IFN-γ and CD8$^+$ T Cells Restore Host Defenses Against *Pneumocystis carinii* in Mice Depleted of CD4$^+$ T Cells[1]

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Host defenses against infection are profoundly compromised in HIV-infected hosts due to progressive depletion of CD4$^+$ T lymphocytes and defective cell-mediated immunity. Although recent advances in antiretroviral therapy can dramatically lower HIV viral load, blood CD4$^+$ T lymphocytes are not restored to normal levels. Therefore, we investigated mechanisms of host defense other than those involving CD4$^+$ T lymphocytes against a common HIV-related opportunistic infection, *Pneumocystis carinii* (PC) pneumonia. Using CD4-depleted mice, which are permissive for chronic PC infection, we show that up-regulation of murine IFN-γ by gene transfer into the lung tissue results in clearance of PC from the lungs in the absence of CD4$^+$ lymphocytes. This resolution of infection was associated with a >4-fold increase in recruited CD8$^+$ T lymphocytes and NK cells into the lungs. The role of CD8$^+$ T cells as effector cells in this model was further confirmed by a lack of an effect of IFN-γ gene transfer in *scid* mice or mice depleted of both CD4$^+$ and CD8$^+$ T cells. Cytokine mRNA analysis revealed that recruited, lung-derived CD8$^+$ T cells had greater expression of IFN-γ message in animals treated with the IFN-γ gene. These results indicate that CD8$^+$ T cells are capable of clearing PC pneumonia in the absence of CD4$^+$ T cells and that this host defense function of CD8$^+$ T cells, as well as their cytokine repertoire, can be up-regulated through cytokine gene transfer. ***The Journal of Immunology,* 1999, 162: 2890–2894.**

Despite current strategies to treat HIV infection and its complications, *Pneumocystis carinii* (PC)[3] pneumonia remains a common clinical problem. Although there is a clear relationship between CD4$^+$ lymphocyte count and the risk of PC infection (1, 2), the role of mononuclear phagocytes, CD8$^+$ cells, NK cells, and their secreted cytokines in host defense against this infection is far less clear. Since it remains unclear whether highly active antiretroviral therapy will result in long-term immune reconstitution of patients with AIDS (3, 4), understanding non-CD4$^+$ T cell-dependent host defense mechanisms operative in opportunistic infections may be critical. Among CD4$^+$ T cell-derived cytokines, IFN-γ is likely to play a key role in host defense against PC infection. Lymphocytes exposed to PC organisms (5) or the major surface glycoprotein of PC (6) in vitro elaborate IFN-γ and lymphocytes recovered from HIV-infected individuals are deficient in IFN-γ production (7). Studies in IFN-γ receptor knockout mice demonstrate that these mice are not permissive to passive acquisition of PC (8). Moreover, studies in IFN-γ knockout mice (9), or in *scid* mice reconstituted with splenocytes from IFN-γ knockout mice (10), demonstrate that IFN-γ is not essential for clearance of PC. However, previous studies from our group have demonstrated that aerosolized, rIFN-γ can ameliorate PC infection in mice depleted of CD4$^+$ T lymphocytes (11). Based on these data, we postulated that in mice depleted of CD4$^+$ T cells using a mAb, overexpression of IFN-γ in the lung might substitute for CD4$^+$ T lymphocytes and mediate clearance of PC pneumonia.

