IL-13 Is a Key Regulatory Cytokine for Th2 Cell-Mediated Pulmonary Granuloma Formation and IgE Responses Induced by Schistosoma mansoni Eggs

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IL-13 Is a Key Regulatory Cytokine for Th2 Cell-Mediated Pulmonary Granuloma Formation and IgE Responses Induced by *Schistosoma mansoni* Eggs

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*Schistosoma mansoni* egg-induced pulmonary granuloma formation is a cell-mediated inflammatory response associated with dominant Th2-type cytokine expression, tissue eosinophilia, and high levels of serum IgE. In the present study, we show that in vivo blockade of the Th2 cytokine IL-13, using soluble IL-13R α2-Fc fusion protein, significantly reduced the size of pulmonary granulomas in unsensitized as well as egg-sensitized mice. Blocking IL-13 also significantly reduced total serum IgE levels. Interestingly, however, IL-13 blockade did not affect the evolving egg-induced Th2-type cytokine response. IL-4, IL-5, as well as IL-13 responses were indistinguishable in control-Fc- and soluble IL-13R α2-Fc fusion protein-treated animals. The smaller granulomas were also phenotypically like the control Fc-treated mice, displaying a similar eosinophil content. Additional studies in IL-4-deficient mice demonstrated that IL-13 was produced, but at much lower levels than in wild-type mice, while IL-4 expression was completely independent of IL-13. Moreover, while granuloma formation was partially reduced in IL-4-deficient mice, blocking IL-13 in these animals almost completely abrogated granuloma development and the pulmonary eosinophilia, while it simultaneously increased IFN-γ production. Together, these data demonstrate that IL-13 serves as an important mediator of Th2-mediated inflammation and plays a role in eliciting IgE responses triggered by schistosome eggs. The *Journal of Immunology*, 1999, 162: 920–930.

Among several factors involved in Th2 cell differentiation, IL-4 appears to play a dominant role in the development of this type of CD4+ Th cell (1). Recent studies have focused on identifying the cofactors such as Ag dose (2), APC population (3), costimulatory molecules (4), cytokine milieu (5), as well as the initial source of IL-4 that drives Th2-associated immune responses in infectious and autoimmune diseases (6, 7). Nevertheless, the early events that promote in vivo Th2 cell development and Th2 cytokine responses remain unclear. Moreover, although recent in vitro studies suggest that specific cytokines (5) or distinct cell subsets (8–11) serve as important mediators in Th2 response development, numerous cell and cytokine depletion and knockout studies in several in vivo Th2 models have failed to identify an indispensable cofactor in the development of this type of CD4+ T cell response (12, 13).

In *Schistosoma mansoni*-infected mice, egg-induced granuloma formation has been characterized as a CD4+ T cell-mediated delayed-type hypersensitivity reaction (14, 15) and is associated with a dominant Th2-type cytokine production profile (16, 17). Depletion of the Th2 cytokine IL-4, both in infected mice (18, 19) and in animals i.v. injected with schistosome eggs (20, 21), revealed an important role for the cytokine in driving the egg-induced Th2-mediated inflammatory response, as well as the accompanying rise in IgE Ab levels (18). Nevertheless, in these reports as well as in studies performed recently in IL-4-deficient mice, it has become clear that a reduced but significant Th2-type cytokine response can develop and that marked, albeit diminished, granulomatous inflammation is observed even in the absence of IL-4 (20–24). These findings suggest that the host response to schistosome eggs may not be totally dependent upon IL-4 and that other Th2-associated cytokines, such as IL-13, which is induced by schistosome eggs (25), might serve as important mediators in the generation of egg-induced granulomas.

IL-13 is produced by Th2 cells and is closely related to IL-4 in many activities (26, 27). The nucleotide sequence of murine IL-13 is 30% homologous with IL-4, but has limited homology with any other cytokine (26). Although IL-4 and IL-13 share many of the same biological activities, some studies indicate that there are indeed functional differences between the two cytokines (26). Similar to IL-4, IL-13 inhibits the production of inflammatory cytokines (28, 29) and up-regulates IgE class II and CD23 expression on monocytes/macrophages as well as on B cells (29). The cytokine also induces IgE class switch in human cells in vitro and triggers IgG and IgM synthesis (28, 30–33). Most importantly, however, in contrast to IL-4, IL-13 does not affect resting or activated T cells (26). Because almost all of the above studies were performed using in vitro systems, little is known about the immunoregulatory properties of IL-13 in vivo and whether the cytokine plays any role in the establishment of Th2 responses or in regulating the effector functions typically attributed to Th2 cells.

The aim of this study was to assess the role of IL-13 in the inflammatory response induced by *S. mansoni* eggs. We employed

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to prepare cell suspensions for in vitro culture. Serum was obtained for total IgE and Ag-specific Ab measurements.

