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Evaluation of a Killed Rocky Mountain Spotted Fever Vaccine in Cynomolgus Monkeys

JANET C. GONDER,†* RICHARD H. KENYON, AND CARL E. PEDERSEN, JR.‡

U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

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A nonhuman primate model of Rocky Mountain spotted fever infection was developed in cynomolgus monkeys (*Macaca fascicularis*) infected by the subcutaneous route or by aerosol. Clinical responses, hematology and serum chemistry values, and pathological findings were similar to those found in humans ill with Rocky Mountain spotted fever. The clinical model was then used to test the efficacy of a killed Rocky Mountain spotted fever vaccine grown in chicken embryo cells. Monkeys were immunized with varying dilutions of the vaccine with a two-dose schedule and then challenged at 2 months with virulent *Rickettsia rickettsii* by the subcutaneous route or by aerosol. The undiluted vaccine totally protected monkeys against both challenges, even at extremely high doses.

Rocky Mountain spotted fever (RMSF) is an acute febrile infectious disease caused by *Rickettsia rickettsii* that is endemic throughout the continental United States. The infection is naturally transmitted by the bite of a tick, but studies in our laboratory indicated that aerosol exposure is a potential route of infection in laboratory workers (8). At the present time, there is no commercial RMSF vaccine available.

An inactivated chicken embryo cell-grown RMSF vaccine has been developed in this laboratory (4). A guinea pig model was used for preliminary studies with the vaccine (5). Vaccinated guinea pigs challenged by the subcutaneous (s.c.) route or by aerosol were fully protected. RMSF infection has been studied extensively in rhesus monkeys (7, 9, 12, 15), and a suitable nonhuman primate model has been described (10). The vaccine has been tested and found to protect rhesus monkeys challenged s.c. (11). A two-dose schedule provided the best protection (4, 11). However, due to the limited availability of the rhesus monkey, a cynomolgus (*Macaca fascicularis*) monkey model was developed for further vaccine testing.

The purpose of this study is to describe the cynomolgus monkey model of RMSF infection and to test the efficacy of our vaccine against s.c. and aerosol challenges.

MATERIALS AND METHODS

Laboratory animals. Forty-three healthy cynomolgus monkeys of both sexes weighing 3 to 4.5 kg were used. They were housed in individual cages and fed a commercial ration (Ralston Purina Co., St. Louis, Mo.) and water ad libitum. All sampling was done between 8 and 9 a.m. (before feeding). Monkeys were observed several times daily for anorexia and depression. Changes in normal aggressive reactions to handling were noted. Base-line clinical and laboratory values were established during a 2-week period before infection. Mean rectal temperatures were based on 5 sampling days, and base-line hematology and serum chemistry values were determined from four samples. For 3 weeks after challenge, rectal temperatures were recorded daily, and blood samples were obtained from the femoral vein at selected intervals for rickettsemia, hematology, serum chemistry, and serology.

Rickettsiae and experimental infection. The Sheila Smith strain of *R. rickettsii* was propagated and assayed as previously described (9). For s.c. infection, yolk sac-grown rickettsiae were inoculated (1 ml/animal) at doses of 10^1, 10^3, or 10^5 plaque-forming units (PFU)/ml. Aerosol-infected monkeys were exposed for 10 min to small-particle aerosols of 10^3 or 10^5 PFU of rickettsiae by a method previously described (3). Aerosol samples were collected during each exposure in an AGI-30 glass impinger and titrated by plaque assay (13), and the total inspired dose was calculated for each monkey. Rickettsemias were confirmed by the plaque assay method described by Wike and Burgdorfer (14).

To determine a dose-response to *R. rickettsii*, monkeys were divided into three groups of seven each: low dose (10^1 PFU), medium dose (10^3 PFU), and high dose (10^5 PFU). Three monkeys in each group were inoculated s.c., and four monkeys in each group were infected by aerosol.

Vaccine and immunization. The vaccine tested in this study was an inactivated chicken embryo cell-grown product prepared and tested by Kenyon and Pedersen (4) containing 1.3×10^8 *R. rickettsii* per ml. The vaccine was administered in two doses (0.5 ml each), 28 days apart, with undiluted and 1:10, 1:100,

† Present address: 3976 Sunny Vale Dr., DeForest, WI 53532.