Materials and Methods

Adenoviral construction

To selectively overexpress IFN-γ in the lung, we constructed an E1-deleted, replication-deficient adenovirus encoding the murine IFN-γ cDNA (AdIFN) driven by the CMV immediate early promoter (12). The murine IFN-γ cDNA (obtained from Dr. Opdenakker, Rega Institute of Catholieke University, Leuven, Belgium) was subcloned into pACCMV.PLA (obtained from Robert Gerard, University of Texas Southwestern Medical Center, Dallas, TX). AdIFN was generated by cotransfecting with pJM17 (obtained from Frank L. Graham, McMaster University, Hamilton, Ontario, Canada) into 293 cells. Isolated plaques were propagated in 293 cells, and viral DNA was screened by Southern blot analysis. Production of secreted IFN-γ by individual clones and in bronchoalveolar lavage fluid (BALF) was measured by ELISA (Genzyme, Cambridge, MA) with a sensitivity of 5 pg/ml (13). AdLuc (provided by Robert Gerard), which encodes the firefly luciferase gene in the E1 region, was used as a control vector. Both viruses were propagated in 293 cells, purified by ultracentrifugation over a CsCl gradient, and titered by a plaque assay on 293 cells as described previously (14). Viral stocks contained <1 replication-competent adenovirus per 10^7 plaque-forming units (PFU) (as determined by a lack of cytopathic effect on A549 cells, at a multiplicity of infection of 10). The particle:PFU ratio was <100:1, and virus stocks contained <0.01 ng/ml of endotoxin as determined by the QCL-1000 *Limulus* lysate assay (BioWhittaker, Walkersville, MD).

PC Inoculation

The PC inoculum was prepared as previously described (15). Briefly, athymic mice with PC pneumonia were injected with a lethal dose of pentobarbital, and the lungs were aseptically removed and frozen for 30 min in 1 ml of PBS at −70°C. Frozen lungs were homogenized in 10 ml of PBS (Model 80 Stomacher; Tekmar Instruments, Cincinnati, OH), filtered through sterile gauze, and pelleted at 500 × *g* for 10 min at 4°C. The pellet

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[3] Abbreviations used in this paper: PC, *Pneumocystis carinii*; PFU, plaque-forming unit; BALF, bronchoalveolar lavage fluid.

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was resuspended in PBS, and a 1:4 dilution was stained with modified Giemsa stain (Diff-Quik; Baxter, McGaw Park, IL). The number of PC cysts was quantified microscopically (15), and the inoculum concentration was adjusted to 2×10^6 cysts/ml. Gram stains were performed on the inoculum to exclude contamination with bacteria.

Monoclonal Abs

Anti-CD4 Ab was prepared as previously described (15). Briefly, the hybridoma GK1.5, which produces a rat IgG_{2b} Mab against murine CD4 (16), was obtained from the American Type Culture Collection (Manassas, VA). Harvesting of the Ab as ascites from pristane-primed, uninfected athymic mice took place in the Monoclonal Ab Core Laboratory facility at Louisiana State University Medical Center (LSUMC). The Ab was precipitated with an equal volume of saturated ammonium sulfate and dialyzed against PBS overnight, and the IgG content was quantitated by cellulose acetate electrophoresis and densitometry. The Ab was stored at −70°C until use. All lots of Ab contained <0.01 ng/ml of endotoxin as determined by the QCL-1000 *Limulus* lysate assay. Heat denaturation of the Ab ablates its CD4-depleting capacity as well as its ability to modify lung host defenses (17). YTS169.4, a depleting rat anti-CD8 mAb, was provided by Dr. Jim Beck (University of Michigan, Ann Arbor, MI) (18).

Adenoviral gene transfer in PC inoculation

Recipient male, 7- to 8-wk-old BALB/c or *scid* mice were purchased from Hilltop Laboratories (Gilroy, CA) and housed in pathogen-free conditions in the LSUMC vivarium. BALB/c mice were treated with 0.3 mg of rat IgG or a depleting anti-CD4 Ab (GK1.5, ATCC no. TIB 207) i.p. and then randomized to receive 10^9 PFU of AdIFN or AdLuc intratracheally 3 days later. Three days after adenovirus administration, animals were challenged with 2×10^5 PC cysts intratracheally. Mice continued to receive rat IgG or GK1.5 weekly until sacrifice. We have previously shown that in BALB/c mice, weekly GK1.5 maintains a continued state of >97% CD4-depletion in blood and lymphoid tissue for up to 14 wk (15). Mice were sacrificed at serial intervals up to 6 wk. *Scid* mice received identical gene transfer and PC inoculation and were sacrificed at 6 wk. For double depletion experiments, 7- to 8-wk-old BALB/c mice received 0.3 mg of GK1.5 and 0.5 mg of YTS169.4 or an equivalent amount of rat IgG i.p. 3 days before adenoviral-mediated gene transfer. Three days after adenovirus administration, animals were challenged with 2×10^5 PC cysts intratracheally. Mice continued to receive rat IgG or GK1.5/YTS 169.4 weekly until sacrifice at 6 wk. Our laboratory has previously demonstrated that coadministration of these Abs results in >95% depletion in both $CD4^+$ and $CD8^+$ T lymphocytes for at least 6 wk (18).

