

IFN- α inhibits IL-3 priming of human basophil cytokine secretion but not leukotriene C₄ and histamine release

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Background: Innate immune responses play a critical role in determining the course of acquired immunity, including that associated with allergic disease. Type I interferons, which are generated early in these reactions, are important soluble factors that prime for T_H1-like activity.

Objective: Because human basophils secrete IL-4 and IL-13 in response to both IgE-dependent and IgE-independent stimuli, we tested whether IFN- α , a major type I IFN, affects the production of these T_H2 cytokines and/or mediator release from these cells.

Methods: Basophils isolated from blood were treated with IFN- α in the presence and absence of IL-3 priming before stimulating through the IgE receptor to release histamine, leukotriene C₄, and IL-4. Effects of IFN- α on IL-3-mediated IL-13 secretion and basophil survival were also tested. IFN- α receptor expression was determined by RT-PCR.

Results: IFN- α specifically inhibited the effects IL-3 has on basophil cytokine secretion. Enhanced secretion of IL-4 resulting from IL-3 priming was significantly inhibited in cells concurrently cultured with IFN- α . This effect was specific for cytokine generation, because histamine and leukotriene C₄ were unaffected. Furthermore, IFN- α blocked IL-13 secretion directly induced by IL-3. Although IFN- β also possessed some inhibitory activity, IFN- γ (a type II IFN) had no effect on basophil cytokine secretion. Basophils constitutively expressed mRNA for the type I IFN receptor, and IFN- α did not affect basophil viability with regard to inhibition of cytokine secretion. **Conclusions:** These results support the belief that early innate immune responses resulting in IFN- α production negatively regulate allergic responses by also inhibiting priming of basophil cytokine release. (*J Allergy Clin Immunol* 2003;112:944-50.)

Key words: IgE, innate immunity, hypersensitivity, cytokine priming, histamine, leukotriene

Basophils are one of several cell types known to selectively infiltrate allergic lesions, both naturally and when experimentally induced.¹⁻⁴ The significance of these

Abbreviations used

IFN- α R: IFN- α receptor
IMDM: Iscove's modified Dulbecco's medium
LTC₄: Leukotriene C₄
PDC: Plasmacytoid dendritic cell
PIPES: Piperazine-N,N'-bis-[2-ethanesulfonic acid]

cells in allergic inflammation has increased during the past decade with evidence that they secrete large quantities of the pro-inflammatory cytokines, IL-4 and IL-13, in addition to releasing the potent inflammatory mediators, histamine and leukotriene C₄ (LTC₄).⁵ Activation through the IgE receptor plays a critical role in the release of all these products. Furthermore, several cytokines markedly enhance this response in what is often referred to as "priming." Of these priming cytokines, none seem to have greater activity than IL-3, which not only primes for IgE-mediated release but is also known to directly induce the secretion of IL-13, particularly from basophils of allergic subjects.⁶⁻⁸ In contrast to the priming qualities of IL-3, there are currently no reports of cytokines that suppress basophil mediator release and/or cytokine generation.

IFN- α is a major member of the type I IFN family.⁹ Although initially characterized for its potent antiviral activity, there is increasing evidence that IFN- α also plays a significant role early on in the course of T_H1-like immune responses. Among the more recent activities attributed to this cytokine is its ability to induce dendritic cell maturation, enhance the cytotoxic activity of macrophages and natural killer cells, support antibody production by B cells, and promote T_H1-like responses by increasing the expression of IL-12 and IFN- γ .¹⁰ Although the cell most responsible for IFN- α production has remained elusive for many years, there is mounting evidence that plasmacytoid predendritic cells account for more of this cytokine than any other immune cell.¹¹ When stimulated with viral replication products, such as double-stranded RNA or unmethylated CpG motifs of bacterial DNA, these cells are capable of producing 200 to 1000 times more IFN- α than any other white blood cell.¹² These observations, in part, have led to the belief that IFN- α plays an essential role in linking innate and acquired immune responses.¹³

As the first available therapeutic cytokine, IFN- α has also been widely used in the treatment of a variety of

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neoplastic conditions,¹⁴ chronic hepatitis B and C infection,^{15,16} hypereosinophilic syndrome,¹⁷ systemic mastocytosis,¹⁸ and histiocytosis.¹⁹ Moreover, there are recent reports of IFN- α being used in the treatment of steroid-resistant asthma and intractable atopic dermatitis with favorable results, even though no definitive mechanisms of action have been determined.²⁰ In accordance with these latter findings, Bufe et al²¹ have shown a significantly lower amount of virus-induced IFN- α in patients with allergic asthma than in healthy children and patients with nonallergic asthma, suggesting that its decreased expression might account, in part, for these conditions.

