Use of Anti-CD4⁺ Hybridoma Cells To Induce Pneumocystis carinii in Mice

DIANE C. McFADDEN,† MARY ANN POWLES,JEFFREY G. SMITH,AMY M. FLATTERY,KEN BARTIZAL,DENNIS M. SCHMATZ
Parasite Biochemistry and Cell Biology and Antibiotic Discovery and Development,Merck Research Laboratories,Rahway,New Jersey 07065-0900

Received 14 March 1994/Returned for modification 18 May 1994/Accepted 6 August 1994

A reduction in peripheral CD4⁺ cell levels has been correlated with the onset of Pneumocystis carinii pneumonia in AIDS patients. Most in vivo drug discovery and development for P. carinii have been conducted in corticosteroid-treated rats. There is need for the development of new small animal models with more selective methods of immunosuppression. This study outlines a new mouse model in which specific depletion of the CD4⁺ T-lymphocyte population was achieved by subcutaneous injection of GK1.5 hybridoma cells into C3HeB/FeJ mice. A significant reduction in splenic CD4⁺ cells was maintained over a 10-week period following a single injection of cells. Circulating anti-CD4⁺ antibody was detected throughout the 10-week period in hybridoma-injected mice, while circulating antibody was undetectable 4 weeks after repeated injection of purified monoclonal antibody. There was no significant increase in the CD8⁺ cell populations of the hybridoma-injected mice. P. carinii cysts increased in the lungs of CD4⁺ T-cell-depleted mice, with the number of cysts detected comparable to levels in dexamethasone-treated mice. High levels of cysts were detected when CD4⁺ cell populations in the spleen remained below 5% and decreased when CD4⁺ populations increased above the 5% level. In mice whose CD4⁺ population was not reduced below 5%, there was no significant increase in P. carinii cysts detected. This study presents a new mouse model with specific immunosuppression requiring a minimum of animal manipulation for use in discovery and development of potential new therapeutics for P. carinii pneumonia.

Pneumocystis carinii is an opportunistic pathogen that causes a fatal pneumonia in immunocompromised patients if left untreated. It is characterized by the presence of P. carinii organisms and an eosinophilic infiltrate within the lung alveoli. The incidence of P. carinii pneumonia (PCP) has become more common as a result of the AIDS epidemic, and it has been found that there is a correlation between peripheral CD4⁺ T-lymphocyte levels in AIDS patients and the onset of PCP (15). As a result, CD4⁺ T-cell levels are monitored in patients diagnosed as human immunodeficiency virus positive to determine whether they are at risk of developing PCP. In most cases, patients with CD4⁺ levels below 200/mm² are put onto prophylactic regimens (21). Since agents currently used to treat and prevent PCP have a high incidence of adverse reactions, especially in AIDS patients, new chemotherapeutics with improved safety and efficacy are still needed. Animal models are required for the discovery and development of these potential new therapeutics.

In vitro propagation of P. carinii has proven difficult, and most research has been conducted in vivo, largely in corticosteroid-treated rats. While this model has proven reliable and predictive of drug efficacy in humans (12, 13), it depends on the use of nonspecific immunosuppressants and often requires large quantities of test compound, which in many cases is restrictive in the early stages of drug discovery. A mouse model for PCP would offer advantages over the rat model, since much less test compound would be needed and more animals could be included in test groups. In addition, the variety of inbred mouse strains currently available would provide opportunities to conduct epidemiological and immunological studies which cannot be achieved in the rat.

Initial studies undertaken by Walzer et al. (24), conducted with various strains of chemically immunosuppressed mice, found the level of P. carinii infection highly variable. More recent models achieve consistent and predictable P. carinii infections by inoculating mice with infected lung tissue (2, 8, 25) or through cohabitation with P. carinii-infected mice (16). However, these models still require immunosuppression with corticosteroids. This nonspecific immunosuppression predisposes animals to a high incidence of other opportunistic infections and may have metabolic side effects, resulting in a reduction in survival time. In addition, it is likely that corticosteroids also limit the extent of pneumonia as a result of their anti-inflammatory effects, and therefore these models may not be true representations of PCP. The use of corticosteroids to overcome respiratory distress in patients with acute PCP has been reported (22).

