)

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(Introduced by Samuel Saslaw)
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Active immunization against airborne infection by inhalation of attenuated microorganisms has been proved with experimental animals(1-7). The potential for mass immunization of man by aerogenic vaccination with single or combined live vaccines has been recognized in the USSR(4, 5). In the U.S.A., aerogenic vaccination has not received widespread attention. The present study was initiated to explain the role of 2 routes of vaccination, dermal and respiratory, on the clinical acceptability and immunogenicity of live tularemia vaccine(8, 9) for the monkey and guinea pig.

Materials and methods. The monkeys *(Macaca mulatta)*, weighing between 1.8 and 3.7 kg, were distributed so that each group included animals of comparable weight and sex. The male to female ratio was approximately 3:1. Hartley strain guinea pigs were selected without regard to sex and weighed 475-525 g each. *Pasteurella tularensis* live vaccine strain LVS was cultivated in modified casein partial hydrolyzate (MCPH) liquid medium(8) and suspended in sterile half-strength medium. Aerogenic vaccination with strain LVS and subsequent respiratory challenge with highly virulent *P. tularensis* strain SCHU S5(10) via the respiratory route were performed as described previously(9). For dermal vaccination, 0.2 ml of either a gel-saline (0.1% gelatin and 0.85% sodium chloride) dilution of an MCPH culture of strain LVS or of rehydrated lyophilized LVS live tularemia vaccine, was administered subcutaneously (sc) on the right rear leg of the guinea pig and intracutaneously on the back of the monkey. Monkeys received a respiratory challenge of 750 cells 50 days after vaccination. Guinea pigs received a respiratory challenge of 4×10^3 cells or a sc challenge of 10^3 cells of strain SCHU S5 in the left rear leg 30 days after vaccination. Rectal temperatures of 10 guinea pigs in each group of 40, and of all monkeys, were recorded once or twice daily for 3 days before and 11-15 days after vaccination. Temperatures of all monkeys were recorded for 15-30 days after challenge. Clinical examination of monkeys included a study of chest roentgenograms taken daily for 10 days after vaccination and then every other day until 22 days after challenge. Agglutinin(11) and precipitin(12) titrations were performed on sera obtained 24 days after vaccination from the 10 guinea pigs per group used for the temperature study and from all monkeys 1, 3, and 6 weeks after vaccination. One ml of blood from each monkey was cultured for *P. tularensis* daily for 7 days after challenge and every other day thereafter if the animal remained febrile. Dead animals were autopsied, examined for gross pathology, and the heart blood, lungs, and spleen cultured for *P. tularensis*.

Results and discussion. Live vaccine in the monkey. The simian experiments and their major results are outlined in Table I. Live

TABLE I. Effect of Route of Vaccination on Resistance of the Monkey to Respiratory Challenge with *Pasteurella tularensis*.*

Vaccination	No. of animals	Animals yielding blood culture(s) of challenge strain, %	Survival, %
Intracutaneous, 10^8 viable cells rehydrated lyophilized vaccine	8	50	63
Intracutaneous, 10^6 viable cells culture vaccine	7	71	57
Aerogenic, 10^6 viable cells culture vaccine	8	13	88†
None	8	88	13

* All animals challenged with 750 cells of strain SCHU S5.

† Cause of death in the only animal that died in this group was complicated by peritonitis not attributed to tularemia.

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vaccine was administered intracutaneously to 2 groups of animals. A third group received live vaccine aerogenically. With few exceptions, animals vaccinated intracutaneously became febrile (104-105°F) for 12-48 hr 3-4 days after vaccination. In contrast none of the monkeys vaccinated via the respiratory route became febrile. Rehydrated lyophilized vaccine and vaccine culture administered intracutaneously could not be differentiated on basis of skin reactivity. The vaccine lesions were benign but of greater intensity than those resulting from live vaccine administered by acupuncture(13). Chest roentgenograms showed no changes caused by vaccination. All vaccinated monkeys developed *P. tularensis* agglutinins. In general, aerogenic vaccination resulted in higher agglutinin titers (1:320-1:1,280) than did intracutaneous vaccination (1:20-1:320). Aerogenic vaccination resulted in more and denser precipitin lines on agar double diffusion plates than did sc vaccination(12).

Respiratory challenge of vaccinated and nonvaccinated monkeys was performed 7 weeks after vaccination. All animals exhibited evidence of infection. With the exception of 1 control, all animals became febrile within 7 days after challenge. The exception did not exhibit overt disease but developed a diagnostic agglutinin titer. The incubation period did not vary appreciably in the 4 groups of animals, nor did the time of death. Positive blood cultures were obtained from fewer of the animals vaccinated by the respiratory route than from other animals. Roentgenograms indicated that pathologic pulmonary changes were less severe in this group. Also, these animals exhibited fewer and less extensive signs of physical disability than animals vaccinated by the dermal route.

