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New Rat Model of *Pneumocystis carinii* Infection

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Rats free of latent *Pneumocystis carinii* organisms were immunosuppressed with adrenal corticosteroids and transtracheally injected with *P. carinii*. These animals subsequently developed *P. carinii* pneumonia. Infection was accomplished by using organisms from infected rat lung or from culture. Diffuse infection was produced with no significant differences in the numbers of organisms found in various lobes of the lungs. Infections progressed over time so that by 6 weeks postinoculation all animals were heavily infected. Infection by transtracheal injection has three advantages over current models. First, transtracheal injection provides a reliable model which is not dependent on naturally occurring latent *Pneumocystis* infection. Second, transtracheal injection allows the perpetuation of specific *Pneumocystis* strains. Third, transtracheal injection is a more rapid and economical means of producing severe *Pneumocystis* pneumonia.

The rat model of *Pneumocystis carinii* is the source of organisms for most studies on the biochemistry, immunology, and host-parasite interactions of this protozoan parasite. Organisms derived from the rat have also been used in culture studies (3). In addition, the rat model has been used to assess the efficacy of antimicrobial agents for both therapy and prophylaxis of *Pneumocystis* pneumonia (10). This model has gained in importance with the epidemic of acquired immunodeficiency syndrome in which over half of the patients develop *Pneumocystis* pneumonia during the course of their disease. Although infection apparently is spread by aerosol, infections in patients with acquired immunodeficiency syndrome and in the immune-suppressed rats probably result from the activation of latent *P. carinii*.

Most laboratory rats have latent *P. carinii* infection, and when the animals are immunosuppressed by adrenal corticosteroids overt infection develops (5). The model is inherently variable because animals differ in levels of latent infection, in genetic susceptibility to *P. carinii* infection when immunosuppressed, and in the level of immunosuppression achieved by corticosteroid treatment (2). Recently, animal suppliers have been changing their stock colonies so as to reduce the carriage of viral, fungal, and bacterial pathogens. Some of the virus-free animals have been shown to be *P. carinii* free as well (1). This change has created difficulties for workers using the rat model of *P. carinii* infection for their studies. A new model which does not depend on the activation of latent infection is needed. In this report we describe such a model, based on *P. carinii* infections initiated by the transtracheal injection of *P. carinii* from infected lung homogenate or lavage or from culture.

Transmission of *P. carinii* to cesarean section-derived, barrier-sustained rats and pathogen-free rats by direct contact with standard rats has been described by Hendley and Weller (7). In addition, Hughes (8) and Walzer et al. (11) reported transmission by the airborne route, and Walzer et al. reported successful development of infection in nude mice by percutaneous injection of organisms into the lung. Although intranasal instillation of organisms failed to produce infection in the nude mouse model of Walzer et al. (11), Furuta et al. have described successful transmission by this route (6). The direct transtracheal inoculation of rats has not been reported previously. This technique allows the rapid and consistent development of infection with *P. carinii* and the perpetuation of specific strains of this organism.

MATERIALS AND METHODS

P. carinii-free rats were obtained as virus-free rats from Harlan Sprague-Dawley, Indianapolis, Ind. (1), or were developed by treating pregnant females with trimethoprim-sulfamethoxazole during the last half of gestation and until the pups were weaned. All animals used as *P. carinii* free were housed in cages with filter tops. Both control and experimental animals were immunosuppressed by administration of dexamethasone (Schering Corp., Kenilworth, N.J.) in drinking water at 1.0 mg per liter. Tetracycline 0.5 g per liter was added to the water to prevent bacterial infections. The inoculum for some experiments was prepared by grinding infected rat lung in a 10 Broek grinder containing sterile physiologic salt solution, centrifuging the homogenate at slow speed to settle large tissue fragments, and evaluating the supernatant for viable trophozoites and cysts by ethidium bromide-fluorescein diacetate staining (9). For other experiments the inoculum was organisms from culture, suspended in sterile phosphate-buffered saline, used either as fresh organisms or after being frozen for up to 3 weeks at −70°C. For inoculation, rats were anesthetized with 0.2 ml of ketamine hydrochloride intramuscularly or with ether. A 1-cm midline incision was made over the trachea, and the trachea was exposed by blunt dissection (Fig. 1). Under direct visualization a 0.05- to 0.2-ml inoculum with 0.4 ml of air following in the same syringe was injected into the trachea. The inoculum contained at least 7×10^5 organisms for each animal. The wound was closed with a wound clip or 3-0 catgut suture.

