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Role of Gamma Interferon in the Host Immune and Inflammatory Responses to *Pneumocystis carinii* Infection

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The role of gamma interferon (IFN-γ) in host defense to *Pneumocystis carinii* was investigated by use of three different murine models of infection. C57BL/6 *scid*/*scid* (severe combined immunodeficient [SCID]) mice were given intratracheal inoculations of *P. carinii* and reconstituted with splenocytes from either mice with disrupted IFN-γ genes (IFN-$\gamma^{-/-}$ mice) or homozygous wild-type (IFN-$\gamma^{+/+}$) mice. Unreconstituted SCID mice had $\log_{10}$ 7.08 ± 0.13 *P. carinii* nuclei in their lungs at day 22 postinfection, whereas SCID mice reconstituted with splenocytes from either wild-type or IFN-$\gamma^{-/-}$ mice had cleared the infection. However, there was a prolonged and exacerbated inflammatory response in the lungs of SCID mice reconstituted with IFN-$\gamma^{-/-}$ splenocytes which was characterized by interstitial pneumonia, eosinophilia, and multinucleated giant cell formation. Similar results were found in C.B17 SCID mice reconstituted with $CD4^+$ cells from *P. carinii*-immunized donors treated with neutralizing anti-IFN-γ monoclonal antibody (MAb). These mice resolved their *P. carinii* infections; however, they also exhibited exacerbated lung pathology compared with mice treated with a control MAb. Finally, IFN-$\gamma^{-/-}$ mice challenged intratracheally with *P. carinii* resolved their infection within 56 days as did IFN-$\gamma^{+/-}$ mice. Furthermore, depletion of T cells in vivo with a MAb resulted in IFN-$\gamma^{-/-}$ mice becoming susceptible to *P. carinii* infection. Together, these data indicate that IFN-γ is not required for resolution of *P. carinii* infection; however, in the absence of IFN-γ, there is a prolonged and exacerbated *P. carinii*-driven interstitial pneumonia characterized by eosinophilia and formation of multinucleated giant cells.

Gamma interferon (IFN-γ) has been shown to be critical to host defense against numerous organisms, including *Toxoplasma gondii*, *Chlamydia trachomatis*, and *Listeria monocytogenes* (7, 18, 21). However, the role of IFN-γ in resistance to *Pneumocystis carinii* is not certain. It has been shown that the administration of recombinant murine IFN-γ to long-term cortisone-treated rats enhanced the efficacy of trimethoprim-sulfamethoxazole to resolve *P. carinii* pneumonia (PCP) (17). In addition, mice made susceptible to PCP by the chronic depletion of $CD4^+$ cells significantly lowered their burden of *P. carinii* upon exposure to aerosols of IFN-γ (1). IFN-γ has also been shown to be produced in the lungs of mice after challenge with *P. carinii* (12). In this same study, it was also found that treatment of mouse alveolar macrophages with IFN-γ enhanced their ability to kill *P. carinii* in vitro by increasing nitric oxide synthesis of the macrophages. On the other hand, experiments performed in this laboratory indicate that treatment of *P. carinii*-infected severe combined immunodeficient (SCID) mice with either monoclonal or polyclonal IFN-γ-specific antibodies does not affect resolution of PCP in these mice after reconstitution with immunocompetent spleen cells (2).

The reason for these apparently inconsistent results is not known. However, it is possible that endogenous IFN-γ is not required for resistance to PCP but that treatment of immunocompromised hosts with exogenous IFN-γ may stimulate some mechanism not utilized in resistance to PCP in immunocompetent hosts. Alternatively, the treatment of mice with antibodies that neutralize IFN-γ may not be effective locally in the lungs and thus may not be able to inhibit an IFN-γ-mediated response at this site.

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Because of the potential therapeutic use of IFN-γ in immunocompromised individuals as well as the important role IFN-γ has in certain types of immune response, such as the Th1-mediated response, we undertook studies to further investigate the role of IFN-γ in resistance to PCP. SCID mice, infected with *P. carinii*, were reconstituted with $CD4^+$ cells from immunocompetent mice. The effect of treatment of these mice with a monoclonal antibody (MAb) that neutralized IFN-γ in vivo was determined. Although the MAb treatment did not affect the clearance of *P. carinii* from the reconstituted SCID mice, the treatment did result in exacerbation of the pulmonary inflammatory response. This was characterized by significant increases in the accumulation of eosinophils and multinucleated giant cells (MNGC). In addition, it was found that mice with disrupted IFN-γ genes (IFN-$\gamma^{-/-}$ mice) were no more susceptible to PCP than were their wild-type counterparts. Similarly, SCID mice reconstituted with spleen cells from IFN-$\gamma^{-/-}$ mice resolved their PCP as well as SCID mice reconstituted with spleen cells from homozygous wild-type (IFN-$\gamma^{+/+}$) mice. These results definitively demonstrate that IFN-γ is not required for resistance to PCP but may function in regulation of the inflammatory response to PCP.

