

# Requirement for CD4<sup>+</sup> Cells in Resistance to *Pneumocystis carinii* Pneumonia in Mice

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## Summary

The importance of CD4<sup>+</sup> cells in resistance to *Pneumocystis carinii* (PC) in PC-susceptible severe combined immunodeficient (SCID) mice that were made resistant to PC by immunocompetent spleen cell transfer, and in conventional PC-resistant mice, was investigated. SCID mice with naturally acquired PC pneumonia (PCP) were given infusions of spleen cells from immunocompetent donors. This reconstitution caused the recipients to resolve their PCP. Treatment of reconstituted SCID mice with anti-CD4 monoclonal antibodies (mAbs) to deplete them of CD4<sup>+</sup> cells eliminated their ability to resolve PCP, whereas treating them with anti-CD8 mAb to deplete CD8<sup>+</sup> cells had no effect. The findings indicate, therefore, that resistance to PCP in immunologically reconstituted SCID mice is dependent on CD4<sup>+</sup> cells. To determine whether CD4<sup>+</sup> cells enable conventional mice to resist PCP, B6D2 mice were treated with anti-CD4 mAb to deplete them of CD4<sup>+</sup> cells in an attempt to induce PCP. After 10–11 wk of treatment, these mice developed progressive PCP. Taken together, these results indicate that loss of CD4<sup>+</sup> cells predisposes mice to PC infection.

Whereas *Pneumocystis carinii* (PC)<sup>1</sup> pneumonia (PCP) rarely occurs in immunocompetent individuals, it is a major cause of morbidity and mortality in patients with AIDS (1). However, the basis of susceptibility of immunocompromised individuals to PCP is not understood. It seems to be generally believed that susceptibility is a consequence of the loss of CD4<sup>+</sup> T cells, and AIDS is characterized by a progressive decline in the number and function of CD4<sup>+</sup> T cells (2). Moreover the onset of PCP in AIDS patients generally does not occur until after numbers of circulating CD4<sup>+</sup> T cells have declined to <200 cells/mm<sup>3</sup> of blood (3). However, this evidence for the need for CD4<sup>+</sup> T cells in resistance to PCP is equivocal at best because HIV infection may compromise the function of other cells of the immune system, including macrophages (4, 5). Indeed, it has been suggested that dysfunction of macrophages is a primary cause of susceptibility of AIDS patients to opportunistic infections (4).

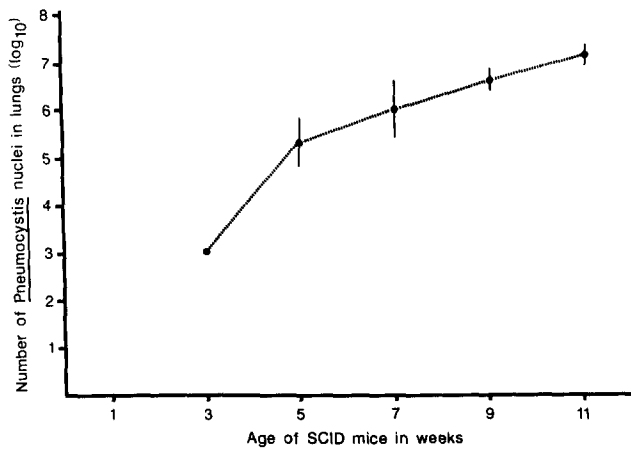
Attempts to determine the basis of susceptibility to PCP in animals have involved the use of chronic treatment with corticosteroids. However, because these compounds have such broad immunosuppressive effects, the results obtained with them have not allowed conclusions to be drawn about the role of a particular cell type in anti-PC resistance. Several studies, however, have been published that did not utilize cortisone-treated animals. Furuta et al. (6) found that the transfer of T cells to PC-infected nude mice enhanced resis-

tance of the recipients to PCP. Another study just recently published (7) shows that conventional mice chronically depleted of CD4<sup>+</sup> cells become susceptible to PCP. These studies then suggest that T cells, and more specifically CD4<sup>+</sup> T cells, play a role in resistance to PCP. To further investigate the role of CD4<sup>+</sup> cells in resistance to PCP and to determine whether a CD4<sup>+</sup> cell-deficient host can be made resistant to PCP by the transfer of CD4<sup>+</sup> cells, we initiated studies in mice with SCID. This paper will show that SCID mice given infusions of spleen cells from immunocompetent mice enables the SCID mice to resolve preexisting PCP, and that depleting these reconstituted SCID mice of CD4<sup>+</sup> T cells by treatment with anti-CD4 mAb eliminated their acquired ability to resolve PCP. This evidence in favor of an essential role for CD4<sup>+</sup> T cells in resistance to PCP is further supported by findings of Shellito et al. (7), as well as our own results shown here that conventional mice chronically depleted of CD4<sup>+</sup> T cells by treatment with anti-CD4 mAb become susceptible to PCP.

## Materials and Methods

**Mice.** Female BALB/c and B6D2 mice, 8–10 wk of age, were obtained from the Trudeau Animal Breeding Facility. Foundation stocks of C.B-17/Icr *scid/scid* (SCID) mice, an Igh congenic partner strain of BALB/c AnIcr (8), were the gift of Dr. Leonard Schultz of The Jackson Laboratory, Bar Harbor, ME. SCID mice were reared and maintained at the Trudeau Institute under barrier-sustained conditions and were shown to be free of most common pathogens, according to tests periodically performed by the diagnostic testing services of Charles River Professional Services, Wilmington, MA.

