Mouse Model for *Pneumocystis carinii* Pneumonia That Uses Natural Transmission To Initiate Infection

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Animal models for *Pneumocystis carinii*, for the most part, have been limited to immunosuppressed rats and ferrets, while a dependable mouse model has been more difficult to develop. A *P. carinii* mouse model has now been established with several strains of mice, including C3Heb/FeJ, C3HeN, BALB/c, DBA/2N, and BALB/c *nu/nu* (athymic). In lieu of using invasive methods for initiating *P. carinii* infections, mice harboring *P. carinii* transmitted the disease to mice without latent infection via short-term cohabitation. After the exposure period, the seed mice were sacrificed to confirm the presence of acute *P. carinii* pneumonia. Acute infections in recipient mice developed at approximately 7 to 8 weeks, while control unseeded littermates remained uninfected. All recipient mice and their littermates were maintained in isolation hoods to eliminate the possibility of exposure to other sources of *P. carinii*. This approach allows investigators to consistently transmit *P. carinii* to mice and to select the strain of mouse desired for use in a particular study. The results presented here suggest that more attention should be given to the potential for patient-to-patient transmission of *P. carinii* in immunocompromised patients such as those with AIDS.

Pneumocystis carinii pneumonia is one of the major life-threatening illnesses in AIDS patients. The causative agent, *P. carinii*, is an opportunistic pathogen which develops in the lungs of immunocompromised patients. Because of the high incidence of adverse reactions in AIDS patients to the currently available antipneumocystis agents, there is an urgent need for a new class of drugs which is safe and effective. Since the organism has not been successfully propagated in vitro, investigators have been restricted, for the most part, to in vivo models for evaluation of candidate compounds. The standard animal models utilize rats and ferrets. *P. carinii* pneumonia has been shown to occur in immunosuppressed mice (15) and genetically immunodeficient athymic (*nu/nu*) and severe combined immune deficient (SCID) mice (5, 8, 11, 13, 14, 17). However, in mice which develop *P. carinii* pneumonia, either as a result of congenic immunodeficiencies or immunosuppression, the infections are variable. Development of infection in athymic and SCID mice is slow and not always predictable because these mice develop *P. carinii* pneumonia naturally as they age.

Host susceptibility and as yet undefined immune responses to *P. carinii* could be more efficiently studied by using mice. Congenic inbred mice are well characterized both genetically and immunologically and many strains differing in specific genetic characteristics are currently available. In addition, when chemotherapeutic studies are conducted, smaller amounts of compound would be required for mice when potential antipneumocystis agents are evaluated (compared, for example, with rats). The focus of this study was to identify a source of mice with latent *P. carinii* and to explore whether these mice could be used to selectively infect other mouse strains through cohabitation. It has been known for some time that *P. carinii* is transmitted via the air and that *P. carinii*-free animals can become infected when kept near infected animals (6). However, this phenom-enon has never been exploited to selectively infect a group of animals for experimental studies.

In this study, mice with latent infections were immunosuppressed with corticosteroids to establish acute *P. carinii* infections (seed mice). These mice were then used to transmit *P. carinii* to a variety of different mouse strains (recipient mice) through 1 to 2 weeks of cohabitation. The approach is simple, is noninvasive, and allows the investigator the ability to choose the recipient strain of mouse. This method establishes consistent infections and allows the investigation of the epidemiology of the disease in a well-characterized system.

MATERIALS AND METHODS

Mice. C3Heb/FeJ mice were obtained from Jackson Laboratories (Bar Harbor, Maine). C3HeN mice were from Sasco Laboratories (St. Louis, Mo.). DBA/2N, BALB/c, athymic (*nu/nu*, congenic with BALB/c) and Fox-Chase SCID-CB-17/ICRTAC mice were supplied by Taconic Laboratories (Germantown, N.Y.). All mice were viral antigen-free inbred strains raised under barrier conditions.