MATERIALS AND METHODS

Mice. C.B17 and C57BL/6 (B6) *scid*/*scid* mice (with SCID) were bred and maintained at the Trudeau Institute animal facility in microisolator cages with sterile food and water. IFN-$\gamma^{-/-}$ and IFN-$\gamma^{+/-}$ mice were obtained from Charles River and maintained at the Trudeau Institute animal facility. These mice were on a mixed 129-C57BL/6 background. C.B17 +/+ mice and C57BL/6 × 129 offspring (B6129/F1) were obtained from the Trudeau Institute Animal Breeding Facility.

Infection of mice with *P. carinii*. Lungs from C.B17 SCID mice maintained in a *P. carinii*-infected colony (9) were used as a source of *P. carinii*. Recipient mice were anesthetized with halothane gas and given intratracheal (i.t.) inoculations of 100 μl of lung homogenates containing 10^8 *P. carinii* nuclei/ml with a blunted 20-gauge needle inserted into the trachea through the oral pharynx as described previously (8). Intranasal inoculations of *P. carinii* were given to mice in some

TABLE 1. Clearance of *P. carinii* and lung inflammatory response at day 22 in SCID mice reconstituted with splenocytes from normal mice or IFN-$\gamma^{-/-}$ mice

Treatment	Log_{10} *P. carinii* nuclei/ right lung[a]	Right lung wt (g)	No. of lung lavage cells (10^5)	
			Eosinophils	$CD4^+$ $CD45RB^{lo}$
B6129/F1	<3.84	0.26 ± 0.04	0.00 ± 0.00	5.84 ± 1.45
IFN-$\gamma^{-/-}$	4.27 ± 0.60	0.37 ± 0.04[c]	29.40 ± 21.32[c]	5.12 ± 1.68
Unreconstituted	7.08 ± 0.13[b]	0.24 ± 0.01	1.92 ± 2.79	0.02 ± 0.01[b]

[a] Values represent the means ± SD for five mice in B6129/F1 or IFN-$\gamma^{-/-}$ reconstituted groups and for four mice in the unreconstituted group (one mouse died).
[b] Significantly different than mice reconstituted with either B6129/F1 or IFN-$\gamma^{-/-}$ splenocytes ($P < 0.05$).
[c] Significantly different than mice either not reconstituted or reconstituted with B6129/F1 splenocytes ($P < 0.05$; $n = 4$ or 5).

experiments. These mice were lightly anesthetized, and a 70-μl droplet of a lung suspension containing 10^7 *P. carinii* nuclei was placed over the nares. The suspension was inhaled since mice are obligate nose breathers. In some experiments, SCID mice were infected with *P. carinii* by cohousing them with mice from the infected colony for 4 to 5 weeks.

Reconstitution of *P. carinii*-infected SCID mice. B6 SCID mice were given i.t. inoculations of 10^7 *P. carinii* nuclei and intravenous injections of 5×10^7 splenocytes isolated from either five B6129/F1 or five IFN-$\gamma^{-/-}$ mice. Spleens were disrupted by passage through mesh in Hanks balanced salt solution (HBSS), and cells in the resulting suspensions were pooled and enumerated. Cells (5×10^7) in 0.5 ml of phosphate-buffered saline (PBS) were injected into the tail veins of recipient mice. *P. carinii*-infected C.B17 SCID mice were reconstituted with fluorescence-activated-cell-sorted (FACS) $CD4^+$ cells obtained from the tracheobronchial lymph nodes of C.B17 +/+ mice immunized against *P. carinii*. C.B17 +/+ mice (10 to 15) were given i.t. injections of lung homogenates of SCID mice infected with *P. carinii* (10^7 nuclei/mouse) 14 and 4 days before their tracheobronchial lymph nodes were excised, pushed through stainless-steel mesh screens in HBSS, and pooled for purification. $CD4^+$ T cells in the suspensions were enriched by passage over preparative columns obtained from R&D Systems (Minneapolis, Minn.). Enriched $CD4^+$ cells were stained with fluorescein isothiocyanate-conjugated anti-CD4 [$F(ab)'_2$, clone GK1.5] and positively sorted with a FACStar Plus (Becton Dickinson, Mountain View, Calif.). Sorted $CD4^+$ cells (2×10^5) in 0.2 ml of PBS, routinely greater than 98% pure, were injected into the tail vein of *P. carinii*-infected C.B17 SCID mice.

Treatment of mice with MAb for neutralization of IFN-γ or depletion of T cells. *P. carinii*-infected C.B17 SCID mice reconstituted with $CD4^+$ T cells were given intraperitoneal injections of either a MAb specific for IFN-γ (2×10^4 neutralizing units) or an equal amount of isotype-matched control MAb (anti-horseradish peroxidase) three times per week beginning on the day of reconstitution. The anti-IFN-γ MAb is a rat-mouse hybridoma (R4 6A2) that secretes rat immunoglobulin G1 capable of neutralizing murine IFN-γ (11). The neutralizing titer was expressed as the highest immunoglobulin G dilution that, when mixed with an equal volume of IFN-γ (20 U/ml), neutralized 50% or more of the antiviral activity on L929B cells infected with vesicular stomatitis virus (2).

IFN-$\gamma^{-/-}$ and homozygous wild-type IFN-$\gamma^{+/+}$ mice were given intraperitoneal injections containing 250 μg of anti-Thy1.2 (clone 30H12; American Type Culture Collection, Rockville, Md.) and 250 μg of anti-CD4 (clone GK1.5; American Type Culture Collection) two times per week beginning 7 days before challenge with 10^7 *P. carinii* nuclei and continuing throughout the experiment. In vivo use of these antibodies results in depletion of T cells bearing the respective surface antigens from the treated mice. Detailed descriptions of the preparation of these antibodies and their activities (in vivo) have been reported previously (9, 10).