An alternative approach to corticosteroid immunosuppression is to selectively deplete mice of CD4⁺ T cells. The first indications that T cells were involved in the development of PCP in mice became apparent when spontaneous PCP outbreaks occurred in severe combined immunodeficiency and athymic mouse colonies (17, 18, 20, 23). Specific evidence of CD4⁺ cell involvement in host defense against P. carinii was supported by studies in which an anti-mouse CD4⁺ monoclonal antibody (MAb) was injected weekly to passively deplete CD4⁺ cells, resulting in P. carinii infections (19). Selective depletion of CD4⁺ cells from immunocompetent mice provides a model for studying PCP which more closely mimics AIDS. The disadvantage of this type of model is the require-
ment for sequential injections of antibody to ensure *P. carinii* development.

The work presented here describes an alternative model for PCP in which mice are also selectively depleted of CD4+ cells. Mice are injected subcutaneously (s.c.) with the GK1.5 hybridoma cells (14) which produce a rat anti-mouse CD4+ MAb (6, 7, 26). Hybridoma cells apparently become established in the mouse and continue to produce anti-CD4+ antibody, resulting in the development of PCP. This eliminates the need to continuously inject antibody to suppress CD4+ levels and provides an alternative model for the discovery and development of new therapeutics for PCP.

**MATERIALS AND METHODS**

**Preparation of GK1.5 cells.** GK1.5 hybridoma cells (ATCC TIB207) were cultured in high-glucose (4.5 g/liter) Dulbecco's modified Eagle's medium (Sigma Chemical Co., St. Louis, Mo.) supplemented with 10% heat-inactivated fetal bovine serum (Sigma), 100 mM L-glutamine (Sigma), 100 U of penicillin (Sigma) per ml, and 100 μg of streptomycin (Sigma) per ml at 37°C under 5% CO2. Cells were passaged and incubated for log-phase growth. Cultures were harvested and centrifuged for 8 min at 450 × g to pellet cells. Cells were washed twice and counted on a hemocytometer. The viability of cell suspensions used for injection into mice was greater than 95%, as measured by trypan blue dye exclusion.

**Production and purification of anti-CD4+ MAb.** Outbred athymic, nu/nu, Swiss Webster mice (Taconic, Germantown, N.Y.) were used for ascites production of anti-CD4+ MAb. Mice were primed by intraperitoneal (i.p.) injection with 0.5 ml of pristane (Sigma). Ten days after pristane priming, mice were injected i.p. with 5 × 10^6 GK1.5 hybridoma cells, prepared as described above.

Ascites fluid collected from mice was centrifuged for 10 min at 450 × g to remove cellular debris and stored frozen at −20°C. Antibody was purified from ascites by passage over a protein G column (MABTrap G kit; Pharmacia LKB, Piscataway, N.J.). Immunoglobulin G (IgG) was collected and dialyzed in a Spectra/Per membrane dialysis bag (10,000 molecular weight cutoff) against phosphate-buffered saline (PBS; Sigma). The concentration of antibody was quantitated by using a Bio-Rad protein assay (Bio-Rad Laboratories, Melville, N.Y.) titrated against a gamma globulin protein standard.

**In vivo study design.** Male C3HeB/FeJ mice (Jackson Laboratories, Bar Harbor, Maine) were housed in standard microisolator cages (Lab Products Inc., Maywood, N.J.) and were provided 23% protein rodent chow (Purina, St. Louis, Mo.) and either tap water or medicated water containing 4 mg of dexamethasone (Butler, Columbus, Ohio) and 1 g of tetracycline (Vedco, St. Joseph, Mo.) per liter ad libitum.

