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Single and Combined Humoral and Cell-Mediated Immunotherapy of *Pneumocystis carinii* Pneumonia in Immunodeficient *scid* Mice

JOHN B. ROTHS† AND CHARLES L. SIDMAN†*

The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609

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Homozygous mutant *scid/scid* (severe combined immunodeficiency) mice (referred to as *scid* mice) lack both specific humoral and cell-mediated immune functions and are exemplary in vivo models for analysis of host-parasite relationships. In our colony, *scid* mice routinely and predictably develop spontaneous *Pneumocystis carinii* pneumonia (PCP) with high morbidity. Previous studies have identified both T cells (specifically, $CD4^+$ cells) and antibody as independent mechanisms of effective anti-*P. carinii* resistance; however, $CD4^+$ T cells also cause an often fatal hyperinflammatory reaction. The current study has explored the optimal application of these immune components for conferring protection against *P. carinii*. Anti-*P. carinii* hyperimmune serum was highly effective at reducing the number of *P. carinii* organisms in early, intermediate, and advanced stages of PCP and was capable of increasing the mean life expectancy of *P. carinii*-infected *scid* mice by more than threefold if provided on a continuing basis. When a short course of hyperimmune-serum therapy was provided prior to transfer of *P. carinii*-sensitized normal lymphocytes, *scid* mice were rendered permanently free of *P. carinii* without the pathological sequelae of the hyperinflammatory reaction. These findings are discussed in the contexts of mechanism and clinical relevance.

The clinical importance of *Pneumocystis carinii* has expanded greatly since about 1980 in parallel with the increasing numbers of cases of human immunodeficiency virus-associated AIDS (17). Prior to the identification of its human immunodeficiency virus etiology, AIDS was defined primarily by the manifestation of *P. carinii* pneumonia (PCP), and PCP remains a major proximal cause of death in AIDS patients (14, 22). Opportunistic infection by *P. carinii* has also been found in infants and children with primary immune deficiency diseases (30), in patients with protein-calorie malnutrition (20), and in patients undergoing intensive immunosuppressive therapy (18, 19).

Experimental models of PCP have been sought to permit studies of the biology of the host-parasite relationship and therapeutic trials. Only limited progress has been achieved in the propagation of *P. carinii* organisms in vitro (26, 31). To date, most in vivo studies of the host-*P. carinii* relationship have employed corticosteroid-induced immunosuppression of euthymic rats (8) and mice (34) or genetically athymic (nude) rats (10) and mice (35). These models suffer from a limited time span of experimental accessibility, additional disabling of nonspecific immunity, or restriction of the deficit primarily to the cell-mediated specific immune system. In contrast, Roths et al. (27) described the natural history and pathobiology of spontaneously acquired PCP in immunodeficient mutant *scid* (severe combined immunodeficiency) mice. For reasons of predictability, unequivocal expression, high morbidity, well-defined genetic basis, natural mode of infection, and extended clinical course and accessibility, mutant *scid* mice may be the in vivo animal model of choice for studies of PCP.

The *scid* mouse, which is essentially devoid of functional humoral or cell-mediated immune responses, was identified and described by Bosma et al. (2, 3) as a model for human congenital severe combined immune deficiency. The lack of specific immune reactivity is thought to be due to abortive differentiation and death of B and T cells consequent to a defective recombinase system that results in abnormal rearrangements of *Ig* and *Tcr* genes (3). Components of the nonspecific immune system, including myeloid lineage cells (6), NK cells (7), splenic antigen-presenting cells (5), and macrophages (1), appear to function normally.

The activities of anti-*P. carinii* antibodies and $CD4^+$ but not $CD8^+$ T cells in conferring long-term protection from *P. carinii* have been described elsewhere (12, 28); however, $CD4^+$ T cells also induce an acute life-threatening hyperinflammatory response (HIR) to *P. carinii* in *scid* mice (28). The present report describes in detail (i) the therapeutic and prophylactic effectiveness of anti-*P. carinii* serum (in the absence of T cells) and (ii) the role of serum pretreatment in preventing the life-threatening HIR associated with transfer of immunocompetent lymphocytes in the chronic *scid* mouse model of PCP.

MATERIALS AND 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 B6 were propagated in a barrier-maintained breeding colony. All of these *scid* mice spontaneously develop PCP, even in the barrier colony, and are thus considered clinically infected with *P. carinii*. At weaning, mice destined for experimental use were transferred to a conventional colony and remained there until necropsy. Because the degree of *P. carinii* infection of *scid* mice is age dependent (see Results and Fig. 1), we used closely age-matched individuals in all experiments. The age ranges of *scid* mice at the start of each therapeutic trial are indicated in the appropriate figure legends and were generally 6 to 8 weeks. Mice of all litters

* Corresponding author.
† Present address: Department of Molecular Genetics, Biochemistry and Microbiology (ML524), University of Cincinnati College of Medicine, 231 Bethesda Ave., Cincinnati, OH 45267-0524.

were distributed evenly into all groups within each experiment. No differences were found in the responses of male and female *scid* mice to anti-*P. carinii* therapy. 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 (27). These studies were conducted at The Jackson Laboratory, which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

***P. carinii* antigen preparation.** *scid* mouse lung digest was prepared from a large number of lungs obtained from sick *scid* mice and frozen at −70°C until needed; the process used was similar to that described by Gradus and Ivey (15). Infected lung was diced, passed through no. 60 wire mesh, and collected in sterile Hanks' balanced salt solution (GIBCO BRL, Grand Island, N.Y.) supplemented with penicillin, streptomycin, and amphotericin B (GIBCO). Ten millimoles of the wetting agent G-acid (2-naphthol-6-8-disulfonic acid, dipotassium; Eastman Kodak Co., Rochester, N.Y.) was added to reduce clumping of *Pneumocystis* organisms. The crude *P. carinii* 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 *P. carinii* enrichment was obtained by biphasic Percoll density gradient centrifugation (15) of the above preparation. Non-Percoll-processed *P. carinii* antigen was used as the in vivo immunogen, while Percoll-refined material was used in enzyme-linked immunosorbent assays (ELISA) of anti-*P. carinii* activity (see below).