Immunosuppression. Male mice of various strains, 7 to 9 weeks of age, were used in these studies. Mice were reared in standard microisolator cages (Lab Products Inc., Maywood, N.J.), in groups of 10 to 15 mice per cage. The *nu/nu* and SCID mice received autoclaved food, water, and bedding throughout the experiments; the other mouse strains did not. With the exception of the severely congenitally immunodeficient *nu/nu* and SCID strains, the mice used in these studies were immunosuppressed with dexamethasone (Butler, Columbus, Ohio) in their drinking water. The dexamethasone was titrated at levels of 1 to 8 mg/liter. The water also contained 1 g of tetracycline (Pfizer Inc., New York, N.Y.) per liter to reduce bacterial infection. Mice were fed either 8% low-protein diet (ICN Biochemicals, Cleveland, Ohio) or standard 23% protein rodent chow formula 5008 (Purina, St. Louis, Mo.). Food and water were supplied ad

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libitum for the duration of the experiment. Figure legends give the specific parameters of each experiment.

P. carinii transmission. *P. carinii* was transmitted to recipient mice by cohabitation with infected C3Heb/FeJ seed mice that were previously immunosuppressed for 5 to 8 weeks prior to use. Two seed mice were placed with 10 to 15 recipient mice and were allowed to cohabitate for 1 to 2 weeks. Recipient and seed mice continued to receive dexamethasone for the duration of the experiment. The seed mice were 12 to 14 weeks of age, and the recipient mice were 7 to 9 weeks of age; however, since the seed mice had been receiving dexamethasone, which suppresses weight gain in mice, there was not a significant difference in the size of the animals during cohabitation. Transmission studies were conducted within laminar air flow isolation hoods. Unseeded control groups were immunosuppressed and placed in separate hoods to prevent exposure to seeded mice. Seed mice were sacrificed after the seeding period, and *P. carinii* infection was confirmed by toluidine blue staining of cysts (see below).

Evaluation of lung tissue. Excised lungs were homogenized in 5.0 ml of phosphate-buffered saline (Sigma Chemical Co., St. Louis, Mo.) with a homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.) at speed 3 for approximately 5 s. Homogenates were centrifuged (Beckman GPR centrifuge) at 1,700 × g for ten minutes. The erythrocytes were lysed by resuspending the pellet in 5.0 ml of a 0.85% ammonium chloride (Mallinckrodt, Paris, Ky.) solution, incubating the suspension for 5 min in a 37°C water bath, and then centrifuging the suspension at 1,700 × g (10 min). The pellets were washed once in saline, and the final pellets were resuspended in 100 μl of saline. A 5.0-μl aliquot of each sample was evenly applied and air-dried onto Teflon-coated microscope slides with a fixed surface area (11-mm circles; Carlson Scientific, Peotone, Ill.). The extent of disease for each animal was determined by microscopic analysis of stained slides. The total number of cysts per animal lung was determined by quantitating the number of cysts in 20 to 50 microscope fields (1,000×) of homogenized lung tissue on slides fixed with ether-sulfuric acid and stained with toluidine blue O (2). The total number of organisms per mouse lung was determined as a function of the surface area on the slide, the volume of the applied sample, and the total volume of the processed homogenate.

RESULTS

Identification of mice with latent *P. carinii*. A survey of several mouse strains from various sources was performed by using an immunosuppression regimen of 8 mg of dexamethasone per liter in the drinking water (ad libitum) and an 8% protein diet for 6 weeks. Mouse strain C3Heb/FeJ was identified as having a latent *P. carinii* infection (data not shown).

Establishment of conditions for an optimal *P. carinii* infection. When C3Heb/FeJ mice were maintained on an 8% protein diet, it was found that the optimal dose of dexamethasone required to achieve an acute *P. carinii* infection in 6 to 8 weeks was 4 mg/liter (Fig. 1). Mice maintained on an 8% protein diet alone were mildly infected with *P. carinii* (Fig. 1, 0 mg/liter). To evaluate the independent effect of dexamethasone, mice were immunosuppressed while receiving 23% protein rodent chow (Fig. 2). Treatment with dexamethasone alone established an optimal *P. carinii* infection at the 4-mg/liter dose. The 8-mg/liter dose did not
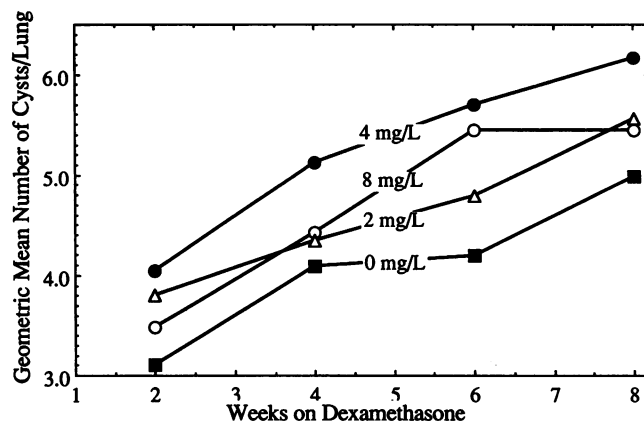

