

## Exposure of Immunocompetent Adult Mice to *Pneumocystis carinii* f. sp. *muris* by Cohousing: Growth of *P. carinii* f. sp. *muris* and Host Immune Response

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Received 5 August 2002/Returned for modification 10 September 2002/Accepted 13 January 2003

There has been emerging evidence that immunocompetent hosts can harbor *Pneumocystis* in their lungs. The purpose of this study was to determine the kinetics of *Pneumocystis carinii* f. sp. *muris* infection in adult immunocompetent mice and the host immune response to the organisms. To accomplish this, we exposed adult immunocompetent mice to SCID mice infected with *P. carinii* f. sp. *muris* by cohousing. We found that *P. carinii* f. sp. *muris* was detectable in the lungs of cohoused immunocompetent mice by PCR by 3 weeks after the beginning of cohousing. At about 4 weeks of cohousing, *P. carinii* f. sp. *muris* was readily detectable in the lungs of mice by microscopic techniques. Also at this time, *P. carinii* f. sp. *muris*-specific immunoglobulin G was found in the sera of the mice, and CD62<sup>low</sup> CD4- and CD8-positive T cells accumulated in the lungs. Shortly after this immune response, the *P. carinii* f. sp. *muris* organisms were cleared from the lungs. Adult mice cohoused for only 1 week also contained *P. carinii* f. sp. *muris* cysts detectable by silver staining at 5 and 6 weeks after the beginning of cohousing. We also found that the *P. carinii* f. sp. *muris* organisms grew to greater numbers in the lungs of BALB/c mice than in those of C57BL6 mice. This indicates that immunocompetent hosts develop a mild infection with *P. carinii* f. sp. *muris* which resolves in 5 to 6 weeks when there is a detectable immune response to the organism. Once an acquired immune response was initiated, the *P. carinii* f. sp. *muris* organisms were quickly eliminated without clinical signs of disease.

*Pneumocystis* sp. is an important pathogen in immunocompromised individuals. It has been shown that a loss of CD4-positive T cells is the predominant cause of susceptibility to these organisms (13, 22). Thus, it is believed that immunocompetent hosts are resistant to infection by *Pneumocystis*. This belief is further enforced by the fact that *Pneumocystis* rarely causes overt disease in immunocompetent individuals.

Recent studies suggest that children may frequently harbor *Pneumocystis* in their lungs (17, 24, 26), and animal experiments have shown that neonatal mice, pigs, and rabbits can harbor substantial numbers of *Pneumocystis* organisms (3, 7, 15, 23). However, after intratracheal instillation of *Pneumocystis carinii* f. sp. *muris* into immunocompetent adult mice, the organisms grow in number in the lungs for only about 1 week and are essentially cleared from the lungs in another 1 to 2 weeks (2; our unpublished data). Because the natural mode of transmission of *Pneumocystis* is by an aerosol route (14), we reasoned that immunocompetent adult mice might harbor *P. carinii* f. sp. *muris* for a longer period after exposure if the organisms are transmitted by the natural aerosol mode. We thought this to be possible because with a natural aerosol exposure, very few organisms would be deposited in the lungs; this should not induce an inflammatory response as does the intratracheal instillation of a suspension of a large number of *P. carinii* f. sp. *muris* in a fluid vehicle that by itself can cause

inflammation when instilled intratracheally. This may be an important point, because infection of SCID mice by cohousing (aerosol exposure) does not result in an inflammatory response (27, 28). In addition, most studies of the host immune response to *Pneumocystis* have been done on animals after intratracheal instillation of *P. carinii* f. sp. *muris*. With this route of administration of the organism, the host's immune system would be exposed to a bolus of *P. carinii* f. sp. *muris* organisms that are not adherent to host lung epithelial cells, whereas with cohousing, a few organisms probably reach the lung epithelium undetected and then expand in number as adherent organisms. These adherent organisms could present the host immune surveillance mechanisms with a much different profile than non-adherent organisms coming down the airways after intratracheal instillation.

The purpose of this study was twofold. First, we wished to determine the ability of *P. carinii* f. sp. *muris* to grow in the lungs of immunocompetent mice for an extended period of time after the mice were exposed by being cohoused with *P. carinii* f. sp. *muris*-infected mice. Secondly, we investigated the immune response to *P. carinii* f. sp. *muris* induced in these cohoused mice. We found that *P. carinii* f. sp. *muris* grows in the lungs of immunocompetent mice for about 5 weeks after the beginning of cohousing. At about 4 weeks of cohousing, *P. carinii* f. sp. *muris*-specific immunoglobulin G (IgG) was found in the sera of the mice and CD4- and CD8-positive T cells accumulated in the lungs. Shortly after this immune response, the *P. carinii* f. sp. *muris* organisms were cleared from the lungs. This indicates that immunocompetent hosts harbored *P. carinii* f. sp. *muris* in small numbers for up to 5 weeks after the beginning of cohousing. However, in the cohoused mice, once

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an acquired immune response was initiated, the *P. carinii* f. sp. *muris* was quickly eliminated.