Survival in animals vaccinated aerogenically was 88% in comparison with 57-63% in animals vaccinated intracutaneously and 13% in nonvaccinated controls. Only 1 of 8 animals vaccinated by the respiratory route died subsequent to challenge. The cause of death in this animal was complicated by a perforated colon, not attributable to tularemia, which resulted in peritonitis. These results are in agreement with a previous study in which 9 of 17 monkeys vaccinated by the intracutaneous route survived a virulent respiratory challenge of 1,000 or more organisms and all nonvaccinated animals died(13).

Data on survival of vaccinated monkeys after respiratory challenge together with clinical observations obtained during the course of disease show that aerogenic vaccination with live vaccine afforded comparable if not greater protection against pulmonary tularemia than vaccination by the intracutaneous route.

Live vaccine in the guinea pig. Groups of 40 guinea pigs each were vaccinated by the respiratory or sc route with 10-20, 10^3, or 10^5 cells of live vaccine culture. Sixty % of the animals inhaling 10-20 cells of live vaccine and 100% of those receiving 10^3 or 10^5 cells became febrile 6-10 days later. After sc vaccination none of the animals inoculated with 10-20 cells became febrile whereas 60% of those administered 10^3 cells and 100% of those having received 10^5 cells exhibited fever in 4-8 days. Vaccine by either route proved innocuous except for a febrile response of 1-5 days duration and a benign skin lesion in animals inoculated sc. Table II shows that a greater number of animals vaccinated by the respiratory route developed *P. tularensis* agglutinins than animals vaccinated sc; also, the quantitative serological response of these animals was higher than observed in animals vaccinated sc.

TABLE II. Agglutinin Response of the Guinea Pig 24 Days after Administration of Live Tularemia Vaccine.

Viable cells in vaccine inoculum	Route of vaccination: Respiratory No. animals	Respiratory Titer	Subcutaneous No. animals	Subcutaneous Titer
10-20	4	Neg	9	Neg
	1	1:80	1	1:160
	4	1:160	—	—
	1	1:320	—	—
10^3	1	Neg	4	Neg
	2	1:80	2	1:80
	2	1:160	3	1:160
	1	1:320	1	1:320
	3	1:640	—	—
	1	1:1,280	—	—
10^5	6	1:320	1	1:10
	2	1:640	1	1:20
	2	1:1,280	1	1:80
	—	—	6	1:160
	—	—	1	1:320

Respiratory challenge of one-half of each group of 38-40 guinea pigs was performed 30

TABLE III. Effect of Route of Vaccination on Resistance of Guinea Pig to Respiratory or Subcutaneous Challenge with *Pasteurella tularensis*.

Viable cells in vaccine inoculum	Respiratory challenge Survival on indicated day after challenge, %*				Subcutaneous challenge Survival on indicated day after challenge, %*			
	7	15	7	15	7	15	7	15
	Respiratory vaccination		Subcutaneous vaccination		Respiratory vaccination		Subcutaneous vaccination	
10^5	100	60	30	5	100	90	90	30
10^3	75	45	35	0	100†	68	89†	11
10-20	30	0	5	0	75	15	45	10

* 1-4 x 10^3 cells of SCHU S5; no survival in non-vaccinated animals 7 days after challenge.
† 19 animals per group, all others 20 per group.

days after vaccination with approximately 4 x 10^3 cells of strain SCHU S5. The remaining animals received 10^3 cells of the same culture sc. Two additional groups of 20 nonvaccinated animals each were challenged either by the respiratory or sc route and served as controls.

Table III shows that aerogenic vaccination provided greater protection against both respiratory and sc challenge than did vaccine administered sc. All nonvaccinated animals were dead within 1 week after challenge. Aerogenic vaccination with 10^5 cells protected 60% and 90% of the guinea pigs against the respiratory and sc challenge, respectively, whereas sc vaccination with the same dosage resulted in only 5% and 30% survival, respectively, 15 days after challenge. Lower order survival comparable in pattern was observed in animals that received a vaccine dosage of 10^3 cells. Vaccination with as few as 10-20 viable cells resulted in a low grade immunity demonstrated by survival of a small percentage of the animals or by an increase in survival time.

These data support results obtained with the monkey. The combined animal data strongly suggest that aerogenic vaccination affords greater immunity against tularemia than does vaccination by the dermal route.

Summary. Studies were made on the clinical acceptability and immunogenicity of live tularemia vaccine administered to the monkey and guinea pig by the dermal or respiratory route. Aerogenic as well as dermal vaccination proved innocuous. Animals vaccinated aerogenically showed higher levels of antibody and comparable if not greater protection against challenge with a highly virulent strain of *P. tularensis* than animals vaccinated dermally.

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