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Protection against *Pneumocystis carinii* Pneumonia by Antibodies Generated from Either T Helper 1 or T Helper 2 Responses

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To determine whether different antibody isotypes associated with T helper 1 (Th1) or Th2 responses are protective against *Pneumocystis carinii*, mice with disrupted interleukin 4 genes (IL-4$^{-/-}$ mice) or gamma interferon genes (IFN-$\gamma^{-/-}$ mice) along with wild-type C57BL/6 mice were immunized intratracheally against *P. carinii*, depleted of T cells in vivo by use of monoclonal antibodies, and rechallenged intratracheally with 10^7 viable *P. carinii* organisms. Nearly all immunized mice resolved their lung *P. carinii* infections (limit of detection, $\log_{10}$ 4.06) within 21 days of challenge even though they were depleted of T cells. Unimmunized mice depleted of T cells had significant lung infections ($>\log_{10}$ 5.5) at day 21 post-*P. carinii* challenge. IFN-$\gamma^{-/-}$ and wild-type mice developed *P. carinii*-specific immunoglobulin primarily of the immunoglobulin G1 (IgG1) subclass with relatively little *P. carinii*-specific IgG2a, IgG2b, or IgG3 in their sera, characteristic of a Th2-type response. In contrast, IL-4$^{-/-}$ mice had primarily an IgG2b *P. carinii*-specific antibody response in their sera with very little IgG1. Although IgG2b was the predominant isotype in IL-4$^{-/-}$ mice, optical density values of IgG2a and IgG3 were significantly higher in these mice (two and three times, respectively) than in IFN-$\gamma^{-/-}$ mice, characteristic of a Th1-type response. Together, these data indicate that resolution of *P. carinii* infection can be mediated by specific antibody responses and that the antibody response can be either a predominantly Th1 or Th2 type. Furthermore, although wild-type mice mounted a Th2-like antibody response, IL-4$^{-/-}$ mice could resolve *P. carinii* pneumonia, indicating that resistance to *P. carinii* can occur in the absence of IL-4.

It has been known for some time that gamma interferon (IFN-γ) supports the production of immunoglobulin G2a (IgG2a) from lipopolysaccharide (LPS)-stimulated B cells and inhibits the production of IgG1, IgG2b, and IgG3 (22). However, in response to specific antigen, IFN-γ stimulates both IgG2a and IgG3 production by B cells (12, 21). In contrast, interleukin 4 (IL-4) supports the production of IgG1 and IgE but inhibits the production of IgG2a, IgG2b, and IgG3 by LPS-stimulated B cells (22, 25). More recently, production of Ig isotypes has been associated with T helper (Th) responses. Th1 cells produce IFN-γ and IL-2 and promote IgG2a production, and Th2 cells produce IL-4, IL-5, and IL-10 and promote IgG1 and IgE production. Although there is a significant amount of evidence that resolution of some infections is dependent on the host response being biased toward Th1 or Th2 in vivo (14), it is unclear whether these biases are related to mounting specific cellular immune responses or if specific antibody isotypes are also critical for resolution of infections.

Pneumocystis carinii-specific antibodies (Ab) are found in the sera of most children over 2 years of age (17). Immunocompetent individuals are able to efficiently resolve *P. carinii* infections in the lungs (7), which is dependent on the presence of functional CD4$^+$ T cells (11, 18, 20). Although it has recently been reported that B-cell-deficient mice are susceptible to *P. carinii* pneumonia (PCP) (13), it is unclear whether this is due to the lack of specific Ig, deficient antigen presentation, inadequate secondary signals for T cells, or some other function provided by competent B cells. Indirect evidence that specific Ig is capable of resolving *P. carinii* infection has been provided by a model in which immunocompetent mice are immunized against *P. carinii*, depleted of T cells with monoclonal Ab (MAb) in vivo, and challenged with *P. carinii*. By use of this protocol, it was found that in the absence of CD4$^+$ cells and in the presence of *P. carinii*-specific Ab, *P. carinii* was efficiently cleared from the lungs (10), indicating that specific Ab can play a decisive role in providing protection against *P. carinii*. It is unknown whether different Ig isotypes have different protective effects on *P. carinii*, although data from this laboratory indicated that passive immunization with a mouse IgG1 MAb specific for the major glycoprotein (gpA) on *P. carinii* was partially protective in infected severe combined immunodeficient (SCID) mice (8). This is of specific interest since it has been found that some IgG subclasses are more protective than others against such pathogens as *Cryptococcus neoformans* (15, 19), lymphocytic choriomeningitis virus (1), *Trypanosoma musculi* (26), and *Plasmodium chabaudi* AS (28).

