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Active Immunity to *Pneumocystis carinii* Reinfection in T-Cell-Depleted Mice

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Immunocompetent C.B-17 mice were immunized against *Pneumocystis carinii* by several intratracheal inoculations with infective *P. carinii*. These mice and another group of C.B-17 mice naive to *P. carinii* were then depleted of $CD4^+$ cells by treatment with both anti-CD4 and anti-Thy1 monoclonal antibodies. Both groups of mice were then challenged with an infective inoculum containing 10^7 *P. carinii* organisms by intratracheal instillation. The mean $\log_{10}$ counts of *P. carinii* nuclei in the lungs of the nonimmune mice were 4.98, 5.89, and 6.77 when they were killed at 4, 10, and 19 days, respectively, after challenge. The *P. carinii* counts in the lungs of the immune mice were significantly lower at each time point and below detectable levels at 10 and 19 days. Analysis of *P. carinii* DNA by PCR revealed no detectable *P. carinii* in the lungs of the immunized mice at either 10 or 19 days, whereas all of the nonimmunized mice contained *P. carinii* DNA at all time points. The sera of immune but not nonimmune mice contained *P. carinii*-specific immunoglobulin G. These results indicate that immunization of an immunocompetent host against *P. carinii* can protect against *P. carinii* pneumonia even after the host is depleted of $CD4^+$ cells. In addition, the results are consistent with the possibility that antibodies were responsible for the observed protection against *P. carinii*.

That the majority of the human population have antibodies to *Pneumocystis carinii* indicates that most persons have a history of previous exposure to this organism (21). This suggests that *P. carinii* is a ubiquitous organism and that many immunocompromised patients are not immunologically naive to it. Despite this observation, many immunocompromised patients, especially those with AIDS, develop *P. carinii* pneumonia (PCP). Therefore, natural exposure to *P. carinii* is probably not sufficient to protect certain immunodeficient patients from developing PCP. However, if it was possible to augment an immunocompromised patient's immunity to *P. carinii*, this might afford protection, at least for a limited period of time. This might be sufficient, for example, to carry a patient with cancer through the period of chemotherapy or to transiently protect an AIDS patient. One way to augment immunity to *P. carinii* would be through active immunization.

The concept of immunizing an immunocompromised patient, while seemingly counterintuitive, has been explored for other microorganisms. Children receiving chemotherapy for acute lymphocytic leukemia have been shown to develop protective levels of antibody to *Haemophilus influenzae* type b after immunization with a polysaccharide conjugate vaccine (8). Vaccine response was best in those children vaccinated early in the course of their chemotherapy. Response to bacterial polysaccharides has also been studied in patients infected with human immunodeficiency virus (17, 25). While the response to vaccination of human immunodeficiency virus-infected patients was less than that observed in healthy control patients, the majority of patients immunized with *H. influenzae* type b vaccines did achieve an antibody concentration generally considered to be protective. Vaccine efficacy has been demonstrated for an attenuated varicella vaccine in children immunized after completing induction chemotherapy for acute leukemia (9). Thus, the potential to protect at-risk patients from PCP by active immunization exists.

Previous attempts to demonstrate that animals can be protected from PCP by active immunization have been unsuccessful. Hughes et al. (16) immunized rats against *P. carinii* by injections of mixtures of *P. carinii* cysts and Freund's adjuvant. Although the rats developed significant serum antibody titers to *P. carinii*, all of the animals became infected with *P. carinii* upon treatment with cortisone acetate. However, it is possible that the broad-spectrum immunosuppression induced with the cortisone acetate inhibited immune mechanisms that would not be affected by a treatment that specifically depleted $CD4^+$ lymphocytes. Alternatively, the route of immunization may not have been the correct route for the induction of optimal resistance.

The objective of this study was to determine whether immunity to PCP acquired by mice while immunocompetent is persistent when the animals are depleted of $CD4^+$ lymphocytes. The approach used was to immunize immunocompetent C.B-17 mice against *P. carinii* by intratracheal inoculations of infective *P. carinii*. The susceptibilities of *P. carinii*-immune and *P. carinii*-naive C.B-17 mice to a *P. carinii* challenge were then determined after the mice were depleted of $CD4^+$ lymphocytes.

