

## Both Immunity and Hyperresponsiveness to *Pneumocystis carinii* Result from Transfer of CD4<sup>+</sup> but not CD8<sup>+</sup> T Cells into Severe Combined Immunodeficiency Mice

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### Abstract

The opportunistic pathogen *Pneumocystis carinii* (*Pc*) is considered to be the leading cause of morbidity in patients with AIDS. It is important, therefore, to determine the immunological mechanisms of resistance to *Pc*. We have taken advantage of the lack of both T and B lymphocytes in severe combined immunodeficiency (*scid*) mice to determine the critical factors in resistance to spontaneously acquired *Pc* pneumonia. Using adoptive transfer of unfractionated or fractionated lymphocyte subsets or hyperimmune serum from congenic normal donors, we have demonstrated that effective immunity to *Pc* results from the action of CD4<sup>+</sup> but not CD8<sup>+</sup> T cells (in the absence of antibody) or from humoral immunity (in the absence of T cells). However, responses of CD4<sup>+</sup> T cells (but not antibody) to already well-established burdens of *Pc* are often accompanied by a fatal hyperinflammatory reaction. The activity of CD4<sup>+</sup> T cells against *Pc* thus illustrates a broadly applicable principle that T cell immunity represents a critical balance between consequences beneficial and harmful to the host. (*J. Clin. Invest.* 1992, 90:673–678.) Key words: AIDS • pneumonia • opportunistic infection • antibody • therapy

### Introduction

*Pneumocystis carinii* (*Pc*)<sup>1</sup> is the major diagnosed cause of morbidity in patients with AIDS (1), and has recently been reported to cause morbid pulmonary disease in mice homozygous for

the recessive mutant gene *scid* (2–4). *Scid* mice are essentially devoid of functional T or B lymphocytes and both primary and secondary lymphoid tissues are rudimentary (5–6). The immune defects of *scid* mice do not extend to macrophages (7–8), natural killer (NK) cells (9–11), or cells of myeloid lineage (12).

Roths et al. (2) have described both positive and negative sequelae to reconstitution of *scid* mice with congenic normal bone marrow. Following marrow transfer, a formidable hyperinflammatory reaction (HIR) develops with 30% to 50% of these recipients becoming morbidly ill between 4 and 6 wk after transplantation. Of those that survive, resolution of the spontaneously acquired *Pc* pneumonia (PCP) is essentially complete. The present study was undertaken to define the role of antibody and specific lymphocyte subsets in the anti-*Pneumocystis* immune response.

### Methods

**Mice.** The *scid* mutation was transferred by us to the inbred strain C57BL/6J (B6) by 10 backcrosses from the original C.B-17-*scid/scid* stock imported from the Institute for Cancer Research, Philadelphia, PA. Both C57BL/6J-*scid/scid* (B6-*scid*) (maintained by continued breeding of homozygotes) and its congenic normal partner strain C57BL/6J (B6) were propagated in a barrier breeding colony. At weaning, mice destined for experimental use were transferred to a conventional colony and remained there until necropsy. The husbandry and animal health characteristics of these colonies were similar to those described in detail in our original study of naturally acquired pneumocystosis in C.B-17-*scid* mice (2). The extent (cyst density) and kinetics of infectivity by *Pc* and the time of morbidity caused by spontaneously acquired *Pc* pneumonia (PCP) in these mice are nearly identical to those described in that previous study. In brief, these mice have carried a stable and endemic *Pc* infection for more than 4 yr, developed plateau levels of organisms in ~ 2 mo, lived an average of 5 mo, and were selected at random for inclusion in these studies.

***Pc* antigen preparation.** *Scid* mouse lung digest (SMLD) was prepared from a large number of lungs obtained from sick *scid* mice (frozen at -70°C until needed) using a process similar to that described by Gradus and Ivey (13). Infected lung was diced and passed through No. 60 wire mesh and collected in sterile HBSS (Gibco BRL, Grand Island, NY) supplemented with penicillin, streptomycin, and amphotericin B (Gibco). 10 mM of the wetting agent "G-acid" (2-naphthol-6-8-disulfonic acid, dipotassium; Eastman Kodak Co., Rochester, NY) were added to reduce clumping of *Pneumocystis* organisms. The crude *Pc* preparation was further manipulated by enzymatic digestion with hyaluronidase Type 1-S, collagenase Type 1-A and DNase I from beef pancreas (Sigma Chemical Co., St. Louis, MO) followed by high speed washing. An additional level of *Pc* enrichment was obtained by biphasic percoll density gradient centrifugation of the above SMLD. SMLD was used as an in vivo immunogen, while PSMLD (percoll-refined SMLD) was used in ELISA assays of anti-*Pc* activity (below).