Histology

After a lethal dose of pentobarbital, mice were exsanguinated by aortic transection. The trachea was exposed through a midline incision and cannulated with a polyethylene catheter. The lungs were fixed by administration of 10% neutral formalin to 20 cm H_20. Paraffin-embedded sections were stained with hematoxylin and eosin or Gomori-methenamine silver (GMS) and scored blindly for alveolar and perivascular inflammation and PC infection score, respectively, as previously described (19–21). Briefly, tissue blocks were sectioned at 5-μM thickness and stained with GMS. The extent of infection with PC was scored using a semiquantitative scale ranging from 0 (no visible infection) to 4 (cysts throughout most alveolar regions). This method has been shown to correlate in a double-blind fashion with organism counts from homogenized lung tissue (18).

FACS analysis

At the time of sacrifice, cells from BALF were recovered by centrifugation at $500 \times g$, washed with sterile PBS, and resuspended at 10^6 cells/ml. Cells (50,000) were used for cytospins and stained with Diff-Quik. BALF cells were seeded at a density of 450,000 cells per well in a Limbro/TiterTek microtiter plate (Flow Laboratories, McLean, VA). Cells were incubated with PBS or stained with FITC anti-mouse CD3e (145-2C11), phycoerythrin (PE) anti-mouse CD4 (RM4-4), PE-anti rat CD8a (53-6.7), or respective isotype controls (all Abs from PharMingen, San Diego, CA). NK cells were detected by staining with Dx-5 (PharMingen, San Diego, CA). A minimum of 5,000 double-labeled cells were analyzed on an EPICS elite cell sorter (Coulter, Hialeah, FL). Nonlymphocyte cells were omitted from analysis by forward-angle scatter.

Cytokine mRNA analysis in $CD8^+$ T cells by cDNA-equalized RT-PCR

$CD8^+$ T cells were purified from both BALF and hilar lymph nodes at both 14 and 28 days after PC inoculation using murine-specific CD8 Dynabeads (Dynal, Lake Success, NY). Briefly, lavaged cells or cells derived from hilar lymph nodes were pooled from three animals in each group, washed, counted, and then incubated with the correct number of Dynabeads, following the manufacturer's protocol. Cells and beads were mixed at 4°C for 20 min. Tubes were placed in a Dynal magnetic particle concentrator magnet for 2 min, and the supernatant was removed by pipetting. Remaining cells and beads were washed twice more with RPMI 1640 and recovered by centrifugation. Beads were detached by resuspending the beads in detachment buffer (2 parts human serum + 2 parts 2% EDTA + 1 part RPMI 1640) and gently shaking for 20 min at room temperature. Detached cells were recovered after placing the tube in the magnet for 1 min. The purity of the $CD8^+$ T cell preparation was >98% from both BALF and node specimens as measured by flow cytometry.

