Outbreaks of *Pneumocystis carinii* Pneumonia in Colonies of Immunodeficient Mice

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Outbreaks of *Pneumocystis carinii* pneumonia occurred in colonies of *nu/nu* and *scid/scid* mice at four different institutions. The disease, which was characterized by chronic wasting and respiratory insufficiency, was more severe in older mice and in animals housed in cages with special protective tops. Histopathologic features included alveolar filling with the typical foamy honeycomb material and a mild, nonspecific host inflammatory response. Immunofluorescence and immunoblotting studies suggested the *P. carinii* isolate was of mouse rather than of rat or human origin, and the outbreaks could be related to each other by common vendor or source of breeding animals. Once *P. carinii* became established in a mouse colony, the organism tended to persist for long periods of time. The principal control measure was depopulation of the colony, although limited experience with the administration of trimethoprim-sulfamethoxazole was encouraging. Thus, outbreaks of pneumocystosis are a serious problem among colonies of immunodeficient mice, with important implications for the use of these animals in biomedical research. Data obtained by studying these outbreaks should enhance understanding of the pathogenesis of *P. carinii* pneumonia and be helpful in formulating improved methods of detection and control.

*Pneumocystis carinii* is an important opportunistic pulmonary pathogen. The organism exists widely in nature as a saprophyte in the lungs of humans and a variety of animals; while isolates from these sources are morphologically identical, antigenic studies suggest that species and strain differences exist (9, 20, 34, 39). Among humans, *P. carinii* causes pneumonia in premature, malnourished infants; children with primary immunodeficiency diseases (particularly severe combined immunodeficiency disease); patients receiving corticosteroids or other immunosuppressive drugs; and persons with the acquired immunodeficiency syndrome (AIDS) (26, 35). The specific host immune defects which predispose to the development of pneumocystosis are poorly understood. Animal challenge studies involving exogenous *P. carinii* have been severely hampered by the low virulence of the organism (2, 3, 27, 36). Rats, mice, and other animals, when administered corticosteroids, spontaneously develop *P. carinii* pneumonia with histologic features closely resembling the human form of the disease by a process which involves reactivation of latent infection (3, 27–29, 37, 38, 46). This model system has been widely used to study *P. carinii* but has not permitted direct analysis of the relationship between the organism and host immune function. Sporadic cases of pneumocystosis have been reported in animals with suspected or proven immunodeficiency disorders, and a few outbreaks of the disease have occurred in colonies of athymic (nude) mice (31, 32, 45). Such "experiments of nature" provide unusual opportunities to study the epidemiology and pathogenesis of *P. carinii* pneumonia.

The Cincinnati VA Medical Center has had a long-term interest in clinical and experimental *P. carinii* infection. Over the past several years we have had the opportunity to participate in the investigation of large outbreaks of pneumocystosis in colonies of immunodeficient mice at four different institutions. In the current study we present the clinical and pathological features of the disease, antigenic characteristics of the *P. carinii* isolates, host serologic responses, control measures, and the potential impact of these outbreaks on the use of immunodeficient mice in biomedical research.

MATERIALS AND METHODS

Immunodeficient mice. The outbreaks of pneumocystosis occurred in colonies of immunodeficient mice at the following institutions: (i) Crl:CD1 (ICR) *nu/nu* mice obtained from a commercial vendor and housed at the University of Michigan, Ann Arbor; (ii) BALB/c *nu/nu* and C B-17/ICr *scid/scid* mice bred and housed at the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pa. (the *scid/scid* mice are lymphopenic, lacking both B and T cells, and serve as a model of severe combined immunodeficiency disease in humans [1]); (iii) Crl:CDI (ICR) *nu/nu* mice obtained from the same commercial vendor as those at the University of Michigan and housed at Smith Kline & French Laboratories, Philadelphia, Pa.; and (iv) C B-17/ICr *scid/scid* mice bred and housed in the Research Animal Facility at The Jackson Laboratory, Bar Harbor, Maine. The original breeding pairs of these mice were obtained from the Fox Chase animal colony.

Investigators at each institution assumed primary responsibility for evaluating their own outbreak of pneumocystosis.