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1. **Abbreviations used in this paper:** ALL LYM, scatter-sorted lymphocytes; GMS&LG, Grocott's methenamine silver and light green; HIS, hyperimmune anti-*Pc* serum; HIR, hyperinflammatory reaction; LNC, lymph node cells; NK, natural killer (cells); *Pc*, *Pneumocystis carinii*; PCP, spontaneously acquired *Pc* pneumonia; *scid*, severe combined immunodeficiency; S&LC, spleen and lymph node cells; SMLD, *scid* mouse lung digest; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

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*Pc* immunization for derivation of hyperimmune anti-*Pc* serum (HIS) and sensitized lymphocytes. Male and female B6 mice were twice or three times immunized with SMLD as a *Pc* antigen source. Mice received injections of 0.2 ml i.p. of an emulsion prepared by polytron homogenization of SMLD (200 µg protein per mouse) in sterile saline and Freund's complete (primary immunization) or incomplete (subsequent immunizations) adjuvant. A large pool of HIS was obtained from multiple bleedings of these mice. The reactivity and specificity of HIS was determined by (a) ELISA (described below) and (b) immunohistochemistry using paraffin sections of lung from normal and *scid* mice incubated sequentially with HIS or *scid* serum (non-Ig containing negative control), horseradish peroxidase-conjugated goat anti-mouse Ig (Southern Biotechnology, Birmingham, AL), and finally 3-amino-9-ethylcarbazole (AEC) chromogen substrate (Zymed Laboratories, San Francisco, CA). Incubation of HIS on lung sections from B6 mice failed to produce detectable, specific immunostaining, whereas sections of *scid* lung revealed intense substrate conversion with product distribution and density consistent with *Pneumocystis* infection as seen in hematoxylin and eosin stained sections. Several of the *Pc*-sensitized B6 mice were sacrificed, and their lymph nodes and spleens were removed to obtain lymphoid cells used in adoptive transfer experiments (see below).

**ELISA assay for determining levels of anti-*Pc* antibody and serum Ig.** Quantitation of serum anti-*Pc* antibody was performed by ELISA assay using *Pneumocystis* preparation (PSMLD)-coated (0.5 µg/well, followed by BSA blocking) polystyrene microtiter plates. The coated and blocked wells were incubated with test serum (1:16 dilution) for 30 min, washed, and then incubated with alkaline phosphatase-conjugated anti-mouse isotype (IgG2a or IgM) specific antisera. Wells were washed and filled with a 1 mg/ml solution of *p*-nitrophenyl phosphate substrate, and absorbance (405 nm) was measured after 30 min incubation. For a positive control, a sample of pooled anti-*Pc* HIS was assayed over the range of 6 (1:64) to 22 doubling dilutions. At 1:64, HIS produced ODs for anti-*Pc* antibodies of the IgM and IgG2a classes of 1.14 and 0.89; the ODs resulting from diluent (PBS/Tween) alone were 0.053 and 0.043. Serum IgM levels (µg/ml) were quantitated by ELISA as previously described (2).

**Quantitation of Ig-producing cells in the lung.** The numbers of intracytoplasmic immunoglobulin-containing cells were determined using 5 µM sections of *scid* lungs that were incubated sequentially with biotinylated goat anti-mouse Ig-kappa antibody (Southern Biotechnology), streptavidin-conjugated horseradish peroxidase, and finally AEC (Biomedica Corp., Foster City, CA), using a code-on automated immunostainer (Fisher Scientific Co., Pittsburgh, PA). Sections were examined (20–30 fields) and the numbers of Ig<sup>+</sup> (peroxidase-positive) cells were counted and converted to density (no./mm<sup>2</sup>).

***Pc* cyst density.** Quantitation of *Pc* cysts was performed as previously described (2). Briefly, 5 µM sections of the left lung were sectioned, stained with Grocott's methenamine silver and light green (GMS&LG), and examined using a Leitz Orthoplan microscope with a 63× plano objective and 10× widefield eyepieces fitted with a rectangular reticle (0.0187 mm<sup>2</sup>). Unambiguous GMS<sup>+</sup> cysts were counted (25–35 fields per section) and the cyst density (cysts/mm<sup>2</sup>) was calculated. The identity of the specimen was unknown during this analysis phase.

