Pneumocystis carinii Infection in Transgenic B Cell–Deficient Mice

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Pneumocystis carinii is an important cause of pneumonia in immunocompromised hosts. Both cellular and humoral immunity seem important in resistance to this pathogen, but the specific role of each component is poorly understood. An outbreak of P. carinii pneumonia in transgenic B cell–deficient mice (μMT) was studied. Over 4 months, >50% of 41 μMT/μMT mice maintained in a sterile environment died of pneumonia. Some mice had concurrent infection with Pasteurella pneumotropica. Homozygous μMT/μMT mice had no detectable serum immunoglobulins, while their heterozygous μMT/+ counterparts had normal levels of IgM, IgG, and IgA and did not develop pneumonia. The infection was controlled by treating the mice with trimethoprim-sulfamethoxazole, and the pathogen was eliminated by cesarean rederivation. These observations suggest an important role for B cells in the host defense against P. carinii.

The importance of antibodies in host defenses against P. carinii has been suggested by the occurrence of pneumocystosis in patients with B cell defects, by the role of antibodies as opsonins in the phagocytosis of the organism, by the beneficial effect of the passive administration of a monoclonal antibody against P. carinii, and by the presence of immunoglobulins on the surface of P. carinii [2, 6, 7].

In the present study, we report an outbreak of P. carinii infection in transgenic μMT mice, which have a B cell deficiency [8], suggesting an important role for B cells in host defense against P. carinii.

Materials and Methods

Mice. Three pairs of 2-month-old C57/BL6 μMT/μMT mice were obtained from B & K Universal (Hull, UK). These mice were produced by Kitamura et al. [8] by disrupting one of the genes encoding the μ-chain constant region by gene targeting in mouse embryonic stem cells. B cell development in homozygous (μMT/μMT) mice is arrested at the stage of pre–B cell maturation, while it is normal in heterozygous (μMT/+ ) mice [8]. T cell numbers in μMT mice were normal [8]. The health report furnished by B & K revealed that the μMT mice were positive for Pasteurella pneumotropica and mouse encephalomyelitis virus (GD-VII) on arrival.

Mice were housed under a vertical laminar flow hood with sterile wood chip bedding (Sani-chips; P.J. Murphy Forest Products, Montville, NJ) and fed sterile chow (Prolab RMH 4018; Agway, Syracuse, NY) and sterile water ad libitum. The colony was developed by mating the 3 pairs of μMT mice. Six normal 6- to 8-week-old female C57/BL6 mice were obtained from Charles River...
Laboratories (St. Constant, Quebec) and mated with male ρMT/μMT mice to obtain the heterozygous counterpart (μMT/+).

Histopathology. Moribund mice were euthanized with carbon dioxide. All mice were necropsied, and lungs from 8 clinically ill mice were fixed in 10% buffered formalin. The lungs were then embedded in paraffin, and 4-μm sections were cut. Sections were stained with hematoxylin-phloxine-saffron, which provides a general view of pulmonary architecture, and with Churukian silver impregnation, which selectively stains the cyst wall of P. carinii.

Bacteriology. Abscesses observed on the lungs of some mice were swabbed. The samples were seeded on Colombia blood agar and MacConkey agar, and the plates were incubated (35°C, 48 h) in a controlled atmosphere (5% CO₂). Gram's staining, catalase, oxidase, and other biochemical tests were done on the organism isolated. Acid production was evaluated on triple sugar iron slants.

Serologic testing. CD-1 mice (Charles River Laboratories) served as sentinels for the serologic test. They were kept in the same room as the μMT mice, outside the sterile flow hood, and were given bedding, food, and water from the cages housing the μMT mice. Serologic examinations of sentinel mice were periodically done by Charles River Biotechnical Services (Wilmington, MA) for the diagnosis of the following pathogens: Sendai virus, mouse pneumonia virus, mouse hepatitis virus, mouse adenovirus FL/K87, cytomegalovirus, mouse pneumonitis virus, and polyomavirus.

Control of infection. Different approaches were used to control the outbreak of P. carinii in our colony. First, female ρMT mice were backcrossed with normal male C57BL6 mice to save the gene deletion in phenotypically normal ρMT/μMT+ mice. Also, permanent treatment of mice with trimethoprim-sulfamethoxazole (TMP-SMZ) was tested [9]. TMP-SMZ was administered in drinking water for periods of 3 consecutive days with 4-day intervals without drug prophylaxis [9]. The dosage was calculated on the basis of 100 mg/kg/day TMP and 500 mg/kg/day SMZ. In parallel, cesarean derivations were done to eliminate the pathogens. The μMT/μMT and μMT/+ litter mice obtained by hysterectomy were fostered onto specific-pathogen-free CD-1 nu/nu mice (Charles River Laboratories, Wilmington, MA). CD-1 mice are good foster mothers, and this nude colony was known to be free from P. carinii. Cesarean-derived mice were maintained in a sterile environment in a separate room.

Immunoglobulin levels. Levels of total serum IgM, IgG, and IgA were evaluated in 10 μMT/μMT and 6 μMT/+ mice by a previously described ELISA [10]. Serum samples were assayed up to a dilution of 1:5000. Standard IgM, IgG, and IgA curves were determined using a reference mouse serum (lot 0024; ICN Biomedicals, St. Laurent, Canada).

Results

Clinical observations. The outbreak appeared 2 months after the breeders arrived. Over a 4-month period, we lost 22 of 41 μMT mice. Ages at death ranged from 4 to 28 weeks, and half of the mortalities occurred before the age of 12 weeks. Clinical signs included rough hair coat, hunched posture, and dyspnea. Some of the mice showed only a few hours of laborious respiration before death; others had clinical signs for longer periods (1–4 days). On necropsy, the lungs were densely consolidated with a firm rubbery consistency typical of P. carinii infection [9]. In 3 of the 22 mice, lung abscesses were observed.