Enumeration of *P. carinii* nuclei. The intensity of *P. carinii* infections in mouse lungs was determined by enumeration of *P. carinii* nuclei as described previously (6, 9). Briefly, lungs (either whole or right lobes) were excised and pushed through stainless-steel mesh screens in HBSS. Aliquots of the lung homogenates were spun onto glass slides with a cytocentrifuge, and smears were stained with Diff-Quik. The number of *P. carinii* nuclei per 10 (for heavy infections) to 50 oil immersion fields were counted, and the total *P. carinii* nuclei per lung or right lobes of the lung were calculated. The limit of detection by this method was $\log_{10}$ 4.00 for whole lung homogenates or 3.84 for right lobe homogenates. Slides from individual mice were coded and counted blindly by a single investigator for each experiment.

Assessment of cellular infiltration into the lungs. Mice were deeply anesthetized with halothane gas and exsanguinated, and lung lavages were performed as described previously (8) with 5×1 ml of HBSS containing 3 mM EDTA. Lung lavage cells were enumerated, and aliquots were spun onto glass slides and stained with Diff-Quik for differential counting. Aliquots of cells were incubated with anti-CD4 fluorescein isothiocyanate (clone GK1.5) and anti-CD45RB biotin (clone 16A; PharMingen, San Diego, Calif.) followed by streptavidin-CyChrome (PharMingen) and analyzed with a FACScan (Becton Dickinson). Four thousand cells were gated with forward and side light scatter, and lymphocytes were analyzed for fluorescence.

Lung histopathology. Right lung lobes were tied off and excised, and the left lobes were inflated with 10% neutral buffered formalin through a tracheal cannula as described previously (3). Lungs were removed en bloc and immersed in fixative for at least 24 h before paraffin embedding. Sections were cut 5 μm thick and stained with hematoxylin and eosin.

Statistics. Differences between experimental groups were determined by Student's *t* test or one-way analysis of variance followed by the Student-Newman-Keuls post-hoc test. Where appropriate, nonparametric Mann-Whitney U or Kruskall-Wallis tests were used. Means ± standard deviations (SD) are reported and considered significantly different when P is <0.05.

RESULTS

Clearance of *P. carinii* from the lungs of SCID mice reconstituted with splenocytes from IFN-$\gamma^{-/-}$ mice. B6 SCID mice were given intranasal inoculations of 10^7 *P. carinii* nuclei and reconstituted on the same day with splenocytes from either B6129/F1 or IFN-$\gamma^{-/-}$ mice. Mice were sacrificed on day 22 postreconstitution and examined for lung *P. carinii* burden, lung pathology, and inflammatory response. SCID mice which were unreconstituted had right lung *P. carinii* burdens of $\log_{10}$ 7.08 ± 0.13 (mean ± SD). By comparison, mice given B6129/F1 splenocytes had undetectable numbers (<$\log_{10}$ 3.84) of *P. carinii* nuclei in their lungs as did most of the mice given IFN-$\gamma^{-/-}$ splenocytes (Table 1).

Interestingly, mice reconstituted with IFN-$\gamma^{-/-}$ splenocytes had significantly higher lung weights than either unreconstituted mice or those reconstituted with B6129/F1 splenocytes (Table 1). This increased lung weight in mice reconstituted with IFN-$\gamma^{-/-}$ cells corresponded to a large infiltration of eosinophils (and neutrophils [data not shown]) into the lungs compared with that of mice reconstituted with B6129/F1 cells (Table 1). However, the numbers of $CD4^+$ CD45RB cells with low fluorescence intensity ($CD45RB^{lo}$) in the lung lavages were not different between the two reconstituted groups of mice. There was also no difference in the number of $CD4^+$ CD45RB cells with high fluorescence intensity ($CD45RB^{hi}$) (approximately 5% of $CD4^+$ cells) nor in the number of $CD8^+$ cells (approximately 3×10^5) in the lung lavages of mice reconstituted with B6129/F1 cells compared with that of mice reconstituted with IFN-$\gamma^{-/-}$ cells. Examination of lung sections indicated that mice given splenocytes from IFN-$\gamma^{-/-}$ mice had large areas of edema and cellular infiltration consisting of lymphocytes, macrophages, and polymorphonuclear cells (PMN) in the alveolar septa and alveolar spaces, as well as lymphocytic infiltration around blood vessels and bronchioles (Fig. 1A). Airway epithelial cell hyperplasia was evident in the mice treated with anti-IFN-γ, and large numbers of MNGC were seen in the alveolar spaces (Fig. 1A). In contrast, the lungs of mice given B6129/F1 splenocytes were unremarkable except for some residual lymphocytic infiltrates around the blood vessels and bronchioles (Fig. 1B).