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Systematic steps were taken to define the clinical features of the illness, etiology, and extent of involvement of the animal population. Procedures included autopsies, histopathologic examination, cultures, and serologic studies. After documented cases of P. carinii pneumonia were found, one of us (P.D.W.) was contacted about further studies. The timing of this contact in relation to the stage of the outbreak had a major impact on the number of animal specimens available for investigation. In general, attempts were made to obtain fixed lung specimens for histologic examination, frozen lungs for immunofluorescence staining and immunoblotting analysis, and serum specimens for the determination of antibody titers to P. carinii.

**Histopathology.** Histopathologic examination of the lungs of the mice was performed by the veterinary pathologist at each institution. Specimens were also requested from the institutions so that the histologic features of pneumocystosis in the different outbreaks would be compared by a single pathologist (C.K.K.). Samples included already stained slides as well as frozen and fixed sections. Since freezing artifacts made detailed histologic examination difficult, the frozen lung sections were usually not included in the data analysis. To prepare permanent specimens, lung tissues were fixed in 4% buffered formaldehyde, embedded in paraffin, sectioned at 4 to 6 μm, and stained with hematoxylin-eosin and Grocott methenamine silver stains. Specimens from 31 mice (11 nude and 20 scid mice) were examined in detail. The severity of pneumocystosis was graded using a previously described scoring system (19, 37, 38) with minor modification: light infection (<25% alveoli involved); moderate infection (25 to 50% alveoli involved); heavy infection (>50% alveoli involved).

**Immunofluorescence.** Immunofluorescence studies were performed on infected lungs in an attempt to determine the source of the outbreaks (i.e., whether the P. carinii organisms were of mouse, rat, or human origin). Lung specimens sent to us in the early stages of the study varied considerably in the quality and quantity of material, storage conditions, method of preparation, and intensity of P. carinii infection. Later, a more uniform protocol was devised in which investigators usually sacrificed the mice, removed and froze the lungs, and shipped them to us on dry ice; in a few instances, live mice were shipped directly to us to provide fresh lungs.

The lung samples were studied by an indirect fluorescent antibody (IFA) technique as described previously (39, 40, 42), with slight alteration. Specimens were prepared for analysis either as frozen sections or as a preparation homogenized in a Stomacher (Tekmar, Inc., Cincinnati, Ohio), added to the wells of a glass slide, air dried, and heat or acetone fixed. Lung specimens from immunosuppressed normal mice, rats, and humans with documented P. carinii pneumonia, prepared in a similar manner, served as positive controls. The presence of P. carinii was documented in frozen sections by methenamine silver stains and in lung homogenates by the cresyl eht violet stain.

Reagents used in the IFA technique were prepared according to procedures outlined in earlier studies (25, 34, 41). Pooled serum specimens from different groups of normal mice with prolonged environmental exposure to mouse P. carinii served as the source of primary antibody to mouse-derived organisms. These specimens had good antibody titers to mouse P. carinii but showed little or no reactivity (titer, <1:4) to rat or human P. carinii. Rabbits immunized with rat P. carinii obtained from infected lungs or grown in tissue culture, and with human P. carinii from infected lungs, served as the source of primary antibody for these studies; the rabbit antisera were adsorbed with normal lung or uninfected tissue culture cells before use. Both rabbit antisera showed good reactivity against rat, human, and mouse P. carinii, suggesting cross-reacting determinants. Secondary antibodies included fluorescein-conjugated F(ab')2 fragment goat anti-mouse immunoglobulin G (IgG) (heavy and light chain) (Cappel Labs, Malvern, Pa.), adsorbed with normal mouse lung, and fluorescein-conjugated F(ab')2 fragment goat anti-rabbit IgG (heavy and light chain) (Cappel Labs).

Frozen lung sections or lung homogenates were incubated with pooled mouse serum, rabbit antiserum to rat P. carinii, or rabbit antiserum to human P. carinii in a moist chamber for 30 minutes, washed with phosphate-buffered saline (PBS), incubated with the fluorescein-conjugated goat anti-mouse or anti-rabbit IgG for 30 min, washed with PBS, mounted with glycerol, and examined with a fluorescence microscope. P. carinii organisms stained brightly with a typical rim pattern of fluorescence. If P. carinii in a lung specimen reacted by IFA with pooled mouse serum, the organisms were considered to be of mouse origin. Reactivity with either or both of the rabbit antiserum did not necessarily imply species specificity. Negative controls included the lack of fluorescence staining when lung specimens from normal or immunodeficient mice without P. carinii were substituted as target antigen or when PBS was used as the primary antibody.

