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Latency Is Not an Inevitable Outcome of Infection with *Pneumocystis carinii*

WANGXUE CHEN,[1]* FRANCIS GIGLIOTTI,[2] AND ALLEN G. HARMSEN[1]

Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, New York 12983,[1] and Departments of Pediatrics, Microbiology, and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642[2]

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Severe combined immunodeficiency (SCID) mice resolve naturally acquired *Pneumocystis carinii* pneumonia after reconstitution with immunocompetent spleen cells and can therefore be used as a model to study latent *P. carinii* infection. Neither *P. carinii* nor amplified *P. carinii* DNA was detected in the lungs of SCID mice killed 21 days after spleen cell reconstitution. Furthermore, SCID mice that recovered from *P. carinii* infection failed to reactivate the infection after they were either depleted of $CD4^+$ cells for up to 84 days or depleted of $CD4^+$ cells and treated with corticosteroid for 35 days. These results indicate that an immune response to *P. carinii* can completely clear the organism from the host. This supports the hypothesis that *P. carinii* pneumonia that develops in immunocompromised patients may be a new infection resulting from exposure to an exogenous source of *P. carinii* and not necessarily from reactivation of latent infection.

Pneumocystis carinii pneumonia (PCP) is a major cause of morbidity and mortality in immunocompromised individuals, especially in AIDS patients (22). An important, unresolved question in the pathogenesis and epidemiology of PCP concerns the mode of transmission of this infection. It is generally assumed that the PCP that develops in AIDS patients with severe destruction of $CD4^+$ cells represents the reactivation of latent infection acquired as early as childhood (1, 16, 23). This assumption has been supported by experimental induction of PCP in animals with immunosuppressive agents (6, 20, 24). However, neither the manner of reactivation of latent infection nor the site at which the organism lies dormant has been determined. Moreover, attempts to demonstrate latent *P. carinii* in the lungs (14, 15) and bronchoalveolar lavage fluids (21) of immunocompetent hosts, aged between 15 and 75 years, were unsuccessful even when techniques such as DNA amplification by the polymerase chain reaction (PCR) were used. These results suggest that asymptomatic carriage of *P. carinii* in the lungs of immunocompetent hosts is rare. Therefore, PCP that develops in immunocompromised patients might be from a new infection resulting from exposure to exogenous sources of *P. carinii* through contact with infected persons or other environmental sources. However, the reported occurrence of extrapulmonary *P. carinii* infection (5) suggests that the lung is not the only tissue that can harbor *P. carinii*. Thus, whether the development of PCP in AIDS patients results from the reactivation of latent infection dormant in either pulmonary or extrapulmonary sites or, indeed, results from a new infection is still not clear. In the present study, we examined whether *P. carinii* could exist as a latent infection in severe combined immunodeficiency (SCID) mice that resolved PCP as a result of spleen cell reconstitution. The findings presented in this report show that *P. carinii* or specific *P. carinii* DNA was not detected in the lungs of reconstituted SCID mice killed at 21 days postreconstitution or thereafter. Furthermore, depletion of $CD4^+$ cells in reconstituted SCID mice that had resolved PCP failed to reactivate the infection. These results suggest that latent *P. carinii* infection was not present in either the lungs or the extrapulmonary sites of these mice.

