Immunodeficient and Immunosuppressed Mice as Models To Test Anti-*Pneumocystis carinii* Drugs

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Congenitally immunodeficient and immunosuppressed normal mice with naturally acquired *Pneumocystis carinii* infection were compared as models for testing anti-*P. carinii* drugs. Among the immunodeficient mice, mice with severe combined immunodeficiency disease (*scid*), which lack B and T cells, had higher levels of *P. carinii* pneumonia than did μ MT mice, which lack B cells. Normal mice administered dexamethasone in the drinking water had more extensive pneumocystosis than mice administered parenteral methylprednisolone or hybridoma cells making a monoclonal antibody to CD4 cells. The standard anti-*P. carinii* drugs trimethoprim (TMP)-sulfamethoxazole (SMX), pentamidine, and atovaquone, which work well in rats and humans, worked well in the mice. Clindamycin and primaquine were effective in the *scid* and μ MT mice but not in the immunosuppressed normal mice. High doses of epiroprim, an analog of TMP, appeared to enhance the activities of low doses of SMX and dapsone, while high doses of TMP did not; however, further studies are needed before definitive conclusions about the actions of these drugs can be drawn. Taken together, the data obtained in this study support the growing body of literature suggesting that the mouse is a valid alternative to the rat as a model for testing anti-*P. carinii* drugs. Additional differences involving the activities of individual drugs in these models will probably emerge as more experience is gained.

Pneumocystis carinii is an opportunistic pulmonary pathogen in patients infected with the human immunodeficiency virus and other immunocompromised hosts. The anti-*P. carinii* drugs in current clinical use have significant limitations, and there is a need for more potent and less toxic agents. Drug development and testing have primarily been performed in animal models because of the lack of a continuous in vitro culture system. Rats immunosuppressed with corticosteroids have been the standard model for three decades (10, 14, 31). Pneumocystosis develops in about 8 weeks and is histologically indistinguishable from the disease in humans; drugs which show activity against rat-derived *P. carinii* are usually active against human-derived *P. carinii*.

In recent years, mice have begun to attract attention as models for testing anti-*P. carinii* drugs (1–3, 5–7, 9, 41). Mice are only about 1/10 the size of rats, and thus require smaller quantities of drugs; mice also have highly developed genetic and immune systems which provide a variety of animal strains and reagents for investigational use. One type of mouse model has involved congenitally immunodeficient animals such as athymic (nude) mice, which lack T cells; μ MT mice, which lack B cells; or mice with severe combined immunodeficiency disease (*scid*), which lack B- and T-cell function (5, 25, 41). The other model has involved normal mice which are immunosuppressed by the administration of corticosteroids or anti-CD4 antibody (2, 18, 19, 24). Although the results obtained so far with mice are promising, further studies with different experimental conditions are needed before mouse models can be accepted as reliable predictors of activity in humans. Such

information is of more than academic interest, because the U.S. Food and Drug Administration has traditionally relied on the rat model as the “gold standard” of animal efficacy testing before granting approval for use of new anti-*P. carinii* drugs in clinical trials.

We have had considerable experience in using immunodeficient and immunosuppressed mice to investigate the immunopathogenesis of pneumocystosis (4, 20–23, 25, 33, 35, 37–39). We have used mice which have become infected with *P. carinii* through natural environmental exposure rather than by intratracheal or intranasal inoculation, because this mode of transmission more accurately reflects the situation in humans. Over the past few years, we have extended this work to investigating anti-*P. carinii* drugs. We undertook the present study with the following goals: (i) to compare newer (μ MT) with existing (*scid*) immunodeficient mice as models for testing anti-*P. carinii* drugs, (ii) to compare different methods of immunosuppressing normal mice to evaluate drugs, (iii) to compare the efficacies of the same anti-*P. carinii* drugs in different mouse models, and (iv) to determine if mice can be used for specialized drug development purposes (e.g., to study combinations of antifolate compounds).

