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Susceptibility to *Pneumocystis carinii* Infection: Host Responses of Neonatal Mice from Immune or Naive Mothers and of Immune or Naive Adults

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Mice from either naive or immunized dams were given intranasal inoculations of *Pneumocystis carinii* as neonates (24 to 48 h old). Lung *P. carinii* burdens increased through day 13 postinoculation in all pups and declined to nearly undetectable numbers by day 23 in pups from immune mothers. However, *P. carinii* numbers in pups from naive mothers did not begin to decline significantly until after day 33, and *P. carinii* organisms were still detectable in low numbers through day 45. In contrast, the lungs of naive or immunized adult mice contained detectable numbers of *P. carinii* organisms only up to 9 or 3 days, respectively, after inoculation. The onset of clearance of *P. carinii* organisms from the lungs of neonatal mice and naive adults was coincident with infiltration of neutrophils and $CD4^+$ $CD45RB^{lo}$ cells into the alveolar spaces and increased titers of *P. carinii*-specific antibody in sera. Immunized dams had high levels of *P. carinii*-specific antibody in both their sera and milk, and pups from these dams had higher titers of *P. carinii*-specific antibody than did pups from naive dams. These data indicate that *P. carinii* survives for a much longer period in neonates than in adult mice, which is the result of a delay in the onset of the immune response in neonates. Furthermore, immunized mothers contributed to an early clearance of *P. carinii* organisms by their offspring presumably because of the transfer of *P. carinii*-specific antibody. However, the passively acquired antibody did not seem to have an effect until the neonates began to mount their own responses.

Most healthy children have developed antibodies to *Pneumocystis carinii* by 2 to 3 years of age, suggesting exposure to this organism at an early age (20). Although children with normal intact immune systems rarely develop clinical *P. carinii* pneumonia, this disease is a problem for children with primary immunodeficiency diseases and AIDS (21). In addition, patients with AIDS who are less than 1 year of age have a higher incidence and a more fulminant course of *P. carinii* pneumonia than do older children with AIDS (21).

Although little is known about the life cycle of *P. carinii*, it is currently thought that airborne organisms are inhaled early in life, resulting in a subclinical infection that persists until host cellular and humoral responses clear the *P. carinii* organisms from the lungs (20). It is currently unknown whether *P. carinii* enters a latent stage until some sort of immunosuppression allows it to reappear (13). However, if the initial immune response to *P. carinii* is sterilizing, there may be another, as-yet-unidentified reservoir in nature in which this organism exists until an immunosuppressed host is located. Because of the immaturity of the neonatal immune system, it is possible that *P. carinii* can exist in infants until specific immunity is sufficiently developed for clearance to take place.

Neonates lack mature $CD4^+$ T cells (1, 2, 30), which have recently been shown to be absolutely necessary for *P. carinii* resistance (14, 25). Furthermore, blockage of CD40L in vivo with a monoclonal antibody results in decreased resistance to *P. carinii* in T-cell-reconstituted adult SCID mice (29). In this regard, T cells from neonates have been reported to have diminished CD40L expression (3, 10, 12, 19). Tumor necrosis factor alpha has also been shown to play an important role in host resistance against *P. carinii* (6). It has been reported that neonatal T cells produce less tumor necrosis factor alpha than do T cells from adults under similar conditions (30). T cells from neonates have also been recently shown to have diminished Th1-like responses (1), require greater accessory cell signalling than do T cells from adults (1), and have diminished cytokine production upon stimulation (gamma interferon, tumor necrosis factor alpha, etc.) (30). Thus, decreased function of T cells coupled with decreased numbers compared with those of adults may lead to inadequate host resistance to *P. carinii* in neonatal mice. However, the susceptibility to *P. carinii* of neonatal mice compared with adult mice has not been reported.

Although the clearance of *P. carinii* organisms from the lungs of adult mice is known to be T cell dependent (14, 25), there is only indirect evidence suggesting that humoral responses play a role in resistance to *P. carinii*. Recently, it was reported that adult mice immunized against *P. carinii* were capable of clearing a subsequent challenge even after in vivo depletion of $CD4^+$ T cells (13). These results suggest that *P. carinii*-specific antibody found in the sera of these mice was integral in clearance of this organism. Antibody responses to various antigens have been reported to be low in neonates compared with those in adults and restricted to the immunoglobulin M (IgM) isotype (4, 10), which is most likely due to inadequate T-cell help (28). Since neonates are born with inadequate cellular and humoral immune functions, it was of interest to determine if *P. carinii*-specific antibody acquired from mothers would assist in the clearance of organisms from the lungs of *P. carinii*-challenged neonates. In this regard, immunization of mothers has proven effective in protecting neonates from challenge with other pathogens, such as influenza virus and *Streptococcus pneumoniae* (11, 15, 16, 18). However, whether neonates can passively acquire resistance to *P. carinii* from an immune mother is not known.

