Environmental Antigen-Induced IL-13 Responses Are Elevated Among Subjects with Allergic Rhinitis, Are Independent of IL-4, and Are Inhibited by Endogenous IFN-γ Synthesis

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*J Immunol* 1998; 161:7007-7014;
http://www.jimmunol.org/content/161/12/7007
Environmental Antigen-Induced IL-13 Responses Are Elevated Among Subjects with Allergic Rhinitis, Are Independent of IL-4, and Are Inhibited by Endogenous IFN-γ Synthesis

Yan Li,* F. Estelle R. Simons,*† and Kent T. HayGlass2*†

Human immediate hypersensitivity diseases represent the most common example of chronic excessive Th2-like activation in developed nations. While IL-13 shares many functional properties with IL-4, the intensity and regulation of environmental Ag-stimulated IL-13 synthesis by allergic vs nonallergic individuals remain ill defined. Here, we examine the intensity of polyclonally and Ag-stimulated IL-13 production by PBMC of 20 subjects with seasonal allergic rhinitis and 20 healthy controls. Polyclonally driven IL-13 responses did not differ significantly (Mann-Whitney U test, p = 0.68). In contrast, the median CD4-dependent IL-13 response among atopics was markedly stronger than nonatopics in Ag-stimulated primary culture (p = 0.0031) and exhibited a strong correlation with IL-5 (r = 0.76, p = 0.0009), but not IL-4 (r = 0.14, p > 0.05), responses. IL-13 production was unaffected by blocking endogenous IL-4 or IL-5 activity or by addition of rIL-4 or rIL-5. In contrast, it was inhibited by low levels of rIFN-γ and strongly enhanced upon addition of neutralizing anti-IFN-γ mAb. Collectively, the data are consistent with a negative regulatory role for endogenous IFN-γ synthesis in controlling the intensity of systemic IL-13 responses evoked in both atopic and nonatopic populations following exposure to common Ags. They also suggest that the elevated levels of IL-4 and IL-5 characteristic of type 2-dominated responses in vivo are without detectable impact on the maintenance of recall Ag-stimulated IL-13 production.


Immediate hypersensitivity diseases represent the most common human immunologic disorder (1). Excessive production of Th2-associated cytokines, in particular IL-4 and IL-5, is thought to be fundamental in their pathogenesis (2–4). IL-13 shares several important characteristics with IL-4, including its ability to enhance MHC class II expression, CD23 expression, B cell switching to IgE, mast cell differentiation, and, via inhibition of monocyte-derived IL-12 synthesis, inhibition of Th1-associated T cell differentiation in vitro (5–12). These findings, taken with recent advances in understanding the degree of overlap in IL-4 and IL-13 receptor usage (13–16), intracellular signaling components, and possible sharing of enhancer elements (17, 18) as well as genetic organization (19, 20) provide strong, albeit indirect, evidence for the participation of IL-13 in promoting or maintaining human immediate hypersensitivity in vivo. At the same time, IL-13 differs from IL-4 in several important respects, notably its production by a broader array of cell types, the extended time course over which it is generated following T cell activation, the absence of IL-13 receptors on T cells, and several functional properties (5, 21–31).

Although naive and Ag-experienced (CD45RA and RO) CD4 and CD8 T cells, activated mast cells, and basophils have all been reported to produce IL-13, the in vitro conditions used to study regulation of this cytokine have been dominantly polyclonal (5–7, 18–29, 32–36). Thus, while anti-CD3, anti-CD28, PMA, calcium ionophore, and other stimuli elicit intense, readily quantified IL-13 responses, the levels of IL-13 protein synthesis observed, and their regulation in healthy and atopic subjects upon Ag-mediated activation, remain ill-defined. Physiologic T cell activation and different polyclonal activators frequently trigger different intracellular signaling pathways (37–41) and, consequently can evoke responses leading to qualitatively different conclusions (42). In this study, we investigated the production of IL-13 in short-term, Ag-driven primary culture of PBMC obtained from healthy, nonatopic subjects and those with seasonal allergic rhinitis. Atopic subjects were found to exhibit markedly stronger IL-13 responses in response to Ag-dependent but not polyclonal activation. The intensity of IL-13 production was negatively regulated by endogenous IFN-γ synthesis in both atopic and nonatopic subjects. The data indicate the complexity of the Th2-associated skewing of responsiveness to environmental Ags seen in atopic individuals and demonstrate that while both IL-4 and IL-13 are markedly elevated among atopics, and can be utilized as indicators of excessive type 2 activation, the levels of production of these two cytokines appears largely independent in Ag-driven responses.

