Antibody to *Pneumocystis carinii* Protects Rats and Mice from Developing Pneumonia

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Received 23 June 1997/Returned for modification 2 October 1997/Accepted 10 November 1997

Well-proven mouse and rat models were used to show that polyclonal antisera to *Pneumocystis carinii* protect against *P. carinii* pneumonia. Antibodies were obtained from animals that were allowed to recover from severe *P. carinii* pneumonia after immunosuppression had been stopped and which then were given a booster injection of *P. carinii* from the same animal species. Mice immunosuppressed with corticosteroids or antibodies to L3T4⁺ lymphocytes (which are comparable to CD4 cells of humans) and transtracheally inoculated with mouse *P. carinii* did not develop *P. carinii* pneumonia if they were passively immunized with antisera, while mice immunosuppressed and inoculated by identical procedures but not given antibodies developed severe infections. Rats immunosuppressed with corticosteroids and inoculated with rat *P. carinii* had less severe infections if they were given rat anti-*P. carinii* antiserum. The polyclonal antiserum developed in mice provided greater protection for the mice than the polyclonal rat antiserum did for the rats; however, the potencies and compositions of the antisera were not quantitated and probably differed. Since both rats and mice can be protected from *P. carinii* infections with polyclonal antiserum, it may be possible to develop vaccines that will elicit protective antibodies in humans.

*Pneumocystis carinii* is a leading cause of pneumonia in patients with immune deficiencies, including individuals infected with human immunodeficiency virus and those receiving chemotherapy for malignancies or for transplantation. Although antimicrobics are available for treatment and prophylaxis of *P. carinii* pneumonia (20), many patients have adverse reactions or fail to respond to the most effective antimicrobial agents (28). In addition, even with prophylaxis, *P. carinii* continues to be a major cause of illness (23, 24). If a vaccine could be developed that would prevent infections from developing, or reduce infections so that they would be mild or occur later in the course of AIDS, it would be of great clinical utility.

The role of host defenses in preventing the development of *P. carinii* pneumonia is not well defined. Since individuals with human immunodeficiency virus develop *P. carinii* pneumonia when their CD4 cell counts fall below 200, cell-mediated immunity has been considered the most important host defense. However, reports of *P. carinii* pneumonia in children often describe hypogammaglobulinemia or agammaglobulinemia as predisposing factors (7, 19, 23, 24, 30). The role of antibodies in preventing or controlling disease has not been well explained, although a study by Gigliotti and Hughes (13) reported a decrease in severity of *P. carinii* infection in ferrets given antibody to ferret *P. carinii*. Also, a study by Harmsen et al. (15) showed that immunized mice cleared *P. carinii* from their lungs and suggested the possibility that antibodies were responsible for protection. Studies by several researchers have demonstrated the role of T lymphocytes in controlling infections (10, 11, 14), but conclusive studies demonstrating the effects of antibodies have been lacking.

Since a great deal of work with *P. carinii* was carried out in latently infected rats immunosuppressed for long periods of time with corticosteroids, assessing the contributions of host defenses in the course of disease was not possible because corticosteroids suppress all lymphocytes, including T and B lymphocytes, and influence phagocytosis and inflammation. Immunized animal models, used in this study, develop severe infections more rapidly and reproducibly than latently infected animals (1, 4). By using them, it has been possible to more clearly determine the effects of drugs on infections (2, 3), study immune responses during the development of infections (5), and test the usefulness of antiserum in providing protection. This report describes the use of polyclonal antiserum from convalescent animals that had received a booster injection to prevent or diminish infections in these well-established models.

MATERIALS AND METHODS

**Animal models.** To develop *P. carinii* infections, immunosuppressed animals were transtracheally inoculated as described previously (1, 4, 5). Briefly, BALB/c mice were immunosuppressed and transtracheally inoculated with mouse *P. carinii*, and virus-free Sprague-Dawley rats from Harlan Colony 202, Indianapolis, Ind., were immunosuppressed and transtracheally inoculated with rat *P. carinii*. Prior to inoculation, mice were immunosuppressed for 14 days with monoclonal antibody from clone GK1.5, which is directed to L3T4⁺ cells (comparable to human CD4 cells) (6), in one study and with dexamethasone for 10 days at 1.2 mg/kg of body weight/day in the second study. Prior to inoculation, the rats were immunosuppressed with dexamethasone at 0.36 mg/kg/day for 7 days. Mice were transtracheally inoculated with 10⁷ mouse *P. carinii* organisms in 0.05 ml of saline, and rats were transtracheally inoculated with 10⁵ rat *P. carinii* organisms in 0.2 ml of saline; the wounds were closed with clips.