HIS (anti-*P. carinii*). A large pool of hyperimmune serum (HIS) was obtained from multiple bleedings of 58 male and female B6 mice that had been twice or thrice immunized with the non-Percoll-processed *P. carinii* antigen preparation. Mice received intraperitoneal (i.p.) injections of 0.2 ml of an emulsion prepared by Polytron homogenization of 200 μg of protein per mouse in sterile saline and Freund's complete adjuvant (primary immunization) or incomplete adjuvant (subsequent immunizations). The reactivity and specificity of the HIS were determined by (i) ELISA (similar to the method described by Nielsen and Mojon [24], who used *P. carinii*-antigen-coated [0.5 μg of protein per well] 96-well polystyrene microtiter plates [modifications are described in reference 28] and (ii) immunohistochemistry using paraffin sections of lungs from normal B6 control mice and *scid* mice incubated sequentially with HIS or *scid* serum (non-immunoglobulin [Ig]-containing negative control), horseradish peroxidase-conjugated goat anti-mouse Ig (Southern Biotechnology, Birmingham, Ala.), and finally, 3-amino, 9-ethylcarbazole (AEC) chromogen substrate (Zymed Laboratories, San Francisco, Calif.). Incubation of HIS on lung sections from B6 mice failed to produce specific immunostaining, whereas sections of *scid* lung revealed intense substrate conversion, with product distribution and density consistent with detection of both cyst and trophozoite forms of *P. carinii* as seen in sections stained with Grocott's methenamine silver-light green (GMS-LG) and hematoxylin and eosin (H&E).

HIS immunotherapy. The reference protocol for anti-*P. carinii* serum therapy was one i.p. injection of 0.25 ml of HIS mixed with 0.25 ml of sterile saline per week.

LNC adoptive transfer. Lymph node cells (LNC) were obtained from the axillary, brachial, cervical, and mediastinal lymph nodes of several *P. carinii*-immunized male B6 mice (also used to acquire HIS). Cells were disaggregated, expressed through NYTEX bolting cloth, and washed twice in Earle's balanced salt solution with 1% HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) at 4°C. Recipient B6-*scid* mice were injected i.p. with 5×10^6 viable LNC.

Necropsy and histopathology. Mice were necropsied at predetermined times following the start of each experiment or when individuals were judged to be moribund. In all experiments, weekly body weights (BW) were obtained, since the decrease in BW of *scid* mice is a good clinical marker of advanced pneumocystosis (27). Following CO_2 asphyxiation, the thorax was exposed, the mediastinal mass was removed, and the left lung was carefully dissected and immediately weighed; the right lobes were frozen for later use. The noninflated lungs were fixed in Bouin's solution for 24 h and transferred to 70% ethanol prior to conventional paraffin embedding. Sections of lung were stained with GMS-LG or H&E.

***P. carinii* cyst density.** Quantitation of *P. carinii* cysts was performed as previously described (27). Briefly, 5-μm-thick sections of the left lung were sectioned, stained with GMS-LG, and examined with a Leitz Orthoplan microscope with a 63× plano objective and 10× wide-field eyepieces fitted with a rectangular reticle (0.0187 mm^2). Unambiguous GMS^+ cysts were counted (25 to 35 fields per section), and the cyst density (cysts per square millimeter) was calculated. The identity of the specimen was unknown during this analysis phase.

Statistics. All results are expressed as the arithmetic mean ± standard error of the mean. Two-tailed Student's *t* tests for comparison of unpaired samples were performed, in all cases comparing an experimental group with its appropriate control group. Probability values of ≤0.05 were considered to indicate significant differences between sample means.

RESULTS

Age of appearance of *P. carinii* cysts in lungs of B6-*scid* mice. Therapeutic trials or other experiments based on natural (noninduced) exposure to infectious agents require that the kinetics of infection be defined. To this end, we determined the level of spontaneous *P. carinii* infection of B6-*scid* mice from 9 to 143 days of age (Fig. 1A). No *Pneumocystis* cysts were identified in 9-day-old *scid* pups. In 39-day-old animals, only 12 ± 5 cysts per mm^2 could be identified; however, this and all subsequent cyst levels were significantly higher than the essentially none found in +/+ animals. Between 39 and 47 days of age, there was a dramatic 14-fold increase in cyst levels to 172 ± 52/mm^2. The density of cysts in *scid* mice 57 days and older was generally in the 200 to 400/mm^2 range. Our experience over several years indicates that this ontogenic course of *P. carinii* infection is highly predictable. We have conducted comparable kinetic studies on *scid* mice of genetically dissimilar strains such as C.B-17 (27) and C3H/HeSnJ (data not shown) and found a very similar pattern of infection as that described above; in all three *scid* stocks, the age of demarcation between none or few to modest or high numbers of cysts was between 40 and 60 days.

The increase of lung mass in *P. carinii*-infected mice is a reliable indicator of the progression and clinical severity of disease and has well-defined histopathological correlates (27). This increase is due to both cellular inflammatory reactions (including large numbers of macrophages, exudates, epithelial and endothelial hyperplasia, and fibrosis) and the load of *P. carinii* organisms. The change with age of