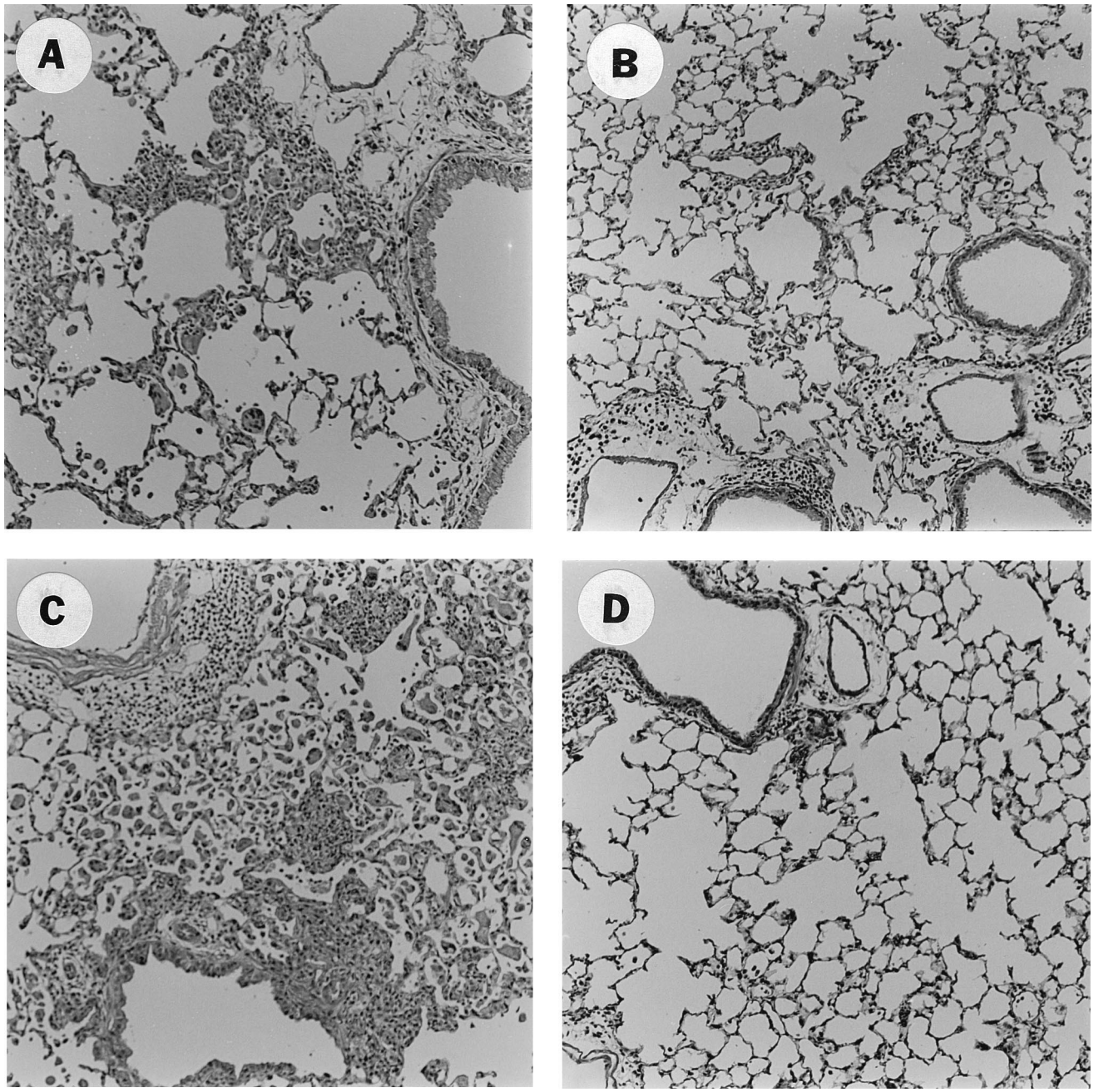

FIG. 1. Lung histopathology in SCID mice reconstituted with IFN-$\gamma^{-/-}$ splenocytes or sorted $CD4^+$ T cells from immunized donors. *P. carinii*-infected SCID mice were reconstituted with IFN-$\gamma^{-/-}$ splenocytes (A), wild-type B6129/F1 splenocytes (B), or sorted $CD4^+$ T cells from *P. carinii*-immunized donors (C and D) and then treated either with anti-IFN-γ MAb (C) or control MAb (D). Lung sections in panels A and B are from day 22 postreconstitution, and those in panels C and D are from day 47 postreconstitution. Magnification, ca. ×100. Hematoxylin and eosin stained.

Clearance of *P. carinii* from the lungs of SCID mice reconstituted with $CD4^+$ cells and treated with anti-IFN-γ MAb. To examine the effect of IFN-γ on the cellular response to *P. carinii*, infected C.B17 SCID mice were reconstituted with purified (98.7%) $CD4^+$ cells isolated from the draining lymph nodes of immunocompetent mice previously immunized by challenge with *P. carinii*. This protocol has been shown previously to result in clearance of *P. carinii* from the lungs of infected SCID mice in the absence of B cells or *P. carinii*-specific antibody (16, 20). These mice were treated either with anti-IFN-γ MAb or isotype-matched control MAb. A third group of mice was unreconstituted and not treated with antibody. Lung *P. carinii* burdens were significantly lower in the two groups of SCID mice reconstituted with T cells compared with that in the unreconstituted mice by day 25 (Fig. 2). Although *P. carinii* continued to grow in the lungs of unreconstituted mice through day 47, lung burdens were 100-fold lower in the two groups of mice that had been given $CD4^+$ T cells (Fig. 2A). Lung *P. carinii* numbers in the lungs of reconstituted SCID mice given anti-IFN-γ increased somewhat through day 47, whereas there was a consistent decrease in lung *P. carinii* burden in reconstituted mice given control MAb (Fig. 2A).

