IL-13 and IFN-γ: Interactions in Lung Inflammation

Jean G. Ford, Donna Rennick, Debra D. Donaldson, Rajeev Venkayya, Cliff McArthur, Elisabeth Hansell, Viswanath P. Kurup, Martha Warnock and Gabriele Grünig

*J Immunol* 2001; 167:1769-1777; doi: 10.4049/jimmunol.167.3.1769

http://www.jimmunol.org/content/167/3/1769

**Why The JI?**

- **Rapid Reviews!** 30 days* from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

**References**  This article cites 67 articles, 30 of which you can access for free at: http://www.jimmunol.org/content/167/3/1769.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2001 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
IL-13 and IFN-γ: Interactions in Lung Inflammation

Jean G. Ford,* Donna Rennick,† Debra D. Donaldson,‡ Rajeev Venkayya,§ Cliff McArthur,¶ Elisabeth Hansell,‖ Viswanath P. Kurup,§ Martha Warnock,¶ and Gabriele Grünig‡##

Chronic inflammatory diseases of the lungs, such as asthma, are frequently associated with mixed (Th2 and Th1) T cell responses. We examined the impact of critical Th1 and Th2 cytokines, IFN-γ and IL-13, on the responses in the lungs. In a mouse model of airway inflammation induced by mixed T cell responses, the number of Th1 (IFN-γ-positive) cells was found to be negatively correlated with airway hyperreactivity. In these mice, blockade of IL-13 partially inhibited airway hyperreactivity and goblet cell hyperplasia but not inflammation. In contrast, in mice that responded with a polarized Th2 response to the same Ag, blockade of IL-13 inhibited airway hyperreactivity, goblet cell hyperplasia, and airway inflammation. These results indicated that the presence of IFN-γ would modulate the effects of IL-13 in the lungs. To test this hypothesis, wild-type mice were given recombinant cytokines intranasally. IFN-γ inhibited IL-13-induced goblet cell hyperplasia and airway eosinophilia. At the same time, IFN-γ and IL-13 potentiated each other’s effects. In the airways of mice given IL-13 and IFN-γ, levels of IL-6 were increased as well as numbers of NK cells and of CD11c-positive cells expressing MHC class II and high levels of CD86. In conclusion, IFN-γ has double-sided effects (inhibiting some, potentiating others) on IL-13-induced changes in the lungs. This may be the reason for the ambiguous role of Th1 responses on Th2 response-induced lung injury.

kept in barrier facilities at DNAX Research Institute (Palo Alto, CA), at the Gladstone Institute (University of California, San Francisco, CA), or in the Antenucci Building, St. Luke’s Roosevelt Hospital (New York, NY). By using monitoring with sentinel mice, the colonies were shown to be free of commonly tested mouse pathogens.

Isolation of donor T cells

Splenic CD4⁺ T cells were isolated as described (8, 29, 33). Briefly, single-cell suspensions were prepared by pressing tissues through 100-μm steel mesh filters followed by straining through 70-μm nylon filters. Red cells was lysed by following magnetic depletion using lineage-specific mAbs (B220 (B cells), 8C5 (neutrophils), Mac-1 (monocytes and macrophages), Ter119 (red blood cells), anti-CD45 (B9-T4 cell line), and CD11b (Gr-1)) and goat anti-rat IgG (Fc-) and anti-rat IgG (H and L)-coated magnetic beads (PerSeptive Diagnostics, Cambridge, MA). Remaining cells were labeled with CD4-FITC and Thy-1.2-PE (BD Pharmingen, San Diego, CA) and sorted using instruments from Becton Dickinson (DNAX Research Institute and Columbia University, New York, NY) or a MoFlo multi-laser sorter high speed sorter, Cytomation (University of California). The purity of the CD4⁺ T cells was typically >97%. In experiments that analyzed cells for the activation marker, CD62L L-selectin (CD62L), 82.44% ± 0.98% was of the CD4⁺ T cells expressed CD62L at high levels, and 17.5% ± 0.97% expressed CD62L at low levels. For some experiments, positive magnetic bead selection was used (Miltenyi Biotech, Auburn, CA). Briefly, spleen cell suspensions were labeled with anti-CD4 mAb coupled to paramagnetic microbeads. Magnetic retained cells were >94% CD4⁺. Purified CD4⁺ T cells were injected i.p. at 2–5 × 10⁷/mouse. The immunization protocol was started 3–6 days after T cell transfer.