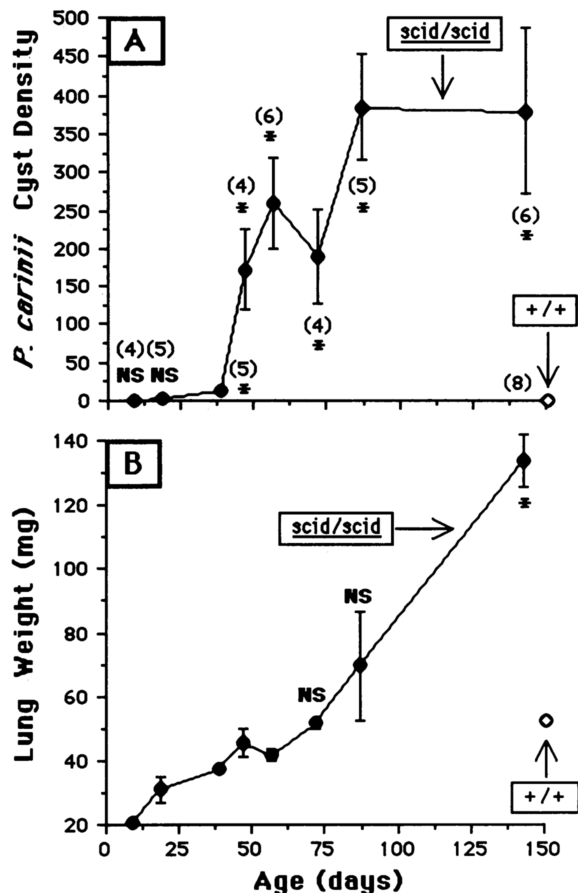

FIG. 1. Kinetics of development of pneumocystosis in B6-*scid* mice. Nineteen female and 20 male *scid* mice (closed circles) were necropsied at ages ranging from 9 to 168 days of age. As controls, eight B6−+/+ mice (open circle) were necropsied at 153 ± 6 days of age. *P. carinii* cyst density (in cysts per square millimeter) (A) and wet weights of left lungs (in milligrams) (B) are presented (means ± standard errors of the means). Numbers of mice at each time point are indicated in panel A. *, significantly different; NS, not significantly different from 153-day +/+ controls. *t* tests were not conducted for lung weights on nonadult (<2 months of age) groups of mice.

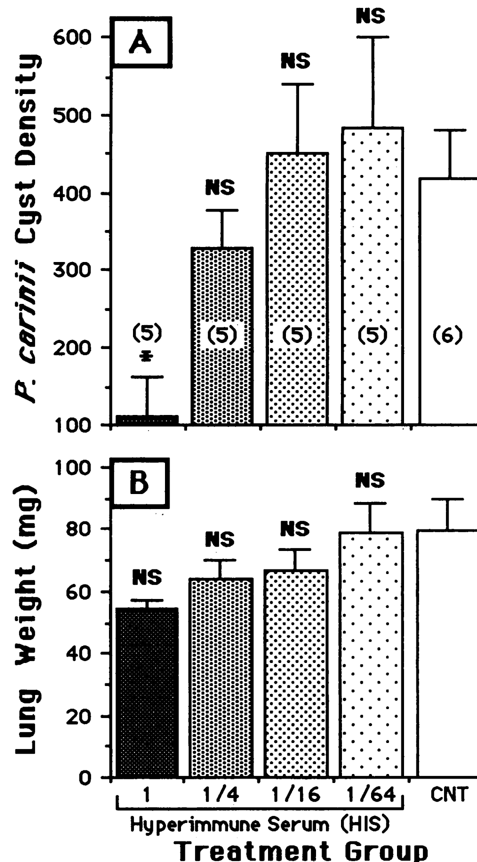

FIG. 2. Treatment of B6-*scid* mice with dilutions of anti-*P. carinii* HIS. Twenty-six *scid* mice (6 ± 1 week old) were distributed among four HIS-treated and one control group. Mice were treated once per week for 8 weeks with neat or diluted (1/4, 1/16, 1/64) HIS or saline (control [CNT]). Neat serum was the equivalent of 0.25 ml of pooled HIS. All mice were necropsied 2 weeks following the last treatment. *P. carinii* cyst density (in cysts per square millimeter) (A) and wet weights of left lungs (in milligrams) (B) are presented (means ± standard errors of the means). Numbers of mice are indicated in panel A. *, significantly different; NS, not significantly different from control group.

lung weight in B6-*scid* mice is documented in Fig. 1B. For comparison, we examined the lungs of eight adult male and female B6 mice (average age, 153 days). The mean weight of healthy left lungs was 54 ± 3 mg; no *P. carinii* cysts or other pulmonary pathologies were identified. At 143 days of age, the left lungs of six B6-*scid* mice weighed significantly more (2.5 times more, or 134 ± 8 mg) than those of their congenic normal counterparts.

Therapeutic effectiveness of titrated doses of anti-*P. carinii* HIS. Twenty-six *scid* mice (6 ± 1 week old) were treated with fourfold serial dilutions of HIS or saline once per week for 8 weeks. Neat HIS was effective in significantly reducing cyst density to 26% of untreated levels ($P < 0.005$) in this experiment (Fig. 2A) and to 16 ± 6% of control levels in four experiments. Treatments using 1:4 or greater dilutions of neat HIS were ineffective in reducing cyst density. Neat HIS also reduced the lung weights of *scid* recipients (Fig. 2B) (although not to the point of statistical significance compared with saline-treated *scid* controls [$P < 0.10$] because of the lower-than-usual untreated lung weights in this experiment's control group; compare with the control group in Fig. 3B). In this and other experiments, examination of H&E-stained sections confirmed the practical disappearance of trophic forms of *P. carinii* and restoration of normal lung architecture.

Frequency of injection of HIS. Sixteen B6-*scid* mice (6 ± 2 weeks old) received i.p. injections of neat HIS once weekly for 2 or 6 weeks or received saline for 6 weeks. The results of this experiment (Fig. 3) support previous evidence that multiple (6 to 8) weekly injections of neat HIS are highly effective in reducing *P. carinii* cyst density and restoring lung size, function, and histology (assayed but not shown for this experiment). To determine the effectiveness of a shorter course of anti-*P. carinii* serum therapy and whether a "rebound" of residual *Pneumocystis* organisms would occur following cessation of treatment, groups receiving only two weekly HIS injections were necropsied at 1 or 5 weeks following the last treatment. These data indicate that two doses of HIS did not reduce *P. carinii* cyst density at either 1 or 5 weeks after the last treatment, nor were lung weights significantly reduced (at 5 weeks after treatment) compared

