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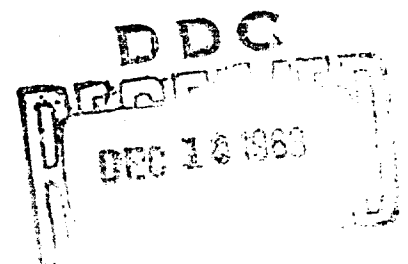
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TECHNICAL MANUSCRIPT 51

HOST-PARASITE RELATIONSHIP IN MONKEYS
ADMINISTERED LIVE TULAREMIA VACCINE
VIA THE DERMAL OR RESPIRATORY ROUTE
AND SUBSEQUENTLY CHALLENGED

OCTOBER 1963



UNITED STATES ARMY
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Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 51

HOST-PARASITE RELATIONSHIP IN MONKEYS ADMINISTERED
LIVE TULAREMIA VACCINE VIA THE DERMAL OR
RESPIRATORY ROUTE AND SUBSEQUENTLY CHALLENGED

Jerry J. Tulis

Henry T. Eigelsbach

Robert W. Kerpsack

Medical Bacteriology Division
DIRECTOR OF BIOLOGICAL RESEARCH

Project 1C022301A068

October 1963

A portion of the work reported here was performed under Project 4B11-02-066, "Bacterial and Fungal Agent Research," Task -02, "Bacterial and Fungal Agent Laboratory Research." The expenditure order was 2200702. This material was originally submitted as manuscript 5097.

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ABSTRACT

We have previously reported on the host-parasite relationship in monkeys vaccinated intracutaneously or aerogenically with live tularemia vaccine. A corollary investigation has been made in vaccinated monkeys exposed via the respiratory route to 10^3 cells of highly virulent Pasteurella tularensis SCHU S4 and serially sacrificed from one hour to 28 days thereafter. Earlier and more extensive dissemination as well as greater proliferation of the challenge strain occurred in nonvaccinated controls than in vaccinees. Focal accumulation of monocytic cells in the pulmonary parenchyma was first observed in nonvaccinated controls, animals vaccinated dermally, and animals vaccinated aerogenically, on the first, third, and fifth day respectively, after challenge. In contrast to septicemia and fatal disease in nonvaccinated controls within seven days after challenge, a reduction in number or disappearance of SCHU S4 organisms occurred in the lungs, tracheobronchial lymph nodes, spleen, and liver of vaccinees between 14 and 28 days. Nonvaccinated controls and animals vaccinated dermally exhibited enlarged caseous tracheobronchial lymph nodes and moderate pulmonary involvement by the fifth day whereas animals vaccinated aerogenically did not show comparable pathology until seven to ten days after challenge. Less extensive histological changes occurred at secondary infection sites, including the spleen, liver, and various lymph nodes, in both groups of vaccinees in comparison with nonvaccinated controls; however, involvement was least severe in animals vaccinated aerogenically.

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HOST-PARASITE RELATIONSHIP IN MONKEYS ADMINISTERED
LIVE TULAREMIA VACCINE VIA THE DERMAL OR
RESPIRATORY ROUTE AND SUBSEQUENTLY CHALLENGED

We have reported previously on the host-parasite relationship resulting from administration of live tularemia vaccine to Macaca irus by either the dermal or respiratory route.* Tissue changes were mild and consisted primarily of the proliferation of histiocytes without formation of granulomas regardless of the inoculation route. Vaccine strain LVS was isolated from the inoculation site of animals vaccinated intracutaneously, from the lungs of animals vaccinated aerogenically, and from the regional lymph nodes, liver, and spleen of both groups of vaccinees; no isolations were made from the blood or bone marrow. Proliferation of LVS was observed in both groups of vaccinees within 24 hours. Peak viable populations were reached within three days and maintained through Day 10; clearance had begun by Day 14. Intracellular antitularensis gamma globulin (ATGG) persisted at least 90 days in vaccinated animals. Plasmacytes containing ATGG were located in the interstitial tissue adjacent to the respiratory bronchioles, peribronchial and perivascular lymphoid aggregates, tracheobronchial lymph nodes, splenic pulp, and liver of aerogenically vaccinated animals, and in the tracheobronchial lymph nodes, splenic pulp, and liver of dermally vaccinated animals.