MATERIALS AND METHODS

Animal model. (i) Immunodeficient mice. C.B17 and BALB/c *scid* mice were developed as described previously (20). μ MT mice were established by targeted disruption of the gene encoding the μ -chain constant region in mouse embryonic stem cells (16). The disrupted μ allele was transferred to C57BL/6Snm mice by multiple backcrosses as described previously (20). The *scid* and μ MT mice were housed in positive individual ventilated cages (Lab Products, Inc., Maywood, N.J.) with autoclaved bedding, food, and water in a barrier facility. Breeding of the mice was done by mating homozygotes.

For both types of immunodeficient mice, *P. carinii* infection was endemic in their colonies. The mice used to test anti-*P. carinii* drugs were transferred to another barrier facility when they reached about 8 to 10 weeks of age. The animals were housed in microisolator cages in a plastic unit with laminar, HEPA-

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filtered air (Biobubble; CSA Fluid Dynamics, Fort Collins, Colo.) and were given autoclaved food, water, and bedding. After a period of acclimation in which sentinel mice were sacrificed to check the progress of *P. carinii* pneumonia, the animals were randomly divided into treatment and control groups of about 10 mice each. The drugs to be tested were administered parenterally or by oral gavage on a milligram-per-kilogram basis in single or divided doses for 3 weeks. Control mice received no drugs or a placebo. The mice were observed daily for food and water intake, appearance, and activity. Animals that appeared ill or moribund were sacrificed by an overdose of halothane or carbon dioxide anesthesia, and their lungs were processed as described below. If infection with another organism or drug toxicity was suspected, tissue specimens for culture and/or histologic examination were obtained from the lungs or other organs as deemed appropriate. At the end of 3 weeks, the remaining mice were sacrificed.

(ii) Normal mice. C3HeB/FeJ mice (Jackson Laboratory, Bar Harbor, Maine) that were about 5 to 7 weeks old and that had latent *P. carinii* infection (19) were used. The animals were housed in the Biobubble as described above, allowed to acclimate, and then started on different immunosuppressive regimens to induce the development of *P. carinii* pneumonia. One regimen involved the administration of different doses of dexamethasone (Butler, Columbus, Ohio) along with cephadrine (0.5 mg/ml; Velosef; Bristol-Myers Squibb, Princeton, N.J.) in the drinking water (19). A second protocol, which was based on our experience with rats (31), replaced the dexamethasone with different doses of methylprednisolone (Depo-Medrol; The Upjohn Co., Kalamazoo, Mich.) administered subcutaneously (s.c.) once weekly; cephadrine remained in the drinking water. The third regimen involved the use of GK1.5 hybridoma cells (ATCC TIB207), which produce a monoclonal antibody to CD4 cells; the hybridoma cells were cultured and harvested, and 5×10^6 cells were injected s.c. as described previously (18).

The immunosuppressive regimens were continued for 9 to 10 weeks, at the end of which time the mice were sacrificed and their lungs were examined for *P. carinii* organisms. Once the optimal immunosuppressive regimen was chosen, drug treatment studies were conducted. The mice were immunosuppressed for about 6 to 7 weeks and were then randomly divided into control or treatment groups as described above; the drugs were administered for 3 weeks, during which time the animals remained on the immunosuppressive regimen. The mice were then sacrificed, and their lungs were examined as described above.

Drugs. Trimethoprim (TMP), sulfamethoxazole (SMX), and dapsone were obtained from Sigma Chemical Co. (St. Louis, Mo.). Pentamidine was supplied by Fujisawa USA, Inc. (Deerfield, Ill.). Atovaquone was obtained from Glaxo-Wellcome Co. (Research Triangle Park, N.C.). Epiroprim was supplied by Hoffmann-La Roche, Inc. (Nutley, N.J.), and R. L. Then and A. Polak, F. Hoffmann-La Roche, Ltd. (Basel, Switzerland). Clindamycin was obtained from Upjohn, and primaquine was obtained from Sigma. The drugs administered orally were prepared as a suspension in 2% ethanol.