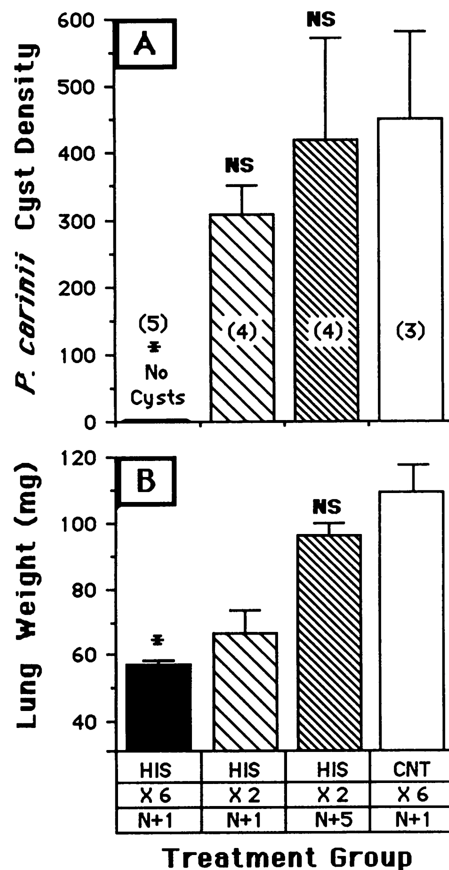

FIG. 3. Treatment of B6-*scid* mice with two versus six doses of anti-*P. carinii* HIS. Thirteen *scid* mice (6 ± 2 weeks at start) received weekly i.p. injections of neat HIS, and three others received sterile saline injections (control [CNT]). One group received six injections of HIS and was necropsied 1 week after the last treatment (HIS, X6, N+1), a second group was injected twice with HIS and necropsied 1 week later (HIS, X2, N+1), a third group was injected twice with HIS and necropsied 5 weeks following the last treatment (HIS, X2, N+5), and a fourth group received six weekly injections of saline and was necropsied 1 week later (CNT, X6, N+1). *P. carinii* cyst density (in cysts per square millimeter) (A) and wet weights of left lungs (in milligrams) (B) are presented (means ± standard errors of the means). Numbers of mice are indicated in panel A. *, significantly different; NS, not significantly different from control group. *t* test was not conducted for lung weights of HIS, X2, N+1 group of mice; this group was necropsied 2 rather than 6 weeks after initiation of the experiment, and since lung weight increases steadily during the life of *scid* mice (Fig. 1), no nontreated comparison was available in this experiment for this group.

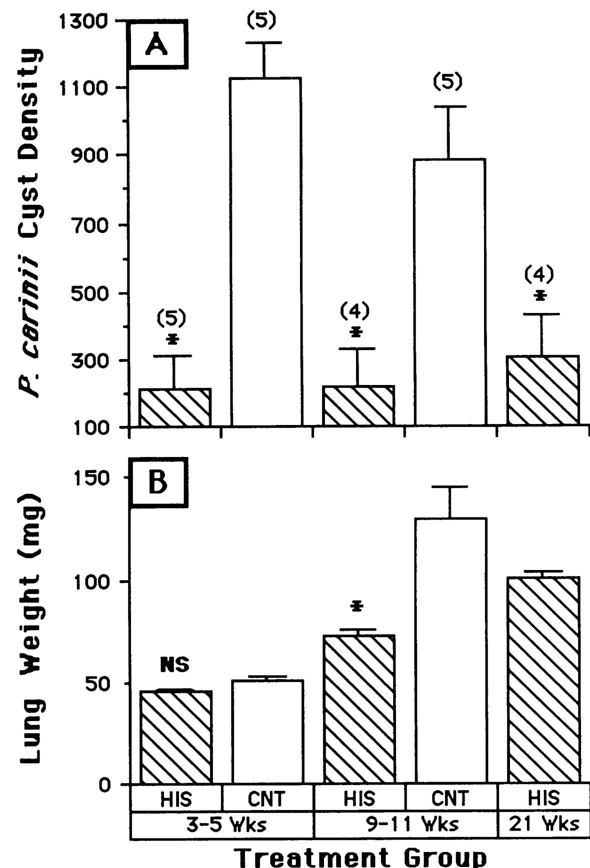

FIG. 4. Treatment of B6-*scid* mice with anti-*P. carinii* HIS at different stages of pneumocystosis. Ten 3- to 5-week-old, 10 9- to 11-week-old, and 12 21-week-old *scid* mice were divided such that half received six weekly i.p. injections of neat HIS and the remainder received saline injections (control [CNT]). All of the control and four of eight HIS-treated 21-week-old mice died prior to scheduled necropsy; one of five HIS-treated 9- to 11-week-old *scid* mice also died. All surviving mice were necropsied 1.5 weeks following the last treatment. *P. carinii* cyst density (in cysts per square millimeter) (A) and wet weights of left lungs (in milligrams) (B) are presented (means ± standard errors of the means). Numbers of mice are indicated in panel A. *, significantly different; NS, not significantly different from untreated, age-matched control group. *t* test for cyst density of HIS-treated 21-week-old group was compared with control 9- to 11-week-old group, since cyst numbers plateau in untreated *scid* mice (Fig. 1). All collateral untreated controls for group HIS, 21 Wks died; therefore, no *t* test for lung weights was conducted for this group (see the legend to Fig. 3).

with those of untreated *scid* mice. In view of the continued presence of *P. carinii* in the lungs of *scid* mice treated twice and analyzed 1 week later, the near-normal lung weights of this group probably reflect the reduced opportunity (these mice were necropsied 4 weeks earlier [younger] than all others) for development of the interstitial hyperplasia and chronic inflammatory changes characteristic of PCP.

Anti-*P. carinii* serum therapy of *scid* mice at early, intermediate, and advanced stages of PCP. Ten 3- to 5-week-old, 10 9- to 11-week-old, and 12 21-week-old *scid* mice were divided into groups such that one-half of each age group received six weekly injections of neat HIS while the other half received saline (control), after which the mice were necropsied and their lungs were assayed as described above. HIS treatment was effective in reducing cyst density to 19% ($P < 0.001$) and 25% ($P < 0.025$) of that of untreated controls in *scid* mice with early- and intermediate-stage PCP, respectively (Fig. 4A). The lung weights of HIS- and saline-treated young (3 to 5 weeks old at start) *scid* mice were not significantly different from each other or from those of control B6 mice (Fig. 4B and 1B). However, HIS treatment was effective in reducing the lung weights of intermediate-stage *scid* mice to 55% of untreated control levels ($P < 0.025$).