‡ Present address: HQDA SGRD-PL (LTC Pedersen), Fort Detrick, Frederick, MD 21701.

and 1:250 dilutions. Monkeys were challenged 1 month after the second s.c. inoculation.

In the first vaccine study, 10 monkeys were divided into five groups of 2 each for vaccination and subsequent s.c. challenge. Two groups were immunized with the undiluted vaccine, and one group each was immunized with the three dilutions. One group immunized with undiluted vaccine was challenged with 10^5 PFU of *R. rickettsii*, and the other was challenged with 10^3 PFU. In the same study, four nonvaccinated control monkeys were inoculated s.c., two with 10^3 PFU and two with 10^5 PFU.

In the second vaccine study, four monkeys were vaccinated with undiluted vaccine. Two were then challenged with 10^3 PFU and two were challenged with 10^5 PFU of *R. rickettsii* given in small-particle aerosol. Four nonvaccinated monkeys were similarly infected to serve as controls.

Hematology and serum chemistry. Blood samples collected in ethylenediaminetetraacetic acid were analyzed for total (Coulter Electronics, Hialeah, Fla.) and differential leukocyte counts, hematocrit, total protein (TS Meter, American Optical, Atlanta, Ga.), and fibrinogen (1). Blood urea nitrogen, serum glutamic oxalacetic transaminase, and serum alkaline phosphatase values were determined with commercially available kits (Mallinckrodt, Inc., Hazelwood, Mo.).

Serology. In the s.c. and aerosol challenge study, serum samples were obtained before challenge and 21 days after infection. In the vaccine study, serum samples were collected before vaccination, 1 and 2 months after the first vaccine dose, and 1, 3, and 6 weeks after challenge. The microagglutination assay for rickettsial antibody was performed as described by Fiset et al. (2). The rickettsial test antigen was prepared as previously described (11). All animals were seronegative at the start of the study.

Pathology. Monkeys that died were examined for gross and microscopic lesions. Tissues were fixed in neutral phosphate-buffered Formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for examination.

Statistical analyses. Analysis of variance for repeated measurements was used to detect differences significantly greater than base-line variability. The lowest level of significance was set at $P < 0.05$.

RESULTS

Clinical response. The clinical responses of 21 cynomolgus monkeys infected with *R. rickettsii* are summarized in Table 1. One monkey did not become ill when challenged with 10^1 PFU by aerosol. All other monkeys became ill with anorexia, depression, and fever. One monkey in each of the two higher-dose groups developed a rash 10 days after aerosol exposure; dyspnea was observed only occasionally.

In general, the mean incubation time decreased with an increase in infecting dose. The time to onset of fever was slightly longer after aerosol exposure than after s.c. challenge with 10^1 and 10^3 PFU; at 10^5 PFU, no difference in incubation time was noted. There was no difference in incubation time between dying and surviving monkeys in each dose group.

The duration of fever after aerosol exposure was shorter at the two lower dosages than was observed at the same dosages given s.c. At the high dose, the duration of fever was longer after aerosol than after s.c. challenge. Duration of fever in the monkeys that died was shorter than in those that survived. Temperatures decreased rapidly 1 to 3 days before death.

There was no difference in the magnitude of fever between routes of infection or dose. Rectal temperatures increased more than 2°C above base-line values of 38.0 to 38.5°C in all febrile monkeys ($P < 0.001$).

Fifty percent of the monkeys in each aerosol group died. All of the monkeys inoculated s.c. with 10^1 PFU died, as did two of three in each of the other two dose groups. The mean time to death in the low- and medium-dose groups was approximately 11 days, compared with 8 to 10 days in the high-dose group. There was a tendency for the monkeys in all dose groups exposed by aerosol to live longer than those infected s.c.