Treatments
Animals were treated either with a human control-Fc or with the sIL-13Rα2-Fc by i.p. injection in 0.5 ml PBS. In primary pulmonary granuloma experiments, 200 µg/mouse/day was administered on days −1, 0, 2, 5, 8, and 11 postchallenge. There was no effect observed with the control-Fc alone. In the secondary granuloma experiments, mice were treated every other day during the 2-wk i.p. egg sensitization period, only at the time of challenge, or throughout the period of sensitization and challenge. Either 200, or in some experiments 400 µg/mouse/day was administered. The optimal concentration for in vivo use was based on kinetic assays and on dose-response experiments in mice. The 200 µg dose was chosen as the optimal dose, although 400 and 800 µg doses were equally effective at suppressing granuloma formation. In additional experiments, egg-sensitized mice were treated with mAbs to CD4 or CD8 to deplete CD4+CD8+ T cells at the time of i.v. egg challenge. Mice were injected i.p. with 0.5 mg GL113 (anti-βGal control mAb), 0.5 mg GK1.5 (anti-CD4 mAb), or 0.25 mg clone 2.43 (anti-CD8 mAb) diluted in 0.5 ml PBS on days −4, −1, and +3 post-i.v. egg challenge.

Histopathology
For measurement of granulomas, the left lung was inflated with Bouin-Holland fixative and processed as previously described (18). The size of the pulmonary granulomas was determined in histologic sections stained by Wright’s Giemsa stain (Histopath of America, Clinton, MD). The diameters of each granuloma containing a single egg were measured with an ocular micrometer, and the volume of each granuloma was calculated assuming a spherical shape. Two diameters from each granuloma are used, using the longest and shortest diameter of each lesion for the measurement. An average of 30 granulomas per mouse was included in the analysis. All histologic examinations were scored by the same individual in a blinded fashion to control for consistency. The percentage of eosinophils and other cell types was evaluated in the same sections.

Isolation and purification of RNA
The right lung from each animal was placed in 1 ml of RNA-STAT 60 (Tel-Test, Friendswood, TX), frozen on dry ice, and kept at −70°C until use. Tissues were homogenized using a tissue polymer (Omni International, Waterbury, CT), and total RNA was extracted following the recommendations of the manufacturer. The RNA was resuspended in diethylpyrocarbonate-treated water and quantitated spectrophotometrically.

RT-PCR detection of cytokine mRNA
A RT-PCR procedure was used to determine relative quantities of mRNA for IL-4, IL-5, IFN-γ, IL-1β, TNF-α, and hypoxanthine-guanine phosphoribosyl transferase (HPRT). The cDNA was obtained after reverse transcription of 1 µg of RNA, as described (21). The primers and probes for all genes except CD4 and CD8 were previously published (21, 25). The primers and probes used to amplify CD4 and CD8 were as follows: CD4 upstream primer, 5′-TGT GCC GAG CCA TCT CTC TTA GG-3′; CD4 downstream primer, 5′-GCC AGA TGC TGG CTG G-3′; CD8 upstream primer, 5′-ATG CCA TGG CTC TGG CTG G-3′; CD8 downstream primer, 5′-GCA TCG AGA TGC TGG CGA AC-3′; CD8 probe, 5′-GTC TCT AAC CCC TTG ACA GAG-3′; and CD8 primer, 5′-GTC TCT AAC CCC TTG ACA GAG-3′. The PCR cycles used for each cytokine were as follows: IL-4 (33), IL-5 (31), IFN-γ (29), IL-1β (29), TNF-α (38), CD4 (33), CD8 (33), and HPRT (23).

Analysis and quantification of PCR products
The amplified DNA was analyzed by electrophoresis, Southern blotting, and hybridization with nonradioactive cytokine-specific probes, as previously described (21). The PCR products were detected using a ECL detection system (Amersham). The chemiluminescent signals were quantified using a flat-bed scanner (Microtek model 600 ZS, Torrance, CA). Arbitrary densitometric units were calculated by dividing the cytokine or cell surface marker OD units by the individual HPRT OD units and multiplying the result by 100.

In vitro cultures
Lung-associated lymph node (LALN) cells (thoracic/mediastinal) and spleens were extracted from the mice, and single cell suspensions were prepared. RBC were lysed by osmotic treatment with ACK lysing buffer (Biofluids, Rockville, MD). Cells were placed in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, 100

Materials and Methods

Animals
Six- to eight-week-old female C57BL/6 mice were purchased from the Division of Cancer Treatment, National Cancer Institute (Frederick, MD). IL-4-deficient mice were constructed by gene targeting in embryonic stem cells, as previously described (35), and were generously provided by Dr. Werner Müller (Institute for Genetics, University of Cologne, Germany). The mice were used at the twelfth generation backcross to C57BL/6.