Of the advanced-stage *scid* mice, all four saline-treated and four of eight HIS-treated mice died (without necropsy) within 1 to 2 weeks after the start of the experiment. (The

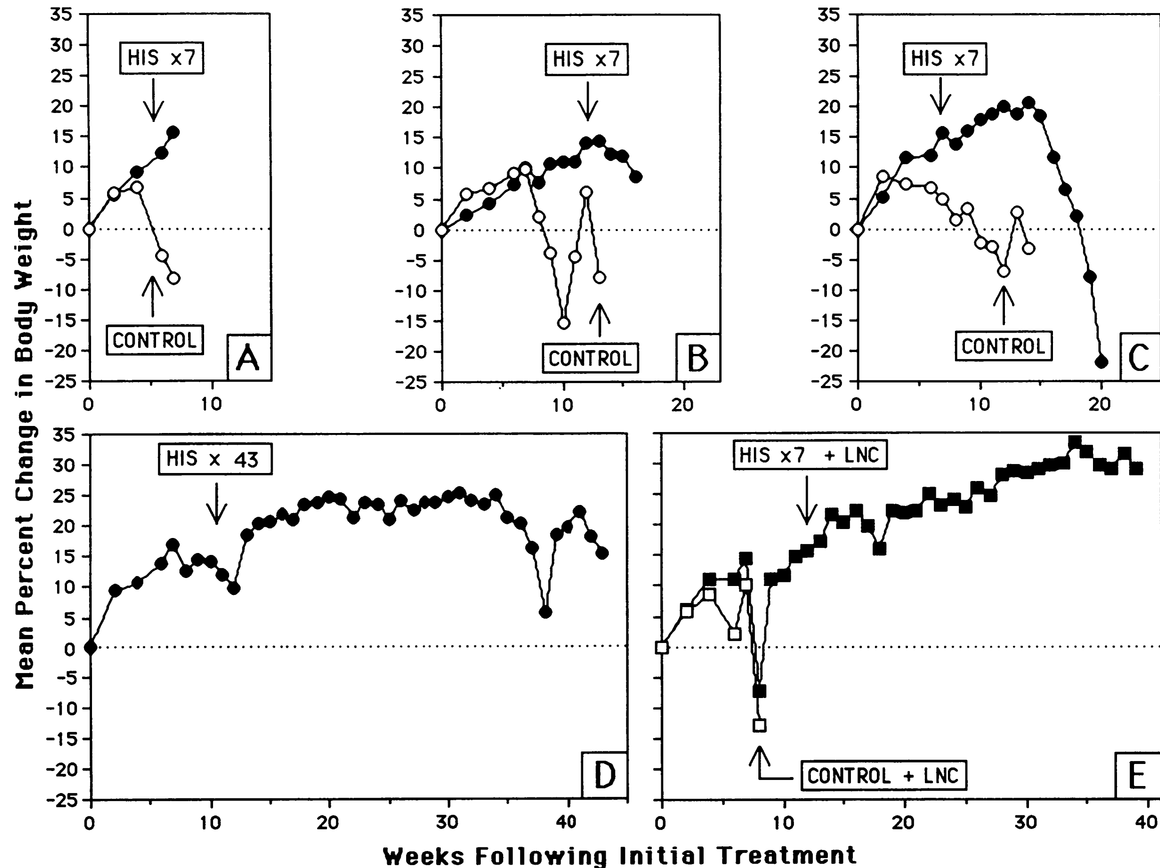

FIG. 5. BW of mice receiving different forms of anti-*P. carinii* immunotherapy. Datum points represent group means of individual differences (percent gain or loss) in BW of surviving mice at various times from the start of experiment. The declines followed by upturns in BW (for example, group B control at 10 versus 12 weeks) reflect the death of one or more members of the group; after a death, the mean BW of the remaining group increases. Fifty-eight B6-*scid* mice (9 ± 2 weeks of age initially) were treated with seven weekly injections of neat HIS (closed symbol) or saline (control; open symbol). The time of initial treatment was designated week 0; the seventh injection of HIS was done in week 6. In addition, group E mice were injected i.p. with LNC from a *P. carinii*-sensitized male B6 donor in week 7. Group A and B mice were necropsied 1 (week 7) and 10 (week 16) weeks following the last treatment. Group C mice were necropsied only when moribund. Group D mice continued to receive HIS weekly until necropsied. Mice of groups D and E were necropsied when moribund or at the conclusion of the experiment. See Results for additional details. Numbers of mice per group are indicated in Fig. 6.

age of these *scid* mice at the start of the experiment [21 weeks] approximates the median age at death for this stock. Among 75 female and 42 male B6-*scid* mice held for life span analysis, we determined the median ages at death to be 20 and 21 weeks, respectively [unpublished data].) However, the surviving four of eight HIS-treated advanced-PCP individuals had significant reductions in numbers of *P. carinii* cysts (to 34% of those of untreated intermediate-age controls) (Fig. 4A). The mean lung weight of these mice was also modestly reduced (23% less than that of intermediate-age untreated controls [Fig. 4B]). The healthy appearance and normal body weights (22 ± 0.8 g) of these four individuals demonstrate a positive therapeutic effect of humoral anti-*P. carinii* therapy even in advanced stages of PCP.