Evaluation of drug activity. Analysis of the effectiveness was performed by techniques adapted from our studies with rats (15, 28–32). Anti-*P. carinii* activity was based on a reduction in the severity or extent of pneumocystosis in the lungs rather than on survival, because the mice sometimes died from factors such as drug toxicity or other opportunistic infections. The animals usually had to receive the drugs for at least 10 days because it ordinarily takes this long to detect an effect. To determine the extent of pneumocystosis, the right lung was homogenized and stained with cresyl echt violet, which selectively stains *P. carinii* cysts; in selected instances the homogenate was stained with Diff-Quik (American Scientific Products, McGaw Park, Ill.), a rapid variant of the Wright-Giemsa stain, which stains all stages of the organism. Initially, the lower limit of detection was 5.58×10^4 ($\log_{10}$ 4.75)/lung; subsequently, the dilution of the lung homogenate for quantitation was changed to reduce the limit of detection to 2.23×10^4 ($\log_{10}$ 4.35)/lung. The left lung was used for histologic studies, which were performed on a selective basis. Sections were stained with methenamine silver and hematoxylin-eosin; the severity of *P. carinii* pneumonia was scored from 0 to 4+ on the basis of the degree of alveolar involvement. All specimens were read in a blinded fashion.

Drug efficacy was determined by comparing the *P. carinii* burden in the treatment groups with those in the controls in the same experiment. Organism counts were log transformed and compared by analysis of variance; individual group comparisons were performed by the Student *t* test with a multicomparison (Newman-Keuls) correction by using INSTAT (Graph Pad Software for Science, San Diego, Calif.). Significance was accepted when the *P* value was <0.05 .

RESULTS

Studies with immunodeficient mice. (i) *scid* mice. The natural history of *P. carinii* infection, which was endemic in the *scid* mouse colony, has been described in detail previously (20). Pneumonia caused by the organism was minimal or undetectable in animals younger than 5 to 6 weeks of age, increased until about 15 weeks, reached a plateau, and then persisted until the time of death. Twenty-four *scid* mice sacrificed at a mean age of 97.5 ± 17.6 days had a mean organism burden (*P. carinii* cyst count) of $\log_{10}$ 7.19 ± 0.21 /lung, indicating heavy

infection. No significant differences were found in the organism burden of *scid* mice with two similar genetic backgrounds (BALB/c and C.B17 mice).

The *scid* mice were then used to test standard anti-*P. carinii* drugs at doses previously shown to be effective (Table 1, study 1). Pentamidine and TMP-SMX were highly active, lowering the mean organism count 186- and 102-fold, respectively, from $\log_{10}$ 7.21/lung in the control group. The combination of clindamycin and primaquine was also active, reducing the count 23-fold.

On histopathologic examination, lung sections stained with hematoxylin-eosin revealed the foamy, vacuolated alveolar exudate similar to that found in human pneumocystosis (Fig. 1A). In some areas, there were foci of polymorphonuclear leukocytes but few other host inflammatory changes. No differences in the features of *P. carinii* in the *scid* mice on different backgrounds were noted. Lungs stained with methenamine silver revealed moderate to numerous organisms within alveoli (Fig. 1B), although the infection was not as dense as we have observed in rats. With treatment with TMP-SMX, the organisms were eliminated and the lung histology was restored to normal.

The major limitation to the use of *scid* mice in drug studies was their lack of availability. Breeding of *P. carinii*-infected stocks was difficult, and only small litters were obtained. Despite attempts to improve the situation, there were not enough mice to place into control and experimental groups for adequate sampling and statistical analysis.

(ii) μ MT mice. μ MT mice were able to breed better than the *scid* mice, and thus there was no shortage of animals for study. The natural history of pneumocystosis in these animals has been described in a separate report (22). The disease paralleled that in the *scid* mice until the animal reached about 15 weeks of age; subsequently, the mice developed a marked inflammatory response which reduced the extent of *P. carinii* infection in the lungs and which persisted until the time of death. Thirty-seven μ MT mice sacrificed at a mean age of 98.6 ± 10.5 days had a mean organism burden of $\log_{10}$ 6.41 ± 1.10 /lung. These data indicated that μ MT mice have a lower (about sixfold) and more variable *P. carinii* burden than *scid* mice.

