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Sources of Rats Free of Latent *Pneumocystis carinii*

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Two sources of rats free of latent *Pneumocystis carinii* are described. First, rats from a virus-free colony failed to develop infection after 8 weeks of immune suppression unless they were housed with previously infected rats. Second, pregnant rats (non-virus free) received trimethoprim-sulfamethoxazole from day 10 of gestation until the pups were weaned. Pups raised in filter-topped cages and immunosuppressed for 8 weeks were free of *P. carinii* infection.

Investigators working with *Pneumocystis carinii* depend on the rat model (2) both as a source of organisms and for evaluation of drugs. *P. carinii* infection is latent in most rat colonies, and pneumonia caused by this organism develops if the animals are immunosuppressed with adrenal corticosteroids. Only recently has the variability of the model been appreciated (1). Even rats from the same supplier differ because most suppliers maintain several independent colonies of the same rat strain. We noted that Sprague-Dawley rats from two different colonies from the same supplier (Harlan Laboratories, Indianapolis, Ind.) developed different levels of infection with *P. carinii*. Animals from the virus-free colony (colony 202; cesarean derived) were less likely to develop infection than were animals from the less protected colony (colony 205). We hypothesized that the virus-free rats lacked the latent *P. carinii* infection.

To test this hypothesis, 20 rats from virus-free colony 202 of Harlan Laboratories were brought directly to the virus-free room of our animal facility, where they were randomly divided into two groups of 10 each. One group remained in the virus-free facility, and the other group was moved to a room housing our colony of immunosuppressed rats used for ongoing investigations of *P. carinii*. All rats were housed in open cages (one per cage), and all were given dexamethasone in the drinking water (2 mg/liter). The water also contained 1 mg of tetracycline per ml. In addition, all received a special complete low-protein diet (ICN Biomedicals Inc., Costa Mesa, Calif.) containing 8% protein; this diet was developed after it was noted that regular low-protein diets are deficient in methionine and zinc. The only difference between the two groups of rats was the room in which they were housed.

We also attempted to develop rats lacking latent *P. carinii* infection. Two pregnant females were given 24 mg of trimethoprim and 120 mg of sulfamethoxazole daily in the drinking water beginning on day 11 of gestation and continuing until the pups were weaned. The 14 weaned pups were housed in filter-topped cages, and at 10 weeks immunosuppression was begun for two males and two females by adding dexamethasone to the drinking water (2 mg/liter).

After 8 weeks of immune suppression, all rats from the virus-free colony and the immunosuppressed pups were sacrificed, and the lungs were evaluated for *P. carinii* infection. Impression smears stained with Giemsa solution or methenamine silver nitrate and sections stained with methenamine silver nitrate were scored on a scale of 0 to 4, as described elsewhere (1).

None of the 10 rats from the virus-free colony housed in the virus-free room developed demonstrable infection with *P. carinii*. All of the rats that were transferred to the room housing rats with *P. carinii* infection developed infection, with numerous trophozoites and cysts of *P. carinii* in the impression smears. The infectivity scores were 3.1 ± 0.2 for Giemsa-stained smears and 3.5 ± 0.2 for silver-stained smears. Sections stained with silver had a mean score of 3.2 ± 0.2.

None of the four rats born to and nursed by mothers treated with trimethoprim-sulfamethoxazole had *P. carinii* infection; all impression smears and sections were negative.

P. carinii-free animals are required for transmission studies and for developing libraries of *P. carinii* strains maintained by inoculation of organisms from various sources into the uninfected rat host. The results reported here show that it is possible to obtain *P. carinii*-free animals for the experiments described above and for other investigations when latent infection with *P. carinii* would be detrimental (5). Conversely, investigators using the Sprague-Dawley rat model for development of *P. carinii* infection need to know that rats from Harlan colony 202 do not have latent infection and when immunosuppressed will not develop infection with *P. carinii* unless housed with previously infected rats.

Germfree rats have been reported to harbor *P. carinii* (3). In response to these reports, the Lobund conventional colony was studied and found to be free of *P. carinii* when the rats were immunosuppressed with cyclophosphamide (4). The few animals kindly supplied by M. Wagner, which we treated with 250 mg of cortisone per kg, had rare cysts in imprints of lungs. Although the rats were not housed in the same room with infected rats and were maintained with barriers in filter-topped cages, we cannot rule out the possibility of infection during shipping or introduction into our facility.

In summary, we showed that animals can be obtained or developed to be free of *P. carinii* and that the virus-free Sprague-Dawley animals from Harlan colony 202 are susceptible to *P. carinii* and can be infected by airborne transmission.

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