### Lymphocyte adoptive transfer

**Experiment 1.** Lymph node cells (LNC) were obtained from pooled lymph nodes of donor mice. Cells were disaggregated, expressed through Nytex bolting cloth and washed twice in Earle's Balanced Salt Solution with 1% Hepes at 4°C. Three groups of recipient B6-*scid* mice were injected IP with 5 × 10<sup>6</sup>, 5 × 10<sup>5</sup>, or 5 × 10<sup>4</sup> viable LNC obtained from twice *Pc*-immunized B6 mice; three other groups received similar numbers of LNC from nonimmunized donors; and one group received an IP injection of saline alone.

**Experiment 2.** Spleen and lymph node cells (S&LC) were obtained from twice-*Pc*-immunized B6 mice. These cells were washed as above and aliquots underwent additional staining and flow cytometry processing (see below) to obtain populations of scatter-sorted (ALL LYM), CD4-positive (CD4<sup>+</sup>) and CD8-positive (CD8<sup>+</sup>) lymphocytes. Groups

of recipient *scid* mice were injected intraperitoneally with 3 × 10<sup>5</sup> unstained and unsorted S&LS, 8 × 10<sup>4</sup> scatter-sorted lymphocytes (ALL LYM), 1.1 × 10<sup>5</sup> sorted CD4<sup>+</sup> or 1.2 × 10<sup>5</sup> sorted CD8<sup>+</sup> lymphocytes.

**Flow cytometry.** Sterile sorting was performed on a FACStar-Plus flow cytometer (Becton Dickinson Immunocytometry Sys., Mountain View, CA). CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes were isolated based on two-parameter fluorescence after staining with phycoerythrin-conjugated anti-L3T4 (clone GK1.5; Becton Dickinson) and FITC-conjugated anti-Lyt2 (clone 53-6; Becton Dickinson). Sorted populations were reanalyzed and a purity of > 90% for each subset was confirmed. The individually sorted CD4 and CD8 T cell subsets showed up to 10% of unstained cells, which were either cells that had adhered to stained cells or cells that had lost their staining antibodies during sorting. Most importantly, however, these sorted populations showed no (0.0%) detectable contamination by the alternate subset of T cells.

**HIS immunotherapy.** In experiment 2, the reference protocol for anti-*Pc* antibody treatment was one injection of 0.25 ml i.p. of HIS mixed with 0.25 ml of sterile saline per week for 8 wk. An additional control group received one injection of 0.25 ml i.p. of sterile saline (CNT).

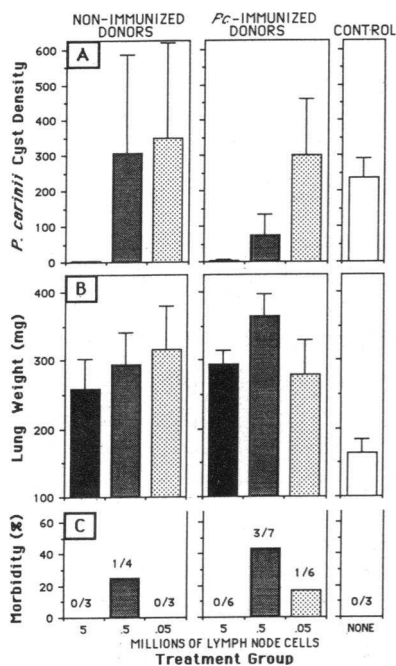
**Necropsy and histopathology.** Mice were necropsied at 28 d (experiment 1) or 53 d (experiment 2) after the start of each experiment or when individuals were judged to be moribund (see Results). After CO<sub>2</sub> asphyxiation, the thorax was exposed, the mediastinal mass was removed, and the whole lung (experiment 1) or left lung (experiment 2) was carefully dissected and immediately weighed. The noninflated lungs were fixed in Bouin's solution for 24 h and transferred to 70% ethanol before conventional paraffin embedding. 5-µM sections of lung were stained with (a) GMS&LG or (b) hematoxylin and eosin (H&E).

**Statistics.** All results are expressed as the arithmetic mean ± SE of the mean. Two-tailed Student's *t* tests for comparison of unpaired samples were performed. *P* values ≤ 0.05 were considered to indicate significant differences between sample means.