The first drug study with μ MT mice examined pentamidine, clindamycin-primaquine, and TMP-SMX at the same doses used with *scid* mice (Table 1, study 2). All regimens proved to be effective, lowering the organism count 15- to 28-fold from a level of $\log_{10}$ 6.39 ± 1.21 /lung in the controls.

Subsequent experiments examined atovaquone and inhibitors of folic acid synthesis (Table 1, studies 3 to 5). Atovaquone reduced the *P. carinii* count about 10-fold from $\log_{10}$ 6.25/lung in the control group (Table 1, study 3). The studies of the antifolate drugs involved the use of SMX or dapsone alone or in combination with inhibitors of dihydrofolate reductase (DHFR). These experiments were based on our previous analysis of antifolate drug combinations in rats which found that in order to demonstrate a beneficial effect of the DHFR inhibitor, a large dose of this drug had to be used relative to the dose of the sulfonamide or sulfone used (29, 30). TMP and epiroprim, the DHFR inhibitors used here, were only investigated as part of the drug combinations because previous studies showed that these drugs had no anti-*P. carinii* activity when they are used alone to treat rats or mice (5, 6, 14, 29). In study 3, SMX used at a dosage of 3 mg/kg of body weight per day lowered the *P. carinii* count sixfold from 6.25/lung in the controls, but this was not statistically significant; the addition of TMP at 100 mg/kg/day to the regimen did not improve efficacy. By contrast, TMP-SMX at its standard dose reduced the *P.*

TABLE 1. Treatment of pneumocystosis in immunodeficient mice

Study no., Mouse type	Treatment	Dosage regimen ^a	No. of mice	Cyst count ^b	P value
1, <i>scid</i>	Control		7	7.21 ± 0.22	
	Pentamidine	10 mg/kg, three times/wk i.m.	6	4.94 ± 0.34	<0.001
	Primaquine-clindamycin	2/225 mg/kg/day p.o.	5	5.84 ± 0.63	<0.01
	IMP-SMX	50/250 mg/kg/day p.o.	8	5.20 ± 0.93	<0.001
2, μ MT	Control		10	6.39 ± 1.21	
	Pentamidine	10 mg/kg three times/wk i.m.	10	5.22 ± 0.57	<0.05
	Primaquine-clindamycin	2/225 mg/kg/day p.o.	13	5.11 ± 0.62	<0.05
	TMP-SMX	50/250 mg/kg/day p.o.	15	4.94 ± 0.52	<0.001
3, μ MT	Control		10	6.25 ± 1.08	
	Atovaquone	100 mg/kg/day p.o.	10	5.23 ± 1.02	<0.05
	SMP	3 mg/kg/day p.o.	10	5.47 ± 0.78	NS ^c
	SMP-TMX	3/100 mg/kg/day p.o.	10	5.77 ± 0.83	NS
	SMP-TMX	250/50 mg/kg/day p.o.	4	4.87 ± 0.2	<0.05
4, μ MT	Control		7	6.55 ± 1.17	
	SMP	2 mg/kg/day p.o.	6	6.31 ± 0.90	NS
	SMP-TMX	2/100 mg/kg/day p.o.	6	6.35 ± 0.93	NS
	SMP-epiroprim	2/100 mg/kg/day p.o.	6	5.14 ± 0.81	NS
5, μ MT	Control		10	6.48 ± 0.97	
	Dapsone	25 mg/kg/day p.o.	11	5.37 ± 0.54	<0.01
	Dapsone-TMP	25/100 mg/kg/day p.o.	9	5.26 ± 0.76	<0.01
	Dapsone-epiroprim	25/100 mg/kg/day p.o.	9	4.78 ± 0.10	<0.001
	SMP-TMX	250/50 mg/kg/day p.o.	6	4.83 ± 0.20	<0.01

^a i.m., intramuscular; p.o., oral.^b Values are mean ± standard deviation log₁₀ cyst count/lung.^c NS, not significant.

carinii count by 24-fold. In study 4, SMX administered alone at a dosage of 2 mg/kg/day or combined with TMP at 100 mg/kg/day had little or no effect on the organism burden. In contrast, the combination of SMX and epiroprim, a more potent analog of TMP (29), lowered the organism count 26-fold from log₁₀ 6.55/lung in the controls. However, this result was not significantly different from that for the controls or the group administered SMX alone.