Mice receiving tap water were either injected s.c. over the hind quarter with various amounts of GK1.5 hybridoma cells in 0.5 ml of Dulbecco's modified Eagle's medium on day 0 or injected i.p. with 0.5 mg of purified anti-CD4+ MAb per 0.5 ml of Dulbecco's modified Eagle's medium on days 0 and 7 of the study.

Mice were euthanized by CO2 inhalation at weekly intervals through week 6 and bimonthly thereafter to week 10. Five animals per group per time point were evaluated for splenic CD4+ (L3T4+), CD8+ (Lyt-2+), and Thy1.2+ cells and for pulmonary *P. carinii* cysts. One animal from each of five cages per group was sampled at each time point to account for possible intergroup differences in *P. carinii* infection.

**Fluorescence-activated cell sorter (FACS) analysis.** Spleens were removed and teased apart by using the frosted end of a glass microscope slides in 3 ml of PBS (Gibco, Grand Island, N.Y.). Cells were centrifuged for 5 min at 400 × g. The supernatant was decanted and cells were resuspended in 1 ml of ACK lysing buffer (Gibco) to lyse erythrocytes, diluted in 3 ml of PBS, and centrifuged for 5 min at 400 × g. Cells were resuspended in 1 ml of PBS, and 100 μl was either double stained with 10 μl (containing 1 μg) of fluorescein isothiocyanate-conjugated L3T4+ (CD4+) MAb (PharMingen, San Diego, Calif.) and 10 μl (containing 1 μg) of R-phycocerythrin-conjugated Lyt-2+ (CD8a+) MAb (PharMingen) or single stained with 10 μl (containing 1 μg) of fluorescein isothiocyanate-conjugated Thy 1.2+ MAb (PharMingen). The cells and labeled antibody were incubated for 30 min at room temperature. Samples were diluted with 3 ml of PBS and centrifuged at 400 × g for 5 min. The supernatant was decanted and cells were resuspended in 200 μl of propidium iodide (1 μg/ml; Sigma). Samples were analyzed on a FACSscan analyzer (Becton Dickinson, San Jose, Calif.).

**P. carinii cyst determination.** Lungs from mice were excised, and the number of *P. carinii* cysts per lung was determined as previously described (16). The limit of detection with this method was 10^3.6 *P. carinii* cysts.

**ELISA.** A sandwich enzyme-linked immunosorbent assay
TABLE 1. Rat anti-mouse IgG, detected by ELISA, in sera of mice following injection of either purified GK1.5 MAb or hybridoma cells a

<table>
<thead>
<tr>
<th>Wk post-injection</th>
<th>MAb treatment</th>
<th>5 x 10^6 GK1.5 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of animals positive for antibody/no. tested</td>
<td>Mean antibody titer ± SEM (ng/ml)</td>
</tr>
<tr>
<td>1</td>
<td>5/5</td>
<td>55.09 ± 2.06</td>
</tr>
<tr>
<td>2</td>
<td>5/5</td>
<td>76.76 ± 4.86</td>
</tr>
<tr>
<td>3</td>
<td>5/5</td>
<td>20.05 ± 6.61</td>
</tr>
<tr>
<td>4</td>
<td>2/5</td>
<td>4.27 ± 0.58</td>
</tr>
<tr>
<td>5</td>
<td>0/5</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>6</td>
<td>0/5</td>
<td>651.2</td>
</tr>
<tr>
<td>8</td>
<td>NT b</td>
<td>4/5</td>
</tr>
<tr>
<td>10</td>
<td>NT</td>
<td>4/5</td>
</tr>
</tbody>
</table>

a Mice received either 500 µg of purified antibody i.p. on days 0 and 7 or 5 x 10^6 cells s.c. on day 0.

b NT, not tested.

