

Interleukin 1: An Important Mediator of Host Resistance Against *Pneumocystis carinii*

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Summary

The importance of endogenous interleukin 1 (IL-1) in resistance to *Pneumocystis carinii* infection was examined in a SCID mouse model. Naturally acquired pulmonary infection of *P. carinii* in SCID mice was completely cleared by reconstitution of the infected mice with immunocompetent spleen cells. IL-1 activity in the lung homogenate supernatant of these mice increased significantly after reconstitution and returned to baseline level after the clearance of *P. carinii*. Treatment of reconstituted SCID mice with 35F5, a monoclonal antibody against murine type I IL-1R almost completely inhibited the clearance of *P. carinii*. In contrast, treatment with control rat immunoglobulin G had no detectable effect. Further study revealed that for the complete clearance of *P. carinii*, IL-1 must be present at the early stage of immune responses induced by reconstitution, since clearance could be blocked by a single injection of 35F5 into SCID mice at 2 d, but not at either 8 or 13 d postreconstitution. Furthermore, pulmonary recruitment of neutrophils, macrophages, and lymphocytes was significantly inhibited in mice that received 35F5 treatment. These findings strongly suggest that, in reconstituted SCID mice, endogenous IL-1 is important in host resistance to *P. carinii* infection and that IL-1 may function early in the host response possibly by the recruitment of inflammatory cells into the lungs.

Pneumocystis carinii pneumonia is a major cause of morbidity and mortality in AIDS patients, whereas the pneumonia rarely occurs in immunocompetent hosts (1). CD4⁺ T lymphocytes are essential both in resistance to and recovery from the disease (2–4). However, CD4⁺ cells on their own are unlikely to function directly as effector cells in *P. carinii* killing, and mechanisms by which these cells function are unknown. On the basis of limited in vitro and in vivo studies, we (5) and others (6, 7) have postulated that CD4⁺ cells may function in resistance to *P. carinii* by regulating interactions between cytokines and effector cells.

IL-1 is an important cytokine that mediates inflammation and other host physiological responses to a variety of infections (8), including acute bacterial infection (9–11). The high incidence of *P. carinii* pneumonia in AIDS patients (1) and the significant reduction of IL-1 secretion by human macrophages infected with HIV-1 (12) suggest that this cytokine may be important in resistance to *P. carinii* infection. The development of a specific mAb against murine IL-1 type I (35F5) has permitted examination of the importance of IL-1 in vivo. The 35F5 prevents binding of both IL-1 α and IL-1 β to the type I receptor, and thus blocks a number of IL-1-de-

pendent effects in mice (10, 11, 13–15). In studies described in this report, this antibody was used to investigate the importance of endogenous IL-1 in host defenses against *P. carinii* infection in a SCID mouse model. SCID mice spontaneously develop detectable *P. carinii* infection at about 3-wk of age, and resistance against the infection can be adoptively transferred by reconstitution of the animals with spleen or bone marrow cells from immunocompetent mice (3, 5, 16). Clearance of the organisms from infected lungs is completed in the majority of animals by 19 d after reconstitution of spleen cells (3, 5). Findings presented in this report show that endogenous IL-1 is essential for resistance to *P. carinii* infection in this host.

Materials and Methods

Mice. 6–8-wk-old C.B-17 +/+ and C.B-17 *scid/scid* mice were obtained from the Trudeau Animal Breeding Facility. A foundation stock of SCID mice was originally obtained from Dr. Leonard Schultz of The Jackson Laboratory (Bar Harbor, ME). They were bred and housed in microisolator cages containing sterilized food and water, and were shown to be free of most common pathogens (3).

Assessment of *P. carinii* Infection. The intensity of the *P. carinii* infection in mouse lung was determined by counting the number of *P. carinii* nuclei as described previously (3, 17). In brief, the lungs were pushed through a stainless steel screen into HBSS (Gibco Laboratories, Grand Island, NY), and the resulting suspension was diluted for making cytocentrifuge prepared smears. The smears were stained with Diff-Quik (Baxter, Miami, FL), and the number of *P. carinii* nuclei per 10–30 oil immersion fields was counted. This number was used to calculate total *P. carinii* nuclei per lung. With this method, $10^{3.98}$ nuclei per lung represented the detection limit. The mean percent variance among counts done by individual investigators was 11% (3). All counts done within an experiment were conducted by a single investigator without knowledge of the origin of the slides.