A group of 58 male and female B6-*scid* mice (9 ± 2 weeks old at the start) were divided among nine treatment and control groups (A through E; Fig. 5 and 6) in order to explore three questions relative to immunotherapy of PCP: (i) does PCP return after cessation of a short-term regimen of humoral therapy, (ii) can continued humoral therapy control PCP over an extended period, and (iii) can humoral therapy and cellular immunotherapy be productively combined? Thus, one-half of the mice in groups A, B, and C received seven weekly i.p. injections of 0.25 ml of neat HIS per injection while the remaining mice in these groups were injected with sterile saline. Groups A and B were necropsied at 1 and 10 weeks, respectively, after the cessation of humoral therapy, while group C was permitted to continue until the mice were moribund. Group D received continued weekly humoral therapy, while group E tested the effectiveness of combining a brief course of humoral therapy with cellular reconstitution. Figure 5 presents the mean percent change (from the initiation of treatment) in BW of the surviving mice, since as described previously (27), the loss of BW is an excellent marker of the severity and progression of PCP. The left lobe of the lung was weighed at necropsy, and the *P. carinii* cyst density was determined as described above; these data are presented in Fig. 6.

Rebound of PCP following cessation of continued anti-*P. carinii* serum therapy. As shown above, the regimen of seven weekly injections of HIS provided effective short-term anti-*P. carinii* therapy. At 1 week following their last injection, HIS-treated *scid* mice had increased their initial BW by 16%, while saline-treated control mice lost 8% of their initial BW

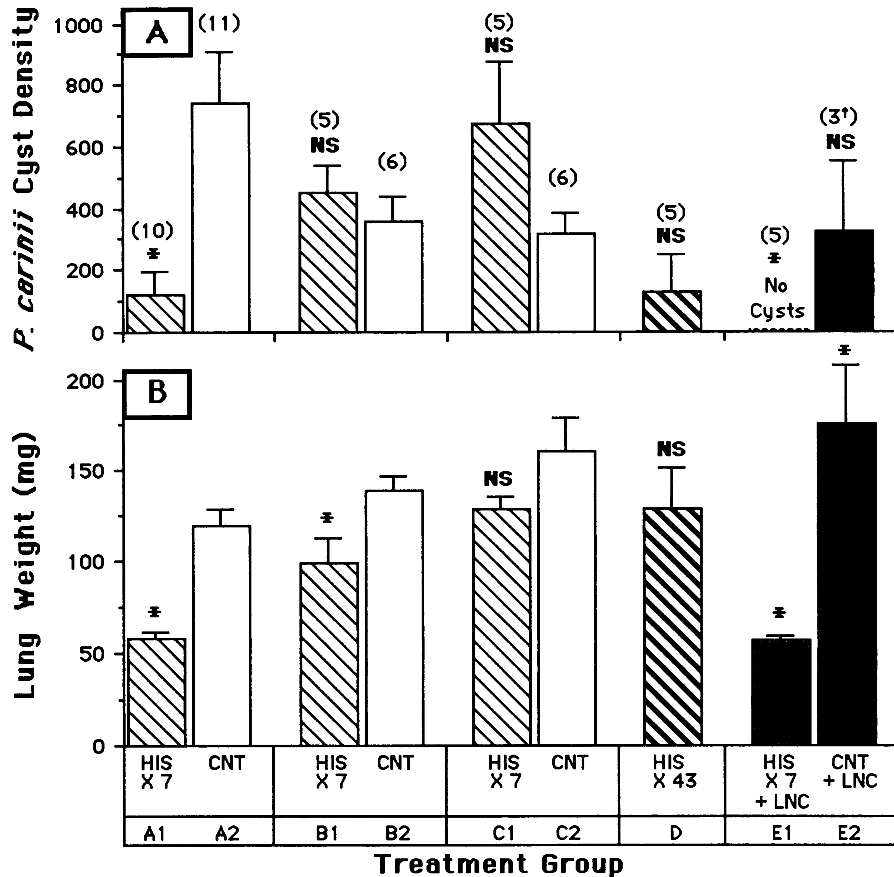

FIG. 6. Analysis of *P. carinii* cyst densities and lung weights of necropsied B6-*scid* mice after various forms of anti-*P. carinii* immunotherapy. Groups and treatments are described in the legend to Fig. 5. *P. carinii* cyst density (in cysts per square millimeter) (A) and wet weights of left lungs (in milligrams) (B) are presented (means ± standard errors of the means). Numbers of necropsies performed are indicated in panel A. No cysts were observed in mice of group E1. †, two mice in group E2 died without necropsy; *, significantly different; NS, not significantly different. *t* tests for groups A1, B1, and C1 were based on comparisons with their matched (A2, B2, and C2) controls (CNT) for both cyst density and lung weight. Cyst densities of groups D and E2 were not significantly different, while that of group E1 was significantly different from the composite set of controls, A2 + B2 + C2. The lung weights of group D mice were not significantly different from each individual group or the entire composite control group A2 + B2 + C2 or from those of the group of 143-day-old B6-*scid* mice (Fig. 1). In contrast, the lung weights of group E1 mice were significantly lower than that of any of these same control groups. The lung weights of the short-lived mice of group E2 were significantly greater than those of group A2 mice.

(Fig. 5A). The density of *P. carinii* cysts in these HIS-treated *scid* mice was significantly reduced ($P < 0.004$) to 17% that of untreated controls (Fig. 6A, groups A1 and A2), while their mean lung weight was about half that of control *scid* mice ($P < 0.0001$) (Fig. 6B, groups A1 and A2). These mice confirm the effectiveness of the 7-week regimen of humoral immunotherapy in this experiment.

All five HIS-treated but none of six saline-treated *scid* mice of group B survived to be necropsied 10 weeks following the last treatment. The average survival time from last treatment (T_s) of group B control mice was only 4.6 weeks. Control *scid* mice rapidly lost weight beginning 8 weeks after the start of the experiment, whereas HIS-treated mice continued to increase their BW until 3 weeks prior to planned necropsy (Fig. 5B). The effectiveness of anti-*P. carinii* therapy documented 1 week after the last of seven weekly HIS injections (see treatment group A, Fig. 5A and Fig. 6A and B) thus disappeared with time. When mice were necropsied 10 weeks following the completion of HIS treatment, cyst density (Fig. 6A, groups B1 and B2) had risen to levels similar to those of untreated control *scid* mice. The lung weights of these treated mice remained slightly but significantly less ($P < 0.025$) than those of untreated control *scid* mice (Fig. 6B, groups B1 and B2).