Reagents
sIL-13Rα2-Fc. A pED expression vector containing DNA encoding the murine sIL-13Rα2-Fc extracellular domain, fused in frame to the hinge CH2/CH3 regions of human IgG1, was transfected into CHO cells (36). IL-13Rα2-Fc is the high affinity binding chain for IL-13 and is closely related to the murine low affinity IL-13 binding subunit IL-13Rα1 (34). Transfected CHO cells were grown in serum-free medium, and culture supernatants that contained secreted sIL-13Rα2-Fc were filtered through a Millipore Millidisk and concentrated 20-fold with a Millipore Pellicon 30,000 m.w. cutoff membrane, then adsorbed to recombinant protein A-Sepharose that had been equilibrated with PBS, pH 7.4, washed with PBS, and eluted with 20 mM citrate, 0.2 M NaCl, pH 3. The eluate was neutralized to pH 7.5 with an equal volume of 0.2 M potassium phosphate, pH 8, and was formulated into PBS, pH 7.3, using an Amicon stirred cell with a YM30 membrane. The final product was 95% pure by SDS-PAGE analysis and had a concentration of 3 mg/ml (determined using A280 and a theoretical extinction coefficient of 1.93 ml/mg·cm). Endotoxin contamination was <2 EU/mg, as determined with the Cape Cod Associates limulus amebocyte lysate assay (Woods Hole, MA). The in vitro ID₅₀, as determined by ability to neutralize 3 ng/ml of murine IL-13 in the B9 proliferation assay (37), was approximately 10 ng/ml. Human IgG (control-Fc), which was used as a control for sIL-13Rα2-Fc, was affinity purified by recombinant protein A-Sepharose chromatography, as described for sIL-13Rα2-Fc, from a 10% solution of human Ig that is commercially available for i.v. administration (Miles, Elkhart, IN). The final preparation had an endotoxin concentration of <0.4 EU/mg. Both reagents were provided by Genetics Institute (Cambridge, MA).

Induction of pulmonary granulomas
The induction of synchronous egg-induced granulomas was performed as described previously (21). S. mansoni eggs were separated from the livers of infected mice (Biomedical Research Institute, Rockville, MD) and enriched for mature eggs. To induce primary pulmonary granulomas, mice were injected with 5000 eggs i.v. Secondary granulomas were induced in i.p. egg-sensitized mice (sensitized with 5000 eggs). Mice were sensitized 2 wk before the i.v. egg challenge. Animals were killed on days 3, 6, or 9 postchallenge. At the time of sacrifice, the lungs were removed for histology and RNA extraction. The lymph nodes draining the lung and spleens were removed

Abbreviations used in this paper: sIL-13Rα2-Fc, soluble IL-13 receptor α2-Fc fusion protein; HPRT, hypoxanthine-guanine phosphoribosyl transferase; LALN, lung-associated lymph node; SEA, soluble egg antigen; WT, wild type.
mM streptomycin, 25 mM HEPES, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 50 μM 2-ME at 37°C in 5% CO₂. Cells were plated in 24-well plates (3 × 10⁶/ml) and stimulated with soluble egg Ag (SEA) at 20 μg/ml or with a combination of SEA and 50 μg/ml of anti-CD4 mAb. Supernatants were collected at 72 h, and IL-4 (B) and IL-13 (C) production was measured by ELISA. These experiments were repeated multiple times with similar results.

Serum IgE and Ab isotype analysis

Total IgE in serum was determined using a specific sandwich ELISA (PharMingen, San Diego, CA) and quantified according to a reference IgE standard (anti-trinitrophenol IgE; PharMingen). Plates were coated with anti-mouse IgE capture mAb from clone R35-72 in 0.1 M NaHCO₃, pH 8.2, overnight at 4°C. The secondary mAb was a biotinylated anti-mouse IgE (PharMingen). Plates were coated with 10 μg/ml of SEA (50 μg/ml) diluted in PBS, and serum samples were analyzed using serial twofold dilutions. Second-step horseradish peroxidase-conjugated rabbit anti-mouse IgG, IgG1, IgG2a, and IgG2b Abs (Zymed Laboratories, San Francisco, CA) were employed at a 1/1000 dilution. The absorbance in the wells was read at 405 nm using a Vmax Kinetic Microplate Reader (Molecular Devices, Palo Alto, CA) after adding 100 μl of ABTS:H₂O₂ one-step substrate.

Statistics

Statistical significance was determined by Student’s t test, and p < 0.05 was considered significant.

Results

S. mansoni eggs trigger a robust IL-13 and IL-4 response with similar kinetics in naive and egg-sensitized mice

Initially, we determined to what extent IL-13 and IL-4 were induced during the initiation of an immune response to eggs (primary response) and in animals that had been sensitized to parasite eggs before experimental challenge (secondary response). The production of IL-4 and IL-13 both in vitro and in vivo was directly compared with changes in granuloma development. In these studies, naive or i.p. egg-sensitized mice were injected i.v. with eggs, and at 1, 3, 6, and 14 days, animals were
sacrificed and the LALN cells were processed for cytokine analysis. Granuloma size was determined and lung tissues were also processed to determine the changes in IL-13 and IL-4 mRNA expression in the local milieu of granuloma formation.