Reconstitution of SCID Mice with Immunocompetent Spleen Cells. Spleens were collected aseptically from 6–8-wk-old C.B.-17 +/+ mice, diced into small pieces, gently pushed through a stainless steel screen into HBSS, and triturated with a Pasteur pipette. After removal of debris, the cells were washed twice with PBS (pH 7.2), counted, and resuspended in PBS at a concentration of 5×10^7 nucleated cells per ml. Recipient SCID mice were given 1 ml of the cell suspension by injection into a tail vein.

Kinetics of IL-1 Production in Reconstituted SCID Mice. Groups of four reconstituted SCID mice were killed at the time of reconstitution (0 d postreconstitution [DPR 0])¹, or at predesignated time points as indicated in Fig. 1 A. In addition, four *P. carinii*-free unreconstituted SCID mice were killed to determine baseline IL-1 production. The mice were anesthetized, bled by cardiac puncture, and the serum collected. After the bleeding, mice were killed and the lungs were removed and processed for enumeration *P. carinii* nuclei and IL-1 assay. Sera and lung suspensions for IL-1 assay were stored at -70°C until use. Sera from mice killed at the same time point were pooled and diluted 1:8 with RPMI 1640 (Gibco Laboratories) before use. Lung suspensions were thawed, homogenized with a motorized Teflon pestle, and centrifuged at 10,000 g at 4°C for 30 min. The supernatant was carefully decanted and sterilized by filtration (0.22- μm pore-size filter) before being assayed.

IL-1 Assay. The amount of IL-1 (U) in the lungs and serum was determined by the murine D10.N4.M cell proliferation assay (18). The IL-1 titre was defined as the reciprocal of the highest dilution of test sample that produced a half-maximal proliferative response of D10.N4.M cells. The detection limit of the assay was 20 U per ml for the serum and 100 U per lung. Selected samples were also assayed for IL-1 activity in the presence of different concentrations of 35F5 to assure the specificity of assay. A dose-dependent inhibition of D10.N4.M cell proliferation was noted (data not shown).

Treatment of Reconstituted SCID Mice with 35F5 mAb The 35F5 was generated and purified from serum-free hybridoma cell culture supernatants by protein G affinity chromatography (13). Detailed descriptions of 35F5 mAb and its activities in vivo and in vitro have been reported previously (10, 11, 13–15). Recipient mice were given intraperitoneal injections of 200 μg of pure 35F5 or control rat IgG (Sigma Chemical Co., St. Louis, MO) in 0.2 ml of pyrogen-free saline. This dose of 35F5 was based on that used in murine models of acute listeriosis (10) and turpentine-induced sterile abscess (14). The number of animals used and regimes of those treatments are detailed in Table 1. All mice were killed at DPR 19 and the numbers of *P. carinii* nuclei in their lungs were determined.

In an additional experiment, groups of four reconstituted SCID mice receiving 200 μg of either 35F5 or rat control IgG at DPR 2 and 8 were killed at the predetermined time points as shown (see Fig. 2). The lungs were lavaged (19) before being processed for *P. carinii* nuclear counts. Performance of such lavage in *P. carinii*-infected mice does not markedly alter the number of *P. carinii* nuclei per lung (unpublished data). Total lavage cell numbers were counted in a hemacytometer, and differential cell counts were done by examination of cell smears produced with a cytocentrifuge and stained with Diff-Quik. The lavage cells were also stained with FITC-conjugated F(ab')₂ fragments of anti-Thy-1.2, -CD4, -CD8, and -Ig for analysis of lymphocyte phenotypes by using a FACScan[®] cytofluorometer (Becton Dickinson & Co., Sunnyvale, CA) (3).

