

Interleukin-13 Fusion Cytotoxin Arrests *Schistosoma mansoni* Egg-Induced Pulmonary Granuloma Formation in Mice

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***Schistosoma mansoni* egg-induced lung pathology requires the actions of interleukin (IL)-4 and IL-13. Because receptors for IL-4 and IL-13 share chains, we examined the effect of a fusion protein comprised of IL-13 and *Pseudomonas* exotoxin (IL13-PE) on the development of pulmonary granulomas in mice. At day 8 after an intravenous injection of live *S. mansoni* eggs, whole lung samples from IL13-PE-treated mice exhibited significantly lower IL-4 and IL-13 gene expression, smaller granulomas, decreased collagen levels, and increased IL-13 receptor α 2 gene expression compared to controls. The therapeutic effects of IL13-PE were also observed at day 16 despite the termination of IL13-PE treatment at day 8. These studies demonstrate that targeting IL-4- and IL-13- responsive cells with IL13-PE effectively arrests *S. mansoni* egg granuloma formation. (Am J Pathol 2002, 161:1283–1297)**

IL-4 and IL-13 are a duo of pleiotropic cytokines that exert similar and divergent effects in immune responses.^{1–4} Conserved or overlapping functions of these cytokines include Ig isotype switching to IgE, eosinophilia, regulation of inflammatory responses, and protective immunity against helminth infections.^{3,5} The functions of these cytokines diverge in key areas such as Th2 cell development, tissue fibrosis, and endotoxemia.^{6,7} The complex roles of IL-4 and IL-13 are further compounded by their interaction with an array of cell surface receptors. IL-4 directly binds the IL-4 receptor α (IL-4R α) and IL-13 specifically binds the IL-13R α 1 chain,⁸ but IL-4R α and IL-13R α 1 can form a functional receptor complex that binds both ligands.⁹ IL-13 also binds with 100-fold higher affinity for IL-13R α 2 than IL-13R α 1, but the IL-13R α 2 chain does not seem to signal and may actually function as an *in vivo* inhibitor of IL-13 function.¹⁰ IL-4 and IL-13 receptors are expressed on a variety of immune and

nonimmune cells including B cells, natural killer cells, monocytes, mast cells, endothelial cells, and fibroblasts,^{8,11,12} but naïve and memory T cells do not appear to respond to the extracellular presence of IL-13.^{13,14}

Previous studies have shown that the granulomatous response elicited by *Schistosoma mansoni* via infection^{15–20} after intravenous injection of live *S. mansoni* eggs^{16,21–26} or after intravenous injection of *S. mansoni* egg-antigen-coated Sephadex beads^{27,28} is dependent on the Th2-dominant cellular events mediated by IL-4 and IL-13. These previous findings were derived from studies that used cytokine immunoneutralization, gene-knockout technology, or receptor decoy strategies designed to target IL-4, IL-13, both cytokines, or their receptors. For example, anti-IL-4 treatment markedly inhibited granuloma formation in the lungs of mice intravenously challenged with eggs.²¹ Similarly, IL-4 knockout or deficient ($-/-$) mice have been observed to generate a modestly smaller granulomatous response to intravenously introduced *S. mansoni* eggs compared with appropriate wild-type mice.²² The specific role for IL-13 after *S. mansoni* infection¹⁷ and pulmonary embolization of *S. mansoni* eggs²⁴ was elucidated using a soluble IL-13R α 2-Fc; this IL-13 inhibitor markedly attenuated granuloma development, pulmonary eosinophilia, and pathological hepatic and pulmonary fibrosis. However, the greatest attenuation of the synchronous pulmonary granulomatous response to *S. mansoni* eggs has been observed in signal transducer and activator of transcription (Stat)-6-deficient mice¹⁶ and in IL-4/IL-13 $-/-$ mice.²⁵ Together, these previous studies highlight that IL-4 and IL-13 play significant roles during *S. mansoni*-induced granuloma formation.

Given the exclusive and mutual roles of IL-4 and IL-13 during the granulomatous response to *S. mansoni* eggs, the exploration of therapeutics that targets the action of both cytokines is warranted. In the present study, we examined the effect of IL-13 cytotoxin-PE38QQQR (IL13-PE), which is a chimeric molecule containing human IL-13 and a derivative of *Pseudomonas* exotoxin (BH Joshi and RK Puri, unpublished results)^{29,30} during synchronous *S. mansoni* egg-induced pulmonary granuloma

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formation. We have previously used IL13-PE to successfully reverse all of the Th2-associated features of *Aspergillus fumigatus*-induced allergic airway disease including airway hyperreactivity and airway remodeling.^{31,32} In addition, IL13-PE markedly reduced or abolished the whole lung expression of IL-4R α and IL-13R α 1 during *Aspergillus*-induced allergic airway disease demonstrating that this IL-13 immunotoxin targets cells that are responsive to IL-4 and IL-13.^{31,32} The present study showed that when given immediately after or as late as 8 days after the intravenous injection of live *S. mansoni* eggs, IL13-PE treatment significantly arrested the development of the Th2-mediated pulmonary granulomatous response. Interestingly, the IL13-PE treatment seemed to be mediated through the inhibition of IL-4, IL-13, and a concomitant dramatic increase in IL-13 decoy receptor (ie, IL-13R α 2) expression.

Materials and Methods

S. mansoni Egg Pulmonary Granuloma Model

Primary, synchronous pulmonary granulomas were induced in female, CBA/J mice (6 to 8 weeks old; Jackson Laboratories, Bar Harbor, ME) after the intravenous injection of 3000 live, mature *S. mansoni* eggs. Live *S. mansoni* eggs were derived from Swiss-Webster mice heavily infected with *S. mansoni* (courtesy of Dr. Fred Lewis, Biomedical Research Laboratory, Rockville, MD) according to previously described methods.³³ The primary model of *Schistosoma* egg-induced lung inflammation has been used extensively to characterize the synchronous pulmonary granulomatous response^{34,35} and model the evolution of the Th2 response.³⁶

IL13-PE Treatment during Pulmonary Granulomatous Responses to Embolized *S. mansoni* Eggs

IL13-PE has been previously used *in vivo* to target and kill tumor cells expressing IL-4R α , IL-13R α 1, and IL-13R α 2.^{37,38} We have previously shown that IL13-PE (at a dose of 200 ng/dose) ameliorated chronic fungal asthma in two independent studies.^{31,32} The dosing protocols used in the present study are outlined in Figure 1. In the first protocol, groups of 15 mice received a 200 ng/dose of IL13-PE dissolved in 10 μ l of phosphate-buffered saline (PBS) containing 0.25% human serum albumin, or diluent, via an intranasal bolus starting immediately after and every other day until day 8 after the *S. mansoni* egg challenge. Mice were then examined at days 8 and 16 after the induction of the pulmonary granulomatous response. In the second protocol, groups of 15 mice received a 200 or 1000 ng/dose of IL13-PE, or diluent starting at day 8 and continuing every other day until day 16 after the egg challenge, at which time the whole lungs from each mouse were subjected to lung histological and morphometric analysis (see below). In both protocols, control groups received the appropriate volume of diluent alone throughout the same time course.

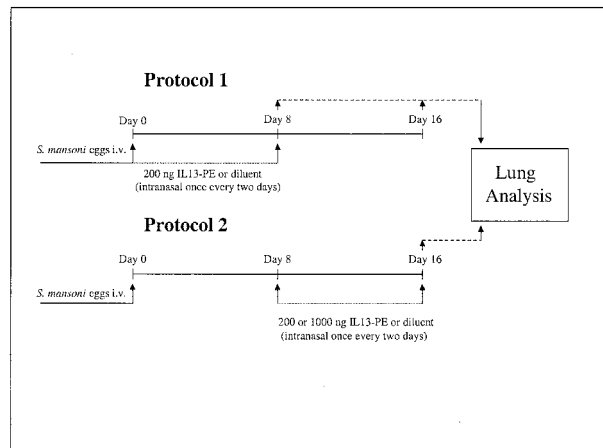


Figure 1. Summary of the treatment protocols used in the present study.

Lung Histological and Morphometric Analysis

Whole lungs from IL13-PE and control groups of mice ($n = 10$ /group) at days 8 and 16 after the *S. mansoni* egg challenge were fully inflated with 10% formalin, dissected, and placed in fresh formalin for 24 hours. Routine histological techniques were used to paraffin-embed the entire lung, and 5- μ m sections of whole lung were stained with hematoxylin and eosin (H&E) or with Masson trichrome. Inflammatory infiltrates and structural alterations were examined around pulmonary granulomas containing a single egg using light microscopy at a magnification of $\times 200$. Morphometric analysis of egg granuloma size and Masson trichrome staining was performed as previously described in detail.³⁹ A minimum of 15 granulomas per lung section was analyzed for granuloma size and collagen deposition. Once the mean granuloma size in each mouse was statistically analyzed, these measurements were then used for the group mean analysis. Granuloma eosinophils were counted in high-powered fields at a magnification of $\times 1000$.

Culture of Dispersed Lung and Spleen Cells

Whole lungs from IL13-PE and control groups of mice ($n = 5$ /group) were removed at days 8 and 16 after *S. mansoni* egg embolization. A portion of the right lobe and the spleen from each mouse was then processed according to methods described in detail elsewhere.⁴⁰ Briefly, lung and spleen were mechanically dissociated by grinding each organ over fine steel mesh, individual cells were quantified using a hemocytometer, and 2×10^6 dispersed cells were added to each well of six-well tissue culture plates (Corning Inc., Corning, NY). Triplicate wells were left unstimulated or exposed to 5 μ g of purified *S. mansoni* egg antigen. Tissue culture plates containing dispersed lung or spleen cells were then maintained in a 37°C incubator for 48 hours, after which time cell-free supernatants were removed for enzyme-linked immunosorbent assay (ELISA) analysis (see below).