## Results

**Adoptive transfer of *Pc*-sensitized and nonsensitized lymphocytes from B6 to B6-*scid* mice (experiment 1).** Initially, we compared the effectiveness of titrated doses of LNC from either deliberately immunized or nonimmunized B6 immunocompetent donors upon transfer to congenic mutant B6-*scid* mice with spontaneous pneumocystosis. 5 million LNC from either donor were effective in eliminating *Pc* 4 wks after cell transfer (Fig. 1 A); cyst density was reduced in both cases by > 98%. Ten times fewer LNC from immunized donors provided a 68% reduction, while an equal number of nonimmunized donor cells did not cause a significant reduction in *Pc* cyst density. Two orders of magnitude fewer LNC (5 × 10<sup>4</sup>) from either source had no effect on the number of *Pc* cysts. However, the transfer of as few as 5 × 10<sup>4</sup> LNC from either donor was sufficient to cause a doubling of the lung mass of *scid* recipients (Fig. 1 E), and 5 × 10<sup>5</sup> LNC of either type yielded significant morbidity (Fig. 1 C). Histologically, these lungs contained large numbers of "activated" mononuclear phagocytes and an accumulation of edema fluid within the alveoli. These findings are consistent with that of the hyperinflammatory reaction previously described (2) after transfer of normal bone marrow to *scid* recipients. Transfer of immunocompetent lymphoid cells thus produced both beneficial and harmful effects for the *Pc*-infected host, with the beneficial effects requiring more cells than the harmful ones.

Serological studies were undertaken to determine whether Ig-producing cells in general and anti-*Pc* antibody specifically were transferred to the *scid* recipients by unfractionated lym-



**Figure 1.** The effects of titrated doses of LNC from B6 donors on pneumocystosis in recipient B6-*scid* mice. 33 6-7-wk-old *scid* mice were distributed among seven groups as follows: Three groups received  $5 \times 10^6$ ,  $5 \times 10^5$ , or  $5 \times 10^4$  LNC from B6 mice that had been previously immunized twice with PSMLD, three other groups received similar numbers of LNC from nonimmunized donors, and one group received an intraperitoneal injection of saline alone. Cyst density (cysts/mm<sup>2</sup>) was determined from microscopic counts of cysts from GMS-stained 5- $\mu$ m sections of mid-

cross sections of lung (A). The entire lung was dissected and weighed (B). The values in A and B represent the mean  $\pm$  SE. Three mice treated with  $5 \times 10^5$  LNC from Pc-immunized donors died without necropsy during the third week following treatment. All other mice were bled to obtain serum and necropsied 4 wk after treatment. The percent morbidity (C) was calculated by dividing the number of mice that died or were moribund at the time of necropsy by the total number of mice at risk.

phocytes (Table I). Unlike untreated *scid* mice, which had essentially no detectable serum immunoglobulin or B cells, *scid* recipients of +/+ LNC had modest to near normal levels of serum IgM and intracytoplasmic Ig<sup>+</sup> cells in the lung. Serum IgM levels and Ig<sup>+</sup> cell numbers were similar in recipients of LNC from either Pc-immunized or nonimmunized donors, and were highest in recipients of the largest numbers of transferred LNC. Note that control *scid* mice had negligible levels of serum IgM attesting to the "non-leaky" (5) status of the *scid* mice employed. Increases in specific anti-Pc antibody (both IgM and IgG2a classes) occurred only in recipients of the highest dose of LNC from Pc-immunized donors. A significant reduction in numbers of Pc cysts could thus occur without detectable titers of anti-Pc antibody (e.g.,  $5 \times 10^5$  LNC from immu-

nized donors or  $5 \times 10^6$  LNC from nonimmunized donors). These data indicate that (a) the B cell compartment of recipient *scid* mice can be restored by transfer of peripheral LNC from congenic normal donors; (b) specific anti-Pc antibody is detectable in recipients of large numbers of LNC from immunized donors; and (c) the density of Pc cysts can be reduced without a concomitant increase in specific anti-Pc antibody.

**Adoptive transfer of lymphocyte subsets and passive HIS therapy (experiment 2).** To determine which lymphoid cells other than or in addition to B cells are capable of exerting an effective anti-*Pneumocystis* response, we examined the separate capacities of immune serum (antibody) or FACS<sup>®</sup>-sorted CD4<sup>+</sup> or CD8<sup>+</sup> T cell subsets to provide an anti-Pc response (Fig. 2). Unsorted spleen plus lymph node cells, as well as ALL LYM were effective in reducing Pc cyst densities to 9% and 16% of control (untreated) numbers, respectively. Similar numbers of sorted CD4<sup>+</sup> T cells reduced the cyst density to 5% of control, while transferred CD8<sup>+</sup> T cells were ineffective (Fig. 2 A). Animals treated with anti-Pc HIS once per week for 8 wk showed a significant reduction in Pc cysts (to 23% of untreated controls).