Transcripts for IFN-γ, IL-2, IL-4, IL-5, and β-actin were measured in these cell preparations by semiquantitative RT-PCR analysis as previously described (22, 23). Briefly, RNA was isolated by lysing 10^6 cells in Tri-zol (Life Technologies/BRL, Gaithersburg, MD) following the manufacturer's protocol. Reverse transcription was conducted in 40-μl reactions with a final concentration of 500 pM random hexamer as primer, 1 mM dATP-dGTP-dTTP, and 0.03 mM dCTP (all from Pharmacia), 1× PCR buffer (50 mM KCl, 25 mM $MgCl_2$, and 10 mM Tris (pH 8.3)), 40 U RNasin (Promega, Madison, WI), 1 mM DTT, and 400 units Moloney murine leukemia virus-reverse transcriptase. cDNA synthesis was quantified by electrophoresing a 3-uL aliquot on a 6% polyacrylamide gel and exposing the gel on a PhosphorImager screen (Molecular Dynamics, Mountain View, CA). Synthesized cDNA mass was calculated using ImageQuant software (Molecular Dynamics) as previously described (22) and further assessed by the ability to amplify β-actin by PCR using actin-specific primers. After this analysis, equivalent masses of cDNA were analyzed by PCR using gene specific primers (IFN-γ-A, 5′-AGCGGCTGACTGAACTCAGATTGTAG-3′; IFN-γ-B, 5′-GTCACAGTTTTCAGCTGTATAGGG-3′; IL-2-A, 5′-TGATGGACCTACAGGAGCTCCTGAG-3′; IL-2-B, 5′-GAGTCAAATCCAGAACATGCCGCAG-3′; IL-4-A, 5′-CGAAGAACACCACAGAGAGTGAGCT-3′; IL-4-B, 5′-GACTCATTCATGGTGCAGCTTATCG-3′; IL-5-A, 5′-ATGACTGTGCCTCTGTGCCTGGAGC-3′; IL-5-B, 5′-CTGTTTTTCCTGGAGTAAACTGGGG-3′; β-actin-A, 5′-TGGAATCCTGTGGCATCCATGAAAC-3′; β-actin-B, 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′). β-actin was coamplified in a parallel reaction to control for PCR efficiency and cDNA integrity. After PCR, a 10-μl aliquot of the reaction was electrophoresed on a 6% denaturing polyacrylamide gel. The gel was dried, exposed, and analyzed on a PhosphorImager. Bands representing the cytokine genes of interest were quantitated and normalized to β-actin expression.

Statistical analysis

Data were analyzed using StatView Software (Carlsbad, CA). Differences between means were analyzed by analysis of variance with Fisher's follow-up testing.

Results

Construction and pharmacokinetics of AdIFN

To selectively overexpress IFN-γ in the lung, we constructed an E1-deleted, replication-deficient adenovirus encoding the murine IFN-γ cDNA (AdIFN) driven by the CMV-immediate early promoter (12). When given intratracheally, this vector resulted in dose-dependent levels of IFN-γ in the BALF of rats (12) and mice. Pilot experiments revealed that intratracheal inoculation of 10^9 or 10^8 PFU of AdIFN into 6- to 8-wk-old BALB/c mice ($n = 4$/dose) resulted in peak levels of 1400 ± 436 or 256 ± 39 pg/ml of IFN-γ in BALF 3 days after gene transfer. No IFN-γ was detected in the BALF of mice receiving vehicle or equal doses of AdLuc (data not shown). However, the 10^9 PFU dose resulted in the strongest induction of class II MHC (Ia) expression on alveolar macrophages, and thus, this dose was used for all subsequent experiments. BALB/c mice were randomized to receive 10^9 PFU of AdIFN or AdLuc followed by PC inoculation 3 days after gene transfer. Expression of IFN-γ was determined by ELISA in the BALF in both $CD4^+$ and $CD4^-$ mice on days 4, 7, 14, and 28 after vector administration. AdIFN-transduced animals had almost two orders of magnitude greater IFN-γ levels in the BALF at day 4, 7, and 14 (Fig. 1). As we have previously observed (23), the expression of IFN-γ was prolonged in CD4-depleted mice, presumably due to a