FIG. 1. Effects of various doses of dexamethasone on the level of *P. carinii* infection in C3HeB/FeJ mice receiving an 8% protein diet. All points represent five animals with the exception of the 0 mg of dexamethasone per liter group, in which 3 animals were sampled. The detection limit is 2.96 cysts per lung (log_{10}).

significantly enhance the infection and the 2-mg/liter level produced suboptimal infection.

Although an 8% protein diet did contribute to the level of infection at the 1- and 2-mg/liter levels, as seen in Fig. 1 and 2, the degree of *P. carinii* infection at the 4-mg/liter level was comparable for the 23 and the 8% protein diet. As a result, the 8% protein diet was eliminated from use in subsequent experiments.

Levels of dexamethasone greater than 4 mg/liter increased the incidence of mortality in both steroid titrations. There was no mortality observed in mice administered 0, 2, and 4 mg of dexamethasone per liter in the 23% protein rodent chow steroid titration; however, a 13.3% mortality was found in mice administered 8 mg of dexamethasone per liter. (Mortality was also observed in mice who received 8 mg of dexamethasone per liter and were maintained on the 8% protein diet, but it was not documented.)

Transmission of *P. carinii* to other immunosuppressed mice. *P. carinii* was successfully transmitted from infected C3Heb/FeJ mice to recipient C3HeN mice during a single week of cohabitation. After cohabitating, recipient mice remained on
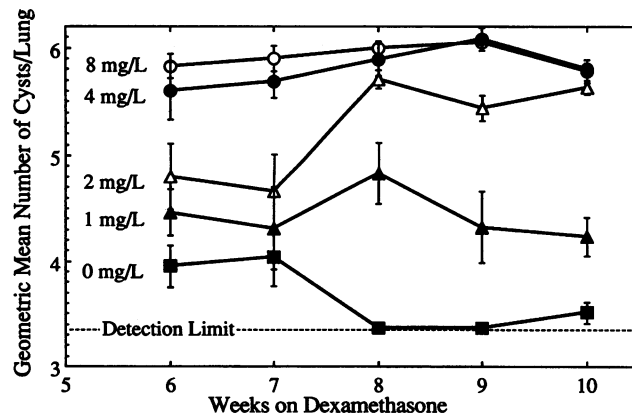

FIG. 2. Effects of various doses of dexamethasone on the level of *P. carinii* infection in C3HeB/FeJ mice receiving a 23% protein rodent chow. All points represent 5 to 10 animals. The detection limit is 3.36 cysts per lung (log_{10}).

TABLE 1. Transmission of *P. carinii* to various mouse strains by short-term exposure to C3HeB/FeJ mice with acute *P. carinii* pneumonia

Recipient[a]	Dexa-methasone (mg/liter)	Seed period (weeks)	Incubation postseeding (weeks)	Mean no. of cysts (± SEM)[b] in mice which were:	
				Seeded	Not seeded
C3HeN	8	1	6	5.41 ± 0.15	0
DBA/2N	4	2	5	5.71 ± 0.04	0
BALB/c	4	2	5	5.55 ± 0.05	0
nu/nu	0	2	6	5.63 ± 0.06	0

[a] There were 6 to 10 animals per group, except for the *nu/nu* group, which had 2 animals. All animals were fed 23% protein rodent chow, except for the C3HeN mice, which were on an 8% protein diet.