C.B-17 *scid/scid* (SCID) mice (Trudeau Institute Animal Breeding Facility, Saranac Lake, N.Y.) were 6 to 8 weeks of age, and the mice were housed in microisolator cages containing sterilized food and water (10). All SCID mice in this colony develop spontaneous *P. carinii* infection that is cytologically detectable at about 4 weeks of age, and this infection progresses with time (10). At the beginning of each experiment, two to five mice were killed to determine the *P. carinii* burden, and the remaining mice were reconstituted with 5×10^7 spleen cells from congenic C.B-17+/+ mice in 1.0 ml of phosphate-buffered saline (day 0 postreconstitution [DPR 0]) (10). Four mice were killed on either DPR 21 or DPR 28 to examine the resolution of *P. carinii* infection. The remaining mice were given weekly injections of 0.5 mg of a monoclonal antibody to CD4 (clone GK 1.5, rat immunoglobulin G2b; American Type Culture Collection) to deplete $CD4^+$ cells (9, 10). One group of these mice were killed between 42 and 84 days after the depletion of $CD4^+$ cells. The numbers of *P. carinii* nuclei in the lungs of mice killed at different times were determined as described previously (10). The results of a representative experiment are summarized in Table 1. Reconstitution of *P. carinii*-infected SCID mice with immunocompetent spleen cells resulted in resolution of *P. carinii* infection by DPR 28, in that there was no detectable *P. carinii* in their lungs. Moreover, *P. carinii* was not detected in the lungs of reconstituted SCID mice that had been depleted of $CD4^+$ cells for 42 to 84 days starting from DPR 28. To verify the absence of latent *P. carinii*, the lungs of SCID mice killed on DPR 21 or at different times after the depletion of $CD4^+$ cells were analyzed for *P. carinii*-specific DNA. This was accomplished by utilizing the PCR technique, as previously described (7), with a pair of primers (pAZ102-E and pAZ102-H) that amplify a portion of the gene encoding the mitochondrial rRNA of *P. carinii* isolated from all hosts studied to date (15). This assay was able to detect *P. carinii* DNA at a concentration of approximately 10 organisms per ml of freshly prepared *P. carinii*-infected mouse lung homogenate which was serially diluted into an unin-

* Corresponding author.

TABLE 1. Numbers of *P. carinii* nuclei in the lungs of SCID mice with spleen cell reconstitution and anti-CD4 treatment[a]

Treatment	Time (DPR)	No. of *P. carinii* nuclei per lung (*n*)[b]
No reconstitution (control mice)	0	5.40 (2)
Spleen cell reconstitution	28	≤3.98 ± 0.00 (4)
Spleen cell reconstitution + anti-CD4 treatment (42 days)[c]	70	≤3.98 ± 0.00 (3)
Spleen cell reconstitution + anti-CD4 treatment (84 days)[c]	112	≤3.98 ± 0.00 (4)

[a] Six-week-old SCID mice were reconstituted with immunocompetent spleen cells on DPR 0.

[b] Data are $\log_{10}$ of means ± standard deviations (numbers of mice), except for datum for unreconstituted controls, which is presented as an average of scores for two mice ($\log_{10}$, 5.00 and 5.79). The results are from one of three experiments with similar results. The limitation of detection of *P. carinii* nuclei was $10^{3.98}$ nuclei per lung.

[c] Treatment reagents were given intraperitoneally starting from DPR 28 and then once a week. The numbers in parentheses indicate the duration of anti-CD4 treatment.

fected lung homogenate (8). This level of detection is similar to the detection limit of 100 *P. carinii* organisms per g of lung tissue reported by the laboratory group which originated these primers (15). Despite the sensitivity of PCR, no amplified *P. carinii* DNA was detected in the lungs of SCID mice killed on DPR 21 and thereafter or at different times (42 to 72 days) following anti-CD4 treatment (Fig. 1). These results indicate that the failure to detect *P. carinii* in the lungs of SCID mice killed at different times after the depletion of $CD4^+$ cells was because of the absence of latent *P. carinii* infection rather than because the numbers of organisms were below the limit of cytological detection.

It was unlikely that the failure to reactivate *P. carinii* infection by depletion of $CD4^+$ cells in the present study was due to the duration of $CD4^+$ cell depletion or to incomplete depletion of $CD4^+$ cells. The decision to kill animals between 42 and 84 days after anti-CD4 treatment was based on the results of previous studies of experimental *P. carinii* infection in $CD4^+$ cell-depleted mice (2, 9, 10, 19). In these studies, mice developed cytologically detectable spontaneous or experimentally induced *P. carinii* infections after they had been treated with anti-CD4 monoclonal antibody for 42 to 84 days (2, 10, 19). The efficacy of $CD4^+$ cell depletion in the anti-CD4-treated mice in the present study was more than 99% in all but one of the mice, as determined by analysis of single cell suspensions prepared from the spleens or mesenteric lymph nodes by using a FACScan cytofluorometer (Becton Dickinson, Sunnyvale, Calif.) (9).