LALN cells were cultured in vitro and restimulated with SEA, and 72-h culture supernatants were analyzed for IL-13 and IL-4 by ELISA. As shown in Fig. 1, B and C, Ag-specific IL-4 and IL-13 responses were first detected in i.v. egg-challenged naive mice as early as day 3 following egg embolization. Peak expression for both cytokines occurred on day 6 and then declined markedly by day 14. Peak granuloma size (Fig. 1A) correlated with the peak in cytokine expression. The cytokine response in egg-sensitized mice followed a similar kinetic pattern, except that the overall levels of IL-4 and IL-13 were much higher than in the naive mice, and the granulomas were larger, confirming an anamnestic-type response in sensitized animals. Again, the peak in the cytokine response corresponded with the peak in granulomatous inflammation (Fig. 1, right panels).

Although these data show that lymphocytes from egg-injected mice produced IL-13 and IL-4 after restimulation with Ag in vitro, they do not demonstrate to what extent the cytokines were produced locally, at the site of granuloma formation. Therefore, to address this issue, we performed RT-PCR analysis on total RNA isolated from the lungs of egg-challenged mice and examined changes in IL-13/IL-4 mRNA expression. Similar to the cytokine response in the draining lymph nodes, mice displayed significant increases in both IL-13 and IL-4 mRNA following a primary i.v. egg challenge (Fig. 2A). IL-4 levels peaked on day 6 and were markedly decreased by day 14, while IL-13 mRNA remained at similarly elevated levels on both days 6 and 14. In egg-sensitized mice, peak induction IL-4 and IL-13 mRNA occurred at much earlier time points than in the unsensitized animals (Fig. 2B). IL-4 and IL-13 mRNA reached maximal levels as early as 1 day following egg embolization, and while IL-13 remained at high levels at all time points, IL-4 mRNA expression dropped rapidly after day 3 and almost reached baseline levels between days 6 and 14. These data suggest that IL-4 mRNA expression is more tightly regulated, while the induction of IL-13 mRNA, at least at the site of granuloma formation, is more sustained. Again, similar to the lymph node data, the peak in IL-13 mRNA expression closely correlated with the peak in granuloma size (Fig. 1A).
IL-4 and IL-13 production in response to S. mansoni eggs is in large part dependent on CD4+ T cells

IL-4 and IL-13 were both initially described as Th2 cytokines, yet recent studies examining the cellular sources of IL-4 have suggested that CD4+ T cells may not be the only source of these important Th2-associated cytokines (6). Therefore, to begin to address the cellular source of IL-4 and IL-13, and HPRT mRNA. This illustrates the average densitometric units/HPRT ± SEM for each cytokine or cell marker. An asterisk indicates that the value is significantly different from the GL113 control mAb-treated group, with p < 0.05 by Student’s t test.

IL-4 and IL-13 mRNA expression. Interestingly, however, only the CD4-depleted mice showed a significant reduction in CD4 mRNA expression following egg embolization. As expected, CD4-depleted mice showed a highly significant reduction in CD4 mRNA and no change in their CD8 levels, while CD8-depleted mice showed an opposite and equally significant reduction in CD8 mRNA expression. Interestingly, however, only the CD4-depleted mice showed a significant reduction in IL-4 and IL-13 mRNA expression, again demonstrating that IL-4 and IL-13 mRNA expression in vivo is highly dependent on CD4+ T cells (Fig. 3).

sIL-13Ra2-Fc reduces granulomatous inflammation in naive and egg-sensitized mice

To assess the role of IL-13 in the development and modulation of granuloma formation, we treated mice with a soluble IL-13 fusion protein to block the activity of the cytokine. The murine IL-13Ra2 capable of binding IL-13, but not IL-4, IL-2, IL-7, IL-9, or IL-15, was fused with a human Fc and effectively blocks IL-13 activity both in vitro and in vivo (34).

Different protocols were employed in these studies so that we could address the contribution of IL-13 to the initiation of a Th2-mediated inflammatory response, as well as determine whether

**FIGURE 3.** IL-4 and IL-13 mRNA expression is highly dependent on CD4+ T cells. C57BL/6 mice were sensitized with 5000 eggs i.p., and 4 wks later, challenged i.v. with the same number of eggs. Mice were also injected i.p. with 0.5 mg GL113 (anti-βGal control mAb), 0.5 mg GK1.5 (anti-CD4 mAb), or 0.25 mg clone 2.43 (anti-CD8 mAb) diluted in 0.5 ml PBS on days −4, −1, and +3 post-i.v. egg challenge. Five mice per group were sacrificed on day 8 postchallenge, and lung mRNA from individual mice was analyzed by RT-PCR for changes in the expression of CD4, CD8, IL-4, IL-13, and HPRT mRNA. This illustrates the average densitometric units/HPRT ± SEM for each cytokine or cell marker. An asterisk indicates that the value is significantly different from the GL113 control mAb-treated group, with p < 0.05 by Student’s t test.