[b] Numbers are the geometric mean numbers of cysts per lung. Detection limit is 3.36 cysts per lung ($\log_{10}$).

the immunosuppression regime for an additional 6 weeks. All recipients developed *P. carinii* infections, and there was no difference in the level of PCP infections observed in recipient mice whether seed animals were previously immunosuppressed 5 or 7 weeks prior to cohabitation (geometric mean numbers ± standard errors of the mean of cysts per lung were 5.41 ± 0.15 and 5.27 ± 0.16, respectively). Cyst levels were comparable to those shown in Fig. 2 for C3Heb/FeJ mice at the same time. However, seed mice which had never been given dexamethasone, except during the cohabitation period, did not efficiently transmit the infection to the recipient mice, even though low numbers of cysts (3.18 ± 0.11; limit of detection, 2.96 cysts per lung) were found in these recipients, indicating exposure to *P. carinii* had occurred. Littermates which had not been exposed to the seed mice did not have detectable *P. carinii* infections.

P. carinii was also successfully transmitted to BALB/c and DBA/2N mice immunosuppressed with dexamethasone (Table 1). The level of infection at 7 weeks was again comparable to that seen in the C3Heb/FeJ mice after 7 weeks of immunosuppression with 4 mg of dexamethasone per liter in the drinking water (Fig. 2). A 2-mg/liter level of dexamethasone was also tested with BALB/c mice, and infections were comparable to those seen at the 4-mg/liter level (data not shown). Control mice, which had not been exposed to seed mice, did not develop *P. carinii* infections, indicating that the experimental recipient animals were free of *P. carinii* prior to exposure to seed mice.

Time course study of BALB/c recipient mice. A time course study of BALB/c recipient mice (Fig. 3) shows gradual development of *P. carinii* infection after seeding. The infection was similar to that seen in C3Heb/FeJ mice (Fig. 2) at weeks 7, 8, and 9. However, infections in BALB/c mice at week 10 surpassed infections seen in the C3Heb/FeJ mice at the same time. At week 13 the *P. carinnii* pneumonia infections were still increasing. Low standard errors of means in groups demonstrate consistency of infection in individual mice in this experiment.

Transmission to immune incompetent mice. Transmission of *P. carinii* from C3Heb/FeJ seed mice to athymic (*nu/nu*) mice was also achieved (Table 1). Since athymic mice lack a functional thymus and are deficient in cell-mediated immunity, dexamethasone was not necessary to establish *P. carinii* infections. Infections seen at 8 weeks in athymic mice were similar to those seen in the C3HeN mice at 7 weeks. Data obtained from seeded groups at weeks 7 and 9 were
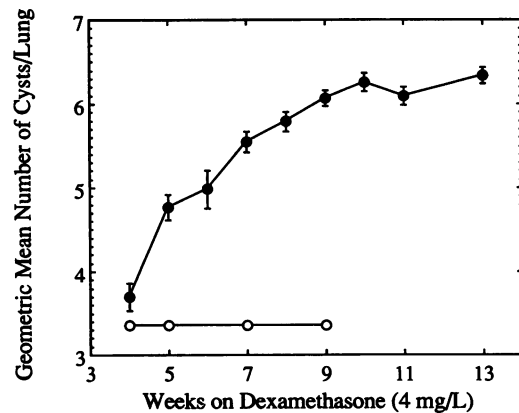
FIG. 3. Time course study showing the development of *P. carinii* infection in recipient BALB/c mice exposed to infected C3Heb/FeJ mice (●). Control mice (○) were removed at weeks 4, 5, 7, and 9. (Times include a 2-week cohabitation period.) All points represent five animals. The detection limit is 3.36 cysts per lung ($\log_{10}$).

4.58 ± 0.38 and 5.62 cysts per lung, respectively (limit of detection, 3.36 cysts per lung).

Successful transmission of *P. carinii* to SCID mice was also performed. Recipient SCID mice that were sacrificed 5 weeks after cohabitation showed *P. carinii* cysts (4.38 ± 0.15 cysts per lung; limit of detection, 3.36 cysts per lung) while control unseeded SCID mice were all negative for *P. carinii*. Unfortunately, none of the mice in either the control or the seeded groups survived to 7 weeks postcohabitation. Presumably these mice died as a result of secondary infections due to their severely compromised immune state and their exposure to seed mice which were not caesarean derived and kept under sterile conditions.