It has recently been reported that mice with disrupted IFN-γ genes (IFN-$\gamma^{-/-}$ mice) are resistant to *P. carinii* infection (6). This is consistent with data from this laboratory indicating that neutralization of IFN-γ in vivo has no effect on the resolution of *P. carinii* from the lungs of SCID mice reconstituted with splenocytes from immunocompetent donors (3). It is unknown whether IL-4 is critical for resolution of PCP. To determine whether different *P. carinii*-specific Ab composed of isotypes produced by either Th1- or Th2-like responses are effective at resolving PCP, IFN-$\gamma^{-/-}$ mice and mice with disrupted IL-4 genes (IL-4$^{-/-}$ mice) were utilized for producing polarized Th responses. Mice were immunized with *P. carinii*, depleted of T cells, and rechallenged. This strategy produced mice with either predominantly *P. carinii*-specific IgG1 (IFN-$\gamma^{-/-}$ and wild type mice) or IgG2b, IgG2a, and IgG3 (IL-4$^{-/-}$ mice). Regardless of the Ab isotype profile produced, all mice resolved their PCP.

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MATERIALS AND METHODS

Mice. Eight- to 10-week-old male IFN-$\gamma^{-/-}$ (C57BL/6-Ifg<tm1Ts>) and IL-$4^{-/-}$ (C57BL/6J-I14<tm1Cgn>) mice which had been backcrossed >10 times to C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, Maine). C57BL/6J mice were obtained from the Trudeau Institute animal breeding facility. The mice were maintained under standard husbandry conditions. *P. carinii*-infected C57BL/6 SCID and C.B17 SCID mice were maintained at the Trudeau Institute (4) in microisolator cages containing sterilized food and water.

Experimental design. C57BL/6, IFN-$\gamma^{-/-}$, and IL-$4^{-/-}$ mice were immunized by intratracheal (i.t.) inoculation of 10^7 *P. carinii* organisms 2 weeks and 4 days prior to T-cell depletion. T cells were depleted by twice weekly intraperitoneal injections of 200 μg of anti-Thy1.2 (clone 30H12; American Type Tissue Culture, Rockville, Md.) and 200 μg of anti-CD4 (clone GK1.5; American Type Tissue Culture). Ab for depletion were raised as ascites in mice. After 1 to 2 weeks of T-cell depletion, mice were challenged with i.t. inoculations of 10^7 *P. carinii* organisms. Mice continued to get anti-Thy1.2 and anti-CD4 treatment for 3 weeks after challenge with *P. carinii*, when the mice were sacrificed. Control groups included mice which were sham immunized with lung material from uninfected C.B17 mice, T cell depleted, and challenged with *P. carinii*.

Inoculations with *P. carinii*. Infected SCID mice were used as a source of *P. carinii*. Lungs were excised after the mice were sacrificed by exsanguination under heavy anesthesia. The lungs were pushed through wire screens in Hanks balanced salt solution (HBSS) to form homogenates, and *P. carinii* nuclei were enumerated as described below and adjusted to 10^8 *P. carinii* nuclei per ml of HBSS. In some experiments, C.B17 SCID mice were used as a source of *P. carinii*; therefore, *P. carinii* organisms were partially purified as described previously (8) to remove lung cellular debris. In some experiments, control mice were given inoculations of lung homogenates from uninfected C.B17 mice to control for responses to alloantigens. The mice were anesthetized with halothane gas and given i.t. inoculations of 10^7 *P. carinii* organisms in 100 μl of HBSS. Experimental results were identical whether *P. carinii* was obtained from C57BL/6 SCID or C.B17 SCID mice. Furthermore, there were no detectable antibody responses in the *P. carinii*-specific enzyme-linked immunosorbent assay (ELISA) of the sera of mice given sham infections.