The purpose of the studies described here was to compare

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the growth of *P. carinii* organisms in the lungs of inoculated neonates and adult mice. In addition, whether pups from immune dams acquire resistance to *P. carinii* from their mothers was investigated. The findings reported here indicate that *P. carinii* organisms inoculated into normal immunocompetent neonatal mice persist until after weaning age. $CD4^+$ T cells are conspicuously absent from the alveolar spaces of infected mice until about 3 weeks of age. Furthermore, immunizing mothers resulted in earlier clearance of *P. carinii* organisms from the lungs of their offspring.

MATERIALS AND METHODS

Mice and experimental design. Adult C.B17 male and female mice (8 to 10 weeks old) were obtained from the Trudeau Animal Breeding Facility. Mice to be used for breeding in experiments and immunized adult mice were given two intratracheal inoculations with about 10^7 *P. carinii* nuclei at 21 and 4 days before breeding. The mice then received an intranasal (i.n.) inoculation of about 5×10^6 *P. carinii* nuclei on day 10 of gestation. Naive females and males used in experiments were housed separately from immunized mice at the animal breeding facility until day 20 of gestation. On day 0 of the experiment, neonatal and adult mice were given i.n. challenge doses of about 10^6 and 10^7 *P. carinii* nuclei, respectively. These inocula were approximately equal on the basis of body weights for neonates (1.5 to 2 g) and adults (18 to 25 g). Neonatal mice were routinely 24 to 48 h old upon inoculation (day 0).

Preparation of *P. carinii* for inoculations. Immunization of dams and adult male mice was performed with lung homogenates from *P. carinii*-infected C.B17 *scid/scid* mice. Lungs were removed and pushed through a stainless steel screen in 5 ml of phosphate-buffered saline (PBS). Samples were spun onto glass slides, fixed in methanol, and stained in Diff-Quik (Baxter Healthcare Inc., Miami, Fla.). *P. carinii* nuclei were enumerated microscopically. Adult mice were immunized with 10^7 *P. carinii* nuclei in 100 μl of Hanks balanced salt solution (HBSS). Mice were anesthetized deeply with holothane gas and placed in a vertical position. A blunted 20-gauge needle was passed through the oral pharynx into the trachea, and *P. carinii* organisms were injected into the trachea through the needle.

Challenge of neonates and adults on day 0 was performed with *P. carinii* organisms partially purified from lung homogenates of infected C.B17 *scid/scid* mice. Homogenates were obtained in HBSS supplemented with 0.5% glutathione as described above, aspirated through 22.5- and 26-gauge needles to disperse clumps, spun at low speed to remove cell debris, and pelleted. Erythrocytes were lysed in H_2O via hypotonic shock, and each isolate was incubated in buffer with 100 μg of DNase for 30 min at 37°C. *P. carinii* organisms were washed by centrifugation and filtered through 5-μm nylon mesh. *P. carinii* nuclei were enumerated as described above, and the concentration was adjusted so that neonates received 10^6 *P. carinii* nuclei in 10 μl of HBSS and adults received 10^7 *P. carinii* nuclei in 50-μl inoculations. Mice were lightly anesthetized with halothane gas to inhibit the divers reflex. The *P. carinii* inoculum was placed over both nares. Mice aspirated the entire volume since they are obligate nose breathers. This method of inoculation resulted in consistent infections in both neonates and adults with low standard deviations (see Fig. 1 and 2) and was better tolerated in neonates than were attempts at intratracheal inoculations.

Comparison of cellular infiltration into the alveolar spaces. Mice were sacrificed by exsanguination under deep halothane anesthesia. The lungs of neonates up to day 13 postinoculation were lavaged with five 0.1-ml aliquots of PBS containing 3 mM EDTA. Thereafter, the pup lungs were lavaged with five 0.2-ml aliquots and the adult lungs were lavaged with five 1-ml aliquots of PBS-EDTA. Aliquots of the lung lavage fluids were spun onto glass slides by using a cytocentrifuge (Shandon, Sewickley, Pa.), fixed in methanol, and stained with Diff-Quik, and differential counts were performed microscopically.