ELISA Analysis

Murine RANTES/CCL5, macrophage-derived chemokine (MDC/CCL22), C10 chemokine/CCL6, macrophage inflammatory protein-1 α (MIP-1 α /CCL3), and IL-12 levels were measured in 50- μ l samples from cell-free supernatants using a standardized sandwich ELISA technique previously described in detail.⁴¹ Each ELISA was screened to ensure antibody specificity and recombinant murine cytokines, and chemokines were used to generate the standard curves from which the concentrations present in the samples were derived. The limit of ELISA detection for each cytokine was consistently more than 50 pg/ml. The cytokine levels in each sample were normalized to total protein levels measured using the Bradford assay.

Serum levels of total IgE, IgG1, and IgG2a in the IL13-PE treatment and control groups were analyzed using complementary capture and detection antibody pairs for IgE, IgG1, and IgG2a according to the manufacturer's directions (PharMingen, San Diego, CA). Duplicate sera samples were diluted to 1:100 for IgE determination and 1:1000 for determination of IgG1 and IgG2a levels. Immunoglobulin levels were then calculated from optical density readings at 492 nm, and Ig concentrations were calculated from a standard curve generated using recombinant IgE, IgG1, or IgG2a (all three standard curves ranged from 5 to 2000 pg/ml).

Pulmonary Fibroblast Culture

The left lung from the IL13-PE-treated and control groups ($n = 5$ /group) at days 8 and 16 after the *S. mansoni* egg challenge was prepared as previously described⁴² to obtain pure primary cultures of lung fibroblasts. In brief, the whole lung sample from each group was finely dispersed on steel mesh and the dispersed cells were then placed into 150-cm² cell culture flasks (Corning Inc.). These cultured lung cells were serially passaged a total of five times to yield pure populations of lung fibroblasts.⁴² The purified fibroblasts were then added to 24-well (at 1×10^3 cells/well) or 6-well (at 1×10^5 cells/well) tissue culture plates. To triplicate wells (in the six-well plates) the following was added: medium alone, or medium plus IL13-PE (0, 10, 200, or 1000 ng/ml). To other triplicate wells (in the 24-well plates) the following were added: 10 ng/ml of cytokine [IL-4, interferon (IFN)- γ , IL-10, IL-12, or IL-13] for 48 hours with the addition of 200 ng/ml of IL13-PE at 24 hours. Fibroblast proliferation was assessed in 24-well plates via [³H]thymidine incorporation (10 μ Ci/well) and RNA was isolated from lung fibroblasts in the six-well tissue culture plates.

Preparation of cDNA and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Amplification

Total RNA was prepared from the right lung samples removed from mice in the IL13-PE-treated and control groups ($n = 3$ /group) at days 8 and 16 after the egg challenge, and

from the cultured pulmonary fibroblasts grown from the same groups at day 16 after the *S. mansoni* egg challenge. RNA was isolated using TRIzol Reagent according to the manufacturer's (Life Technologies, Inc., Rockville, MD) directions. The purified RNA was subsequently reverse-transcribed into cDNA using a BRL reverse transcription kit and oligo (dT) 12-18 primers. The amplification buffer contained 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.3, and 2.5 mmol/L MgCl₂. Specific oligonucleotide primers were added (200 ng/sample) to the buffer, along with 1 μ l of reverse-transcribed cDNA sample. The following murine oligonucleotide primers (5' to 3' sequences) were used for RT-PCR analysis: IL-13 receptor α 1 sense, GAATTTGAGCGTCTGTGCGAA; IL-13 receptor α 1 anti-sense, GGTTATGCCAAATGCACTTGAG; IL-13 receptor α 2 sense, ATGGCTTTTGTGCATATCAGAT; IL-13 receptor α 2 anti-sense, CAGGTGTGCTCCATTTCATTCT; procollagen I sense, TCGTGACCGTGACCTTGCG; procollagen I anti-sense, GAGGCACAGACGGCTGAGTAG; procollagen III sense, AGCCACCTTGTCAGTCCTA; procollagen III anti-sense, TTCCTCCCACTCCAGACTTG.

These mixtures were then first incubated for 4 minutes at 94°C and amplified using the following cycling parameters: IL-4R α : procollagen I, and procollagen III cycled 38 times at 94°C for 30 seconds, 55°C for 45 seconds, and elongated at 72°C for 60 seconds; IL-13 α 1R: cycled 38 times at 94°C for 30 seconds, 66°C for 60 seconds, and elongated at 72°C for 60 seconds; and IL-13 α 2R: cycled 38 times at 94°C for 30 seconds, 66°C for 60 seconds, and elongated at 72°C for 60 seconds. After amplification the samples were separated on a 2% agarose gel containing 0.3 μ g/ml of ethidium bromide and bands visualized and photographed using a translucent UV source.

Real-Time TaqMan PCR Analysis

Total RNA was isolated from right lobes of the lung ($n = 5$ /group) and cultured pulmonary fibroblast samples at days 0, 8, and 16 after the *S. mansoni* egg challenge as described above. A total of 0.5 μ g of total RNA was reverse-transcribed to yield cDNA, and IL-13R α 2, IL-4, IL-13, IFN- γ , tumor necrosis factor (TNF)- α , IL-5, MCP-1/CCL2, and MIP-1 α gene expression was analyzed by real-time quantitative RT-PCR procedure using an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). GAPDH was analyzed as an internal control. All primers and probes were purchased from Applied Biosystems. Gene expression was normalized to GAPDH before the fold change in gene expression was calculated. The fold increases in receptor, cytokine, and chemokine gene expression were calculated via the comparison of gene expression of these cytokines and cytokine receptor during the granulomatous response to that detected before the intravenous injection of eggs (ie, T = 0). All mRNA levels before egg embolization were assigned an arbitrary value of 1.

Statistical Analysis

All results are expressed as mean \pm SEM (SE). A one-way analysis of variance and a Dunnett's multiple comparisons test or a Student's *t*-test were used to reveal statistical differences between the control group and the IL13-PE treatment groups before and at days 8 and 16 after the *S. mansoni* egg challenge; *P* < 0.05 was considered statistically significant.

Results

IL13-PE Treatment Arrested *S. mansoni* Egg-Induced Pulmonary Granuloma Formation

Morphometric analysis of pulmonary granulomas area is summarized in Figure 2, A (protocol 1) and B (protocol 2). After treatment according to protocol 1, pulmonary granulomas in IL13-PE-treated mice were significantly (*P* < 0.01) smaller at day 8 (the final day of the 200 ng/dose-IL13-PE treatment) and (*P* < 0.001) at day 16 compared with control-treated mice at the same times after the *S. mansoni* egg challenge (Figure 2A). Specifically, the day 8 and day 16 granulomas were 44% and 67% smaller, respectively, in the IL13-PE treatment groups compared with the control groups at the same time after the egg challenge. Mice treated with IL13-PE according to protocol 2, also exhibited significantly (*P* < 0.01) smaller granulomas, but this effect was only observed in the group of *S. mansoni* egg-challenged mice that received the 1000 ng/dose of IL13-PE every other day from day 8 to day 16 (Figure 2B). Mice that received the higher dose of IL13-PE in protocol 2 had lung granulomas that were 33% smaller than the granulomas in the control group. Thus, IL13-PE treatment using protocol 1 markedly attenuated and appeared to interfere with the ongoing development of pulmonary granulomas around *S. mansoni* ova in naïve mice. Given that IL13-PE seemed to have a greater preventive (ie, protocol 1) than therapeutic (ie, protocol 2) effect on *S. mansoni* egg granuloma development we focused the remainder of our study on protocol 1.

IL13-PE Treatment Significantly Increased Whole Lung Gene Expression of IL-13R α 2

RT-PCR analysis revealed that the expression of IL-13R α 1, a receptor chain that binds both IL-4 and IL-13,⁹ was constitutively present in whole lung samples from naïve mice (Figure 3A). After *S. mansoni* egg embolization, IL-13R α 1 gene expression was marginally increased at days 8 and 16 in the PBS control groups (densitometry analysis is shown in Figure 3B). In contrast, gene expression for IL-13R α 1 gene was not increased at either day after the egg challenge in the IL13-PE-treated groups (Figure 3, A and B). At day 16 after the egg challenge, the level of gene expression for IL-13R α 1 was significantly decreased in the IL13-PE-treated group compared with the control group. Because we found that RT-PCR analysis was not sufficiently sensitive enough to detect IL-

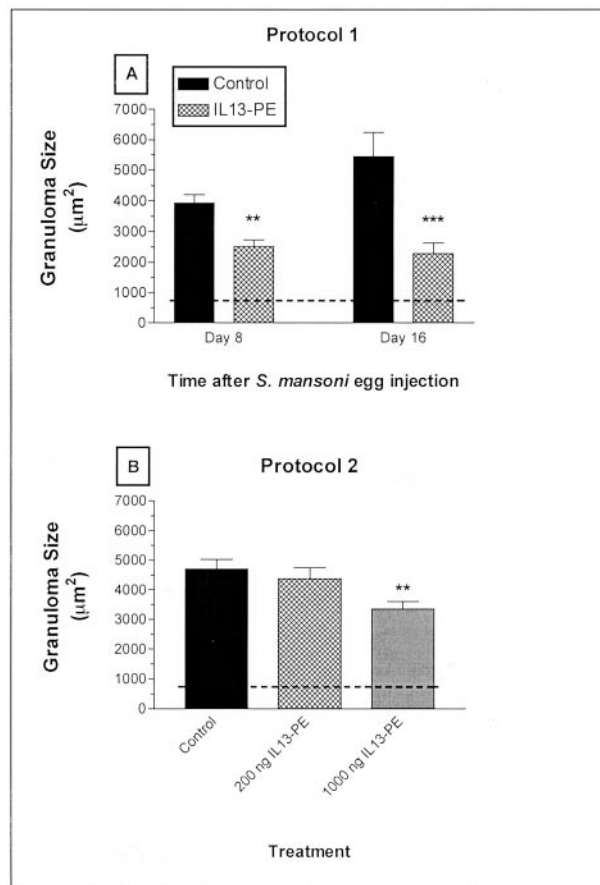


Figure 2. Morphometric analysis of pulmonary granuloma areas. Cohorts of 10 mice/group were injected intravenously with 3000 live *S. mansoni* eggs. **A:** *S. mansoni* egg-challenged mice then received diluent or 200 ng/dose of IL13-PE every other day starting at day 0 and concluding at day 8 after the egg challenge (see protocol 1 in Figure 1). **B:** In other cohorts of *S. mansoni* egg-challenged mice, diluent or IL13-PE was administered once every other day from days 8 to 16 after the egg challenge (see protocol 2 in Figure 1). At least 10 granulomas/mouse were measured per group. The **dashed line** demarcates the average area of an egg. Values are expressed as mean \pm SE; *n* = 5 mice/group. **, *P* \leq 0.01; ***, *P* \leq 0.001 compared with the granuloma size in the control group at the same time after *S. mansoni* egg challenge.