The initial phase of the study having been completed, host-parasite relationship data were obtained on vaccinated monkeys subsequent to challenge. The present report concerns the serological, bacteriological, and histological studies in the monkey vaccinated dermally or aerogenically with LVS and subsequently challenged aerogenically with Pasteurella tularensis SCHU S4.**

Sixty cynomolgus monkeys were administered live tularemia vaccine; 24 received 1×10^5 cells of live vaccine intracutaneously in the deltoid area of the left arm and 36 received a mean inhaled dose of 7.6×10^5 cells. Two months after vaccination, all immunized monkeys and 16 non-vaccinated controls received an inhaled dose of approximately 1000 viable SCHU S4 organisms. Groups of two dermally vaccinated, three aerogenically vaccinated, and two nonvaccinated monkeys were sacrificed 1, 6, and 12 hours and 1, 2, 3, 5, and 7 days after challenge. In addition, two dermally vaccinated and three aerogenically vaccinated monkeys were sacrificed at 10 and 14 days after exposure to SCHU S4; one dermally vaccinated and two aerogenically vaccinated monkeys were sacrificed at 28 days after exposure.

* Eigelsbach, H.T.; Tulis, J.J.; McGavran, M.H.; and White, J.D.
"Live tularemia vaccine. I. Host-parasite relationship in monkeys vaccinated intracutaneously or aerogenically," J. Bact. 84:1020-1027, 1962.

** Animals maintained in compliance with the "Principles of Laboratory Animal Care" as promulgated by the National Society for Medical Research.

The agglutinin response of monkeys after dermal or aerogenic vaccination is shown in Figure 1. Peak mean agglutinin titers, 1:1440 for animals vaccinated intracutanecusly and 1:3810 for animals vaccinated aerogenically, were attained in both groups 28 days after administration of live vaccine organisms and declined gradually thereafter. At time of challenge, two months after vaccination, mean agglutinin titers of 1:940 and 1:1980 were recorded for monkeys vaccinated dermally and aerogenically, respectively.

Data on the number of SCHU S4 organisms recovered from the lungs of vaccinated and nonvaccinated monkeys at 1, 12, and 24 hours after respiratory challenge are shown in Table I. Within one hour after aerogenic exposure, organisms were isolated from the lungs of all animals sacrificed and examined except one dermally vaccinated monkey. This animal received the lowest number of challenge organisms and, based on 40 per cent retention, had only 60 cells dispersed in 14.6 grams of lung tissue. Recovery of SCHU S4 from the right apical or diaphragmatic lobes of the lung one hour after exposure to a mean inhaled dose of 880 viable cells ranged from one to 20 organisms per gram; corresponding retention was two to 35 per cent and the mean retained dose was 132 cells. Twelve and 24 hours after exposure, isolations from the lung were sporadic and no significant difference in population of SCHU S4 was observed between groups of vaccinees or between vaccinees and controls.

Three days after challenge, SCHU S4 organisms were cultured from the lungs of all vaccinated and nonvaccinated animals. Viable populations ranged from 100 to 100,000 organisms per gram of lung tissue. At this time, SCHU S4 was isolated from the spleen of both nonvaccinated monkeys and from the liver of one. Vaccinees failed to yield positive cultures.

Viable populations of 10^5 to 10^6 SCHU S4 per gram were recovered from the lungs of the aerogenically vaccinated monkeys between three and ten days after challenge. Data shown in Table II indicate clearance in most of these animals as early as 14 days after exposure. However, 10^7 SCHU S4 per gram were cultured from the lungs of one of two dermally vaccinated monkeys 14 days after challenge. During the acute phase of tularemic infection (three to 14 days after challenge), *P. tularensis* was isolated from both apical and diaphragmatic lobes of the right aspect of the lung in six of 14 aerogenically vaccinated and all eight of eight dermally vaccinated monkeys.

As shown in Table III, recovery of SCHU S4 from the regional lymph nodes was sporadic until the fifth day after challenge. Higher counts were obtained from the nonvaccinated controls on Days 5 and 8 in comparison with vaccinees. Clearance was apparently complete in vaccinees within 28 days.