(ELISA) was performed to measure rat anti-mouse IgG antibody present in the sera of mice receiving MAB or GK1.5 cells. Immulon II 96-well microtiter plates (Dynatech, Chantilly, Va.) were coated overnight at 4°C with 100 µl per well of 1 µg of rabbit anti-rat IgG (Pel-Freeze Biologicals, Rogers, Ark.) per ml in pH 9.6 bicarbonate buffer. Plates were blocked with a 1% bovine serum albumin (ICN Biochemicals, Cleveland, Ohio) solution, incubated for 30 min at 37°C, and washed three times with 0.05% Tween 20–PBS. Rat IgG whole molecule (Pel-Freeze Biologicals) was used for the standard curve. Serum samples were added to microtiter plates and serially diluted (twofold). Following a 1- to 2-h incubation at 37°C, plates were washed three times and 100 µl of goat anti-rat IgG conjugated to alkaline phosphatase (FisherBiotech, Pittsburgh, Pa.) was added to each well. Plates were incubated for 1 h at 37°C, and then 100 µl per well of a 1-mg/ml nitrophenyl phosphate solution was added to each well. After 20 min at room temperature, the color reaction was stopped with 0.75 N NaOH and the A405 was read.

Statistics. The Student t test for sample groups with unequal variances was used for analysis of the FACS results.

RESULTS

Effect of GK1.5 MAB on CD4+ cells. The MAB, whether produced in vivo by s.c. injected GK1.5 hybridoma cells at levels of 5 x 10^6 and 10^7 or administered as purified MAB, resulted in a selective depletion of CD4+ lymphocytes and a decrease in overall Thy1.2+ cells in C3HeB/FeJ mice as determined by FACS analysis. The FACS analysis data for the 5 x 10^6 hybridoma cell level and purified antibody groups are presented in Fig. 1. There was no significant effect on CD8+ lymphocytes in any of the three experimental groups.

ELISA results. An ELISA was performed to assess the amount of rat anti-mouse antibody detectable in the sera of mice injected with either MAB or 5 x 10^6 GK1.5 hybridoma cells. Table 1 shows that the level of antibody present in the MAB-treated group increased from 5.59 ± 2.06 ng/ml at week 1 to 76.76 ± 4.86 ng/ml at week 2 following the second MAB injection. Detectable MAB gradually decreased from circulation, being detected only at low levels in two of five mice tested during week 4 of the study, and was completely undetectable in all mouse sera tested in weeks 5 and 6 of the study.

ELISA results for the group that received a single injection of 5 x 10^6 GK1.5 cells demonstrated an increase in the amount of circulating rat antibody over time (Table 1). One week after the injection of cells, the amount of circulating antibody (38.90 ± 6.12 ng/ml) was lower than in the mice that received the injection of purified MAB (55.09 ± 2.06 ng/ml). However, by week 2 the GK1.5 hybridoma cell-injected group had levels of circulating rat antibody similar to those measured in the sera of mice that had received both doses of MAB (80.27 ± 15.53 ng/ml in the hybridoma mice compared with 76.76 ± 4.86 ng/ml in the antibody-treated mice). By week 3, antibody levels in the 5 x 10^6 GK1.5 cell group continued to increase (123.37 ± 56.30 ng/ml), while levels in the MAB-treated group had begun to decrease (20.05 ± 6.61 ng/ml). The variability in circulating rat antibody was greater in the 5 x 10^6 GK1.5 cell group than in the MAB-treated group, and at some time points only some of the mice had detectable antibody. Most animals with any level of rat antibody detected by ELISA had less than 1% CD4+ cells as measured by FACS analysis. The 4- and 6-week time points, when antibody was detected in only some of the mice, corresponded with increases in the CD4+ T cells (see Fig. 4), and at weeks 5 and 8 extremely high levels of antibody (>1,000 ng/ml), accompanied by reduced CD4+ splenic T-cell populations, were found. At week 10, a reduction in detectable antibody in the sera of mice (127.37 ± 61.17 ng/ml), accompanied by increasing CD4+ splenic T cells, was found.