Immunization

Aspergillus fumigatus Ag extract (Asp Ag) was prepared free of living organisms as described (30). Ag sensitization was conducted by priming with Asp Ag (100 μg in 200 μl PBS) given i.p. three times at 4-day intervals followed by intranasal (i.n.) challenge 4 days later (31). Challenges with Asp Ag (100 μg in 50 μl PBS) or control (PBS) were given to mice lightly anesthetized with isoflurane or methofane. Challenges were given i.n. two times (6 days apart) or three times (4 days apart).

Inhibition of IL-13

Inhibition of IL-13 was done as previously described (8). Groups of T cell-reconstituted RAG1⁻/⁻ mice (C57BL/6 background) or of C57BL/6 wild-type mice were primed with Asp Ag i.p. on days −11, −7, and −4. On days 0, 1, 4, 5, 6, 8, 9, and 10, the mice were given 0.325 mg of IL-13 (Sigma), a recombinant protein (IL-13-Fc) (32) or control human Ig i.p. Challenges with Asp Ag i.n. were given on days 0 and 8. On day 11, the mice were analyzed for a variety of parameters as indicated in the results section.

Challenge with recombinant cytokines

Challenge with recombinant cytokines was done as previously described (8). Groups of naive, wild-type C57BL/6 mice were given in a 50-μl volume of PBS i.n. one of the following: 1) control BS A (low in endotoxin; Sigma, St. Louis, MO) 2) murine rIL-13 (Genetics Institute) and BSA, 3) murine rIFN-γ (R&D Systems, Minneapolis, MN) and BSA, or 4) rIL-13 and rIFN-γ. IL-13 was used at a dose of 5 μg/mouse at days 1, 3, and 5 as described (7, 8). IFN-γ was given at a dose of 1.25 μg/mouse with IL-13 and was additionally given (mixed with BSA) at days 0, 0.5, 2, 2.5, 4, and 4.5. The groups of mice that did not receive IFN-γ were given BSA on days 0, 0.5, 2, 2.5, 4, and 4.5.

Airway hyperreactivity

Airway hyperreactivity was determined as described (8, 31, 33). Briefly, mice were anesthetized with etomidate (30 mg/kg), and the tracheas were cannulated. The mice were ventilated with 100% oxygen at physiologic tidal volumes (9 μl/g) and paralyzed using pancuronium bromide. A catheter was inserted into the tail vein, and the mice were placed inside of a plethysmograph. Increasing doses of acetylcholine (0.01–10 μg/g body weight) were administered i.v. until airway resistance reached at least 200% over baseline. From these data, the concentration of acetylcholine that produced a 200% increase in airway resistance over baseline (PC200) was calculated.

Bronchoalveolar lavage (BAL)

BAL was obtained as described previously (8, 31, 33). Samples were obtained by washing the lungs three times with 1 ml HBSS. Total cells were counted in undiluted samples. Cytospin preparations were stained with Wright and Giemsa solutions and a differential cell count performed on 300 cells or more. BAL supernatants were stored frozen (−70°C). Cytokine levels were determined in unconcentrated wash fluids using specific ELISAs. Flow cytometric analysis of BAL cells was performed using FITC-labeled anti-CD8, PE-labeled anti-CD4, CyChrome-labeled anti-CD3, allophycocyanin (APC)-labeled anti-Thy1.2, PE-labeled anti-NK1.1, FITC-labeled anti-CD11c, PE-labeled anti-CD86, and biotinylated anti-I-A¹ mAbs. The biotinylated mAb was visualized using CyChrome-labeled streptavidin. All reagents were obtained from BD Pharmingen. The cells were analyzed on a FACS Calibur using CellQuest software (BD Biosciences, San Jose, CA). Using forward and side scatter, gates were set for lymphocytes or for all other cells except lymphocytes.