Statistical Analysis. Data are presented as means \pm SEM of results calculated from four animals. Differences in the observations made between the groups of mice treated with 35F5 and rat control IgG were analyzed using Mann-Whitney U test (20). The difference was considered significant if $p < 0.05$.

Results and Discussion

Effect of Type I IL-1R Blockade on the Clearance of *P. carinii* from Lungs. To acquire evidence that endogenous IL-1 is required for *P. carinii* clearance in reconstituted SCID mice, these mice were treated with 35F5 or rat IgG as control. Table 1 shows the numbers of *P. carinii* nuclei in the lungs of unreconstituted control SCID mice and reconstituted SCID mice treated with 35F5 mAb or control rat IgG. In agreement with our previous studies (3, 5), all unreconstituted SCID mice had substantial numbers of *P. carinii* in their lungs when they were killed (about 9-wk-old), whereas reconstituted SCID mice had cleared *P. carinii* by DPR 19. Intraperitoneal injection of 35F5 into reconstituted SCID mice on DPR 2, 8, and 12 almost completely inhibited the clearance of *P. carinii* seen on DPR 19 in reconstituted SCID mice that had received control rat IgG ($p < 0.05$, Table 1). We then determined when endogenous IL-1 was required for the clearance

Table 1. Effect of 35F5 Treatment on *P. carinii* clearance from the Lungs of SCID Mice 19 d after Reconstitution with Immunocompetent Spleen Cells

Spleen cell reconstitution	Antibody treatments	Treatment time (DPR)	No. of <i>P. carinii</i> nuclei/lung (\log_{10})
No	No	–	7.00 ± 0.11
Yes	No	–	$<3.98 \pm 0.00$
Yes	Rat IgG	2,8,12	$<3.98 \pm 0.00$
Yes	35F5	2,8,12	$6.31 \pm 0.15^*$
Yes	35F5	2	$5.77 \pm 0.65^*$
Yes	35F5	8	$<3.98 \pm 0.00$
Yes	35F5	12	$<3.98 \pm 0.00$

* $p < 0.05$ versus group treated with rat IgG.

¹ Abbreviation used in this paper: DPR, days postreconstitution.

of *P. carinii*. Significant inhibition of *P. carinii* clearance was seen in mice treated with 35F5 on DPR 2 ($p < 0.05$, Table 1), however, a single injection of 35F5 on either DPR 8 or 12 had no significant effect on the clearance. These results indicate that for clearance of *P. carinii* in SCID mice, endogenous IL-1 is required mainly in the early stage of the immune response induced by the reconstitution. The inhibition of clearance was not due to depletion of lymphocytes (particularly CD4⁺ cells) by 35F5, because it was previously determined that treatment of normal or *Listeria*-infected mice with the amount of 35F5 used in the current experiments did not significantly change the numbers of splenic Thy 1.2⁺, CD4⁺, CD8⁺, or Ig⁺ cells (10).

Kinetics of IL-1 Production in Reconstituted SCID Mice. The inhibition of *P. carinii* clearance from the lungs of reconstituted SCID mice by treatment with 35F5 but not control IgG, suggests the importance of endogenous IL-1 in resistance to *P. carinii*. It was therefore considered important to determine whether there is an association between resistance to *P. carinii* and local and/or systemic production of IL-1. Fig. 1 A shows IL-1 activity in SCID mice that were either free of *P. carinii* or infected with *P. carinii*, and that either had or had not been reconstituted with immunocompetent spleen cells. The amount of IL-1 in the lungs of *P. carinii*-free mice was 519 ± 135 U/lung, whereas *P. carinii*-infected SCID mice had $6,719 \pm 3,677$ U/lung at the time of reconstitution. Reconstitution of *P. carinii*-infected SCID mice significantly increased the local production of IL-1 that peaked on DPR 7 at $68,268 \pm 21,717$ U/lung and remained at about this level until DPR 12 (Fig. 1 A). The amount of IL-1 then declined rapidly at the end of the experiment (DPR 19), when the infection was cleared, to a level comparable with that in the lungs of *P. carinii*-free SCID mice. No IL-1 activity (<20 U/ml) was detected in the serum of any animals, regardless of their status of infection or immunocompetence (data not shown). The appearance and maintenance of high IL-1 levels in the lungs of reconstituted SCID mice and its rapid decrease in concordance with *P. carinii* clearance, suggests that this cytokine may participate in host defenses against *P. carinii*.