MATERIALS AND METHODS

Mice. Six- to 8-week-old C.B-17 +/+ and C.B-17 *scid*/*scid* (severe combined immunodeficient [SCID]) mice were obtained from the Trudeau Animal Breeding Facility (Saranac Lake, N.Y.). SCID mice were bred and housed in microisolator cages containing sterilized food and water and shown to be free of most common pathogens (15). They were infected with *P. carinii* as described previously (4). These mice were then used as a source of *P. carinii* for inoculations and challenge of C.B-17 +/+ mice. The animal studies were approved by the Trudeau Institute Animal Care and Use Committee.

Assessment of *P. carinii* infection. The intensity of the *P. carinii* infection in the mouse lung was determined by counting the number of *P. carinii* nuclei as described previously (7, 15). In brief, the lungs were pushed through a stainless steel screen into Hanks' balanced salt solution (GIBCO, Grand Island, N.Y.), and the resulting suspension was diluted for making cytocentrifuge-prepared

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smears. The smears were stained with Diff Quik (Baxter, Miami, Fla.), and the number of *P. carinii* nuclei per 10 (for heavy *P. carinii* infections) to 30 (for light *P. carinii* infections) oil immersion fields was counted. This number was used to calculate the total nuclei per lung. With this method, $\log_{10}$ 4.00 nuclei per lung represented the detection limit. Some of the lungs were lavaged before lung homogenates were made. We have found that with our method, the few *P. carinii* organisms that are lavaged from the lung do not significantly reduce the counts in the lung homogenates.

***P. carinii*-specific DNA amplification by PCR.** *P. carinii*-specific DNA in clarified boiled lung homogenates of SCID mice under different experimental conditions was amplified by the PCR technique with primers specific for either the *P. carinii* mitochondrial rRNA gene (pAZ102-E, 5′-GATGGCTGTTTCCAA GCCCA-3′; pAZ102-H, 5′-GTGTACGTTGCAAAGTACTC-3′) or surface glycoprotein A [Cys-sense, 5′-AGAG(T/C)AGC(G/C)TG(C/T)TA(T/C)AA(A/G) AA(A/G)GG-3′; Cys-antisense, 5′-ACA(C/T)T(T/G)CTCCTTCAA(C/T)TCA ACACA-3′] as described previously (26). Reactions were done in a volume of 100 μl containing 0.2 mM deoxynucleoside triphosphates, 50 mM KCl, 3 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 1 μM primer, and 2.5 U of AmpliTaq (Perkin-Elmer, Norwalk, Conn.). For the rRNA gene primers, samples were denatured at 94°C for 90 s, annealed at 55°C for 90 s, and extended at 72°C for 120 s for 35 cycles. Conditions were the same for the *P. carinii* glycoprotein A gene primers except that annealing at 60°C and 32 cycles of amplification were performed. Amplified DNA products were separated by agarose gel electrophoresis (1.4% agarose), stained with ethidium bromide, and visualized by UV transillumination. In titer determination experiments, we were able to detect mouse *P. carinii* organisms at a concentration of approximately 10 nuclei per ml of lung homogenates (4).

***P. carinii* inoculation.** The *P. carinii* inoculum was prepared from the lung homogenates of *P. carinii*-infected SCID mice as described previously (11). Immunocompetent mice were inoculated intratracheally with 0.1 ml of the inoculum containing about 1.0×10^8 *P. carinii* nuclei per ml and then received a second inoculation 10 days after the first. Intratracheal inoculations were done as described previously (11). Inocula prepared by this method are typically 70 to 80% viable as determined by the metabolism of fluorescein diacetate (18). That the inocula used in these experiments were viable and infective is indicated by the growth of the inocula in the nonimmune mice depleted of Thy1.2$^+$ cells.