**Development of mouse antiserum.** The animals developed infections for 10 weeks, at which time severe infections had developed; immunosuppression was stopped, and the animals were allowed to recover. During the second week after the cessation of immunosuppression, the animals were injected intraperitoneally with 0.1 ml of solubilized *P. carinii* prepared as follows. Heavily infected lung tissue was ground in phosphate-buffered saline (10 mg/ml) and centrifuged slowly (300 × g) to remove large lung debris. The supernatant, containing approximately 10⁸ organisms, was centrifuged at 5,000 × g for 10 min, and the pellet was suspended in 0.1 ml volume of 1 M urea with 10 mg of dithiothreitol/ml in water, solubilized at 4°C overnight, and diluted 1:10 in phosphate-buffered saline. After an additional 17 days, the animals were anesthetized and exsanguinated by cardiac puncture and sera were evaluated for antibody by enzyme-linked immunosorbent assay (ELISA) by a method that was developed to detect *P. carinii* in cultures and in animals (9). Briefly, ELISAs were performed in...
of antiserum pool, and rats were given 400 μl intraperitoneally. For the first mouse study, which used L3T4- antibody immunosuppression, antiserum was given to a group of mice 1 day prior to P. carinii transtracheal inoculation and at 2 and 4 weeks postinoculation while antiserum was given to a second group of mice only at 2 weeks after P. carinii inoculations. For the second mouse study, which used dexamethasone immunosuppression, antiserum was given 1 day prior to transtracheal inoculation and at 2 and 4 weeks after inoculation. Immunossuppressed rats were given antiserum 1 day prior to transtracheal inoculation with P. carinii and at 2 and 4 weeks after inoculation. For each of the above studies, there were 10 mice or 8 rats in each antiserum-treated group, 8 in control groups, and 10 mice treated with trimethoprim plus sulfamethoxazole at 50 and 250 mg/kg/day, respectively, in drinking water.

### Table 1. P. carinii infection scores with various treatments

<table>
<thead>
<tr>
<th>Animal</th>
<th>Immunosuppression</th>
<th>Antiserum</th>
<th>Score* when treated with:</th>
<th>TMP + SMX† (wk 3-6)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No treatment</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>L3T4</td>
<td>0.5 ± 0.2*</td>
<td>1.2 ± 0.3**</td>
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<tr>
<td>Mouse</td>
<td>Dexamethasone</td>
<td>0.3 ± 0.2***</td>
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<td>4.7 ± 0.1 0.0 ± 0.0</td>
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* Giemsa stain score ± standard error. P values for differences between treated and untreated animals: *, P = 0.003; **, P = 0.023; ***, P = 0.000; ****, P = 0.006. ND, not done.
† TMP + SMX, trimethoprim plus sulfamethoxazole.

Development of rat antisera. Rat antisera were developed in the same manner as that used for mouse antiserum. Transtracheally-inoculated Sprague-Dawley rats developed P. carinii infections for 6 weeks; then the dexamethasone immunosuppression was stopped, and the rats were allowed to recover. At the end of the first week after dexamethasone was stopped, the rats were given solubilized rat P. carinii prepared in the same way as solubilized mouse P. carinii except that the pellet contained approximately 10⁹ organisms and the final volume given was 0.3 ml per rat intra-peritoneally. The rats were exsanguinated by cardiac puncture 2 weeks after the P. carinii injection, and the individual rat sera were evaluated by ELISA and Western blotting. The rat anti-P. carinii antibody was primarily IgG. Rat sera shown to have antibodies to rat P. carinii antigens were pooled.

Development and evaluation of infections. After inoculation, the animals were continued on immunosuppression for 6 weeks, allowing infections to become severe. The severity of infections was determined by scoring numbers of organisms in histologically stained impression smears of lung samples. Animals were anesthetized and exsanguinated by cardiac puncture, and their lungs were removed. A portion of the left lower lobe of each lung was used for the preparation of smears for Giemsa and methenamine-silver nitrate staining. The smears were examined for unknowns by two experienced microscopists and scored according to the following roughly logarithmic scheme (organisms per representative 1,000× microscopic field): 5+ > 100; 4+ ≥ 10 to 100; 3+ ≥ 1 to 10; 2+ ≥ 2 to 9 (in 10 fields); 1+ ≥ 1 (in 10 to 50 fields); 0, 0 ≥ 0 (in >50 fields).

Evaluation of antisera for major surface glycoprotein specificity. The mouse serum pool that provided protection was used to blot a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (18) gel of rat P. carinii recombinant major surface glycoprotein (MSG) (21). For controls, normal mouse serum, rat convalescent-phase serum, and normal rat serum were included in the blot. The mouse serum pool also was blotted against a mouse serum or normal mouse serum (Fig. 1). The mouse antiserum did not react with rat P. carinii recombinant MSG. The Western blot showed two bands with the control rat convalesce-antiphase antiserum, but there was no reaction with normal rat serum or with the polyclonal mouse serum or normal mouse serum (Fig. 1). The mouse serum did react to a mouse P. carinii protein of about 55 kDa as well as the MSG of about 130 kDa and some other constituents of the pool (Fig. 2).