To examine whether 35F5 treatment altered production of IL-1, the IL-1 levels in the lungs of 35F5-treated mice were also determined and compared with those from control IgG-treated animals. In Fig. 1 B, it can be seen that the amounts of IL-1 in the lungs of mice treated with either 35F5 or control rat IgG were similar at the time of reconstitution (DPR 0) and on DPR 3. After DPR 3, 35F5 treatment did not change the kinetics of IL-1 production, despite the fact that measurable IL-1 levels were slightly lower in the lungs of 35F5-treated mice as compared with control IgG-treated mice. Although these results might be predictable, there are certain technical problems that could interfere with the IL-1 assay employed. Rather than by binding directly to, and neutralizing IL-1, 35F5 mAb inhibits IL-1 action by binding to IL-1 type I receptors, and prevents IL-1 binding to its cellular receptors (13). The only assays available to us for murine IL-1 α and IL-1 β is either an IL-1-dependent cell proliferation assay (used in the present study), or an IL-1 competitive inhibition binding assay (10). Because both these assays require direct binding of IL-1 in samples to IL-1 receptors on cells, any residual 35F5 mAb in the samples derived from 35F5-treated mice could possibly interfere with the IL-1 assays. Therefore, certain cautions should be taken in the interpretation of these results.

35F5 mAb Treatment Inhibits Recruitment of Inflammatory Cells into the Lungs. The finding that 35F5 treatment was effective only if given well before *P. carinii* were cleared from the lungs (Table 1), and that a substantial time lag existed between the local appearance of peak IL-1 activity and *P. carinii* clearance (Fig. 1 A), suggested that IL-1 functions by mechanisms other than a direct effect on *P. carinii*. IL-1 elicits local accumulation and activation of inflammatory cells (8, 11, 21). These cells are considered important in host defenses against *P. carinii* (3, 4, 6, 22–26) and, indeed, reconstitution of *P. carinii*-infected SCID mice with immunocompetent spleen cells induces significant recruitment of inflammatory cells into bronchoalveolar lumina (3, 5). The effect of 35F5 treatment on pulmonary inflammation in reconstituted SCID mice was therefore investigated. At DPR 2, there was no significant difference in the composition of cell populations in bronchoal-

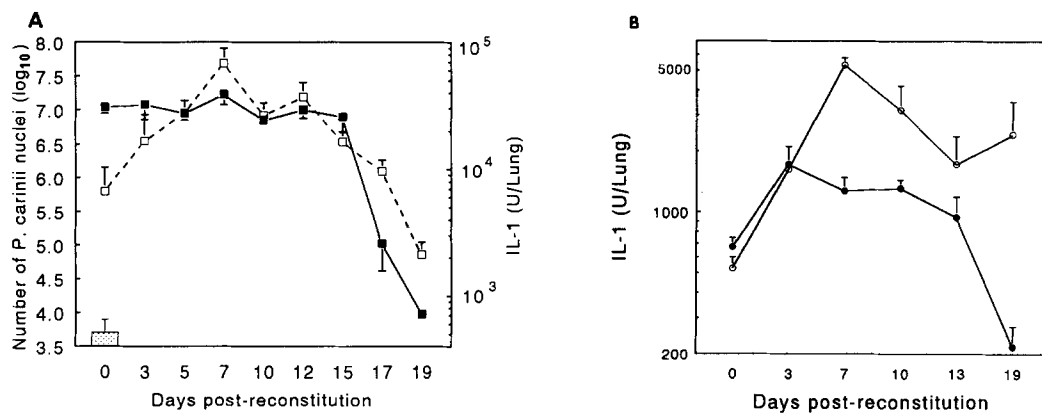


Figure 1. (A) Kinetics of the clearance of *P. carinii* (■) and production of IL-1 (□) in the lungs of SCID mice reconstituted with immunocompetent spleen cells. (Bar) IL-1 activity in the lungs of *P. carinii*-free SCID mice. (B) Measurable IL-1 levels in the lungs of reconstituted SCID mice treated with either 35F5 mAb (●) or control of rat IgG (○).