Enumeration of *P. carinii* nuclei in the lungs of neonatal and adult mice. The numbers of *P. carinii* nuclei in the lungs of neonatal and adult mice were determined as previously described (14), with some modifications. Lungs were excised and pushed through a screen in 1 to 3 ml (pups) or 5 ml (adults) of PBS as described above. Lung homogenates were diluted 1:10 (pups) or 1:20 (adults), and 100 μl was spun onto a 28.3-mm^2 area of a glass slide, fixed in methanol, and stained in Diff-Quik. The number of *P. carinii* nuclei per 20 to 50 oil immersion fields was used to calculate total *P. carinii* nuclei per lung homogenate. Previously in our laboratory we have observed that up to 5 days after instillation, *P. carinii* nuclei can be visualized in lung lavage fluids (unpublished observations). Therefore, the total number of *P. carinii* nuclei per pair of lungs was obtained by adding *P. carinii* nuclei in 10 to 50 oil immersion fields enumerated from the lung lavage slides and added to the number calculated for the lung homogenate. As pups aged, the volumes used for lung lavages and lung homogenates were adjusted to accommodate the increasing sizes of the lungs. The limit of detection of *P. carinii* nuclei per pair of lungs ranged from $\log_{10}$ 3.55 to 3.84 for pups and 3.84 to 4.08 for adults.

Flow cytometric analysis of lung lavage lymphocytes. Lung lavage cells were washed in PBS with 0.1% bovine serum albumin and 0.02% NaN_3 and stained with appropriate concentrations of fluorescein isothiocyanate-conjugated anti-CD4 (hybridoma GK1.5) and biotinylated anti-CD45RB (clone 16A; PharMingen) followed by streptavidin-CyChrome (PharMingen). The proportion of lung lavage cells which were positive for specific fluorescence was determined by using a FACScan cytofluorometer (Becton Dickinson, Mountain View, Calif.). Naive adult mouse splenocytes were included in all analyses in order to assist in distinguishing $CD45RB^{hi}$ cells from $CD45RB^{lo}$ cells.

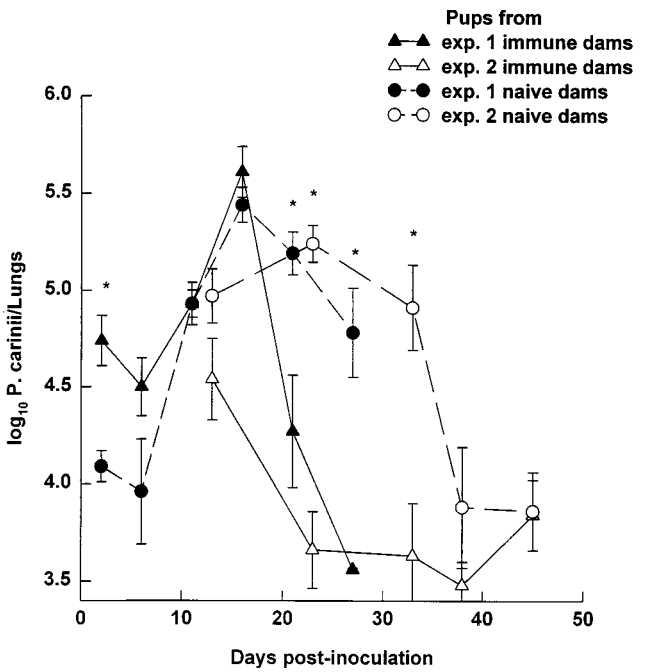

FIG. 1. *P. carinii* clearance from the lungs of pups from immunized dams was more rapid than clearance in pups from naive dams. Neonatal mice (24 to 48 h old) from either immunized or naive dams were given i.n. inoculations of about 10^6 *P. carinii* nuclei. Lung *P. carinii* burdens were determined at the times indicated by microscopic examination of cytospin preparations of lung homogenates stained with Diff-Quik. Data from two separate kinetics experiments (days 2 to 27 and days 13 to 45) are shown. Data are the mean ± standard error of the mean (SEM) $\log_{10}$ numbers of *P. carinii* nuclei in the lungs of four to five mice per experimental group. *, $P \leq 0.05$, compared with result for pups from immunized dams. exp., experiment.

Analysis of *P. carinii*-specific antibodies in serum and milk. Blood samples were collected from adults and neonates at the time of sacrifice by severing the abdominal aorta. Blood samples from dams were collected from the retro-orbital sinus vein at day 2 of the experiment or from the abdominal aorta at the termination of the experiment. Sera were frozen at −20°C until needed. Milk samples were collected from dams on day 2 of the experiment. Dams were physically, but not visually, separated from pups for 4 h and then injected with 0.5 U of oxytocin (Sigma) intravenously 10 min prior to milk collection. Milk was manually expressed, diluted 1:5 in PBS containing 3 mM EDTA, and spun at high speed for 5 min in a microcentrifuge. The clear liquid between the pelleted cells and floating fat was collected and frozen at −20°C until needed. Antibodies (IgG) specific for *P. carinii* were detected from 1:50 dilutions of serum or milk by a standard enzyme-linked immunosorbent assay (ELISA) as described elsewhere (5).