Materials and Methods

Subject evaluation

This study, approved by the University of Manitoba Faculty Committee on the use of Human Subjects in Research, was conducted in 40 subjects ages 18–35 yr who gave prior written informed consent to participation. Twenty subjects had grass pollen allergic rhinitis and 20 were healthy nonatopic controls. Atopic subjects were recruited on the basis of 1) a history of seasonal allergic rhinitis of at least 2 yr duration warranting regular daily treatment with an intranasal glucocorticoid and/or histamine receptor antagonists (except in the 24- to 48-h period immediately before skin testing), and 2) a positive epicutaneous test (wheat diameter >4 mm that of the negative control) to the grass pollen mix used for in vivo and in vitro testing (grass mix 1649, consisting of June/Kentucky blue grass, timothy grass, bromegrass and red-top grass; Bayer, Etobicoke, Ontario, Canada).
None of the subjects had previously received allergen immunotherapy. Control subjects had no history of allergic rhinitis, asthma, or other allergic disorders and exhibited negative epicutaneous tests to the grass pollen mixture used in this study as well as to a panel of 12 common aeroallergens.

**Cell culture**

Whole blood (20 ml) was collected into 1 ml of 2.7% EDTA. PBMC were obtained by centrifugation with Histopaque-1077 (Sigma, St. Louis, MO) within 30 min of blood collection. Cells collected from the interface were washed three times in saline, counted (> 99% viability as determined by trypan blue exclusion), and used for culture immediately or following storage in liquid N₂. Only frozen samples in which viability exceeded 90%, the great majority, were used. PBMC were cultured at a final concentration of 2.5 x 10⁶/ml for Ag stimulation or 1.5 x 10⁶/ml with PHA using 96-well U-bottom plates (Nunc, Naperville, IL) in RPMI 1640 supplemented with 10% FCS, 10 mM l-glutamine, 2 x 10⁻⁵ M 2-ME, and antibiotic-antimycotic (Life Technologies, Burlington, Canada). For each subject, a minimum of two wells were set up for each condition tested. Cells were cultured 1) in the absence of stimuli; 2) with grass pollen extract (Bayer) at 400 µg/ml alone and in the presence of anti-CD4 (PharMingen, Mississauga, Canada), anti-DR, DP, DQ (PharMingen), or normal mouse IgG (Sigma, St. Louis, MO). All samples were evaluated in at least two assays, with the results of three points falling on the linear portion of titration curves calibrated against human IFN-γ standards of sp. act. 10⁶ U/mg (PharMingen). Each assay was conducted using internal standards of an IFN-γ-containing, PHA-stimulated (Difco) PBMC supernatant, calibrated against a standard (PharMingen) set in each plate. Assay sensitivity was typically 0.25–1.0 pg/ml of IL-4 as calibrated against WHO IL-4 interim standard 88/656 run on each assay plate.

**Cytokine assays**

**IL-13.** A sandwich ELISA using a purified monoclonal rat anti-human IL-13 as capture reagent and a purified, biotinylated polyclonal anti-human IL-13 (PharMingen) in combination with streptavidin–alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA) was used. Internal IL-13 standards of sp. act. 10⁶ U/mg (PharMingen) were included on each plate. The lower limit of detection was typically 8.4 pg/ml.