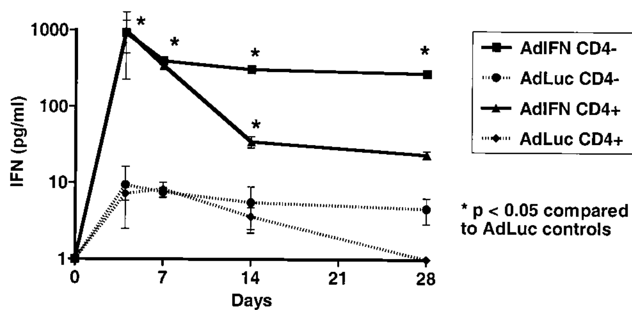

FIGURE 1. Male, 6- to 8-wk-old BALB/c mice were transduced with 10^9 PFU of AdIFN or AdLuc. Three days later, all mice were inoculated intratracheally with PC, and mice were sacrificed at serial intervals. AdIFN resulted in significantly higher IFN-γ levels in both $CD4^+$ and CD4-depleted mice and more sustained levels in CD4-depleted mice, compared with AdLuc-transduced controls (n = 4–6). This is a representative experiment of three experiments. Data are mean ± SEM.

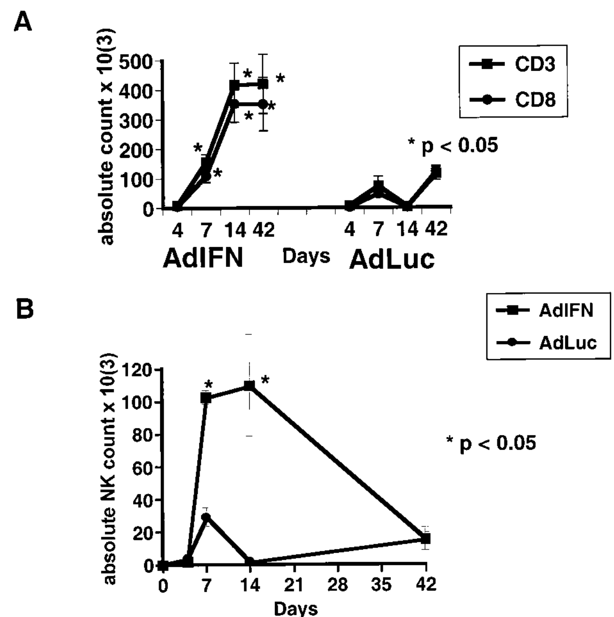

FIGURE 3. *A*, BALF $CD3^+$ and $CD8^+$ T cell populations. CD4-depleted mice were transduced with AdIFN or AdLuc and then challenged with PC as described in *Materials and Methods*. At serial intervals, $CD3^+$, $CD4^+$, and $CD8^+$ BALF cells were analyzed and counted by flow cytometry. No $CD4^+$ cells were detected in BALF of either AdIFN- or AdLuc-treated animals (data not shown). *B*, BALF NK cell counts in PC pneumonia. NK cell counts were determined in BALF at serial intervals by gating on the lymphocyte population using forward and light scatter and staining for DX-5 (a Pan-NK marker in mice). Data are mean ± SEM. *, statistically significant compared with AdLuc controls (n = 3–4/time point). This is a representative experiment of three experiments.

reduced anti-adenoviral immune response in this group of animals (23, 24). Small amounts of IFN-γ were detected in both $CD4^+$ and CD4-depleted ($CD4^-$) animals transduced with AdLuc (Fig. 1). In $CD4^+$ animals that clear the infection over this time, IFN-γ levels became undetectable by day 28. However, IFN-γ remained elevated in AdLuc-transduced CD4-depleted animals coincident with ongoing PC infection.

Time course of PC infection

To determine whether IFN-γ overexpression enhanced resolution of PC infection, mice were pretreated with 10^9 PFU of AdIFN, AdLuc, or an equal volume of PBS intratracheally. Three days later, all mice were challenged with 2×10^5 PC cysts. Control CD4-depleted mice developed progressive, severe infection after inoculation of PC (Fig. 2, PBS data not shown). In contrast, CD4-depleted mice that received the AdIFN vector followed by inoculation with PC showed moderate levels of PC infection during the first 2 wk, but then subsequently cleared the infection within 4–6 wk (Fig. 2). Both PBS- and AdLuc-treated animals showed similar levels of infection throughout the study (data not shown).