For evaluation of severity of *P. carinii* infection, rats were anesthetized with ketamine or ether and sacrificed by exsanguination. The lungs were removed, and separate por-

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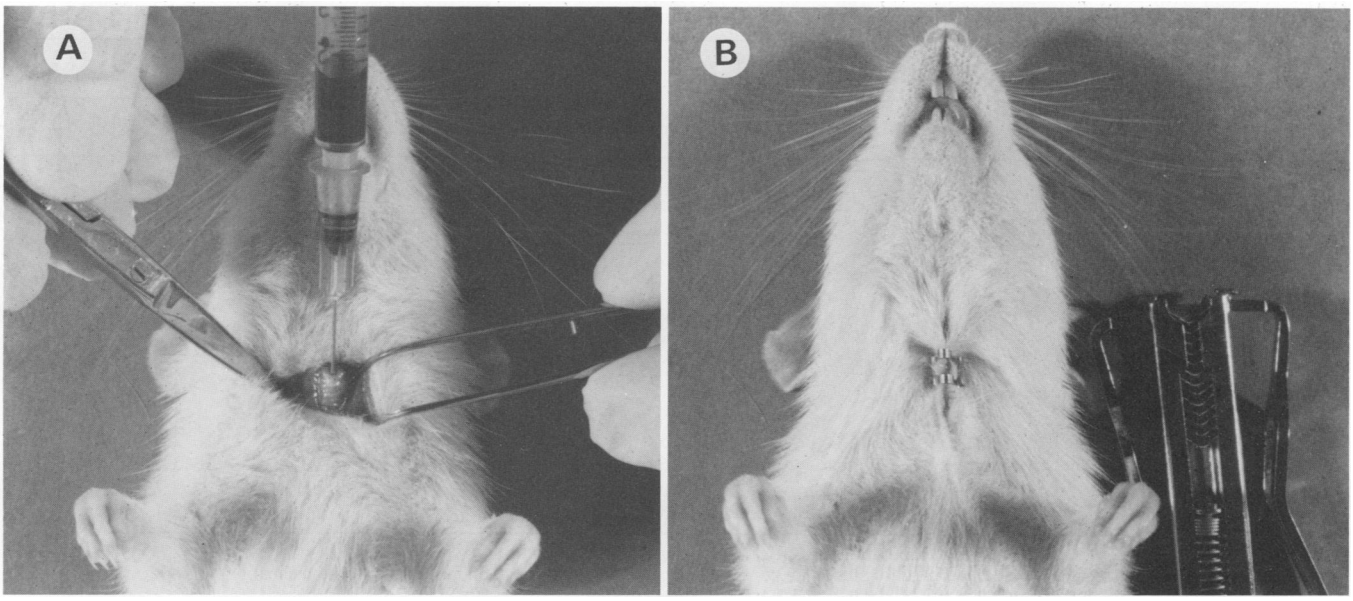

FIG. 1. (A) The trachea of this rat has been exposed, and the needle is inserted into the trachea. The syringe contains dye for photographic clarity which is followed by air (see text). (B) The incision has been closed with a wound clip. The apparatus used for applying the wound clip is beside the rat.

tions were used for impression smears or fixed in 10% Formalin for histologic sections. For rats in the third set, portions of left and right upper and lower lobes were used. Separate impression smears were stained with Giemsa and methenamine-silver nitrate (4), and sections were stained with methenamine-silver nitrate and hematoxylin-eosin. Slides were evaluated as unknowns by three examiners, and infectivity scores were determined according to the following scale with the number of organisms in 1,000× microscopic fields: greater than 100, 5+; 11 to 100, 4+; 1 to 10, 3+; 2 to 9 in 10 fields, 2+; 1 in 10 or more fields 1+; and no organisms seen in 50 fields, 0.

Three sets of rats were evaluated. The first set was eight rat pups from trimethoprim-sulfamethoxazole-treated mothers (described above). At six weeks of age, these weanling rats were started on immunosuppression which was continued for 8 weeks, at which time four of the eight were sacrificed and examined for the presence of *Pneumocystis* organisms. The other four, two males and two females, were transtracheally injected with rat lung infected with *P. carinii* and immunosuppressed for an additional 8 weeks then sacrificed.

The second set was 10 adult male rats weighing an average of 220 g, (Sprague-Dawley; Taconic Farms, Germantown, N.J.), of which 5 were injected transtracheally with cultured organisms and 5 were injected with organisms lavaged from lungs of *P. carinii*-infected rats lung after 2 weeks of immunosuppression. Two animals from each group died and could not be evaluated. The remaining animals were evaluated 4 weeks after the instillation of organisms by impression smears and histology.

The third set was 20 virus-free rats purchased from Harlan Sprague-Dawley. They were immunosuppressed for 4 days, transtracheally injected with *P. carinii* from infected lungs as described above, and continued on immunosuppression. Groups of rats were evaluated for severity of infection at 4, 5, and 6 weeks postinoculation. In addition, the severity of infection in various lobes of lungs (left and right, upper and lower) was evaluated in each animal to assess the uniformity of the infection. Rats immunosuppressed but not transtracheally injected served as rat colony controls.

RESULTS

In the first group, the four pups of trimethoprim-sulfamethoxazole-treated mothers sacrificed after 8 weeks of immunosuppression had no *P. carinii* detected in any smear or section in any stain. The littermates, two males and two females which had been transtracheally injected with *P. carinii* and immunosuppressed an additional 8 weeks, had heavy infections with an average severity score of 3.5.