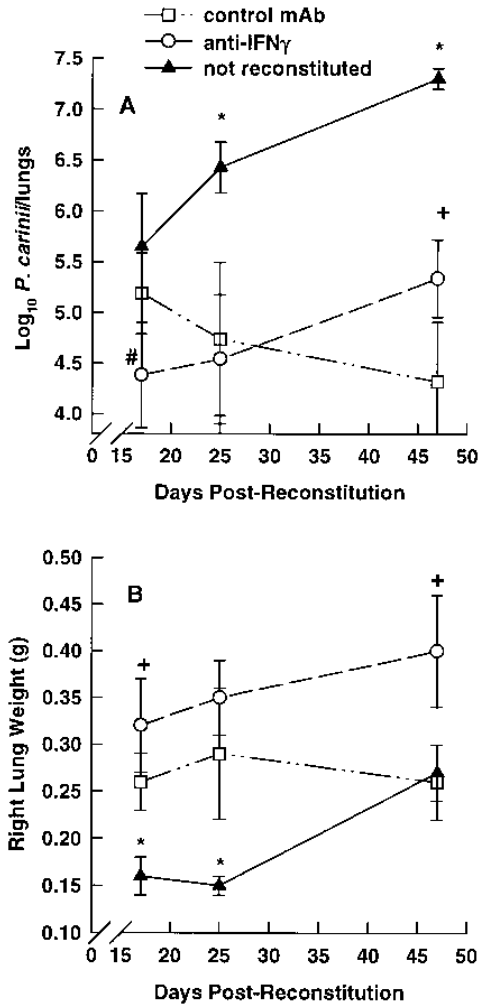

FIG. 2. Lung *P. carinii* burden and weight in SCID mice reconstituted with $CD4^+$ cells and treated with anti-IFN-γ MAb or control MAb. $CD4^+$ cells were sorted from draining lymph nodes of *P. carinii*-immunized mice and transferred to *P. carinii* SCID mice. (A) The *P. carinii* burden in the right lobes of the lungs was assessed at various times after reconstitution. The limit of detection was $\log_{10}$ 3.84. (B) Wet weights of right lung lobes were determined at the times indicated. Data points represent the means ± SD for five mice per group. Data are representative of two separate experiments. *, $P < 0.05$ compared with mice treated with either anti-IFN-γ MAb or control MAb. #, $P < 0.05$ compared with mice not reconstituted or reconstituted and treated with control MAb. †, $P < 0.05$ compared with mice treated with control MAb.

Since the experiment was terminated at day 47, it is unknown whether the *P. carinii* would have completely cleared from the lungs of anti-IFN-γ-treated mice; however, in a second experiment, three of five SCID mice reconstituted with sensitized $CD4^+$ cells and treated with anti-IFN-γ had cleared *P. carinii* from the lungs by day 32 (data not shown).

As was seen in SCID mice reconstituted with splenocytes from IFN-$\gamma^{-/-}$ mice, there was a significant amount of lung pathology found in the SCID mice reconstituted with $CD4^+$ cells and treated with anti-IFN-γ. These mice had cellular infiltrates consisting of lymphocytes, macrophages, and PMN in the alveolar septa and alveolar spaces, edema, and large numbers of eosinophils and MNGC forming in the alveolar spaces which persisted through day 47 (Fig. 1C). In addition, there was a significant amount of airway epithelial cell hyperplasia in mice treated with anti-IFN-γ (Fig. 1C). In contrast, SCID mice reconstituted with $CD4^+$ cells and treated with control MAb had cellular infiltrates in the alveolar spaces at days 17 and 25 (data not shown); however, by day 41, the alveolar spaces appeared normal, with some residual lymphocytic infiltration in the peribronchiolar and perivascular areas (Fig. 1D).

The prolonged lung pathology in the reconstituted mice given anti-IFN-γ was associated with significantly higher lung weights at day 47 in these mice compared with those of reconstituted mice treated with control MAb or unreconstituted mice (Fig. 2B). Lung weights in reconstituted mice were significantly greater than those of unreconstituted mice at days 17 and 25. However, by day 47, lung weights of unreconstituted mice had increased, presumably due to increased *P. carinii* load, to levels similar to those of reconstituted mice given control MAb (Fig. 2B).

