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Serological Cross-Reaction and Cross-Protection in Guinea Pigs Infected with *Rickettsia rickettsii* and *Rickettsia montana*

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Antisera produced in guinea pigs inoculated with *Rickettsia rickettsii* or *Rickettsia montana* were cross-reactive but sufficiently specific to identify the primary infecting agent. Guinea pigs immunized with *R. montana* were protected from fatal infection with *R. rickettsii*, although a few (25%) developed mild fever of short duration.

Rickettsia rickettsii and *Rickettsia montana* are antigenically related spotted fever group rickettsiae (3). Serological studies indicate frequent exposure of the canine population on Cape Cod, Massachusetts, (W. C. Feng et al., Am. J. Trop. Med. Hyg., in press), to *R. montana*. *R. rickettsii*, the etiological agent of Rocky Mountain spotted fever, has been shown to be responsible for a similar and more frequently occurring disease in canines on Cape Cod (1). Interpretation of serological tests for infection with *R. rickettsii* may be difficult if the patient has been exposed previously to the more common but nonpathogenic *R. montana*. More significantly, there could be implications for the control of Rocky Mountain spotted fever if protective immunity to *R. rickettsii* could be conferred by exposure to *R. montana*. The serological responses to individual and dual infections of *R. rickettsii* and *R. montana* were investigated, and cross-protection studies were conducted in a guinea pig model to address these questions.

The strain of *R. rickettsii* (77H1207) used to produce antisera or immunize guinea pigs was serologically identical to the Sheila Smith strain (1) but was less pathogenic for male guinea pigs. *R. montana* (strain M/5-6B) was obtained from Willy Burgdorfer and was not pathogenic for male guinea pigs (3).

Hartley male guinea pigs, weighing approximately 800 g each, were inoculated intraperitoneally with 1 ml of a 0.5% suspension of yolk sac obtained from eggs infected with *R. rickettsii* or *R. montana*. The inoculum contained approximately 8×10^5 organisms of *R. rickettsii* or *R. montana* per ml as determined with Gimenez strain (2) and counted at ×800. The animals were bled by cardiac puncture for 15 consecutive weeks; sera were separated and stored at −20°C until tested for antibody. To test the nature of the antibody response on challenge with the heterologous strain of rickettsiae, three guinea pigs originally inoculated with *R. rickettsii* (strain 77H1207) were re-inoculated intraperitoneally with *R. montana* and four guinea pigs originally inoculated with *R. montana* were re-inoculated intraperitoneally with *R. rickettsii* at 8 weeks after the primary inoculations. All sera were tested for antibodies to *R. rickettsii* or *R. montana* or both by the indirect immunofluorescence assay previously described (1).

Cross-protection experiments were conducted by inoculating eight guinea pigs with *R. rickettsii* (strain 77H1207) and eight with *R. montana* as described above; seven guinea pigs were inoculated with yolk sac suspensions from uninfected eggs. After 24 days, all of the guinea pigs were inoculated intraperitoneally with five 50% lethal doses of the Sheila Smith strain of *R. rickettsii*. The temperature of the animals was measured and scrotal reactions were observed daily for 12 days; the animals were observed for fatalities for 21 days after infection, and the surviving guinea pigs were bled for sera 2 days later. Pre-inoculation and post-inoculation sera were tested for antibodies to the two rickettsiae.

Antibodies to *R. rickettsii* in guinea pigs were detectable by week 2, the titers rising to a mean of 1:640 3 weeks after primary inoculation and then to approximately 1:2,560 by week 6 (Fig. 1). After re-inoculation with *R. montana*, antibody titers to both rickettsiae increased, with *R. rickettsii* remaining in the range of 1:2,150 until week 14. Antibody activity to *R. montana* followed essentially the same pattern as that to *R. rickettsii* except that titers were 4- to 16-fold lower than those to *R. rickettsii*.

Antibody activity to *R. montana* was detectable by week 2 after primary inoculation (Fig. 2). The antibodies to *R. rickettsii* initially fol-

lowed the same pattern except the titers were fourfold lower than those to *R. montana*. However, after the challenge with *R. rickettsii*, the titers to *R. rickettsii* were approximately the same as those to *R. montana*.