Study 5 explored dapsone used alone and in combination with TMP or epiroprim (Table 1). Dapsone administered alone at a dosage of 25 mg/kg/day lowered the *P. carinii* count 13-fold from log₁₀ 6.48/lung in the controls. The addition of TMP to the regimen had a marginal effect on drug activity, lowering the organism count 16-fold. By contrast, the combination of dapsone and epiroprim lowered the organism burden 50-fold to log₁₀ 4.78/lung. However, this result was not significantly different from the result achieved with dapsone used alone.

Pneumocystosis in the μ MT mice produced a varied histologic picture ranging from the typical foamy material within alveolar lumens, to foci of numerous polymorphonuclear leukocytes (Fig. 2A), to no inflammatory response. *P. carinii* cysts were readily apparent on silver staining (Fig. 2B). Treatment with anti-*P. carinii* drugs cleared the organism and restored the lung histology to a normal or near-normal appearance.

These experiments suggested that μ MT mice might be a useful model for testing anti-*P. carinii* drugs. However, over time we noticed that the level of *P. carinii* infection began to decline in the colony. Mean organism counts in the control μ MT mice in the next six studies ranged from log₁₀ 4.56 ± 0.56 to log₁₀ 5.68 ± 0.89/lung; these levels were too low to demonstrate the effects of the anti-*P. carinii* drugs. The reasons for this fall in organism burden were unclear. Efforts to increase

the *P. carinii* infection in the mouse colony by maneuvers such as using different breeders, changing the housing procedures, or administering corticosteroids were unsuccessful. However, we were able to reintroduce *P. carinii* infection into μ MT mice by housing them with immunosuppressed C3H mice with pneumocystosis (see below).

Studies with immunosuppressed normal mice. Studies with immunosuppressed normal mice used C3HeB/FeJ mice with naturally acquired latent infection which had been found by other investigators to develop good levels of pneumocystosis with immunosuppression (19). Initial experiments compared different immunosuppressive regimens (Table 2). The first study (study 6) compared methylprednisolone acetate administered once per week s.c., the regimen that we used for rats (31), with dexamethasone dissolved in the drinking water (19). Methylprednisolone acetate at a dose of 0.4 mg resulted in a slight weight loss, but no change in *P. carinii* counts. Since the 0.8-mg dose was also well tolerated by the animals, it was increased to 1.2 mg after several weeks; this resulted in an organism burden of only log₁₀ 5.18/lung. Mice receiving dexamethasone at 4 mg/500 ml of drinking water developed such marked weight loss and shortened survival that the dose had to be reduced to 2 mg/500 ml (0.004 mg/ml); these animals developed a *P. carinii* count of log₁₀ 5.77/lung. Mice receiving dexamethasone at a dose of 2 mg/500 ml in the water exhibited moderate weight loss (about 8 g), slightly shortened survival (4.5 days), and the highest organism counts (log₁₀ 6.74/lung).

Other experiments compared dexamethasone at a dose of 2 mg/500 ml with s.c. injection of the hybridoma producing the monoclonal antibody to CD4 cells (study 7) and methylprednisolone at 1.6 mg/week given s.c. (study 8) (Table 2). Dexamethasone again resulted in larger organism burdens with acceptable weight loss and survival.

FIG. 1. (A) Lung section of C.B17 *scid* mouse with untreated *P. carinii* pneumonia. Hematoxylin-eosin stain was used. Magnification, $\times 100$. (B) Foamy material fills the alveoli and reveals numerous organisms on methenamine silver staining. Magnification, $\times 100$.