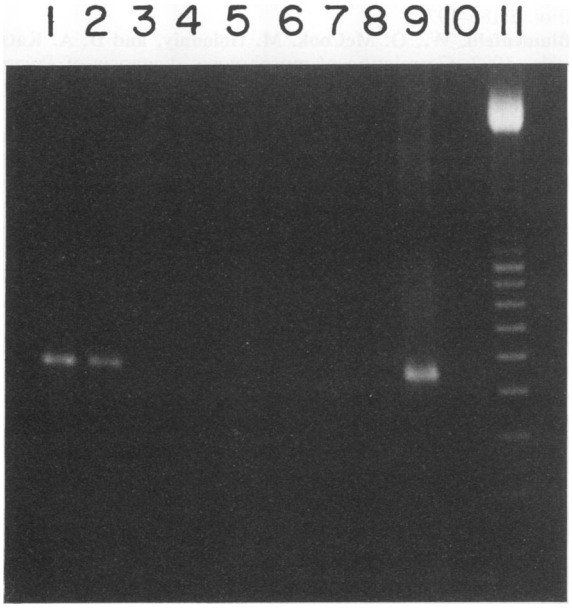

FIG. 1. Ethidium bromide-stained agarose gel electrophoresis of DNA amplification reactions from lung homogenates of SCID mice with different treatments. The primers used were pAZ102-E (5′-GATGGCTGTTTCCAAGCCCA-3′) and pAZ102-H (5′-GTGTA CGTTGCAAAGTACTC-3′). Lanes: 1 and 2, lungs of *P. carinii*-infected SCID mice killed on DPR 17; 3 and 4, lungs of *P. carinii*-infected SCID mice killed on DPR 21; 5 to 7, lungs of *P. carinii*-infected SCID mice that had been depleted of $CD4^+$ cells for 56 days after they resolved infection; 8, normal mouse lung genomic DNA (0.05 μg) (*P. carinii*-negative control); 9, *P. carinii*-infected SCID mouse lung genomic DNA (0.02 μg) (*P. carinii*-positive control); 10, no template DNA control; 11, molecular weight markers in 100-bp increments.

The results from the experiments described above suggest that latency is not an inevitable outcome of infection with *P. carinii* in the SCID mouse model. However, it was possible that the reconstituted SCID mice failed to reactivate latent *P. carinii* infections because they remained resistant to *P. carinii* infections after the depletion of their $CD4^+$ cells. Preliminary experiments did in fact show that when SCID mice were infected with *P. carinii* at the time at which they were reconstituted, $CD4^+$ cell depletion by itself was not sufficient to make the mice susceptible to *P. carinii* infections after intranasal inoculation (4). Further preliminary experiments indicated that when the reconstituted SCID mice were treated with corticosteroid in conjunction with $CD4^+$ cell depletion, the mice did become susceptible to intranasal inoculation with *P. carinii*. Therefore, groups of *P. carinii*-infected SCID mice were reconstituted with spleen cells from C.B-17 +/+ mice on DPR 0 and were treated with weekly injections of anti-CD4 monoclonal antibody, starting from DPR 19 and extending to the end of the experiment. In addition, these mice also received 2.5 mg of hydrocortisone acetate (Hydrocortone; Merck Sharp & Dohme, West Point, Pa.) twice weekly as subcutaneous injections, starting at 19 days after the first anti-CD4 treatment. One group of mice (n = 5) were then given intranasal inoculations of 2.5×10^7 *P. carinii* nuclei at 4 and 11 days after the first corticosteroid treatment. The remaining group of mice (n = 5) were left uninoculated. The numbers of *P. carinii* nuclei in the lungs of these mice were determined 4 weeks later. By that time, four of five inoculated mice developed moderately severe *P. carinii* infections ($\log_{10}$, 5.61 ± 1.04 nuclei per lung), whereas in uninoculated mice that otherwise received the same treatments, there were no *P. carinii* organisms detected ($\log_{10}$, ≤3.98 ± 0.00 nuclei per lung) (Table 2). These results, together with the finding that no *P. carinii*-specific DNA was amplified in the lungs of reconstituted SCID mice