#### MATERIALS AND METHODS

**Infection of mice.** CB.17 *scid/scid* (SCID), C57BL/6 (B6), CB.17 and BALB/c mice were obtained from the Trudeau Institute animal breeding facility (Saranac Lake, N.Y.). The SCID mice were maintained in microisolator cages and fed sterilized food and water. Beginning at 3 weeks of age, the *P. carinii* f. sp. *muris*-free SCID mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice. After 6 weeks of cohousing, the mice newly infected with *P. carinii* f. sp. *muris* were removed to their own cages and were used as *P. carinii* f. sp. *muris* source mice after an additional 2 weeks. At this time, the SCID mice contained about  $10^7$  *P. carinii* f. sp. *muris* nuclei in their lungs (data not shown). For infection experiments, 2 *P. carinii* f. sp. *muris*-infected SCID mice were cohoused with 10 adult BALB/c or B6 mice (7 to 9 weeks of age) for a period of 6 weeks.

**Microscopic enumeration of *P. carinii* f. sp. *muris* organisms in mouse lungs.** The numbers of *P. carinii* f. sp. *muris* organisms in the lungs of the mice were determined microscopically as described previously (13), except that besides staining with Diff-Quick, in some experiments, the *P. carinii* f. sp. *muris* organisms were also stained by silver stain or by immunohistochemical staining, as previously described (9). With all of these microscopic techniques, the limit of detection of *P. carinii* f. sp. *muris* is determined by the amount of lung homogenate placed on the slide (as determined by the dilution and volume) and the fraction of the stained slide that is read. Thus, the limit of detection for the Diff-Quick stain was  $4.2 \log_{10}$  nuclei, whereas the limit of detection with the immunostaining was  $3.0 \log_{10}$  organisms (each cyst and each trophozoite is considered one organism) and that for the silver stain was  $2.7 \log_{10}$  cysts. Although the silver stain was sensitive, it detected cysts only, and because the cysts comprised only about one-tenth of the total number of organisms, the immunostaining method was the most sensitive of the microscopic methods of detection used, since it was sensitive to both cysts and trophozoites.

***P. carinii* f. sp. *muris*-specific DNA amplification by PCR.** A previously described PCR technique (4, 10) was used to amplify *P. carinii* f. sp. *muris*-specific DNA in clarified boiled lung homogenate of mice with primers pAZ102-E (5'-GATGGCTGTTTCCAAGCCCA-3') and pAZ102-H (5'-GTGTACGTTGC AAAGTACTC-3'), which are specific for a portion of the *P. carinii* f. sp. *muris* mitochondrial rRNA gene. PCR conditions were as follows. A hot start was done at 94°C for 2 min, followed by denaturing at 94°C for 90 s, annealing at 65°C for 90 s, and extension at 72°C for 120 s; a total of 35 cycles was done. In titer determination experiments, we were able to detect mouse *P. carinii* f. sp. *muris* at a concentration of approximately 10 nuclei per ml of lung homogenate.

**Measurement of *P. carinii* f. sp. *muris*-specific IgG.** A previously described enzyme-linked immunosorbent assay (5, 11) was used to determine *P. carinii* f. sp. *muris*-specific IgG in mouse serum. Flat-bottom microtiter plates (Flow Laboratories, McLean, Va.) were coated with a *P. carinii* f. sp. *muris* soluble total protein preparation from the lungs of SCID mice infected with *P. carinii* f. sp. *muris* (10 µg of protein per ml). 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* f. sp. *muris*-specific glycoprotein A, normal mouse serum, and mouse hyperimmune serum produced by immunizing immunocompetent mice with subcutaneous injections of *P. carinii* f. sp. *muris*.

**Lung lavage and analysis of cells by fluorescence-activated cell sorting.** Lungs were subjected to lavage, as previously described (12), with five 1-ml aliquots of phosphate-buffered saline containing 0.3 mM EDTA. Total cells in the bronchoalveolar lavage fluids (BALF) were determined with a hemocytometer, and cells were spun onto a slide with a cytocentrifuge and stained with Diff-Quick for differential counts. Aliquots of the cells were then stained with Cy-Chrome-conjugated anti-CD4 (clone GK1.5), phycoerythrin-conjugated anti-CD8 (clone TIB 210), and fluorescein isothiocyanate-conjugated CD62 (clone Mel-14). Fluorescence-labeled cells were analyzed by using a FACScan sorter (Becton Dickinson, Mountain View, Calif.), and 5,000 events were routinely acquired.