<sup>1</sup> Abbreviations used in this paper: PC, *Pneumocystis carinii*; PCP, PC pneumonia; TBLN, tracheobronchial lymph node.



**Figure 1.** Numbers of PC nuclei in the lungs of SCID mice at different ages. Points are means  $\pm$  1 SD;  $n = 4$  animals. The limit of detectability was  $10^3$  PC nuclei per animal.

B6D2 mice were maintained in open-topped cages in a standard animal room and were given food and acidified water ad libitum.

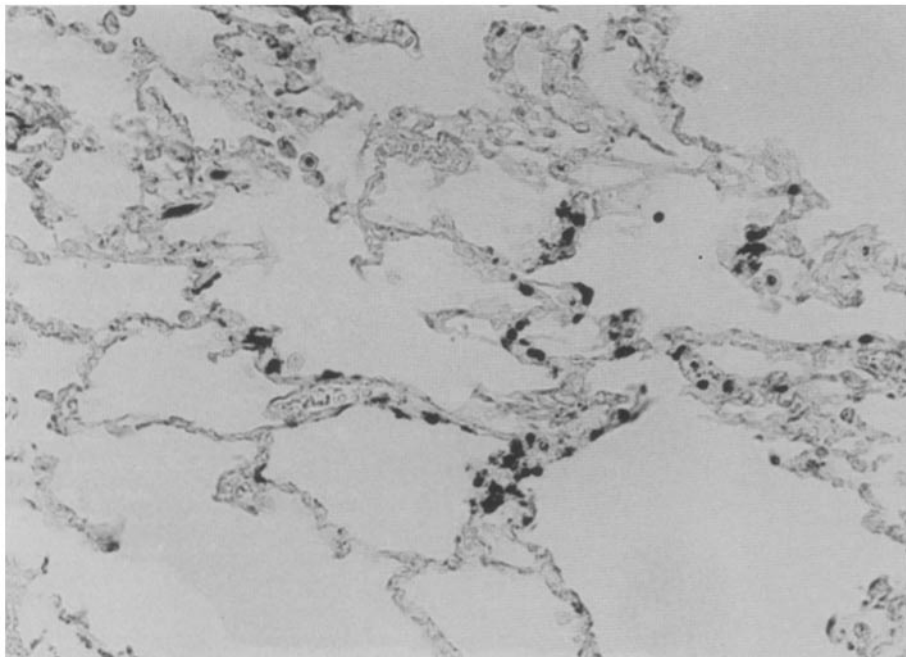
**Antibodies and FACS Analysis.** The hybridoma GK1.5 (Dr. Frank Fitch, University of Chicago, Chicago, IL) secreting rat IgG2b anti-CD4 mAb, hybridoma TIB-210 (American Type Culture Collection, Rockville, MD) producing rat IgG2b anti-CD8, and hybridoma HB92 (American Type Culture Collection) producing rat IgG2b anti- $\gamma$  chain were grown as ascites in pristane-primed, irradiated BALB/c mice. The IgG2b content of the ascites fluid was quantified by radial immunodiffusion, and the fluids were stored at  $-70^\circ\text{C}$  until needed. Some of the SCID mice infused with spleen cells (hereafter referred to as reconstituted mice) received intravenous injections of 0.5 mg anti-CD4 or anti-CD8 1, 6, and 12 d after injection of spleen cells. The extent of T cell depletion was determined by cytofluorometric analysis with a FACScan cytofluorometer (Becton Dickinson & Co., Mountain View, CA). Spleen

cells, tracheobronchial lymph node (TBLN) cells, and lung lavage cells were stained with FITC-conjugated anti-CD4, anti-CD8 (9), anti-mouse Ig, and anti-rat Ig (Cappel Laboratories, Westchester, PA).

**Enumeration of PC in the Lungs of Mice.** The number of PC nuclei in the lungs of mice was determined as described elsewhere (10), but with some modifications. Mice were anesthetized, exsanguinated, and their lungs excised. The lungs were pushed through a stainless steel screen into 5 ml of PBS, diluted 1:20, and 0.1 ml of the resulting suspension was fixed onto a  $28.3\text{-mm}^2$  area of a glass slide by the use of a cytocentrifuge (Shandon, Sewickly, PA), and stained with Diff-Quik. The number of PC nuclei per 20-100 oil emersion fields was determined, converted into total nuclei in 0.1 of lung homogenate, and used to calculate total PC nuclei per lungs. With this method, one nuclei counted in 100 fields equates to slightly more than  $10^3$  PC nuclei per lung. Therefore,  $10^3$  nuclei per lung was considered the limit of detectability. The mean percent variance among counts done by individual investigators in this lab when counting the same PC preparation is 11%. All counts done within an experiment were done by a single investigator reading the slides in a random, blinded manner.

Some lungs were fixed by intravenous perfusion of buffered formalin, processed, embedded in paraffin, and sectioned. Sections were stained with hematoxylin + eosin or with Gomori-Grocott silver stain (11).

