Nude Mouse: A New Experimental Model for *Pneumocystis carinii* Infection

Abstract. *Experimental infection with both human- and rat-derived* Pneumocystis carinii *has been produced in nude mice by intrapulmonary injection of lung homogenates that contained* P. carinii *and by environmental transmission. Most infected mice did not appear ill, and their lungs exhibited a mild and nonspecific inflammatory response. Thus,* P. carinii *can be transmitted from one animal species to another, and this result suggests that a defect in thymic-dependent lymphocytes is important in the development of the infection. Experimental work with* P. carinii *in the nude mouse should be performed in isolators because of the communicability of the organism.*

Pneumocystis carinii is an important cause of pneumonia in immunosuppressed patients (*1*). It is found in nature as a saprophyte in the lungs of various animals (*2, 3*), yet fundamental questions remain concerning the organism's taxonomic position and its epidemiologic features. Rats and, to a lesser extent, rabbits have been successful experimental models (*4, 5*); pneumocystis pneumonia can be regularly produced in rats in 8 to 12 weeks by the administration of corticosteroids and antibiotics and apparently represents reactivation of latent infection. Since corticosteroids have broad immunosuppressive and anti-inflammatory properties, the specific host immune factors important in the pathogenesis of *P. carinii* infection cannot be clearly delineated. It is unclear whether pneumocystis organisms found in humans or other animals, while morphologically indistinguishable, are indeed the same. Attempts to transmit *P. carinii* from one animal species to another have failed (*4, 6*), and antigen studies of rat and human *P. carinii* have produced conflicting results (*7, 8*).

Our study was undertaken to develop a new animal model for *P. carinii* infection. We used congenitally athymic (nude) mice, which have served as experimental models of infection for several microorganisms (*9*).

Outbred Swiss nude (*nu/nu*) mice raised in a conventional closed colony or in an isolator colony with defined bacterial flora at Memorial Sloan-Kettering Cancer Center were used in the experiments. The mice were at least 5 weeks old, of either sex, and were housed in standard cages with a paper cover to filter out particulate matter, while permitting free flow of air. Free access to standard laboratory food and tap water containing ampicillin (1 mg/ml) or tetracycline (1 mg/ml) in sterilized bottles was provided.

Initial inoculation experiments were performed in a room that housed (i) nude mice and (ii) treated rats (that is, given cortisone acetate subcutaneously twice weekly, or whose drinking water contained dexamethasone). Lungs from rats that received corticosteroids for 8 to 12 weeks (*4, 10*) and from patients with histologically proven *P. carinii* pneumonia served as sources of organisms. Small pieces of lungs were ground up in a balanced salt solution; 0.1 ml of the homogenate was injected percutaneously into both lungs, or 0.5 to 1.0 ml was inoculated intranasally. An estimate of the inoculum size was made by placing 0.001 ml on a slide, staining by the Gram-Weigert method (*11*), and counting the number of cysts. Nude mice inoculated with lung homogenates without *P. carinii*, uninoculated nude mice, and haired littermates served as controls. When the mice were killed 4 to 8 weeks after inoculation, the lungs were aseptically removed and cultured for bacteria, fungi, human viruses, and mycoplasmas; histopathologic sections were stained by the hematoxylin and eosin, periodic acid–Schiff (PAS), methenamine silver, Giemsa, and Gram-Weigert techniques.

The results (Table 1) indicated that intrapulmonary injection of human (patient A) or rat lung homogenates, either freshly obtained or frozen at −70°C for up to 3 months, could produce *P. carinii* infection in nude mice. Injection of human or rat lung homogenates, frozen for longer periods of time, rarely produced *P. carinii* infection in the mice; intranasal instillation did not result in infection. In selected experiments (not shown), three of seven nude mice injected only in the right lung with fresh rat lung homogenates developed *P. carinii*; the resultant infection was bilateral in two of the three mice. Pneumocystis infection was found in 6 of 39 control nude mice housed in the room with other infected mice and the cortisone-treated rats, but was absent in 12 nude mice raised in the closed

Table 1. Initial inoculation studies with *Pneumocystis carinii*.

