Host-Parasite Relationship in Monkeys Administered Live Tularemia Vaccine

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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.[1] Tissue changes were mild and consisted primarily of the proliferation of histiocytes without formation of granulomas regardless of inoculation route. *Pasteurella tularensis* vaccine strain LVS [2] 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 hr. Peak viable populations were reached within 3 days and maintained through Day 10; clearance had begun by Day 14. Intracellular antitularensis γ-globulin (ATGG) persisted at least 90 days in vaccinated animals.[3,4] 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 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. This report is concerned with the serologic, bacteriologic, and histologic studies conducted in the monkey vaccinated dermally or aerogenically with LVS and subsequently challenged aerogenically with highly virulent *P tularensis*.

Materials and Methods

P tularensis Strains. Live vaccine strain LVS[2] and strain SCHU S4[5] were cultivated in a modified case in partial hydrolyzate liquid medium.[6] Cultures after 14–16

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hr incubation at 37°C contained approximately 3.5×10^{10} viable organisms/ml.

Monkeys. *Macaca irus* monkeys were caged individually and conditioned for 6 months prior to use. Animals weighed between 2 and 5 kg and were distributed randomly in regard to weight and sex.

Intracutaneous Vaccination. Twenty-four monkeys were administered LVS intracutaneously in the deltoid area of the left arm after the hair had been clipped and the region cleansed with alcohol. LVS culture was diluted with gelatin-saline to give 10^5 viable cells/vaccine dose (0.2 ml). Inoculations were performed with a 25-gauge needle; a bleb approximately 1 cm in diameter was formed.

Aerogenic Vaccination with LVS or Challenge with SCHU S4. Aerosols were generated with a nebulizer that produced particles primarily in the range of 1–5μ in diameter. On the basis of aerosol sampler data, 35 monkeys received a mean inhaled dose of 7.6×10^5 viable LVS. Two months after vaccination, all dermally and aerogenically vaccinated monkeys and 16 nonvaccinated controls were challenged with a mean inhaled dose of 1×10^3 viable SCHU S4.

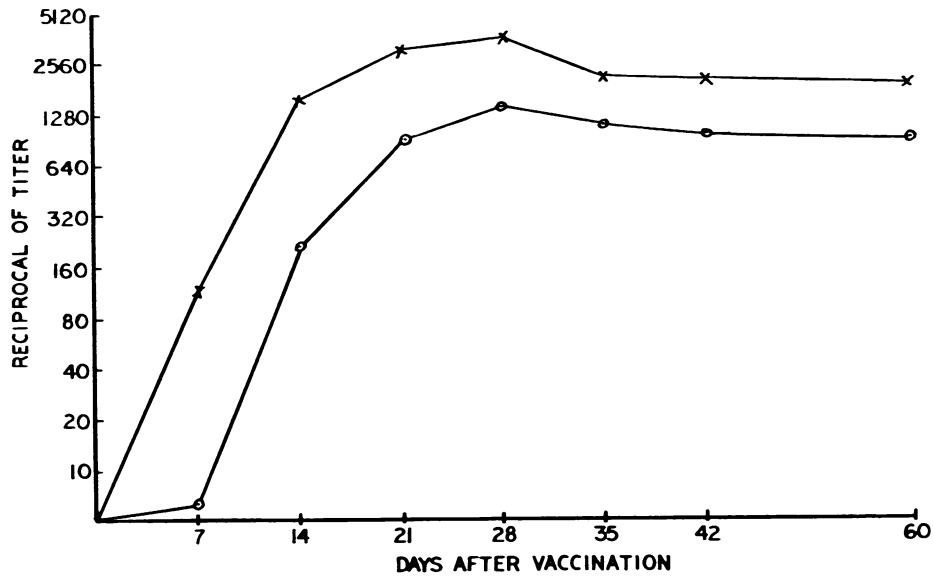
Bacteriological Studies. Groups of 2 dermally vaccinated, 3 aerogenically vaccinated, and 2 nonvaccinated monkeys were sacrificed 1, 6, and 12 hr and 1, 2, 3, 5, and 7 days after challenge. In addition, 2 dermally vaccinated and 3 aerogenically vaccinated monkeys were sacrificed at 10 and 14 days and 1 dermally vaccinated and 2 aerogenically vaccinated at 28 days after exposure to SCHU S4.