Analysis of lung sections

Analysis of lung sections was performed as described previously (8). After BAL, the lungs were distended by injecting 1 ml formalin (4% formaldehyde solution in PBS), removed, and fixed in formalin. Parasagittal slices were paraffin-embedded and cut at 4–6 μm thickness. Sections were stained with H&E or with the periodic acid Schiff (PAS) methods. The sections were assigned a random code to blind the examiner to the identity of each specimen. The lungs were first evaluated for the general nature of the lesions. Scoring was then performed at a magnification of ×250 by examining at least 50 consecutive fields.

Goblet cells

Medium-sized airways were assessed in sections stained with PAS. Each lung was assigned a unit by computing the mean of the numerical scores. The numerical scores for the abundance of PAS-positive goblet cells (8) in each airway were determined as follows: 0, <5% goblet cells; 1, 5–25%; 2, 25–50%; 3, 50–75%; 4, >75%.

Inflammation

Lung sections stained with H&E were assigned a unit value for peribronchio-olar/perivascular inflammation or for alveolar inflammation by computing the means of the numerical scores.

Peribronchiolar and perivascular inflammation

The numerical scores for each view-field were determined as follows: 0, normal; 1, few cells; 2, a ring of inflammatory cells 1 cell layer deep; 3, a ring of inflammatory cells 2–4 cells deep; 4, a ring of inflammatory cells of >4 cells deep.

Alveolar inflammation

The numerical scores for each view-field were determined as follows: 0, normal; 1, alveolar walls normal, few macrophages in alveoli; 2, mild thickening of alveolar walls and increased alveolar macrophages and eosinophils; 3, marked thickening of alveolar walls and alveolar multicellular giant cells and eosinophils in 30–50% of the field; 4, same as 3 but in >50% of the field; 5, complete consolidation.

Intracellular cytokine staining

Intracellular cytokine staining was performed as described previously (31). The lung tissues were passed through 100-μm steel mesh filters, and the cells were then strained through 70-μm and 40-μm cell strainers. Total cell numbers were determined by manual counting using a Neubauer chamber. The cells were put into culture with PMA/ionomycin for 2 h followed by addition of brefeldin A for 2 h. DNase I (Sigma) was added during the last 5 min of culture. The cells were recovered by vigorous pipetting, stained (70-μm cell strainer), and fixed with formaldehyde. Intracellular labeling was performed using PE-labeled 11B11 mAb for IL-4 (BD Pharmingen), FITC-labeled AN18 mAb for IFN-γ (a generous gift from A. O’Garra, DNAX Research Institute), and biotinylated anti-IL-13 Ab (R&D Systems) followed by streptavidin-PE and in cells permeabilized with saponin. Cell surface staining was performed with CyChrome-labeled anti-CD3 and APC-labeled anti-Thy1.2 mAbs (BD Pharmingen). The cells were analyzed using FACS Calibur using CellQuest software (Becton Dickinson).

For each lung sample, the negative gates for the intracellular staining were set using cells expressed intracellularly to FITC- and PE-labeled iso-type control mAbs (BD Pharmingen) followed by surface labeling with anti-CD3 and anti-Thy1.2 mAbs. For the staining with 11B11 and with AN18, the background was very low, 0.5% fluorescing cells. The specificity of staining with the anti-IL-13 Ab was tested using soluble IL-13 given in excess. Using this method, we determined that the background for this Ab was at 1.5% fluorescing cells.