13R α 2 expression in whole lung samples, we used quantitative TaqMan PCR analysis to examine temporal changes in this decoy receptor for IL-13.⁴³ As shown in Figure 3C, whole lung expression of IL-13R α 2 was not altered in the control group during the course of the *S. mansoni* egg challenge in the control group. Conversely, IL-13R α 2 gene expression was increased at day 8 and statistically significantly increased at day 16 when compared to the control group at the same time after the egg embolization. Taken together, these data suggested that IL13-PE targeted cells expressing a receptor that can recognize IL-4 and IL-13 while concomitantly increasing the pulmonary expression of an IL-13-specific decoy receptor.

IL13-PE Treatment Did Not Significantly Reduce Circulating Levels of IgE, IgG1, or IgG2a

Serum levels of total IgE, IgG1, and IgG2a are summarized in Table 1. When administered according to proto-

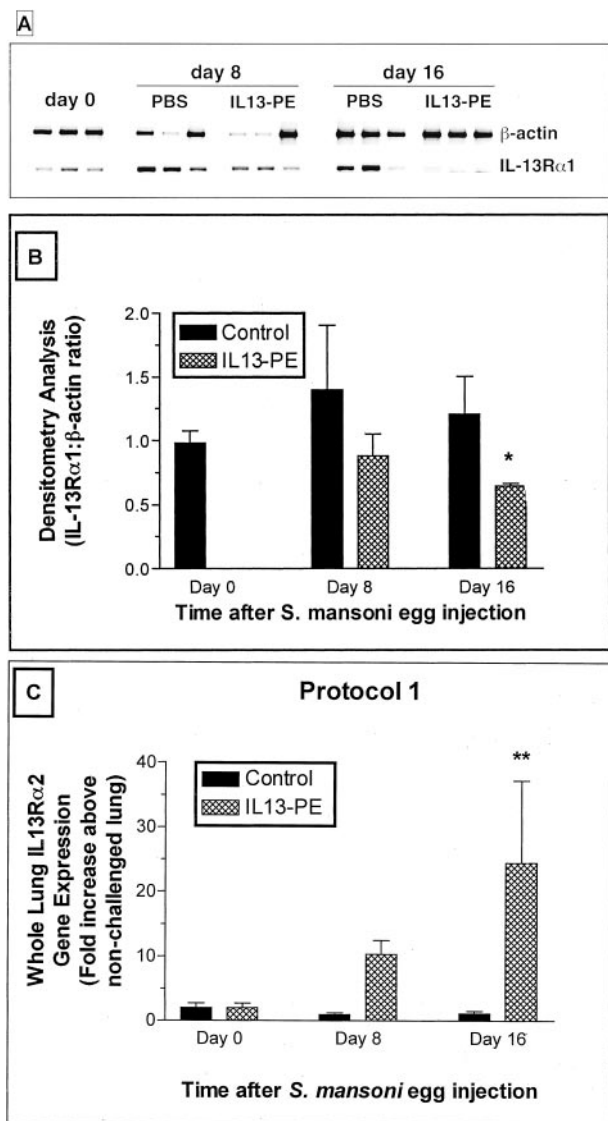


Figure 3. A: Representative RT-PCR analysis of β -actin and IL-13 receptor α 1 (IL-13R α 1) in whole lung samples from *S. mansoni* egg-challenged mice at days 0, 8, and 16 after the egg challenge. *S. mansoni* egg-challenged mice then received diluent or a 200 ng/dose of IL13-PE every other day starting at day 0 and concluding at day 8 after the egg challenge (see protocol 1 in Figure 1). **B:** Densitometry analysis was used to determine the ratio of IL-13R α 1: β -actin mRNA in the treatment groups. These ratios confirmed that the mRNA levels for IL-13R α 1 were significantly reduced in the IL13-PE treatment group compared with the control group (at day 16 after the egg challenge). *, $P \leq 0.05$. Values are expressed as mean \pm SEM; $n = 3$ mice/group. **C:** Because IL-13R α 2 mRNA was expressed in minuscule quantities in the whole lung samples using RT-PCR analysis, we used quantitative TaqMan PCR to monitor changes in IL-13R α 2 expression. Temporal significant changes in IL-13R α 2 gene expression were noted in the IL13-PE-treated groups but not in the control groups after the egg challenge. Values are expressed as mean \pm SE; $n = 5$ mice/group. **, $P \leq 0.01$ compared with IL-13R α 2 gene expression in the control group at the same time after the egg challenge.

col 1, IL13-PE treatment from day 0 to day 8 after *S. mansoni* egg embolization did not significantly affect circulating levels of IgE, IgG1, and IgG2a. Serum levels of IgG1 were significantly increased at day 16 after egg embolization in the IL13-PE-treated group compared with the control group at the same time. Thus, these data suggested that IL13-PE did not specifically target Ig-producing cells during *S. mansoni* egg challenge.

The IL13-PE Treatment Significantly Reduced the Presence of Eosinophils and Collagen in Pulmonary Granulomas

S. mansoni egg granulomas are composed of a number of immune and nonimmune cells including eosinophils, macrophages, lymphocytes, neutrophils, mast cells, and fibroblasts.¹⁵ Given the strong Th2-driven response invoked by the *S. mansoni* egg, eosinophils typically comprise between 50 to 70% of the cells present in the circumoval granulomas.²⁴ Representative H&E-stained histological sections from the control and IL13-PE treatment groups included in protocol 1 are shown in Figure 4 (A and B represent day 8; C and D represent day 16). The average pulmonary granuloma size was markedly reduced at both times after the egg challenge in the IL13-PE treatment groups (Figure 4, B and D, respectively). However, despite the reduced size of granulomas at day 8 in the IL13-PE-treated group, eosinophils were prominent around *S. mansoni* eggs at this time. Quantification of eosinophils around pulmonary granulomas confirmed that similar numbers of eosinophils were present in both groups at day 8, despite the significant differences in the size of the granulomas (Figure 5). The diminution of granuloma size in the IL13-PE-treated groups appeared to be because of the marked absence of mononuclear cells in these lesions. However, significantly fewer eosinophils were detected in pulmonary granulomas from the IL13-PE treatment group at day 16 after the *S. mansoni* ova challenge (Figure 5).

Previous studies have shown that IL-13 is a major mediator of fibroblast activation and tissue fibrosis.^{17,44,45} In the present study, the peribronchial distribution of extracellular matrix and fibroblasts, revealed by the Masson trichrome stain, was pronounced in the granulomas of the control groups at days 8 and 16 after the *S. mansoni* egg challenge (Figure 4, E and G). In contrast, Masson trichrome staining appeared to be much less prominent in granulomas in the IL13-PE treatment groups particularly at day 16 after the egg challenge given the markedly smaller granulomas present in these mice (Figure 4, F and H). RT-PCR confirmation of this observation is shown in Figure 6, A and B, in which the levels of procollagen I mRNA expression were significantly lower in the IL13-PE treatment groups at day 16 after the egg challenge. Taken together, these data suggest that targeting lung cells that recognize IL-4 and IL-13 significantly reduced the degree of lung fibrosis associated with *S. mansoni* egg-induced pulmonary granulomas.

IL13-PE Treatment Prevented the Significant Increase in Whole Lung Gene Expression of IL-13 and IL-4 after *S. mansoni* Egg Challenge

We next examined by TaqMan real-time PCR whether the IL13-PE treatment used in protocol 1 affected the gene expression of IL-13, IL-4, and IFN- γ in whole lung samples at days 0, 8, and 16 after *S. mansoni* egg challenge. At day 8 after the egg challenge in the control group,

Table 1. Serum Levels of IgE, IgG1, and IgG2a at days 0, 8, and 16 after *S. mansoni* egg injection

| Ig isotype ($\mu\text{g/ml}$) | Time after <i>S. mansoni</i> Egg Injection | | | | |
|------------------------------------|--|--------------------|---------------------|---------------------|----------------------|
| | Day 0 | Day 8 (control) | Day 8 (+IL13-PE) | Day 16 (control) | Day 16 (+IL13-PE) |
| IgE | N.D. | N.D. | N.D. | 6.5 \pm 0.2 | 8.3 \pm 1.4 |
| IgG1 | 11.4 \pm 1.8 | 21 \pm 3.9 | 18.3 \pm 3.7 | 16.8 \pm 0.6 | 29 \pm 0.6* |
| IgG2a | 34 \pm 5.8 | 88.7 \pm 15 | 78.2 \pm 2.2 | 69.2 \pm 5.1 | 90 \pm 24 |

N.D., none detected.