**IL-5.** A sandwich ELISA was also used for IL-5 detection with a purified anti-human IL-5 mAb (TRFK5, PharMingen, ND 50 60 ng/ml to neutralize the activity of 1 ng/ml rIL-5); rIFN-γ (PharMingen) in combination with streptavidin–alkaline phosphatase (PharMingen) as capture reagent and a purified, biotinylated polyclonal anti-human IL-5 (PharMingen) for Ag capture and biotinylated JES1–5A10 (PharMingen). IL-5 levels in each sample were calculated against a standard (PharMingen) set in each plate. Assay sensitivity was typically 8.4 pg/ml.

**IL-4.** Levels were evaluated using CT.h4S cells in an MTS assay (43) that detected 0.25–1.0 pg/ml of IL-4 as calibrated against WHO IL-4 interim standard 88/656 run on each assay plate.

**IFN-γ.** IFN-γ was determined in ELISA using mAbs obtained from PharMingen. Each assay was conducted using internal standards of an IFN-γ-containing, PHA-stimulated (Difco) PBMC supernatant, calibrated against human IFN-γ reference reagent G3 29-901-530 (1 National Institutes of Health unit = 115 pg, provided by Dr. C. Laughlin, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD). All samples were evaluated in at least two assays, with the concentration of cytokine in each supernatant calculated from a minimum of three points falling on the linear portion of titration curves calibrated against recombinant cytokine standards serially diluted on each plate. SE typically ranged from 3 to 10%.

**Statistical analysis**

For individual subjects, the mean (±SEM) is shown. Median values for each group, indicated by a bar, and range are shown for each population. Statistical significance was evaluated using the Mann-Whitney U test. Correlations were determined using nonparametric statistics (Spearman’s rank sum).

**Results**

**Kinetics of allergen-driven IL-13 and IL-5 production**

We first examined the time course of IL-13 production in response to Ag-specific and polyclonal stimulation. Culture supernatants were harvested at day 1, 2, 3, 5, and 7. PHA-stimulated IL-13 was readily detectable by 24 h and increased for the duration of the experiment (not shown), whereas grass pollen-stimulated IL-13 (Fig. 1) was undetectable until 48–72 h, peaking at 5–7 days of culture (Fig. 1). Data shown are from four of eight subjects for whom extensive time course analyses were conducted. The kinetics of IL-13 responses to Ag did not differ between atopic and nonatopic subjects. Similarly, Ag-stimulated IL-5 production was first detectable by day 3–5, peaking at days 5–7 (Fig. 1). As previously reported for IL-10 (42, 44), cultures conducted in the absence of antigenic stimulation exhibited low but detectable cytokine production that increased over the duration of culture with peak median responses of 13 and 21 pg/ml for IL-5 and IL-13, respectively, in these experiments. This is in marked contrast to a report that atopic dermatitis subjects spontaneously release large amounts of IL-13 (median >4000 pg/ml at 24 h) and IL-4 (>100 pg/ml) in the absence of in vitro activation while nonatopic subjects had responses that were lower but still well above that seen here (45).

**Polyclonally stimulated IL-13 synthesis is equivalent among PBMC from grass pollen-sensitive and control subjects**

To date, IL-13 synthesis by fresh PBMC, T cell or basophil populations has primarily been studied in response to polyclonal activators such as various combinations of anti-CD3, anti-CD28, Con A, PHA, PMA, and/or Ca²⁺ ionophore. IL-13 production to PHA is of more rapid onset, is markedly more intense than that seen following Ag stimulation, and, most importantly, is of equivalent intensity in atopic and nonatopic groups (Fig. 2. Mann-Whitney p > 0.05). Similar data were obtained at day 7 (medians: atopic 26,233 vs nonatopic 16,751 pg/ml, n = 20/group, Mann-Whitney p > 0.05). A statistically significant but weak elevation of polyclonally evoked IL-5 synthesis was observed in the allergic population at day 5 (medians 227 vs 107 pg/ml, p = 0.013). We previously reported that polyclonally elicited IL-4 responses in a similar comparison did not differ significantly between atopics and nonatopics (42).