Determination of inhibitory activity of IL-13 in BAL samples

The B9 mouse cell line, in which growth is dependent on different cytokines, including IL-13, which has been used to determine IL-13 bioactivity...
was analyzed for airway hyperreactivity and intracellular cytokines expressed by lung T cells. For analysis, the data from Ag-sensitized, T cell-reconstituted

cells and no growth factor. Inhibitor activity was expressed as nanograms

FIGURE 1. Airway hyperreactivity and T cell responses induced by sensitization with Asp Ag in wild-type and T cell-reconstituted RAG−/− mice. A) Intracellular Cytokines of Lung T cells

Results

Allergen-induced lung disease in T cell-reconstituted RAG−/− mice is characterized by mixed T cell responses

As we had previously found that reconstitution of T- and B cell-deficient RAG−/− mice with CD4+ T cells is necessary and suf-
ficient for the induction of the signs of asthma by allergen (33), this system was used further to study the mechanisms involved in the process. RAG−/− mice of the 129SvEv or C57BL/6 strains were reconstituted with highly purified syngeneic CD4+ T cells from naive wild-type mice and then sensitized with Asp Ag (33). This Ag induces marked airway hyperreactivity and a uniform Th2 response in wild-type mice (Ref. 31 and Fig. 1). In contrast to wild-
type mice, the lung T cell response to Asp Ag in reconstituted RAG−/− mice was nonuniform (Fig. 1A). Eighty percent of the mice developed a mixed lung T cell response (simultaneous presence of IL-4+ and IFN-γ+ T cells); 10% a Th2-like response (IL-4+ T cells); and 10% a Th1-like response (IFN-γ+ T cells). The mixed T cell responses in the lungs of sensitized, T cell-
reconstituted RAG−/− mice were characterized by a pronounced increase in the numbers of Th1 (IFN-γ+) cells (10.65 ± 1.83 × 10^5 vs 0.4 ± 0.1 × 10^5 IFN-γ+ T cells per lung (p < 0.0001) in T cell-reconstituted RAG−/− vs wild-type mice). In contrast, similar numbers of Th2 cells (IL-4+ or IL-5+) were present in Ag-challenged T cell-reconstituted RAG−/− mice and in wild-type mice (4.3 ± 0.6 × 10^5 vs 2.4 ± 0.4 × 10^4 IL-4+ T cells per lung). The total numbers of T cells in the lungs (Thy1.2, CD3 double-

Airway hyperreactivity was induced to a much more variable degree in reconstituted RAG−/− mice relative to wild-type mice (Fig. 1B). There was a significant, but nonlinear, negative correlation between the number of Th1 cells (IFN-γ+) in the lungs and airway hyperreactivity (Spearman’s rank correlation test, p < 0.05; Fig. 1B). Airway hyperreactivity was induced to a significant

Downloaded from http://www.jimmunol.org/ by guest on October 22, 2017
degree in those sensitized RAG−/− mice that had <5 × 10^5 IFN-γ+ T cells in their lungs. Airway hyperreactivity was also induced in some sensitized, reconstituted RAG−/− mice that had >5 × 10^5 IFN-γ+ T cells in the lungs; however, the group mean was not different from unsensitized controls. Numbers of Th2 cells (IL-4+ or IL-5+) in the lungs were not correlated with airway hyperreactivity, or with numbers of Th1 (IFN-γ+) cells in the lungs (Fig. 1B).

Both strains of T cell-reconstituted RAG−/− mice responded similarly. RAG1−/− mice (C57BL/6 background) or RAG2−/− mice (129SvEv background) were reconstituted with CD4+ T cells from syngeneic, naive, wild-type mice. Groups of 8–15 mice were used for this comparison. Upon sensitization, both strains of mice developed a robust Th2 response in the lungs (mean ± SEM: 3.5 ± 0.8 × 10^5 or 3.8 ± 0.5 × 10^5 IL-4+ T cells; 8.2 ± 1.2 × 10^5 or 6.6 ± 1.1 × 10^5 IL-5+ T cells). Both strains of mice showed a variable Th1 response in the lungs (mean, range: 5.5, 0.1–23.1 × 10^5 or 5.9, 0.3–24.5 × 10^5 IFN-γ+ T cells). Airway hyperreactivity was induced to a similar variable degree (mean, range of PC200: 0.89, 0.2–2.0 or 0.57, 0.1–2 µg/g). The variability in the immune response to Asp Ag seen in both strains of T cell-reconstituted RAG−/− mice is most probably due to the innate immune system (immaturity of dendritic cells and presence of a large population of NK cells relative to numbers of T cells (our unpublished data).