Of seven guinea pigs immunized and challenged with *R. rickettsii*, none died or had fever or scrotal reaction (Table 1). Of eight guinea pigs immunized with *R. montana* and challenged with *R. rickettsii*, none died or had scrotal reaction, although two (25%) had a mild fever of 103.5°F (39.7°C) for 3 to 4 days (Table 1). All of the normal guinea pigs infected with *R. rickettsii* had fever greater than 104°F (40°C) and had hemorrhagic-to-necrotic scrotal reactions; four (57%) animals died from 6 to 9 days after the infection (Table 1). All surviving guinea pigs had serological evidence of rickettsial infection.

Antibody titers were consistently greater to the primary immunizing agent, although titers were generally greater in sera from guinea pigs with *R. rickettsii* infections; the increased immunogenicity may reflect the pathogenicity of *R. rickettsii*. Additionally, antisera of guinea pigs inoculated initially with *R. rickettsii* were more specific than those resulting from a primary *R. montana* inoculation; cross-reactivity was not significantly increased after re-inoculation with *R. montana*. Sera from guinea pigs inoculated first with *R. montana*, however, showed greater cross-reactivity with *R. rickettsii*; re-inoculation with *R. rickettsii* resulted in the formation of antisera that could not discern the primary infecting agent. Whether these serological observations can be extrapolated to the serology of dogs and humans is problematical. Potential hosts may vary in antibody responses because of innate differences in their susceptibilities to infection or pathogenesis by these organisms or both.

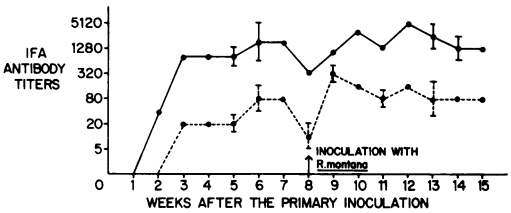

FIG. 1. *Antibody titers to R. rickettsii* (——) *and R. montana* (- - -) *of guinea pigs inoculated with R. rickettsii and re-inoculated with R. montana. Each point represents the geometric mean titer of the sera from three guinea pigs. IFA, Indirect immunofluorescence assay.*

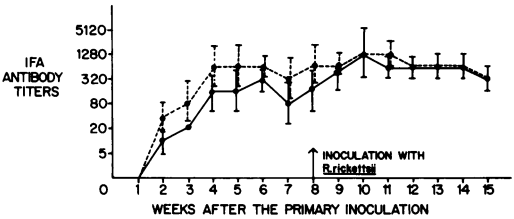

FIG. 2. *Antibody titers to R. rickettsii* (——) *and R. montana* (- - -) *of guinea pigs inoculated with R. montana and re-inoculated with R. rickettsii. Each point represents the geometric mean titer of the sera from four guinea pigs. IFA, Indirect immunofluorescence assay.*

TABLE 1. *Protection of guinea pigs against R. rickettsii infection by prior immunization with R. rickettsii or R. montana*

Immunizing agent	No. of animals immunized	No. of animals responding to challenge with *R. rickettsii*		
		Fever[a]	Scrotal reaction[b]	Death
R. rickettsii	7	0	0	0
R. montana	8	2	0	0
None	7	7	7	4

[a] Temperature > 103°F (39.4°C).
[b] Swelling, hemorrhage, or necrosis.

If the protection conferred to guinea pigs by exposure to *R. montana* occurs in other hosts of *R. rickettsii*, then *R. montana* might be utilized in measures to control Rocky Mountain spotted fever, particularly in areas where *Dermacentor variabilis* is a major vector. Although the virulence of *R. montana* in humans has not been established, *R. montana* has not been associated with human disease. In other studies, *R. rickettsii* was the only one of the spotted fever group rickettsiae associated with human or canine spotted fever disease on Cape Cod (1), although it was not isolated from any of the 6,956 *D. variabilis* organisms examined. *R. montana*, however, was identified in approximately 1% of these ticks, indicating a far greater potential for exposure to humans and canines (Feng et al., in press). Conceivably, *R. montana* could be used as a vaccine. Further, the introduction of *R. montana* into an ecosystem might competitively diminish the prevalence of *R. rickettsii* by eliciting the formation of protective antibody in those animal populations that normally support the occurrence of *R. rickettsii*. The efficacy of the proposal could be tested initially by looking for spotted fever group rickettsiae with antigens in common with *R. rickettsii*, such as *R. montana*, in areas highly endemic for *R. rickettsii*. If absent, it could be postulated that *R. rickettsii* is prevalent because competition from a related

spotted fever group rickettsiae does not occur. Local introduction of nonpathogenic *R. montana* could then be considered for evaluation as a biological competitor of *R. rickettsii*.

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