The IFA technique was also used to measure serum antibodies to P. carinii among the mice. Lung homogenates of mouse, rat, or human P. carinii added to the wells of a glass slide were used as antigens. Serial dilutions of the test serum (beginning at 1:4) were added to the wells, the slide was washed with PBS, fluorescein-conjugated goat anti-mouse IgG was added, and the slide was washed and prepared for examination as described above. The fluorescence intensity was graded on a scale from 0 (negative) to 4+ (maximum), and the highest serum dilution with a 1+ intensity was considered to be the peak antibody titer. No fluorescent staining occurred when PBS was substituted for the mouse serum.

**Immunoblotting.** Immunoblotting studies were performed by procedures described in detail in earlier reports (34, 44). P. carinii-infected mouse lung homogenates served as antigen and were compared with rat and human P. carinii lung homogenates prepared in a similar manner. Organisms were solubilized for polyacrylamide gel electrophoresis using a lysis buffer of 2% sodium dodecyl sulfate–0.06 M Tris (pH 6.8)–1% glycerol–5% 2-mercaptoethanol, boiled for 3 min, cooled, and centrifuged at 9,000 × g for 20 min, and the supernatants were stored at −20°C. The specimens were electrophoresed in discontinuous 0.1% sodium dodecyl sulfate–12.5% polyacrylamide gels by the method of Laemmli (22).

Protein blotting was performed by the method of Towbin et al. (30) with slight modification. Separated proteins were transferred from the gel to nitrocellulose paper in a Trans-Blot cell (Bio-Rad Laboratories, Richmond, Calif.) overnight at 30 V and then for 1 h at 60 V. The resulting protein blots were either stained with ponceau red or blocked with 3% gelatin and then incubated overnight at 4°C with the same primary antibody used in the IFA studies (i.e., pooled mouse serum or rabbit antiserum to rat or human P. carinii). The blots were then washed, incubated with affinity-purified goat anti-mouse IgG or goat anti-rabbit IgG (Kirkegaard and Perry Laboratories, Gaithersburg, Md.), washed, and reacted with Tris-buffered saline containing 0.6 mg of 4-chloro-
RESULTS

Clinical features of the outbreaks. (i) University of Michigan. One outbreak occurred in a colony of 100 Crl:CDI (ICR) nu/nu mice maintained under microbiologic barrier conditions at the University of Michigan. The mice were all obtained from the same commercial vendor and were group-housed on autoclaved corn cob bedding in 22 plastic shoebox cages with filter tops. They were provided autoclaved commercial rodent Chow and water ad libitum. The cages were distributed among two positive-pressure, laminar flow, mass air displacement racks within a barrier colony which had always been free of P. carinii. These were densely consolidated with hectic coughing, weight loss, and dyspnea which progressed to cyanosis. The mice were frequently ill for several months before they became moribund. The lungs of infected nu/nu mice had the same morphologic features as the lungs of infected scid/scid mice. P. carinii was eliminated from nu/nu mouse stocks by caesarean redeviration and isolator maintenance.

(ii) Fox Chase Cancer Center. The Fox Chase Cancer Center outbreak began in November, 1985, in one room of a barrier operated facility containing Crl:CDI (ICR) nu/nu and nu/+ mice which had been obtained from the same vendor as the mice at the University of Michigan. The nu/nu mice were group-housed in polycarbonate shoebox cages with microisolator lids (Lab Products, Maywood, N.Y.) and autoclaved hardwood chip bedding, food, and water. All animal manipulations and cage changing was performed under a biological safety cabinet by personnel wearing protective clothing (e.g., masks, gloves, etc.). Ultimately, about 75% of the 300 nu/nu mice housed in this room were involved in the outbreak. A survey of nu/nu mice housed in other rooms revealed that P. carinii had spread to these locations as well. The clinical manifestations of pneumocystosis included wasting, diarrhea, and cyanosis. The illness seemed to be more severe in mice more than 4 months old than in younger animals and more severe in mice housed in microisolator cages than in animals housed under conventional conditions. Lung histology and cultures, as well as serologic testing of nu/nu and sentinel nu/+ mice, failed to reveal an etiology other than P. carinii. The outbreak was controlled by depopulation and restocking the colony with mice obtained from a separate breeding facility maintained by the vendor known to be free of P. carinii infection. These mice have remained free.