* $P \leq 0.05$ compared with serum IgG1 levels measured in the control group at day 16.

Mice were treated with IL13-PE or diluent according to protocol 1. Values are expressed as mean \pm SE; $n = 5$ mice/group.

IL-13 and IL-4 gene expressions were increased 70- and 125-fold, respectively, compared with levels of these genes in whole lung samples at day 0 (Figure 7, A and B). In addition, whole lung gene expression of IL-13 was significantly lower in the IL13-PE-treated group compared with the control group at this time. At day 16 after the egg challenge, IL-4 and IL-13 gene expressions were significantly increased in the control group above levels measured at day 0, and gene expression for both Th2 cytokines was significantly decreased compared with the control group at this time after the egg challenge. Because IL-4/IL-13 $^{-/-}$ mice exhibit a marked increase in IFN- γ levels during intrapulmonary *S. mansoni* egg challenge,²⁵ we determined whether a similar shift in IFN- γ levels occurred in IL13-PE-treated mice. As shown in Figure 7C, significantly ($P = 0.0329$) less whole lung IFN- γ gene expression was observed in the IL13-PE-treated group compared with the control group at day 8 after the egg challenge. At day 16, both groups exhibited similar gene expression for IFN- γ . These data showed that IL13-PE targeted cells that generated IL-13 and IL-4, but this same treatment also negatively affected IFN- γ -expressing cells in the lung during *S. mansoni* egg challenge.

IL13-PE Treatment Prevented the Significant Increase in Whole Lung Gene Expression of TNF- α and MCP-1/CCL2 after S. mansoni Egg Challenge

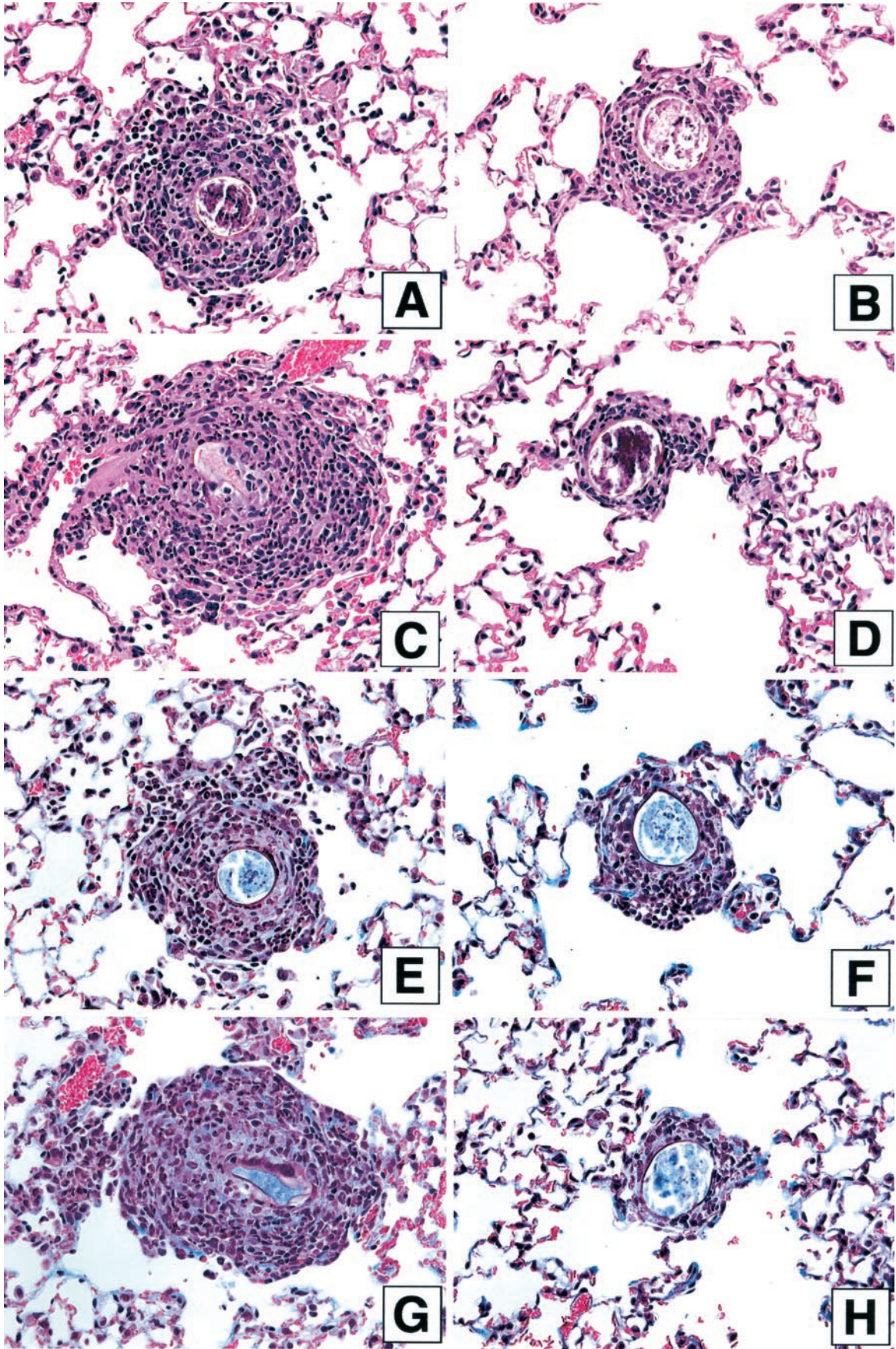
TNF- α , IL-5, MCP-1/CCL2, and MIP-1 α /CCL3 gene expression were examined in whole lung samples using TaqMan real-time PCR (Figure 8; A to D). TNF- α (Figure 8A) and MCP-1/CCL2 (Figure 8C) gene expression was significantly increased in whole lung samples in the control group at day 8 compared with corresponding gene expression at day 0. Similar significant increases in TNF- α and MCP-1/CCL2 were not detected in IL13-PE-treated mice at either day 8 or 16 after the egg challenge. In addition, elevations in gene expression for IL-5 (Figure

8B) and MIP-1 α /CCL3 (Figure 8D) were detected in the control groups at both days after the egg challenge, but these increases were not statistically different from gene expression measured at day 0. Whole lung gene expression for these two mediators was also lower in the IL13-PE-treated groups at days 8 and 16 after *S. mansoni* egg challenge. These data suggested that the IL13-PE treatment also targeted cells with the capacity to generate cytokines and chemokines that have been previously shown to contribute to the granulomatous response to *S. mansoni* eggs.^{35,46}

IL13-PE Treatment Significantly Reduced S. mansoni Antigen-Induced Chemokine Synthesis by Cells Cultured from Whole Lung Samples

A number of previous studies have shown that the granulomatous response to *S. mansoni* eggs and antigens requires the concerted actions of chemotactic cytokines or chemokines.⁴⁷ The analysis of chemokine generation in cultures of dispersed lung cells is summarized in Figure 9. These experiments addressed whether IL13-PE targeted cells in the lung that could respond to *S. mansoni* egg antigen and subsequently generate chemokines. The incubation of dispersed lung cells from control mice at day 8 after egg challenge with *S. mansoni* egg antigen resulted in a significant elevation in RANTES/CCL5 (Figure 9A), MDC/CCL22 (Figure 9B), C10/CCL6 (Figure 9C), and MIP-1 α /CCL6 (Figure 9D) in cell-free supernatants compared with supernatants from similarly challenged and equivalent numbers (2×10^6 cells/well) of dispersed lung cells from naïve mice (ie, day 0). Two million *S. mansoni* egg antigen-challenged lung cells from IL13-PE-treated mice at day 8 after egg challenge did not exhibit similar increases in chemokine generation, and C10/CCL6 and MIP-1 α /CCL3 levels were actually significantly decreased compared with *S. mansoni* egg antigen-challenged lung cells from naïve mice. These data suggested that IL13-PE targeted *S. mansoni* egg antigen-

Figure 4. Representative photomicrographs of H&E-stained (A–D) and Masson trichrome-stained (E–H) whole lung sections at days 8 and 16 after *S. mansoni* egg injection in the control (human serum albumin-PBS vehicle or diluent) and IL13-PE38QQR (IL13-PE) treatment groups. *S. mansoni* egg-challenged mice received diluent or a 200 ng/dose of IL13-PE every other day starting at day 0 and concluding at day 8 after the egg challenge (see protocol 1 in Figure 1). Histological analysis of whole lung samples from both groups at days 8 and 16 after the egg challenge was conducted as described in Materials and Methods. A marked accumulation of inflammatory leukocytes was apparent around egg granulomas at days 8 (A) and 16 (C) in naïve mice that received diluent alone from days 0 to 8 after the egg challenge. In contrast, fewer leukocytes were apparent around egg granulomas in IL13-PE-treated mice at days 8 (B) and 16 (D) corresponding to the smaller sized granulomas measured in these mice (see Figure 3). Collagen (light blue-stained material) was prominent around *S. mansoni* eggs at days 8 (E) and 16 (G) in the control groups. Collagen was also detected around *S. mansoni* eggs in IL13-PE-treated mice at day 8 (F), but markedly less collagen was observed around granulomas in this group at day 16 (H), presumably because of the much smaller size of the granulomas measured in these mice (see Figure 3). Original magnifications, $\times 200$.