Measurement of *P. carinii*-specific IgG. *P. carinii*-specific immunoglobulin G (IgG) in mouse serum was determined by enzyme-linked immunosorbent assay as described previously (6). Flat-bottom microtitration plates (Flow Laboratories, McLean, Va.) were coated with a mouse *P. carinii* soluble total protein preparation from the lungs of *P. carinii*-infected SCID mice (10 μg of protein per ml). For the preparation of lung protein, the lungs were first homogenized and sonicated. After centrifugation, each supernatant was removed and pushed through a 0.45-μm filter, and the remaining protein was quantified with a commercial kit obtained from Bio-Rad (Richmond, Calif.). Test sera were diluted 1:100 in phosphate-buffered saline–0.05% Tween 20. Controls for this assay included a monoclonal antibody (MAb; 90-3-2B5) specific for mouse *P. carinii* glycoprotein A (10), mouse hyperimmune sera produced by immunizing immunocompetent mice with subcutaneous injections of *P. carinii* preparations and Freund's incomplete adjuvant obtained from Difco (Detroit, Mich.), and normal mouse sera.

In vivo depletion of CD4$^+$ cells and other T-cell subsets. C.B-17 +/+ mice immune or naive to *P. carinii* were depleted of CD4$^+$ cells by treatment with 1.0 mg of anti-CD4 MAb given weekly as an intraperitoneal injection, starting 10 days after the second inoculation with *P. carinii*. Some of the anti-CD4-treated mice also received weekly intraperitoneal injections of anti-Thy1.2 MAb (1.0 mg). All mice remained on antibody treatments for the duration of the experiments. The MAbs to CD4 (clone GK1.5, American Type Culture Collection) and Thy1.2 (clone 30H12, American Type Culture Collection) are rat-mouse hybridomas that secrete rat IgG2b capable of lysing cells bearing the respective surface antigens. Detailed descriptions of the preparation of these antibodies and their in vivo activities have been reported previously (5, 14, 15).

The extent of cell depletion was determined by cytofluorometric analysis of cells obtained by lung lavage (13) and tracheal bronchial lymph node cells prepared from individual mice obtained as described previously (15). Total cell numbers in the lavage fluids or lymph node suspensions were determined with a hemocytometer. The cells were stained with fluorochrome-conjugated F(ab′)$_2$ fragments of anti-Thy1.2, anti-CD4, and anti-CD8 or anti-mouse immunoglobulins obtained from Cappel Laboratories (Westchester, Pa.) as well as anti-B220 from PharMingen (San Diego, Calif.) and anti-rat IgG from Sigma. Simultaneous three-color staining was done with fluorescein isothiocyanate-conjugated anti-CD4 and phycoerythrin-conjugated anti-CD45RB together with biotinylated anti-CD44 and Streptavidin-Cy-chrome obtained from PharMingen. Cells were analyzed on a FACScan cytofluorometer with the LYSIS II software from Becton Dickinson (Sunnyvale, Calif.). Total numbers of lymphocytes of specific phenotypes were then calculated from the total number of cells in lung lavages or lymph nodes and the percentage of stained cells as determined by fluorescence-activated cell sorter (FACS) analysis.

Statistics. The significance of difference in means of *P. carinii* or cell numbers for groups of mice was calculated by Student's *t* test. Differences with *P* values of less than 0.05 were considered significant.

TABLE 1. Numbers of lymphocytes in lungs of *P. carinii*-immunized or -naive mice 4 days after challenge with *P. carinii*: effect of treatment with anti-CD4 MAb or anti-CD4 plus anti-Thy1.2 MAbs

Mouse type and treatment[a]	No. of cells in lung lavage fluids (10^4)[b]			
	CD4$^+$	CD4$^+$ CD45^{low}	CD4$^+$ CD44^{high}	CD4$^+$ CD45^{low} CD44^{high}
Immune; CD4 MAb	4.8 ± 3.2	3.8 ± 3.0	2.0 ± 1.6	1.7 ± 1.2
Naive; CD4 MAb	0.5 ± 0.5	<0.05[c]	0.4 ± 0.4	<0.05
Immune; CD4 plus Thy1.2 MAb	<0.05	<0.05	<0.05	<0.05
Naive; CD4 plus Thy1.2 MAb	<0.05	<0.05	<0.05	<0.05

[a] *P. carinii*-immune or -naive mice were treated with either CD4 MAb or CD4 and Thy1.2 MAbs beginning 15 days before challenge with *P. carinii.*
[b] Values are means ± standard deviations (n = five mice).
[c] The limit of detection was considered to be 0.05×10^4 because a smaller number of cells could not be distinguished from background staining.