MAB treatment. The administration of two injections of 0.5 mg of antibody 1 week apart rapidly decreased the level of CD4+ T cells in C3HeB/FeJ mice (Fig. 2). Administration of purified antibody resulted in a significant (P < 0.001) reduction 1 week after the first injection, with less than 1% CD4+ cells detected in the spleens of all mice sampled. Non-antibody-treated C3HeB/FeJ mice had an average of 23% ± 1.29% CD4+ spleen cells. Thus, the MAB treatment depleted greater than 95% of the CD4+ cell population. Although all mice sampled up to week 4 of the study had less than 1% CD4+ cells, CD4+ cells gradually increased during the subsequent weeks of the study. The reduced CD4+ T-cell levels, while increasing, remained significantly (P < 0.001) depressed below normal levels throughout the entire study period.

The course of P. carinii infection was quantitated in conjunction with FACS analysis (Fig. 2). While the percentage of CD4+ cells was significantly reduced 1 week after the first injection (day 0) of MAB, P. carinii was not detected in the...
lungs of mice. After a second injection of purified MAb on day 7, \textit{P. carinii} cysts were detected in the lungs of mice at week 2 of the study (log mean, 4.66 ± 0.183). During subsequent weeks, without further MAb injections, the number of \textit{P. carinii} cysts did not continue to increase significantly. Between weeks 8 and 10, \textit{P. carinii} was successfully cleared by the animals, and few cysts were detected at week 10 (log mean, 3.45 ± 0.10).

**Injection of GK1.5 cells** s.c. Injection of \(10^7\) GK1.5 hybridoma cells resulted in significant depletion of CD4+ cells in the spleen and detectable levels of \textit{P. carinii} cysts in the lungs (Fig. 3). The five mice sampled at week 1 had a mean of 0.2% ± 0.0% CD4+ T cells, a significant reduction \((P < 0.001)\) from the normal level of 23% ± 1.29%. The percent CD4+ cells remained significantly reduced \((P < 0.001)\) throughout the 10 weeks of study. The levels of CD4+ spleen cells remained below 1% through week 5 of the study but gradually increased during the later time periods. By week 10, the mice still had significantly reduced CD4+ cell levels (7.4% ± 1.7%).

\textit{P. carinii} cysts were not detected in the lungs of mice until 3 weeks after the cells were injected (log mean, 3.99 ± 0.31) (Fig. 3). From weeks 3 to 8, the level of \textit{P. carinii} cysts increased to a peak log mean value of 5.37 ± 0.29. The log mean number of cysts at week 10 (log mean, 4.97 ± 0.43) had decreased, though not significantly, compared with the levels of infectivity measured at the 6 (log mean, 5.35 ± 0.34)- and 8 (log mean, 5.12 ± 0.29)-week time points.

Injection of \(5 \times 10^6\) GK1.5 hybridoma cells s.c. resulted in significant depletion of CD4+ cells in the spleen and detectable levels of \textit{P. carinii} cysts in the lungs (Fig. 4). This level of hybridoma cells significantly reduced \((P < 0.001)\) the CD4+ population to 0.2% ± 0.0% 1 week following injection. The percent CD4+ cells remained undetectable until week 4, when they rose to 3.0% ± 1.7%, still significantly reduced \((P < 0.001)\) from controls; however, the CD4+ cells were again completely undetectable in the spleens by week 5. This fluctuation in percent CD4+ cells continued throughout the study, with a rise in the level at week 6 (3.8% ± 1.9%), reduction in week 8 (0.2% ± 0.1%), and a third rise in the splenic CD4+ population at week 10 (2.6% ± 2.4%). CD4+ levels in the spleen were significantly reduced \((P < 0.001)\), compared with controls, at all time periods tested and levels did not rise above 4% throughout the 10-week study period.