Influx of $CD8^+$ T cells and NK cells

Clearance of infection observed in CD4-depleted, AdIFN-treated mice was associated with significant recruitment into lung of $CD3^+$ T cells, which were principally of the $CD8^+$ phenotype in the BALF. (Fig. 3*A*). Moreover, AdIFN treatment resulted in significant increases in recruited NK cells in BALF of CD4-depleted animals (Fig. 3*B*). Approximately 45% of these NK cells were $CD3^+$, which explains, in part, the discrepancy between the total $CD3^+$ and $CD8^+$ population (Fig. 3*A*). Increases in T cells and NK cells in the BALF paralleled peribronchiolar and perivascular accumulations of lymphocytes as determined by blindly scored hematoxylin and eosin-stained lung sections, as previously described (Refs. 15, 20, 25, and data not shown).

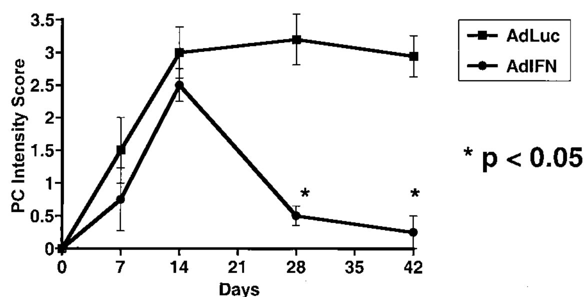

FIGURE 2. AdIFN-mediated clearance of PC. Male BALB/c mice were CD4-depleted by i.p. administration of GK1.5 followed by transduction with 10^9 PFU of AdIFN or AdLuc. Three days later, all mice were challenged with 2×10^5 PC cysts and sacrificed at serial intervals for organism burden (n = 6–8/time point). This is a representative experiment of three experiments. Data are mean ± SEM.

Studies in scid *mice; double-depletion of $CD4^+$ and $CD8^+$ T cells*

To determine whether recruited $CD8^+$ T cells associated with AdIFN were effector cells in AdIFN-induced clearance of PC, we investigated AdIFN in 6- to 8-wk-old, male *scid* mice, which lack both $CD4^+$ and $CD8^+$ T cells, or mice depleted of both $CD4^+$ and $CD8^+$ T cells (using mAbs). Mice were pretreated with 10^9 PFU of AdIFN, AdLuc, or PBS 3 days before PC challenge. Mice were analyzed for intensity of PC infection at 6 wk. In both *scid* and double-depleted mice, AdIFN did not enhance the clearance of PC pneumonia (Fig. 4, *A* and *B*), despite persistent expression of IFN-γ in the BALF at the 6-wk sacrifice point (data not shown).

Cytokine profiles in lung $CD8^+$ T cells

To investigate whether AdIFN was influencing the lung/cytokine microenvironment, $CD8^+$ T cells were purified from BALF and hilar lymph nodes at 14 and 28 days after PC inoculation and analyzed for cytokine mRNA expression. We focused our analysis on the Tc1 cytokines IL-2 and IFN-γ, and the Tc2 cytokines IL-4 and IL-5. Using cDNA-equalized PCR, we observed a significant