When permitted to age to the point of morbidity, *scid* mice injected with seven weekly doses of HIS showed a mean T_s after their last treatment of 13 ± 0.8 weeks (19 weeks after initiation of treatment). The first remarkable decline in BW was noted 17 weeks after the first treatment, which is considerably later than that of saline-treated controls (Fig. 5C). (The mean T_s of saline-treated *scid* mice [combined groups B2 and C2] was 11 ± 0.6 weeks from the start of the experiment.) The cyst densities and lung weights of these HIS-treated *scid* mice (group C1) were not significantly different from those of animals that received saline alone (group C2) (Fig. 6). Thus, although this frequency and dosage of HIS were highly effective in temporarily reducing the number of *P. carinii* organisms and the clinical indicators of infectivity, the effect was transient. Longevity was extended only by approximately the number of weeks of HIS treatment. Whether the returning *P. carinii* resulted from

reexpansion of temporarily checked (see below) or de novo infecting organisms is not yet clear.

Long-term humoral anti-*P. carinii* therapy of *scid* mice. Five *scid* mice (group D) were treated continuously with weekly injections of HIS until they were moribund or the experiment was ended. Three of these five individuals survived, in apparent good health, until the experiment was terminated 43 weeks after the first treatment. In general, this group maintained a significant gain in BW (Fig. 5D). Two of these five individuals became moribund and were necropsied at 12 and 38 weeks from the start of the experiment (coinciding with the negative deflections in mean percent change of BW in Fig. 5D).

The mean cyst density of all necropsied group D mice was 25% that of saline-treated *scid* controls (A2, B2, and C2 in Fig. 6A), thus approaching ($P < 0.079$) but not attaining a statistically significant difference. Although the individual surviving only 12 weeks after the start of the experiment had a cyst density of 623 cysts per mm^2, the four individuals surviving to ≥38 weeks had only 3, 3, 0, and 6 cysts per mm^2. Histologically, the lungs of these four were unlike those of mice with PCP. Trophic forms of *P. carinii* were rare or nonexistent, interstitial hyperplasia with some fibrosis was common, and small (nonactivated) macrophages as well as polymorphonuclear leukocytes were common but widely scattered. The individual that was necropsied at 38 weeks into the experiment had a diffuse alveolitis, piecemeal parenchymal necrosis, and extensive sloughing of bronchiolar epithelium. The rather heavy lungs of these mice (Fig. 6B, group D) (not significantly different from any or all of groups A2, B2, or C2 or from similarly aged B6-*scid* mice evaluated in Fig. 1) may have been due in part to chronic inflammatory reactions to very low but persistent levels of *P. carinii*. Alternatively, these *scid* mice may have suffered from infection by another, unidentified pulmonary pathogen.

Albeit not uniformly successful, long-term weekly treatment of *scid* mice with HIS was definitely effective in lengthening T_s from the start of the experiment to more than three times that of saline-treated *scid* mice (>39 versus 11 weeks).

Combined humoral and cellular immunotherapy of PCP. Previous studies have described the deleterious sequelae associated with transplantation of normal bone marrow (27) or immunocompetent ($CD4^+$) T cells (28) into *P. carinii*-infected *scid* mice. HIR to *P. carinii* is usually fatal within the first 4 weeks following cellular transfer, depending on (i) the source of cells (lymph node, spleen, or bone marrow), (ii) the dose of cells, and (iii) the state of *P. carinii* sensitization of the donor. Individuals surviving this period of acute HIR typically become and remain *Pneumocystis* free and have greatly increased longevity. The goal for the last pair of experimental groups was to determine the efficacy of reducing the burden of *P. carinii* with anti-*P. carinii* HIS prior to transfer of *P. carinii*-sensitized lymphocytes on minimizing the deleterious effects of the anti-*P. carinii* HIR.

All five saline-treated B6-*scid* mice that received only *P. carinii*-sensitized LNC 7 weeks after the start of the experiment (group E2) experienced a precipitous loss of BW (from +10 to −13%) within a 1-week period (Fig. 5E) and became morbidly ill (mean T_s from the start of the experiment was 8 weeks). Two members of this group died without necropsy. Of the three that were necropsied, large numbers of *P. carinii* cysts (not significantly different from that of the composite saline-treated controls A2, B2, and C2) were found (Fig. 6A). Increased lung weight, previously described for mice undergoing this HIR, was evident (Fig. 6B). The lungs of these *scid* mice were 44% ($P < 0.046$ level of significance) heavier than those of their most appropriate (based on age) comparison group (E2 versus A2, Fig. 6B).

None of the five HIS-treated *scid* mice that then received LNC (group E1) died. Although they had a sharp initial decline in BW (from +14 to −7%) similar to that of saline-treated and LNC-receiving mice (Fig. 5E), they did not exhibit similar signs of morbidity (cachexia, inactivity, unkempt pelage, etc.). Mice in group E1 exhibited an equally remarkable gain in BW (from −7 to +11%) during the following week and continued to gain BW and remain thrifty in appearance for the remainder of the experiment. At the time the experiment was terminated, these *scid* mice, having survived nearly four times (T_s of 39 weeks from the start of the experiment) as long as untreated *scid* controls (groups B2 and C2), were uniformly in apparent good health. Absolutely no *P. carinii* cysts were identified in these five individuals (Fig. 6A) ($P < 0.018$ compared with composite controls A2, B2, and C2), and their mean lung weight (57 ± 1.7 mg) (Fig. 6B) was significantly lower than that of each group or of the composite group (A2, B2, and C2) ($P < 0.001$) of saline-treated *scid* controls. Additionally, the lungs of this group were significantly less massive ($P < 0.001$) than those of similarly aged untreated B6-*scid* mice and not different ($P > 0.3$; not significant) from that of similarly aged B6+/+ mice (Fig. 1B). The combination of HIS and LNC treatments (group E1) was also significantly more effective in reducing lung weight ($P < 0.016$) than long-term HIS treatment (group D). Thus, adoptive transfer of LNC 1 week following the last of seven weekly injections of HIS was fully curative of PCP in *scid* mice without the morbidity of an anti-*P. carinii* HIR. It should be noted that an anti-*P. carinii* HIR results from transfer of either naive or deliberately sensitized T cells in the absence of prior HIS treatment (28).