(iii) Smith Kline & French. The Smith Kline & French outbreak began in October, 1984, in one room of a barrier operated facility containing Crl:CDI (ICR) nu/nu and nu/+ mice which had been obtained from the same vendor as the mice at the University of Michigan. The nu/nu mice were group-housed in polycarbonate shoebox cages with microisolator lids (Lab Products, Maywood, N.Y.) and autoclaved hardwood chip bedding, food, and water. All animal manipulations and cage changing was performed under a biological safety cabinet by personnel wearing protective clothing (e.g., masks, gloves, etc.). Ultimately, about 75% of the 300 nu/nu mice housed in this room were involved in the outbreak. A survey of nu/nu mice housed in other rooms revealed that P. carinii had spread to these locations as well. The clinical manifestations of pneumocystosis included wasting, diarrhea, and cyanosis. The illness seemed to be more severe in mice more than 4 months old than in younger animals and more severe in mice housed in microisolator cages than in animals housed under conventional conditions. Lung histology and cultures, as well as serologic testing of nu/nu and sentinel nu/+ mice, failed to reveal an etiology other than P. carinii. The outbreak was controlled by depopulation and restocking the colony with mice obtained from a separate breeding facility maintained by the vendor known to be free of P. carinii infection. These mice have remained free.

(iv) The Jackson Laboratory. C B-17 scid/scid mice were introduced into a research colony at the Jackson Laboratory by caesarean derivation from homozygotes kindly supplied by Gayle and Mel Bosma at the Fox Chase Cancer Center in 1984. The mice were housed at four to five animals per cage in pine shavings under conventional conditions in a research animal room. Lexon filter tops were used to minimize airborne infections. The diet consisted of pasteurized Wayne Lab Blox and acidified water ad libitum. Although 60 scid/scid mice raised under the above conditions showed a mean life span of 337 ± 13.3 days over the first 18 months, there was a gradual decrease in longevity associated with clinical signs of debilitation and chronic respiratory disease. The mean life span of scid/scid mice in this research colony decreased to 133 ± 2.98 days. Analysis of lung tissue by Gomori methenamine silver stain and by a sensitive immunoperoxidase stain of paraffin-embedded sections confirmed the presence of P. carinii. The age of affected animals varied from 6 to 30 weeks.

To protect other immunocompromised mice from P. carinii infection and to minimize spread of this pathogen, the scid/scid mice were placed in a Maxi-Miser positive individually ventilated (PIV) cage rack (Thoren Caging System,
Hazelton, Pa.) which had a high-efficiency particulate air (HEPA)-filtered air supply and exhaust system and was under positive pressure relative to the outside. Rather than improving the health of the scid/scid mice, housing these animals in the PIV rack had a detrimental effect on life span. Within 6 months of placing scid/scid mice in the PIV rack, the mean life span had dropped further to only 91.2 ± 2.64 days. Histopathological examination of stained lungs confirmed severe pneumonia caused by infection with *P. carinii*.

Since trimethoprim-sulfamethoxazole (TMP-SMZ) suspension had been previously shown to be effective in the prevention and treatment of *P. carinii* pneumonia in humans and in corticosteroid-suppressed rats (15-17), scid/scid mice were placed on a regimen of TMP-SMZ in the drinking water. To assess the efficacy of TMP-SMZ treatment in a conventional mouse room environment, breeding pairs of scid/scid mice were removed from the PIV rack and placed in conventional mouse boxes. A dose of 500 of SMZ and 100 mg of TMP per kg per day was supplied in the drinking water as Sufartrim suspension (Goldline Laboratories). The mice were supplied with TMP-SMZ suspension in drinking water for 3 days per week and were provided with water alone for the remainder of the week in a manner similar to the intermittent chemoprophylactic regimen used in rats (18).

Treatment with TMP-SMZ had a remarkable effect on the health of the scid/scid mice, with improvement in their overall appearance and an increase in their life span. The overall mortality has been shown to be assessed by the histopathological evaluation of methenamine silver-stained lung sections revealed widely scattered *P. carinii* organisms with focal accumulations of macrophages. Moreover, the breeding performance of homozygotes did not appear to be affected by the TMP-SMZ therapy. New generations of mice are currently being derived by hysterectomy. On the other hand, six young scid/scid mice were first placed in the PIV rack for 3 weeks and then begun on TMP-SMZ; analysis of the lungs of these animals killed at 6 to 7 months of age revealed severe *P. carinii* pneumonia.