RESULTS

In vivo depletion of CD4$^+$ cells with anti-CD4 MAb. Mice that were immunized by inoculations of *P. carinii* were treated with anti-CD4 MAb beginning 10 days after the second *P. carinii* inoculation. Mice naive to *P. carinii* were started on the same treatment simultaneously. After an additional 15 days, all of the mice were challenged with 10^7 *P. carinii* nuclei given intratracheally. Mice were killed 4, 10, or 19 days after challenge with *P. carinii*. The numbers of cells of different T-cell subsets in lung lavage fluids of the mice at 4 days after challenge were determined, and the results are shown in Table 1. Despite the infusion of anti-CD4 MAb, the *P. carinii*-immune mice had a significant accumulation of CD4$^+$ cells in their lung alveoli as compared with that of the *P. carinii*-naive mice. Furthermore, most of the CD4$^+$ cells demonstrated the staining characteristics of activated cells (1, 2) in that they were CD45RB^{low}, CD44^{high}, or both CD45RB^{low} and CD44^{high}. Results of FACS analysis of lung lavage cells obtained at 10 days and of tracheal bronchial lymph node cells obtained at either 4 or 10 days after challenge also indicated that the anti-CD4 treatment was not effective in eliminating activated CD4$^+$ cells from the immune mice (data not shown). No significant numbers of B cells were found in the lavage fluids, whereas CD8$^+$ cell accumulation was observed in the lungs (data not shown).

In vivo depletion of CD4$^+$ cells by using anti-CD4 plus anti-Thy1.2 MAbs. In an effort to deplete all CD4$^+$ cells from the immunized mice, the experiment described above was repeated except that the mice were treated with both anti-CD4 and anti-Thy1.2 MAbs. The numbers of cells of different T-cell subsets in lung lavage fluids of the mice at 4 days after challenge with *P. carinii* are shown in Table 1. The addition of

TABLE 2. Effect of immunization on resolution of a challenge with *P. carinii* in CD4$^+$ Thy1.2$^+$ cell-depleted mice: *P. carinii* counts postchallenge

Mouse type	*P. carinii* count ($\log_{10}$) postchallenge[a]		
	4 days	10 days	19 days
Immune	4.10 ± 0.23[b]	<4.0[c]	<4.0[c]
Nonimmune	4.98 ± 0.52	5.89 ± 0.23	6.77 ± 0.29

[a] Values are means ± standard deviations (n = four or five mice).
[b] Significantly different from nonimmune mice, $P < 0.05$.
[c] The limit of detectability by microscopy was 4.0.

TABLE 3. Effect of immunization on resolution of a challenge with *P. carinii* in $CD4^+$ $Thy1.2^+$ cell-depleted mice: number of mice positive for *P. carinii*

Mouse type and primer[a]	No. of mice positive/total no.[b]		
	4 days	10 days	19 days
Immune; gpA	1/5	0/5	0/4
Nonimmune; gpA	5/5	5/5	4/4
Immune; rRNA	2/5	0/5	0/4
Nonimmune; rRNA	5/5	5/5	4/4

[a] gpA, glycoprotein A.
[b] The number of mice positive for glycoprotein A DNA or rRNA DNA of *P. carinii* was measured.

anti-Thy1.2 to the anti-CD4 treatment completely eliminated the accumulation of $CD4^+$ cells in the lungs of the mice. The results of FACS analysis (data not shown) of lung lavage cells obtained at 10 or 19 days and of tracheal bronchial lymph node cells obtained at 4, 10, or 19 days after challenge also indicated that the anti-Thy1.2 and anti-CD4 treatments together depleted $CD4^+$ cells to below detectable numbers. Also, neither B cells nor $CD8^+$ cells were detectable in the lavage fluids (data not shown).