**FIGURE 4.** IL-13 blockade modulates granuloma formation after schistosome egg injection. A, C57BL/6 mice were treated with control-Fc (200 μg/dose, open bars) or sIL-13Ra2-Fc (200 μg/dose, filled bars) at the time of i.v. challenge with 5000 eggs, as described in Materials and Methods, and three to five mice per group were sacrificed on day 14 postchallenge. B, C57BL/6 mice were sensitized with 5000 eggs i.p. and treated with control-Fc (200 μg/dose, open bars) or sIL-13Ra2-Fc (200 μg/dose, filled bars) during the period of sensitization and challenge (Expts. 1 and 2) or only at the time of challenge (Expt. 3). In experiment 3, the mice were treated with 400 μg/dose of control-Fc or sIL-13Ra2-Fc. Four to five mice per group were sacrificed on day 8 postchallenge. In all studies, the left lung from each animal was placed in fixative and examined histologically to evaluate the size of the granulomas and the degree of tissue eosinophilia. Each bar represents the mean ± SE of three to five mice/group. * Denotes a significant difference between the groups, p < 0.05 by Student’s t test. A total of five separate studies was performed in secondary egg-challenged mice, and granuloma sizes in sIL-13Ra2-Fc-treated mice decreased between 28 and 50% when compared with control-Fc-treated animals.
IL-13 blockade could affect established Th2 responses. As shown in Fig. 4A, sIL-13Rα2-Fc significantly reduced primary pulmonary granuloma formation in unsensitized mice. In additional experiments, mice were sensitized with eggs i.p. to establish an egg-specific Th2 response, and then 2 to 3 wk later, animals were challenged i.v. with eggs. Mice were treated with sIL-13Rα2-Fc during both the period of sensitization and again at the time of challenge (Fig. 4B, Expts. 1 and 2) or only at the time of challenge (Fig. 4B, Expt. 3). With both protocols, sensitized mice showed significant reductions in lesion formation. In a final adjuvant-type protocol, we wanted to determine whether treatment with sIL-13Rα2-Fc during the period of sensitization alone would modify subsequent granulomatous responses. In this situation, IL-13 blockade had little or no effect (data not shown).

**IL-13 blockade does not modulate Th2-type cytokine responses**

Although in vitro studies suggested that IL-13 does not directly influence Th2 cell development, it is possible that it may indirectly influence CD4+ Th2 cell differentiation in vivo, due to its role as a regulator of APC monokine secretion and surface Ag expression (26, 28, 30). Therefore, to begin to address the mechanism for the reduced granulomatous response, we analyzed the effects of IL-13 blockade on related responses during Th2-dominated reactions, including eosinophil differentiation and IgE production. In addition, changes in the expression of Th1- and Th2-associated cytokines were monitored both in vitro and in vivo after sIL-13Rα2-Fc treatment.

Previous studies have established that alterations in granuloma formation are often accompanied by significant changes in the local and systemic cytokine response (25, 38–40). Therefore, to examine whether IL-13 blockade influenced Th cell cytokine production, LALN cells were isolated from control-Fc- and sIL-13Rα2-Fc-treated mice at multiple time points following i.v. egg challenge. Isolated lymphocytes were placed in culture and restimulated with either SEA or mitogen (Con A), and the production of IFN-γ, IL-4, IL-5, and IL-13 was examined in the 72-h supernatants. As shown in Fig. 5, marked increases in IL-4, IL-5, and IL-13 were observed in all SEA- and Con A-stimulated cultures prepared from egg-challenged mice, and no major differences were detected among the control-Fc- and sIL-13Rα2-Fc-treated animals at any time point. There was only minor IFN-γ expression in response to SEA, and the peak Con A-induced IFN-γ response detected at early time points (day 3) was down-regulated significantly at days 6 and 9 postchallenge (Fig. 5). However, in two separate experiments in unsensitized mice (primary response), treatment with sIL-13Rα2-Fc induced a modest increase in SEA-specific IFN-γ production on day 14 postchallenge (data not shown). Nevertheless, this was not accompanied by decreases in
FIGURE 6. IL-13 blockade does not alter cytokine mRNA response at the site of granuloma formation. C57BL/6 mice were sensitized with 5000 eggs i.p. and treated with control-Fc (200 μg/dose, open bars) or sIL-13Ra2-Fc (200 μg/dose, filled bars) for 2 wk before and again after i.v. challenge with 5000 eggs. On day 8 postchallenge, mice were sacrificed and the left lung was placed in RNA STAT-60 for total mRNA extraction. Cytokine mRNA levels were measured by RT-PCR analysis and expressed as arbitrary OD units of cytokine mRNA/HPRT mRNA (housekeeping gene). Each bar represents the mean ± SEM of five mice/group. Similar results were obtained in repeat experiments.