**Histologic features:** The histologic features of the different outbreaks of pneumocystosis were very similar, although the extent of disease varied among individual mice. Of the 11 nude mice graded by a single examiner, 3 had light, 5 had moderate, and 3 had severe *P. carinii* pneumonia; of 20 scid mice analyzed, 5 had light, 4 had moderate, and 11 had severe disease.

Representative histologic features of pneumocystosis are presented in Figure 1. In light infection, the underlying lung architecture was not altered except for slight interstitial thickening and mild chronic inflammatory cell infiltrates. Methenamine silver stain depicted *P. carinii* cyst forms along the alveolar walls. As the disease progressed, the alveolar lumens contained the characteristic foamy honeycomb material (Fig. 1A to C) which revealed various numbers of organisms (Fig. 1D). In some areas, the alveoli contained prominent collections of macrophages but few *P. carinii* organisms (Fig. 1E and F). Severe pneumocystosis was characterized by extensive alveolar filling with the foamy material and masses of *P. carinii*, interstitial thickening with edema and mild fibrosis, and foci of atelectasis with compensatory hyperinflation; however, host inflammatory cell infiltrates were still rather inconspicuous.

**Immunofluorescence.** Initial IFA studies were performed on *nu/nu* mice obtained from the University of Michigan and on scid/scid mice obtained from Fox Chase. Since we were contacted late in the course of these outbreaks, we were only able to obtain four mice (two nude, two scid) with sufficient lung tissue and *P. carinii* infection available for testing. All specimens reacted with pooled mouse serum, suggesting a mouse strain of organism. However, discrepancies (e.g., variation in the intensity of the immunofluorescent staining) occurred between lung homogenates and frozen sections when tested with rabbit antisera to rat and human *P. carinii*, thus making further data interpretation difficult.

The outbreak at Smith Kline & French provided a larger number of mice to study as well as the opportunity to develop a more uniform protocol. Lung homogenates were chosen as the standard antigen preparation because the immunofluorescent staining was consistently easier to interpret than were the frozen sections. Specimens from all nine *nu/nu* mice examined reacted with pooled mouse serum, rabbit antisera to rat *P. carinii*, and rabbit antisera to human *P. carinii* by IFA. In contrast, no lung homogenates from six *nu/+* mice reacted with any source of antibody.

The results of serologic testing in 22 mice by IFA are presented in Figure 2. Serum antibodies to mouse *P. carinii* were detected in 10 of 12 *nu/+* mice housed in the same room as the *nu/nu* mice; 6 of these animals had a reciprocal titer of >8. Serum antibodies to rat *P. carinii* were detected in only four of the *nu/+* mice, and the titers were lower than those to mouse *P. carinii*. No antibodies to human *P. carinii* were detected. Only 1 of 10 *nu/nu* mice had detectable serum antibodies to any source of *P. carinii*, and this was a low titer of antibodies to mouse-derived organisms.

When we heard about the problem of pneumocystosis in the nude mouse colony at Fox Chase, we obtained lung specimens from five *nu/nu* mice. The lung homogenates from four of these animals had *P. carinii* by cresyl echt violet stain; all of these specimens reacted with pooled mouse serum as well as with rabbit antisera to rat and human *P. carinii* by IFA.

Serum antibodies to *P. carinii* were measured among *nu/nu* and *nu/+* mice; because of the small amounts of serum available, specimens from one to three animals were pooled for analysis. All three serum specimens from the *nu/+* mice had reciprocal antibody titers of 128 (Fig. 2). Only one of these mice had antibodies to rat *P. carinii*, and none had antibodies to human organisms. No serum antibodies to any source of *P. carinii* were found in two specimens from *nu/nu* mice.

Eight scid/scid mice were obtained from the Research Animal Facility at The Jackson Laboratory for analysis. The animals had been housed for 3 to 5 months and appeared clinically ill. The lung homogenates from all eight scid/scid mice reacted with pooled mouse serum and rabbit antisera to rat and human *P. carinii* by IFA.