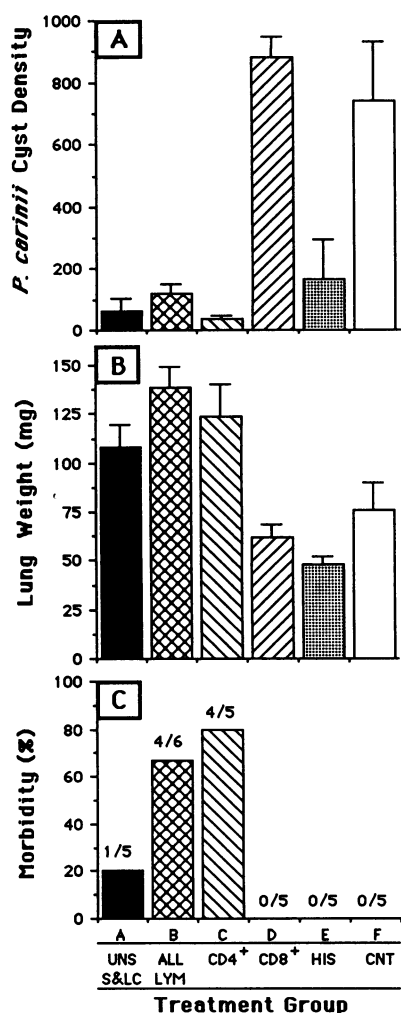
All recipients of unfractionated or CD4<sup>+</sup> lymphocytes (i.e., those transfers that cleared Pc from the lungs) had an increase in lung mass (Fig. 2 B) that was attributable to an extreme HIR (Fig. 3, A and B). This process and the presence of well-differentiated lymphoid cells in the perivascular and peribronchial regions of the lung were identical to those described for *scid* recipients of immunocompetent bone marrow (2). The Pc levels and histological features of the lungs of *scid* recipients of CD8<sup>+</sup> T cells were indistinguishable from those of untreated (Pc infected) *scid* lungs (Fig. 3 C). Morbidity correlated with effective Pc clearance and increased lung weights in recipients of immune lymphocytes, with the highest morbidity in recipients of CD4<sup>+</sup> T cells (80%) (Fig. 2 C). In contrast to the results of cell transfer, HIS-treated *scid* mice suffered no mortality. The lungs of these mice did not show HIR, and in fact were reduced (by 37%) in weight compared to untreated controls (Fig. 2 B), reflective of a striking reduction of Pc cysts, foamy matrix and numbers of macrophages. The septal interstitium appeared thin and alveoli increasingly clear in recipients of both hyperimmune serum and CD4<sup>+</sup> T cells (Fig. 3 D).

As expected, recipients of HIS had significant elevations of anti-Pc antibody with a mean OD for the IgG2a class of 0.58 compared to 0.11 for untreated *scid* controls. None of the recipients of unfractionated or specific T cell subsets, which also effectively cleared Pc organisms, had detectable anti-Pc anti-

**Table I.** Humoral Immune Responses of *scid* Mice following Adoptive Transfer of Lymphoid Cells from Pc-sensitized or Nonsensitized Normal Donors

No. cells ( $\times 10^6$ ):	Lymph node cell donor*						
	Nonimmunized			Pc-immunized			Control
	5	0.5	0.05	5	0.5	0.05	None
Serum IgM	395 $\pm$ 64 <sup>‡</sup>	36 $\pm$ 14	61 $\pm$ 52	318 $\pm$ 46	86 $\pm$ 37	65 $\pm$ 29	$\leq$ 3
Serum < Pc (IgM)	0.15 $\pm$ .01 <sup>§</sup>	0.12 $\pm$ .02	0.11 $\pm$ .004	0.31 $\pm$ .06	0.16 $\pm$ .03	0.11 $\pm$ .01	0.13 $\pm$ .01
Serum < Pc (IgG2a)	0.13 $\pm$ .01	0.11 $\pm$ .02	0.09 $\pm$ .01	0.73 $\pm$ .13	0.16 $\pm$ .03	0.13 $\pm$ .02	0.11 $\pm$ .004
IC Ig <sup>+</sup> cells (lung)	23 $\pm$ 11 <sup>  </sup>	0.2 $\pm$ 0.1	0	15 $\pm$ 3.3	1.5 $\pm$ 1.0	.03 $\pm$ .03	0

\* Experimental groups defined in Fig. 1 (legend). All data expressed as the mean  $\pm$  SE. <sup>‡</sup> Serum IgM ( $\mu$ g/ml) was quantitated by ELISA. <sup>§</sup> Serum anti-Pc antibody, expressed as OD, was determined by ELISA. <sup>||</sup> Intracytoplasmic immunoglobulin-containing cells (no./mm<sup>2</sup>) quantitated microscopically based on immunoperoxidase histochemistry.