The dexamethasone regimen of 2 mg/500 ml was used in study 9 to induce pneumocystosis for testing anti-*P. carinii* drugs (Table 3). Pentamidine and TMP-SMX were the most active agents, lowering the organism counts 19- to 60-fold. Atovaquone also displayed activity, but the clindamycin-primaquine regimen was ineffective.

The histologic features of pneumocystosis in mice conditioned with dexamethasone were generally similar to those seen in the immunodeficient mice except that there was less of a host inflammatory response (Fig. 3A). The *P. carinii* organism burden was moderate to heavy (Fig. 3B) and cleared with treatment. Mice treated with the anti-CD4 hybridoma exhibited somewhat lighter infection and little host inflammatory response. Rarely, perivascular mononuclear cell infiltrates were observed, but their etiology was unclear.

On the basis of this experience, we decided to establish a model of normal mice with latent *P. carinii* infection and immunosuppressed with dexamethasone, our standard mouse model for testing anti-*P. carinii* drugs. However, implementation of this plan became more complicated when we unexpectedly learned that the vendor (Jackson) which supplied these animals had rederived the breeding colony. We then obtained C3H mice from five other vendors (Charles River, Taconic, Harlan, Sorensen, and B&K Universal) and placed them on the dexamethasone immunosuppressive regimen. Only the animals from B&K Universal developed pneumocystosis, with a mean organism count of $\log_{10} 6.49 \pm 0.54/\text{lung}$. However,

these mice were also infected with a parvovirus, and the colony was slated to be rederived. We did not wish to risk a repeat of the scenario that we had with the first vendor; thus, we decided to establish our own breeding colony, as we had done with rats. Since we only had a few mice with active pneumocystosis, we wanted to infect our colony with *P. carinii* obtained from someone with an ongoing mouse *P. carinii* research program in case our initial efforts were less than fully successful. Allen Harmen, of the Trudeau Institute, who had conducted studies of pneumocystosis for many years, kindly supplied us with heavily infected animals. We were able to transmit *P. carinii* infection to naive C3H mice immunosuppressed with dexamethasone both by intratracheal inoculation with infected lung homogenates and by housing these animals with mice with active pneumocystosis (the "seeding" technique) (19, 41). We chose the seeding technique to develop a breeding colony of latently infected normal mice because it is easier and involves the natural mode of transmission. We are now using C3H mice with pneumocystosis to test investigational anti-*P. carinii* drugs. In addition, we have used these C3H mice with pneumocystosis to reintroduce *P. carinii* infection into the μMT mouse colony.

DISCUSSION

Mice are becoming increasingly popular as experimental models for studying anti-*P. carinii* drugs. This popularity is based more on the characteristics of mice as hosts (e.g., their

FIG. 2. (A) Lung section of C57BL/6 μ MT mouse with untreated pneumocystosis with focal collections of polymorphonuclear leukocytes. Hematoxylin-eosin stain was used. Magnification, $\times 100$. (B) Organisms seen on methenamine silver staining. Magnification, $\times 100$.

small size) rather than on any unique properties of mouse-derived *P. carinii*. Whereas rat-derived *P. carinii* has been studied extensively (10, 13, 14, 31), little is known about mouse-derived organisms. There is accumulating evidence supporting species and/or strain differences in *P. carinii* (26a); thus, it cannot be assumed a priori that mouse-derived *P. carinii* will have the same pattern of susceptibility to antimicrobial drugs as has been found with rat-derived *P. carinii*. Additional studies

are needed to determine whether mice are a valid alternative to rats for testing anti-*P. carinii* drugs.

The present report has investigated both congenitally immunodeficient and immunosuppressed normal mice as drug testing models. The common denominator among these animals is that they acquired their *P. carinii* infection via natural environmental exposure. Such a system is simple, requires no invasive procedure, and probably reflects the mode of transmission of