Higher lung weights in the reconstituted mice treated with anti-IFN-γ, compared with those of mice treated with control MAb, were associated with increased numbers of $CD4^+$ cells with an activated phenotype ($CD4^+$ $CD45RB^{lo}$) (13) isolated from the lung lavage fluids (Fig. 3A). Notably, over 80% of $CD4^+$ cells in the lung lavages in both reconstituted groups were also $CD45RB^{lo}$ (data not shown). This increased number of $CD4^+$ $CD45RB^{lo}$ cells was statistically significantly higher in the anti-IFN-γ-treated mice at day 17. Although there was a 2.5-fold increase in these cells in the lungs of mice treated with control MAb by day 25, their numbers were never quite as high as those in the mice given anti-IFN-γ (Fig. 3A). The higher lung weights in the reconstituted mice treated with anti-IFN-γ were also associated with large numbers of eosinophils infiltrating the lungs. Eosinophils were nearly undetectable in number in the lung lavages of reconstituted SCID mice treated with control MAb and in unreconstituted SCID mice (Fig. 3B). However, there were greater than 10^6 eosinophils found in the lung lavage fluids of mice treated with anti-IFN-γ through day 47 (Fig. 3B). Large numbers of eosinophils could also be seen in the alveolar spaces by examining the lung sections by high-power microscopy (data not shown). Reconstituted SCID mice had large numbers of neutrophils in the lung lavages but only at day 17 (data not shown). Mice treated with control MAb had $(15.7 \pm 10.5) \times 10^5$ neutrophils in the lung lavages at day 17, whereas mice treated with anti-IFN-γ had $(30.1 \pm 16.9) \times 10^5$ neutrophils in the lung lavages. By day 25, neutrophil numbers in the lung lavages of both groups of reconstituted SCID mice had decreased to less than 6×10^5 (data not shown).

Clearance of *P. carinii* from the lungs of IFN-$\gamma^{-/-}$ mice. To determine whether IFN-γ was important for clearance of *P. carinii* from the lungs of otherwise immunologically competent mice, IFN-$\gamma^{-/-}$ mice were challenged i.t. with 10^7 *P. carinii* nuclei. At day 8 postchallenge, both IFN-$\gamma^{-/-}$ and IFN-$\gamma^{+/-}$ mice had over $\log_{10}$ 6.0 *P. carinii* nuclei in the lungs (Fig. 4). The lung *P. carinii* burden decreased steadily through day 56 in IFN-$\gamma^{+/-}$ mice, when all of the mice had cleared the organisms. In contrast, the lung *P. carinii* burden remained constant through day 16 in IFN-$\gamma^{-/-}$ mice before decreasing through day 56. At day 56, four of five IFN-$\gamma^{-/-}$ mice had undetectable numbers of *P. carinii* nuclei in their lungs. For comparison, C.B17 SCID mice inoculated with *P. carinii* at the same time as the IFN-$\gamma^{-/-}$ mice had a lung *P. carinii* burden of $\log_{10}$ 7.35 nuclei at day 56 (Fig. 4).

To confirm that IFN-$\gamma^{-/-}$ mice are susceptible to *P. carinii* when immunosuppressed, IFN-$\gamma^{-/-}$ mice were depleted of T cells by treatment twice a week with a MAb specific for Thy1.2 and CD4. Mice were given i.t. inoculations of 10^7 *P. carinii* nuclei 1 week after the beginning of in vivo T-cell depletion. *P. carinii* numbers were below the limit of detection in the lungs of IFN-$\gamma^{-/-}$ mice given control MAb by day 21 postchallenge (Table 2). However, T-cell-depleted IFN-$\gamma^{-/-}$ mice had $\log_{10}$

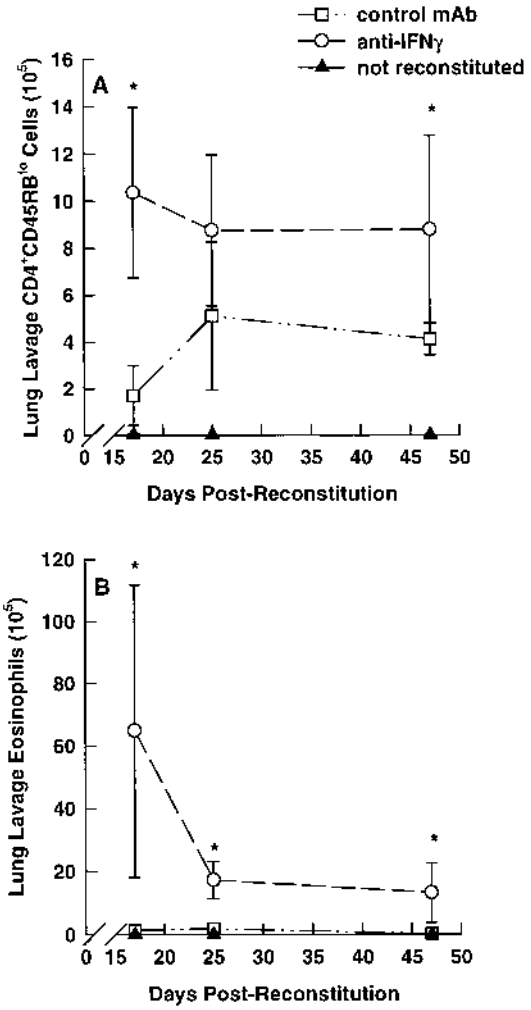

FIG. 3. Number of $CD4^+$ $CD45RB^{lo}$ cells and eosinophils in the lung lavage fluids after reconstitution of SCID mice with $CD4^+$ cells from *P. carinii*-immunized mice. *P. carinii*-infected SCID mice were reconstituted with $CD4^+$ cells isolated from the draining lymph nodes of *P. carinii*-immunized mice and treated with either anti-IFN-γ MAb or control MAb. (A) Number of $CD4^+$ $CD45RB^{lo}$ cells in lung lavage fluids determined at various times after reconstitution by flow cytometry; (B) number of eosinophils determined microscopically from cytospin preparations of lung lavage fluids stained with Diff-Quik. Data points represent the means ± SD for five mice per group. Data are representative of two separate experiments. *, $P < 0.05$ compared with mice either not reconstituted or reconstituted and treated with control MAb. Cells from mice not reconstituted were near the limit of detection and significantly different from the reconstituted groups at all time points tested.