FIGURE 7. Total serum IgE levels are reduced in egg-injected mice after IL-13 blockade. A. C57BL/6 mice were treated as described in Fig. 4. At the time of sacrifice, 4–14 mice per group were bled, and total IgE serum levels were measured by ELISA. The data represent the IgE levels in μg/ml for individual animals. The * indicates that the groups are significantly different, p < 0.05 by Student’s t test. B. On day 8 postchallenge, five mice per group were bled, and anti-SEA IgG1 and IgG2b Ab titers were measured by ELISA. Each curve represents the mean values of pooled sera from each group. Similar results were obtained in several additional experiments.

IL-13 blockade suppresses serum IgE levels, but does not alter the cellular phenotype of the smaller egg-induced lesions

It is well known that Ab isotype switching in B cells is greatly influenced by the cytokine milieu. In mice, high IgG1/IgE and low IgG2 Ab isotype titers associate with Th2 responses, while IgG2 and low IgG1/IgE titers are observed in Th1-type cellular immune responses (44, 45). Tissue eosinophilia is similarly regulated by the Th2/Th1 cytokine balance, with IL-5 positively and IFN-γ negatively regulating eosinophil accumulation (46). Because IL-13 blockade down-regulated the overall Th2-mediated inflammatory response, we wanted to determine whether these related responses were similarly affected by IL-13 antagonism.

To address this issue, we compared the cellular composition of granulomas in both control-Fc- and sIL-13Ra2-Fc-treated mice and monitored their serum Ab response. While lesion size was decreased significantly after IL-13 blockade, the cellular composition of the granulomas did not vary significantly. In all experiments, both control-Fc- and sIL-13Ra2-Fc-treated mice had granulomas that were composed of between 50–70% eosinophils, with the remaining cells in all groups being predominantly macrophages with moderate numbers of lymphocytes. Although the overall composition of granulomas did not change, the total numbers of eosinophils recruited to the lungs must be decreased in proportion to the reduction in granuloma size. There was also no obvious difference in the amount of collagen deposition in or around the granulomas, as determined by microscopic analysis of sections stained with picrosirius red, which stains collagen specifically (data not shown).

Total serum IgE titers were reduced significantly as a result of IL-13 blockade (Fig. 7A). However, the reduction in total IgE Ab expression was only observed in mice that were treated with the sIL-13Ra2-Fc both at the time of sensitization and again at challenge. There was no significant reduction in IgE titers in mice that were treated with the sIL-13Ra2-Fc only at the time of sensitization or during the i.v. challenge alone (data not shown). Marked SEA-specific IgG1 and IgG2b Ab responses were also induced by schistosome eggs; however, no significant differences in Ab titers were detected among the two groups (Fig. 7B).
IL-4 expression in egg-challenged mice is independent of IL-13, while IL-13 is in large part dependent on IL-4

While the role of IL-4 in regulating granuloma formation is still incompletely understood (6), IL-4 clearly plays a key role in IgE production (47), and most likely indirectly influences IL-5, and thus eosinophil differentiation through its role as a differentiation factor for CD4\(^+\) Th2 cells (1). Thus, IL-13 blockade could indirectly affect granuloma formation and IgE production by down-regulating IL-4 responses. It should be noted, however, that sIL-13R\(_a\)2-Fc does not block IL-4 activity directly (34). To rule out the possibility that sIL-13R\(_a\)2-Fc was playing an indirect role by modulating IL-4 levels, LALN cells from egg-challenged control-Fc- and sIL-13R\(_a\)2-Fc-treated mice were examined for IL-4 production after restimulation with SEA or Con A. As was observed with the other Th2-associated cytokines, continuous in vivo treatment with the sIL-13R\(_a\)2-Fc had no significant effect on the capacity of LALN cells to produce IL-4 at any time point (Fig. 5). Comparable findings were also observed with spleen cells obtained from similarly treated animals (data not shown).

Studies were also conducted in IL-4-deficient mice to examine whether IL-13 production was modulated or controlled by IL-4. As shown in Fig. 8, Ag-specific IL-13 production was decreased almost 10-fold in IL-4-deficient mice when compared with the levels observed in WT egg-injected animals. A modest IL-13 response was detected, however, in the IL-4-deficient animals, confirming that a low, but significant, IL-13 response can develop in the absence of IL-4 (22–24).