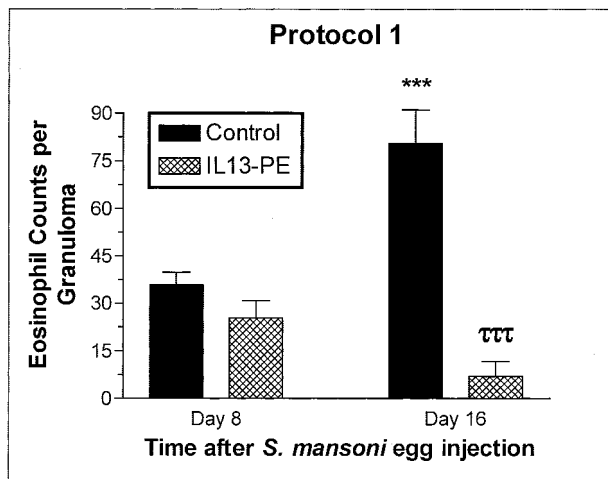


Figure 5. Eosinophil counts in individual granulomas at days 8 and 16 after *S. mansoni* egg injection in the control (human serum albumin-PBS vehicle or diluent) and IL-13-PE38QQR (IL13-PE) treatment groups. *S. mansoni* egg-challenged mice received diluent or a 200 ng/dose of IL13-PE every other day starting at day 0 and concluding at day 8 after the egg challenge (see protocol 1 in Figure 1). Histological analysis of whole lung samples from both groups at days 8 and 16 after the egg challenge was conducted as described in Materials and Methods. Values are expressed as mean \pm SE; $n = 5$ mice/group. ***, $P \leq 0.001$ compared with eosinophils counted at day 8 in the control group. TTT, $P \leq 0.001$ compared with eosinophils counted at day 16 in the control group.

responsive cells typically associated with this pulmonary granulomatous response.

IL13-PE Significantly Increased IL-12 Production by Cells Cultured from Whole Lung and Spleen Samples

Previous studies have shown that IL-12 is a potent inhibitor of the granulomatous and Th2 responses evoked by *S. mansoni*.^{18,48-51} The effect of IL13-PE on IL-12 synthesis by dispersed lung cells and spleen cells is shown in Figure 10, top and bottom panels, respectively. Dispersed lung cells (2×10^6 cells/well) from the control and IL13-PE-treated groups at day 8 after the egg challenge produced significantly higher amounts of IL-12 on *S. mansoni* egg antigen challenge compared with similar number of cells from naïve mice activated in the same manner. However, it was noteworthy that unstimulated cultures of dispersed lung cells from IL13-PE-treated mice also released significantly increased amounts of IL-12 compared with naïve lung cells. The effect of IL13-PE on IL-12 synthesis appeared to be limited to the lung because no significant differences were detected among the spleen cell cultures examined (Figure 10, bottom). These data suggested that IL13-PE significantly altered the generation of IL-12 in the lung, and this effect may have partly accounted for the effect of this chimeric protein on the granulomatous response to *S. mansoni* eggs.

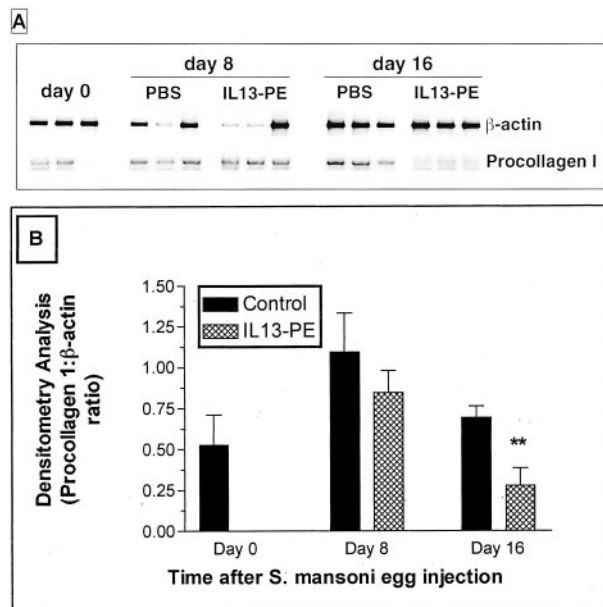


Figure 6. Whole lung procollagen I mRNA expression (A) and percentage of collagen content at days 8 and 16 after *S. mansoni* egg injection in the control (human serum albumin-PBS vehicle or diluent) and IL-13-PE38QQR (IL13-PE) treatment groups (B). Densitometry analysis was used to determine the ratios of procollagen I:β-actin mRNA in the treatment groups. Values are expressed as mean \pm SE; $n = 3$ mice/group. **, $P \leq 0.01$ compared with the densitometry analysis of the control group at day 16 after the egg challenge.

IL13-PE Inhibits Proliferation and Procollagen III Expression in Cultured Pulmonary Fibroblasts Derived from *S. mansoni* Egg Granulomas

The direct effect of IL13-PE on cultured pulmonary fibroblast proliferation and receptor expression is summarized in Figure 11. Pulmonary fibroblasts were purified from whole lung samples removed from untreated mice at day 16 after the *S. mansoni* egg embolization. These cultured pulmonary fibroblasts exhibited aggressive proliferative characteristics, but as shown in Figure 11A the exposure of these cultured fibroblasts to 200 or 1000 ng/ml of IL13-PE significantly reduced their proliferation as determined by [³H]thymidine incorporation. RT-PCR analysis of mRNA from cultured pulmonary fibroblasts after their exposure to IL13-PE revealed that this chimeric protein also significantly reduced the gene expression of procollagen III; the greatest inhibitory effect on procollagen III gene expression was observed when these cells were exposed to 1000 ng/ml of IL13-PE (Figure 11B). Thus, these findings demonstrated that IL13-PE targeted fibroblasts and markedly inhibited the proliferative and procollagen III synthesis responses by these cells.

IL13-PE Induces IL-13Rα2 Gene Expression in Cultured Pulmonary Fibroblasts Derived from *S. mansoni* Egg Granulomas

The intriguing finding that IL13-PE treatment significantly increased IL-13Rα2 expression in whole lung samples led us to examine whether this effect was related to

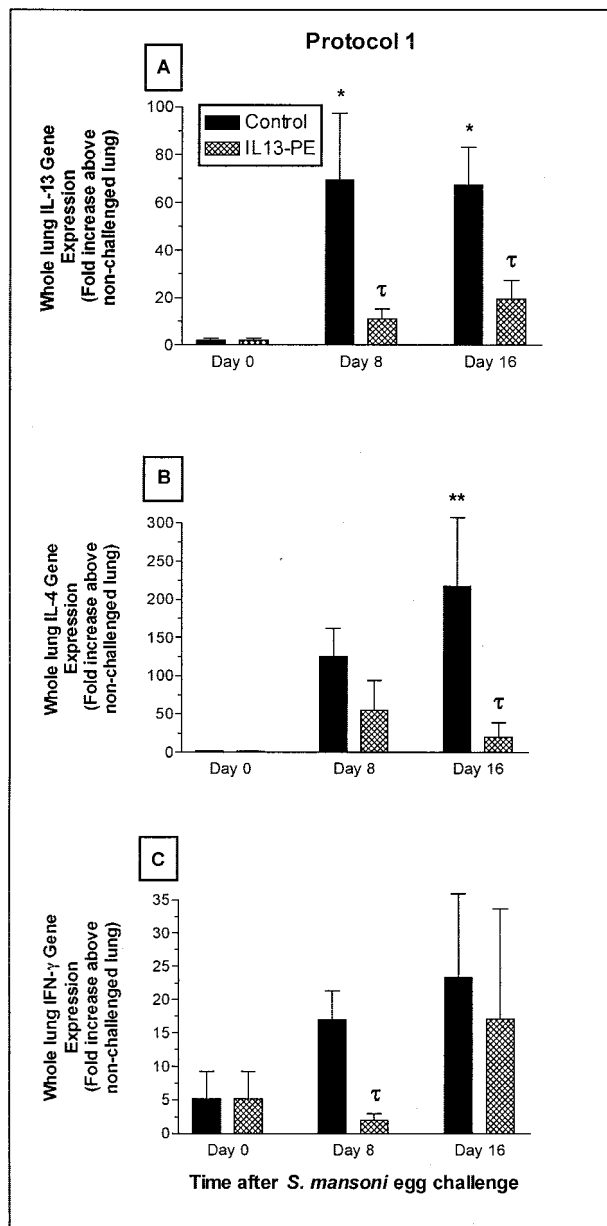


Figure 7. Quantitative TaqMan PCR analysis of whole lung gene expression of IL-13 (A), IL-4 (B), and IFN- γ (C) at days 0, 8, and 16 after *S. mansoni* egg challenge in naïve mice. RNA was isolated from whole lung samples and cytokine gene expression was quantified as described in Materials and Methods. Values are expressed as mean \pm SE; $n = 5$ mice/group. *, $P \leq 0.05$; **, $P \leq 0.01$ compared with cytokine gene expression at day 0. τ , $P \leq 0.05$ compared with cytokine gene expression in the control group at day 8 or day 16 after the egg challenge.

changes in pulmonary fibroblasts. We therefore examined the effect of IL13-PE on the proliferation of cultured lung fibroblasts and the ability of these cells to express IL-13R α 2 after their exposure to IL13-PE. Of some surprise was the finding that those fibroblasts that remained after IL13-PE exposure displayed a significant, dose-related increase in IL-13R α 2 gene expression (Figure 12, A and B). This increase may have been related to an effect mediated by IL-13 in this fusion protein considering that recombinant IL-13 was a potent inducer of IL-13R α 2 gene expression in pulmonary fibroblasts when IL13-PE

(200 ng/ml) was present in the culture (Figure 12C). Together, these data provided evidence that IL13-PE targeted pulmonary fibroblasts (presumably accounting for the significant decrease in fibroblast proliferation) and induced IL-13R α 2 gene expression in surviving fibroblasts (presumably accounting for the marked increase in IL-13R α 2 in whole lung samples from IL13-PE-treated mice).

