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New Rat Model of *Pneumocystis* Pneumonia Induced by Anti-CD4⁺ T-Lymphocyte Antibodies

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The CD4⁺ T lymphocyte plays a central role in host defense against *Pneumocystis pneumonia* but has received only limited attention in rats. CD4⁺ T-cell-depleting (OX-38) and nondepleting (W3/25) monoclonal antibodies, which recognize an identical or adjacent epitope, were administered for up to 14 weeks to Lewis rats that had been exposed to *Pneumocystis*. While OX-38 produced a greater decrease in circulating CD4⁺ cells than W3/25, both antibody treatments resulted in similar effects on the health of the rats and the levels of *Pneumocystis pneumonia*, which were milder than those found with corticosteroids. W3/25 also did not enhance the severity of *Pneumocystis pneumonia* achieved with corticosteroids alone. We conclude that CD4⁺ cell function is more important than CD4⁺ cell number in host defense against *Pneumocystis* in the rat and that this new model permits study of opportunistic infections in the rat without the confounding effects of corticosteroids.

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Pneumocystis is an extracellular fungus of low virulence that causes pneumonia in immunocompromised individuals, such as human immunodeficiency virus-positive patients and cancer and organ transplant patients. Various immunodeficient and immunosuppressed rat and mouse models have been used to study the interaction between *Pneumocystis* and host (9, 11, 31, 32). The corticosteroid (CS)-treated rat is the original animal model used to study *Pneumocystis pneumonia* and is the source of most available information on the epidemiology, immunopathogenesis, diagnosis, and therapy of the disease (3, 9). However, this model is limited by the broad immunosuppressive effects of steroids on the immune system, including lymphocyte depletion and impairment of function, reduced leukocyte chemotaxis and phagocytosis, and deficient antibody (Ab) production (38).

Clinical and experimental studies have shown that CD4⁺ T lymphocytes play a central role in host defenses against *Pneumocystis* (14, 25, 29, 37). *Pneumocystis pneumonia* can be induced in mice exposed to the organism by the administration of GK1.5, a rat immunoglobulin G2b (IgG2b) monoclonal antibody (MAb) specific for CD4⁺ cells (31). This model has proven to be popular because it circumvents the need for CS immunosuppression; however, no such model exists in rats. Given the increasing evidence of the genetic diversity and host specificity of *Pneumocystis* (6, 33), it cannot be assumed that the results obtained in one animal species can be applied to another. The development of a CD4⁺ depletion model in the rat would be important in studying the role of CD4⁺ T cells in the rat.

MAbs have been produced to rat CD4⁺ cells, and their properties have been analyzed by in vitro or short-term in vivo studies (2, 5, 26, 39). These Abs are of two general types: depleting Abs, which trigger cell lysis; and nondepleting Abs, which cause downregulation of CD4 antigen expression resulting in inadequate T-cell receptor (TCR)-antigen-major histocompatibility complex class II interaction (34). These Abs have been used in a variety of autoimmune or other disease studies, including studies of rat adjuvant arthritis (23), organ transplantation (17), and allergic encephalomyelitis (34). Yet, there is little published information about the use of these Abs in an infectious disease model.

We undertook the present study to determine if the administration of MAbs to CD4⁺ cells can induce *Pneumocystis pneumonia* in rats. We chose two widely used MAbs that recognize an identical or adjacent epitope on the CD4⁺ molecule (15): W3/25 (mouse IgG1), a nondepleting MAb that downregulates CD4⁺ cell function, and OX-38 (mouse IgG2a), a depleting MAb.

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**MATERIALS AND METHODS**

**Animals.** Male Lewis rats were acquired from Charles River Laboratories (Hollister, Calif.). Male and female Long-Evans rats were bred and raised at the Veterans Affairs Medical Center, Cincinnati, Ohio. All rats were 6 to 8 weeks of age and weighed 125 to 150 g at the beginning of the experiments. The animals were housed in microisolator cages in a bioBuble (Fort Collins, Colo.) to control aerosol contamination and were nourished with autoclaved food and water. Ampicillin (1 mg ml⁻¹; TEVA Pharmaceuticals, Sellersville, Pa.) was given in the water to control secondary bacterial infections. All rats used in this study were exposed to *Pneumocystis* by being housed with CS-treated rats with active *Pneumocystis* pneumonia. *Pneumocystis pneumonia* was induced in rats by subcutaneous injections of methylprednisolone acetate (4.0 mg/0.2 ml/week; Depo-Medrol; Pharmacia and Upjohn Co., Kalamazoo, Mich.), as described previously (35). All animals were handled according to institutionally recommended guidelines.