Pathology. Splenomegaly was noted on gross pathological examination. The most common lesions microscopically were vasculitis and thrombosis of the capillaries, arterioles, and venules of the facial skin, ears, and nares. The lesions were characterized primarily by perivascular infiltration of lymphocytes and plasma cells. Vasculitis was also seen in the palpebral

TABLE 1. *Clinical responses of cynomolgus monkeys to R. rickettsii infection*

Challenge		No.	No. ill	No. with		Fever (days)		No. dead	Mean days to death (range)
Dose	Route			Rickettsemia	Rash	Time to onset (range)	Duration (range)		
10^1	s.c.	3	3	3	0	6 (5–7)	5.7 (4–7)	3	11 (10–11)
	Aerosol	4	3	2	0	7.3 (7–8)	2.7 (2–4)	2	11.5 (11–12)
10^3	s.c.	3	3	3	0	3 (2–4)	7.3 (5–9)	2	10
	Aerosol	4	4	4	1	5.7 (5–6)	4.0 (3–5)	2	12 (11–13)
10^5	s.c.	3	3	3	0	4	5	2	8
	Aerosol	4	4	3	1	3	6.7 (5–9)	2	10

conjunctiva, testes, uterus, thymus, heart, skeletal muscle, and abdominal mesentery. Varying degrees of interstitial pneumonia (no difference was seen in monkeys infected by either route) and minimal adrenitis were noted. Two monkeys infected s.c. had fibrin thrombi in glomerular tufts, suggesting disseminated intravascular coagulation.

Hematology and serum chemistry. The total leukocyte counts increased 2,000 to 4,000 cells per/mm^3 above base-line values in all but the afebrile monkey. These increases were due to an absolute neutrophilia and occurred during the febrile period. Increases were not significant at $P < 0.05$. The hematocrit decreased slightly in all groups due to repeated bleeding. There was no significant change in total protein values, although plasma fibrinogen increased significantly ($P < 0.01$) during the febrile period (as high as 700 mg/100 ml).

Blood urea nitrogen and serum glutamic oxalacetic transaminase values were elevated (>2 standard deviations) on days 4 and 6 of the febrile period in the medium- and high-dose groups. Only a 1-day elevation was noted in the low-dose groups (on day 6). There were no changes in serum alkaline phosphatase values.

Rickettsemia and serology. All monkeys infected by the s.c. route developed rickettsemia. The mean time to onset in the high-dose group was 4 days and, in the other two groups, 6 days. The duration of rickettsemia in the two surviving monkeys was 4 days. In those that died after s.c. challenge, rickettsemia persisted until death.

After aerosol exposure, only two of four monkeys in the low-dose group developed rickettsemia. These two monkeys died. Rickettsemia was first detected on day 7. Deaths occurred on days 11 and 12 with rickettsemia persisting until death (mean duration = 5.5 days).

All four monkeys in the medium-dose group demonstrated rickettsemia. Mean time to onset was 6.2 days (range = 5 to 7 days). Rickettsemia persisted until death in the two monkeys that died (duration = 7 days). Duration of rickettsemia in both surviving monkeys was 3 days.

Three of the four monkeys in the high-dose group developed rickettsemia after aerosol exposure. The mean time to onset was 5.3 days (range = 5 to 6 days). The rickettsemia persisted until death in the two monkeys that died (duration = 5 and 6 days). The duration of rickettsemia in the surviving monkey was 5 days.

All monkeys in the s.c. and aerosol studies had microagglutination titers of at least 1:64 on day 21. The aerosol-challenged monkey that did not become ill had a titer of 1:64. Titers correlated with increasing challenge doses. The geometric mean titers of the low-, medium-, and high-dose groups were 1:64, 1:107, and 1:341, respectively.

Vaccine studies. Results of the vaccine studies are summarized in Table 2. All eight nonvaccinated, control monkeys became ill and seven died. All controls had demonstrable leukocytosis due to absolute neutrophilia. Decreases in hematocrit occurred due to repeated bleeding. Slight increases were noted in blood urea nitrogen and serum glutamic oxalacetic transaminase values, with no significant changes in serum alkaline phosphatase.

One monkey vaccinated and challenged with an aerosol dose of 10^5 PFU was mildly febrile on days 2 and 3 (1.0°C above base line). One monkey in each of two groups receiving diluted vac-

TABLE 2. *Clinical responses of vaccinated and unvaccinated cynomolgus monkeys (n = 2/group) after challenge with R. rickettsii*

Vaccine and route	Challenge dose (PFU)	No. with		Fever (days)		No. dead
		Rickettsemia	Fever	Time to onset (range)	Duration	
s.c.						
None	10^5	2	2	3	0[a]	2
	10^3	2	2	5.5 (5–6)	9	1
1:250	10^3	0	1	6	8	0
1:100		0	1	6	6	0
1:10		0	0			0
Undiluted	10^3	0	0			0
	10^5	0	0			0
Aerosol						
None	10^5	2	2	4.5 (4–5)	0[a]	2
	10^3	2	2	6	0[a]	2
Undiluted	10^5	0	1	2	2	0
	10^3	0	0			0

[a] Died.

cine (1:100 and 1:250) became febrile after s.c. challenge. The magnitude and duration of fever were comparable to those seen in the nonvaccinated controls. The three vaccinated monkeys that became febrile showed no signs of anorexia or depression. None of the vaccinated monkeys showed any alterations from base-line values in hematology or serum chamistry values, except that the hematocrit decreased due to repeated bleeding.