#### RESULTS

**Susceptibility of immunocompetent CB.17 mice to *P. carinii* f. sp. *muris* after exposure by cohousing.** CB.17 mice were cohoused for 6 weeks with CB.17 SCID mice infected with *P. carinii* f. sp. *muris*. Groups of these mice, and control mice not cohoused with infected SCID mice, were euthanized at 3, 5,

TABLE 1. Development of *Pneumocystis* infection in CB.17 immunocompetent mice cohoused with infected mice

Length of exposure (wks)	No. of <i>P. carinii</i> f. sp. <i>muris</i> nuclei ( $\log_{10}$ )		PCR result for <i>P. carinii</i> f. sp. <i>muris</i> <sup>a</sup>	
	Cohoused mice	Control mice	Cohoused mice	Control mice
3	$<4.3 \pm 0.0^b$	$<4.3 \pm 0.0$	++	–
5	$5.1 \pm 0.8^c$	$<4.3 \pm 0.0$	+++	–
6	$<4.3 \pm 0.0$	$<4.3 \pm 0.0$	+	–

<sup>a</sup> +, light band; ++, medium band; +++, heavy band, –, no band.

<sup>b</sup> Limit of detection  $4.3 \log_{10}$  *P. carinii* f. sp. *muris* nuclei per mouse.

<sup>c</sup> Significantly different than control ( $P > 0.05$ ).

and 6 weeks after the start of cohousing. The lungs of the mice were subjected to lavage, the numbers of *P. carinii* f. sp. *muris* nuclei and DNA in their lung homogenates were determined by Diff-Quick staining and PCR, respectively, and serum was collected. Results of *P. carinii* f. sp. *muris* nuclear counts are shown in Table 1. No *P. carinii* f. sp. *muris* nuclei were detected in any of the control mice at any time point by either microscopy or PCR. In the cohoused mice, no *P. carinii* f. sp. *muris* were detected at 3 weeks by microscopy but *P. carinii* f. sp. *muris* DNA was found by PCR (medium density bands consistently found). At 5 weeks, most of the cohoused mice had microscopically detectable numbers of *P. carinii* f. sp. *muris* nuclei in their lungs as well as *P. carinii* f. sp. *muris* DNA detectable by PCR (heavy bands). By 6 weeks after the end of cohousing, the cohoused mice did not have microscopically detectable *P. carinii* f. sp. *muris* nuclei in their lungs, but all the mice were still weakly positive (light but consistently detected band) for *P. carinii* f. sp. *muris* DNA by PCR.

**Susceptibility of B6 and BALB/c mice to infection with *P. carinii* f. sp. *muris* by cohousing.** B6 and BALB/c mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice for 6 weeks. As a control, some of each of the two strains of mice were not cohoused with infected mice. Groups of the mice were killed at 4, 5, and 6 weeks after cohousing. The numbers of *P. carinii* f. sp. *muris* cysts in the lungs of the mice as determined by microscopy of silver-stained homogenates is shown in Fig. 1. No cysts were detected in mice of either strain that were not cohoused. In the cohoused mice, *P. carinii* f. sp. *muris* cysts were detected at 4 weeks in both the B6 and BALB/c mice. At 5 weeks, cysts were easily detected in the BALB/c mice but were undetectable in the B6 mice. Cysts were not detected by silver staining at 6 weeks in either strain of mouse.

In order to get a more accurate picture of the kinetics of *P. carinii* f. sp. *muris* growth in immunocompetent B6 and BALB/c mice, the previous experiment was repeated, except groups of the mice were euthanized at 15, 20, 26, 30, 35, and 40 days after cohousing and lung homogenates were stained for *P. carinii* f. sp. *muris* by immunocytochemistry.

Immunocytochemical staining detects both cysts and trophozoites, whereas the silver stain normally only detects cysts, which usually are only about one-tenth the number of trophozoites in these mice. Results are shown in Fig. 2. Most of the BALB/c and B6 mice had *P. carinii* f. sp. *muris* organisms detectable by immunocytochemistry at 30 days after the end of cohousing. The numbers of organisms detected peaked at 35 days and quickly declined by 41 days in both strains. As in the