In this study, we show evidence for the first time that type I interferons, specifically IFN- α , inhibit the effects IL-3 has on basophil cytokine secretion, suggesting one mechanism by which innate immune responses might negatively regulate allergic reactions.

METHODS

Special reagents

The following reagents were purchased: crystallized human serum albumin (Calbiochem-Behring Corp, La Jolla, Calif); piperazine-*N,N'*-bis-[2-ethanesulfonic acid] (PIPES), FBS, and crystallized BSA (Sigma Chemical Co, St Louis, Mo); gentamicin, Iscove's modified Dulbecco's medium (IMDM), nonessential amino acids (100 $\times$ stock), and 0.4% trypan blue (Life Technologies, Inc, Grand Island, NY); Percoll (Pharmacia Biotech, Inc, Piscataway, NJ); recombinant human IL-3, IFN- α 2, IFN- β , and IFN- γ (Biosource, Inc, Camarillo, Calif). The polyclonal anti-human IgE antibody used in these experiments was made in a goat.²²

Special buffers and media

PIPES buffer $\times 10$ (250 mmol/L PIPES, 1.10 mol/L NaCl, 50 mmol/L KCl, pH 7.3) was stored at 4°C as a stock solution. PIPES-albumin-glucose was made by diluting 1 part 10 $\times$ PIPES with 9 parts deionized water and contained 0.003% human serum albumin and 0.1% D-glucose. PIPES-albumin-glucose-EDTA additionally contained 4 mmol/L EDTA. Isotonic Percoll (referred to in this article as 100% Percoll) was prepared by mixing 1 part 10 $\times$ PIPES and 9 parts Percoll. Working solutions of Percoll at 55% (density, 1.072 g/mL) and 61% (density, 1.081 g/mL) were made by mixing the appropriate amounts of 100% Percoll with 1 $\times$ PIPES. Conditioned medium (C-IMDM) consisted of IMDM supplemented with 5% heat-inactivated (56°C for 30 minutes) FBS, 1 $\times$ nonessential amino acids, and 5 μ g/mL gentamicin.

Cell purification and culture

Basophils were purified from either fresh blood or from residual cells of normal donors undergoing leukapheresis. Venipuncture was performed on nonmedicated consenting adults (age range, 21-55 years) by use of the guidelines approved by either the Western Institutional Review Board (Seattle, Wash) or by the Johns Hopkins University Institutional Review Board. Subjects were not selected on the basis of their allergic status. Mixed-leukocyte suspensions containing basophils were prepared by use of double-Percoll density centrifugation, as previously described.²³ The basophils were additionally purified to 95% to 99.9% by negative selection by use of an antibody cocktail and microbead protocol (StemCell Technologies, Vancouver, Canada) followed by gravity filtration through a magnetized LS-column attached to a MidiMACS magnet (Miltenyi Corp, Auburn, Calif). The percentages of basophils were determined by counting Alcian blue-positive and Alcian blue-negative

stained cells on a Spiers-Levy chamber.²⁴ Cells were cultured in 96-well flat-bottom microtiter plates (in duplicate) with C-IMDM and were warmed to 37°C, 5% CO₂ before adding reagents that were also equilibrated to these conditions. For the concurrent detection of histamine, LTC₄, and IL-4 after IgE-mediated activation, basophils were preincubated in C-IMDM alone or with the indicated concentrations of IL-3, interferons, or a combination of both. After 18 hours of incubation, the cells were then activated with an anti-IgE antibody (final concentration of 10-25 ng/mL) and cultured for an additional 4 hours. In experiments investigating IL-3-dependent IL-13 secretion, interferon and IL-3 were added simultaneously, and the cells were cultured for 18 to 20 hours as previously described.⁷ Portions of the cell-free supernatants were analyzed for histamine and LTC₄ by use of automated fluorimetry and RIA, respectively, and according to protocols previously detailed.^{23,25} Cytokine protein was detected by ELISA using IL-4 (e-Bioscience, International, Camarillo, Calif) and IL-13 (Immunotech, Westbrook, Me) commercial kits.