Before challenge the microagglutination titers were relatively high (>1:32) in the monkeys given undiluted and 1:10 dilution of the vaccine. After challenge these monkeys showed a slow rise to peak titers (>1:100) at 21 days. Those receiving 1:100 and 1:250 dilutions had negligible titers (1:4) just before challenge, but exhibited a pronounced antibody response (>1:32) 7 days after s.c. challenge with 10^3 PFU of *R. rickettsii*. The rise in titer from 1 to 6 weeks tended to be slower in the group receiving the 1:250 dilution of vaccine (Fig. 1).

There were no differences in the microagglutination titers after s.c. or aerosol challenge of monkeys given the undiluted vaccine. Antibody response after challenge was more pronounced in those monkeys challenged with the higher dose (10^5), but by 6 weeks the titers in the two dosage groups were essentially the same.

No rickettsiae were isolated from peripheral blood of vaccinated monkeys at any time after challenge.

DISCUSSION

RMSF in cynomolgus monkeys closely resembles the disease in rhesus monkeys (9) and humans (16). Skin rashes were less frequent in cynomolgus than in rhesus monkeys and may be due to the former's dark complexion and the resulting difficulty in recognition of a rash. Compared to the rhesus monkey, the mean incubation time tended to be shorter and the duration of fever tended to be longer after s.c. challenge with 10^3 PFU of *R. rickettsii*. All monkeys infected s.c. in this study became ill, whereas 2 of the 12 rhesus monkeys in the previous study did not. This may reflect the smaller number of animals used here. The mortality rate in cynomolgus monkeys infected with 10^3 PFU was similar to that of rhesus monkeys. Rectal temperatures decreased rapidly before death, as seen previously in rhesus monkeys (10), although mean time to death was somewhat longer in cynomolgus monkeys.

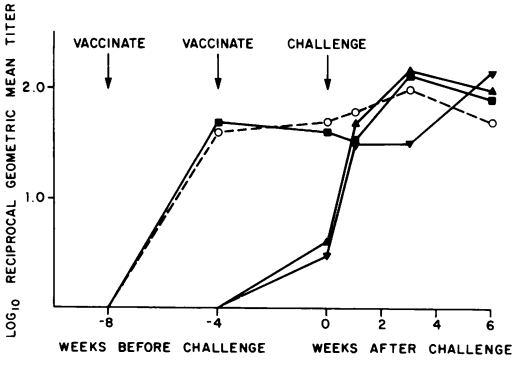

FIG. 1. *Microagglutination antibody responses of cynomolgus monkeys vaccinated with undiluted (○), 1:10 (■), 1:100 (▲), and 1:250 (▼) dilutions of RMSF vaccine and challenged s.c. with 10^3 PFU of R. rickettsii.*

The microscopic lesions of vasculitis and thrombosis of small vessels in numerous tissues seen in the cynomolgus monkeys were similar to those reported in rhesus monkeys (7, 10, 15) and humans (6). The lack of consistent or severe respiratory involvement in the aerosol-infected monkeys has been reported (12, 15).

The changes in hematology and serum chemistry values were similar to those reported by Sammons et al. (10), although decreases in total protein and serum alkaline phosphatase were not seen in the present study.

Although three of the vaccinated monkeys became febrile, they either had been given a low antigen concentration (1:100 or 1:250 dilutions) or an extremely high challenge dose (10^5 PFU). It is important to note that these monkeys showed no other clinical signs of illness, nor did they develop rickettsemia. The hematology and serum chemistry values of all vaccinated monkeys remained normal throughout the study.

Prechallenge microagglutination titers (after two doses of vaccine) were comparable to titers observed with the same schedule in rhesus monkeys (11).