Resolution of *P. carinii* challenge in *P. carinii*-immune and -naive mice depleted of $CD4^+$ $Thy1^+$ cells. The *P. carinii*-immune and -naive mice treated with anti-CD4 and anti-Thy1.2 MAbs were challenged with 10^7 *P. carinii* nuclei by intratracheal inoculation, and groups of mice were killed at 4, 10, or 19 days later. The numbers of *P. carinii* nuclei in the lungs of these mice are shown in Table 2 and represent results of one of two experiments that had similar results. Although 10^7 *P. carinii* nuclei were inoculated into the mice, only about 10^5 were found in the nonimmune mice after 4 days. However, the *P. carinii* then began to grow in the nonimmune mice as indicated by mean *P. carinii* counts of $10^{5.89}$ and $10^{6.77}$ at 10 and 19 days after inoculation, respectively. In addition, PCR detected *P. carinii* DNA in the lungs of all of the nonimmune mice killed at either 4, 10, or 19 days after inoculation (Table 3 and Fig. 1). At 4 days after inoculation, the number of *P. carinii* organisms in the lungs of the immune mice was significantly less than that in the nonimmune mice. At 10 and 19 days after inoculation, the *P. carinii* counts in the immune mice were below the limit of detection. The PCR detected *P. carinii* glycoprotein A DNA in one of five immune mice at 4 days, rRNA DNA in two of five immune mice at 4 days, and in none of the immune mice at either 10 or 19 days.

***P. carinii*-specific IgG in sera of mice.** The levels of *P. carinii*-specific IgG in the sera of immune and nonimmune mice after $CD4^+$ $Thy1^+$ cell depletion and *P. carinii* challenge are shown in Table 4. The optical density values for *P. carinii*-specific IgG were less than 0.010 in all of the nonimmune mice both before (8 days prechallenge) and at 4, 10, or 19 days after challenge. However, the immune mice consistently had *P. carinii*-specific IgG in their sera both before and after *P. carinii* challenge. In addition, the optical density values for the IgG did not wane during the 19 days after challenge.

DISCUSSION

Results of this investigation indicate that mice that have been immunized against *P. carinii* remain resistant to PCP, whereas nonimmune mice become susceptible to PCP after they are depleted of $CD4^+$ $Thy1^+$ cells. Although this initial observation of protection against experimental PCP by active immunization was produced under very specific conditions, it offers guarded optimism that immunization could be utilized to protect immunocompromised patients. There are many clinical situations where the at-risk period for PCP can be anticipated, such as in patients newly diagnosed with a malignancy or who are being prepared for organ transplantation. Under these conditions, early in the course of immunosuppressive therapy, patients may still be responsive to immunization (8, 9). Indirect evidence of the ability of AIDS patients to mount an antibody response to antigens of *P. carinii* after immunization comes from the studies of Peglow et al. (20) and Lundgren et al. (19). In these studies, a serologic response to antigens of *P. carinii* was more likely to be present in patients who had experienced an episode of PCP. Furthermore, many patients demonstrated a rise in antibody titer, despite their immunosuppressed state,