RNA isolation

Total RNA was isolated from 0.5 to 1.0 $\times 10^6$ basophils using the RNazol protocol (Tel-test Inc, Friendswood, Tex). After isopropanol precipitation, the RNA was washed with 70% ethanol and nearly dried under vacuum. Subsequently, the RNA was reconstituted in diethylpyrocarbonate-treated water and stored at -70°C.

Detection of IFN- α receptor mRNA by RT-PCR

RT-PCR was performed using the GeneAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, Conn), as described elsewhere.²⁶ Both the forward primer sequence: 5'-AGTGTAGTGGGCTTG-GATGGTTTAAGC-3' and the reverse primer sequence: 5'-TCTG-CCTTTCACACAATATACAGTCAGTGG-3' for the IFN- α R₁ subunit (accession No. NM 000629) have been previously reported.²⁷ Resolution of the cDNA product (765 bp) after amplification was done in 3% agarose gels. Imaging was performed with an Electrophoresis Documentation and Analysis System 290 (Kodak Scientific Imaging Systems, New Haven, Conn).

Basophil viability

Basophils were cultured in C-IMDM alone, with IL-3 (100 pg/mL) or IFN- α (100 U/mL), and with a combination of these 2 cytokines. Basophil viability under these conditions was determined after 1, 24, 48, 72, 96, and 120 hours incubation using trypan-blue exclusion with enumeration of the cell counts made using a Spiers-Levy chamber.

Statistical analysis

Data are presented as mean $\pm$ SEM unless otherwise indicated. Statistical analysis was performed with SPSS 10.0 software (SPSS, Inc, Chicago, Ill). Nonparametric tests (Wilcoxon signed rank test or Friedman test as indicated) were used to determine the statistical significance of differences. *P* values less than .05 were considered significant.

RESULTS

The effects of IFN- α on IgE-mediated cytokine and mediator release by IL-3-primed human basophils

In the first series of experiments, we tested the effects of IFN- α on IgE-mediated cytokine and mediator release using various culture conditions that we routinely use in screening the effects of pharmacologic and biologic sub-

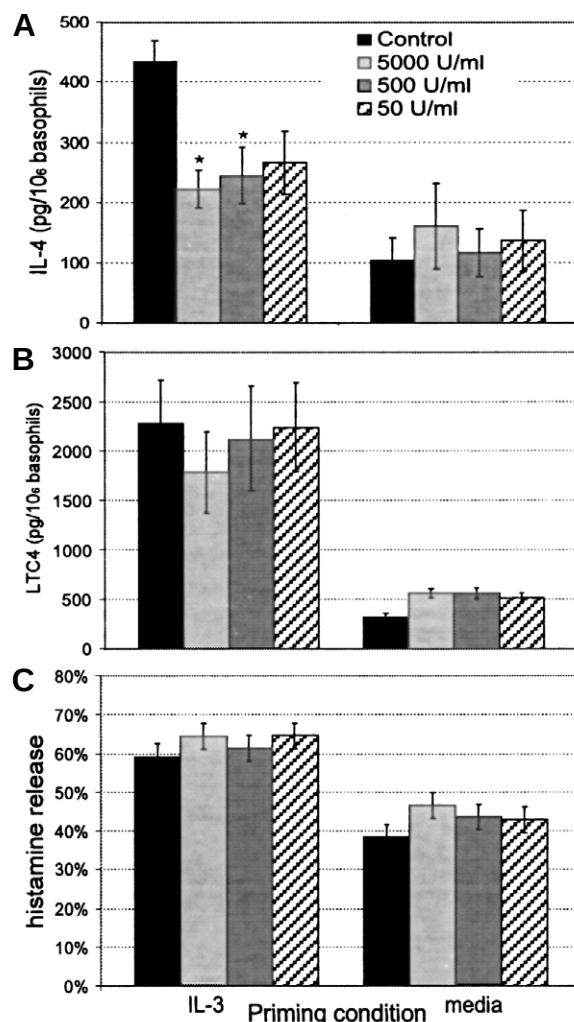


FIG 1. Effect of IFN- α on the IgE-mediated secretion of IL-4 (A), LTC₄ (B), and histamine (C) with and without IL-3 priming. Basophils were cultured with and without IL-3 (100 pg/mL) and increasing concentrations of IFN- α . Cells were activated after 18 hours with the addition of anti-IgE antibody (10–20 ng/mL). Supernatants were harvested 4 hours later for concurrent measures of histamine, LTC₄, and IL-4. Results are the average $\pm$ SEM of 4 experiments using cells from different donors. *Denotes statistical significance ($P < .05$).