**Anti-rat CD4⁺ MAbs.** The W3/25 and OX-38 hybridomas were obtained from the European Collection of Animal Cell Cultures (ECACC) Centre for Applied Microbiology & Research (CAMR) (Wiltshire, United Kingdom) and shipped to...
of penicillin G sodium, 100.0 units, was given i.m. weekly to each of our animals. Weekly i.p. administration of methylprednisolone (4.0 mg/kg) administered either on a milligram-per-rat or milligram-per-kilogram basis. We also found that once a week downregulated CD4+ T cells and CD8+ T cells counted per spleen in a lymphocyte forward-versus-side light-scatter region by flow cytometry analysis. The percentage of CD4+ or CD8+ cells per spleen is given in parentheses.

**RESULTS**

Effects of nondepleting rat CD4+ W3/25 MAb administration and depleting rat CD4+–OX-38 MAb administration. We hypothesized that the CD4+ T-cell-reducing effect of the CD4+–OX-38 double MAbs would render our Lewis rat model susceptible to *Pneumocystis*. To test this, Lewis rats received weekly administration of either 1.0 mg of OX-38, 1.0 mg of W3/25, or a combination of both Abs (1.0 mg each). As positive and negative controls for *Pneumocystis* infection, rats received weekly i.p. administration of methylprednisolone (4.0 mg/kg) or 1.0 ml of PBS, respectively. All rats were exposed to *Pneumocystis*-infected rats and were sacrificed at different time points.

PBS-treated rats were healthy and increased their mean body weight by 130% over 8 weeks (Fig. 1). CS-treated rats experienced weekly administration of either 1.0 mg of OX-38, 1.0 mg of W3/25, or a combination of both Abs (1.0 mg each). As positive and negative controls for *Pneumocystis* infection, rats received weekly i.p. administration of methylprednisolone (4.0 mg/kg) or 1.0 ml of PBS, respectively. All rats were exposed to *Pneumocystis*-infected rats and were sacrificed at different time points.

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increased CD8 T-cell numbers compared to the level in PBS-treated controls; \( P < 0.001 \). A significant increase in the splenic CD8 T-cell population was observed in rats treated with OX-38 (230\% increase; \( P < 0.001 \)), W3/25 (120\% increase; \( P < 0.001 \)), and the combined anti-CD4 MAbs (210\% increase; \( P < 0.001 \)). The increased CD8\(^+\) T-cell numbers seen in both OX-38- and W3/25-treated animals were proportional to the decreased CD4\(^+\) T-cell numbers, indicating that CD4\(^+\) T cells were actually depleted after treatment with the W3/25 Ab. This CD4\(^+\) T-cell reduction effect has been observed in other studies after multiple MAb injections (23).

A comparison of the CD4\(^+\)/CD8\(^+\) ratios is also shown in Table 1. CS-treated rats had a lower CD4\(^+\)/CD8\(^+\) ratio than control rats, as was previously seen in our studies (5). W3/25-treated rats and W3/25–OX-38-treated rats exhibited reduced CD4\(^+\)/CD8\(^+\) ratios in the spleen compared to controls, but the greatest reduction of the CD4\(^+\)/CD8\(^+\) ratio was seen with the OX-38-treated rats compared to controls.

**Mild *P. carinii* infection susceptibility in W3/25- and OX-38-treated Lewis rats.** Our CS-treated Lewis rats typically perished after 9 to 10 weeks of treatment due to the wasting effects of the steroids, so all of the CS-treated rats were sacrificed at the week 8 time point. These steroid-treated rats were infected with *Pneumocystis* (Fig. 2), evidenced by detection of both *Pneumocystis* cysts (log \( 7.0 \pm 0.44 \) per lung [mean \pm standard deviation]) and nuclei (7.2 \( \pm 0.43 \) per lung), while PBS-treated, healthy rats were not infected with *Pneumocystis*. When anti-CD4\(^+\) MAb-treated lung rats were processed for *Pneumocystis* detection at week 8, *Pneumocystis* cysts and nuclei were detected in the OX-38-treated rats (cysts, 5.8 \( \pm 0.40 \) per lung; and nuclei, 6.9 \( \pm 0.23 \) per lung), W3/25-treated rats (5.9 \( \pm 0.17 \) and 6.9 \( \pm 0.44 \) per lung), and OX-38–W3/25-treated rats (5.8 \( \pm 0.09 \) and 7.1 \( \pm 0.26 \) per lung). The effect of anti-CD4\(^+\) MAb administration appeared to be greater on the trophic form of *Pneumocystis* than on the cyst, as evidenced by the similar burden of nuclei (indicating all morphological forms of *Pneumocystis*) between CS- and anti-CD4\(^+\) MAb-treated rats. Similar *Pneumocystis* cyst and nucleus burdens were observed in rats treated with the anti-CD4\(^+\) MAbs for 12 to 14 weeks (data not shown); the cyst and nucleus burdens detected at 12 to 14 weeks were (5.2 \( \pm 0.36 \) cysts per lung and 6.6 \( \pm 0.28 \) nuclei per lung for OX-38-treated rats, 5.2 \( \pm 0.36 \) cysts per lung and 6.0 \( \pm 0.28 \) nuclei per lung for W3/25-treated rats, and 5.3 \( \pm 0.35 \) cysts per lung and 6.6 \( \pm 0.31 \) nuclei per lung for OX-38–W3/25-treated rats.