**Figure 2.** The effects of cellular or antibody treatments on pneumocystosis in B6-*scid* mice. 31 *scid* mice (4–7 wk old) were distributed among the following treatment groups: *A* (UNS S&LC). Injected intraperitoneally with  $3 \times 10^5$  unstained and unsorted splenic plus lymph node cells obtained from twice-immunized B6 mice; *B* (ALL LYM). Injected with  $8 \times 10^4$  stained and scatter-sorted lymphocytes from the above source; *C* (CD4<sup>+</sup>). Injected with  $1.1 \times 10^5$  sorted CD4-positive lymphocytes; *D* (CD8<sup>+</sup>). Injected with  $1.1 \times 10^5$  sorted CD8-positive lymphocytes; *E* (HIS). Received 8 intraperitoneal injections of hyper-immune anti-*Pc* serum once per week; *F* (CNT). Received one 0.25-ml intraperitoneal injection of saline. All surviving mice (22 of 31) were necropsied 53 d after initial treatment. The following morbidity occurred: Group *A* (35 d), *B* (29 d, 35 d, 43

d, 43 d), and *C* (29 d, 48 d, 48 d, 53 d). Only one of these morbidly ill mice (*C*, 48 d) was not necropsied because of severe autolysis after death. Cyst density (*A*) and morbidity (*C*) analyses were done as described above (Fig. 1) and left lungs were dissected and weighed (*B*) before histological processing.

body levels of either the IgM or IgG2a isotypes (data not shown).

## Discussion

In the past, the role of humoral immunity in defense against *Pc* has been controversial because of findings of serum antibodies in both healthy individuals and patients with PCP (14–15). A series of patients with PCP has been described as being hypogammaglobulinemic but having intact T cell immunity (16). Studies by Walzer and Rutledge have demonstrated serum antibody responses to *Pc* (17). The partial success of passive immunoprophylaxis (using anti-*Pc* mAb) in protecting experimental animals against *Pc* (18) showed that humoral immunity can play a role in resistance to *Pc*.