Discussion

Experimental *S. mansoni* egg embolization in the lung initiates a dynamic Th2 cytokine-mediated pathological process that involves the concerted actions of IL-4 and IL-13.³ Previous studies have shown that the simultaneous disruption of IL-4 and IL-13 in mice through a single vector gene targeting strategy abrogates the development of pulmonary granulomas around intravenously injected *S. mansoni* eggs, whereas IL-4 $^{-/-}$ and IL-13 $^{-/-}$ mice retained the ability to mount a limited Th2 response.²⁵ These findings and additional observations in STAT-6 $^{-/-}$ mice¹⁶ were our impetus to explore the effect of a novel chimeric protein, IL13-PE38QQR or IL13-PE, containing human IL-13 and *Pseudomonas* exotoxin on the aggressive granulomatous and Th2 responses driven by intravenously introduced *S. mansoni* eggs.⁵² IL13-PE³⁰ has been previously used to successfully target human cancer cells expressing functional IL-4 and IL-13 receptors *in vitro*^{29,53-55} and *in vivo*.^{37,38,56,57} The utility of this molecule was also recently described in two independent studies of a model of chronic allergic airway disease induced by *A. fumigatus*.^{31,32} Because IL13-PE targeted IL-4R α - and IL-13R α 1-positive cells in the allergic lung^{31,32} and in the lungs of mice challenged with live *S. mansoni* eggs (observed in the present study), this chimeric protein provided an effective treatment for attenuating the granulomatous and Th2 responses invoked by *S. mansoni* eggs. The data shown herein demonstrate that the intranasal administration of IL13-PE, whether given immediately after (according to protocol 1) or at day 8 (according to protocol 2) after *S. mansoni* egg injection, significantly reduced the *S. mansoni* egg-induced granuloma formation. It was apparent that IL13-PE treatment had a pronounced inhibitory effect on the developmental stage of the egg granuloma (ie, from days 0 to 8), and a more modest therapeutic effect in this model once the egg granuloma was established (ie, after day 8). One explanation for the diminished therapeutic effect of IL13-PE may be that this chimeric protein was unable to effectively penetrate and target the leukocytes in the established egg granuloma. Nevertheless, when administered according to protocol 1, IL13-PE significantly reduced most of the major Th2-mediated features of this model including enhanced Th2 cytokine generation, eosinophil persistence in the granuloma, and the fibrotic response around *S. mansoni* eggs. More importantly, these Th2-associated features did not reappear on the cessation of IL13-PE treatment suggesting that IL13-PE effectively targets the effector cells that modulate the ova-induced granulomatous response. Also of major in-

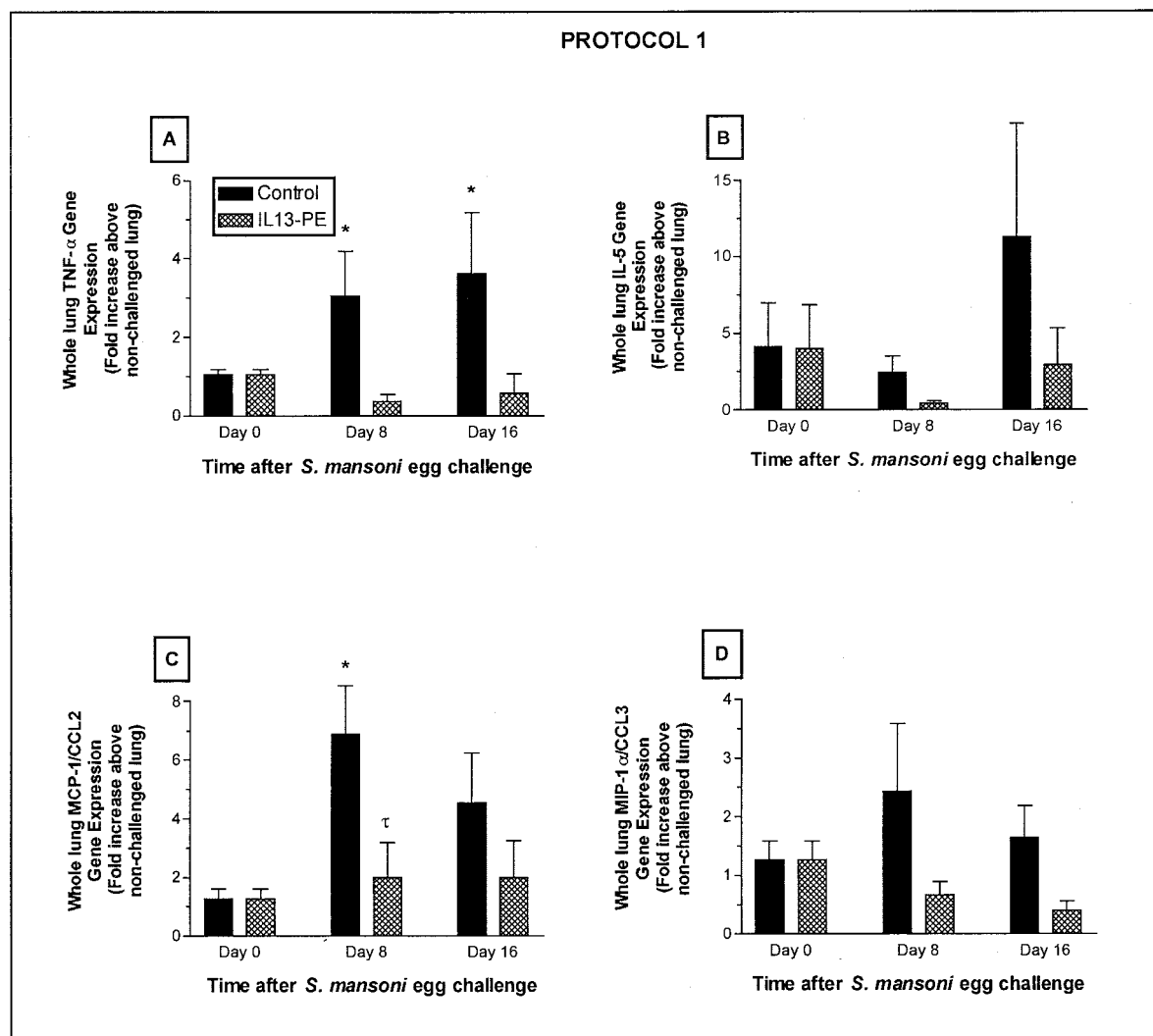


Figure 8. Quantitative TaqMan PCR analysis of whole lung gene expression of TNF- α (A), IL-5 (B), MCP-1/CCL2 (C), and MIP-1 α /CCL3 (D) at days 0, 8, and 16 after *S. mansoni* egg challenge in naïve mice. RNA was isolated from whole lung samples and cytokine gene expression was quantified as described in Materials and Methods. Values are expressed as mean \pm SE; $n = 5$ mice/group. *, $P \leq 0.05$ compared with cytokine gene expression at day 0; τ , $P \leq 0.05$ compared with cytokine gene expression in control group at day 8.

terest, was the finding that IL13-PE up-regulated the expression of the decoy IL-13 receptor (IL-13R α 2) in whole lung samples and in cultured fibroblasts. Although it remains to be confirmed whether the increase in IL-13R α 2 gene expression represents the entire gene or the extracellular domain of IL-13R α 2 chain, these findings demonstrate that IL13-PE not only inhibits the proliferation of IL-13-responsive fibroblasts, it also induces IL-13R α 2 chain on these cells. The IL-13R α 2 chain has been shown to effectively block the effects of IL-13. Taken together these findings demonstrate that IL13-PE significantly attenuates the effects of IL-4 and IL-13 during the immune response to *S. mansoni* ova.

Considerable research attention has been directed at identifying the specific temporal roles of several inflammatory and immune mediators in the development of synchronous *S. mansoni*-induced egg granulomas in the lung. Early studies pointed to distinct roles for arachidonic acid metabolites such as prostaglandins⁵⁸ and leukotrienes⁵⁹ in the pulmonary granulomatous re-

sponse. More recently, attention has turned to cytokines and gene analysis revealed that the introduction of *S. mansoni* eggs into mice initiated a unique pattern of cytokine expression dominated by IFN- γ , IL-1 β , and IL-6 at day 1; IL-2, IL-4, and IL-10 at day 3; and TNF- α and IL-5 at day 6.⁶⁰ When *S. mansoni* ova-challenged mice received either anti-IL-2 or anti-IL-4 antibodies, the size of the egg granulomas were significantly reduced in both groups of treated mice.⁶⁰ Additional studies highlighted that IL-1 β ⁶¹ and TNF- α (via intercellular adhesion molecule-1)⁶² both contribute to the development of *S. mansoni* egg granulomas in the lung. A number of recent studies have addressed the relative importance of the Th2 response to the pulmonary granulomatous response. These studies have revealed that Th2 cells⁶⁰ and eosinophils,⁶³ which generate IL-4, strongly promote the development of granulomas whereas natural killer cells presumably generating IFN- γ and IL-12 suppress the formation of this lesion.⁴⁸ Further studies in cytokine gene-deficient mice outlined the relative importance of