Statistical analysis. Differences between experimental groups were determined by using Student's *t* test or the Mann-Whitney rank sum test, as appropriate. Differences with $P \leq 0.05$ were considered significant.

RESULTS

Clearance of *P. carinii* nuclei from the lungs of offspring from immunized and naive dams. Neonatal C.B17 mice from immunized and naive dams were given an i.n. challenge of 10^6 *P. carinii* nuclei at 24 to 48 h of age. Neonates from immunized dams as well as those from naive dams had over 10^4 *P. carinii* nuclei in their lungs at day 2 postinoculation (Fig. 1). Furthermore, after a small decline at day 6, *P. carinii* organisms grew 10-fold in number in the lungs of both groups of pups through

day 16 postinoculation (Fig. 1). Thus, through day 16, the rate of growth of *P. carinii* organisms in the lungs of pups from immunized dams was similar to that of pups from naive dams. Thereafter, pups from immunized dams began clearing the organisms from their lungs through day 27 postinoculation, at which time the lung *P. carinii* burden was near the limit of detection ($10^{3.55}$) (Fig. 1). In contrast, the lung *P. carinii* burden in pups from naive dams began to decrease only slowly after day 16 and was still nearly 10^5 at day 27 postinoculation (Fig. 1).

A second experiment utilizing later time points was carried out to determine the time necessary to clear *P. carinii* nuclei from the lungs of immunocompetent pups from naive dams. As seen in Fig. 1, lung *P. carinii* burdens reached their peak in pups from immunized dams as well as from naive dams by day 13 postinoculation (Fig. 1). Notably, the *P. carinii* burden was near the limit of detection in the lungs of pups from immunized dams by day 23 postinoculation (Fig. 1), which was consistent with the kinetics of clearance seen in the first experiment (Fig. 1). In contrast, *P. carinii* numbers remained elevated in the lungs of pups from naive dams through day 23, and significant clearance of this organism did not occur until after day 33 postinoculation (Fig. 1). By day 45 postinoculation, the *P. carinii* burden in the lungs of pups from naive dams was near the limit of detection (Fig. 1). These results indicated a significantly faster rate of *P. carinii* clearance from the lungs of pups from immune dams versus the clearance from pups of naive dams.

As a comparison to the kinetics of clearance of *P. carinii* organisms from the lungs of neonatal mice, clearance of this organism from adult lungs was also evaluated. Adult C.B17 mice which either were naive or had been immunized on the same schedule as were the immunized dams received i.n. inoculations of *P. carinii*. A 10-fold-higher dose of *P. carinii* compared with that given to neonates was given to adults since the adults had approximately 10 times the body weight of the pups. Both the naive and immunized adult mice had detectable *P. carinii* organisms in the lungs at day 3 postinoculation (Fig. 2A). The immunized adults had cleared the *P. carinii* organisms from the lungs by day 9, whereas there was no significant change in the lung burden in the naive adults until after day 9 postinoculation. By day 13 postinoculation, the *P. carinii* burden in the lungs of naive adults was near the limit of detection and by day 23 was undetectable (Fig. 2A). *P. carinii* clearance from the lungs of both immunized and naive adults was significantly faster than clearance from mice challenged as neonates.

***P. carinii*-specific antibody in the sera of pups and adults.** The level of *P. carinii*-specific IgG in serum was elevated in the pups from immunized dams compared with that of the pups from naive dams throughout the first 27 days of the first experiment (Fig. 3). Interestingly, the largest difference in the serum *P. carinii*-specific IgG levels between the two groups of pups was at day 6 postinoculation, which was just prior to the spurt of growth of this organism in the lungs of pups from both groups. In the second experiment, the differences in the serum *P. carinii*-specific IgG levels between pups from immunized dams and those from naive dams were even more apparent than they were in the first experiment (Fig. 3). High titers of *P. carinii*-specific IgG were found in the sera of pups from immune dams at day 13 postinoculation (Fig. 3). This corresponded to large amounts of *P. carinii*-specific IgG in both the sera (optical density at 405 nm [OD_{405}] = 0.562 ± 0.102) and milk samples (OD_{405} = 0.392 ± 0.096) of immunized dams compared with those in the sera (OD_{405} = 0.029 ± 0.014) and milk samples (OD_{405} = 0.022 ± 0.012) of naive dams (determined at day 2). The serum *P. carinii*-specific antibody level