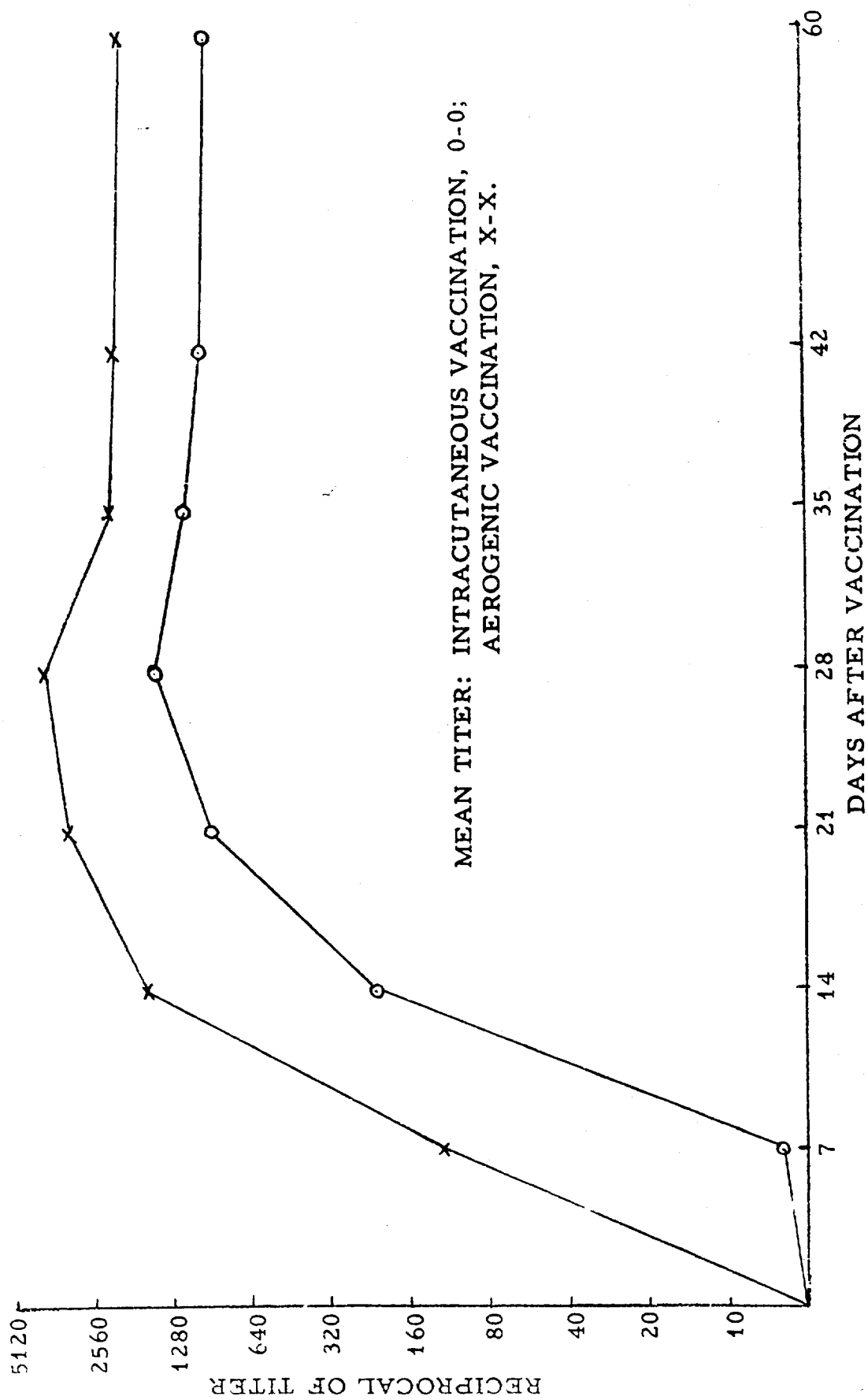


Figure 1. Agglutinin Response of Monkeys Administered Live Tularemia Vaccine.