**Spleen Cell Transfers and In Vivo Depletion of  $CD4^+$  or  $CD8^+$  Cells.** Splens were collected from BALB/c mice, diced into small pieces, gently pushed through stainless steel screens into PBS containing 1% FCS, repeatedly passed through a pasteur pipette to reduce cell clumps, and filtered through sterile gauze to remove debris. The cells were then washed twice in PBS, counted, and resuspended at  $10^8$  nucleated cells per ml of PBS. SCID mice were given 1 ml of the spleen cell suspension intravenously. Some of these reconstituted mice then received injections of 0.5 mg anti-CD4 or anti-CD8 at 1, 6, and 12 d after reconstitution. At different times after reconstitution, the numbers of PC in the lungs of the reconstituted and unreconstituted SCID mice were determined.



**Figure 2.** Photomicrograph of Gomori-Grocott silver-stained section of SCID mouse lung. Note darkly stained PC cysts lining the alveoli ( $\times 400$ ).

**Table 1.** Numbers of PC Nuclei in Lung Homogenates of SCID Mice 19–27 d after Reconstitution with Immunocompetent Spleen Cells and Depletion of T Cells

Treatment	No. of PC nuclei
	$\log_{10}$
Controls (19–27 d)	$7.2 \pm 0.1$ (12)*
Spleen cell-reconstituted (19 d)	$3.7 \pm 0.8$ (12)†
Spleen cell-reconstituted (27 d)	<3.0 (7)‡
Spleen cell-reconstituted + anti-CD4 mAb (19 d)	$7.7 \pm 0.1$ (8)¶
Spleen cell-reconstituted + anti-CD4 mAb (21–27 d)	$8.2 \pm 0.1$ (4)¶
Spleen cell-reconstituted + anti-CD8 mAb (19 d)	$3.9 \pm 1.9$ (3)†

\* Numbers represent means  $\pm$  1 SEM (numbers of mice compiled from two to four experiments).

† Significantly lower than control ( $p < 0.01$ ).

‡ Less than detectable number.

¶ Significantly greater than control ( $p < 0.01$ ).

† Significantly lower than control ( $p < 0.05$ ).

The lungs of some of the mice were lavaged as previously described (12). At the same time, the spleens and tracheobronchial lymph nodes were excised, single cell suspensions prepared, and the numbers of CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> cells in the suspensions and lung lavage fluids determined by cytofluorometric analysis.

**Depletion of CD4<sup>+</sup> Cells by Infusion of Anti-CD4 mAb into Conventional B6D2 Mice.** To test directly whether CD4<sup>+</sup> cells are required for resistance to PCP, an attempt was made to induce PCP in conventional B6D2 mice by depleting them of CD4<sup>+</sup> T cells. Groups of normal and thymectomized B6D2 mice were given 0.5 mg anti-CD4 mAb (GK1.5 ascites) twice a week for 8 wk and once a week thereafter. The mice were maintained under standard husbandry conditions in open-topped cages in a conventional animal room. Mice were killed at progressive times, and PC nuclei in their lungs were enumerated. In addition, CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> cell numbers in the spleens, TBLN, and lung lavage fluids of some of the mice were determined as described above.

**Statistics.** The significance of differences in means of PC numbers in the lungs of groups of mice was calculated utilizing the student's *t* test.

## Results

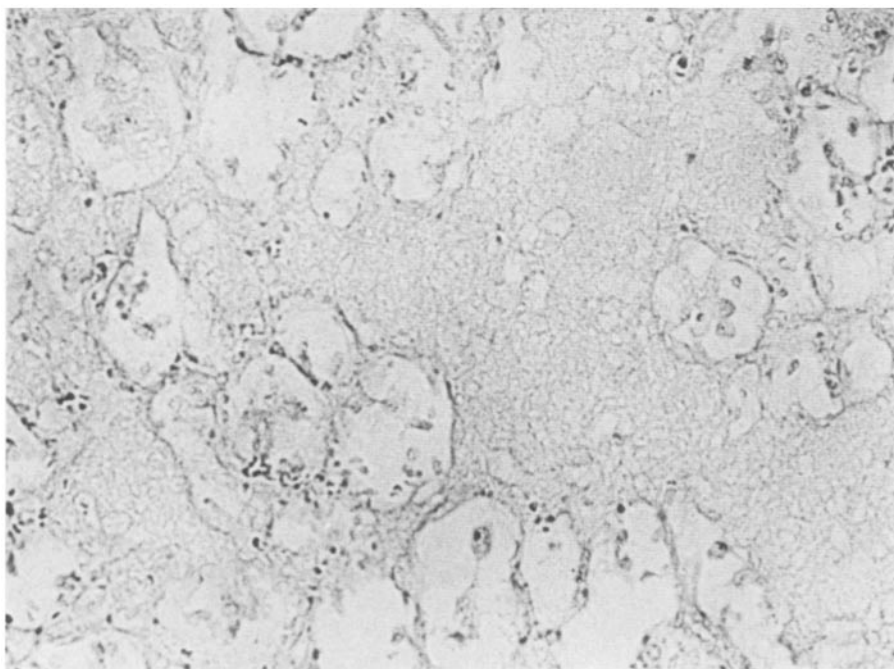
**Colonization of PC in the Lungs of SCID Mice.** Groups of four SCID mice each were killed at 3, 5, 7, 9, and 11 wk of age, and the numbers of PC nuclei in their lungs were determined. As shown in Fig. 1, PC nuclei were not detectable in the lungs of 3-wk-old SCID mice, but  $\sim 10^{5.3}$  nuclei were found in the lungs of 5-wk-old SCID mice. The numbers of PC then gradually increased with time and reached  $\sim 10^{7.1}$  at 11 wk of age. Thus, PC increased in numbers  $\sim 10$ -fold in SCID mice when determined  $\sim 7$  and  $\sim 11$  wk of age. Mice

used in the following experiments were  $\sim 7$  wk of age at the start of the respective experiment.