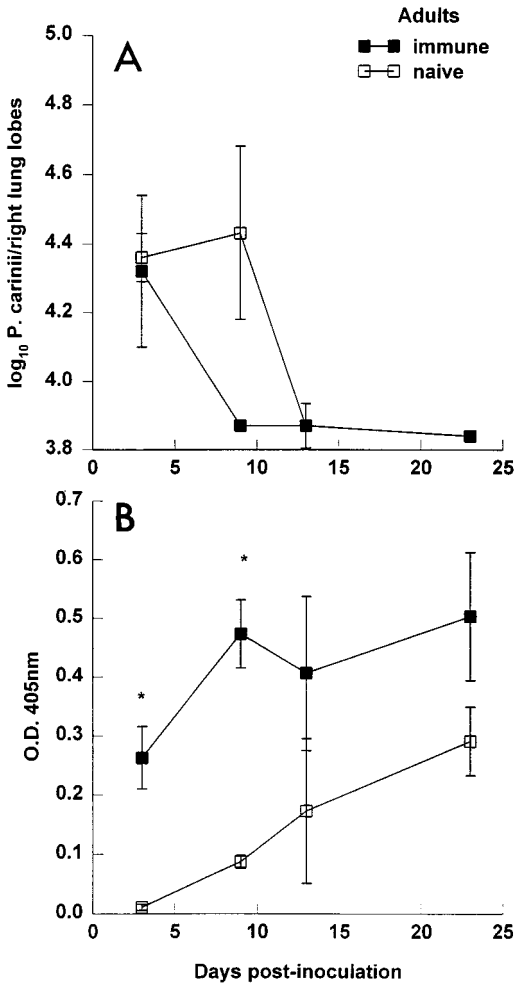

FIG. 2. Faster clearance of *P. carinii* organisms from the lungs of immunized adults, compared with that from the lungs of naive adults, corresponded with higher *P. carinii*-specific antibody levels in sera. Lung *P. carinii* burdens (A) and serum *P. carinii*-specific antibody levels (B) in immunized and naive adults were determined as described in the legends to Fig. 1 and 3. Data are the means ± SEM for four to five mice per group. Data are from one of two experiments with similar results. *, $P \leq 0.05$, compared with result for immunized adults at the same time point.

gradually decreased in pups from immune dams over the course of the 45-day experiment, suggesting that the antibody was obtained from the dams. In contrast, the serum *P. carinii*-specific IgG level in pups from naive dams remained at near-background levels until day 45 postinoculation when there was a slight increase (Fig. 3). This indicated that the presence of high titers of *P. carinii*-specific antibody in sera did not alter the early growth of *P. carinii* organisms in the lungs of pups born to immunized dams. However, these pups began reducing their lung *P. carinii* burden approximately 2 weeks earlier than did pups from naive dams, which had consistently smaller amounts of *P. carinii*-specific IgG in their sera.

By comparison, the serum *P. carinii*-specific IgG level in immunized adult mice was significantly higher than that in naive adult mice at days 3 and 9 postinoculation (Fig. 2B). However, unlike the pups from immunized dams, the serum *P. carinii*-specific IgG level in immunized adults remained elevated until after *P. carinii* clearance (Fig. 2B). Although low at day 3 in naive adults, the serum *P. carinii*-specific IgG level

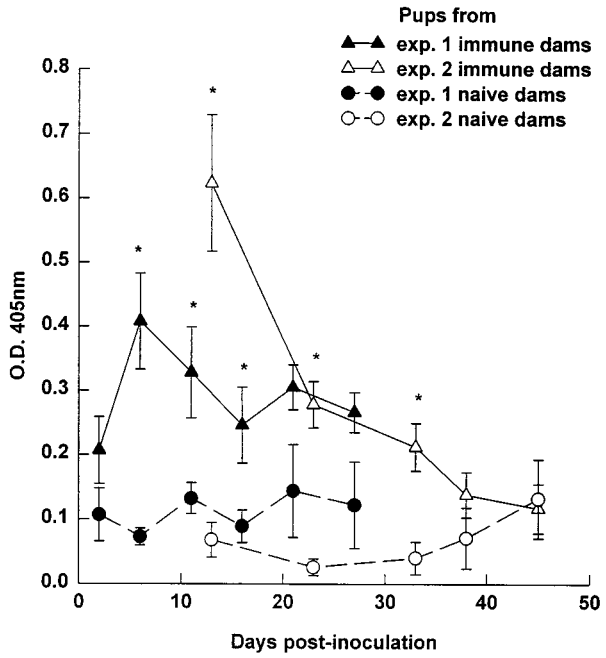

FIG. 3. The serum *P. carinii*-specific antibody level in pups from immunized dams was significantly higher than that in pups from naive dams. Sera were collected from pups at the times indicated. The *P. carinii*-specific antibody levels in sera were determined by ELISA. The mean ± SEM OD_{405} readings for four to five mice per group are reported. Data from two separate kinetics experiments (days 2 to 27 and days 13 to 45) are shown. *, $P \leq 0.05$, compared with result for pups from immunized dams. exp., experiment.