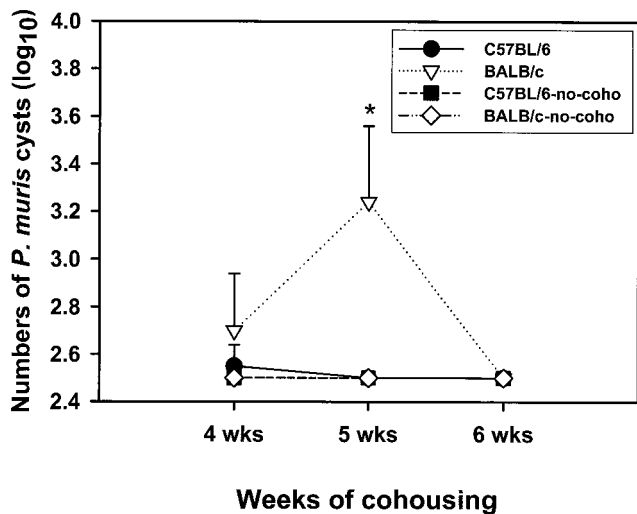


FIG. 1. Numbers of *P. carinii* f. sp. *muris* cysts ( $\log_{10}$ ) in lungs of C57BL/6 and BALB/c mice with and without (no-coho) cohousing with *P. carinii* f. sp. *muris*-infected SCID mice. Mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice for 4, 5, or 6 weeks; the mice were then killed, and the numbers of *P. carinii* f. sp. *muris* cysts in their lungs were determined by silver staining of lung homogenates. Other mice were not cohoused as a control. The minimal level of detection of cysts was  $10^{2.5}$ . Data points are the average number of cysts  $\pm$  standard deviation of the mean at each time point ( $n = 5$ ). \*,  $P < 0.05$  for comparison of cohoused and control mice, as calculated by one-way ANOVA.

previous experiment, significantly greater numbers of *P. carinii* f. sp. *muris* organisms were detected in the lungs of the BALB/c mice than in those of the B6 mice even though the immunocytochemical staining detected more organisms than did the silver staining.

**Immune response to *P. carinii* f. sp. *muris* in BALB/c and B6 mice cohoused with *P. carinii* f. sp. *muris*-infected SCID mice.**

The numbers of neutrophils in BALF of the mice were determined by differential counting. Expression of CD4, CD8, and CD62 by cells in the BALF of the B6 and BALB/c mice cohoused or not cohoused with infected mice and killed at 4, 5, and 6 weeks after the end of cohousing was determined by fluorescence-activated cell sorting. Neutrophils were nearly undetectable in the BALF of either BALB/c or B6 mice (Fig. 3) not cohoused with *P. carinii* f. sp. *muris*-infected SCID mice. However, significant numbers of neutrophils were found in the BALF of both strains of mice at 4 weeks after commencement of cohousing. The numbers of neutrophils in the BALF of both strains of mice peaked at 5 weeks after the beginning of cohousing and dropped to values near those of the controls by 6 weeks. The numbers of CD4<sup>+</sup> CD62<sup>low</sup> cells in the BALF of the mice is shown in Fig. 4, and nearly all CD4<sup>+</sup> cells (>90%) in the BALF of mice in this experiment were typically CD62<sup>low</sup>. Few CD4<sup>+</sup> CD62<sup>low</sup> cells were found in the BALF of mice not cohoused. However, large numbers of these cells were found in the BALF of both strains of mice at 5 weeks after the end of cohousing. At 6 weeks, the number of CD4<sup>+</sup> CD62<sup>low</sup> cells in the BALF of the C57BL6 mice had decrease to numbers near those of the controls, but the numbers of these cells in the BALF of the BALB/c mice remained elevated. The numbers of CD8<sup>+</sup> CD62<sup>low</sup> cells in the BALF of the mice is shown in Fig.