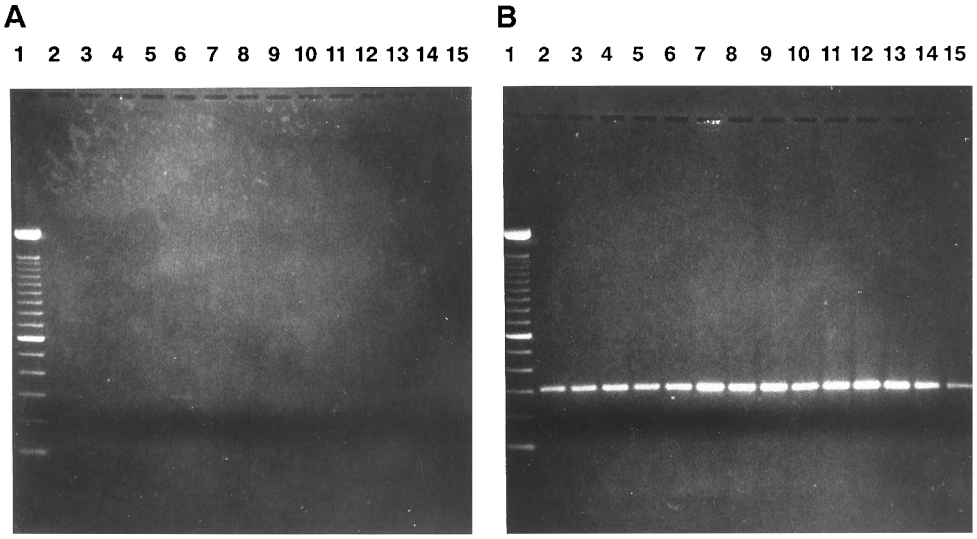

FIG. 1. Detection of *P. carinii* glycoprotein A DNA by PCR in immune (A) and nonimmune (B) mice after challenge with *P. carinii*. Lanes: 1, DNA standards in 100-bp increments; 2 to 6, lung homogenates obtained 4 days postchallenge; 7 to 11, homogenates from lungs obtained 10 days postchallenge; 12 to 15, homogenates from lungs obtained 19 days postchallenge.

TABLE 4. *P. carinii*-specific IgG in the sera of *P. carinii*-immunized or -naive mice depleted of $CD4^+$ $Thy1^+$ cells

Mouse type	OD of *P. carinii*-specific IgG in sera[a]			
	8 days pre	4 days post	10 days post	19 days post
Immune	0.384 ± 0.244^b	0.585 ± 0.235^b	0.536 ± 0.446^b	0.688 ± 0.391^b
Nonimmune	<0.010	<0.010	<0.010	<0.010

[a] Optical density (OD) was measured pre- or postchallenge (pre and post, respectively) with *P. carinii*. Values are means ± standard deviations. The numbers of mice tested were eight for 8 days prechallenge and 4 days postchallenge and five for 10 and 19 days postchallenge.
[b] Significantly different from nonimmune mice, $P < 0.05$.

immediately following an episode of PCP. Passive antibody therapy has been shown to afford some protection in animal models of PCP (12, 23). Therefore, if *P. carinii* antigen(s) could be presented in a sufficiently immunogenic form to immunocompromised patients, protection might be achieved.

The mechanism by which the immune mice cleared the *P. carinii* challenge is not known. Although it is apparent that the responsible mechanism was acquired through immunization, its expression was neither $CD4^+$ cell dependent nor other $Thy1^+$ cell dependent. It is possible that immunization of the mice left their lungs nonspecifically activated, causing an enhanced nonspecific clearance of the *P. carinii* challenge. However, the numbers of neutrophils and macrophages in the lungs of immune and nonimmune mice before and at 4 days after *P. carinii* inoculation were similar (results not shown). Furthermore, in ongoing experiments, we have found that immunization with *P. carinii* by the intraperitoneal route also affords protection in this model (data not shown), indicating that the protection observed is not dependent on intratracheal delivery of antigen(s). Another possibility, and one we favor, is that *P. carinii*-specific antibodies in the immune mice were responsible for the resolution of PCP. Immune mice had significant amounts of *P. carinii*-specific IgG in their sera before $CD4^+$ $Thy1^+$ cell depletion, and the level of these antibodies did not decrease with $CD4^+$ cell depletion or *P. carinii* challenge. In addition, the nonimmune mice had no detectable *P. carinii*-specific antibodies and were in fact susceptible to the growth of *P. carinii*. Thus, a reasonable conclusion would be that antibodies were important for the clearance of the *P. carinii* challenge in the immune mice. However, there is no causal evidence that the resistance was antibody mediated. Even so, it is of interest that the resistance to *P. carinii* acquired in the immunized mice did not require the presence of $CD4^+$ cells. This argues against a purely cell-mediated response being responsible for the resistance observed in this model. However, others have clearly shown that the transfer of purified $CD4^+$ cells can be sufficient to protect SCID mice from PCP (22) and that depletion of $CD4^+$ cells in normal mice makes them susceptible to *P. carinii* (24). It seems likely, therefore, that there are multiple mechanisms of resistance to PCP.