Source of inoculum (lung)	Status of lungs	Method of inoculation	Degree of infection*				Infected/ total
			Focal	Light	Moderate	Heavy	
		Nude mice					
Patient A	Fresh or frozen (3 months)	Lung injection		3	4	1	8/10
Patient A	Frozen (5 months)	Lung injection					0/12
Patients B, C, D†	Frozen (2 to 11 months)	Lung injection	4				4/12
Patients E, F	Frozen (11 to 12 months)	Lung injection					0/5
Rats	Fresh	Lung injection	6	1	1		8/10
Rats	Frozen	Lung injection					0/11
Patient B	Frozen	Intranasal					0/8
Rats	Fresh	Intranasal					0/8
Controls		Same room	4	1	1		6/39
Controls		Closed colony or isolator					0/66
		Haired littermates					
Patients and rats	Fresh or frozen	Lung injection	1				1/20
Controls		Closed colony or isolator					0/10

*Focal indicates less than five alveoli with *P. carinii* per lung section. Light indicates scattered alveolar involvement. Moderate indicates widespread infection, with less than 50 percent of the alveoli involved. Heavy indicates that more than 50 percent of the alveoli contained organisms. †Lungs from each patient resulted in infection in at least one mouse.

Table 2. Transmission of infection with *Pneumocystis carinii* in a nude mouse closed colony.

Source of infection	Degree of infection				Infected/ total
	Focal	Light	Moderate	Heavy	
Lung homogenates*	5	1	3		9/21
Close contact controls†	1	4	2		7/10
Distant contact controls‡	2	4	8	2	16/23

*Mice injected with homogenates from patients G, H, and I. †Mice occupying the same cage. ‡Mice occupying separate cages.

colony and in 54 nude mice raised in the isolator colony.

Because *P. carinii* was found rarely in uninoculated control mice, the next experiments were conducted in the closed colony room housing the nude mice. As indicated, no *P. carinii* had been found in the nude mice raised in the closed colony room where no other animals were permitted. Over a period of 3 months, groups of nude mice were injected with three lung homogenates, which had been obtained fresh or had been frozen for up to 3 days, from patients infected with *P. carinii* (G, H, and I). Some uninoculated nude mice were placed in the same cage as the inoculated nude mice to study mouse-to-mouse transmission of human *P. carinii* by close contact. Other nude mice occupying separate cages in the colony room were randomly selected as "distant contact" controls. As is seen in Table 2, *P. carinii* developed in all three groups of nude mice, suggesting that environmental transmission of human *P. carinii* occurred throughout the closed colony of nude mice.

Nude mice raised only in the isolator colony were used in the third series of experiments. A group of these mice was placed in the same cage with rats treated with cortisone and separated by a metal screen, in order to study rat-to-mouse transmission of *P. carinii* by close contact. Two of these cages, each containing three rats and five mice, were placed in a portable germfree isolator with unidirectional airflow. Another group of nude mice was divided among two standard mouse cages, which were placed on top of the rat-mouse cages. The control group of nude mice remained within the isolator colony. The animals were killed at 7 weeks, and *P. carinii* was found in all six rats (Table 3) and also in both groups of nude mice exposed to the rats in the germfree isolator. These results indicated that *P. carinii* could be transmitted from cortisone-treated rats to nude mice by close contact and perhaps by the airborne route as well. However, the possibility of small particles (for example, bedding, excreta) getting into nude mouse cages during changing or other cage manipulations could not be ruled out. Of note, two nude mice in the rat-mouse cages died attempting to get across the metal screen 2 and 6 days after the experiment began; both mice demonstrated focal infection with *P. carinii*.