**IL-13 production to environmental Ags is significantly higher among allergic individuals than healthy nonatopic subjects**

Reasoning that Ag-mediated activation would provide a more biologically relevant comparison of responses in atopic and normal...
subjects, we evaluated IL-13 and IL-5 synthesis in grass pollen-stimulated primary culture (Fig. 3). Ag-elicited IL-5 responses were markedly elevated among individuals with seasonal allergic rhinitis \( (p = 0.0008) \). In marked contrast to IL-4 (data not shown and 46), IL-13 was produced at detectable levels by every individual in both atopic and nonatopic groups upon Ag-mediated stimulation. Median IL-13 responses were 609 pg/ml vs 238 pg/ml at day 5 and 874 pg/ml vs 406 pg/ml at day 7 for atopic and nonatopic subjects, respectively. Thus, environmental Ag-driven IL-13 production was consistently higher among atopic subjects \( (p = 0.0031 \) and 0.0087 on day 5 and day 7, respectively). The median cytokine responses in the absence of Ag ranged from 32–84 pg/ml for IL-13 and 17–39 pg/ml for IL-5. They did not differ significantly between atopic and nonatopic subjects for either cytokine at either time point examined.

To ensure that the cytokine synthesis observed was truly Ag-stimulated, further analysis was conducted for three subjects in each group. As evident from Fig. 4, the addition of anti-CD4 mAb or anti-DR,DP,DQ strongly inhibited IL-13 (and IL-5, data not shown) synthesis while control murine IgG was without effect, indicating that the vast majority of the grass pollen-stimulated IL-13 production observed is CD4 dependent and requires activation via the MHC class II pathway.

**Elevated IL-13 synthesis among subjects with allergic rhinitis is independent of the intensity of the IL-4 response but is closely associated with IL-5 responses**

We previously demonstrated that the allergen-specific cytokine response in grass pollen allergic subjects is characterized by markedly elevated IL-4 synthesis and reduced IFN-\( \gamma \) synthesis relative to normal, nonatopic subjects (46, 47). In marked contrast to IL-13 or IL-5 production, where the difference between these populations is quantitative (Fig. 3), IL-4 responses were undetectable in virtually all healthy subjects and ranged from 2 to 30 pg/ml in atopics (data not shown). Statistical analysis of the relationship between inhalant Ag-dependent IL-4, IL-5, and IL-13 synthesis in primary culture revealed that while IL-5 and IL-13 are highly correlated, the intensity of the IL-4 response observed was not correlated with either IL-13 (Fig. 5) or IL-5 (data not shown). If subjects are stratified for clinical phenotype (atopic vs nonatopic), the intensity of the Ag-driven IL-4 responses remains independent of IL-13 levels (Spearman’s \( p > 0.05 \) in both instances).
In light of the role that IL-4 plays in shaping initial commitment to the Th1/Th2 phenotypes, we subsequently investigated the impact on recall IL-13 responses of 1) the addition of physiologically relevant levels of rIL-4 and 2) blocking endogenous IL-4 activity in vitro during grass pollen-mediated activation. Thus, PBMC were stimulated with grass pollen Ag in the presence of rIL-4 vs neutralizing anti-IL-4 Ab or, in subsequent experiments, rIL-5/anti-IL5 mAb. Atopic and nonatopic subjects were selected to represent the range from those yielding virtually undetectable (<8 pg/ml) to very high levels of Ag-dependent IL-13 production. As detailed in Fig. 6 for six representative subjects of the 16 examined, Ag-driven IL-13 production was not affected either by blocking endogenous IL-4 activity nor by supplementing cultures with rIL-4, even at levels greatly in excess of those seen upon Ag stimulation (i.e., 500 pg/ml). In independent experiments, rIL-5 added at ~20-fold the median Ag-driven response or neutralizing mAb to IL-5 also did not affect recall Ag-driven IL-13 responses in the six subjects examined (Table I).