Silver-stained lung sections of CS-treated rats revealed numerous *Pneumocystis* cysts arranged in typical clusters within alveoli (Fig. 3). In contrast, lung sections of the anti-CD4\(^+\) MAb-treated (OX-38, W3/25, and OX-38 plus W3/25) rats only showed scattered cysts, and no cysts were seen in the PBS-treated animals. The frequencies of cysts found in lung sections from rats treated with CD4\(^-\) depleting and -nondepleting MAbs were similar. Lung sections stained with H+E revealed typical intra-alveolar foamy exudates with increased macrophages in the CS-treated rats but not in the anti-CD4\(^+\) MAb- or PBS-treated rats. The host inflammatory response in all groups was modest and characterized by a nonspecific mononuclear cell infiltrate with occasional perivascular inflammation (Fig. 3).

Another experiment was performed to investigate the effects of W3/25 in a different rat species and to determine if this MAb increased the level of *Pneumocystis* infection in CS-treated rats. Long-Evans rats were treated with weekly administrations of W3/25 (1.0 mg), CS (4.0 mg), or a combination of W3/25 and
Rodent models of *Pneumocystis* pneumonia have been powerful tools to study the disease. Due to the plentiful supply of inbred strains and ease of genetic manipulation, mouse models of *Pneumocystis* pneumonia are more numerous than rat models (3). Besides the immunosuppressed (CS treated) rat, the only other rat model of *Pneumocystis* pneumonia is the athymic (nude) rat. While there are two 1993 reports of spontaneous Pneumocystis only other rat model of *Pneumocystis* pneumonia in inbred strains and ease of genetic manipulation, mouse models are more powerful tools to study the disease. Due to the plentiful supply of inbred strains and ease of genetic manipulation, mouse models of *Pneumocystis* pneumonia are more numerous than rat models (3). Besides the immunosuppressed (CS treated) rat, the only other rat model of *Pneumocystis* pneumonia is the athymic (nude) rat. While there are two 1993 reports of spontaneous *Pneumocystis* pneumonia in athymic Rowett nude (Han:RNU rnu/rnu) rats and New Zealand nude (Han:NZNU rnu/rnu) rats (10, 27), there have been no further published results concerning the development of this model. More recently, the rnu/rnu rat strain has been used in *Pneumocystis* studies, but these nude rats were treated with dexamethasone before inoculation with *Pneumocystis* (1, 7).

The present study was undertaken to develop a new rat model of *Pneumocystis* pneumonia by administration of MAbs to CD4⁺ cells. These MAbs have mainly been used in short-term experiments and to study autoimmune and related disorders. W3/25 and OX-38, the MAbs selected for analysis, recognize an identical or adjacent epitope (21) and thus provided a unique opportunity to compare the effects of CD4⁺ depletion and CD4⁺ antigen downregulation on this disease. Since *Pneumocystis* replicates slowly in the lungs, the different effects of these MAbs on CD4⁺ cells could be monitored over a lengthy period of time.

The data in the present study have shown that the OX-38 MAb had a greater effect on the number of circulating and splenic CD4⁺ cells than the W3/25 MAb. Yet, the use of both MAbs resulted in a modest level of *Pneumocystis* pneumonia that was considerably lower than the level that could be achieved with CS. The *Pneumocystis* organism burden was not increased by coadministration of W3/25 and OX-38 or by longer periods of Ab treatment (up to 14 weeks). These MAbs also did not enhance the level of *Pneumocystis* pneumonia induced by CS alone. There were discrepancies observed in the effectiveness of anti-CD4⁺ MAb treatment on *Pneumocystis* nucleus burdens in our two different animal models, and these may be explained by rat strain *Pneumocystis* susceptibility differences or by the diverse burdens of *Pneumocystis* infection found in the different experiments. Other reports have shown that variations in *Pneumocystis* cyst and nucleus burden susceptibility can occur following certain treatments, such as immune cell adoptive transfer and echinocandin drugs (30, 41).