Serologic, bacteriologic, and histologic procedures were conducted as previously described in detail.[1]

Results and Discussion

The antibody response of monkeys after dermal or aerogenic vaccination is shown in Text-fig 1. Peak mean agglutinin titers, 1:1440 for animals vaccinated intracutaneously and 1:3810 for animals vaccinated aerogenically, were attained in both groups approximately 28 days after administration of live vaccine organisms and declined gradually thereafter. At time of challenge, 2 months after vaccination, mean agglutinin titers of 1:940 and 1:1980 were recorded for monkeys vaccinated dermally or aerogenically, respectively.

Data on the number of SCHU S4 organisms recovered from the lung of vaccinated and nonvaccinated monkeys at 1, 12, and 24 hr after respiratory challenge are shown in Table 1. Within 1 hr after aerogenic exposure, organisms were isolated from the lungs of all animals sacrificed and examined, except 1 dermally vaccinated monkey. This animal received the lowest number of challenge organisms and based on known retention had only 60 cells dispersed in 14.6 g of lung tissue. Recovery of SCHU S4 from the right apical or diaphragmatic lobes of the lung 1 hr after exposure to a mean inhaled dose of 880 viable cells ranged from 1 to 20 organisms/g; corresponding retention was 2–35% and the mean inhaled dose was 132 cells. Twelve and 24 hr after exposure, isolations from the lung were sporadic and no significant difference in



TEXT-FIG 1. Mean agglutinin titers of *M irus* administered live tularemia vaccine. Intracutaneous route, O—O; aerogenic route, X—X.

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 10^2 to 10^5 organisms per g of lung tissue. At this time SCHU S4 was isolated from the spleen of both nonvaccinated monkeys and from the liver of one, whereas vaccinees failed to yield positive cultures from these tissues.

Table 1. Recovery of P tularensis from the Lung of M irus after Respiratory Challenge*

Time after challenge (hr)	Vaccine route	No. SCHU S4/g of lung tissue	
		Apical lobe	Diaphragmatic lobe
1	Aerogenic	0, 6, 0	21, 5, 10
	dermal	8, 0	10, 0
	control	5, 20	1, 0
12	Aerogenic	0, 0, 0	0, 0, 0
	dermal	0, 900	0, 0
	control	0, 0	0, 0
24	Aerogenic	0, 0, 0	18, 300, 0
	dermal	0, 0	0, 0
	control	400, 40	20, 0

* Approximately 10^3 SCHU S4.

Viable populations of 10^5 to 10^6 SCHU S4/g were recovered from the lungs of the aerogenically vaccinated monkeys between 3 and 10 days after challenge. Data shown in Table 2 indicate clearance in most of these animals as early as 14 days after exposure to SCHU S4. However, 10^7 SCHU S4/g were cultured from the lungs of 1 of 2 dermally vaccinated monkeys 14 days after challenge. During the acute phase of tularemic infection (3–14 days after challenge), *P tularensis* was isolated from both apical and diaphragmatic lobes of the right aspect of the lung in 6 out of 14 of the aerogenically vaccinated and 8 out of 8 of the dermally vaccinated monkeys.

As shown in Table 3, 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 Day 5 and 7 in comparison with vaccinees. Clearance was apparently complete in vaccinees within 28 days.

Information on dissemination and proliferation of SCHU S4 organisms in other lymphatic tissue of vaccinated and control animals is presented in Table 4. *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.

Earlier involvement and higher concentrations of SCHU S4 were demonstrable in the spleen and liver of controls in comparison with vaccinees (Table 5). During the experiment, 10–10^3 cells/organ were

Table 2. Clearance of **P tularensis** SCHU S4 in the Lung of Vaccinated **M irus** after Respiratory Challenge*

Time after challenge (days)	Vaccine route	No. SCHU S4/g of lung tissue	
		Apical lobe	Diaphragmatic lobe
7	Aerogenic	0, 10^4, 10^6	0, 0, 10^6
	dermal	10^2, 10^2	10^4, 10^5
	control	10^7, 10^7	10^7, 10^7
10	Aerogenic	10, 10^3, 10	10^6, 0, 0
	dermal	10^3, 10	10^3, 10^4
14	Aerogenic	0, 10, 0	10^3, 0, 0
	dermal	10^7, 10^2	10^7, 10^3
28	Aerogenic	0, 0	0, 0
	dermal	0	0

* Approximately 10^3 SCHU S4.