stances on basophil secretion. Although short (eg, 15 minutes) preincubations with IFN- α produced no identifiable effects on the IL-3 priming of mediator release and cytokine secretion (data not shown), this type I IFN did affect basophil function after an 18-hour preincubation. **Fig 1, A** shows the effects of IFN- α on the IgE-mediated release of IL-4 in both the presence and absence of priming with low concentrations of IL-3 (100 pg/mL) for 18 hours. Whereas increasing concentrations of IFN- α had no effect on the IL-4 secreted in response to anti-IgE alone (ie, without IL-3 priming), the 4.5-fold increase in IL-4 achieved with IL-3 priming was significantly inhibited at concentrations of 500 and 5000 U/mL IFN- α . In contrast to this inhibitory effect of IFN- α on the priming of IgE-mediated IL-4 secretion, only a marginal, yet

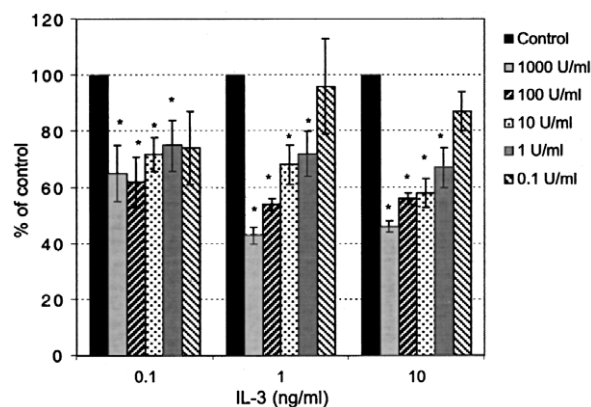


FIG 2. Effect of IFN- α on the IL-3-mediated production of IL-13. Basophils were cultured with the indicated concentrations of IL-3 and/or IFN- α . Supernatants were harvested after 18 to 20 hours incubation and analyzed for IL-13 protein by ELISA. The results from 6 to 8 experiments are expressed as the mean percentage $\pm$ SEM of the control responses obtained in the absence of IFN- α , which were 20 ± 9 , 49 ± 11 , and 107 ± 23 pg/10⁶ basophils using IL-3 at 0.1, 1, and 10 ng/mL, respectively. *Denotes statistical significance ($P < .05$).

insignificant, inhibition of LTC₄ was observed even with the highest (5000 U/mL) concentration tested (**Fig 1, B**). Moreover, IFN- α produced no effects on the histamine released in these cultures, either in the presence or absence of IL-3 priming (**Fig 1, C**).

Effect of IFN- α on IL-3-mediated IL-13 production

Because IFN- α precluded IL-3 priming of IgE-mediated IL-4 secretion, we decided to investigate whether this type I IFN would also affect other IL-3-dependent basophil responses. In particular, we, and others, have shown that basophils secrete large amounts of IL-13 when exposed to IL-3 alone.^{6,28} Therefore, we tested a wide range of IFN- α concentrations on this response, adding them simultaneously with several concentrations of IL-3 and culturing basophils for 18 to 20 hours to measure IL-13 protein. As shown in **Fig 2**, IFN- α inhibited IL-13 production from basophils in a dose-response fashion when activated with IL-3 at either 1 or 10 ng/mL. In fact, a significant 20% to 30% inhibition was observed with as low as 1 U/mL IFN- α , and a nearly 60% inhibition was seen with 1000 U/mL. Although significant inhibition by IFN- α was also seen when IL-13 was induced with 0.1 ng/mL IL-3, the much lower levels of IL-13 (20 ± 9 pg/mL) secreted under these conditions likely account for the lack of discernible dose-response inhibition.