DISCUSSION

The experiments reported here demonstrate that *P. carinii* infections can be successfully established in several strains of mice by short-term cohabitation with previously infected mice. This eliminates the need for invasive methods to infect mice with *P. carinii* (1), as well as the requirement for a viable source of cryopreserved organisms, which must be derived from infected animals. In addition to allowing for the development of *P. carinii* in a chosen strain of mouse, this method should also be useful for the continuous propagation of *P. carinii* infection within a colony of immunosuppressed mice. By utilizing recipient mice with developed *P. carinii* infections to seed subsequent groups of immunosuppressed mice, one is able to maintain established infection indefinitely. This eliminates dependence on an animal supplier with a consistent source of mice possessing latent *P. carinii*. Also, mice used for seeding can still be used for studies after the cohabitation period, allowing for more efficient animal usage.

The consistent level of infection in the various strains of mice used demonstrates that *P. carinii* develops to the same degree regardless of strain. It could be argued that the consistency of infection is due to the use of microisolators; however, there were at least three cages of animals per group in these experiments. When mice were sampled, they were randomly removed from all the boxes within the group; infections were consistent within the groups as well as within cages. This, however, does not indicate that all strains of mice are equally susceptible to *P. carinii* in the immune-

competent state, and this is likely to have some bearing on the type of mice which can be initially identified with latent *P. carinii*. This has been demonstrated by the work of Walzer et al. (15) in which eight strains of mice were immunosuppressed and graded on their intensity of *P. carinii* infection. The C3H/HeN mice achieved the highest level of infection, while BALB/c mice had an intermediate level and DBA/2N mice developed light infections. Although the actual source of *P. carinii* is not known, the transmission experiments described in this paper indicate that similar levels of infection can be obtained in these mice. Further studies using this transmission model with immune-competent recipient mice may help to identify *P. carinii*-susceptible and -resistant mouse strains. The shortest cohabitation period used in these studies was 1 week; it is possible that shorter periods may also be effective and should be investigated.

Athymic mice used in these studies appeared to take slightly longer to develop equivalent infections; the number of mice in this experiment prevents definite interpretation at this time. However, if confirmed, this slightly longer time may occur as a result of the presence of non-T-cell-mediated immune mechanisms that are absent or dramatically reduced in dexamethasone-treated mice. *P. carinii* pneumonia has been reported to occur in athymic mouse colonies as they age (14). These experiments suggest that it is possible to establish a more consistent infection by transmitting *P. carinii* through cohabitation. As a result, the source of the *P. carinii* appearing in older athymic mouse colonies must be questioned. A latent infection would be expected to be established sooner, so athymic immunodeficient mice may be exposed to *P. carinii* at some point as they age. The sample size in this experiment was small because of incompatibility of the C3Heb/FeJ mice and the athymic (*nu/nu*) mice. Most of the seeds were not able to survive the 2-week seed period. However, it is evident from the results that transmission did occur by this method.

The relationship between development of infection in mice with latent *P. carinii* and seeded mice can be examined at the C3Heb/FeJ 4-mg/liter steroid level (Fig. 2) and in the BALB/c time course study (Fig. 3). While mice with latent infections had an increased level of *P. carinii* cysts initially, the seeded animals were only 1 week behind the latently infected animals in developing *P. carinii* pneumonia by week 6. Since the BALB/c time points include the seed period, this substantiates the claim that transmission occurs in a 1-week seed period. The time course study also demonstrates the uniform infection level seen in the recipient mice at different times (see standard errors), a major improvement over claims in the literature of widely variable *P. carinii* infections within groups of immunosuppressed mice (16). The levels of dexamethasone used in these experiments have been optimized for C3Heb/FeJ mice, and different levels may be optimal for other types of mice. Many of the strains used in these studies were not able to tolerate 8 mg of dexamethasone per liter, while the C3Heb/FeJ mice did. Also, the BALB/c mice developed comparable levels of infection whether on 4 or 2 mg of dexamethasone per liter. Optimization for a particular mouse strain should be investigated before a dexamethasone level is used routinely. Since water consumption was not determined for these experiments, it is possible that the various mouse strains differ in the amount of water they consume.

These cohabitation experiments support claims that human transmission of *P. carinii* between immunocompromised individuals in the clinical setting is likely to occur (3, 4, 6, 7, 9, 10, 12). Although *P. carinii* is considered endemic, the results of the initial seeding study indicate that seed mice not immunosuppressed prior to cohabitation are able to transmit infection at low levels. This suggests that the level of initial exposure to *P. carinii* has a major bearing on the rate at which an infection is established. A closer look at the epidemiology of *P. carinii* in the hospital setting is warranted.

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