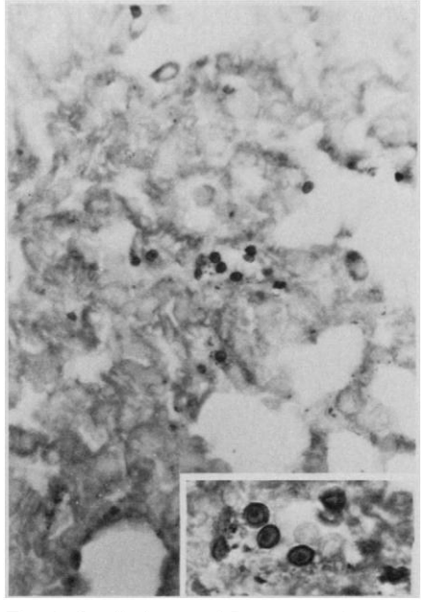

Fig. 1. Small cluster of *Pneumocystis carinii* cysts in nude mouse lung stained with methenamine silver (original magnification × 350), with typical targetlike appearance (× 1000).

Although the intensity of *P. carinii* infection in the nude mice varied considerably (Tables 1 to 3), there was little evidence of clinical illness. A notable exception was seen in mice inoculated with lung homogenate from patient A, who died from a heavy infection with pneumocystis pneumonia without treatment. Six of the eight nude mice inoculated became ill and died 4 to 8 weeks later. Lung specimens from patients B to I were obtained earlier in the course of the illness by biopsy, or at autopsy after treatment with pentamidine isethionate. The development of *P. carinii* infection in the nude mice after intrapulmonary injection of these homogenates was more closely related to the freshness of the lung homogenate than to the size of the inoculum, which was usually 10^3 to 10^5 organisms.

In histopathologic sections of the lungs from the nude mice, *P. carinii* organisms were best demonstrated by methenamine silver stain (Fig. 1). The individual cysts, whether from human or rat sources, were morphologically identical and were also indistinguishable from *P. carinii* seen in patients with the disease or as reported in the rat model (*3*). However, in contrast to most human infections, the cysts were usually present singly or in small groups, and invariably were located at the periphery of alveoli. The inflammatory changes, whether to human or rat *P. carinii*, were generally inconspicuous, and did not correlate with the number of cysts demonstrated in corresponding sections with special stains. When an interstitial reaction was present, it was composed of histiocytic cells and lymphocytes. The foamy or vacuolated alveolar exudate typical of the human infection was not observed. Consequently, it was not possible to determine which animals had or were likely to have *P. carinii* infection from the H and E sections. The contribution of other organisms to these pathologic changes is unclear. Bacteria (for example, *Klebsiella pneumoniae*, *Enterococci*) were often cultured from nude mouse lungs, regardless of the presence of *P. carinii*. Fungi (such as *Aspergillus* sp., *Candida albicans*, *Torulopsis glabrata*) were isolated infrequently and were not seen in histopathologic sections.

Our study demonstrates that the nude mouse can be used as an experimental model for *P. carinii* infection without the need for immunosuppressive agents, and that both human and rat organisms can infect athymic mice. It provides additional direct evidence that *P. carinii* is infectious and supports the policy of isolating hospitalized patients with pneumocystis pneumonia. Clinical evidence for the communicability of *P. carinii* has been derived from outbreaks of the disease in orphanages and hospitals, and from the development of serum antibodies among close contacts of patients (*8*, *12*). The incubation period has been

Table 3. Transmission of *Pneumocystis carinii* from the rat to the nude mouse in germfree isolator. Abbreviations: F, focal; L, light; M, moderate; H, heavy.

Nude mice group	Degree of infection				Infected/ total
	F	L	M	H	
Close contacts*	4	2	3		9/10
Airborne†	5		1		6/10
Isolator controls‡	0	0	0		0/18

*Mice occupying same case with cortisone-treated rats. †Mice occupying separate cages placed on top of rat-mouse cages. ‡Mice remaining in isolator colony.

estimated to be 4 to 8 weeks (*2*). *Pneumocystis carinii* has been transmitted from cortisone-treated rats to cortisone-treated germfree rats by close contact and airborne routes (*10, 13*). Heavier degrees of infection were found after prolonged periods (3 or more weeks) of exposure.