Enumeration of *P. carinii* nuclei. The number of *P. carinii* nuclei in the lungs of mice was determined as described previously (5, 11). Aliquots of lung homogenates or purified *P. carinii* was spun onto glass slides with a cytocentrifuge and stained with Diff-Quik (Baxter Scientific Products, Miami, Fla.). The number of *P. carinii* nuclei was determined by microscopic examination of the slides.

Lung lavage and cell type enumeration. Mice were deeply anesthetized with halothane gas, and their lungs were lavaged with HBSS containing 3 mM EDTA (9). Cells were spun onto glass slides with a cytocentrifuge and stained with Diff-Quik. Differential counts were performed by microscopic examination of the slides. The number of $CD4^+$ and $CD8^+$ cells in the lavage fluids was determined by incubating the cells with anti-CD4-fluorescein isothiocyanate (clone GK1.5) and anti-CD8-phycoerythrin (PharMingen, San Diego, Calif.) followed by analysis with a FACScan cell sorter (Becton Dickinson, Mountain View, Calif.).

Analysis of *P. carinii*-specific IgG subclasses in the sera. Blood was collected from the abdominal aorta upon sacrifice of the mice, and sera were frozen at −20°C. Relative titers of *P. carinii*-specific IgG, IgG1, IgG2a, IgG2b, or IgG3 in sera were determined by use of a modification of an ELISA as described previously (10). Flat-bottom microtitration plates (Flow Laboratories, McLean, Va.) were coated with a crude preparation of *P. carinii* antigens prepared by sonicating homogenized *P. carinii*-infected C.B17 SCID mouse lungs, removing particulate material by centrifugation at 14,000 × *g*, and passing the clarified supernatant through a 0.45-μm-pore-size filter. Plates were blocked with 5% nonfat dry milk in phosphate-buffered saline, and serum samples, diluted 1:100, were incubated in the wells overnight at 4°C. After the plates were washed, the IgG subclass recognition pattern of the *P. carinii*-specific Ab response was determined by adding rabbit anti-mouse IgG subclass-specific antisera (Bionetics, Charleston, S.C.) diluted 1:10,000 to the plates for 3 h at 37°C followed by goat anti-rabbit Ig conjugated to alkaline phosphatase diluted 1:5,000 (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) for an additional 3 h at 37°C. The plates were developed with *p*-nitrophenylphosphate as a substrate and read at 405 nm. This assay was standardized and validated with *P. carinii*-specific MAb of known class and subclass. MAb were isolated from the spleens or lymph nodes of mice after infection or immunization with *P. carinii*. The isotype of the individual *P. carinii*-specific MAb (90-3-2B5 [IgG1], 95-1-1G12 [IgG2b], and 96-1-F1[IgG2a]) was determined by use of Ouchterlony immunodiffusion against three rabbit anti-mouse subclass-specific MAb (Accurate Chemical and Scientific Corporation, Westbury, N.Y.). The specificity of the ELISA was determined by testing MAb and polyclonal Ab on sonicates prepared from C.B17 mouse lungs not infected with *P. carinii*. Sera from experimental mice did not bind above background levels with sonicates from uninfected C.B17 lungs (data not shown).

Statistics. Data are expressed as the means ± standard deviations (SDs) for seven to eight mice per experimental group and are representative of two separate experiments. Differences between experimental groups were determined by the Kruskall-Wallis nonparametric test followed by Dunn's post hoc test where appropriate. Differences were considered significant when P was <0.05.

TABLE 1. Lung lavage cell counts 21 days after rechallenge in T-cell-depleted mice

Exptl mouse group	Total no. of lung lavage cells (10^5)[a]		
	PMN	$CD4^+$	$CD8^+$
C57BL/6			
Immunized	0.03 ± 0.04	0.01 ± 0.01	<0.01
Naive	0.02 ± 0.04	<0.01	<0.01
IFN-$\gamma^{-/-}$			
Immunized	6.36 ± 11.65[b]	0.04 ± 0.05	0.15 ± 0.09
Naive	2.10 ± 2.53	0.01 ± 0.01	0.03 ± 0.03
IL-$4^{-/-}$			
Immunized	0.02 ± 0.03	<0.01	<0.01
Naive	0.08 ± 0.04	<0.01	<0.01

[a] All data are the means ± SDs for five to seven mice per group. No deaths occurred in any of the experimental groups.