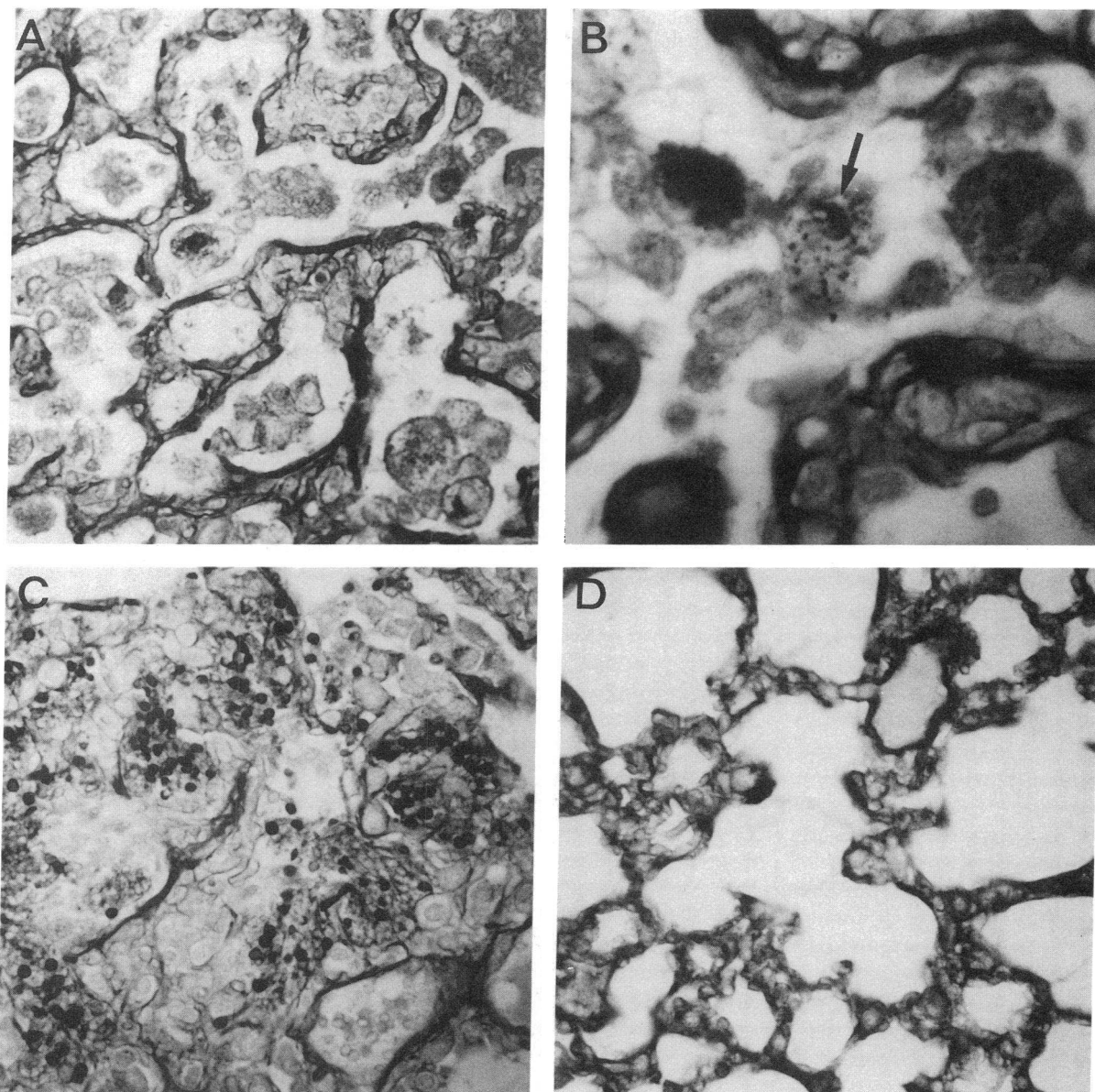
A large body of evidence also indicates that T cells, and CD4<sup>+</sup> T cells in particular, are required for resistance to PCP. The identification of *Pc* in athymic *nu* (nude) rats and mice (19–20) suggested that intact cell-mediated immunity is important in resistance to *Pc*. The experiments by Furata et al. (21) on steroid-induced PCP in mice showed that adoptive transfer

of splenic T cells (those associated with the delayed-type hypersensitivity reaction toward *Pc* antigen) could enhance recovery from infection. CD4<sup>+</sup> lymphocytes appear to be the primary target of HIV infection in man (22) and in most cases (84%) the occurrence of PCP is limited to patients whose CD4<sup>+</sup> lymphocyte counts have fallen from normal numbers ( $\sim 1000/\text{mm}^3$ ) to  $< 100/\text{mm}^3$  (23). PCP has also been identified in patients with low CD4<sup>+</sup>, high CD8<sup>+</sup> counts as a result of cancer chemotherapy (24). Recently, Shelito et al. (25) have shown that BALB/c mice could be rendered susceptible to PCP (following intratracheal inoculation of *Pc*) by selective depletion of CD4<sup>+</sup> T cells. Similarly, Harmsen and Stankiewicz (26) demonstrated that *scid/scid* mice that were reconstituted with immunocompetent spleen cells and then selectively depleted of CD4<sup>+</sup> T cells (using mAb GK1.5) lost their ability to resolve naturally acquired PCP. These experiments indicated that CD4<sup>+</sup> T cells are necessary for resistance to *Pc*, but did not identify their mechanisms of action or interactions with other cell types. The description by Roifman et al. (27) of an infant presenting PCP with normal numbers of CD4<sup>+</sup> but lacking CD8<sup>+</sup> lymphocytes suggested that CD8<sup>+</sup> T cells may also play a role in resistance to *Pc*.

The results reported here demonstrate that CD4<sup>+</sup> T cells are both necessary and sufficient for resistance to *Pc*. In addition, CD4<sup>+</sup> T cells cause an intense HIR that is life-threatening to the host, and can presumably also collaborate with B cells to produce anti-*Pc* antibody, a second effective means of resistance to *Pc*. Thus, antibody to *Pc* in the absence of cell-mediated immunity, or CD4<sup>+</sup> T cell-mediated processes independent of humoral immunity, are each effective for resistance to and control of *Pc*. CD4<sup>+</sup> T cells seem essential for both of these mechanisms. In contrast, CD8<sup>+</sup> T cells appear completely ineffective and uninvolved in both processes.