DISCUSSION

In spite of the findings of a high frequency of anti-*P. carinii* antibodies in healthy people (23, 25) and animals (9, 32, 33), which suggests a role for antibody in protection against *P. carinii*, there has been controversy in the literature concerning a protective effect of humoral immunity in PCP. In one study, Furata et al. (11) concluded that immune serum was not effective for recovery from *P. carinii* infection. It should be noted that their protocol used intranasally infected ICR strain mice (immunocompromised by cortisone) that were necropsied 2 to 3 weeks following only two antiserum treatments, negative results that are consistent with and explainable by the findings presented here.

In contrast, we have described here and previously (28) the successful immunotherapy of naturally acquired PCP in mutant *scid* mice by the administration of isologous anti-*P. carinii* HIS. It is important to recognize that at this time, the contribution of antibody or other serum components such as cytokines has not been definitively determined in this system; experiments are in progress to confirm the role of antibody and to identify the effective isotype(s). However, the demonstration by Gigliotti and Hughes (12) of definite protection from *P. carinii* in immunocompromised (steroid-induced) ferrets and rats by monoclonal antibody M5E12 clearly establishes the efficacy of antibody for *P. carinii* resistance. It is clear that humoral immunotherapy, either alone or with cellular therapy, is of clear and dramatic utility in rendering *scid* mice healthy and free of *P. carinii* for two or three times their usual life span.

From these experiments, we have determined that a

regimen of 6 to 8 weekly i.p. injections of 0.25 ml of anti-*P. carinii* HIS was therapeutically effective when the lungs were analyzed 1 to 2 weeks following the last HIS treatment. Even mice with advanced PCP and on the verge of death were successfully treated by this means. More-conservative treatments (fewer doses or more-dilute HIS) were ineffective, and a rebound in numbers of organisms and severity of PCP rapidly followed cessation of HIS treatment. However, continued weekly anti-*P. carinii* HIS treatments produced at least a threefold increase in the average survival time of *scid* mice. Two of five long-term HIS-treated mice died despite continued treatment by HIS. One of these mice died showing typical PCP only 12 weeks following the start of treatment. This individual could have had an unusually high initial burden of *P. carinii* or could have been coinfected with another (unknown) pathogen, or the organism infecting this particular host could have undergone an alteration of surface antigenic determinants (little is known about the extent of variability of the antigenic repertoire of *P. carinii*). A second individual, moribund 38 weeks into continuing anti-*P. carinii* serum treatment, did not have demonstrable PCP.

The combination of humoral therapy followed by transfer of LNC was fully curative of PCP. Importantly, this regimen greatly diminished the vigor of the HIR previously described (27, 28), presumably by reducing the burden of *P. carinii* organisms which the later cellular reconstitution encounters and permanently resolves. Although there was a period of transient weight loss, no mortality or cachexia was evident. Even the temporary weight loss may have been preventable by shortening the gap between cessation of HIS treatment and transfer of LNC to less than 1 week. The ability to reduce or eliminate undesirable HIR occurring upon restoration of full immune competence to formerly immunodeficient individuals may be clinically important after courses of immunosuppression for transplantation or chemotherapy as well as in the future, when means of restoring immune competence to AIDS patients have been developed.

Further enhancement of the therapeutic effectiveness of anti-*P. carinii* serum might be achieved by increasing the dose or frequency of HIS administered, using Ig fractions of HIS, affinity purifying Ig with specificity towards *P. carinii* epitopes, or using single or pooled anti-*P. carinii* monoclonal antibodies. The use of homologous monoclonal antibodies to *P. carinii* would predictably have fewer immunological complications than heterologous antiserum, since serum sickness and anaphylactic reactions are considered significant risk factors in heterologous passive immunotherapy. However, in many systems, these negative sequelae are exceedingly rare; for example, purified equine anti-rabies Ig has been shown to be a safe and effective alternative to human anti-rabies IgG, producing only minor serum sickness-like reactions in less than 2% of patients (37). Given that the population at risk for developing PCP has significant immune deficiencies, the risk of developing serum sickness after use of heterologous anti-*P. carinii* serum may be quite low. Additionally, in some systems, polyclonal antibody has advantages over monoclonal antibody. For example, heterologous polyclonal rabbit anti-human thymocyte globulin has a significantly greater effect than monoclonal antibody OKT3 in dampening the cell-mediated immune rejection of a transplanted heart (16).

At present, there are three approved drugs and several investigational drugs in clinical trials for use in preventive or therapeutic regimens directed against PCP. The approved drugs, including trimethoprim-sulfamethoxazole, parenteral pentamidine, and pyrimethamine-sulfadiazine, are imperfect with regard to efficacy, safety, and convenience (21). For example, potentially severe adverse reactions to trimethoprim-sulfamethoxazole (13) or parenteral pentamidine (13, 36) occur with extraordinarily high frequency (60 to 100%) in patients infected with human immunodeficiency virus. Moreover, after a conventional course of such chemotherapy, nearly 90% of AIDS patients in one study (based on transbronchial biopsy) had persistent *P. carinii* cysts and characteristic inflammation (29). Several authors have suggested that there is a pressing need for the development of new immunologic approaches (including passive anti-*P. carinii* humoral therapy) for prophylaxis against or definitive treatment of ongoing PCP (4, 23). The studies presented here address this need.

In summary, this paper describes the positive effect of serotherapy of naturally acquired PCP in the *scid* mouse model of severe combined immunodeficiency. This study provides unambiguous evidence that in the absence of T-cells, humoral immunity can be used to prevent or treat ongoing PCP. However, such serum treatment is even more effective and long lasting when followed by restoration of cellular immune function. Consideration should thus be given to the efficacy of humoral therapy (with or without T-cell supplementation) in complementing existing, primarily chemotherapeutic approaches to treatment of *P. carinii* infection in humans.

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