\textit{P. carinii} cysts were detected by week 3 (log mean, 4.02 ± 0.17) and steadily increased over time (Fig. 4), with maximum numbers of cysts detected 8 weeks (log mean, 5.96 ± 0.03) after the injection of hybridoma cells. Tissue load at 8 weeks in this group was greater than the peak cyst load measured in any of the other experimental groups, except in the dexamethasone-treated group (log mean, 5.91 ± 0.13), in which the peak cyst load was similar. A slight decrease from the 8-week level was measured at 10 weeks (log mean, 5.46 ± 0.36) but still represented a significant infection. The level of \textit{P. carinii} cysts in this group was more variable over time than in the \(10^7\) hybridoma cell group. \textit{P. carinii} cyst levels corresponded with fluctuations observed in the CD4+ T-cell population.

The CD4+ T-cell population in the spleens of mice injected s.c. with \(10^6\) GK1.5 cells was only partially decreased (Fig. 5) but still represented a significant reduction \((P < 0.01)\) from controls at all time points. One week after injection, mice had a mean CD4+ spleen cell count of 5.2% ± 1.5%. The CD4+ cells remained between 5 and 10% through week 5 of the study and gradually began to approach normal levels by week 10 (18.7% ± 0.8%). The CD4+ spleen cell population did not fall below 5% at any time point during the study period.

**FIG. 3.** FACS analysis of CD4+ spleen cells and \textit{P. carinii} cysts (PC) in the lungs of C3HeB/FeJ mice following s.c. injection of \(10^7\) GK1.5 hybridoma cells.

**FIG. 4.** FACS analysis of CD4+ spleen cells and \textit{P. carinii} cysts (PC) in the lungs of C3HeB/FeJ mice following s.c. injection of \(5 \times 10^6\) GK1.5 hybridoma cells.

**FIG. 5.** FACS analysis of CD4+ spleen cells and \textit{P. carinii} cysts (PC) in the lungs of C3HeB/FeJ mice following s.c. injection of \(10^6\) GK1.5 hybridoma cells.
**P. carinii** infection occurred with the injection of $10^6$ GKL.5 cells (Fig. 5). **P. carinii** cysts were detected in the lungs of mice at week 2 (log mean, $3.58 \pm 0.09$). The log mean of **P. carinii** cysts in lungs of mice in this group did not rise above 4.2 at any time point tested during the study.

**Dexamethasone treatment.** Continued administration of dexamethasone in the drinking water resulted in the development of **P. carinii** infection (Fig. 6). Cysts were detected 1 week (log mean, $3.50 \pm 0.14$) after initiation of treatment and increased to $3.85 \pm 0.33$ by week 3. The level of **P. carinii** cysts continued to increase and reached a log mean of $6.01 \pm 0.09$ by week 10 of the study. FACs analysis was not conducted for this experimental group because the dexamethasone treatment resulted in small spleens with large numbers of dead cells and accurate FACs analysis was not possible.

**DISCUSSION**

These studies were undertaken to develop a new small animal model for PCP with a specific deficiency in CD4+ T-lymphocyte function with minimum animal manipulation. GKL.5 hybridoma cells are well known for the production of a rat IgG2b MAb directed against mouse L3T4+ (CD4+) T lymphocytes (6, 7, 26). This MAb has been used to deplete CD4+ cells in many different animal models of infectious disease (1, 4, 5, 9, 11, 19). Injection of the GKL.5 MAb has provided significant evidence for the critical role of CD4+ T lymphocytes in protection from and clearance of **P. carinii** (3, 10). Selective depletion of murine CD4+ cells provides a mouse model with advantages over the commonly used corticosteroid-treated rat model and more closely represents the clinical situation. This new mouse model provides an alternative to those previously described and eliminates the need for frequent MAb injections. GKL.5 hybridoma cells injected once provide continuous MAb secretion that depletes CD4+ cells in vivo.