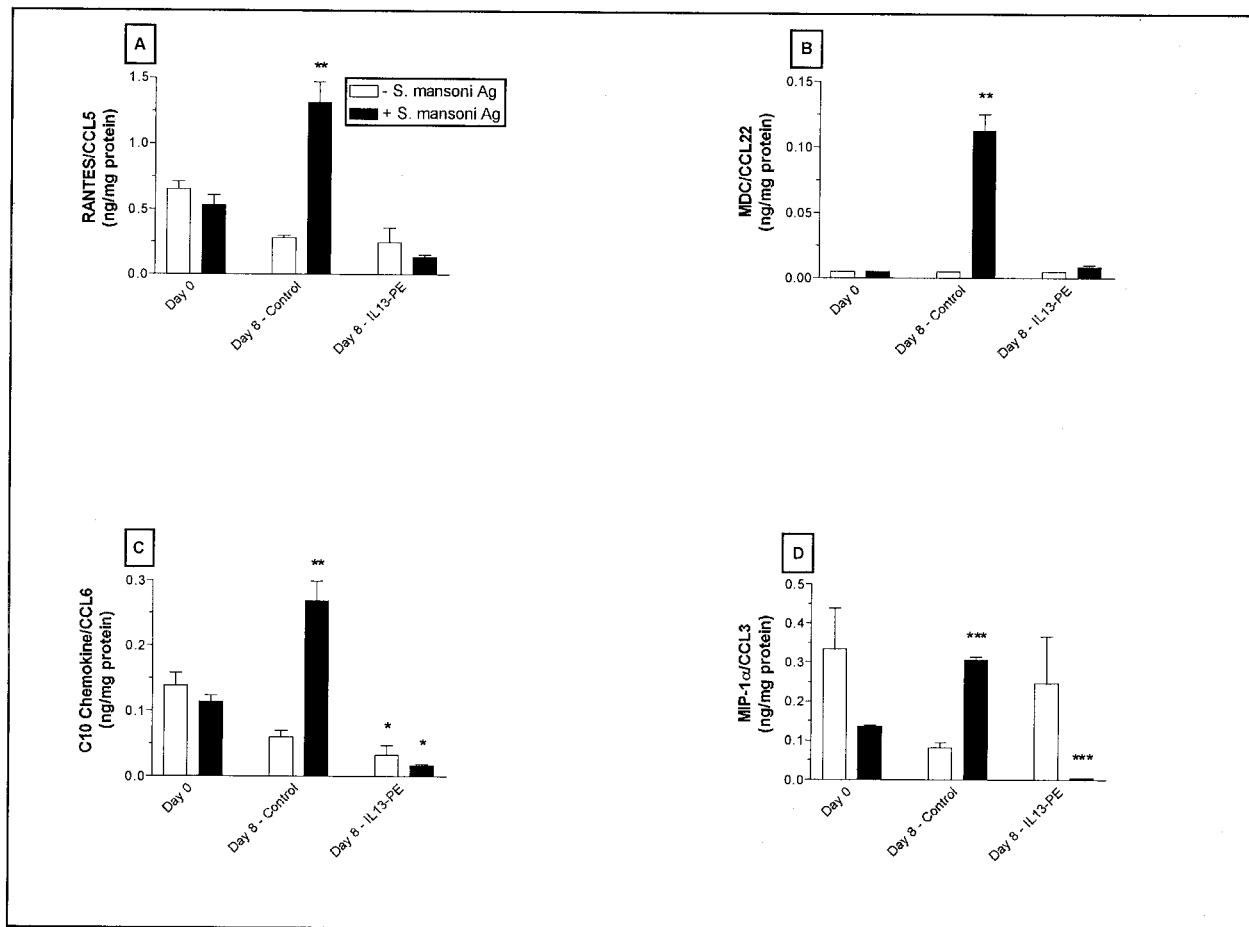


Figure 9. Chemokine generation by cultured lung cells with or without *S. mansoni* egg antigen rechallenge. Lung cells were dispersed from whole lung samples removed at day 0 or day 8 after *S. mansoni* egg challenge, cultured for 48 hours in the presence or absence of *S. mansoni* egg antigen, and cell-free supernatants were removed for ELISA analysis. Values are expressed as mean \pm SE; $n = 5$ mice/group. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ compared with levels measured at day 0 (ie, before *S. mansoni* egg injection).

individual as well as synergistic actions of these cytokines in the pulmonary granulomatous response: IL-10 $^{-/-}$ mice exhibited a mixed Th1 and Th2 response with reduced granulomas; IL-4 $^{-/-}$ mice exhibited diminished Th2 response and modest reductions in granuloma size; IL-4/IL-10 $^{-/-}$ mice exhibited a completely defective pulmonary granuloma formation process and a Th1 cytokine response; IL-12/IL-10 $^{-/-}$ mice exhibited an exaggerated Th2 cytokine response.⁶⁴ The granulomatous and Th2 response to *S. mansoni* eggs has also been shown to be dependent on the contribution of B7-2 (a CD28/CTLA4 ligand),²³ Stat-6 $^{-/-}$,¹⁶ and IL-13.²⁴ Ultimately, it was the finding that IL-4/IL-13 $^{-/-}$ mice fail to develop any evidence of a granulomatous or Th2 response to *S. mansoni* eggs that clearly demonstrated the unequivocal role of both Th2 cytokines in this response.²⁵

Clearly, the data derived from gene knockout mice show that IL-4 and IL-13 act in concert to promote an aggressive response to *S. mansoni* eggs revealing the need to explore therapeutics that target these cytokines or the cells they activate.^{16,25} The recent studies by Chiramonte and colleagues²⁴ also highlighted this fact in showing that a soluble IL-13R α 2-Fc fusion protein administered by intraperitoneal injection significantly reduced

serum IgE levels and the size of egg granulomas, but this soluble IL-13 antagonist only attenuated the Th2 cytokine response, eosinophilia, and the fibrotic response when it was administered to IL-4 $^{-/-}$ mice after *S. mansoni* egg challenge. In the present study, IL13-PE treatment appeared to target or modulate the function of IL-4- and IL-13-responsive cells because IL-13R α 1 gene expression was markedly reduced in mice that received this chimeric protein. At this point the characteristics of the targeted cells are unknown, but considering that IL-4 and IL-13 gene expression were all markedly reduced at days 8 and 16 after the egg challenge it is probable that CD4 $^{+}$ T cells were targeted by IL13-PE. Previous studies have shown that the production of both cytokines is primarily dependent on the presence of CD4 $^{+}$ T cells.²⁴ In addition, we have previously observed that IL13-PE treatment significantly reduced the numbers of CD4 $^{+}$ T cells in the lung during chronic fungal asthma (CM Hogsaboam, unpublished findings). However, other cells that concurrently express IL-4 and/or IL-13 receptors, and generate these cytokines include mast cells, basophils, natural killer cells, B cells, and monocytes.¹⁴ The targeting of these other cells may also explain the reduction in whole lung gene expression of TNF- α , and in chemokines such

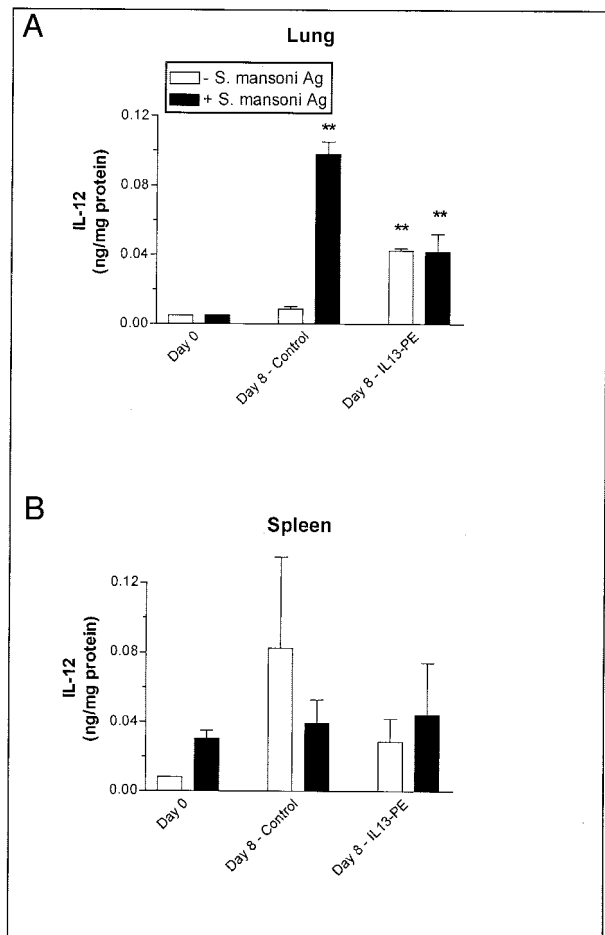


Figure 10. IL-12 levels in cultures of dispersed cells derived from whole lung homogenates (A) and whole spleens (B) at day 8 after *S. mansoni* egg challenge in the control (human serum albumin-PBS vehicle or diluent) and IL-13-PE38QQR (IL13-PE) treatment groups. *S. mansoni* egg-challenged mice received diluent or a 200 ng/dose of IL13-PE every other day starting at day 0 and concluding at day 8 after embolization of eggs (see Materials and Methods). Immunoreactive levels of IL-12 in cell-free supernatants were measured using a specific ELISA as described in the Materials and Methods section. Values are expressed as mean \pm SE; $n = 5$ mice/group. **, $P \leq 0.01$ compared with levels measured at day 0 (ie, before *S. mansoni* egg injection).

as macrophage inflammatory protein-1 α (MIP-1 α /CCL3) and monocyte chemoattractant protein-1 (MCP-1/CCL2). The latter two chemokines contribute to the development of synchronous pulmonary granulomas after *S. mansoni* egg challenge.^{33,65,66} Other deficiencies in chemokine generation were noted in experiments designed to assess the antigen recall of mixed cultures of lung cells. These studies showed that lung cells from day 8 (after egg challenge) lungs exposed to IL13-PE had a markedly reduced capacity to generate RANTES/CCL5, MDC/CCL22, C10/CCL6, and MIP-1 α /CCL3 compared with day 8 lungs cells from the control group. The lack of chemokine generation in the IL13-PE-treated group may explain the diminished number of leukocytes that were present in the granulomas of these treated mice. Specifically, the diminished numbers of eosinophils in the granulomas of IL13-PE-treated mice may have reflected the diminished levels of major eosinophils chemoattractants such as C10/CCL6, RANTES/CCL5, and MIP-1 α /CCL3.⁶⁷