**Immunoblotting.** In the immunoblotting studies, lung homogenates from mice in the different outbreaks were probed with different sources of antibody and compared with the antigenic profile of rat and human *P. carinii*. Data obtained using rabbit antiserum to rat *P. carinii* are presented in Figure 3. As in our earlier reports (34), multiple bands were found with rat *P. carinii*, the most prominent of which were moieties of about 45, 50, and 116 kilodaltons (kDa) (Fig. 3, lane 8). The principal antigen recognized in human *P. carinii* was a broad-based band of about 40 kDa (lane 9). The major antigens recognized in mouse *P. carinii* were bands of about 45 and 50 kDa, as seen in specimens from Fox Chase (lanes 2 and 3), Smith Kline (lanes 4 and 5), and Jackson Laboratory (lanes 6 and 7); these moieties were similar to the 45-
and 50-kDa bands seen in rat *P. carinii*. Other bands (e.g., 92-kDa moiety) that were consistently found in *P. carinii* from mice also appeared to be present in rat *P. carinii*. However, low-molecular-weight antigens detected in *P. carinii* of Smith Kline mice (Fig. 3, lanes 4 and 5) were present only weakly or not at all in specimens of other animals. No immunoreactivity was found in lung homogenate from a Smith Kline mouse without *P. carinii* infection (lane 1).

The results obtained using rabbit antiserum to human *P. carinii* as the source of antibody are presented in Fig. 4. As noted in our previous studies (34), the antiserum detected the broad-based 40-kDa antigen in human *P. carinii* as well as less prominent bands of about 66 and 116 kDa (Fig. 4, lane 9). The antiserum reacted with the 45- and 50-kDa antigens along with other moieties in rat *P. carinii* (lane 8), but the 116-kDa band was not detected. Mouse *P. carinii* specimens from Smith Kline (Fig. 4, lanes 4 and 5), Fox Chase (lanes 2 and 3), and Jackson Laboratory (lanes 6 and 7) all demonstrated bands of 45 and 50 kDa of varying intensity; immunoreactive moieties of other molecular masses (e.g., 92 kDa) were also detected in most of the mouse *P. carinii* specimens. No bands were found in the mouse lung without *P. carinii* (Fig. 4, lane 1).

Probing the specimens with pooled mouse serum as the source of antibody revealed a different pattern of antigen
recognition (Fig. 5). As in the immunofluorescence experiments, there was no reactivity with rat P. carinii (lane 8) or human P. carinii (lane 9). All six mouse P. carinii lungs (lanes 2 through 7) demonstrated at least one band of varying intensity of about 50 kDa; a doublet was found in specimens from Smith Kline (lanes 4 and 5). The mouse serum also reacted with a band of 116 kDa in P. carinii specimens from Fox Chase (lanes 2 and 3) and the Jackson Laboratory (lanes 6 and 7); this antigen was very similar to the 116-kDa moiety in rat and human P. carinii recognized by the rabbit antisera. In contrast, the 116-kDa antigen was not recognized in P. carinii of five Smith Kline mice: two specimens presented here (Fig. 5, lanes 4 and 5) and three specimens analyzed separately (data not shown). No immunoreactivity occurred with mouse lung homogenate without P. carinii (Fig. 5, lane 1).

**FIG. 3.** P. carinii antigen and control preparations analyzed by immunoblotting using rabbit antiserum to rat P. carinii diluted 1/100. Lane 1, Smith Kline & French mouse lung homogenate without P. carinii (control preparation). Lanes 2 through 9, Lung homogenates, infected with P. carinii, from the following: (2 and 3) two separate Fox Chase nulnu mice; (4 and 5) two separate Smith Kline & French nulnu mice; (6 and 7) two separate Jackson Laboratory scid/scid mice; (8) rat; (9) human.

**FIG. 4.** P. carinii antigen and control preparations analyzed by immunoblotting using rabbit antiserum to human P. carinii diluted 1/100. Lanes are the same as in Fig. 3.

**FIG. 5.** P. carinii antigen and control preparations analyzed by immunoblotting using pooled mouse serum diluted 1/100. Lanes are the same as in Fig. 3.