Injection of hybridoma cells specific to T-cell subsets offers a novel approach for specific immunosuppression of the animal host. Two key issues arose in this experiment. First, since the cells injected were viable, the development of s.c. tumors was a distinct possibility. However, tumors were not readily apparent in any of the mice in this study at the cell levels injected. It may have been that small tumors did arise but were not observed. Second, mice may recognize the hybridoma cells as non-self and then mount an immune response and subsequently reject them. A slight, but not significant, rise in CD8+ T cells was observed in the mice after injection of hybridoma cells, suggesting that a cytotoxic immune response to the tumor cells was mounted. A continued high level of circulating antibody detected through 10 weeks in these mice would indicate that some of the hybridoma cells had survived and were still secreting antibody. Circulating anti-CD4+ antibody in the sera of the MAb-injected mice decreased over time and was undetectable in all mice tested by 4 weeks after the final injection. Since a reduction in circulating antibody would be expected on the basis of antibody half-life but was not observed at any time point in the hybridoma-injected mice, this would imply that the hybridoma cells were not completely eliminated and continued to secrete antibody. However, there is no direct evidence of cell survival or clearance from the mice throughout the period of study.

In a similar study, when hybridoma cells were injected into BALB/c mice, depletion of CD4+ T cells and detectable circulating antibody were both much shorter in duration than in the C3HeB/FeJ mice (data not shown). The observed rise in splenic CD8+ T cells was also greater in the BALB/c mice following hybridoma injection. The reasons for the observed differences between the mouse strains remain unexplained at this time. Selection of the C3HeB/FeJ mouse was important for the functional success of the hybridoma cells in this model, resulting in significant reduction of CD4+ T-cell levels over an extended period of time.

When using monoclonal MAbs directed against cell markers for specific cell depletion, it is important to determine whether the observed reduction is an actual depletion of the cells or just blockage of the antigenic binding site for that marker. FACs analysis for Thyl.2+, CD4+, and CD8+ T lymphocytes was performed to address this issue (Fig. 1). Thyl.2+ T-cell numbers in mice from all groups, hybridoma or MAb injected, paralleled CD4+ T-cell decreases and increases. CD8+ T-cell percentages remained at normal or marginally above normal levels throughout the study. If the CD4+ antigenic marker was just being blocked, there would have been no observed decrease in percent total Thyl.2+ T cells, with the CD8+ T-cell percentage remaining essentially unchanged. Therefore, CD4+ T-cell levels were eliminated from the circulation by the MAb being either injected or secreted.

Overall, the degree of **P. carinii** infection as measured by cyst levels parallels the absence of CD4+ cells. The $10^7$ and $5 \times 10^6$ hybridoma cell levels adequately depleted CD4+ T cells with increasing **P. carinii** cysts in the lungs. Both hybridoma cell concentrations maintained CD4+ T cells at levels below 5% throughout 8 weeks of study, with the mice in the $5 \times 10^6$ cell group remaining below 5% CD4+ throughout the study. The level of **P. carinii** obtained in the $5 \times 10^6$ cell group was comparable to that in the dexamethasone-treated mice, while the **P. carinii** cyst level in the $10^7$ cell group was approximately 0.5 log lower. The level of 5% CD4+ T cells would appear to be a critical functional level for **P. carinii** infection in these mice. Mice receiving either MAb or $10^7$ or $10^6$ hybridoma cells exhibited **P. carinii** cysts during the period when CD4+ T-cell numbers remained below the 5% level. When CD4+ T-cell levels rose above 5%, the number of **P. carinii** cysts detected was reduced. There did appear to be a minimum level of hybridoma cells required to adequately deplete CD4+ T-cell function, allowing PCP development. In the $10^6$ hybridoma cell group, the number of splenic CD4+ T cells was not observed to fall below 5% at any time during the course of study and no significant increase in cysts was observed. The CD4+ T-cell level in the $10^7$ hybridoma cell group was observed to increase.

![Figure 6](http://iai.asm.org)
above 5% at 10 weeks after injection along with a concomitant
decrease in P. carinii cysts.