Histological examination of hematoxylin- and eosin-stained sections of the lungs of SCID mice (results not shown), at 7–11 wk of age, revealed essentially normal lung architecture, and the presence of PC was not readily apparent. However, examination of silver-stained sections of the lungs of these mice did confirm the presence of PC cysts (Fig. 2). The SCID mice of 11 wk of age and less had a normal, healthy appearance, but by 15 wk of age, the mice appeared to have lost weight and had respiratory difficulties. These mice usually contained  $\sim 10^8$  PC in their lungs (results not shown), and histological examination revealed that many of their lung alveoli were filled with an eosinophilic exudate composed of clumps of PC trophozoites and cysts. These lungs appeared to have the same histological appearance as has been previously described in SCID mice with heavy PC burdens (13).

**Resolution of PCP by Reconstituted SCID Mice.** The numbers of PC nuclei in lung homogenates of SCID mice after reconstitution with immunocompetent spleen cells are shown in Table 1. SCID mice not reconstituted with spleen cells had  $\sim 10^{7.2}$  PC nuclei in the homogenates of their lungs, whereas reconstitution of SCID mice reduced their lung PC burden to  $10^{3.7}$  nuclei ( $p < 0.01$ ) at 19 d and to  $< 10^{3.0}$  (less than a detectable number) nuclei at 27 d after reconstitution. Treatment of reconstituted SCID mice with anti-CD8 mAb had no effect on the ability of the recipients to clear PC from their lungs. In contrast, treatment with anti-CD4 eliminated the ability of reconstituted SCID mice to resolve their PCP. Examination of sections of reconstituted SCID mice to resolve their PCP. Examination of sections of paraffin-embedded tissue stained with Gomori-Grocott silver confirmed that reconstitution of SCID mice caused clearance of PC cysts from the lungs (Figs. 2 and 3).

The numbers of CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> cells in spleens and TBLN of mAb-treated SCID mice at 19 d after reconstitution are shown in Tables 2 and 3, respectively. These results are of one of three experiments performed but represent similar results found in all three experiments. SCID mice that were not reconstituted (controls) had few CD4<sup>+</sup>, CD8<sup>+</sup>, or Ig<sup>+</sup> cells in their spleens. In fact, the number of stained cells were similar to the numbers of cells that stained with anti-rat Ig (results not shown). No values for cell numbers in the TBLN of control mice are given since the TBLN of these mice were too small to be located. CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> cells were detected in both spleen and TBLN suspensions of SCID mice reconstituted with immunocompetent spleen cells. Reconstituted SCID mice that received injections of anti-CD4 mAb had CD8<sup>+</sup> and Ig<sup>+</sup> cells but only very few CD4<sup>+</sup> cells in both their spleen and TBLN suspensions. Reconstituted SCID mice that received injections of anti-CD8 mAb had CD4<sup>+</sup> and Ig<sup>+</sup> cells but only a few CD8<sup>+</sup> cells in both their spleen and TBLN suspensions. These results show that injections of immunocompetent spleen cells into SCID mice at least partially reconstitute their lymphoid tissue. In addition, treatment of the reconstituted SCID mice with anti-CD4 mAb eliminated CD4<sup>+</sup> cells, and treatment with anti-CD8 mAb eliminated CD8<sup>+</sup> cells.



**Figure 3.** Photomicrograph of Gomori-Grocott silver-stained section of SCID mouse lung at 19 d after reconstitution with immunocompetent spleen cells. Note absence of darkly stained PC cysts ( $\times 400$ ).

Cells in the lung lavage fluids of unreconstituted SCID mice contained predominantly macrophages with very few neutrophils and lymphocytes (Fig. 4). However, the lung lavage fluids of SCID mice at 19 d after reconstitution contained large numbers of lymphoblasts, lymphocytes, neutrophils, enlarged macrophages, and giant cells (Fig. 5). The numbers and type of leukocytes in lung lavage fluids of reconstituted and anti-CD4- or anti-CD8-treated SCID mice were

**Table 2.** Numbers of CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> Cells in Spleens of mAb-treated SCID Mice 19–27 d after Reconstitution with Immunocompetent Spleen Cells

Treatment	No. of cells per spleen		
	CD4 <sup>+</sup>	CD8 <sup>+</sup>	Ig <sup>+</sup>
Controls (19–27 d)	0.18*	0.03	0.03
Spleen cell-reconstituted (19 d)	2.50	0.29	3.08
Spleen cell-reconstituted (27 d)	1.26	1.35	7.78
Spleen cell-reconstituted + anti-CD4 mAb (19 d)	0.02	0.30	1.10
Spleen cell-reconstituted + anti-CD4 mAb (21–27 d)	0.02	1.21	4.60
Spleen cell-reconstituted + anti-CD8 mAb (19 d)	1.10	0.02	0.86

\* Numbers are means of measurements from two or three spleens.