**DISCUSSION**

This study has described outbreaks of pneumocystosis in two types of immunodeficient mice at four different institutions. The clinical features of the disease were similar in each outbreak and also resembled those found in previous reports (31, 45). P. carinii pneumonia presented as a subacute or chronic illness, lasting weeks to months and characterized by wasting, debilitation, and respiratory distress. Older mice were more severely affected than were younger mice. On histopathologic examination, the alveoli showed the typical foamy eosinophilic material, but the host inflammatory response was mild and nonspecific. These features are similar to those found in previous studies of P. carinii pneumonia in nude mice and in corticosteroid-treated normal mice and rats (37, 38, 43).

Immunofluorescence was used in an attempt to identify the source of P. carinii. Initial efforts were complicated by the paucity of specimens and by such technical factors as the amount of available lung tissue, intensity of P. carinii infection, and method of storage or preparation. Despite these limitations, the IFA technique proved to be helpful in determining whether organisms were mouse, rat, or human in origin once standard procedures for examining specimens were established. Data from our previous studies revealed a relationship between source of antibody and recognition of P. carinii antigenic determinants and emphasized specificity of the mouse serum antibody response (39, 41). Rats exposed to rat P. carinii developed serum antibodies to both rat and mouse organisms, whereas mice exposed to mouse or rat P. carinii developed serum antibodies to each source of organisms with little or no cross-reactivity. Pooled mouse serum specimens used in the present study exhibited a similar immunoreactive pattern and were thus interpreted as reasonably specific for mouse P. carinii.

All infected lung homogenates from immunodeficient mice in the present study reacted with pooled mouse serum by IFA, suggesting the organisms were derived from mice. It was not possible to distinguish among individual P. carinii isolates in these outbreaks because they all reacted with rabbit antisera to rat and human P. carinii and exhibited a similar immunofluorescent staining pattern. Monoclonal antibodies might be helpful in analyzing species differences in P. carinii (8, 9, 21, 23, 24); yet, since these reagents have
mainly been prepared using rat- or human-derived organisms as antigen and are not yet widely available, their applicability to studies of mouse *P. carinii* is unclear.

Serum antibody results indicated that the principal serologic response of *nul/+* mice at Smith Kline and Fox Chase was directed towards mouse *P. carinii*, thus lending further support to the hypothesis that mouse-derived organisms were the cause of illness in these outbreaks. As in our previous studies (41), nude mice were unable to mount a meaningful antibody response to any source of *P. carinii*.

In recent years, immunoblotting has been used to compare the antigenic characteristics of rat and human *P. carinii* (8–10, 20, 23, 34). The present study has applied this technique to mouse *P. carinii*, and overall, the data are consistent with the results obtained with immunofluorescence. Rabbit antiserum to rat and human *P. carinii* reacted with bands of 45 kDa, 50 kDa, and other molecular masses in infected-lung homogenates of three different groups of mice, suggesting shared or cross-reacting determinants. The antigenic characteristics of mouse *P. carinii* appear to be more closely related to those of rat than to those of human *P. carinii*. The specificity of the pooled mouse serum for mouse *P. carinii* was again demonstrated by its lack of reactivity with rat- or human-derived organisms. This serum did react with a 116-kDa band and other moieties in infected mouse lungs. The 116-kDa antigen in mouse *P. carinii* is very similar to the 116-kDa bands in rat and human *P. carinii*. It is of interest that, when tested with different reagents in this study, these antigens only reacted with the homologous source of antibody. Whether the differences in immunoactivity among the Smith Kline & French *nunu* mice represent true species or strain differences in *P. carinii*, or perhaps are merely due to technical factors, is unclear; however, all lung specimens were prepared and analyzed in a similar manner.

Introduction of *P. carinii* into a colony of immunodeficient mice may be related to the manner in which these animals are obtained. Nude mice at the University of Michigan and Smith Kline & French were purchased from the same commercial vendor. This breeder had been implicated as the source of a previously reported outbreak of pneumocystosis in *nunu* mice (45), and conversations among veterinarians revealed that several additional institutions were experiencing problems with *P. carinii* among nude mice obtained from this company. Investigations by the vendor indicated that *P. carinii* was present among mice in certain breeding facilities but not in others. This information is important not only for researchers but also because it can enable a vendor to adjust breeding activities to ensure an adequate supply of mice free of *P. carinii* infection.