Table 3. Viable *P tularensis* SCHU S4 in the Tracheobronchial Lymph Node of *M irus* after Respiratory Challenge*

	No. organisms/g of tissue at indicated time										
	Hr			Day							
Animal group	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	10^5	10^4	10^3	10^2	0
	0	0	0	0	10	0	—	10^5	10^4	10^3	—
Dermal	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	—	—	—

* Approximately 10^3 SCHU S4.

recovered from 4 out of 11 aerogenically vaccinated animals and 10^2–10^4 cells/organ from 5 out of 6 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 5 days after challenge. Viable populations of 10^6–10^7 organisms/g of lung, spleen, or liver and 10^8/g of tracheobronchial

Table 4. Viable *P tularensis* SCHU S4 in Lymph Nodes of *M irus* after Respiratory Challenge*

		No. organisms/g of tissue at indicated day					
Tissue	Vaccine route	1 hr–3 days	5	7	10	14	28
Axillary	Aerogenic	0, 0, 0	0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0
	Dermal	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
	Dermal	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
	Dermal	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
	Dermal	0, 0	—	0, 0	0, 0	0, 10^2	10^3
	Control	0, 0	10^4, 10^4	10^4, 10^4	—	—	—

* Approximately 10^3 SCHU S4.

Table 5. Viable **P tularensis** SCHU S4 in the Spleen and Liver of **M irus** after Respiratory Challenge*

Tissue	Vaccine route	No. organisms per organ at indicated day						
		1 hr–2 days	3	5	7	10	14	28
Spleen	Aerogenic	0	0	0	0	0	0	0
	Aerogenic	0	0	0	10^3	10	0	0
	Aerogenic	0	0	—	10^3	10^2	0	—
	Dermal	0	0	—	0	10^3	10^3	0
	Dermal	0	0	—	10^3	10^3	10^3	—
	Control	0	10	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	—
	Dermal	0	0	—	0	10^3	10^3	0
	Dermal	0	0	—	10^2	10^3	10^4	—
	Control	0	0	10^6	10^7	—	—	—
	Control	0	10^2	10^6	10^6	—	—	—

* Approximately 10^3 SCHU S4.

lymph node, and 10^4–10^6/g of other tissues were recovered from the 4 control animals sacrificed 5 and 7 days after challenge.

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 to nonvaccinated controls. Moreover, systemic involvement was minimized to a greater extent in the aerogenically vaccinated monkeys compared to 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 Day 5 in the dermally vaccinated and nonvaccinated monkeys; similar pathologic changes were not seen in aerogenically vaccinated monkeys until Day 10.

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 reduced by the self-limiting granulomatous response in the lungs.

This study affords evidence which indicates that monkeys vaccinated aerogenically are more resistant to virulent respiratory challenge with *P tularensis* than animals vaccinated dermally. Serologically, both agglutinin and precipitin titers [7] 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 aborting the effects of virulent challenge. Nevertheless, the acute, fulminating, necrotic disease process terminating in a fatal septicemia, as seen in nonvaccinated animals, was prevented.

Summary

Host-parasite studies conducted with monkeys vaccinated against tularemia, challenged aerogenically approximately 60 days later and subsequently sacrificed, indicated that earlier and more extensive 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 observed initially in control, dermally vaccinated, and aerogenically vaccinated animals on Day 2, 3, and 5, respectively, after challenge. In contrast to the septicemia and fatal tularemic disease observed in control animals within 7 days after challenge, a reduction in number or disappearance of challenge organisms occurred in the lungs, tracheobronchial lymph nodes, spleen, and liver of vaccinees between 14 and 28 days. Controls and animals vaccinated dermally displayed enlarged caseous tracheobronchial lymph nodes and moderate pulmonary involvement by Day 5 whereas animals vaccinated aerogenically did not exhibit comparable pathology until Day 10 after challenge. Also, less extensive histological changes were observed at secondary infection sites in vaccinees, with least involvement being exhibited by aerogenically vaccinated animals. Serologic studies demonstrated that antibody titers were higher in aerogenically vaccinated animals. Therefore, on the basis of bacteriolo-

gic, histologic, and serologic observations, dermal vaccination of monkeys was less effective than aerogenic in aborting the effects of virulent challenge. However, the fulminating tularemic disease associated with nonvaccinated challenged animals was averted.

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