This model was then used to examine which molecules are critical for the airway physiologic and inflammatory changes in allergen-induced lung disease characterized by mixed T cell responses. We hypothesized that IL-13 would be the critical effector molecule as shown in Th2-induced asthma models (7, 8).

**IL-13: a partial mediator of allergen-induced lung injury in T cell-reconstituted RAG−/− mice**

Groups of T cell-reconstituted RAG1−/− and wild-type mice were primed i.p. with Asp Ag followed by challenge i.n. An IL-13 inhibitor (IL-13R-Fc) (32) or control protein was given during the i.n. challenge period. 

**Airway hyperreactivity (Fig. 2A).** IL-13R-Fc potently inhibited airway hyperreactivity in wild-type mice. In T cell-reconstituted RAG−/− mice, Asp Ag induced airway hyperreactivity in some mice. Because of the variability of airway hyperreactivity, and the relative small number of animals examined, the group means did not reach statistical significance. T cell-reconstituted RAG−/− mice that received IL-13R-Fc had a trend to reduced airway hyperreactivity that was statistically not significant.

**Goblet cell hyperplasia (Fig. 2B).** IL-13R-Fc inhibited Ag-induced goblet cell hyperplasia in wild-type mice potently. Similarly, IL-13R-Fc significantly inhibited goblet cell hyperplasia in Ag-sensitized, T cell-reconstituted RAG−/− mice. However, the relative inhibition of goblet cell hyperplasia by IL-13R-Fc was smaller in reconstituted RAG−/− mice as compared with wild-type mice.

**BAL eosinophilia (Fig. 2C).** IL-13R-Fc significantly inhibited Ag-induced BAL eosinophilia in wild-type mice. In contrast, IL-13R-Fc did not reduce BAL eosinophilia in T cell-reconstituted RAG−/− mice.

**BAL neutrophilia (Fig. 2D).** In wild-type mice, neutrophils were an appreciable constituent of BAL fluid on the first day after giving Ag (data not shown), but neutrophilia was mostly resolved 4 days after Ag challenge (Fig. 2D). In T cell-reconstituted RAG−/− mice, Ag induced prolonged BAL neutrophilia (Fig. 2D). Injections of IL-13R-Fc did not affect BAL neutrophilia.

**Inflammation (Figs. 2, E and F, and 3, A–F).** Control wild-type mice did not show signs of inflammation. Control T cell-reconstituted RAG−/− mice demonstrated mild inflammation of the lungs. This inflammation could reflect an increased state of activation of the transferred T cells as has been observed in T cell transfer models used to study inflammatory bowel disease (35). Ag sensitization induced peribronchial, perivascular, and alveolar inflammation of similar type and extent in wild-type and in T cell-reconstituted RAG−/− mice (different scales of y-axes). E, Alveolar inflammation. F, Peribronchial and perivascular inflammation. Data of individual mice are shown; medians are indicated by bold lines. Statistical analysis was conducted for the comparison between groups of mice given control protein or IL-13R-Fc (∗, p < 0.01; #, p < 0.05).

**Airway inflammation (Figs. 2E and 3, C–F).** In wild-type mice, IL-13R-Fc potently reduced alveolar inflammation. In contrast, IL-13R-Fc did not affect alveolar inflammation in T cell-reconstituted RAG−/− mice.