In this model, we were able to selectively deplete CD4+ cells
from immune competent mice without the use of broad-
spectrum immunosuppressants or frequent injection of anti-
body. The level of P. carinii infection achieved was similar to
that attained by dexamethasone treatment. This represents a
novel mouse model which more closely mimics the immuno-
logical predisposition of human immunodeficiency virus- posi-
tive patients at risk of developing PCP.

ACKNOWLEDGMENT

We acknowledge Anna Sirota for the excellent technical assistance
provided for the FACS analysis.

REFERENCES

prevents development of resistance to Toxoplasma gondii in mice.

2. Bartlett, M. S., S. F. Queener, M. M. Durkin, M. A. Shaw, and

1993. Host defenses against Pneumocystis carinii in mice selectively
depleted of CD4+ lymphocytes. Chest 103:116S–118S.


1989. Role of L3T4+ lymphocytes in protective immunity to
systemic Candida albicans infection in mice. Infect. Immun. 57:
3581–3587.

6. Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans,
M. R. Loken, M. Pierres, and F. W. Fitch. 1983. Characterization of
the murine T cell surface molecule, designated L3T4, identified by
monoclonal antibody GK1.5: similarity of L3T4 to the human

W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and
F. W. Fitch. 1983. Characterization of the murine antigenic determinant,
designated L3T4a, recognized by monoclonal antibody GK1.5:
expression of L3T4a by functional T cell clones appears to
correlate primarily with class II MHC antigen-reactivity. Immunol.
Rev. 74:29–56.

Cellular and humoral responses of mice subclinically infected with

1988. Role of L3T4+ cells in host defense against Histoplasma capsulatum.

CD4+ cells in resistance to Pneumocystis carinii pneumonia in

to a pulmonary Cryptococcus neoforms infection requires both
CD4+ and CD8+ T cells. J. Exp. Med. 173:793–800.

in the prevention of Pneumocystis carinii pneumonia. Antimicrob.

1974. Efficacy of trimethoprim-sulfamethoxazole in the prevention
Agents Chemother. 5:289–293.

Pneumocystis carinii pneumonia, abstr. 6. 8th International
Congress of Immunology, Budapest, Hungary.

15. Phair, J., A. Monoz, R. Detels, R. Kaslo, C. Rinaldo, A. Sanh, and
the Multicenter AIDS Cohort Study Group. 1990. The risk of
Pneumocystis carinii pneumonia among men infected with human

1992. Mouse model for Pneumocystis carinii pneumonia that
uses natural transmission to initiate infection. Infect. Immun. 60:
1397–1400.

17. Schultz, L. D., P. A. Schweitzer, E. J. Hall, J. P. Sundberg, S.
Taylor, and P. D. Walzer. 1989. Pneumocystis carinii pneumonia in

survey of Pneumocystis carinii infection in research mouse colonies

19. Shellito, J., V. V. Suara, W. Blumenfeld, J. M. Beck, H. J. Steiger,
infection in mice selectively depleted of helper T lymphocytes. J.

fatal pneumocystosis in nude mice. J. Exp. Med. 147:475–482.

and prophylaxis, p. 439–466. In P. Walzer (ed.), Pneumocystis


23. Walzer, P. D., C. K. Kim, M. J. Linke, C. L. Pogue, M. J.
Huerkamp, C. E. Chrisp, A. V. Lerro, S. K. Wixon, E. Hall, and
L. Schultz. 1989. Outbreaks of Pneumocystis carinii pneumonia in

Pneumocystis carinii pneumonia in different strains of cimtized


Fitch. 1983. Evidence implicating L3T4 in class II MHC antigen
reactivity; monoclonal antibody GK1.5 (anti-L3T4a) blocks class
II MHC antigen-specific proliferation, release of lymphokines, and
binding by cloned murine helper T lymphocyte lines. J. Immunol.
131:2178–2183.