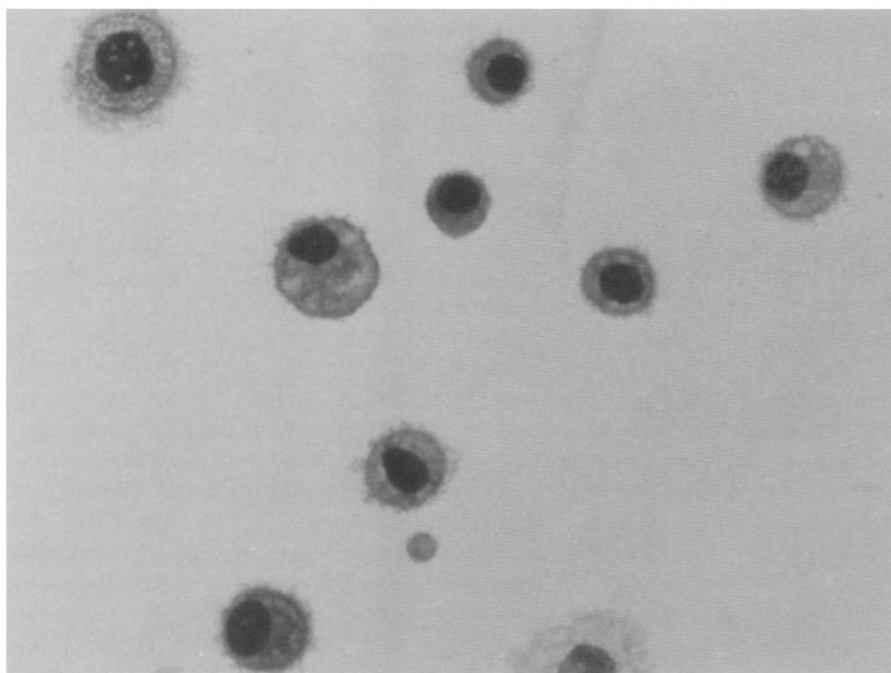
similar to that observed for reconstituted SCID mice not treated with mAb. However, the lung lavage fluids of reconstituted and anti-CD4 mAb-treated SCID mice also contained large numbers of RBC (Fig. 6). The presence of the RBC in these mice could indicate severe pulmonary damage, and indeed, these mice appeared to be very ill before they were

**Table 3.** Numbers of CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> Cells in TBLN of mAb-treated SCID Mice 19–27 d after Reconstitution with Immunocompetent Spleen Cells

Treatment	No. of cells per TBLN		
	CD4 <sup>+</sup>	CD8 <sup>+</sup>	Ig <sup>+</sup>
Controls (19–27 d)	ND*	ND	ND
Spleen cell-reconstituted (19 d)	2.25†	0.63	1.78
Spleen cell-reconstituted (27 d)	1.33	1.28	4.27
Spleen cell-reconstituted + anti-CD4 mAb (19 d)	0.02	3.15	1.20
Spleen cell-reconstituted + anti-CD4 mAb (21–27 d)	0.01	3.40	2.40
Spleen cell-reconstituted + anti-CD8 mAb (19 d)	0.88	0.02	1.48

\* TBLN were not visible in SCID mice that had not been reconstituted with spleen cells so that the presence of T cells could not be determined.

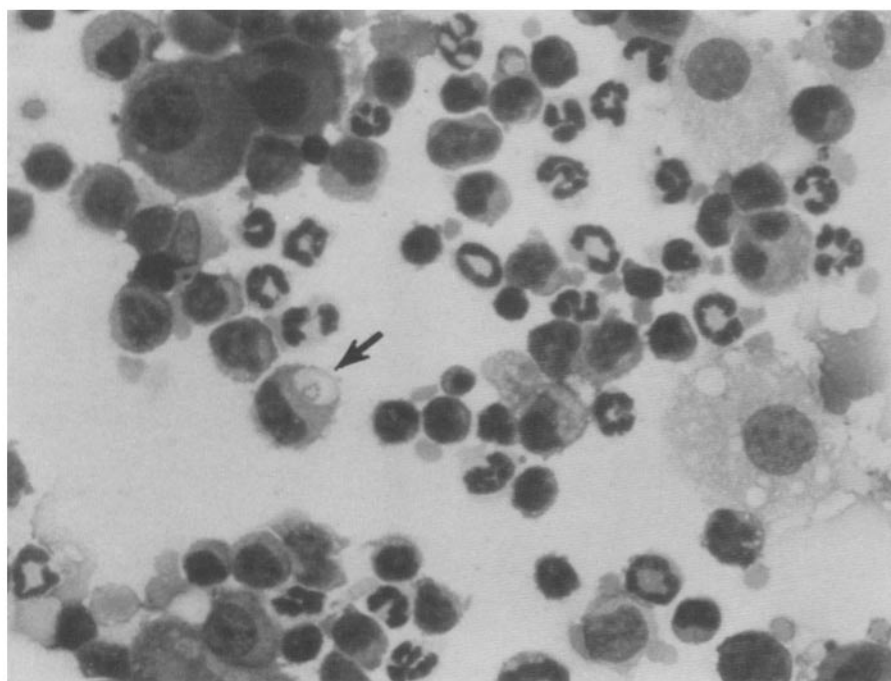
† Numbers are means of two separate pools of cells from TBLN.