TABLE I. RECOVERY OF P. TULARENSIS FROM THE LUNG OF M. IRUS
AFTER RESPIRATORY CHALLENGE WITH 880 SCHU S4 ORGANISMS

Time After Challenge, hours	Vaccine Route	Number SCHU S4 Per Gram Of Lung Tissue	
		Apical Lobe	Diaphragmatic Lobe
1	Aerogenic	0, 6, 0	12, 5, 10
	Intracutaneous	8, 0	10, 0
	Control	5, 20	1. 0
12	Aerogenic	0, 0, 0	0, 0, 0
	Intracutaneous	0, 900	0, 0
	Control	0, 0	0, 0
24	Aerogenic	0, 0, 0	18, 300, 0
	Intracutaneous	0, 0	0, 0
	Control	400, 40	20, 0

TABLE II. CLEARANCE OF P. TULARENSIS SCHU S4 IN THE LUNG
OF VACCINATED M. IRUS AFTER RESPIRATORY CHALLENGE

Time After Challenge, days	Vaccine Route	Number SCHU S4 Per Gram Of Lung Tissue	
		Apical Lobe	Diaphragmatic Lobe
7	Aerogenic	0, 10^4 , 10^6	0, 0, 10^6
	Intracutaneous	10^2 , 10^2	10^4 , 10^5
	Control	10^7 , 10^7	10^7 , 10^7
10	Aerogenic	10 , 10^3 , 10	10^5 , 0, 0
	Intracutaneous	10^3 , 10	10^3 , 10^4
14	Aerogenic	0, 10 , 0	10^3 , 0, 0
	Intracutaneous	10^7 , 10^2	10^7 , 10^3
28	Aerogenic	0, 0	0, 0
	Intracutaneous	0	0

TABLE III. VIABLE P. TULARENSIS SCHU S4 IN THE TRACHEOBRONCHIAL LYMPH NODE OF M. IRUS AFTER RESPIRATORY CHALLENGE

Animal Designation	NUMBER ORGANISMS PER GRAM OF TISSUE AT INDICATED TIME AFTER CHALLENGE										
	Hour			Day							
	1	6	12	1	2	3	5	7	10	14	28
Aerogenic	0	0	0	0	0	0	10^3	10	10^2	10	0
Vaccinees	0	0	0	0	0	0	10^6	10^4	10^3	10^2	0
	10^2	0	0	0	10	0		10^5	10^4	10^3	
Intracutaneous	0	0	0	0	10^2	0		10^4	10^4	10^4	0
Vaccinees	0	0	10^3	0	10^2	10^5		10^5	10^4	10^6	
Nonvaccinated	0	0	0	0	0	0	10^7	10^8			
Controls	0	10^2	0	0	10^3	10^6	10^7	10^7			

Data on dissemination and proliferation of SCHU S4 organisms in other lymph nodes of vaccinated and control animals are presented in Table IV. P. tularensis was not cultured from the axillary, inguinal, or coeliac lymph nodes of vaccinees until Day 14; both axillary and inguinal nodes of the controls were positive as early as Day 5. No isolations were obtained from the cervical lymph nodes of vaccinees during the course of the study.

As seen in Table V, earlier involvement and higher concentrations of SCHU S4 were demonstrated in the spleen and liver of controls in comparison with vaccinees. During the experiment, 10 to 10^3 cells per organ were recovered from four of eleven aerogenically vaccinated animals and 10^2 to 10^4 cells per organ from five of six dermally vaccinated monkeys.

In contrast to the self-limiting disease in vaccinated animals, the pattern of tularemic infection in nonvaccinated controls was characterized by rapid and persistent multiplication, systemic involvement, and morbidity. From these animals, P. tularensis was readily isolated from all tissues assayed five days after challenge. Viable populations of 10^5 to 10^7 organisms per gram of lung, spleen, or liver and 10^3 per gram of tracheobronchial lymph node, and 10^4 to 10^6 per gram of other tissues were recovered from the four control animals sacrificed five and seven days after challenge.