Rats in the second group, the six surviving rats inoculated with lavaged organisms or with freshly harvested or frozen cultured organisms, developed heavy *P. carinii* infection with no detectable differences in the severity of infection from the different inocula. All had infectivity scores of over 3.5. The infection was uniformly distributed throughout the lungs. Animals from the same supplier placed on immune suppression but not inoculated with *P. carinii* developed *Pneumocystis* pneumonia from latent infection, but it was uniformly less severe than that in inoculated animals immunosuppressed for a comparable period of time.

The third group, virus-free rats immunosuppressed, inoculated, and evaluated over time for the location and severity of infection, all demonstrated *Pneumocystis* infection (Table 1). The severity of infection increased with time after the inoculation, and there was no significant difference in severity in different lobes of the lung by either Giemsa or methenamine-silver staining techniques. Four rats in group 3 died during or shortly after the inoculation procedure. No infections developed at the surgical sites in any animals in any group, and in many instances the animals had sloughed the wound clips with complete healing at the surgical site despite corticosteroid administration. None of the noninjected animals which were immunosuppressed for the same period of time as the transtracheally injected animals developed infection.

TABLE 1. Infectivity scores by lung area of group 3 rats evaluated at 4, 5, and 6 weeks after inoculation[a]

Wk postinoculation	Impression smear, Giemsa stain[b]				Impression smear, silver stain[c]				Sections, silver stain[d]				Avg for stains[e]		
	LU	LL	RU	RL	LU	LL	RU	RL	LU	LL	RU	RL	Impression, Giemsa	Impression, silver	Section, silver
4	2	2	2	1.5	2.5	2	2.5	2	2.5	2	1.5	2	1.9	2.2	2
	1.5	1	1.5	1.5	2.5	2.5	2	3	2	1.5	2	3	1.4	2.5	2.1
	3.5	3	2.7	3	3.5	3	3.5	3	3	3	3	3.5	3	3.2	3.1
	3	2.5	2	2.5	4	3	3	3	2	3	3	3	2.5	3.2	2.8
	3	3	2	2	3.5	3	3	4	3	2	3	2.5	2.5	3.4	2.6
5	3.5	3.5	3.5	3.7	4	3	3.5	3.5	3.5	4	4	3.5	3.6	3.5	3.8
	2	2	2	2	3	3			2.5	3	2.5	3	2	3	2.7
	2	1.5	2.5	2.5	3.5	3	2	3	3	2.5	3	3	2.1	3	2.8
6	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	3	2.5	3	2.5	3.5	4	0.5	2.5	4	3.5	3.5	3	2.7	2.6	3.5
	3.7	3	3.5	4	3.5	4	4	2	3	4	3.5	3.5	3.6	3.4	3.5
	3.7	4	4	4	3.5	3.5	4	4	4	4	3.5	3.3	3.9	3.7	4
	3.5	3	2.5	4	3.5	3.5	3	3.5	4	4	3	4	3.2	3.4	3.8

[a] Lung lobes: LU, left upper; LL, left lower; RU, right upper; RL, right lower.
[b] Averages at weeks 4, 5, and 6 were 2.3 ± 0.2, 2.6 ± 0.2, and 3.5 ± 0.1, respectively (means ± standard errors of the means).
[c] Averages at weeks 4, 5, and 6 were 2.9 ± 0.1, 3.1 ± 0.2, and 3.4 ± 0.2, respectively.
[d] Averages at weeks 4, 5, and 6 were 2.5 ± 0.1, 3.1 ± 0.2, and 3.7 ± 0.1, respectively.
[e] Averages at weeks 4, 5, and 6 were 2.6 ± 0.1, 2.9 ± 0.2 (not significantly different from value at 4 weeks), and 3.6 ± 0.1 ($P < 0.001$).

DISCUSSION

The transtracheally infected rat model for *P. carinii* pneumonia adds to the research tools available for the study of this organism and will become increasingly important as rats which are latently infected are more difficult to obtain. Transtracheal inoculation of organisms has produced consistent *P. carinii* infection with a uniform distribution of organisms throughout the lungs. This model has a number of advantages. All inoculated animals become infected with *P. carinii*, and the infection is more uniform in severity from animal to animal than in the conventional model. The numbers of organisms present initially are greater than in latent infection, so severe infection develops more rapidly. Transtracheal injection allows the investigator to select specific *Pneumocystis* strains for study and, by adjusting the inoculum, the severity of immunosuppression, and the length of immunosuppression, to have some control over severity of infection. The propagation of specific *Pneumocystis* strains will allow comparisons among strains and the selection of strains with specific characteristics of interest such as antimicrobial resistance. This animal model can be used in the search for better antimicrobial agents for therapy of *P. carinii* pneumonia. It has been used in ongoing studies of antibiotic therapies for *P. carinii* pneumonia. Animals infected by transtracheal inoculation have a response to antibiotic therapy that is comparable to that of animals with similar levels of infection on the conventional model (unpublished data).

In summary, the transtracheally infected rat model is a useful new technique for the study of *P. carinii*. It allows the propagation of infections with specific strains of *P. carinii* so that these strains may be perpetuated. The model also assures the availability of *P. carinii*-infected animals for various in vivo and in vitro studies. Finally, this model allows for a greater uniformity of the initial infection and shorter times for the development of more severe infections. Further studies of this model are underway.

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