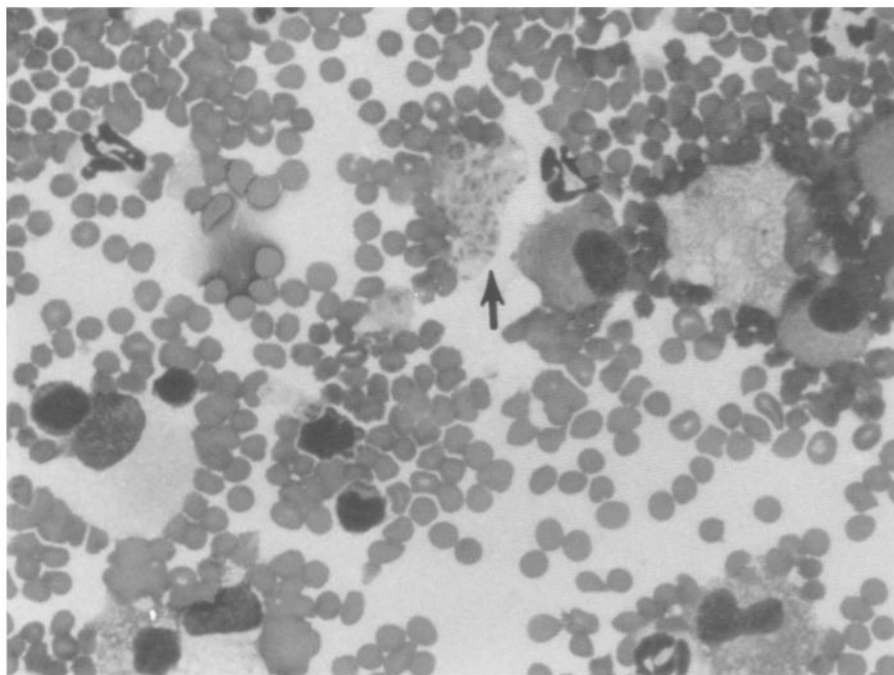
**Figure 4.** Lung lavage cells from a control SCID mouse. Note that cells are predominantly macrophages. Cytocentrifuge preparation was stained with Diff-Quik ( $\times 400$ ).

killed. At 27 d after reconstitution, the cells in the lung lavage fluids of SCID mice were similar in numbers and types to those of unreconstituted SCID mice, except that a few lymphocytes were present and many more of the macrophages had indented nuclei and lightly stained cytoplasm (Fig. 7). These macrophages were similar in appearance to blood monocytes.

Cytofluorometric analysis of lung lavage fluid cells showed that at 19 d after reconstitution, SCID mice had large numbers of  $CD4^+$  cells in their lungs and relatively few  $CD8^+$  cells (Table 4, representative results of one of three experiments performed). Reconstituted SCID mice treated with anti- $CD8$  mAb also contained large numbers of  $CD4^+$  cells in their lungs and few  $CD8^+$  cells, whereas anti- $CD4$  mAb-treated



**Figure 5.** Lung lavage cells from a SCID mouse 19 d after reconstitution with spleen cells. Note the presence of numerous lymphocytes, neutrophils, and enlarged basophilic macrophages. The arrow points to a macrophage containing a PC cyst. Cytocentrifuge preparation was stained with Diff-Quik ( $\times 400$ ).

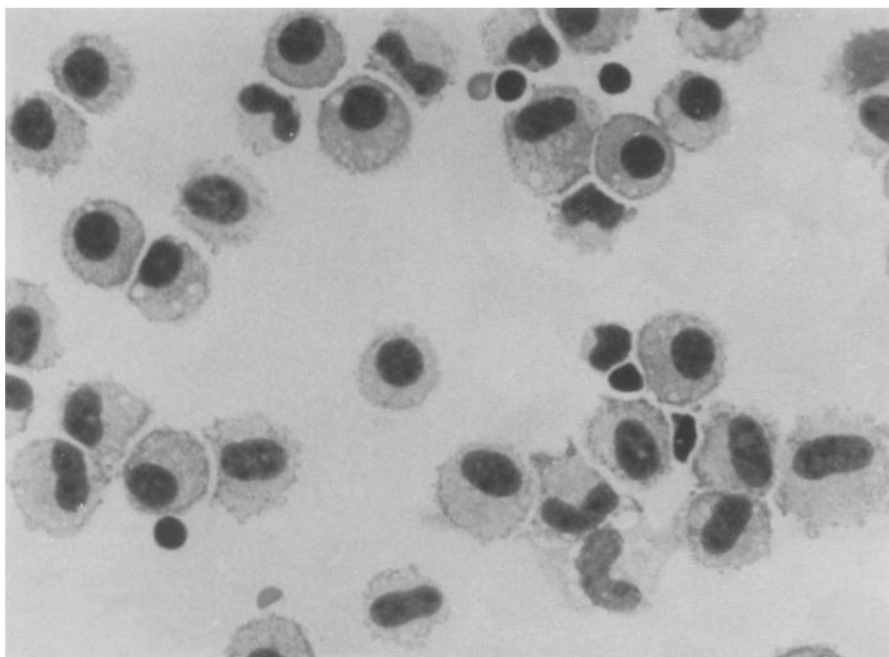


**Figure 6.** Lung lavage cells from a SCID mouse 19 d after spleen cell reconstitution and CD4<sup>+</sup> cell depletion. The cell populations present are similar to those in Fig. 2, except that numerous erythrocytes are also present. The arrow points to a clump of PC. Cytocentrifuge preparation was stained with Diff-Quik ( $\times 400$ ).

recipients contained large numbers of CD8<sup>+</sup> cells but few CD4<sup>+</sup> cells. At 27 d after reconstitution, the numbers of CD4<sup>+</sup> cells and CD8<sup>+</sup> cells in lung lavage fluids of reconstituted SCID mice had decreased to values similar to those of untreated SCID mice.