There is general acceptance, supported by the present histological findings, that the macrophage is a critical effector cell in anti-*Pc* responses. Opsonic antibody enhances the ingestion, vacuolization, and dissolution of *Pneumocystis* by peritoneal and alveolar macrophages (28). It is also known that the cytokines IFN- $\gamma$  (29) and colony-stimulating factors (30) are produced by CD4<sup>+</sup> T cells and activate alveolar macrophages during infection by opportunistic pathogens. IFN- $\gamma$  has been shown to enhance IgG2a production *in vivo* (31), and other studies in our laboratory have shown IgG2a to be the predominant isotype generated in mouse anti-*Pc* humoral responses (manuscript in preparation). Activated macrophages produce as well as respond to tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (32). *Pc* has been shown to stimulate the *in vitro* production of TNF- $\alpha$  by human macrophages (33). A recent clinical study suggested that TNF- $\alpha$  produced by alveolar macrophages may play a major role in the control of PCP in AIDS patients (34). In addition to the enhancement of macrophage activation, TNF- $\alpha$  has been shown to be directly toxic to *Pneumocystis* (29).

The HIR-related morbidity associated with T-cell transfer, as well as the wasting syndrome associated with chronic pneumocystosis in *scid* mice, may reflect the known capacity of TNF- $\alpha$  to produce cachexia *in vivo* (35) and in patients with parasitic disease (36). The fact that *in vivo* administration of IL-2, a cytokine produced by type 1 T helper (CD4<sup>+</sup>) cells (37), results in increased microvascular permeability with resultant pulmonary edema (38) provides a possible mechanistic explanation for our finding of intraalveolar fluid accumulation during HIR following T cell transfer to *Pc*-infected *scid* mice. The



**Figure 3.** Histological observations on the effects of transferred CD4<sup>+</sup> or CD8<sup>+</sup> T cells, or multiple injections of HIS, on *Pneumocystosis* in B6-*scid* mice (from experiment 2). Microscopic sections of lung (5  $\mu$ m) were stained with GMS&LG. (A, C, D)  $\times$ 440. (B)  $\times$ 1100. (A) hyperinflammatory response in *scid* recipients of CD4<sup>+</sup> T cells. Note the extreme state of activation and number of alveolar macrophages, alveolar fluid exudate and GMS-positive particulate matter (remnants of *Pc* cysts) within the macrophages. (B) Higher magnification of the lung of a second *scid* recipient of CD4<sup>+</sup> T cells showing extensive phagocytosis and degraded remnants (intracellular GMS<sup>+</sup> particles) of *Pneumocystis*. Note the as yet undigested *Pc* cyst (arrow). (C) Section obtained from *scid* recipient of CD8<sup>+</sup> T cells, is indistinguishable from untreated *scid* mouse. Present are numerous *Pc* cysts in a field showing widely scattered macrophages, foamy matrix and thickening of the alveolar interstitium. (D) The lungs of *scid* mice that received HIS are essentially free of *Pneumocystis* and have regained thin alveolar septa and patent alveoli. It should be noted that recipients of CD4<sup>+</sup> lymphocytes that survive the crisis of HIR resolve PCP to the extent represented in this panel.

local action of prostaglandins, leukotrienes, and other vasoactive mediators, present during the acute phase response typical of parasitic infection (39), including pneumocystosis (40), are additional possible causes for this fluid accumulation.

Our histological analysis of *scid* recipients of unsorted or sorted CD4<sup>+</sup> T cells found well-differentiated lymphocytes located primarily in the perivascular and peribronchial regions of the lung. Studies by Breel et al. (41) have shown that the bronchus-associated lymphoid tissue of mice is populated by macrophages and lymphocytes, with the majority of T cells belonging to the CD4<sup>+</sup> subset. This localization suggests that both the

host-beneficial and -harmful effects of anti-*Pc* CD4<sup>+</sup> T cells may be expressed "at a distance" rather than by direct contact in the affected parenchyma. Ongoing efforts are thus directed at studying the effects and role of action of cytokines (especially IFN- $\gamma$  and TNF- $\alpha$ ) in response to *Pneumocystis* in *scid* mice.

Most generally, the results reported here dramatically illustrate the nature of the immune system as a "double-edged sword" capable of either protecting or harming its host. CD4<sup>+</sup> T cells may be particularly noteworthy in this regard since they produce a broad range of cytokines involved in both protection of the host and immunopathological processes. These observa-



tions provide a possible theoretical basis for the paradoxical and surprising benefits of administering immunosuppressive steroids to already immunocompromised AIDS patients in respiratory failure due to PCP (42), and suggest that both enhancing and suppressing the activity of the immune system should be considered as therapies in immunological disease.

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