Incomplete depletion of $CD4^+$ cells could have led us to the erroneous conclusion that the protection we observed was independent of this cell type. In fact, our initial studies revealed that mice treated with anti-CD4 MAb contained few detectable $CD4^+$ cells and that the immunized mice contained significantly more than the nonimmunized mice. In addition, it was found that most of the $CD4^+$ cells that remained in the immunized mice that had been treated with anti-CD4 MAb only were $CD45RB^{low}$ and/or $CD44^{high}$. Both of these markers are expressed on activated $CD4^+$ cells and not naive cells (1–3). The findings that (i) there were significant numbers of $CD4^+$ cells in immune mice treated with anti-CD4 MAb and not in nonimmune mice and (ii) nearly all of the $CD4^+$ cells that remained expressed surface antigens associated with activation indicate that anti-CD4 MAb treatment depleted naive $CD4^+$ cells efficiently but that some activated $CD4^+$ cells remained. This result is consistent with that reported previously by Chace et al. (3), who found that anti-CD4 MAb treatment preferentially depletes resting, naive $CD4^+$ cells and spares activated $CD4^+$ cells. Why naive but not activated $CD4^+$ cells are depleted by this treatment is not known. However, in the experiments in this investigation in which naive mice were treated with anti-CD4 MAb only, the mice were depleted of $CD4^+$ cells and were susceptible to *P. carinii* infection (data not shown). Thus, the results reported here are consistent with those reported previously by this laboratory (14, 15) and with those of others (24) that treatment of naive mice with MAb GK1.5 makes mice susceptible to *P. carinii* infection.

To completely deplete $CD4^+$ cells, we treated the immune and nonimmune mice with anti-CD4 MAb as well as anti-Thy1 MAb, since the Thy1.2 marker is present on activated $CD4^+$ cells. Mice so treated had no detectable $CD4^+$ cells in either their lung alveoli or tracheal bronchial lymph nodes after challenge with *P. carinii*. This is a crucial point because we have shown previously that during $CD4^+$ cell-mediated resolution of PCP, large numbers of $CD4^+$ cells accumulate at these two sites (15). In addition, no lymphocytes with rat immunoglobulin attached to them were detected in the mice. This indicates that no viable $CD4^+$ cells that had rat anti-CD4 MAb bound to their surface were present. This is important because such binding could cause cells to escape detection by fluorescein isothiocyanate-conjugated anti-CD4 MAb. Thus, it is apparent that $CD4^+$ cells were depleted from the mice and that, therefore, the increased resistance to *P. carinii* challenge in the immunized mice was not dependent on the presence of these cells.

It is also of interest that the numbers of *P. carinii* organisms in the lungs of both immune and nonimmune mice at 4 days were less than 1% of the initial inoculum. Thus, regardless of whether the mice were immune or nonimmune, most of the inoculum was cleared quickly. This suggests that nonspecific defense mechanisms are very efficient at eliminating *P. carinii* inoculated into the lungs. Alternatively, it is possible that only a small fraction of the *P. carinii* inoculum is infective. For example, only a certain life cycle stage may be infective, and this stage may represent a small fraction of the inoculum. It may also be possible that instilling the *P. carinii* organisms results in few of them gaining access to the appropriate microenvironment in the lung. In this regard, the *P. carinii* organisms may need to adhere to alveolar epithelial cells to grow, and this process may be inefficient in the instilled *P. carinii* organisms. In any case, it is apparent that both immune and nonimmune mice initially cleared the bulk of the *P. carinii* inoculum but that the few organisms remaining were able to grow in the nonimmune mice and not in the immune mice. In fact, already by 4 days after inoculation, the lungs of nearly all of the immune mice had no detectable *P. carinii* DNA. Mice in the immune group remained PCR negative for *P. carinii* even at 19

days after challenge. We have shown previously that *P. carinii*-infected SCID mice that were immunologically reconstituted and had become PCR negative for *P. carinii* DNA had in fact completely resolved their *P. carinii* infection (4). This suggests that the immune mice in this investigation retained very efficient acquired immune mechanisms against *P. carinii* after they were depleted of $CD4^+$ $Thy1.2^+$ cells. This raises the compelling possibility that immunity to *P. carinii* acquired either naturally or artificially by immunization could persist after the loss of $CD4^+$ cell function.

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