**Peribronchial and perivascular inflammation (Figs. 2F and 3, C–F).** IL-13R-Fc did not affect peribronchial and perivascular inflammation in wild-type or T cell-reconstituted RAG−/− mice.
Correlation of scores of lung inflammation and airway hyperreactivity in individual T cell-reconstituted RAG−/− mice

To control for the possibility that in this series of experiments, low airway hyperreactivity would be the result of an insufficient immune response, the data were analyzed for correlation with lung inflammation. There was no correlation between airway hyperreactivity and peribronchial inflammation (p = 0.728); or alveolar inflammation (p = 0.148); or goblet cell hyperplasia (p = 0.755). For example, the mice with the least inflammation (alveolar inflammation scores of 0.857 and 1.472; peribronchial inflammation scores of 0.667 and 1.382) had PC200 values of 0.222 (range) in wild-type mice and ~12.4 μg/ml in T cell-reconstituted RAG−/− mice. However, the exact amount of IL-13-inhibitory activity could not be determined in the T cell-reconstituted RAG−/− mice because 7 of 10 BAL samples contained IL-6 by ELISA (group mean ± SEM, 0.56 ± 0.22 ng/ml). In contrast, IL-6 could not be detected in any of the BAL samples from wild-type mice given IL-13R-Fc. Because IL-6 is another growth factor for B9 cells, most BAL samples from reconstituted RAG−/− mice promoted growth of B9 cells, even in the presence of the anti-IL-6 Ab. However, at the highest BAL dilution tested, all samples from RAG−/− mice given IL-13R-Fc inhibited the growth of B9 cells completely.

These data show that the relative diminished potency of IL-13R-Fc to inhibit pathology in T cell-reconstituted RAG−/− mice relative to wild-type mice was not due to a lack of IL-13-expressing T cells in the lungs or to an insufficient availability of the IL-13 inhibitor in the lungs. Therefore, we hypothesized that a molecule made by Th1 cells during the course of the mixed T cell response in the lungs would modulate the function of IL-13. We hypothesized that this molecule was IFN-γ because IFN-γ has been shown to inhibit allergen-induced lung injury (36–42). To test this hypothesis, wild-type mice were given recombinant cytokines.

IFN-γ inhibits and at the same time potentiates effects of IL-13 in the lungs

Wild-type mice were given recombinant IFN-γ and IL-13, or each cytokine alone, or BSA i.n. The mice were analyzed 1 day after the last cytokine challenge. BAL fluids were analyzed for IL-13 and IFN-γ to ensure that differences between groups of mice were not due to insufficient exposure to cytokines. Both IL-13 and IFN-γ levels were similar in groups of mice that were challenged with either IL-13, IFN-γ, or with IL-13 and IFN-γ (mean ± SEM: 17.3 ± 1.2 vs 17.7 ± 2.6 ng/ml IL-13; and 14.8 ± 3.9 vs 10.8 ± 4.8 ng/ml IFN-γ comparing mice challenged with respective single cytokines vs both cytokines together). Differences between groups of mice were also not due to a large induction of TNF-α or IL-12 production, because these cytokines could not be detected in BAL samples by ELISA (data not shown).

Inhibitory effects

IFN-γ inhibited goblet cell hyperplasia (Fig. 4A), BAL eosinophils (Fig. 4B), and BAL neutrophils (Fig. 4B) induced by IL-13. There was a trend toward a decrease in the numbers of BAL− CD4+ T cells in mice challenged with IL-13 and IFN-γ relative to mice challenged with IL-13 (mean ± SEM: 0.66 ± 0.13 × 104, or 1.03 ± 0.20 × 104 CD4+ T cells/BAL, respectively).

Potentiating effects

There was a trend to increased peribronchiolar and alveolar inflammation in mice given IL-13 and IFN-γ relative to mice challenged with IL-13 (Figs. 4C and 5, A–D). Both parameters of inflammation were significantly increased relative to mice given BSA or mice challenged with IFN-γ. Numbers of NK cells in the BAL were significantly increased in mice given IL-13 and IFN-γ relative to all other groups of mice (Fig. 4B). Levels of IL-6 were
increased in mice given IL-13 and IFN-γ relative to all other groups of mice (Fig. 4D). Numbers of cells expressing high levels of Ag-presentation molecules were increased in mice given IL-13 and IFN-γ relative to all other groups of mice (Fig. 6). These cells were detected using anti-CD11c (a molecule expressed by dendritic cells), MHC class II, and CD86 (B7-2) Abs. These Abs marked cell subpopulations that were distinct for each group of mice (Fig. 6A). The percentage of cells that coexpressed MHC class II, CD11c, and high levels of CD86 was significantly higher in mice (Fig. 6C). The percentage of cells coexpressing CD11c, MHC class II, and CD86 for each BAL sample, the number of total cells was determined using a Neubauer chamber. This information and the percentages of cells within the gate for large granular cells and the gates depicted in A were used to calculate numbers of cells coexpressing CD11c, MHC class II, and CD86. Equation used was: cell number × cells in gate for large granular cells (%) × cells in the gates shown in A, α–d (%), × cells in the gates shown in A, e–h (%). Each dot represents data of individual mice or of a sample of pooled cells from two to three mice. Medians are indicated by bold lines. Statistical analysis was performed using Bonferroni multiple comparison’s test; *p < 0.05 for the comparison between groups of mice given IL-13 and IFN-γ, or IL-13 alone.