[b] PMN (neutrophils and eosinophils) numbers for the immunized IFN-$\gamma^{-/-}$ mice were significantly different from those values for the C57BL/6 and IL-$4^{-/-}$ mice (both immunized and naive groups; $P < 0.05$). PMN numbers for the immunized IFN-$\gamma^{-/-}$ mice were not different from those values for the naive IFN-$\gamma^{-/-}$ mice.

RESULTS AND DISCUSSION

Resolution of PCP was independent of the presence of IL-4 or IFN-γ. Wild-type C57BL/6, IFN-$\gamma^{-/-}$, and IL-$4^{-/-}$ mice were all immunized with i.t. inoculations of 10^7 *P. carinii* organisms and subsequently depleted of T cells in vivo prior to rechallenge with *P. carinii*. As has been previously reported (10, 11, 20), treatment of mice with intraperitoneal injections of MAb against Thy1.2 and CD4 resulted in depletion of T cells and susceptibility to *P. carinii*. Mice treated with MAb had nearly undetectable numbers of $CD4^+$ and $CD8^+$ cells in their lung lavage fluids 21 days postchallenge (Table 1). Figure 1 shows the lung *P. carinii* burdens of mice from two separate experiments. Depletion of T cells in naive wild-type mice resulted in lung burdens of $\log_{10}$ 6.0 or greater *P. carinii* nuclei 21 days postchallenge (Fig. 1). Depletion of T cells in naive IFN-$\gamma^{-/-}$ or IL-$4^{-/-}$ mice also resulted in the inability of the mice to resolve PCP (Fig. 1). However, wild-type as well as IFN-$\gamma^{-/-}$ and IL-$4^{-/-}$ mice which were immunized with *P. carinii* prior to T-cell depletion were protected from rechallenge (Fig. 1). None of the IL-$4^{-/-}$ mice had detectable *P. carinii* nuclei in the lungs 21 days postchallenge, and one of eight wild-type and three of eight IFN-$\gamma^{-/-}$ mice had only low numbers of *P. carinii* nuclei in the lungs (Fig. 1A). In a second experiment, all of the immunized C57BL/6 and IL-$4^{-/-}$ mice and six of seven IFN-$\gamma^{-/-}$ mice had cleared their lung infections by day 20 postchallenge (Fig. 1B).

As has been reported previously, IFN-$\gamma^{-/-}$ mice were resistant to development of PCP (6). Furthermore, IFN-$\gamma^{-/-}$ mice developed an effective secondary response capable of resolving PCP in the absence of immunocompetent T cells. These data provide further evidence that IFN-γ is not critical for clearance of PCP nor for developing a strong IgG response to *P. carinii*. Interestingly, IL-$4^{-/-}$ mice also effectively resolved their PCP and, in addition, developed a protective IgG response. It should be noted that mice were immunized by challenge with live *P. carinii* organisms and therefore were able to clear both a primary infection in the presence of $CD4^+$ cells and a secondary infection in the absence of $CD4^+$ cells. Clearly, neither IFN-γ nor IL-4 alone is necessary for clearance of a primary *P. carinii* infection; however, specific recognition by $CD4^+$ T cells is required. Furthermore, $CD4^+$ cells are not required for