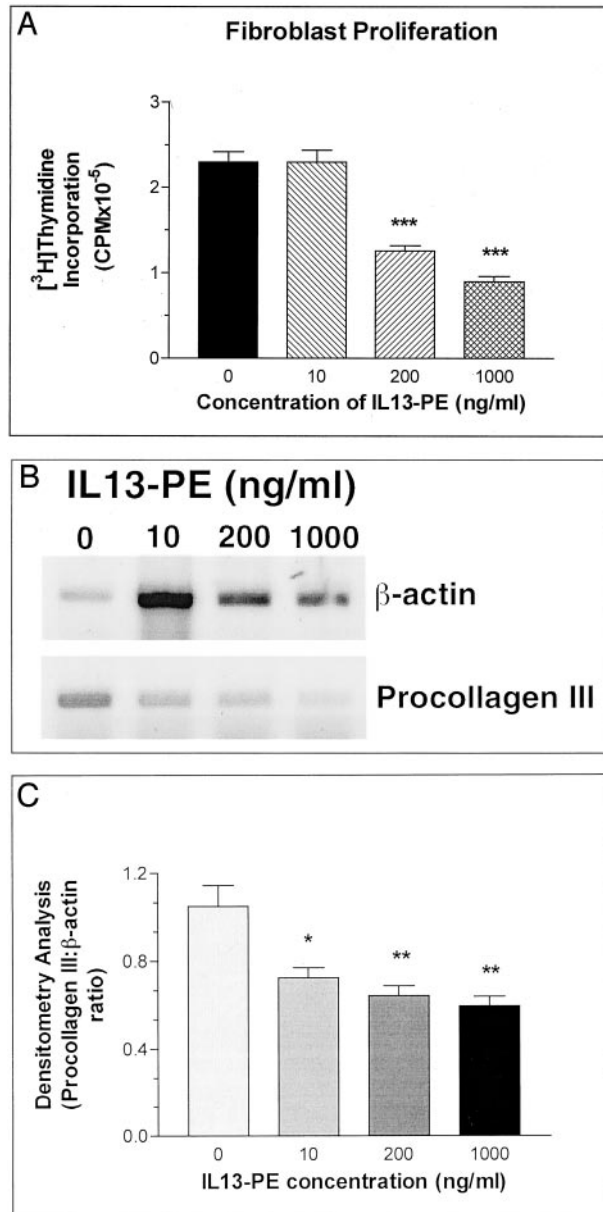


Figure 11. Fibroblast proliferation (A) and procollagen III gene expression (B) in cultured lung fibroblasts. Densitometry analysis was used to determine the ratios of procollagen III: β -actin mRNA in the treatment groups (C). Pulmonary fibroblasts were isolated from untreated mice at day 16 after egg embolization and exposed to increasing (0 to 1000 ng/ml) amounts of IL13-PE for 48 hours. After this time, [3 H]thymidine incorporation was monitored in some cultures whereas others were used to isolate mRNA for RT-PCR analysis. Values are expressed as mean \pm SE; $n = 5$ mice/treatment. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ compared with proliferation measured in control or untreated fibroblasts.

Because naïve Stat-6 $^{-/-}$ ¹⁶ and IL-4/IL-13 $^{-/-}$ ²⁵ mice appear to develop a *S. mansoni* egg antigen-specific Th1 response when intravenously challenged with *S. mansoni* eggs, we examined whether IL13-PE treatment promoted a similar Th1 response. Our previous studies using IL13-PE treatment in a model of chronic fungal asthma showed that targeting IL-4 and IL-13-responsive cells resulted in significantly increased levels of IFN- γ in the lung.³¹ In the present study, examination of whole lung

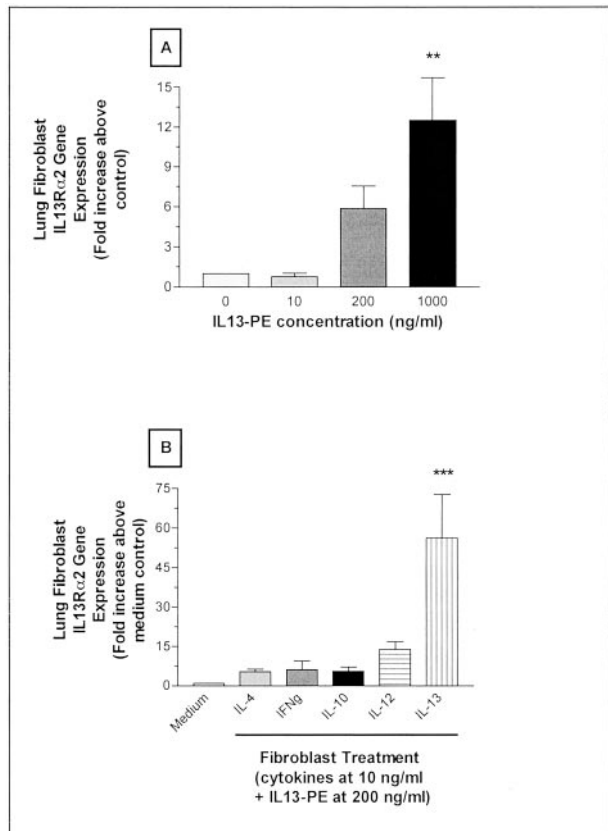


Figure 12. Quantitative TaqMan PCR analysis of IL-13Rα2 gene expression in cultured pulmonary fibroblasts. Pulmonary fibroblasts were cultured and treated as detailed in Materials and Methods. IL13-PE treatment alone induced the expression of IL-13Rα2 in pulmonary fibroblasts. A significant increase in IL-13Rα2 gene expression was observed in pulmonary fibroblasts exposed to 1000 ng/ml of IL13-PE (A). Pulmonary fibroblasts also exhibited a significant increase in IL-13Rα2 gene expression after exposure to IL-13 for 48 hours and the addition of 200 ng/ml of IL13-PE at 24 hours. Values are expressed as mean ± SE. **, $P \leq 0.01$; ***, $P \leq 0.001$ compared with levels measured in cultures of fibroblasts exposed to medium alone.

samples revealed that IFN-γ gene expression levels were significantly decreased (at day 8) by the IL13-PE treatment used in protocol 1. However, analysis of dispersed cells from whole lungs of IL13-PE-treated mice at day 8 after *S. mansoni* egg challenge revealed enhanced *S. mansoni* egg antigen-induced IL-12 production. This finding is particularly interesting in light of the potent down-regulatory effect of IL-12 on the development of *S. mansoni* egg-induced granulomatous responses.^{49,68,69}

However, these findings are somewhat tempered by recent findings from Stat-4-/- mice, which lack IL-12-mediated immune responses,⁷⁰ in which pulmonary granuloma formation was modestly inhibited during *S. mansoni* infection.¹⁶ Regardless of the precise role invoked by Th1-type cytokines during *S. mansoni* egg granuloma formation, it was apparent from the present study that IL13-PE treatment did not appear to promote an aggressive Th1 cytokine response as a result of its diminution of the Th2 cytokine response.

The decrease in procollagen I gene levels in whole lung samples from IL13-PE-treated mice compared with their appropriate controls suggests that this chimeric protein targets the survival and actions of pulmonary fibro-

blasts. Although the fibrotic response was significantly decreased in IL13-PE-treated mice (corresponding to the significantly smaller granulomas in these mice), the fibrotic response did not appear completely inhibited by the targeting of IL-4- and IL-13-responsive lung cells with this chimeric protein. Examination of the relative contribution of collagen to the cross-sectional area of the egg granulomas confirmed that collagen remained a major component of the diminished granuloma in IL13-PE-treated mice. This finding is clearly important in light of the finding that IL-4-/- and IL-4/IL-13-/- mice develop severe liver pathology and die after infection by *S. mansoni* and this susceptibility appeared to be related to the lack of containment of the egg antigens by a fibrotic matrix.²⁰ Thus, any successful treatment directed at IL-4 and IL-13 during a *S. mansoni* egg challenge should limit but not completely inhibit the fibrotic response; IL13-PE appears to meet this criterion in the context of a pulmonary *S. mansoni* egg challenge.

Lung fibroblasts express receptors that recognize IL-4 and IL-13^{12,44,71} and both cytokines promote the proliferation and synthetic activities of these cells. In this study, pulmonary fibroblasts cultured from day 16 lungs (ie, day 16 after egg embolization) exhibited a baseline aggressive proliferative profile. However, IL13-PE at 200 and 1000 ng/ml effectively targeted the proliferation of these cells as evidenced by a decrease in [³H]thymidine incorporation. IL13-PE also effectively inhibited the gene expression of procollagen III, a major procollagen produced by pulmonary fibroblasts during clinical pulmonary fibrosis.⁷² Of particular interest was the observation that IL13-PE induced the expression of IL-13Rα2 in the surviving cells.

In conclusion, the data presented herein demonstrate the beneficial effect of targeting IL-4R- and IL-13R-expressing cells with a chimeric protein comprised of human IL-13 and *Pseudomonas* exotoxin during *S. mansoni* egg-induced pulmonary granuloma formation. IL13-PE effectively arrested the pulmonary granulomatous and the corresponding Th2 response adding further weight to the contention that both IL-4 and IL-13 are important targets in this model. These results also hold promise in the clinical treatment of chronic *S. mansoni* egg pathology with the goal of preventing the development of severe disease.

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