In marked contrast, the addition of rIFN-γ at low levels (i.e., levels similar to and those 5- to 10-fold above those seen in Ag-driven culture (46)) markedly inhibited expression of IL-13 responses (Fig. 7). Moreover, the addition of neutralizing anti-IFN-γ mAb to block the activity of endogenous IFN-γ enhanced IL-13 synthesis in most subjects by several fold. Collectively, these data are consistent with a role for endogenous IFN-γ synthesis in controlling expression of environmental Ag-driven IL-13 synthesis but argue that the elevated levels of IL-4 and IL-5 characteristic of type 2-dominated responses in vivo are without detectable impact on the maintenance of recall Ag-stimulated IL-13 production.

Discussion

IL-13 exhibits a number of characteristics in common with IL-4, suggesting that it may play an important role in vivo in maintenance of immediate hypersensitivity. While numerous studies on the biology of IL-13 activity and regulation are available, the great majority rely exclusively upon polyclonal stimuli. We report here that Ag-mediated stimulation in short-term primary culture reveals that subjects with allergic rhinitis to common environmental inhalant Ags, the most common form of human immediate hypersensitivity, exhibit markedly elevated IL-13 production relative to normal, nonatopic controls. The intensity of IL-13 production is highly correlated with the intensity of Ag-driven IL-5 responses but is essentially independent of IL-4 as demonstrated by 1) the relationship between the levels of cytokine (protein) observed in primary culture, 2) the failure of high levels of rIL-4 or rIL-5 to alter IL-13 recall responses, and 3) the lack of impact that neutralizing endogenous IL-4 or IL-5 activity has on IL-13 synthesis. In contrast, the addition of low levels of exogenous rIFN-γ, or neutralization of endogenous IFN-γ, substantively alters allergen-driven IL-13 synthesis. While it needs to be kept in mind that these conclusions are based on net cytokine protein levels in culture supernatants, with the value obtained for each cytokine affected by
 FIGURE 6. Blocking the activity of endogenous IL-4 or the addition of exogenous rIL-4 does not affect recall IL-13 responses to environmental Ag. PBMC were cultured 5 days with Ag (400 μg/ml) alone, in the presence of 5–500 pg/ml rIL-4, or with 10 μg/ml neutralizing anti-IL-4 polyclonal Ab. IL-13 responses by individual allergic (solid symbols) and normal, nonatopic subjects (open symbols) were assessed by ELISA. Culture of cells with IL-4 or anti-IL-4 in the absence of Ag did not elicit IL-13 responses above those in medium alone (data not shown). Data shown are from 6 subjects typical of 16 examined in independent experiments.

The relationship between IL-4 and IL-13 production has been examined by several groups, with a statistically significant association noted in some reports (20, 27, 32) and others finding that they are not correlated (22, 23, 28). Each of the studies cited relied upon polyclonal activation to elicit IL-4 and IL-13. In one of the few studies using Ag-driven activation, Kroegel et al. investigated IL-13 secretion into airways of 10 mild allergic asthmatics and 4 controls following local allergen challenge. While the intensity of IL-13 responses did not correlate with IL-4 levels, it did with eosinophil numbers in bronchoalveolar lavage, suggesting that IL-13 is actively secreted during the late asthmatic response (48). Taken together, the data argue that while the intensity of IL-5 and IL-13 recall responses in both atopic and nonatopic subjects are tightly correlated, the intensity of Ag-dependent IL-4 and IL-13 responses are essentially independent. Endogenous IFN-γ production appears to play a negative regulatory role, dampening the intensity of consumption and cross-regulation, this applies to any system utilizing in vivo or in vitro restimulation.