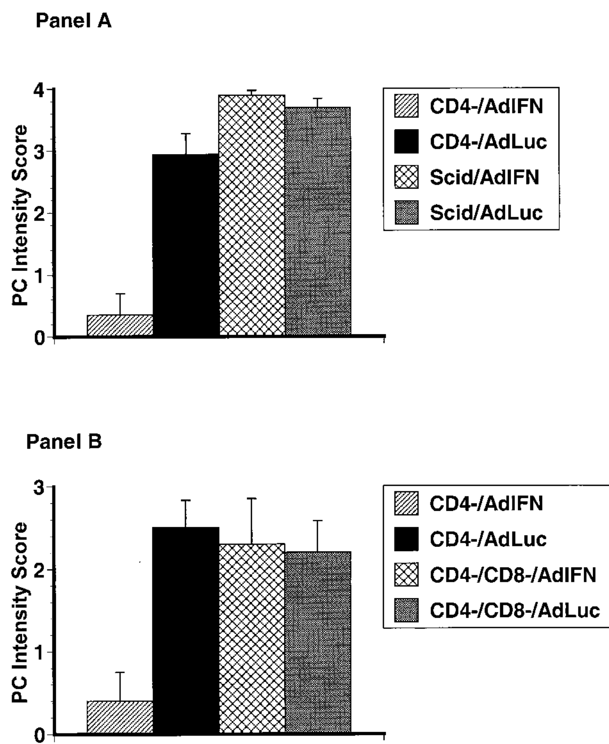

FIGURE 4. Lack of an effect of AdIFN in *scid* or CD4/CD8-depleted mice. *A*, Male, 7- to 8-wk-old CD4-depleted mice or *scid* mice on a BALB/c background were transduced with AdIFN or AdLuc followed by PC inoculation. Mice were sacrificed at 6 wk, and organism burden was quantified as outlined in *Materials and Methods*. Data are mean ± SEM. (n = 6–8). *B*, Male, 7- to 8-wk-old CD4-depleted mice or CD4/CD8-depleted mice were transduced with AdIFN or AdLuc followed by PC inoculation. Mice were sacrificed at 6 wk, and organism burden was quantified as outlined in *Materials and Methods*. Data are mean ± SEM. (n = 6–8). This is a representative experiment of two experiments.

increase in IFN-γ mRNA in purified CD8$^+$ T cells in AdIFN-treated, CD4-depleted mice at both 14 and 28 days after PC infection (Fig. 5). No IL-2, IL-4, or IL-5 mRNA expression was detected in AdIFN-treated or control groups at either of these two time points.

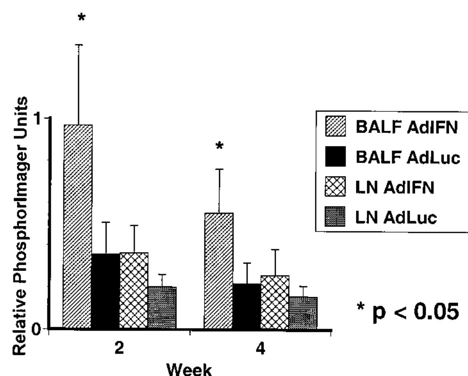

FIGURE 5. Up-regulation of IFN-γ mRNA in lung CD8$^+$ T cells by AdIFN. CD8$^+$ T cells were purified from BALF and hilar lymph nodes 2 and 4 wk after transduction with AdIFN or AdLuc. Expression of IFN-γ mRNA was performed using cDNA-equalized RT-PCR. Data are expressed as relative PhosphorImager units and are from two groups of pooled mice (n = 3 per group). This is a representative experiment of two experiments.

Discussion

CD8$^+$ T cells are part of the inflammatory response to PC in patients and experimental animals (26–28). Our laboratory has demonstrated that, in normal mice, there is an influx of both CD4$^+$ and CD8$^+$ T cells, which parallels clearance of PC (26). Moreover, resolution of PC pneumonia in corticosteroid-treated rats, where the steroid therapy is withdrawn, is associated with recruitment of both CD4$^+$ and CD8$^+$ T cells into the lung (27). In CD4-depleted mice, the predominant mononuclear cell in the lung is the CD8$^+$ T cell (26). Despite an influx of these cells, CD4-depleted mice develop progressive PC infection (26). However, administration of both a depleting anti-CD4 and anti-CD8 Ab results in more intense PC infection, suggesting that CD8$^+$ T cells are important in host defense against PC (18).