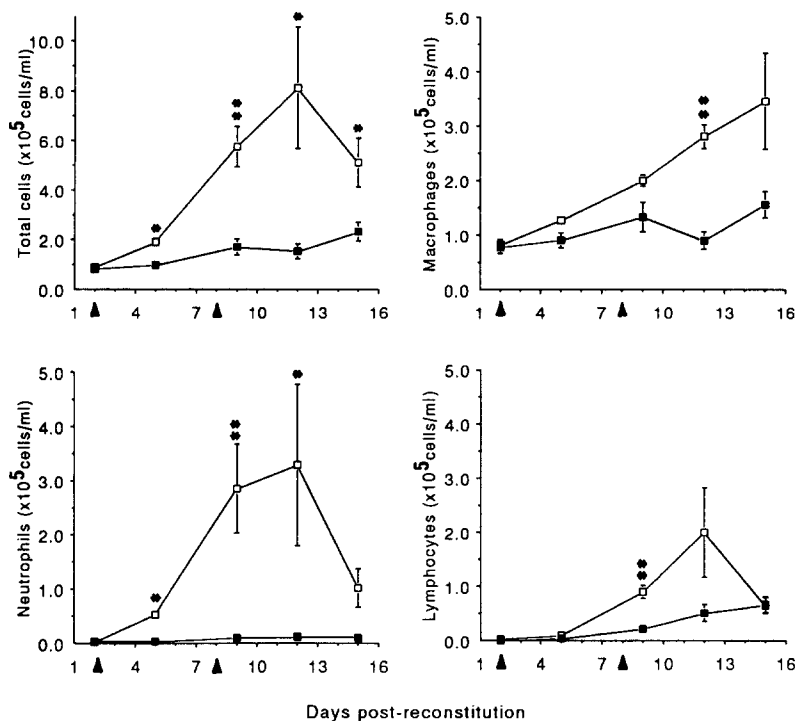


Figure 2. Effect of 35F5 treatment on composition of cell populations in the bronchoalveolar lavage fluids of SCID mice reconstituted with immunocompetent spleen cells. (■) 35F5-treated mice and (□) rat control IgG-treated mice. (▲) Time points when 35F5 or rat IgG was administered. (*) $p < 0.05$ versus rat IgG treated control mice. (**) $p < 0.01$ versus rat IgG treated control mice.

veolar lavage fluids of mice treated with 35F5 or rat IgG (Fig. 2). After DPR 2, the numbers of macrophages, neutrophils, and lymphocytes in the lavage fluids of control mice increased consistently and significantly, whereas pulmonary recruitment

of all types of cells examined was markedly inhibited by 35F5 treatment (Fig. 2). The most striking effect of 35F5 treatment was on the inhibition of neutrophil recruitment that was first evident by DPR 5 and reached maximum inhibi-