As an alternative approach to investigate the role that IL-4 may play in modulating IL-13 synthesis in established responses, Huang et al. used Th2 clones derived from TCR transgenic and, independently, IL-4 knockout mice (49). They found similar IL-13 mRNA levels under neutral conditions, when endogenous IL-4 production or IL-4R function was neutralized, and when substantial amounts of rIL-4 were added at restimulation, leading them to conclude that IL-4 does not play a demonstrable role in expression of established IL-13 responses. Collectively, the data suggest that once CD4 T cells are committed to type 2 patterns of activation, IL-4 and IL-5 may have minimal impact on Ag-driven expression of IL-4, -5, or -13, whereas IFN-γ markedly reduces the intensity of recall type 2 cytokine responses in both atopic and nonatopic situations.

Characterization of the relative intensity of IL-13 responses in atopic and normal subjects has yielded varying conclusions. Freshly obtained naive and memory CD4 and CD8 T cells (22, 23, 27), as well as T cell lines and clones (21, 50, 51), from both atopic and control donors exhibit strong IL-13 synthesis following stimulation with anti-CD3/anti-CD28, PMA plus A23187, or anti-CD3 plus rIL-2. Responses to Ag were not examined in any of these studies. Similarly (24, 26, 36), activated basophils secrete substantial quantities of IL-13, but only polyclonal activators or anti-IgE were used to characterize its production. Several studies of PBMC from atopic and nonatopic subjects indicate similar kinetics and comparable or marginally different levels of IL-13, as well as IL-4 and IFN-γ, in the two populations following in vitro (polyclonal) activation (22, 27, 52). However, in light of the data above (Fig. 2, also using polyclonal activation), comparisons of the relative responsiveness of atopic and nonatopic subjects to such stimuli are difficult to interpret.

Looking at mRNA expression, several groups have obtained evidence of elevated IL-13 in atopic dermatitis lesions (45, 53), allergic rhinitis (54), or in the nasal mucosa of atopic subjects after challenge with diesel exhaust particles, an adjuvant believed to promote Th2-associated disease (55, 56). Esnault et al. used RT-PCR and subsequent autoradiography of dot blots following stimulation with PHA plus PMA (57), reporting substantial IL-13 mRNA in both groups. Responses by PBMC from atopics were slightly (p = 0.02) elevated relative to the normal controls.

In Ag-stimulated systems, Essayan et al. (52) evaluated IL-13 production by eight T cell clones derived from one ragweed allergic and one nonatopic control subject. Huang et al. (58) reported increased IL-13 mRNA and protein levels among allergen-challenged BAL of asthmatic and rhinitic subjects compared with saline-challenged healthy controls. Interestingly, no IL-13 synthesis (mRNA or protein) was seen in the two controls before and following challenge with the locally common environmental Ag mixture used (58). Similarly, Ying et al. (59) examined IL-13 gene expression via in situ hybridization and immunohistochemistry subjects with allergic rhinitis or asthma after allergen challenge in vivo. IL-13 mRNA and intracellular protein levels were undetectable prechallenge and in diluent challenged atopics, while atopics exhibited marked increases in IL-13 and IL-4 at skin test sites (i.e., not restricted to nasal or bronchial mucosa) over the course of the late phase reaction. The four controls exhibited IL-13 responses at or below the limits of detection. Humbert (60), comparing IL-13 mRNA levels in atopic and nonatopic asthmatics, nonasthmatic atopics, and healthy controls, found a weak statistically significant increase in IL-13 mRNA levels in mucosal biopsies (p = 0.02) between asthmatics (both atopic and nonatopic) and healthy controls but, surprisingly, no difference between atopic subjects and healthy controls.