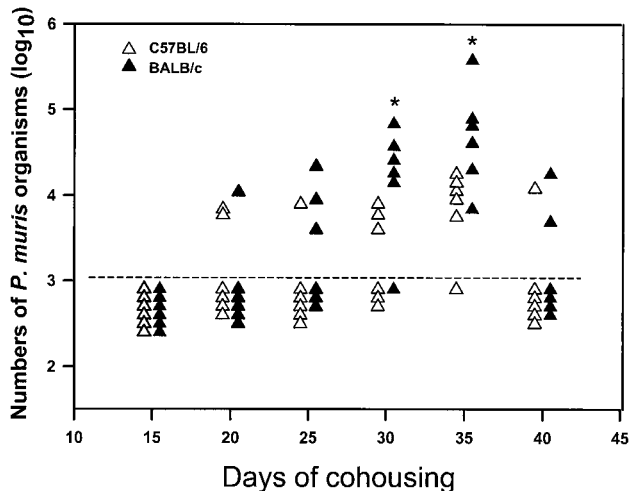


FIG. 2. Numbers of *P. carinii* f. sp. *muris* organisms ( $\log_{10}$ ) in lungs of C57BL/6 and BALB/c mice with and without cohousing with *P. carinii* f. sp. *muris*-infected SCID mice. Mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice for 15, 20, 25, 30, 35, 40, or 45 days; the mice were then killed, and the numbers of *P. carinii* f. sp. *muris* organisms in their lungs were determined by immunostaining of lung homogenates. The minimal level of detection was  $10^{3.1}$  organisms. The numbers of *P. carinii* f. sp. *muris* organisms are the average number of organisms  $\pm$  standard deviation of the mean at each time point ( $n = 5$ ). \*,  $P < 0.05$  for comparison of results at day 15 and those at days 30 and 35, as calculated by one-way ANOVA.

5; nearly all (>90%) of the CD8<sup>+</sup> cells in BALF were CD62<sup>low</sup>. The kinetics of CD8<sup>+</sup> CD62<sup>low</sup> cell accumulation in the lungs of the four groups of mice was similar to that of the CD4<sup>+</sup> CD62<sup>low</sup> cells. Significant numbers of CD8<sup>+</sup> CD62<sup>low</sup> cells were found in the BALF of the cohoused mice at 5 weeks; these cells then waned in number in the C57BL6 by 6 weeks but persisted in the BALB/c mice. The *P. carinii* f. sp. *muris*-

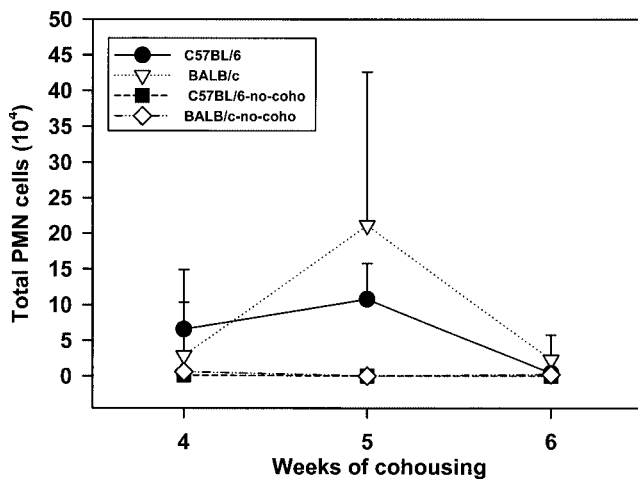


FIG. 3. Numbers of polymorphonuclear neutrophils (PMN) in lung lavage fluids of C57BL/6 and BALB/c mice after cohousing. Mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice for 4, 5, or 6 weeks; the mice were then killed, and the numbers of PMN in their lung lavage fluids were determined. Other mice (no-coho) were not cohoused as control. Data points are the average numbers of PMN  $\pm$  standard deviation of the mean at each time point ( $n = 5$ ).

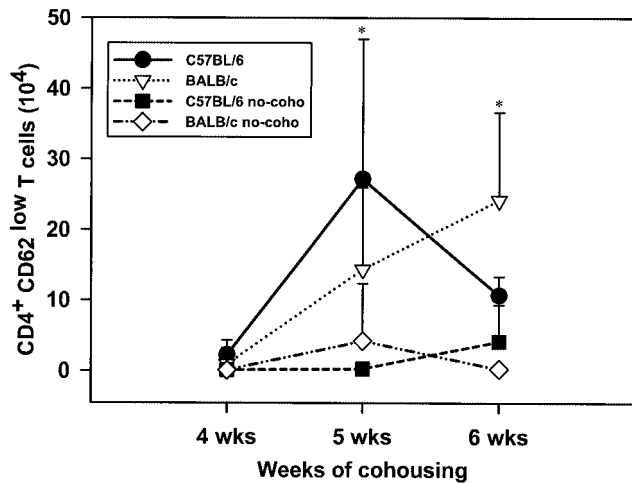


FIG. 4. Numbers of CD4<sup>+</sup> CD62<sup>low</sup> T cells in lung lavage fluids of C57BL/6 and BALB/c mice after cohousing. Mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice for 4, 5, or 6 weeks; the mice were then killed, and the numbers of CD4<sup>+</sup> CD62<sup>low</sup> T cells in their lung lavage fluids were determined. Other mice (no-coho) were not cohoused as a control. Data points are the average numbers of CD4<sup>+</sup> CD62<sup>low</sup> T cells  $\pm$  standard deviation of the mean at each time point ( $n = 5$ ). \*,  $P < 0.05$  for comparison of cohoused and control mice, as calculated by one-way ANOVA.

specific IgG responses in the sera of the four groups of mice are shown in Fig. 6. Compared to the control mice not cohoused, the cohoused mice had detectable levels of *P. carinii* f. sp. *muris*-specific IgG in their sera at both 5 and 6 weeks. There were no significant differences in the amounts of specific IgG in the sera of the cohoused B6 and BALB/c mice.