IL-13 blockade almost completely abrogates granuloma formation and tissue eosinophilia in IL-4-deficient mice and up-regulates IFN-\(\gamma\) production

While previous studies have demonstrated an important role for IL-4 in granuloma formation (20, 21), studies in IL-4-deficient mice have shown that reduced, but significant, egg-induced inflammatory responses persist in these animals (22, 23, 48). Because IL-13 production is maintained at reduced levels in these mice (Fig. 8), we wanted to determine whether the low-grade inflammatory response was influenced by this cytokine. In these experiments, IL-4-deficient mice were sensitized and challenged i.v. with eggs. The knockout mice were treated throughout the period of sensitization and again at challenge with either control-Fc or sIL-13R\(_a\)2-Fc. Granuloma formation in both groups was compared with the levels observed in sensitized WT animals. While granuloma size was reduced in IL-4-deficient animals, lesion formation was almost completely abrogated in sIL-13R\(_a\)2-Fc-treated IL-4 knockout mice (Fig. 9A). Even more striking was the complete absence of eosinophils in the small inflammatory foci of doubly-deficient animals (Fig. 9B).

The cytokine response was also evaluated in the draining lymph nodes of these animals, and as shown in Fig. 10, IFN-\(\gamma\) production was partially up-regulated in control-Fc-treated IL-4-deficient
mice, while IL-5 production was almost completely ablated. Interestingly, the sIL-13Ra2-Fc-treated IL-4-deficient mice showed an even more striking increase in their SEA-specific IFN-γ response, suggesting that IL-4 and IL-13 may act together to suppress type 1 cytokine expression. The latter findings may at least in part explain the marked reduction in granuloma formation and tissue eosinophilia observed in the double IL-4/IL-13-deficient animals (Fig. 9A).

Discussion
To address the role of IL-13 in granuloma formation, we used sIL-13Ra2-Fc to block IL-13 activity in vivo and then examined in detail the effects on the evolving Th2-associated egg response. Different protocols were explored so that we could determine whether IL-13 blockade either would influence the development of a Th2-mediated inflammatory response or could be used to modulate established responses. In naive mice, granulomas peak in size at about 2 wk after i.v. challenge with eggs (21). In these animals, treatment with the sIL-13Ra2-Fc reduced peak granuloma volume by more than 50%. In separate studies, mice were sensitized i.p. with eggs to establish a polarized Th2 response and then challenged i.v. with eggs and treated with the sIL-13Rα2-Fc (200 μg/dose) or sIL-13Rα2-Fc (200 μg/dose) during the period of sensitization and challenge. Mice were sacrificed on day 6 postchallenge, and the LALN were isolated, placed in culture at 3 × 10⁶ cells/ml, and restimulated with SEA (20 μg/ml) or Con A (5 μg/ml). Supernatants were collected at 72 h, and IFN-γ and IL-5 production were measured by ELISA. This illustrates mean ± SD of supernatants analyzed in duplicate.

The collective results from all of the blocking studies clearly show that IL-13 plays a role in the egg-induced inflammatory response.

Schistosome granulomas are rich in eosinophils, and egg deposition is accompanied by marked increases in serum IgE. Interestingly, previous studies have established that IL-13 exhibits chemotactic activity for human eosinophils and may also play a role in their survival by stimulating the production of IL-3 and granulocyte-macrophage CSF (49). IL-13 has also been shown to induce IgG4 and IgE synthesis, and directs IgE isotype switching in human B cells (32). Thus, IL-13 may also participate in other aspects of the egg-induced Th2 response. We therefore wanted to determine whether the altered granulomatous response in sIL-13Ra2-Fc-treated mice was accompanied by changes in eosinophil accumulation or IgE production. Although granuloma size was reduced in both naive and sensitized mice by IL-13 blockade, the cellular phenotype of the lesions was not significantly different. Nevertheless, the total number of eosinophils in the lungs was decreased since the lesions in sIL-13Ra2-Fc-treated mice were smaller.

Total serum IgE responses were also significantly reduced in egg-injected sIL-13Ra2-Fc-treated mice. This finding, however, was restricted to animals treated with IL-13Ra2-Fc both during sensitization and at the time of challenge (Fig. 7A). There was no effect on IgE levels in animals that had been treated with the IL-13 antagonist only at the time of challenge (data not shown). This was surprising since, in contrast to its effects on human Ab responses, IL-13 is not believed to induce IgE production by mouse cells (26). It is possible, however, that the reduced IgE levels are more a consequence of the overall reduction in the host’s reaction to the eggs rather than a direct effect of IL-13 on B cell Ig production. This conclusion seems likely since mouse B cells are believed to lack functional IL-13R (26). Nevertheless, a novel IL-4-independent pathway for IgE switching in the mouse was described (50) that might be influenced by IL-13, and recent studies in IL-13 transgenic animals have confirmed an IL-4-independent role for IL-13 in regulating IgE responses (51).