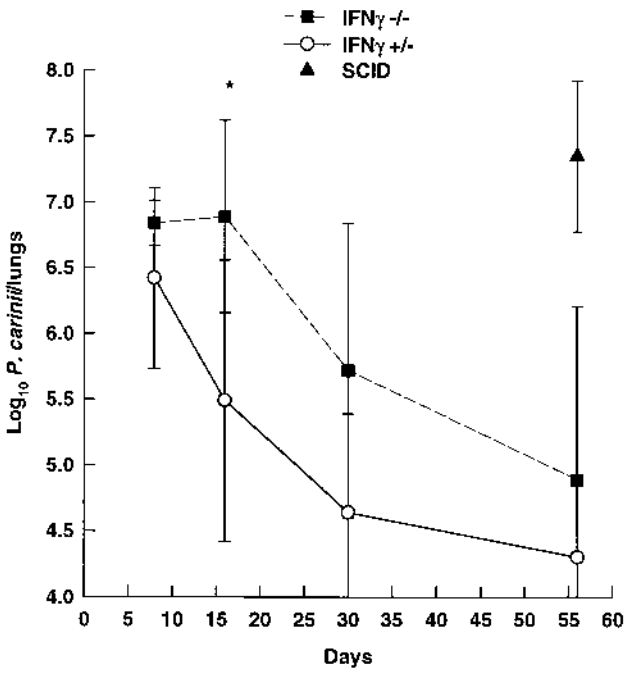

FIG. 4. Lung burden in IFN-$\gamma^{-/-}$ or wild-type mice challenged with *P. carinii*. IFN-$\gamma^{-/-}$ or IFN-$\gamma^{+/-}$ mice were challenged with *P. carinii* i.t. at day 0 of the experiment, and lung burdens were assessed microscopically from cytospin preparations of lung homogenates stained with Diff-Quik at the times indicated. The limit of detection for *P. carinii* was $\log_{10}$ 4.03 nuclei. Data points represent the means ± SD for five mice per group. Data are representative of two separate experiments. *, $P < 0.05$ compared with IFN-$\gamma^{+/-}$ mice.

6.3 and 7.1 *P. carinii* nuclei at days 21 and 42 postchallenge, respectively. Clearance of *P. carinii* in IFN-$\gamma^{-/-}$ mice was associated with a large inflammatory response in the lungs, consisting of macrophages, lymphocytes, and PMN at day 21, which was subsiding by day 42 postchallenge (Table 2). In contrast, T-cell-depleted IFN-$\gamma^{-/-}$ mice had very few macrophages and barely detectable numbers of lymphocytes and PMN in the lung lavage fluids at days 21 and 42 postchallenge (Table 2).

DISCUSSION

The data presented here confirms previously published results from this laboratory indicating that neutralization of IFN-γ in vivo does not affect the efficiency of clearance of *P. carinii* from the lungs of SCID mice reconstituted with splenocytes from immunocompetent donors (2). Furthermore, these results were extended by determining that: (i) SCID mice reconstituted with splenocytes from IFN-$\gamma^{-/-}$ mice were as efficient at resolving *P. carinii* lung infections as were SCID mice reconstituted with splenocytes from wild-type mice; (ii) neutralization of IFN-γ in vivo did not affect the efficiency of clearance of *P. carinii* from SCID mice reconstituted with sensitized $CD4^+$ cells; and (iii) IFN-$\gamma^{-/-}$ mice were no more susceptible to *P. carinii* than were IFN-$\gamma^{+/-}$ mice. Although it is clear from these results that IFN-γ was not critical for resolution of *P. carinii* infection in mice with T cells, it was also apparent that IFN-γ is a critical mediator of the inflammatory response initiated by specific recognition of *P. carinii* by T cells. In all three of the murine models of *P. carinii* infection utilized in the studies presented here, the lung inflammatory response was exacerbated when IFN-γ was absent or neutralized.

It was previously found in this laboratory that neutralization of IFN-γ had no effect on clearance of *P. carinii* from SCID mice reconstituted with splenocytes from immunocompetent donors (2). A possible criticism of this work is that IFN-γ was not completely neutralized or that the neutralization MAb did not reach the alveolar spaces. To address these possibilities, *P. carinii*-infected SCID mice were reconstituted with splenocytes from IFN-$\gamma^{-/-}$ mice. The results indicated that *P. carinii* infection was resolved even in the absence of IFN-γ-producing T cells. However, there was a significant exacerbation of the inflammatory response in mice reconstituted with IFN-$\gamma^{-/-}$ cells compared with those reconstituted with splenocytes from normal mice. This exacerbated inflammatory response was characterized by increased lung weight, eosinophilia, generation of MNGC, and interstitial pneumonia. Eosinophilia is