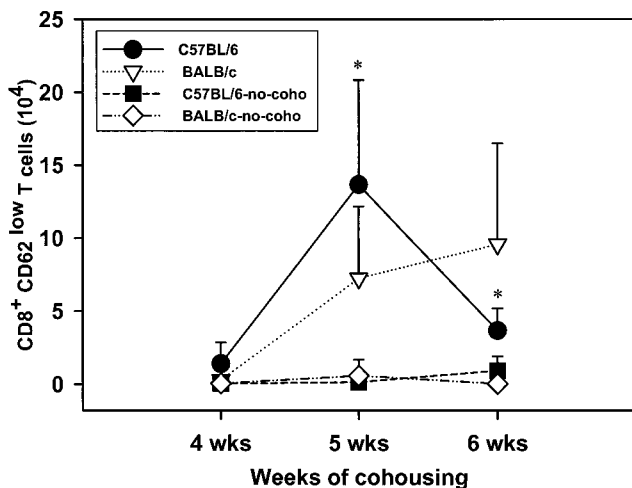


FIG. 5. Numbers of CD8<sup>+</sup> CD62<sup>low</sup> T cells in lung lavage fluids of C57BL/6 and BALB/c mice after cohousing. Mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice for 4, 5, or 6 weeks; the mice were then killed, and the numbers of CD8<sup>+</sup> CD62<sup>low</sup> T cells in their lung lavage fluids were determined. Other mice (no-coho) were not cohoused as a control. Data points are the average numbers of CD8<sup>+</sup> CD62<sup>low</sup> T cells  $\pm$  standard deviation of the mean at each time point ( $n = 5$ ). \*,  $P < 0.05$  for comparison of cohoused and control mice, as calculated by one-way ANOVA.

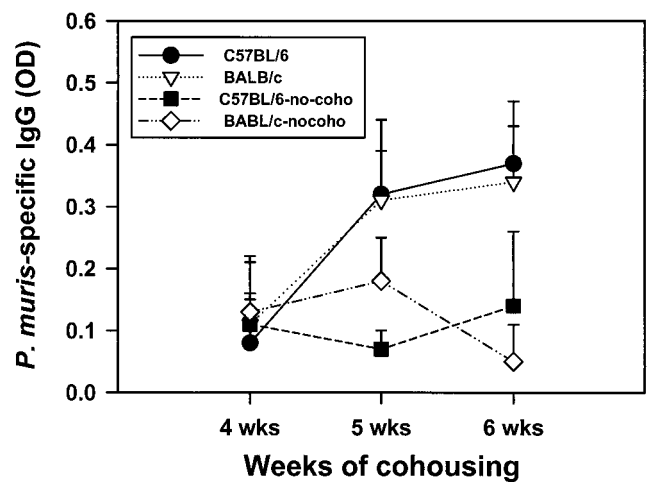


FIG. 6. *P. carinii* f. sp. *muris*-specific IgG in lung lavage fluid of C57BL/6 and BALB/c mice after cohousing. Mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice for 4, 5, or 6 weeks; the mice were then killed, and the levels of *P. carinii* f. sp. *muris*-specific IgG in their lung lavage fluids were determined by ELISA. Other mice (no-coho) were not cohoused as a control. Data points are the average optical densities  $\pm$  standard deviation of the mean at each time point ( $n = 5$ ).

#### Susceptibility of CB-17 mice to infection with *P. carinii* f. sp. *muris* by continuous cohousing or a single week of cohousing.

Adult CB-17 mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice. Half of the mice were removed from cohousing after 1 week, and the rest remained in cohousing for the duration of the experiment. Some of the mice from the two cohoused groups and some mice not cohoused were killed at 3, 5, or 6 weeks after the beginning of cohousing, and the numbers of *P. carinii* f. sp. *muris* cysts in their lungs were determined by silver staining of lung homogenates. Results are shown in Fig. 7. As seen previously, *P. carinii* f. sp. *muris* cysts were found in the lungs of continuously cohoused mice at 5 and 6 weeks after the start of cohousing. Similarly, *P. carinii* f. sp. *muris* cysts were also found in the lungs of mice cohoused for a single week when they were killed at 5 or 6 weeks after the beginning of cohousing. There was no significant difference in *P. carinii* f. sp. *muris* cyst numbers between the mice continuously cohoused and those cohoused for 1 week.