TABLE 2. Numbers of *P. carinii* nuclei in the lungs of reconstituted SCID mice depleted of $CD4^+$ cells and treated with corticosteroid[a]

Treatment	Time (DPR)[b]	*P. carinii* inoculation[c]	No. of *P. carinii*-positive mice/no. of total mice	No. of *P. carinii* nuclei per lung[d]
No reconstitution (control mice)	0	No	3/3	5.59 ± 1.12
Reconstitution	19	No	0/3	≤3.98 ± 0.00
Reconstitution + anti-CD4 + corticosteroid[e]	73	Yes	4/5	≤5.61 ± 1.04
Reconstitution + anti-CD4 + corticosteroid[e]	73	No	0/5	≤3.98 ± 0.00

[a] Six-week-old SCID mice were reconstituted with immunocompetent spleen cells on DPR 0.
[b] Mice were killed at the times indicated.
[c] *P. carinii* inoculation was given intranasally at DPR 42 and DPR 49, i.e., 24 days after the first anti-CD4 treatment and 4 days after the first corticosteroid treatment.
[d] Data are $\log_{10}$ of means ± standard deviations. The limitation of detection of *P. carinii* nuclei was $10^{3.98}$ nuclei per lung.
[e] Anti-CD4 treatment was given intraperitoneally starting from DPR 19 and then once a week. Corticosteroid treatment was given subcutaneously starting from DPR 38 and then twice weekly.

killed on DPR 21 and thereafter, indicate that reconstitution of SCID mice with spleen cells causes the mice to completely eliminate their *P. carinii* burden. Thus, these findings do not support the existence of latent *P. carinii* infections.

The absence of *P. carinii* organisms or *P. carinii*-specific DNA in the lungs of SCID mice that resolved PCP is consistent with recent observations that asymptomatic pulmonary infections of *P. carinii* in immunocompetent humans and animals are not common (15, 17). In addition, use of the SCID mouse model of PCP enabled us to determine the possible importance of extrapulmonary *P. carinii* latency in the development of PCP in immunocompromised hosts. If PCP in the immunocompromised host is the result of reactivation of latent *P. carinii* infection from an extrapulmonary site, then, in our experiments, systemic depletion of $CD4^+$ cells should have resulted in the subsequent development of PCP in SCID mice that had previously resolved *P. carinii* infection through immune mechanisms. No reactivation of *P. carinii* infection, as evaluated both by cytologic and by molecular approaches, was evident in the lungs of mice that were depleted of $CD4^+$ cells for up to 84 days. These findings provide the first in vivo evidence to support the hypothesis that PCP that develops in immunocompromised hosts represents a new infection resulting from exposure to exogenous sources of *P. carinii*. In this regard, epidemiological and experimental studies (11–13) have already established the possibility of horizontal transmission of *P. carinii* through the airborne route. Reported antigenic differences among *P. carinii* isolates from some patients with recurrent episodes of PCP suggest that these patients had acquired new infections (23). Moreover, using both standard cytologic techniques and amplification of *P. carinii*-specific DNA by PCR, Blumenfeld et al. (3) have recently reported that of 24 *P. carinii*-negative human lung specimens, 12 were from patients with previous histories of PCP. During the revision of the present paper, Sepkowitz et al. (18) reported that *P. carinii*-specific DNA disappeared rapidly in both the serum and the lungs of immunocompetent rats after these animals had been isolated from *P. carinii*-infected rats. In conclusion, the data presented here suggest that immunocompetent hosts completely clear *P. carinii* from their lungs and probably from extrapulmonary sites as well. These results, together with those of others, suggest that the first *P. carinii* infection that occurs after the host is immunocompromised is probably the result of infection by exogenous *P. carinii*. However, recurrence of PCP in the immunocompromised host may be the result of the inability of the host to clear all *P. carinii* organisms that the host has harbored.

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