Effect of type I versus type II interferons on IL-3-mediated IL-13 production

IFN- β represents another well-characterized type I IFN, which, like IFN- α , is produced primarily by plasmacytoid dendritic cells (PDCs) in response to viral infection.¹¹ We therefore tested this IFN along with IFN- α to compare whether these substances produced similar inhibitory activity. In addition, we compared the effects

of the type II IFN, IFN- γ , which unlike type I IFN is produced primarily by T cells and natural killer cells and is known to bind receptors other than those for IFN- α and IFN- β . As shown in Fig 3, IFN- α once again significantly inhibited IL-13 secretion when stimulating concentrations of IL-3 at 10 ng/mL were used. Inhibitory activity (~35%) was also observed with IFN- β , but only at the highest concentration tested (ie, 100 U/mL), which did not reach significance ($P = .08$). In contrast, there was no evidence for inhibition of IL-13 mediated by IFN- γ , which is consistent with the belief that suppression of this response is specific only for type I IFN.

Human basophils constitutively express IFN- α R mRNA

Both IFN- α and IFN- β are reported to signal through a common receptor, IFN- α R, for which the former IFN is thought to mediate greater activity. We therefore proceeded to determine whether basophils express this receptor. Fig 4 shows conventional RT-PCR results demonstrating that mRNA for IFN- α R is constitutively expressed in basophils. It is most important to note that these cells were not manipulated in any way other than being purified from blood. Furthermore, an amplicon of 765 bp was detected in all 5 of the basophil preparations and was qualitatively similar to that observed in a T-cell preparation used as a positive control.

Effect of IFN- α on human basophil viability

Evidence exists that therapeutic administration of IFN- α causes a sustained decrease in the circulating counts of various immune cells, including basophils.²⁹ We therefore thought it possible that a mechanism of IFN- α -mediated inhibition of basophil cytokine secretion might simply result from an ability to induce basophil cell death over a prolonged time. Although this seemed unlikely in light of the antiapoptotic and survival-enhancing properties that IL-3 has on basophils, we did test whether coculture with IFN- α might reverse this effect of IL-3. As shown in Fig 5, IL-3 markedly enhanced the survival of basophils cultured during a 5-day period, as expected, with approximately 50% of the cells viable at the end of 5 days compared with 15% survival in medium alone. More importantly, the addition of IFN- α (100 U/mL) did not significantly reduce this IL-3-mediated survival, especially during the first 72 hours of culture. Finally, there was some evidence that IFN- α alone provided slightly better survival conditions than those observed for cells cultured in medium alone, although no significant differences were identified. From these data it was concluded that IFN- α does not significantly affect basophil viability to the extent of inhibiting cytokine secretion.

DISCUSSION

Many studies have been performed during the past 15 years showing that IL-3 is effective in priming human basophils for IgE-mediated responses, including both

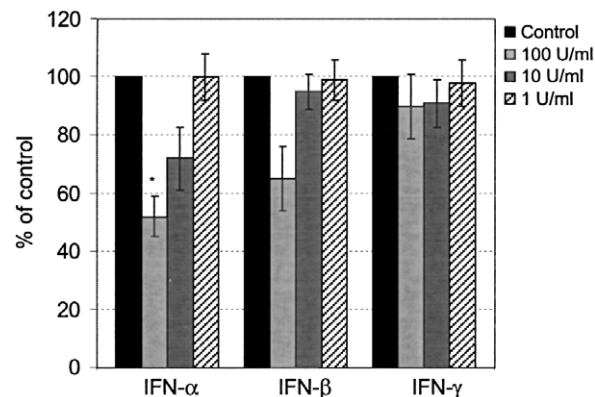


FIG 3. Inhibition of IL-3-mediated IL-13 secretion by type I vs type II IFN. Basophils were stimulated with IL-3 (10 ng/mL) alone or in the presence of the indicated concentrations of IFN- α , IFN- β , and IFN- γ . Supernatants were harvested after 18 to 20 hours of incubation and analyzed for IL-13 protein by ELISA. The results from 5 experiments are expressed as the mean percentage $\pm$ SEM of the control response with IL-3 alone, which was 102 ± 32 pg/ 10^6 basophils. *Denotes statistical significance.