TABLE 2. Level of pneumocystosis in immunosuppressed normal mice

Study no.	Immunosuppression	Dosage regimen	No. of mice	Body wt (g) ^a	Survival	Cyst count ^b
6	Normal control		4	36.5 $\pm$ 3.4	63 $\pm$ 0.0	4.35 $\pm$ 0.0
	Methylprednisolone acetate	0.4 mg/wk, s.c.	11	33.7 $\pm$ 3.0	63 $\pm$ 0.0	4.45 $\pm$ 0.24
	Methylprednisolone acetate	0.8–1.2 mg/wk, s.c.	11	32.2 $\pm$ 2.8	63 $\pm$ 0.0	5.18 $\pm$ 0.85
	Dexamethasone	2 mg/500-ml bottle	11	28.6 $\pm$ 3.1	58.5 $\pm$ 14.2	6.74 $\pm$ 0.44
	Dexamethasone	4–2 mg/500-ml bottle	10	25.4 $\pm$ 3.3	30.8 $\pm$ 22.5	5.77 $\pm$ 1.10
7	Dexamethasone	2 mg/500-ml bottle	11	29.5 $\pm$ 2.5	62 $\pm$ 0.0	6.96 $\pm$ 0.18
	Hybridoma	5 $\times$ 10 ⁶ GK1.5 hybridoma cells, s.c.	9	34.3 $\pm$ 2.3	57.3 $\pm$ 9.2	5.71 $\pm$ 1.05
8	Dexamethasone	2 mg/500-ml bottle	9	31.1 $\pm$ 3.4	57.6 $\pm$ 17.2	6.38 $\pm$ 0.84
	Methylprednisolone acetate	1.6 mg/wk, s.c.	10	32.4 $\pm$ 3.5	64.5 $\pm$ 7.9	5.71 $\pm$ 0.76

^a Values are means $\pm$ standard deviations.

^b Values are mean $\pm$ standard deviation log₁₀ cyst count/lung.

TABLE 3. Treatment of pneumocystosis in immunosuppressed normal mice^a

Treatment	Dosage regimen ^b	No. of mice	Cyst count ^c	P value
Control (steroid)		27	6.13 ± 0.80	
Pentamidine	10 mg/kg three times/wk, i.m.	10	4.86 ± 0.67	<0.001
Atovaquone	100 mg/kg/day, p.o.	10	5.23 ± 0.97	<0.05
Primaquine-clindamycin	2/225 mg/kg/day, p.o.	9	6.11 ± 0.82	NS ^d
TMP-SMX	50/250 mg/kg/day, p.o.	8	4.35 ± 0.00	<0.001
Normal control		4	4.35 ± 0.00	

^a These data are for study 9.^b i.m., intramuscular; p.o., oral.^c Values are mean ± standard deviation log₁₀ cyst count/lung.^d NS, not significant.

P. carinii in humans. The limitations of this model are seen when the presence of *P. carinii* infection in a mouse colony impairs animal breeding or when the organism is eliminated from the colony. Efforts by commercial breeders to rid their rodent colonies of viruses and other pathogens have also resulted in the elimination of *P. carinii*; this has forced investigators to develop alternative methods of propagating the infection in their animals (27).

scid mice of diverse genetic backgrounds in the present study developed extensive pneumocystosis which responded well to standard anti-*P. carinii* drugs. These results confirm and extend the work of other investigators who used immunodeficient mice to evaluate drugs (5–7). One group infected naive *scid*

mice by the intratracheal instillation of live *P. carinii* organisms, a technique which is efficient and which has the advantage of using a standard inoculum (5). Dexamethasone was administered in the drinking water to enhance the development of pneumocystosis; we have not used corticosteroids in our *scid* mice because these drugs were not needed and might increase the risk of other opportunistic infections. Another group established *P. carinii* infection in naive athymic mice by housing them with infected animals (41). Such a seeding technique relies on the same mode of transmission of the organism that occurs in colonies in which *P. carinii* infection is endemic. These studies and our own experience presented here suggest that in order to obtain sufficient numbers of *scid* mice for drug

FIG. 3. (A) Lung section of immunosuppressed C3H mouse with untreated *P. carinii* pneumonia and little host inflammatory response. Hematoxylin-eosin stain was used. Magnification, ×100. (B) Organisms seen on methenamine silver staining. Magnification, ×100.

testing, naive animals must be infected with *P. carinii* by one of the techniques described above.