### Discussion

Polyclonal anti-P. carinii antiserum can protect mice and rats from developing severe P. carinii infections. The mice immunosuppressed by antibody to L3T4⁺ lymphocytes (comparable

**Table**: P. carinii infection scores with various treatments

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Results

Untreated dexamethasone-immunosuppressed mice were severely infected and had approximately 50 P. carinii organisms per 1,000× microscopic field detected by Giemsa staining. Untreated L3T4⁺-immunosuppressed mice also had severe infections, with comparable numbers of organisms detected by Giemsa staining.

Mice immunosuppressed with L3T4⁺ antibody and mice immunosuppressed with dexamethasone were protected with antiserum given prior to transtracheal inoculation. In both immunosuppression groups, zero or few organisms were found in up to 50 1,000× microscopic fields. Differences in infection were statistically significant, with comparisons of the scores of mice treated by antiserum prophylaxis to those of untreated control mice having P values of 0.003 and 0.000, respectively. The group of mice given antiserum only at weeks 3 and 5 had slightly higher infection scores; however, antiserum-treated mice had a statistically significant reduction in numbers of organisms compared to untreated controls (P = 0.023). The drug-treated (trimethoprim plus sulfamethoxazole) control mice were cured (Table 1).

Rats immunosuppressed with dexamethasone and treated with antiserum prior to inoculation had less severe P. carinii infections than untreated rats. Trimethoprim plus sulfamethoxazole cured rats (infection scores of antiserum-treated rats compared to those of untreated control rats had P values of 0.006). Rat scores are summarized in Table 1.

The mouse antiserum did not react with rat P. carinii recombinant MSG. The Western blot showed two bands with the control rat convalescent-phase antiserum, but there was no reaction with normal rat serum or with the polyclonal mouse serum or normal mouse serum (Fig. 1). The mouse serum did react to a mouse P. carinii protein of about 55 kDa as well as the MSG of about 130 kDa and some other constituents of the pool (Fig. 2). The rat serum pool had reactions to the MSG of about 120 kDa and to both a 50- and 40-kDa band (data not shown).

**Statistical analysis.** Data were analyzed with the SigmaStat program and the Mann-Whitney rank sum test for nonparametric data.

**FIG. 1.** (A) Coomassie blue-stained SDS-PAGE gel with molecular mass markers (in kilodaltons) (lane 1) and recombinant MSG from rat P. carinii recombinant major surface glycoprotein (MSG) (21). For controls, normal mouse serum, rat convalescent-phase serum, and normal rat serum were included in the blot. The mouse serum pool also was blotted against a mouse P. carinii antigen prepared from mostly trophozoite forms obtained by differential centrifugation according to a method developed by Merali and Clarkson (22). Individual rat sera were evaluated by ELISA and Western blotting. The mouse serum pool that provided protection was used to blot a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (18) gel of rat P. carinii recombinant major surface glycoprotein (MSG) (21). For controls, normal mouse serum, rat convalescent-phase serum, and normal rat serum were included in the blot. The mouse serum pool also was blotted against a mouse P. carinii antigen prepared from mostly trophozoite forms obtained by differential centrifugation according to a method developed by Merali and Clarkson (22). Individual rat serum samples were tested by ELISA and Western blotting, using as the antigen a rat P. carinii preparation of mostly trophozoite forms prepared by the same differential centrifugation method.

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**DISCUSSION**

Polyclonal anti-P. carinii antiserum can protect mice and rats from developing severe P. carinii infections. The mice immunosuppressed by antibody to L3T4⁺ lymphocytes (comparable...
to CD4 lymphocytes of humans) and given mouse polyclonal antiserum prior to inoculation were most effectively protected from *P. carinii* pneumonia. This antiserum pool was also effective when given after infections had begun to develop, at 3 weeks postinoculation. In dexamethasone-immunosuppressed mice, the antiserum was effective when given prior to inoculation. Rats were less well protected with the available rat antiserum pool than were mice with their antiserum pool. Still, rats that were treated with polyclonal antiserum at the time of inoculation had less severe infections than the rats that were not treated. The rat antiserum pool may not have been administered in sufficient quantity to provide protection, or the amounts of protective antibodies may have been less in the rat serum pool than in the mouse serum pool.

The mouse antiserum did not react with recombinant rat *P. carinii* MSG. Some investigators have suggested that MSG is a dominant antigen, and since a previous study had shown cross-reaction of antibodies to surface glycoproteins with a dominant antigen, and since a previous study had shown that were antigenically different. Infect. Immun. 1:511–516.