Discussion

In a model of lung injury due to mixed T cell responses, the development of airway hyperreactivity was negatively correlated to the presence of IFN-γ^+^ T cells in the lungs. The correlation was such that airway hyperreactivity was induced on a group level in mice that had <5 × 10^5^ IFN-γ^+^ T cells in the lungs. These data confirm reports of many other groups that the presence of large amounts of IFN-γ (by administering IFN-γ, IL-12, or IL-18) in the lungs inhibits the development of airway hyperreactivity induced by Th2 responses in the lungs (38, 39, 41, 43–48).

Our data show that IL-13 was only a partial mediator of lung injury induced by mixed T cell responses. This is in contrast to Th2-mediated asthma models due to OVA sensitization, in which IL-13 has been shown to play a central role (7, 8, 49). Our results confirm this conclusion for Th2 models using a different Ag. In mice that developed polarized Th2 responses to Asp Ag, airway hyperreactivity, goblet cell hyperplasia, and inflammation were all blocked by IL-13R-Fc. However, in mice that developed mixed T cell responses (Th1 and Th2) to Asp Ag, blockade of IL-13 reduced the numbers of mice that developed airway hyperreactivity.
reduced goblet cell hyperplasia, but did not affect inflammation. Furthermore, in the mice with lung injury due to mixed T cell responses, the extent of inhibition of airway hyperreactivity and goblet cell hyperplasia was relatively smaller than that seen in mice that had Th2-induced lung injury. This may be because airway hyperreactivity developed in a smaller percentage of mice, and because goblet cell hyperplasia developed to a smaller degree in mice with mixed T cell responses. Alternately, the decrease in relative inhibition may be due to mediators other than IL-13, which contributed to airway hyperreactivity and goblet hyperplasia in these mice.

IL-13 inhibition clearly reduced airway and parenchymal inflammation in lung injury due to Th2 responses but not in lung injury due to mixed T cell responses (Fig. 2). This confirms published results showing IL-13’s role in airway inflammation (7, 8), in parenchymal inflammation (9), and in emphysema (50). Therefore, the most likely explanation for the inability to demonstrate a role for IL-13 in inflammation in the model of mixed T cell responses was that a molecule made by Th1 cells during the course of the mixed T cell response in the lungs would modulate the function of IL-13. We hypothesized that this molecule was IFN-γ.

Using recombinant cytokines, our data demonstrate that IFN-γ has double-sided effects on IL-13-induced lung injury. IFN-γ inhibited IL-13-induced goblet cell hyperplasia and the infiltration of the airways with eosinophils and neutrophils. This result provides a mechanistic explanation for the down-regulation of the signs of asthma in allergen-induced lung disease by IFN-γ that has been described (36–39, 41, 42, 46, 47). The inhibition of IL-13 functions in the lungs by IFN-γ is analogous to the inhibition of IL-13-mediated worm expulsion by IFN-γ in the intestines (51). The inhibition by IFN-γ of IL-13 functions in the lungs may recapitulate on the level of a whole organ, the IFN-γ-mediated inhibition of IL-13-induced IL-1R-antagonist expression in macrophages (52), or the IL-13-induced IgE production in human B cells (53).