While *P. carinii* infection in *scid* and athymic mice has been studied for many years, the occurrence of pneumocystosis in μ MT mice has only been described recently (17, 22, 25). μ MT mice are potentially valuable experimental models of *P. carinii* infection because they provide a direct way of investigating the role of B cells (e.g., antigen presentation and antibody production) in host defenses against the organism. The present report contains the first description of the use of μ MT mice to evaluate anti-*P. carinii* drugs. The data indicate that μ MT mice develop good levels of pneumocystosis and that the decrease responds to anti-*P. carinii* drugs in a manner similar to that in *scid* mice. The reasons for the disappearance of *P. carinii* from our colony are poorly understood, but our data suggest that the infection can be reintroduced into the animals.

The development of pneumocystosis in normal mice requires some form of immunosuppression. One approach has been to use corticosteroids, which are broadly immunosuppressive, are catabolic, and produce changes in rodents similar to those found in AIDS patients (40). The present report has shown that the regimen of dexamethasone in the drinking water resulted in higher levels of *P. carinii* infection than could be achieved with parenteral methylprednisolone. This was somewhat surprising, because the dose of methylprednisolone that we use for our rats to induce pneumocystosis is 4 mg (30). In our previous studies, we found that a dosage of cortisone acetate of 1 mg twice weekly given s.c. was optimal for mice and 25 mg twice weekly given s.c. was optimal for rats (34, 35).

A more selective approach to immunosuppression involves the depletion of CD4 cells by the administration of a monoclonal antibody or hybridoma cells; this mimics the fall in CD4-cell counts that occurs in human immunodeficiency virus-infected patients (18, 24). Some drugs show greater anti-*P. carinii* activity in mice depleted of CD4 cells, probably because these animals are less immunosuppressed (1). This is not surprising in light of studies with other fungi (e.g., *Cryptococcus neoformans*), which showed that antifungal drugs worked better in normal mice than in immunodeficient mice (11). In the present study, CD4-cell depletion did not result in as high a level of pneumocystosis as that achieved with dexamethasone.

P. carinii pneumonia induced by steroids in the normal mice described here responded well to pentamidine, TMP-SMX, and atovaquone. By contrast, the regimen of clindamycin and primaquine, which displayed good anti-*P. carinii* activity in *scid* and μ MT mice, was ineffective in the immunosuppressed normal mice. Other investigators have reported the lack of efficacy of clindamycin and primaquine in the immunosuppressed rat model (13). The reasons for these inconsistent results are unclear.

One important aim of the present study was to determine if mice could be used to analyze combinations of antifolate drugs. DHFR inhibitors administered alone to rats or mice have little or no anti-*P. carinii* activity, whereas sulfonamides or sulfones used alone are highly effective (4, 5, 31). We found that the beneficial effects of the DHFR inhibitors could only be demonstrated in rats if we used high doses of these agents relative to the dose of the sulfonamide or sulfone (29). In the present study, epiroprim appeared to enhance the activity of SMX and dapsone to a greater degree than TMP did. However, further studies involving more doses of these drugs, used individually and in combination, along with additional statistical techniques are needed before firm conclusions about synergy can be drawn (8, 12, 36). Further studies are also needed to detect differences between the rat and mouse models. The importance of such studies is exemplified by a recent report

which used mice to study the anti-*P. carinii* activity of diacetyldapsone, a dapsone prodrug, because mice are the only rodents capable of metabolizing diacetyldapsone to dapsone (26).

In summary, this study has shown that immunodeficient and immunosuppressed normal mice develop good levels of pneumocystosis, that standard anti-*P. carinii* drugs usually work in the predictable manner, and that antifolate drug combinations can be analyzed. This information, combined with information from previously published reports, lends strong support to the validity of mice as models for testing anti-*P. carinii* drugs. The differences between the mouse and rat models detected so far have more to do with the animal hosts than with the organism which resides in them. Yet, knowledge of these differences will become important as more experience with these models is gained, because it can affect the form, dose, and mode of administration of drugs selected for testing.

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