Although the scid/scid mice at The Jackson Laboratory were originally developed from breeding pairs of animals obtained from Fox Chase, the source of the pneumocystis outbreaks at these two institutions could not be determined. Once *P. carinii* became established at The Jackson Laboratory, the organism apparently spread to other mice as the colony expanded. The fact that *P. carinii* may be present in research colonies of scid/scid or *nunu* mice has important implications for investigators who wish to develop colonies of these immunodeficient animals at their own institutions.

The spread of pneumocystosis among the animal colonies described in the present study emphasizes the communicability of the organism. Previous reports have implicated the airborne route as the mode of transmission (11, 13, 14, 43), but questions about the infective form of *P. carinii* (cyst or trophozoite), environmental sources of the organism, and vertical transmission remain largely unanswered. Data from the present study suggest that cages with special filters or lids protect mice from environmental exposure to *P. carinii*. However, immunodeficient mice already infected with *P. carinii*, housed in these cages, develop a more severe form of pneumocystosis than do animals housed in conventional open cages. It is unclear whether this difference in disease reflects a higher *P. carinii* burden within the inspired air, animal crowding, or other factors (e.g., temperature, humidity, ammonia, carbon dioxide levels) within the milieu of these microisolator cages.

The outbreaks described in this report illustrate the pathogenicity of *P. carinii* for immunodeficient mice under natural conditions. While these animals might serve as experimental models for *P. carinii*, efforts to develop a reliable system for general use have been rather disappointing. Our original studies suggested that nude mice could be infected with rat and human *P. carinii* by different methods of inoculation but developed little overt clinical illness (43). In later studies performed at another institution with a different strain of nude mice and with nude rats, we were unable to achieve the experimental transmission of *P. carinii* with any degree of consistency (36). Similar negative results have been reported to us in unpublished studies by other investigators. On the other hand, Japanese workers have conducted a variety of *P. carinii* transmission experiments among nude mice and nude rats and have used these animals to analyze host immune responses to *P. carinii* and to perpetuate the infection in their colonies (4–7). Yet *P. carinii* replication in the animals was modest, and corticosteroids had to be used in some cases to facilitate development of infection. scid/scid mice, which are even more immunologically impaired than are *nu/nu* mice, might be explored as an alternative animal model.

Control of pneumocystosis within colonies of immunodeficient mice has been difficult. As evidenced by the persistence of *P. carinii* in Fox Chase *nu/nu* mice for over 10 years, once infection with the organism has been established it tends to become endemic. The principal control measure has been to depopulate the colony and repopulate using animals with no known exposure to the organism. Although drugs such as TMP-SMZ have been successful in the treatment and prevention of pneumocystosis in humans and rats (15–18), there has been reluctance to use these agents in immunodeficient mice. These drugs are not microbicidal for *P. carinii* and hence are only effective in chemoprophylaxis as long as they are being given (12). Since TMP-SMZ and related drugs work by inhibiting synthesis of folic acid, there has been concern about their effects on animal breeding, immune function, or use in tumor studies. The apparent success of TMP-SMZ on the scid/scid mice in a conventionally housed research colony at The Jackson Laboratory is encouraging. Since *P. carinii* organisms could still be found in healthy animals, further studies will be necessary to find the optimal dose regimen.

Outbreaks of pneumocystosis constitute a major problem to colonies of immunodeficient mice and may become more common. Successful strategies to meet this challenge should include strictly controlled policies for breeding and maintaining these colonies, careful surveillance for *P. carinii*, and early intervention at the first signs of infection. The only available method for detecting *P. carinii* in mouse colonies has been lung histology, which is cumbersome, time consuming, expensive, and relatively insensitive. The present study has demonstrated the feasibility of using serology as a sensitive test to monitor sentinel heterozygote mice for exposure to *P. carinii* in a manner similar to that used for viruses and mycoplasma. The major impediment to
the use of serology is a lack of a ready supply of antigen. Moreover, serological tests cannot be used to diagnose pneumocystosis in immunodeficient mice since some of these animals lack immunoglobulins or are poor antibody responders. Systems for detecting \( P. \text{ carinii} \) antigens or genetic material in tissues might be explored as alternative diagnostic techniques (33). Regardless of the method used, the heightened interest in \( P. \text{ carinii} \) and widespread use of immunodeficient mice in biomedical research emphasize the need to develop practical methods of detecting and controlling infection with the organism in these animal colonies.

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**LITERATURE CITED**