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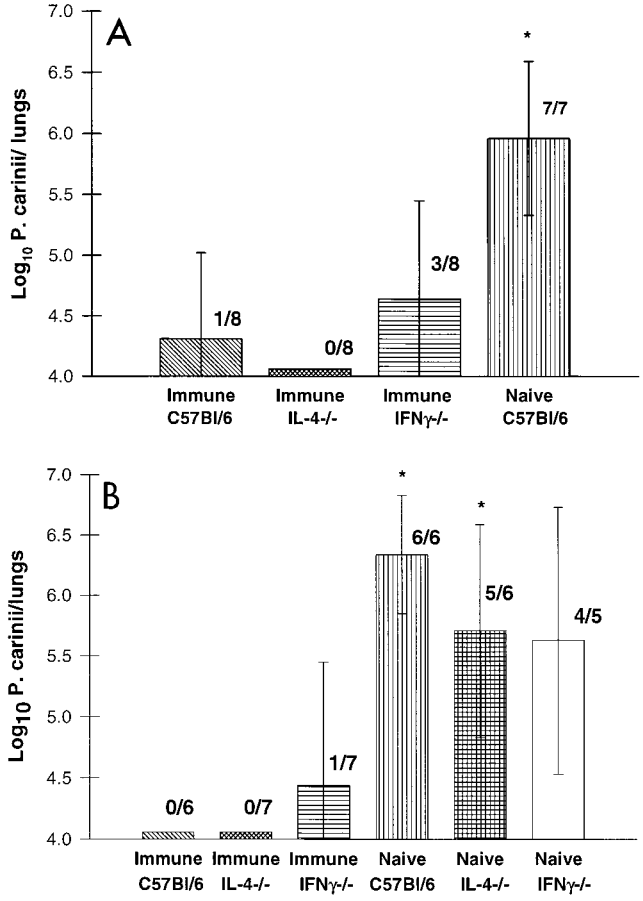

FIG. 1. Lung *P. carinii* burden 21 days postchallenge. Mice were immunized twice with i.t. inoculations of *P. carinii*, depleted of T cells, and challenged with 10^7 *P. carinii* organisms. The *P. carinii* lung burden was determined microscopically at day 21 postchallenge. The limit of detection was $\log_{10}$ 4.06 organisms. Two separate experiments are shown (A and B). Data are expressed as the means ± SDs. The number of mice with detectable *P. carinii* per total number of mice in the group is shown over each bar. *, significantly different from values for immunized groups ($P < 0.05$).

clearance of a secondary *P. carinii* infection in normal, IFN-$\gamma^{-/-}$, or IL-$4^{-/-}$ mice.

Clearance of PCP is independent of the specific Ab isotype profiles produced. To determine whether secondary responses produced by IFN-$\gamma^{-/-}$ or IL-$4^{-/-}$ mice were biased toward Ab isotype production, *P. carinii*-specific ELISAs were performed on sera collected at the time of sacrifice. All immunized mice produced high amounts of *P. carinii*-specific IgG prior to $CD4^+$ T-cell depletion and rechallenge (the optical density [OD] at 405 nm of sera was between 1.0 and 2.5 in immunized mice compared to between 0.04 and 0.09 in unimmunized wild-type, IFN-$\gamma^{-/-}$, and IL-$4^{-/-}$ mice [data not shown]). Naive C57BL/6 and IL-$4^{-/-}$ mice had background levels of *P. carinii*-specific IgG after T-cell depletion and rechallenge (Fig. 2). This corresponded to over log 5.5 *P. carinii* organisms in the lungs (Fig. 1). Two of five naive IFN-$\gamma^{-/-}$ mice had detectable levels of *P. carinii*-specific IgG1 after CD4 T-cell depletion and rechallenge (Fig. 2B). Of these two mice with detectable specific IgG1, one had no detectable *P. carinii* nuclei in the lungs and the other had 10-fold fewer *P. carinii* nuclei than the naive IFN-$\gamma^{-/-}$ mice, with no detectable Ab indicating that *P. carinii*-specific IgG1 was sufficient for reducing lung *P. carinii* burdens. Immunized IFN-$\gamma^{-/-}$ and wild-type mice produced high levels of IgG1, compared to naive C57BL/6; immunized IL-$4^{-/-}$ mice produced very little (i.e., about a 10-fold lower OD than that for immunized IL-$4^{-/-}$ and wild-type mice) (Fig. 2). Conversely, in one experiment, immunized IL-$4^{-/-}$ mice produced significantly more IgG2a, IgG2b, and IgG3 than IFN-$\gamma^{-/-}$ mice (Fig. 2A). In a second experiment, the IL-$4^{-/-}$ mice had OD values of *P. carinii*-specific IgG2a that were two to three times higher than those of the wild-type and IFN-$\gamma^{-/-}$ mice and approximately equivalent levels of IgG2b and IgG3 (Fig. 2B). Importantly, the differences between the patterns of *P. carinii*-specific IgG subclass production for IFN-$\gamma^{-/-}$ and IL-$4^{-/-}$ mice did not affect the ability of mice to resolve *P. carinii* infections.