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<th>Subject</th>
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<td>A2</td>
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<td>958 ± 78</td>
<td>811 ± 47</td>
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* Cultures were set up with PBMC from atopic and nonatopic subjects as described for Fig. 3, but incorporating rIL-5 (2 ng/ml, ~20X the median Ag-driven IL-5 response), neutralizing anti-IL-5 (2.5 μg/ml, sufficient to neutralize the activity of >4 ng/ml), or control rat IgG (2.5 μg/ml) along with Ag stimulation (400 μg/ml). Data shown indicate mean ± SEM IL-13 levels seen in culture at day 5.
nonatopic controls. In contrast, we observed readily detectable Ag-dependent IL-13 (protein) responses in all of the 40 subjects examined following short-term primary culture of unselected PBMC populations, with differences in IL-13 expression being quantitative.

Previous reports demonstrated that CD4 and CD8 T cells, mast cells, and basophils are capable of substantial (polyclonally driven) IL-13 responses (21–31), with several groups (21, 23, 28) finding CD8 cells to be more potent than CD4 cells. Here, incorporation of anti-CD4 or anti-MHC class II mAbs blocked virtually all IL-13 protein synthesis. Whether this difference is attributable to use of different stimuli or whether it reflects CD4-dependent, CD8-mediated IL-13 synthesis remains to be determined. It is clear that polyclonal and Ag-driven triggering initiate distinct signaling pathways and functional consequences, which can include different patterns of cytokine synthesis (40, 41), and that anti-CD3 can stimulate or inhibit IL-13 production depending on the costimulus used (23). The optimal conditions for (polyclonal) activation of IL-13 are actively inhibitory for IL-4 gene expression (21, 23). In light of the extensive literature indicating that the nature of stimulation conditions plays a pivotal role in influencing cytokine production and that the relationship between these polyclonal stimuli and MHC-dependent Ag processing and presentation remains speculative, these data argue for greater use of Ag as a more physiologic means of activation.

An important caveat to this study is that the cellular profile and interactions in PBMC may differ from those at the site of allergen exposure in vivo (i.e., in nasal mucosa). Indeed, one could hypothesize that the increased IL-4, IL-5, and IL-13 synthesis seen in vitro merely reflects an increased frequency of total CD4 cells in the circulation of atopics. We believe this unlikely as extensive studies in the early 1980s, using well in excess of 200 subjects, revealed that there were no detectable differences in major T cell subsets (i.e., CD3, CD4, and CD8) between PBMC of atopic and nonatopic individuals (61–63). Moreover, the frequency of PBMC exhibiting Ag-driven proliferation (64) or estimates of the frequency of cytokine-producing PBMC (total of IFN-γ plus IL-4-producing cell populations) (65), as determined by limiting dilution analysis, is also highly similar in atopic and nonatopic populations.

Finally, we examined the role of other type 1 and type 2 cytokines in regulation of Ag-driven IL-13 recall responses. We previously demonstrated that markedly higher frequencies of atopic individuals are capable of exhibiting inhalant Ag-driven IL-4 responses in primary culture (47) and that the levels of IL-4 production are substantially elevated (Mann-Whitney $p = 0.000001$), while IFN-γ responses are lower ($p = 0.008$) (42). The role played by IFN-γ in regulation of IL-13 synthesis has also been controversial, with some reporting it to be without effect (22) and others demonstrating that addition of exogenous IFN-γ inhibits IL-13 production (66). Our data, demonstrating that exogenous rIFN-γ inhibits IL-13 while neutralization of endogenous IFN-γ-activity increases IL-13 production (Fig. 7), taken with the known differences in the capacity of normals and atopics to generate IFN-γ responses to allergen stimulation (1–4, 46), argues in support of a role for IFN-γ in IL-13 regulation in vivo. Indeed, it raises the possibility that the elevated IL-13 levels seen in atopic subjects may be secondary to deficient responses to IFN-γ-promoting, Th1-associated cytokines such as IL-12 and IL-18. Both consequences may be related to the finding that PBMC of subjects with seasonal
allergic rhinitis exhibit similar levels of IL-12 production but markedly lower responsiveness to rIL-12 stimulation (as measured by IFN-γ synthesis) than do nonatotics (67) (Y. Li et al., manuscript in preparation).

References


