

Interferon- γ Inhibits in vitro Mobilization of Eosinophils by Interleukin-5

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Key Words

Asthma · Eosinophils · Interferon- γ · Interleukin-5 ·
Interleukin-6 · Tumor necrosis factor

Abstract

Background: Th2 cytokines play pivotal roles in allergic inflammation, including eosinophilia, and their actions are antagonized by Th1 cytokines, conferring them therapeutic potential. **Methods:** In this study, we examined the ability of a number of cytokines to suppress the activation of eosinophils that function as effector cells for allergic airway diseases. **Results:** Interleukin (IL)-5, IL-6, and tumor necrosis factor (TNF) induced an eosinophil shape change, whereas interferon (IFN)- γ significantly inhibited the shape change. Other cytokines, including IL-1 β , IL-4, IL-10 and IL-13, had little or only slightly enhancing or reducing effects on the shape change. We further analyzed the IFN- γ effect, showing that pretreatment with IFN- γ strongly suppressed IL-5-induced eosinophil shape change, and cycloheximide (CHX) abrogated the suppression by IFN- γ , suggesting that new protein synthesis is required for the inhibitory effect by this cytokine. In agreement with these results, IFN- γ blocked the eosinophil migration and ERK phosphorylation induced by IL-5, and the addition of CHX restored eosinophil che-

motaxis. **Conclusions:** Collectively, IFN- γ may attenuate eosinophilic inflammation by directly negating eosinophil mobilization.

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Introduction

Polarized immune responses are induced by the cytokine profile and cellular components present at inflammation sites, as well as by the nature of the antigen itself. Allergic airway inflammation is thought to be orchestrated by Th2 cells [1], and is often characterized notably by infiltration of eosinophils that are implicated in airway hyperresponsiveness (AHR) and damage [2]. Numerous proinflammatory (TNF, IL-1 β , and IL-6) and Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) are found in asthmatic lungs, and their levels are increased during exacerbation of asthma after allergen provocation, whereas the levels of anti-inflammatory and Th1 cytokines such as IL-10, IFN- γ , and IL-12 are diminished, providing potential mechanisms for modulation of these cells' function by these cytokines [3, 4]. It is proposed that multiple mediators, including cytokines, lipid mediators, peptides, and nitric oxide, constitute endogenous anti-inflammatory activities that may block the inflammatory processes, and

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deficiencies in such mechanisms may result in increased inflammation and disease severity [5, 6].

The anti-inflammatory cytokines exert their effects at a variety of levels during the inflammatory process. They may not only promote Th1 differentiation and suppress Th2 development at earlier stages, but also direct the functional reorientation of Th2 and effector cells at later stages; Th1 cytokines suppress the differentiation and proliferation of Th2 cells, the release of Th2 cytokines from activated T cells, the production of IgE and local recruitment of eosinophils, and promote production of nitric oxide and apoptosis of T cells and eosinophils [4, 7–9]. The importance of the anti-allergic effects of Th1 cytokines is clearly illustrated by the increased inflammation observed in cytokine- or cytokine-receptor-deficient mice. Mice with targeted disruption of the IFN- γ gene, for instance, exhibited increased airway responsiveness, bronchoalveolar lavage (BAL) eosinophils, and lung CD4 $^{+}$ T lymphocytes compared with wild-type mice, and IFN- γ treatment restores baseline airway responsiveness [10]. Similarly, persisting eosinophilic inflammation and sustained secretion of Th2 cytokines in the lungs are observed in IFN- γ -receptor (IFN- γ R)-deficient mice [11]. However, a few studies show that IFN- γ does not necessarily have anti-allergic effects; mice deficient in IFN- γ exhibit a suppressed allergic response [12]. IFN- γ also promotes eosinophil survival rather than apoptosis *in vitro* [13]. Given that airway eosinophilia is a characteristic feature of airway disease, direct effects of various cytokines, including Th1 cytokines, on eosinophil shape change, which is relevant to eosinophil mobilization and indicative of eosinophil activation, remain to be evaluated. In this study, we focused on the direct effect of the proinflammatory Th1 and Th2 cytokines on eosinophil shape change. Our results reveal that IFN- γ , among the cytokines examined, most strongly inhibits eosinophil shape change and chemotaxis both in the presence and absence of IL-5, and that the inhibitory effect requires protein synthesis.