To begin to address the mechanism behind the reduced granulomatous response in sIL-13Ra2-Fc-treated mice, we first examined whether IL-13 blockade altered the cytokine response. Modulation of granuloma formation is often accompanied by changes in the Th1/Th2-type cytokine response (52). Although in vitro studies established that IL-13 does not directly affect CD4⁺ T cell differentiation as does IL-4 (53), the cytokine could indirectly influence T cell differentiation in vivo by affecting Ag presentation. IL-13 was described initially as a potent down-regulatory cytokine for macrophage function and, like IL-4 and IL-10 (54, 55), modulates cytokine production (56, 57) as well as MHC class II expression in macrophages (58). To determine whether there was any effect on Th1/Th2-type cytokine production, we isolated LALN and spleen cells from egg-injected/sIL-13Ra2-Fc-treated mice and examined IFN-γ production as a marker of a Th1-type response, and IL-4, IL-5, and IL-13 as markers of a Th2-type response. Although we observed modest increases in IFN-γ production in sIL-13Ra2-Fc-treated primary egg-challenged mice (data not shown), this finding was not observed in any of the secondary challenge experiments. At no time point did we detect any significant difference in cytokine production by cultured lymphocytes from egg-sensitized mice, even after continuous treatment with sIL-13Ra2-Fc (Fig. 5).

We also examined the cytokine mRNA response at the site of granuloma formation and extended our analysis to include TNF-α and IL-1β, two inflammatory cytokines induced in both infected
production in the sIL-13R

IL-13 blockade does not directly inhibit IL-4 activity, it was possible that even modest effects on IL-4 production could explain the reduction in granuloma formation and IgE responses in IL-4-deficient/sIL-13R-Fc-treated mice. To rule out the possibility that IL-13 blockade was indirectly affecting IL-4 levels, we examined IL-4 production at the site of granuloma formation (Fig. 6), as well as in the draining lymph nodes and spleens of both control-Fc- and sIL-13Rα2-Fc-treated mice at multiple time points (Fig. 6A). In all of our studies in WT mice, however, we failed to detect any significant effect on the IL-4 response.

In contrast to these findings, however, IL-13 production was highly dependent upon IL-4. SEA-specific IL-13 production was reduced almost 10-fold in egg-injected IL-4-deficient mice (Fig. 6). These findings are thus consistent with the well-established role of IL-4 as the key differentiation factor for Th2 cells (1). Nevertheless, significant IL-13 was detected in the culture supernatants of LALN cells obtained from IL-4-deficient mice. Previous studies have also demonstrated the maintenance of low-level Th2 responses in IL-4-deficient animals (20–24). Interestingly, we showed that this reduced IL-13 response was in large part responsible for the granulomatous response of IL-4-deficient mice. Moreover, the lesions in double-deficient mice, unlike their single cytokine-deficient counterparts, were also completely devoid of eosinophils. These findings further emphasize the IL-4-independent effects of IL-13 in the granulomatous response and suggest that IL-13 may act at the effector end of the Th2 response.

Previous studies addressing the role of IL-13 relied primarily on in vitro studies to examine its role in immunoregulation (26). The development of the sIL-13Rα2-Fc provided a novel approach for studying the effects of IL-13 in vivo. The combined results from this study demonstrate for the first time that IL-13 is an important factor in Th2-mediated inflammation in the lung. The data showing that Th1/Th2-type cytokine responses were not significantly affected by IL-13 blockade suggest that IL-13 may in fact be a key effector cytokine for granuloma formation. Thus, unlike IL-4, whose main role may be as a differentiation factor for egg-induced Th2 responses, IL-13 may play more of a direct role in the inflammatory response. The transient nature of IL-4 mRNA expression and the more sustained IL-13 response at the site of lesion formation might support such a hypothesis (Fig. 2). Indeed, IL-13 has been shown to regulate adhesion molecule expression on human endothelial cells (60), and the expression of ICAM-1 in particular is important to granuloma development (61). The fact that granuloma formation and tissue eosinophilia were only partially reduced by IL-4 or IL-13 deficiencies, but were nearly completely ablated in IL-4-deficient/sIL-13Rα2-Fc-treated mice, suggests that both cytokines are equally important to the overall granulomatous response. The observation that IFN-γ production was partially up-regulated in IL-4-deficient mice, and even more so in double IL-4/IL-13-deficient animals, might suggest an alternative hypothesis whereby IL-4 and IL-13 act together to suppress the antiinflammatory type 1 cytokine response (Fig. 10). Regardless of the exact mechanism, these findings are particularly important for schistosomiasis, since it is chronic egg-associated pathology that leads to the development of severe disease in infected humans. Moreover, these data may also extend to other infectious diseases (62), asthma (63), or allergic-type reactions (64), in which Th2 responses and the production of IL-13 are linked with pathology.

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