In conclusion, the cynomolgus monkey appears to be a suitable substitute for the rhesus monkey for the study of RMSF in nonhuman primates. The clinical and physiological responses and pathological changes are similar in the two species. RMSF resulting from s.c. infection is indistinguishable from the disease acquired by aerosol exposure. The chicken embryo cell vaccine protects cynomolgus monkeys against both s.c. and aerosol challenges.

LITERATURE CITED

1. **Dodds, W. J., and J. J. Kaneko.** 1971. Hemostasis and blood coagulation, p. 179-206. *In* J. J. Kaneko and C. E. Cornelius (ed.), Clinical biochemistry of domestic animals, vol. 2, 2nd ed. Academic Press Inc., New York.
2. **Fiset, P., R. A. Ormsbee, R. Silberman, M. Peacock, and S. H. Spielman.** 1969. A microagglutination tech-

nique for detection and measurement of rickettsial antibodies. Acta Virol. **13**:60–66.

3. **Gonder, J. C., R. A., Kishimoto, M. D. Kastello, C. E Pedersen, Jr., and E. W. Larson.** 1979. Cynomolgus monkey model for experimental Q fever infection. J. Infect. Dis. **139**:191–196.
4. **Kenyon, R. H., and C. E. Pedersen, Jr.** 1975. Preparation of Rocky Mountain spotted fever vaccine suitable for human immunization. J. Clin. Microbiol. **1**:500–503.
5. **Kenyon, R. H., R. A. Kishimoto, and W. C. Hall.** 1979. Exposure of guinea pigs to *Rickettsia rickettsii* by aerosol, nasal, conjunctival, gastric, and subcutaneous routes and protection afforded by an experimental vaccine. Infect. Immun. **25**:580–582.
6. **Lillie, R. D.** 1941. The pathology of Rocky Mountain spotted fever. National Institutes of Health bulletin no. 177. U.S. Government Printing Office, Washington, D.C.
7. **Moe, J. B., G. L. Ruch, R. H. Kenyon, J. D. Burek and J. L. Stookey.** 1976. Pathology of experimental Rocky Mountain spotted fever in rhesus monkeys. Vet. Pathol. **13**:69–77.
8. **Oster, C. N., D. S. Burke, R. H. Kenyon, M. S. Ascher, P. Harber, and C. E. Pedersen, Jr.** 1977. Laboratory-acquired Rocky Mountain spotted fever. The hazard of aerosol transmission. N. Engl. J. Med. **297**:859–863.
9. **Sammons, L. S., R. H. Kenyon, G. T. Burger, W. R. Beisel, and C. E. Pedersen, Jr.** 1977. Studies on *Macaca mulatta* infected with Rocky Mountain spotted fever. Am. J. Vet. Res. **38**:907–910.
10. **Sammons, L. S., R. H. Kenyon, G. T. Burger, C. E. Pedersen, Jr., and R. O. Spertzel.** 1976. Changes in blood serum constituents and hematologic values in *Macaca mulatta* with Rocky Mountain spotted fever. Am. J. Vet. Res. **37**:725–730.
11. **Sammons, L. S., R. H. Kenyon, and C. E. Pedersen, Jr.** 1976. Effect of vaccination schedule on immune response of *Macaca mulatta* to cell culture-grown Rocky Mountain spotted fever vaccine. J. Clin. Microbiol. **4**:253–257.
12. **Saslaw, S., H. N. Carlisle, G. L. Wolf, and C. R. Cole.** 1966. Rocky Mountain spotted fever: clinical and laboratory observations of monkeys after respiratory exposure. J. Infect. Dis. **116**:243–255.
13. **Weinberg, E. H., J. R. Stakebake, and P. J. Gerone.** 1969. Plaque assay for *Rickettsia rickettsii*. J. Bacteriol. **98**:398–402.
14. **Wike, D. A., and W. Burgdorfer.** 1972. Plaque formation in tissue culture by *Rickettsia rickettsii* isolated directly from whole blood and tick hemolymph. Infect. Immun. **6**:736–738.
15. **Wolf, G. L., C. R. Cole, H. N. Carlisle, and S. Saslaw.** 1967. The pathogenesis of Rocky Mountain spotted fever in monkeys. Infected by inhalation. Arch. Pathol. **84**:486–494.
16. **Woodward, T. E.** 1959. Rickettsial diseases in the United States. Med. Clin. N. Am. **43**:1507–1535.