mediator release and cytokine secretion.^{23, 30-34} More recently, IL-3 has also been shown to directly activate basophils for IL-13 secretion and to promote their survival in culture.^{6,8,35} The enhancing effects of IL-3 are further substantiated by its ability to reverse the inhibitory effects that glucocorticosteroids have on mediator release.³⁶ In contrast, we show for the first time that IFN- α , a type I IFN, specifically inhibits the ability of IL-3 to promote cytokine secretion in basophils, an important finding, considering it did not prevent cytokine produced in response to IgE-mediated stimulation alone (Fig 1). In particular, we found that IFN- α reduced IL-3 priming of IgE-mediated IL-4 secretion and inhibited the capacity of IL-3 to directly induce IL-13 generation. Surprisingly, IFN- α did not affect the priming of IgE-mediated histamine release and LTC₄ generation, suggesting that it targets an IL-3-dependent pathway(s) specific only for cytokine production. Furthermore, there was no evidence that IFN- α reversed the survival-enhancing capabilities of IL-3, which might otherwise account for its inhibitory effect on cytokine secretion (Fig 5).

The biologic significance of IFN- α inhibiting basophil cytokine secretion mediated by IL-3 is speculative at this time, yet we believe it relates to the T_H1-like priming capabilities of this cytokine. For example, IFN- α has the ability to “prime” T cells for increased IFN- γ production by making them more responsive to IL-12 through the upregulation of STAT4.³⁷ As mentioned, recent studies show that PDCs account for the greatest production of this cytokine, particularly after viral infection or exposure to certain CpG-DNA oligonucleotides, both of which favor T_H1-like responses. As a result, the data presented in this study are consistent with the T_H1-promoting activities of IFN- α by showing that it suppresses the generation of IL-4 and IL-13, both of which are hallmark

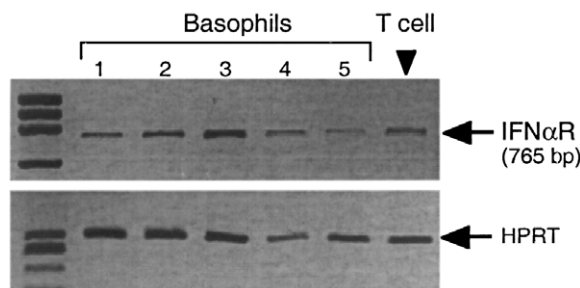


FIG 4. Expression of type I IFN receptor (IFN- α R) in human basophils. Total RNA was extracted from basophil (exceeding 97% purity) and T-cell pellets immediately after isolation from blood. Conventional RT-PCR was performed as described in the "Methods." Shown is a gel image of the amplicons generated from 5 different basophil preparations and 1 T-cell sample. HPRT expression is shown for comparison.

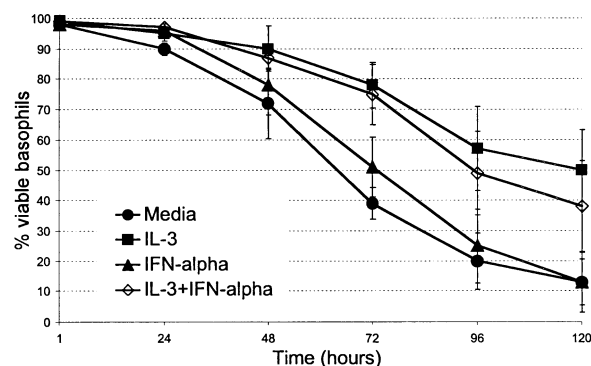


FIG 5. Effect of IFN- α on basophil survival in vitro. Basophils were cultured in medium alone or with the indicated concentrations of IFN- α (100 u/mL) and/or minimal IL-3 (100 pg/mL). Cell viability was assessed at the time points shown. Values represent the mean $\pm$ SEM of 3 experiments.

T_H2 -like cytokines. The fact that this occurs in basophils is most intriguing and suggests a more direct line of communication between basophils and PDC. To add to this complexity, both IL-4 and IL-13 are reported to inhibit IFN- α by PDC.³⁸ Therefore, basophils and PDC each secrete at least 1 regulatory cytokine that potentially down regulates the cytokine-producing capabilities of the other. Although it has long been suspected that such a control mechanism might exist between basophils and T_H1 lymphocytes, we have yet to find a T_H1 -like cytokine possessing inhibitory activity equal to that mediated by IFN- α (JTS, unpublished data). In fact, this is exemplified in this study by the observation that IFN- γ (a classic T_H1 cytokine) had no inhibitory effect on the production of IL-13 by basophils treated with IL-3 (Fig 3).