## DISCUSSION

Results presented here indicate that when immunocompetent mice are cohoused with *P. carinii* f. sp. *muris*-infected SCID mice, the *P. carinii* f. sp. *muris* organisms grow progressively in the lungs of the mice for about 5 weeks and decline to undetectable numbers (by microscopy of silver-stained or immunohistochemically stained slides) by 6 weeks. The kinetics of this infection is in contrast to what occurs in immunocompetent mice infected with *P. carinii* f. sp. *muris* by administration of organisms as a bolus directly into the lungs. In this regard, *P. carinii* f. sp. *muris* organisms grow in numbers in the lungs of immunocompetent mice for only about 1 week after intratracheal instillation of viable organisms (2) and are subsequently cleared by 3 weeks. We have found results similar to those of Beck et al. (2) in our lab (unpublished observations).

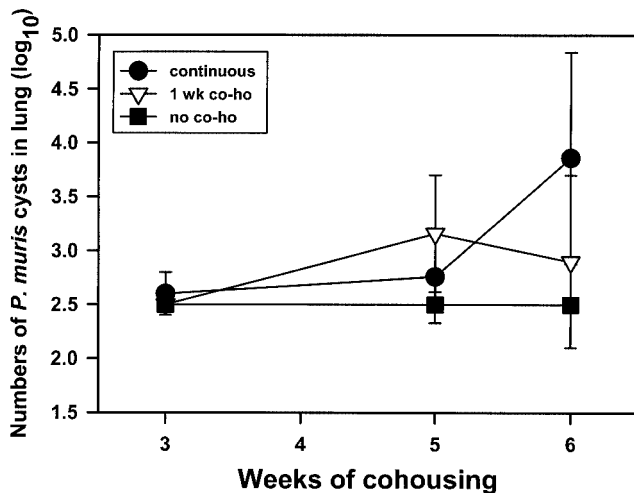


FIG. 7. Numbers of *P. carinii* f. sp. *muris* cysts ( $\log_{10}$ ) in lungs of CB-17 mice with 1 week of cohousing (1 wk co-ho) or continuous cohousing with *P. carinii* f. sp. *muris*-infected SCID mice. Mice were cohoused with *P. carinii* f. sp. *muris*-infected SCID mice for 3, 4, 5, or 6 weeks; the mice were then killed, and the numbers of *P. carinii* f. sp. *muris* cysts in their lungs were determined by silver staining of lung homogenates. Other mice (no co-ho) were not cohoused as a control. The minimal level of detection of cysts was  $10^{2.5}$ . Data points are the average numbers of cysts  $\pm$  standard deviation of the mean at each time point ( $n = 5$ ).

The reason for this difference in persistence of infection with different means of inoculation is not known, but there are several possibilities. Others (16) have shown that by 3 h after intratracheal instillation of *P. carinii* f. sp. *muris* into immunocompetent or CD4-depleted mice, large amounts of tumor necrosis factor and numerous neutrophils are found in the BALF of the mice. This indicates that there is a significant inflammatory response in the lungs of the mice soon after *P. carinii* f. sp. *muris* is introduced by intratracheal instillation. In contrast, our current data indicate that the mice exposed to *P. carinii* f. sp. *muris* by cohousing do not mount a detectable neutrophil response in the lungs until about 4 to 5 weeks after the beginning of cohousing. In addition, we have previously shown that SCID mice infected with *P. carinii* f. sp. *muris* by cohousing do not have a detectable inflammatory response in their lungs, as indicated by a lack of both neutrophil accumulation and the expression of inflammatory cytokine mRNA in the lungs (27) until the infection progresses to the point where the lung alveoli are nearly filled with organisms (about 10 to 12 weeks of infection). This suggests that in contrast with direct intratracheal instillation, exposure to *P. carinii* f. sp. *muris* by cohousing may allow organisms to establish themselves in the lung alveoli without inducing an inflammatory response.

An inflammatory response could be a prerequisite to the induction of the acquired immune response to *P. carinii* f. sp. *muris*. In this regard, it has been shown that lung immune responses are augmented if an inflammagen is deposited in the lung together with the antigen (21). This would account for the observation that the acquired immune response to *P. carinii* f. sp. *muris* is initiated by about 10 days after intratracheal inoculation but not until about 5 weeks after exposure to *P. carinii* f. sp. *muris* by cohousing. An alternative explanation is that

there is a threshold of the quantity of antigen needed to induce an immune response to the *P. carinii* f. sp. *muris*. Such a threshold could be crossed immediately with intratracheal instillation but may not be reached for some time after cohousing. Future experiments will address why, with cohousing exposure, *P. carinii* f. sp. *muris* can grow in the lungs of mice for 5 weeks before an acquired immune response is induced.