TABLE 2. Numbers of *P. carinii* nuclei in lung homogenates and cells in lung lavage fluids of IFN-γ knockout mice with and without depletion of T cells

Treatment of mice (day)	No. of cells in lung lavage fluids (10^5)			No. of *P. carinii* nuclei ($\log_{10}$)
	Macrophages	Lymphocytes	PMN	
T-cell depletion (21)	$8.0 \pm 0.3^{a,b}$	0 ± 0^b	0 ± 0^b	6.3 ± 0.6^b
Control (21)	23.0 ± 11.0	14.0 ± 11.0	56.0 ± 44.0	<4.0
T-cell depletion (42)	5.0 ± 0.8^b	0.1 ± 0.1^b	0.2 ± 0.1^b	7.1 ± 0.2^b
Control (42)	11.0 ± 4.8	12.0 ± 8.3	4.5 ± 4.3	<4.0

[a] Values are means ± SD for four mice.
[b] Significantly different from control ($P < 0.05$).

consistent with the generation of a Th2-type response, which is commonly seen in the lungs as a result of airway hyperreactivity (4). It has been reported that IFN-γ receptor knockout mice had prolonged eosinophilia (more than 2 months) in their lungs after a single dose of aerosolized ovalbumin. This response corresponded to elevated production of interleukin 4 (IL-4) and IL-5 from lung-derived T cells (5). Furthermore, IFN-γ receptor knockout mice developed alveolar and interstitial pneumonia with predominant eosinophilia in response to *Schistosoma mansoni* vaccine, which usually induces a Th1-type response (22). It is unclear from the data presented here whether eosinophils play any role in the clearance of *P. carinii* from the lungs; however, eosinophil infiltration in the lungs is indicative of a significantly different inflammatory response to *P. carinii* than that seen when IFN-γ is present.

IFN-γ has been classically associated with a cellular immune response, whereas IL-4, IL-5, and IL-10 have been associated with a humoral response (14). It was of interest to determine if, in the absence of IFN-γ, $CD4^+$ T cells could mediate clearance of *P. carinii* without B cells or antibody production. It has been shown previously that *P. carinii* can be cleared from the lungs of SCID mice reconstituted with $CD4^+$ T cells isolated from *P. carinii*-immunized mice (16, 20). In vivo neutralization of IFN-γ in *P. carinii*-infected SCID mice reconstituted with $CD4^+$ T cells did not significantly affect clearance of the infection. It should be pointed out that treatment of the mice with the neutralizing antibody did not significantly alter the percentage of $CD4^+$ T cells in the lung lavages that were producing IFN-γ (data not shown) but most likely acted by neutralizing secreted IFN-γ. This is consistent with the notion that the $CD4^+$ cells from immunized donors were committed to a Th1 or Th2 lineage previous to transfer and that they do not convert to a different phenotype once committed (19). It is apparent that IFN-γ is not critical for clearance of *P. carinii*; however, as seen in SCID mice reconstituted with splenocytes from IFN-$\gamma^{-/-}$ mice, IFN-γ is important for resolution of the inflammatory response. Mice treated with anti-IFN-γ had large numbers of eosinophils in the lung lavage fluids and interstitial pneumonia at day 47 after reconstitution. These data indicate that $CD4^+$ T cells can act as effector cells for clearance of *P. carinii* regardless of whether IFN-γ is active. However, in the absence of IFN-γ, these T cells were unable either to down-regulate the inflammatory response or to down-regulate themselves.

Perhaps the most definitive experiment regarding the role of IFN-γ in host defense to *P. carinii* was that showing that IFN-$\gamma^{-/-}$ mice were no more susceptible to the infection than were IFN-$\gamma^{+/-}$ mice. IFN-$\gamma^{-/-}$ mice cleared *P. carinii* from their lungs as efficiently, and within a similar time frame, as did wild-type IFN-$\gamma^{+/-}$ mice. Furthermore, depletion of T cells resulted in IFN-$\gamma^{-/-}$ mice becoming susceptible to infection. It is clear that IFN-γ is not required for clearance of *P. carinii* in immunocompetent mice. However, there have been some reports in which recombinant IFN-γ acted prophylactically in immunosuppressed mice (1) and rats (17). *P. carinii*-infected T-cell-depleted mice treated with aerosolized IFN-γ significantly decreased their lung *P. carinii* burdens after 2 weeks of daily treatment (1). In addition, *P. carinii* infection was prevented in cortisone-treated rats that received infusions of IFN-γ from implanted osmotic pumps for 4 weeks (17). Presumably, treatment with IFN-γ resulted in activation of nonspecific immune responses which were able to resist *P. carinii* infection (15). The data presented in this paper indicate that IFN-γ is not critical for clearance of *P. carinii* in immunocompetent mice; however, that does not preclude the possibility that in the absence of competent T cells, IFN-γ can have a significant prophylactic role in resolving infection. IFN-γ does play an important role in regulating the inflammatory response after resolution of the infection. In the absence of IFN-γ or when IFN-γ was neutralized, the lung inflammatory response was prolonged and exacerbated. It is unclear what signals IFN-γ provides which terminate the lung inflammatory response; however, determining the mechanism will result in a better understanding of host defense in the lungs.

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