In contrast, IFN-γ potentiated the effects of IL-13-induced lung injury. Mice challenged with IL-13 and IFN-γ had increased numbers of cells expressing high levels of Ag-presentation molecules, NK cells, and increased levels of IL-6 in the BAL. Lung inflammation (peribronchiolar and alveolar scores) showed an increased trend relative to mice challenged with IL-13 and was significantly increased relative to mice challenged with IFN-γ. Although we were surprised that mice challenged with IFN-γ did not have higher numbers of cells expressing high levels of Ag-presentation molecules in the BAL, this result is supported by a report by Suda et al. (54), showing that IFN-γ induces increased expression of MHC class II in resident lung dendritic cell precursors but not increased migration of circulating dendritic cells to the lungs. The high levels of IL-6 present in BAL samples from mice challenged with IL-13 and IFN-γ may be the result of a synergistic effect of IFN-γ and IL-13 on IL-6 production in bronchial epithelial cells (55). In these mice, IL-6 is most probably not made by macrophages as IL-13 inhibits IFN-γ-induced IL-6 production in macrophages (56). The presence of high numbers of NK cells in the samples of mice challenged with IL-13 and IFN-γ is most probably due to a very specific regulation of chemokines and chemokine receptors that promotes migration of some cells and inhibits migration of others.

The additive and synergistic effects of IL-13 and IFN-γ in the lungs may regulate subsequent immune and inflammatory response to inhaled Ags. We do not know if the high levels of IL-6 present in the airways of mice exposed to IL-13 and IFN-γ have a pro- or anti-inflammatory role. Although IL-6 has been described to be important for the development of Th2 responses (57), it has also been described to inhibit allergen-induced airway disease (58). The same is true for NK cells, as they can differentiate to secrete distinct patterns of cytokines (59). Although NK cells have been shown to be necessary for the full elicitation of lung injury in some allergen-induced models (60, 61), these cells are also important for protection from aberrant chronic inflammation (62). The increase in the numbers of cells expressing high levels of Ag-presenting molecules, MHC class II, and CD96 may predispose the lungs for increased responses to inhaled Ags because CD86 is a critical costimulatory molecule, particularly for the induction of Th2 responses (63–66). Increased capability of Ag presentation in airways exposed to IL-13 and IFN-γ could explain why inflammation is more intense in Ag-challenged mice that were given Ag-specific Th2 and Th1 cells by cell transfer (22–24) or why IFN-γ is requisite for allergen-induced inflammation in some mouse models (67, 68).

In conclusion, although IL-13 is the major effector cytokine of goblet cell hyperplasia, airway hyperreactivity, and inflammation in lung injury induced by polarized Th2 responses, IL-13 is only a partial mediator of the changes in the lungs induced by mixed T cell responses. This divergence is most probably due to the presence of the inhibitor and potentiator of IL-13-mediated changes in the lungs, IFN-γ. Whereas IFN-γ limits IL-13-induced goblet cell hyperplasia and eosinophilic inflammation, it increases numbers of cells expressing high levels of Ag-presenting molecules, NK cells, and IL-6 in the airways. By this mechanism, IFN-γ and IL-13 induce a distinct type of inflammation and may prepare the lungs for increased responses to inhaled Ags.

Acknowledgments

We thank Drs. D. Corry (Baylor College of Medicine, Houston, TX), A. O’Garra, R. Coffman, (DNAX Research Institute, Palo Alto, CA), R. Locksley (Howard Hughes Medical Institute, University of California, San Francisco, CA), J. McKerrow, (Department of Pathology, University of California, San Francisco), D. J. Erle, D. Sheppard (Lung Biology Center, University of California, San Francisco), J. Lee (Mayo Clinic Arizona, Scottsdale, AZ), and Dr. G. M. Turino (Columbia University, New York, NY) for expert advice and reagents. We also thank E. Callas, M. Cook, D. Polakoff, and Dr. J. Cupp (DNAX Research Institute) for cell sorting and the Research Support Team of Genetics Institute for IL-13Rα2-Fc fusion protein.

References