The lower organism burden in the anti-CD4⁺-treated rats

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**DISCUSSION**

CS for 9 weeks (Fig. 4). Animals were sacrificed 7 to 9 weeks after treatments, and their lungs were examined for organism burden. The CS-treated rats were positive for *Pneumocystis* cysts (7.8 ± 0.50 per lung) and nuclei (9.4 ± 0.86 per lung). The organism burden of CS-treated rats was not increased when W3/25 was administered along with the steroids (7.7 ± 0.65 and 9.2 ± 0.42 per lung, respectively). As in the previous experiment with Lewis rats, the W3/25-treated Long-Evans rats also showed a mild susceptibility to *Pneumocystis* infection (5.8 ± 0.80 and 6.5 ± 0.91 per lung) that was significantly less (*P < 0.001) than that of steroid-treated rats and steroid-W3/25-treated rats. In contrast to the previous experiment shown in Fig. 2, the cyst and nucleus burdens were significantly lower in W3/25-treated rats than in CS-treated rats.

**FIG. 3.** Photomicrographs of representative lung sections from a CS-treated rat and anti-CD4⁺ MAb-treated rat stained with H+E and GMS. A lung section from a CS-treated rat following 8 weeks of treatment is represented in panel A. A lung section from a W3/25 MAb-treated rat following 12 weeks of treatment is represented in panel B. The photos were taken with a Zeiss Axioscope microscope (Carl Zeiss, Inc., Germany) with an attached Spot 2e digital camera (Diagnostic Instruments, Inc., Sterling Heights, Mich.). The larger panels are stained with H+E (magnification, ×200); the inset figures stained with GMS (magnification, ×400). The lower organism burden in the anti-CD4⁺ MAb-treated rat (B). Arrows (insets) indicate cysts; notched arrows (A) indicate macrophages in the vicinity of *Pneumocystis* cysts.

**FIG. 4.** *Pneumocystis* organism burden of Long-Evans rats following W3/25, methylprednisolone, or combined W3/25- methylprednisolone treatment. Rats received either 1.0 mg of W3/25, 4.0 mg of methylprednisolone acetate (Depo-Medrol), or both. Rats were sacrificed after 7 to 9 weeks of treatment. Each data point represents the respective organism burden of one rat. The horizontal bar represents the mean. ***, P < 0.001** compared to steroid-treated controls.

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compared to CS-treated rats suggests that components other than CD4+ cells play an important role in host defenses against Pneumocystis in this model. Components of CD8+ T cells, alveolar macrophages (20), B cells (19), the CD40-CD40 ligand interaction (40), lung surfactant proteins (18), Igs (12), cytokines (24, 42), and chemokines (43) have all been reported in mice and/or rats. Only the CD4+ T-cell contribution to host defense is affected by the anti-CD4+ Abs. While CD4+ reduction or depletion may lead to dysregulation of the immune system (such as CD4+ T-cell–macrophage interactions or cytokine production), the other components in host defense are not directly affected by the anti-CD4+ Abs.

Another potential reason may relate to the level of CD4+ depletion brought on by the anti-CD4+ Abs. In contrast to mice, in which the GKL.5 MAbs used to develop Pneumocystis pneumonia results in no detectable CD4+ cells (31), both MAbs used in the present study did not completely eliminate the CD4+ cells. Although we explored a broad range of doses of W3/25 and a mainstream dose of OX-38 similar to those used by other investigators (9, 30), we found that higher or more frequent doses did not improve the level of CD4+ depletion. The possibility that higher doses of OX-38 or other MAbs could enhance our results will require further investigation. It is also feasible that the pool of CD4+ T cells that remains following anti-CD4+ Ab treatments is sufficient to mount a cellular response to Pneumocystis, as was demonstrated in our ex vivo and in vitro studies.

Despite the different immunosuppressive effects of the Abs used in this study, Pneumocystis susceptibility remained the same following CD4+ molecule downregulation or CD4+ cell depletion. The fact that W3/25 and OX-38 Abs had similar effects in our model suggests that susceptibility to Pneumocystis pneumonia is related not only to the number of CD4+ T cells (25), but also to defects in CD4+ function. Support for this idea comes from the occurrence of Pneumocystis pneumonia in knockout mice that lack TCR function (13) or experience interruption of the CD40-CD40L pathway (22). On the other hand, our study has also shown that W3/25 has some cell-depleting properties in the spleen.

The new rat model of Pneumocystis pneumonia described here offers a number of advantages over the CS-treated model. Since the anti-CD4+ MAbs result in less debilitation than CS, longer experiments can be conducted. The new model permits the study of antibodies and other factors involved in host defenses against Pneumocystis without the confounding effects of corticosteroids. The CD4+ model will now permit comparison of the effects of another form of immunosuppression on Pneumocystis antigenic expression and variation, as well as on the efficacy of anti-Pneumocystis drugs in rats. Finally, the new model may be helpful in studying other opportunistic infections that are better suited for rat than mouse models.

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REFERENCES


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