*Cellular Responses in the Lungs of PC-free SCID Mice Given an Infusion of Spleen Cells.* The infusion of BALB/c spleen cells into SCID mice could possibly result in a cellular response in the lungs of the recipients that is not driven by

the presence of PC. One way to test this possibility would be to examine the cellular response in the lungs of PC-free SCID mice after the mice were given injections of BALB/c spleen cells. PCP-free SCID mice were produced by mating spleen cell-reconstituted male and female SCID that had resolved their PCP. The offspring of the reconstituted SCID were shown to remain free of PCP for 14 wk when kept under barrier conditions (results not shown). These PCP-free mice and PC-infected control SCID mice were then given an infu-



**Figure 7.** Lung lavage cells from a SCID mouse 27 d after reconstitution with spleen cells. Note that the cells are predominantly macrophages with few lymphocytes present. Many of the macrophages contain indented nuclei and have the appearance of blood monocytes. Cytocentrifuge preparation was stained with Diff-Quik ( $\times 400$ ).

**Table 4.** Numbers of CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> Cells in Lung Lavage Fluids of mAb-treated SCID Mice 19–27 d after Reconstitution with Immunocompetent Spleen Cells

Treatment	No. of cells per lung lavage		
	CD4 <sup>+</sup>	CD8 <sup>+</sup>	Ig <sup>+</sup>
		× 10 <sup>6</sup>	
Controls (19–27 d)	<0.01*	<0.01	<0.01
Spleen cell-reconstituted (19 d)	1.93	0.17	0.09
Spleen cell-reconstituted (27 d)	0.02	0.01	<0.01
Spleen cell-reconstituted + anti-CD4 mAb (19 d)	<0.01†	1.95	0.03
Spleen cell-reconstituted + anti-CD4 mAb (21–27 d)	0.03	1.70	0.03
Spleen cell-reconstituted + anti-CD8 mAb (19 d)	0.89	0.05	0.21

\* Numbers represent means of measurements of two to three lung lavage samples.

† <0.01 is less than detectable numbers.

sion of 10<sup>8</sup> BALB/c spleen cells. After 15 d, the mice were killed, and the numbers of CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> cells in their spleens and lung lavage fluids were determined. The results (Table 5) show that reconstituted PC-free SCID mice had few lymphocytes accumulate in their lungs, whereas large numbers of both CD4<sup>+</sup> and CD8<sup>+</sup> cells accumulated in the lungs of PC-infected SCID mice.

*Development of PCP in B6D2 Mice Depleted of CD4<sup>+</sup> Cells by Treatment with Anti-CD4 mAb* As shown in Table 6, mice treated with anti-CD4 mAb did not contain detectable numbers of PC nuclei in their lungs at day 50 of treatment. However, mice that were treated for 81 or 95 d contained 10<sup>6.4</sup> and 10<sup>6.6</sup> PC nuclei, respectively, in their lungs. Again, thymectomized mice treated with anti-CD4 mAb had 10<sup>5.5</sup>

**Table 5.** Numbers of CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> Cells in Lung Lavage Fluids of PC-infected and PC-free SCID Mice at 15 d after Reconstitution with Immunocompetent Spleen Cells

SCIDs	No. of cells per lavage		
	CD4 <sup>+</sup>	CD8 <sup>+</sup>	Ig <sup>+</sup>
		× 10 <sup>6</sup>	
PC-infected	0.7 ± 0.1*	0.4 ± 0.1	0.05 ± 0.01
PC-free	0.01†	0.01	0.01

\* Numbers represent means of measurements of four mice.

† < 0.01 is less than detectable numbers.

**Table 6.** Numbers of PC Nuclei in Lung Homogenates of B6D2 Mice after 50–95 d of Treatment with anti-CD4 mAb

Exp.	Treatment and period	No. of P. carinii nuclei
		log <sub>10</sub>
A	Anti-CD4 mAb (50 d)	<3.0 (3)*†
	Anti-CD4 mAb (81 d)	6.4 ± 0.5 (3)
	Anti-CD4 mAb (95 d)	6.6 ± 0.3 (3)
	Thymectomy + anti-CD4 mAb (71 d)	5.5 ± 0.4 (3)
	Thymectomy + anti-CD4 mAb (85 d)	7.2 ± 0.2 (3)
	Controls (95-d age-matched)	<3.0 (3)
B	Anti-CD4 mAb (98 d)	6.7 ± 0.4 (7)
	Anti-γ mAb (98 d)	<3.0 (5)

\* Mean ± SD (number of mice).

† Below detectable number.

and 10<sup>7.2</sup> PC nuclei in their lungs at 71 and 85 d of treatment. No PC were detected in the lungs of either age-matched controls or B6D2 mice treated with control mAb.