Histopathology. The intensity of the P. carinii infection in the μMT mice ranged from mild to severe. The alveoli were filled with macrophages and characteristic foamy eosinophilic material (figure 1). The alveolar septae were thickened with infiltrating macrophages and lymphocytes (figure 1B). Churukian staining revealed the presence of cysts in the alveoli consistent with the presence of P. carinii (figure 1B). In 3 mice, the pneumocystosis alveolitis was accompanied by a suppurative bronchopneumonia. Abscesses observed in the lungs were composed of a necrotic cellular debris core surrounded by a thick layer of neutrophils and macrophages. The bronchi and bronchioles were filled with neutrophils, mucus, and necrotic debris. P. pneumotropica was isolated from abscesses and was identified as the causal agent of the bronchopneumonia.

Serologic testing. Serologic analysis revealed positive reactions only for GD-VII, a strain of Theiler's murine encephalomyelitis virus. This virus is a pathogen of the central nervous system of mice but rarely causes clinical disease. We did not observe any clinical symptoms associated with this pathogen.

Control of infection. The heterozygous μMT/+ mice did not show clinical symptoms, and histopathologic analyses of the lungs of 2 mice did not reveal the presence of P. carinii or P. pneumotropica. Also, as previously reported [9], intermittent treatment of μMT mice with TMP-SMZ was effective in the prevention of pneumocystosis. Mortality was reduced to zero after the beginning of therapy. The cesarean-derived mice have remained healthy for 1 year without TMP-SMZ administration. Histopathologic analyses done on 2 cesarean-derived mice did not reveal the presence of P. carinii or P. pneumotropica. Serologic analyses were negative for the above-described pathogens, including GD-VII.

Immunoglobulin levels. No immunoglobulins (<0.1 μg/mL) were detected in the serum of μMT/μMT mice. Serum immunoglobulin levels of μMT/+ mice were 110 ± 20 μg/mL (mean ± SD) for IgM, 420 ± 60 μg/mL for IgG, and 1040 ± 160 μg/mL for IgA.

Discussion

Previous reports have described outbreaks of P. carinii infection in immunodeficient nude and SCID mice [9]. Pneumocystosis has also been reported in another μMT mice colony [5], but to our knowledge detailed results have never been published. The source of the organism that infected our mouse colony is unclear. It is not known if the mice were already infected subclinically on arrival or if they acquired the infection at our animal facility. Although the mice were maintained under sterile conditions, our husbandry techniques may have been inadequate in preventing airborne transmission from other rodents. Some studies have shown that nude mice can be contaminated by P. carinii by the airborne route [3].
P. carinii pneumonia in immunodeficient mice usually presents as a subacute or chronic illness, lasting weeks to months, and characterized by wasting, debilitation, and respiratory distress. The acute manifestation observed in our µMT colony could be due to a concurrent infection with P. pneumotropica. It has been observed that concurrent infections may exacerbate the clinical expression of pneumocystosis [5]. Inoculation of microorganisms not usually associated with significant morbidity in immunocompetent mice caused an increased density of cysts, increased P. carinii–associated pathology, and a reduced survival time of SCID mice [5].

P. pneumotropica is described as a secondary pathogen, often acting synergistically with other microbial agents to produce clinical pneumonia in mice [11]. A defect in the immune system might also predispose mice to P. pneumotropica infections. Immunologically incompetent mice, such as athymic nude mice, are more susceptible to infection by this organism [11]. The supplier indicated that the µMT colony harbored P. pneumotropica, but they had never observed any clinical disease outbreaks associated with this bacterial species. It is possible that the outbreak of pneumonia in our colony was due to a synergistic reaction between P. carinii and the opportunistic pathogen P. pneumotropica. Mixed infections with P. carinii and P. pneumotropica have been observed in SCID mice [12]. In contrast, it has been demonstrated that deliberate coinfection with P. pneumotropica does not significantly alter the course of P. carinii infections in SCID mice [5]. To clarify this point, we will follow the evolution of the pneumocystosis in cesarian-
derived μMT mice inoculated with *P. carinii* and treated with antibacterial agents.

Previous studies using knockout mice with a single gene deletion showed that they are valuable, clean tools for studying the effect of the immune system on microbial infections [13]. μMT mice could be an excellent experimental model to study *P. carinii* infections. Contrary to most previous models that have severe immunodeficiencies, μMT mice have normal T cell numbers and only lack B cell and humoral immune functions [8]. In vitro lymphocyte proliferative and delayed-type hypersensitivity responses to *P. carinii* will be evaluated to verify the state of the cell-mediated immunity of μMT mice. There are well-documented clinical cases of *P. carinii* infections in patients with hypogammaglobulinemia and normal cell-mediated immunity [1, 6]. Although these cases remain rare and often occur in infants under 1 year of age, our findings add to these previous clinical reports and suggest an important role for B cells and antibodies in the host defense against *P. carinii*.

It must be considered that *P. carinii* pneumonia in patients with T cell disorders might be due not only to an impairment in cell-mediated immunity but also to a reduced antibody response to *P. carinii*. Antibodies could act as opsonins, enhancing the phagocytosis and killing of *P. carinii* by alveolar macrophages [7]. Alternatively, B cells could also present *P. carinii* antigens to T cells [14]. Further studies using μMT mice will attempt to determine the role of B cells and antibodies in host defense against *P. carinii* pneumonia. For example, adoptive transfer of anti-*P. carinii* immune serum to μMT/μMT mice may reveal whether antibodies in the absence of other B cell functions are important in limiting the infection.

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**References**