There is a long history of reports demonstrating that basophils of allergic subjects possess a primed phenotype and are hyperresponsive in nature.³⁹⁻⁴¹ More recent findings indicate that chronic allergen exposure plays a significant role in this increased responsiveness. For example, we have shown that basophils spontaneously secrete IL-13 after repeated nasal allergen challenge.⁴² Although the simplest explanation for this finding is that allergen exposure induces cytokines (eg, IL-3) that systemically prime basophils, it is intriguing to think that immune responses resulting in IFN- α production might, in fact, inhibit the priming of these cells. In this respect, the ability of IFN- α to inhibit priming is likely not limited to just the basophil. Indeed, several studies have presented evidence that IFN- α also decreases T_H2 cytokine secretion (and mediator release) in eosinophils and mast cells.⁴³⁻⁴⁵

Although there is currently no in vivo evidence supporting IFN- α 's role in preventing the priming of any cell type involved in allergic inflammation, there are clinical studies showing that its expression is reduced in allergic disease compared with nonallergic conditions. In a retrospective study involving 88 children, Bufe et al²¹ reported a significant correlation between the presence of an atopic phenotype and reduced levels of IFN- α . Approximately 2-fold to 3-fold lower levels of IFN- α were produced by mononuclear cells obtained from subjects diag-

nosed with allergic asthma than with normal controls. Interestingly, this reduction was only evident in subjects with allergic asthma with measurable allergen-specific IgE, whereas normal levels of IFN- α were produced by PBMCs of children seen with nonatopic asthma. At least 1 study has also reported reduced serum levels of IFN- α in a patient seen with atopic dermatitis.⁴⁶ Accordingly, these clinical observations have led to the belief that IFN- α plays a significant role in reducing the atopic state. It therefore follows that its ability to antagonize the enhancing effects of IL-3 on basophil IL-4 and IL-13 secretion accounts, in part, for this negative regulation.

Clinical case studies arising from IFN- α therapy also support the concept that this cytokine is an important modulator of T_H1 -type and T_H2 -type immune responses. For example, there have been reports that injections of IFN- α have led to exacerbation of psoriasis and/or autoimmune conditions, both of which are characterized by T_H1 -like responses.^{47,48} As noted previously, IFN- α has been used with success in treating neoplastic conditions such as hypereosinophilia, mastocytosis, and chronic myelogenous leukemia. All these involve the uncontrolled growth of immune cells for which growth factors (such as IL-5, stem cell factor, and IL-3, respectively) likely have a positive influence. The high doses of IFN- α used in treating these conditions are thought to play a role in blocking the growth-enhancing capabilities of these growth factors.²⁹ It therefore seems possible that this is related to our observations of IFN- α inhibiting IL-3 priming for cytokine production. However, it is important to note that the inhibitory concentrations of IFN- α used in our study (ie, 10-100 U/mL) are well below the estimated serum concentration (~ 750 U/mL) achieved with minimal therapeutic doses (eg, 3×10^6 units). Whether low-dose IFN- α therapy would be suitable for allergic disease remains to be seen, although at least 1 study has successfully used this cytokine in treating steroid-resistant asthma.⁴⁹

Finally, it is difficult at this time to predict the mechanism(s) by which IFN- α inhibits IL-3-mediated cytokine secretion in basophils. Once again, the inhibition was specific for cytokine secretion and not mediator release.

Therefore, the recent discoveries regarding the role of IL-3 in modulating mediator release by affecting various intracellular pathways might not fully apply to our findings.^{50,51} Messenger RNA for the IFN- α R subunit of the common type I IFN receptor was detected in basophils and at levels comparable with those found in T cells (Fig 4). Thus, it does seem likely that the IFN- α used in this study mediated signaling through this receptor. This might further explain why some inhibitory activity was observed using IFN- β , which is also known to bind IFN- α R. It is currently not known whether any of the other variants of IFN- α , or other type I IFN's (eg, IFN- ω)⁹ possess similar repressive activity on basophil cytokine secretion.

In summary, these data show that IFN- α is capable of suppressing the enhancing effects IL-3 has on basophil production of IL-4 and IL-13, thus representing the first description of a cytokine possessing this activity. Significantly, this was only true for IL-4 and IL-13 production, because priming for histamine release and LTC₄ synthesis was unaffected. These findings are consistent with the T_H1-like promoting activities of IFN- α , suggesting that this cytokine plays a critical role in down-modulating allergic inflammation.

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