These data are consistent with previously published reports in which IL-$4^{-/-}$ mice produced primarily IgG2a and little IgG1 in response to *Leishmania major* (16) and cholera toxin (24). However, the *P. carinii*-specific IgG2a in the IL-$4^{-/-}$ mice was not as dominant as might be expected in a biased Th1 response. We have observed previously that *P. carinii*-specific Ab responses are not commonly of the IgG2a subclass (unpublished observations). Additionally, the predominant IgG1 re-

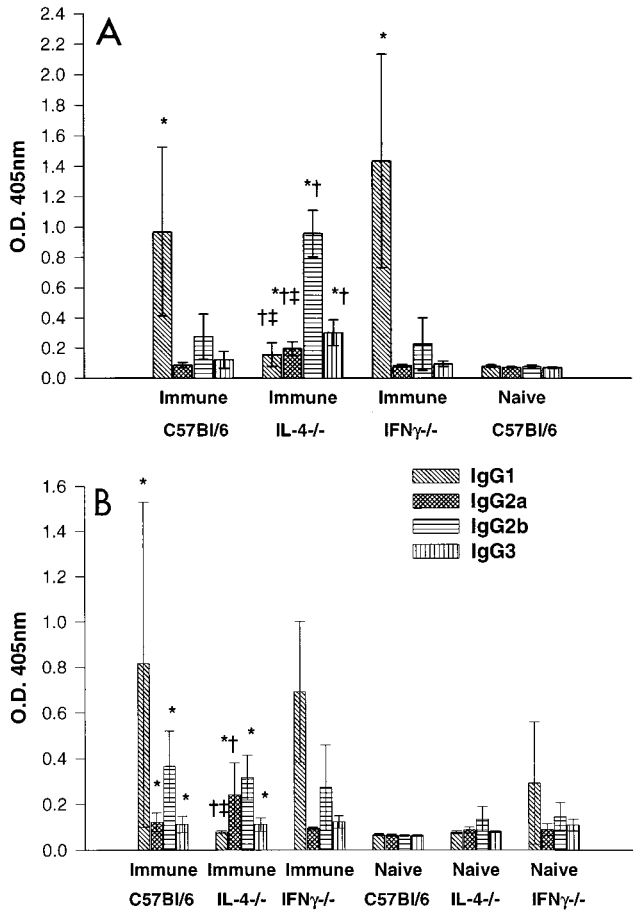

FIG. 2. Relative *P. carinii*-specific serum IgG subclasses 21 days postchallenge. Mice were immunized twice with i.t. inoculations of *P. carinii*, depleted of T cells, and challenged with 10^7 *P. carinii* organisms. Serum collected at time of sacrifice was examined for the presence of *P. carinii*-specific IgG subclasses with an ELISA. Two separate experiments are shown (A and B). Data are expressed as the means ± SDs of OD readings at 405 nm. *, significantly different from values for the same IgG subclass in the naive mice of the same strain ($P < 0.05$); †, significantly different from values for the same IgG subclass in immunized IFN-$\gamma^{-/-}$ mice ($P < 0.05$); ‡, significantly different from values for the same IgG subclass in immunized C57BL/6 mice ($P < 0.05$).

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sponse to *P. carinii* in IFN-$\gamma^{-/-}$ mice is also consistent with published reports in which mice with disrupted IFN-γ receptor genes responded to trinitrophenyl-ovalbumin with a predominantly IgG1 response (12). Notably, the Ab response to *P. carinii* in immunized wild-type C57BL/6 mice was similar to that in the IFN-$\gamma^{-/-}$ mice, suggesting that responses to secondary challenge with *P. carinii* in normal mice are Th2-like. Preliminary data from this laboratory support this contention since lung lavage $CD4^+$ cells produce largely IFN-γ ex vivo after a primary challenge whereas they produce predominantly IL-4 after a secondary challenge (data not shown).