TABLE IV. VIABLE P. TULARENSIS SCHU S4 IN LYMPH NODES
OF M. IRUS AFTER RESPIRATORY CHALLENGE

TISSUE	VACCINE ROUTE	NUMBER ORGANISMS PER GRAM OF TISSUE AT INDICATED TIME AFTER CHALLENGE					
		Day					
		to 3*	5	7	10	14	28
Axillary	Aerogenic	0,0,0	0,0	0,0,0	0,0,0	0,0,0	0,0
	Intracutaneous	0,0		0,0	0,0	0,0	10 ²
	Control	0,0	10 ⁴ , 10 ⁴	10 ⁴ , 10 ⁵			
Inguinal	Aerogenic	0,0,0	0,0	0,0,0	0,0,0	0,0,0	0,10
	Intracutaneous	0,0		0,0	0,0	10 ² , 10 ⁴	10 ⁴
	Control	0,0	10 ⁴ , 10 ⁴	10 ⁴ , 10 ⁴			
Cervical	Aerogenic	0,0,0	0,0	0,0,0	0,0,0	0,0,0	0,0
	Intracutaneous	0,0		0,0	0,0	0,0	0
	Control	0,0	10 ⁴ , 10 ⁴	10 ⁴ , 10 ⁵			
Coeliac	Aerogenic	0,0,0	0,0	0,0,0	0,0,0	0,0,0	0,0
	Intracutaneous	0,0		0,0	0,0	0,10 ²	10 ³
	Control	0,0	10 ⁴ , 10 ⁴	10 ⁴ , 10 ⁶			

* Animals sacrificed at intervals from one hour to three days after exposure.

TABLE V. VIABLE P. TULARENSIS SCHU S4 IN THE SPLEEN
AND LIVER OF M. IRUS AFTER RESPIRATORY CHALLENGE

TISSUE	VACCINE ROUTE	NUMBER ORGANISMS PER GRAM OF TISSUE AT INDICATED TIME AFTER CHALLENGE						
		Day						
		to 2*	3	5	7	10	14	28
Spleen	Aerogenic	0	0	0	0	0	0	0
	Aerogenic	0	0	0	10^3	10^3	0	0
	Aerogenic	0	0		10^3	10^2	0	
	Intracutaneous	0	0		0	10^3	10^3	0
	Intracutaneous	0	0		10^3	10^3	10^3	
	Control	0	10^3	10^7	10^7			
	Control	0	10^3	10^7	10^7			
Liver	Aerogenic	0	0	0	0	0	0	0
	Aerogenic	0	0	10^2	0	0	0	0
	Aerogenic	0	0		10^2	10^3	10^3	
	Intracutaneous	0	0		0	10^3	10^3	0
	Intracutaneous	0	0		10^2	10^3	10^4	
	Control	0	0	10^6	10^7			
	Control	0	10^2	10^6	10^6			

* Animals sacrificed at intervals from one hour to two days after exposure.

Histopathologic changes were manifested on Day 2 in nonvaccinated, Day 3 in dermally vaccinated, and Day 5 in aerogenically vaccinated monkeys by focal accumulation of monocytes and neutrophils in the rudimentary alveoli of the respiratory bronchioles and alveolar ducts of the lungs. Less extensive histologic changes occurred at secondary infection sites (spleen, lymph nodes, etc.) in vaccinated animals compared with nonvaccinated controls. Moreover, systemic involvement was minimized to a greater extent in the aerogenically vaccinated monkeys than in the dermally vaccinated. These observations were made on the basis of fewer infarctions and less focal necrosis and granuloma formation in the spleen and by less involvement of the systemic lymph nodes other than the tracheobronchial lymph nodes. Enlargement and extensive caseous necrosis of the tracheobronchial lymph nodes were observed by the fifth day in the dermally vaccinated and nonvaccinated monkeys; similar pathologic changes were not seen in aerogenically vaccinated monkeys until the tenth day.

Aerogenic challenge of nonvaccinated monkeys produced an acute, progressive, inflammatory response associated with necrosis. Death usually occurred before granuloma formation became manifest. Although a similar focal pulmonary necrotizing response was evoked in vaccinated animals, the disease process was localized and secondary systemic involvement was reduced by the self-limiting granulomatous response in the lungs.

Evidence has been obtained indicating that monkeys vaccinated aerogenically are more resistant to virulent respiratory challenge with P. tularensis than animals vaccinated dermally. Serologically, both agglutinin and precipitin titers were higher in aerogenically vaccinated animals than in dermally vaccinated animals. Bacteriologically, dissemination and proliferation of challenge organisms were less extensive and clearance occurred earlier in aerogenically vaccinated animals. Histologically, involvement of the lung and regional lymph nodes of aerogenic vaccinees was less pronounced and occurred later, and less dissemination to secondary infection sites was observed. Dermal administration of LVS was somewhat less effective than aerogenic in limiting the effects of virulent challenge. Nevertheless, the acute, fulminating, necrotic disease process terminating in fatal septicemia, as seen in nonvaccinated animals, was prevented.