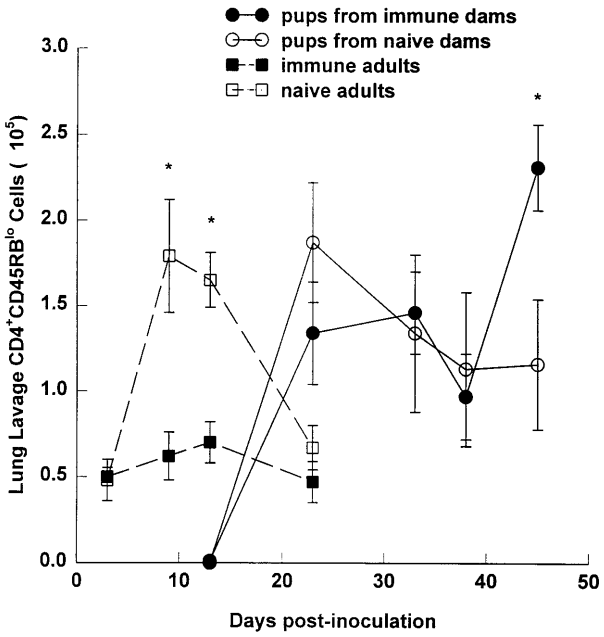

FIG. 4. Infiltration of activated $CD4^+$ cells into the alveoli of pups from immunized dams was similar to that in pups from naive dams in kinetics and intensity. However, the cellular infiltration in pups was delayed compared with that in adults. The number of $CD4^+$ $CD45RB^{lo}$ cells in lung lavage samples from adults or pups was determined by using fluorescently labelled antibodies and fluorescence-activated cell sorter analysis. Data are the means ± SEM for four to five mice per group. Data are from one of three experiments with similar results. *, $P \leq 0.05$, compared with result for the immunized group at the same time point.

increased steadily throughout day 23 postinoculation (Fig. 2B). This steady increase in the *P. carinii*-specific IgG level in naive adults corresponded to the clearance of *P. carinii* organisms from the lungs (Fig. 2).

Inflammatory cell response in the alveolar spaces of *P. carinii*-infected mice. Lung lavages were performed on mice in order to determine the types of cells associated with *P. carinii* clearance. $CD4^+$ T cells with an activated phenotype ($CD45RB^{lo}$) were enumerated by flow cytometry (17). $CD4^+$ $CD45RB^{lo}$ cells were nearly undetectable in the lung lavage samples of pups from either group at day 13 postinoculation (Fig. 4). In other experiments not shown, $CD4^+$ cells were also not detectable in the lungs of neonates at 3, 6, and 11 days postinoculation. However, by day 23, pups from immunized dams as well as those from naive dams had over 10^5 $CD4^+$ $CD45RB^{lo}$ cells in their lung lavage samples. These cells persisted in the lungs of both groups of pups through day 45 of the experiment (Fig. 4). It should be noted that low numbers of $CD8^+$ cells ($<8 \times 10^4$) were present at day 23 in the lungs of pups from both immune and naive dams (data not shown). These cells remained at low levels through day 45.

The numbers of $CD4^+$ $CD45RB^{lo}$ cells in the alveoli of immune and naive adults were similar at day 3 postinoculation; however, the number increased threefold in the alveoli of naive adults by day 9 and remained elevated through day 13 postinoculation before decreasing at day 23 (Fig. 4). In contrast, $CD4^+$ $CD45RB^{lo}$ cells in the lung lavage samples of immunized adults remained constant at around 5×10^4 through day 23 postinoculation (Fig. 4). It should be noted that in uninfected C.B17 mice, macrophages make up over 95% of lung lavage cells and $CD4^+$ cells are rare (data not shown). In both pups and adults from either the immunized or naive group, the appearance of $CD4^+$ $CD45RB^{lo}$ T cells in the lung lavage samples corresponded to a decreased lung *P. carinii* burden.