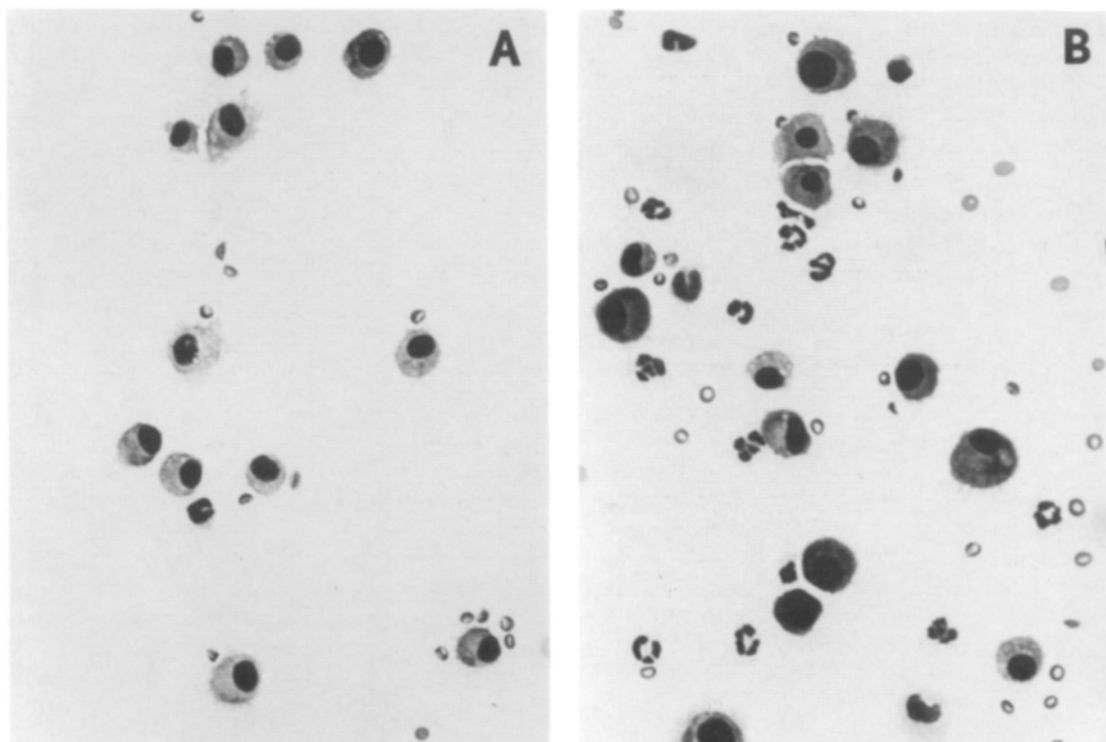


Figure 3. Bronchoalveolar lavage cells from a reconstituted SCID mouse treated with 35F5 (A) or rat IgG (B) on DPR 2 and 8 and killed on DPR 12. In addition to many neutrophils, the bronchoalveolar lavage fluid of the control mouse (B) contains larger macrophages with basophilic cytoplasm than are found in the 35F5-treated animal (A). ($\times 360$).

tion at DPR 12, when the number of neutrophils in lavage fluids of control mice was nearly 30 times that of 35F5-treated mice (Fig. 2). Although the inhibitory effect of 35F5 treatment on macrophage recruitment was not as dramatic as that on neutrophils, macrophages from control mice nevertheless had a more activated appearance than those from 35F5-treated animals (Fig. 3). Treatment with 35F5 also markedly reduced the number of lymphocytes accumulating in the bronchoalveolar lumina. However, flow cytometric analysis of these cells showed no selective reduction of cells with regard to phenotype (Thy 1.2⁺, CD4⁺, CD8⁺, or Ig⁺) (data not shown). These findings suggest that 35F5 may inhibit the clearance of *P. carinii* from the lungs of reconstituted SCID mice by blocking IL-1-dependent inflammatory responses.

Based on the results presented in this paper, we conclude that IL-1 is important in host defenses against *P. carinii* in

the SCID mouse model. It is possible that reduced numbers of CD4⁺ cells may underlie an impaired capacity to induce IL-1 production, thus accounting for the high incidence of *P. carinii* infection seen in AIDS patients (1). In view of earlier results implicating TNF- α (5) as a mediator in host defenses against *P. carinii*, the demonstration that IL-1 is also an important mediator shows that the host defenses against *P. carinii* probably result from combined effects of several cytokines. This conclusion is in accordance with the findings that neither blockade of IL-1 activity (Table 1) nor neutralization of TNF- α activity (5) could completely inhibit the clearance of *P. carinii*. It will be worthwhile to investigate whether IL-1 treatment is of practical therapeutic benefit in human *P. carinii* pneumonia. In addition, the results reported here provide the first in vivo evidence that IL-1 is important in resistance to a protozoan infection.

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