We utilized four different techniques to detect *P. carinii* f. sp. *muris* in the lungs of immunocompetent mice. As expected, PCR was the most sensitive, as it has been previously shown to detect 10 *P. carinii* f. sp. *muris* nuclei per ml of lung homogenate (4). Of the microscopic techniques we utilized, indirect fluorescent-antibody (IFA) staining detected greater numbers of *P. carinii* f. sp. *muris* organisms in the lungs of immunocompetent mice than did either the silver stain or the Diff-Quik stain. This is similar to results reported by Baughman et al. (1), who compared IFA, Diff-Quik, and silver stain sensitivities. We believe that the IFA detected more *P. carinii* f. sp. *muris* organisms than the silver stain because the IFA readily detected both cysts and trophozoites, whereas the silver stain detects only cysts. This difference is important because in our mice, there are approximately 10 times more trophozoites than cysts. Thus, quantitating both forms of *P. carinii* f. sp. *muris* by IFA gives a minimal detection number in the lungs that is 10 times lower than that obtained by using silver stain. This difference is important when trying to quantitate the few organisms present in the lungs of the immunocompetent mice.

Studies by others have shown that immunocompetent infants can harbor *Pneumocystis* organisms in their lungs (17, 19, 24, 26). In addition it was recently shown that healthy adults can, at least transiently, acquire *Pneumocystis* organisms from infected, immunodeficient patients (25). Recent work by others has shown that immunocompetent mice can transmit *P. carinii* f. sp. *muris* to immunodeficient mice (6). Thus, it is becoming apparent that *Pneumocystis* can infect the lungs of immunocompetent individuals. Results of the present studies establish that by 21 days after the beginning of cohousing, the lungs of all exposed immunocompetent mice become positive for *P. carinii* f. sp. *muris* DNA by PCR. By 30 days after the beginning of cohousing, the lungs of most exposed immunocompetent mice contain *P. carinii* f. sp. *muris* organisms, as determined by immunohistochemistry. By 35 days after the beginning of cohousing, activated CD4- and CD8-positive cells begin to accumulate in the lungs of the mice, and their sera contain detectable amounts of *P. carinii* f. sp. *muris*-specific IgG. By 40 to 42 days, most of the exposed mice no longer contain detectable numbers of *P. carinii* f. sp. *muris* organisms in their lungs. Thus, it takes approximately 40 days after the beginning of exposure for the *P. carinii* f. sp. *muris* to be cleared from the lungs of immunocompetent mice by the acquired immune response. The clearance of the *P. carinii* f. sp. *muris* occurs as a *P. carinii* f. sp. *muris*-specific antibody response develops. This is consistent with previous findings (8, 9, 11, 18) that antibody responses to *Pneumocystis* are a significant contributor to resistance to this organism. It is of interest that the cellular response occurring in the lungs of the mice as they clear the *P. carinii* f. sp. *muris* is readily detectable in the mice by analysis of the BALF but is of such a low intensity that it could easily not be noticed by histologic analysis. The minimal amount of inflammation that occurs in the immunocom-

petent mice as they clear the *P. carinii* f. sp. *muris* is consistent with our inability to detect overt clinical signs in these mice.

The present results indicate that *P. carinii* f. sp. *muris* persists in the lungs of immunocompetent adults for about 5 weeks before an acquired immune response clears the organisms. This could be ample time for the infected mouse to infect other mice. It is of interest that neither an environmental source nor an animal reservoir for *Pneumocystis* has been identified, yet the organism appears to be ubiquitous (20). It seems unlikely that in nature, the number of immunodeficient hosts is adequate to perpetuate this organism. It has already been shown that immunocompetent infant animals can acquire *Pneumocystis* from infected mothers (3, 7, 15, 23) and that the organisms persist for several weeks. In addition, recent work by others has shown that immunocompetent mice can transmit *P. carinii* f. sp. *muris* to immunodeficient mice (6). Therefore, our data adds to the growing evidence that immunocompetent adult and infant animals harboring *Pneumocystis* as a subclinical infection may be reservoirs for this organism. Studies to determine the ability of *P. carinii* f. sp. *muris*-harboring immunocompetent mice to transmit *P. carinii* f. sp. *muris* to other immunocompetent mice are currently in progress in our laboratory. In addition, whether *P. carinii* f. sp. *muris* is completely cleared from immunocompetent adult mice infected by cohousing, or whether a latent infection can persist, remains to be determined.

#### ACKNOWLEDGMENTS

We acknowledge the technical assistance of Mike Tighe and Ann Harmsen.

This work was supported by NIH grants HL55002 and HL59833 as well as the Montana Agricultural Experiment Station and USDA Formula Funds.

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