Results of cytofluorometric analysis of lymphocytes from the spleens, TBLN, and lung lavage fluids of anti-CD4-treated B6D2 mice are shown in Table 7. As was expected, thymectomized, anti-CD4 mAb-treated mice had very few CD4<sup>+</sup> cells in their spleens and TBLN compared with untreated B6D2 mice. However, treatment with anti-CD4 mAb had little effect on CD8<sup>+</sup> and Ig<sup>+</sup> cell numbers in the spleens and TBLN of treated mice. The lung lavage fluids of both the anti-CD4 mAb-treated mice and control mice contained

**Table 7.** Numbers of CD4<sup>+</sup>, CD8<sup>+</sup>, and Ig<sup>+</sup> Cells in Spleens, TBLN, and Lung Lavage Fluids of Control B6D2 Mice and B6D2 Mice that Had Been Thymectomized and Treated with anti-CD4 mAb

Organ	Treatment	No. of cells		
		CD4 <sup>+</sup>	CD8 <sup>+</sup>	Ig <sup>+</sup>
			× 10 <sup>6</sup>	
Spleen	Treated	0.04*	4.8	39
	Control	17.0	7.8	46
TBLN	Treated	<0.01	1.0	3.5
	Control	1.7	1.6	0.9
Lung lavage fluid	Treated	0.01	2.0	0.05
	Control	<0.01	<0.01	<0.01

\* Numbers are means of three mice.

only few CD4<sup>+</sup> or Ig<sup>+</sup> cells. However, large numbers of CD8<sup>+</sup> cells were detected in the lung lavage fluids of the treated mice, whereas very few were found in the fluids of the control mice.

## Discussion

Results described in this paper show that SCID mice that have acquired PC infection from their environment resolve preexisting PCP after they are given spleen cells from immunocompetent donors. This is in agreement with results reported by Furuta et al. (6), who showed that infusion of immunocompetent splenic T cells into nude mice with PCP caused the recipients to clear much of their PC burden. We also found that CD4<sup>+</sup> cells are necessary for reconstituted SCID mice to resolve their PCP. This conclusion is based on the finding that treatment of reconstituted SCID mice with anti-CD4 mAb eliminated the ability of recipients to resolve PCP, whereas treatment with anti-CD8 mAb had no effect. Although this finding indicates that susceptibility of SCID mice to PCP can be reversed by giving them immunocompetent spleen cells containing CD4<sup>+</sup> cells, it does not necessarily indicate that resistance of conventional mice to PCP is dependent on CD4<sup>+</sup> cells. However, the finding that conventional mice depleted of CD4<sup>+</sup> cells develop PCP strongly indicates that CD4<sup>+</sup> cells are indeed required for normal host resistance to PCP. This finding was recently reported by Shellito et al. (7) during the writing of this manuscript.

It was not determined whether the splenocytes infused into the SCID mice were previously sensitized to PC. However, the serum of the donor BALB/c mice did contain anti-PC IgG (results not shown), as did the serum of reconstituted SCID mice already at 4 d after reconstitution (data not shown). Together, these two findings suggest that the donor mice were previously exposed to and sensitized to PC antigens.

Although results of this investigation indicate CD4<sup>+</sup> cells play a critical role in resistance to PCP, whether they function as helper cells in the induction of an antibody response to PC, or as contributors to cellular immunity to this organism, is not yet known. In the experiments described in this study, B cells, as well as T cells, were transferred to recipient SCID mice, and large numbers of B cells were found

in lymphoid tissues after reconstitution. The fact that large numbers of Ig<sup>+</sup> cells were not found in the lungs of reconstituted SCID mice need not indicate that PC-specific antibodies are not important in resolving PCP. On the contrary, it has been shown that antigen deposition in the lungs does not always cause antibody-secreting cells to accumulate (14, 15). The likelihood that an antibody response is in progress is instead indicated by the accumulation of B cells in the TBLN (15). In this regard, we found large numbers of Ig<sup>+</sup> cells in TBLN of reconstituted SCID mice resolving PCP.

The report by Furuta et al. (6), which showed that T cells are necessary for resistance to PCP, does not rule out the possibility that humoral immunity plays a role in resistance to PCP. These authors transferred T cells into nude mice that already contained B cells, and by doing so, could have made it possible for the recipient nude mice to mount a T cell-dependent antibody response to PC. The knowledge that PC is an extracellular parasite (16) and that antibodies greatly augment phagocytosis and killing of PC by macrophages (17) also suggests a possible role for antibodies in resistance to the organism. Nevertheless, cell-mediated immunity could also play an essential role in resistance to PCP. Our observation that the lungs of reconstituted SCID mice contained acquired lymphocytes, large numbers of macrophages, and numerous giant cells is consistent with the interpretation that these cells were acquired as part of a cell-mediated immune response in the lungs. Indeed, it is possible that a T cell-mediated response in and of itself could cause clearance of PC. Alternatively, a T cell-mediated response could serve to augment antibody-mediated defense mechanisms, possibly by activating the microbicidal ability of phagocytes.

The finding that resistance of reconstituted SCID and conventional mice to PCP can be eliminated by depleting them of CD4<sup>+</sup> T cells suggests that these mice and AIDS patients may be susceptible to PCP for similar reasons. Therefore, both models may be useful for analyzing the basis for susceptibility of the immunocompromised host to PCP. Experiments designed to determine whether CD4<sup>+</sup> cells function in resistance to PCP by mediating humoral responses, T cell-mediated responses, or both are currently in progress in this laboratory. Some of these experiments are best performed in SCID mice because they lack functional B cells, as well as functional T cells (8).

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