The nude mouse offers the potential for performing definitive epidemiologic studies with *P. carinii*. The light infection produced in many nude mice closely resembles the carrier state that exists in different animals in nature. With this model, it should be possible to determine the incubation period of *P. carinii*, to establish dose-response curves, and study the development of the infection. The nude mouse should provide an in vivo counterpart to the in vitro culture system of *P. carinii* (*14*). The potential problems with the animal model system are illustrated by the spread of *P. carinii* throughout the closed nude mouse colony (Table 2), which has interfered with interpretation of lung injection and serial passage studies performed thus far. We now perform all studies in germfree isolators to avoid problems of cross contamination.

The role of specific host immune factors in the development of *P. carinii* infection is poorly understood. Clinical studies of patients with primary immune deficiency diseases, lymphoreticular neoplasms receiving immunosuppressive agents, and the role of malnutrition suggest that defects in both B and T cells contribute (*15*). Yet, the occurrence of pneumocystis pneumonia after corticosteroids have been tapered and after bone marrow transplantation suggest that some degree of host immune reaction is important for overt disease to develop. Using thymic transplantation and other immunologic manipulations, this animal model offers a method for clarifying the complex factors in the host-parasite relationship of *P. carinii* infection.

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Pineal Vasotocin: Release into Cat Cerebrospinal Fluid by Melanocyte-Stimulating Hormone Release-Inhibiting Factor

Abstract. *The intracarotid injection of both synthetic melanocyte-stimulating hormone release-inhibiting factor (MIF) and purified MIF prepared from bovine hypothalami induces arginine vasotocin release into cerebrospinal fluid of cats and significantly decreases the pineal arginine vasotocin content at 5 minutes after the injection. The present results demonstrate an extrapituitary endocrine effect of synthetic and purified bovine MIF.*

We demonstrated previously that the mammalian pineal gland contains (*1, 2*) and synthesizes (*3*) the nonapeptide arginine vasotocin (AVT), and that very low concentrations of AVT (0.0001 pg) injected into the third ventricle significantly decrease the pituitary melanocyte-stimulating hormone (MSH) content in normal mice, and completely reverse the pituitary MSH increase in pinealectomized mice (*4*). Since at the same time pinealectomy significantly increases the content of a MSH release-inhibiting factor (MIF) in mice hypothalami, and AVT reverses this increase (*5*), it is reasonable to assume that AVT induces alterations in the pituitary MSH content by interfering with the synthesis or release of MIF.

Although several peptides have been proposed as MIF (*6*), there is no agreement about the nature of the physiological MIF. One of the most potent and most investigated MIF's is the tripeptide Pro-Leu-Gly-NH_2 (*7*) also designated MIF-I (*6*). The preferential uptake of radioactivity by the pineal after the intravenous injection of ^{3}H-labeled MIF-I in both mice and rats (*8*), as well as the concentration of radioactivity in the ependymal cells bordering the ventricles after the intracarotid injection of ^{3}H-labeled MIF-I in rats (*9*), prompted us to investigate the possible effects of synthetic MIF-I, and of a purified MIF from bovine hypothalami, on the AVT content of the pineal and cerebrospinal fluid (CSF) of cats.

Bovine MIF, obtained from stalk median eminence tissues, was purified by gel filtration on Sephadex columns as recommended by Schally and Kastin (*10*). The intravenous administration of 0.5 μg of this purified material produces an increase of the pituitary MSH levels in mice equivalent to that of 0.2 μg of Pro-Leu-Gly-NH_2 (Hoechst). The assay methods used have been described (*1*) and included rat antidiuretic, rat uterine, and frog bladder (*Rana temporaria*) assays. Synthetic AVT having a potency of 110 ± 18 rat antidiuretic and 18,000 ± 2000 hydroosmotic units per milligram was used as standard in the biological assays. Highly purified crystalline trypsin (twice crystallized, chymotrypsin-free, Serva) was used for the tryptic digestion of crude pineal extracts and CSF samples. The pineals were extracted in 0.25 percent acetic acid as described (*11*). Seventeen cats (3 to 4 kg) anesthetized with urethane were injected in the left carotid either with 1.5 μg of MIF-I (Hoechst) or with 3.5 μg (the equivalent