In addition to lymphocyte subsets, lung lavage neutrophils were enumerated as an indication of the lung inflammatory response to *P. carinii* in pups and adults. Neutrophils were undetectable in the lung lavage samples of either group of pups at day 13 postinoculation as well as at earlier time points (data not shown); however, there was a large infiltration of neutrophils in pups from both immunized and naive dams by day 23 (Fig. 5). After the peak at day 23 postinoculation, neutrophil numbers in the lung lavage samples of both groups of pups declined through day 45 (Fig. 5). Although statistically significant only on day 23, the number of neutrophils in the lung lavage samples of pups from naive dams overall tended to be greater than that in pups from immunized dams. Furthermore, the appearance and decline of neutrophils in both groups of pups corresponded with the clearance of *P. carinii* organisms from the lungs. Neutrophils also accumulated in adult lungs at day 3 postinoculation and then declined significantly by day 9 (Fig. 5). Interestingly, the accumulation of neutrophils was almost 10-fold higher in the alveolar spaces of naive adults compared with that in immunized adults at day 3 postinoculation. These data indicate that neutrophil accumulation in adult lungs is considerably higher during a primary response to *P. carinii* than during a secondary response.

DISCUSSION

The data presented above demonstrate that *P. carinii* is able to survive and, more importantly, to grow for an extended period (almost 6 weeks) in normal neonatally infected mice. This is in agreement with the work of Soulez et al. that showed

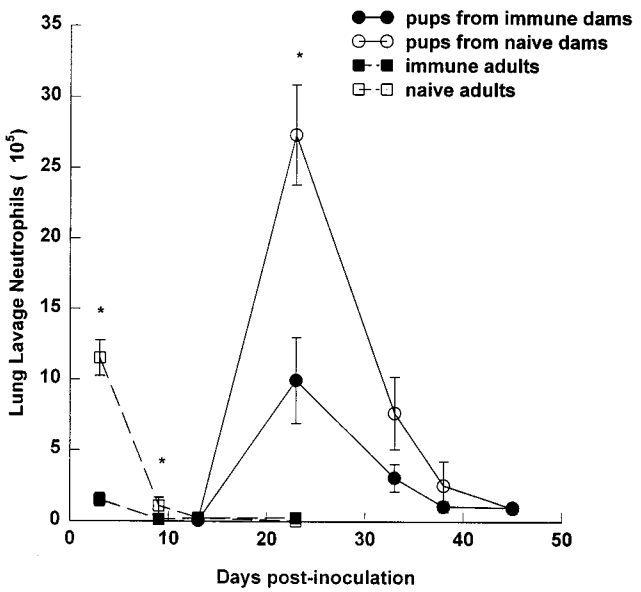

FIG. 5. Infiltration of neutrophils into the alveoli of pups from naive dams was more intense than infiltration into the alveoli of pups from immunized dams or of either group of adults. Lung lavage cells from adults and pups were spun onto glass slides and stained in Diff-Quik, and neutrophils were enumerated microscopically. Data are the means ± SEM of four to five mice per group. Data are from one of three experiments with similar results. *, $P \leq 0.05$, compared with result for the immunized group at the same time point.

weanling rabbits to be susceptible to *P. carinii* infection (26). In contrast, naive adult mice cleared *P. carinii* organisms from their lungs within 2 weeks of infection and the organisms did not increase in number over that time. *P. carinii* is an opportunistic pathogen which is efficiently disposed of in an immunocompetent host but can cause life-threatening pneumonia in individuals with compromised immune systems (27). Although *P. carinii* is thought to be ubiquitous, its precise life cycle and its residence in nature until a susceptible host is located have remained a mystery (8). Thus, neonates from immunocompetent parents may provide a reservoir where *P. carinii* can grow for weeks in mice and, therefore, possibly months in humans until the host immune system becomes mature enough to eliminate this pathogen. Interestingly, although the number of *P. carinii* organisms increased 10-fold in the lungs of neonates over a 2-week period, the pups did not display signs of illness, indicating that such an infection may easily go undetected.

P. carinii clearance in both adults and pups corresponded to increased levels of *P. carinii*-specific antibody in their sera. Furthermore, pups from immunized dams had significantly higher levels of *P. carinii*-specific IgG in their sera than did pups from naive dams during the first 3 weeks after inoculation. Although this high titer of *P. carinii*-specific IgG corresponded to faster clearance of this organism from the lungs, the initial growth of *P. carinii* organisms in the lungs of pups with high levels of *P. carinii*-specific IgG in their sera was similar to the initial growth of this organism in the lungs of pups with negligible serum antibody levels. This indicates that *P. carinii*-specific antibody was ineffective at preventing growth in the first 2 weeks of the infection. In this regard, it has been reported that adult SCID mice clear *P. carinii* organisms from the lungs after reconstitution with purified $CD4^+$ cells from immunized donors without the presence of *P. carinii*-specific antibody (22, 29). Thus, although *P. carinii*-specific antibody is not absolutely required for *P. carinii* clearance in some animal models, the presence of antibody in the pups from immunized dams evidently made a difference in the kinetics of *P. carinii* clearance. These pups cleared the *P. carinii* organisms nearly 2 weeks earlier than did pups from naive dams, even though the cellular response to *P. carinii* was nearly identical in kinetics and intensity for the two groups of pups. Notably, in both groups of pups, *P. carinii* clearance did not take place until neutrophils and $CD4^+$ cells began to accumulate in the lungs. A possible explanation for these data is that maternal *P. carinii*-specific antibody, while present in the sera of pups from immunized dams, may not have entered the alveolar spaces until the initiation of an inflammatory response caused serum transudation. Indeed, once the inflammatory response began, maternal *P. carinii*-specific antibody appeared to contribute significantly to *P. carinii* clearance. It is also conceivable that maternal B or T cells acquired through milk may have contributed to the clearance of *P. carinii* organisms in pups from immunized dams (24); however, further experiments have to be performed to examine this issue.