Our results described in this paper suggest that CD8$^+$ T cells can be driven by the tissue cytokine milieu to migrate into infected lung tissue and become effector cells in PC pneumonia. We observed significantly higher levels of IFN-γ mRNA in CD8$^+$ T cells from AdIFN-treated mice at both 2 and 4 wk after challenge with PC. This is not likely due to adenoviral transduction of these cells, as these cells are not present in the lungs of mice at the time of administration of the adenovirus. Moreover, PCR analysis for CMV promoter sequences in these cells was negative (data not shown). The effector systems utilized by CD8$^+$ T cells in our model remain unclear. Putative effector mechanisms of CD8$^+$ T cells include cytokine elaboration such as TNF-α, TNF-β, or IFN-γ (29, 30) and cytotoxic effector molecules, such as perforin or granzyme (30). CD8$^+$ T cells have recently been shown to have cytokine patterns analogous to Th1 and Th2 CD4$^+$ T cells, termed Tc1 and Tc2 (29, 30). It has also been demonstrated that naïve CD8$^+$ T cells can be induced into a Tc1 phenotype by IFN-γ (29). Thus, we postulate that overexpression of IFN-γ in both normal and CD4-depleted mice causes naïve CD8$^+$ T cells in lung tissue to adopt a Tc1-like cytokine profile, expressing IL-2 and IFN-γ, and that these cells are effector cells in vivo against PC infection. Although we did not detect IL-2 mRNA in analysis of CD8$^+$ T cells at 2 and 4 wk after PC inoculation, IL-2 may only be expressed early at the time of initial Ag presentation (31, 32). Thus, we are in the process of phenotyping cytokine profiles in CD8$^+$ T cells at earlier time points in this model.

Possible cytotoxic mechanisms include elaboration of perforin or granzyme (33, 34) or an effect of CD8$^+$ cells on other effector cells, such as macrophages, which have been recently shown to be required for host defense against PC (35). IFN-γ increases TNF production by rat alveolar macrophages exposed to PC in vitro (36). Moreover, we have previously shown that in vivo neutralization of both TNF and lymphotoxin abrogates host defense against PC in normal mice as well as CD4-depleted mice (25). Thus, these two mediators could be involved in the beneficial results we observed in this model. However, we saw no difference in peak TNF levels in lung lavage fluid between AdIFN and control animals 3 h after PC challenge, which is the time of peak alveolar macrophage release of TNF in this model (Ref. 37, and data not shown). Recently, the roles of granzyme B and perforin have been investigated in another pulmonary pathogen in a mouse model of tuberculosis (38). Although CD8$^+$ T cells are critical to contain growth of *Mycobacterium tuberculosis* in the mouse lung (39), growth was not affected in mice deficient in perforin or granzyme B (38). An important difference, however, is that host defense in *M. tuberculosis* relies on granuloma formation (40, 41), which is not observed in PC pneumonia. While CD8$^+$ T cells are required, the clearance of PC by IFN-γ may be through a combined

CD8$^+$/NK cell mechanism. Reconstituted *scid* mice with homozygous disruption of the IFN-γ gene ultimately clear an acute challenge with PC (10). Moreover, mice lacking the IFN-γ receptor are not permissive to passive acquisition of PC (8). Thus, IFN-γ itself does not appear to be critical for organism clearance in reconstituted *scid* or CD4-replete mice. In a recent study by Rudmann et al. (9), mice lacking both TNF receptor genes (TNFRI and TNFRII), as well as the IFN-γ gene, developed severe PC infection despite the presence of CD4$^+$ T lymphocytes. Taken together, these data suggest that IFN-γ is not essential for host defenses against PC but is part of a cytokine response, which is critical for optimal host defenses. Moreover, our data suggest that IFN-γ can be used pharmacologically to increase host defenses and experimental animals lacking CD4$^+$ T lymphocytes. Our data are also in agreement with a prior study that demonstrated efficacy of rIFN-γ in an aerosol form, delivered to mice with established PC infection (11). However, the mechanism through which IFN-γ works when delivered as an aerosol has not been demonstrated. In this study, lung inflammation was reduced in the IFN-γ-treated group (11), however, it remains unclear if this was secondary to a decrease in organism burden at the end of the course of IFN-γ.

In conclusion, the combination of IFN-γ and CD8$^+$ T cells can effectively eradicate PC pneumonia even in the absence of host CD4$^+$ T cells. These data not only have implications for novel treatment strategies for PC pneumonia but also may apply to other opportunistic infections in HIV-infected hosts.

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