Importantly, these data suggest that there are a variety of *P. carinii*-specific IgG subclasses which are effective in resolving PCP. It has been reported previously by this laboratory that passive transfer of a mouse monoclonal IgG1 specific for gpA, a major *P. carinii* surface glycoprotein, reduced the lung *P. carinii* burden of SCID mice (8). However, IL-$4^{-/-}$ mice resolved their *P. carinii* infections in the absence of $CD4^+$ cells and with background levels of IgG1, indicating that IgG2a, IgG2b, or IgG3 is also effective at clearing *P. carinii*. It is unlikely that IgM or IgA played significant roles in the resolution of PCP since it has been shown that in the presence of *P. carinii*-specific IgM, but not IgG, SCID mice are unable to clear the infection (27). Furthermore, secretory IgA is found in much lower levels in the alveoli than is IgG, suggesting that IgG is more important in host defense in the alveolar spaces of the lungs. It was found that passive immunization of mice with anti-phosphocholine MAb which were of subclass IgG1, IgG2b, or IgG3 gave similar levels of protection against lethal doses of *Streptococcus pneumoniae* (2). However, IgG2a has been found to be most effective in resolving infections such as those with *T. musculi* (23, 26), *C. neoformans* (15, 19), and *Plasmodium chabaudi* AS (28) in mice. In the case of *P. carinii*, it may be unimportant if the Ab present are particularly efficient at complement fixation or binding to Fcγ receptors since killing is most likely mediated through several pathways. This is an important point since it may be possible to immunize at-risk patients against *P. carinii* prior to loss of T-cell function. Results presented here suggest that to accomplish this, it may not be critical to immunize in a way that drives a particular Th-type Ab response to *P. carinii*.

The inflammatory response to *P. carinii* was more intense in IFN-$\gamma^{-/-}$ mice than in IL-$4^{-/-}$ or wild-type mice. Previously, we have reported that IFN-$\gamma^{-/-}$ mice are as efficient as wild-type mice at resolving PCP; however, they develop an exacerbated inflammatory response in the lungs (6). To determine whether inflammation in IFN-$\gamma^{-/-}$ mice was also exacerbated during a secondary response to *P. carinii*, lung lavage polymorphonuclear leukocytes (PMN) were enumerated microscopically. Table 1 indicates that PMN numbers were very low in immunized C57BL/6 and IL-$4^{-/-}$ mice; however, there were on average more than 5×10^5 PMN in the lung lavage fluids of IFN-$\gamma^{-/-}$ mice. This corresponded to about three times more cells in the lung lavage fluids and 20% higher lung weights in IFN-$\gamma^{-/-}$ mice than in IL-$4^{-/-}$ or C57BL/6 mice (data not shown). Notably, this increased inflammatory response in IFN-$\gamma^{-/-}$ mice was in the absence of T cells (Table 1). If T cells were the only source of IFN-γ in the secondary inflammatory response, it would have been expected that the T-cell-depleted C57BL/6 and IL-$4^{-/-}$ mice would have had inflammatory responses similar to that of the IFN-$\gamma^{-/-}$ mice. NK cells are also a source of IFN-γ and may have provided the signals to turn off the inflammatory response in the wild-type and IL-$4^{-/-}$ mice. It is also possible that the increased PMN numbers in the lung lavage fluids of IFN-$\gamma^{-/-}$ mice were a result of prolonged inflammation in response to the primary immunizations before T cells were depleted. Unlike the C57BL/6 or IL-$4^{-/-}$ mice, without the presence of IFN-γ, the IFN-$\gamma^{-/-}$ mice may not have been able to down-regulate the inflammatory response once it was initiated.

P. carinii has proven to be a relatively easy pathogen to kill once specific recognition by $CD4^+$ T cells takes place. *P. carinii*-specific B cells or Ab appears capable of resolving PCP in the absence of T cells (reference 10 and data herein), whereas adoptive transfer of *P. carinii*-specific $CD4^+$ T cells is sufficient for resolving PCP in SCID mice (18, 27). As shown by the data presented here, mice are able to resolve PCP in the absence of either IFN-γ or IL-4. Furthermore, once a *P. carinii*-specific secondary Ab response takes place, $CD4^+$ T cells are no longer required for resolution. It is also not critical which Th-like IgG subclasses are predominantly produced for resolution of PCP to take place. Hyperimmunization prior to loss of function of $CD4^+$ cells in human immunodeficiency virus-positive persons or other at-risk populations may be a viable strategy for protecting these individuals against PCP.

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