The clearance of *P. carinii* organisms in both pups and adults followed the appearance of $CD4^+$ $CD45RB^{lo}$ cells in the alveolar spaces. These cells had a phenotype consistent with being activated (17) and represented over 80% of the $CD4^+$ cells in the lung lavage samples of both pups and adults. Interestingly, approximately three times as many $CD4^+$ $CD45RB^{lo}$ cells accumulated in the lungs of naive adults compared with the accumulation in immunized adults at days 9 and 13 postinoculation. Furthermore, significantly more neutrophils accumulated in the lungs of naive adults than in immunized adults while antibody in sera developed more slowly in naive adults than in immunized adults. Although *P. carinii* organisms were cleared very quickly from both naive and immune adults, there was a significant difference in these two groups of mice in that the *P. carinii* number remained constant in naive mice over the first 9 days but decreased to nearly undetectable numbers in immune mice during the same period. The more efficient clearance of *P. carinii* organisms evident by fewer cells and high titers of specific antibody represents a classic secondary immune response. Unlike the differences found between naive and immunized adults, the cellular responses in pups from naive and immunized dams were virtually identical. Furthermore, the cellular response to *P. carinii* in the lungs of mice infected as neonates was similar in cell type and intensity to that in the primary response observed in naive adults.

A profound difference between the primary response mounted by naive adults and that mounted by pups was the protracted length of time required to initiate the response to *P. carinii* in pups. This delay in T-cell infiltration into the lungs of *P. carinii*-infected pups compared with that in adults may be due to a number of factors in pups, such as diminished adhesion molecule expression, accessory molecule expression, accessory cell function, and cytokine production (1, 3, 7, 12, 23, 28). In this regard, it has been reported that T cells from murine neonates are deficient in T-helper function possibly because of decreased CD40 ligand expression, interleukin-2 production, and signalling through CD28 (1–3, 7, 9, 12, 23, 28). Neonatal T-cell responses to anti-CD3 stimulation in vitro have been shown to approach those of adults in the presence of exogenous cytokines such as interleukin-6 and interleukin-2 and with accessory cell signalling through CD28 (1, 9), suggesting that T cells from neonates lack the accessory signals necessary for responses to various pathogens. Diminished T-cell function may be the reason that *P. carinii* clearance took almost 3 weeks to be initiated in pups from naive dams compared with 2 weeks in naive adults. However, this does not

necessarily explain the 3-week delay in the onset of the inflammatory response in pups compared with a nearly immediate response in adults. One possibility is that the appropriate cytokines for signalling the initiation of the response were not available. Another possibility is that there was no recognition of the *P. carinii* organisms in the lungs of neonates. Although it is known that specific recognition via the T-cell receptor or antibody is required for *P. carinii* clearance (14, 25), it is unclear that recognition took place to signal T-cell and neutrophil infiltration into the lungs initially. It is possible that innate immune defenses were insufficient to signal the beginning of the immune response to *P. carinii*.

The results of experiments presented here clearly demonstrate that *P. carinii* is able to colonize and grow in neonates from immunocompetent dams, whether they have been previously immunized or not. This is important because it suggests that exposure to infants may be a source of transmission of *P. carinii* to immunocompromised individuals. This early, unchecked growth of *P. carinii* may also explain why *P. carinii* pneumonia has such a fulminant course in patients with AIDS who are less than 1 year of age (21). *P. carinii* organisms are cleared from the lungs of mice infected as neonates only after the initiation of an inflammatory response which includes $CD4^+$ T cells and neutrophils. Furthermore, once the inflammatory response has been initiated, the presence of *P. carinii*-specific antibody, acquired from immunized dams, results in accelerated clearance of this organism. This finding is important because it indicates that immunization of mothers could significantly enhance resistance to *P. carinii* and may represent a way to improve resistance to *P. carinii* pneumonia in high-risk infants.

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