Materials and Methods

Materials

RPML-1640, FBS, PBS, human serum albumin, and HBSS were purchased from Gibco BRL (Grand Island, N.Y., USA). Reagents for eosinophil preparation and cycloheximide (CHX) were purchased from Sigma (St. Louis, Mo., USA). Recombinant human IL-1 β , IL-5, IL-6, IL-13, IL-18, and TNF were obtained from R&D Systems (Minneapolis, Minn., USA), and IFN- γ , IL-4, and IL-10 were purchased from Pharmingen (San Diego, Calif., USA). Diff-Quick solution was obtained from American Scientific Products (McGaw Park, Ill., USA).

Preparation of Polymorphonuclear Leukocytes and Eosinophils

Blood donors were atopic patients with eosinophils ranging between 10 and 20% of their peripheral blood leukocytes. They had not received any medication during the 4 weeks before blood sampling. After the erythrocytes had been sedimented in 6% dextran-dextrose in 0.1 M EDTA (pH 7.4), the leukocyte-rich cell suspension was centrifuged at 200 g for 5 min at 4°C and then washed with cold PBS. Percoll, FBS, and Pipes buffer (25 mM Pipes, 11 mM NaCl, 5 mM KCl, 40 mM NaOH, and 5.4 mM glucose) were mixed and layered at the indicated volumes in 140 $\times$ 10 mm polystyrene tubes. The leukocyte-rich suspension in 2 ml of the Percoll solution (1.070 g/ml) was overlaid on top of the gradient and centrifuged at 1,600 g for 20 min at 4°C. The obtained polymorphonuclear leukocytes (PMNL) fraction was used for the determination of eosinophil shape change or further incubated with CD16 monoclonal antibody conjugated-microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany) for eosinophil purification, as previously described [14]. The purity and the viability of eosinophils was greater than 98% at the beginning of assays.

Measurement of Eosinophil Shape Change

Gated autofluorescence forward scatter assay was performed to determine eosinophil shape change as previously described [15]. Briefly, PMNL or purified eosinophils were washed with tissue culture medium and RPMI-1640 medium containing 10% heat-inactivated FBS, and were resuspended at a concentration of 2×10^6 cells/ml. Aliquots of cells (2×10^5 cells) were treated with cytokines in a final volume of 100 μ l. The tubes were incubated at 37°C in a 5% CO $_2$ incubator for the indicated time intervals. Alternatively, IFN- γ and/or CHX were used to pretreat the cells, followed by IL-5 treatment for 30 min or 1 h. After incubation, the cells were fixed with 4% paraformaldehyde and analyzed on a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif., USA). The shape change was expressed in two ways. When eosinophil shape changes induced by cytokines alone were compared with the unstimulated eosinophil shape change, the percentage of change in forward scatter (FSC) was used to estimate the extent of increase in the shape change, as determined below:

Change in FSC (%) = (mean FSC from stimulated eosinophils / mean FSC from unstimulated eosinophils) $\times$ 100.

When the effect of IFN- γ and/or CHX on IL-5-induced eosinophil shape change was measured, the percent change in FSC was calculated as follows:

Change in FSC (%) = [(mean FSC induced in the presence of IL-5 – mean FSC induced by IL-5 in the presence of IFN- γ and/or CHX) / (mean FSC induced by medium)] $\times$ 100.

Measurement of Phosphorylated ERK 1/2

ERK 1/2 phosphorylation was measured as previously described [16]. The freshly purified eosinophils were suspended at a density of 2×10^6 cells/ml in HBSS containing 0.5% HSA, pretreated with or without IFN- γ for 60 min at 37°C and then treated with IL-5 or control buffer at 37°C for 10 min. The cell suspension was washed twice with by centrifugation at 600 g for 8 min. The resulting cell pellets were lysed on ice, and the lysate was prepared from supernatant by centrifugation at 10,000 g for 15 min. The protein in the supernatant was run on a Tris-tricine gel (9.8%), and electrophoretically blotted to PVDF membrane (Millipore, Bedford, Mass., USA). The membrane was probed with a 1:1,000 dilution of anti-phospho-p44/42 MAP kinase antibody (Cell Signaling Technology, Beverly,

Table 1. Effect of cytokines on eosinophil shape change (n = 3–5)

Treatment	FSC, mean $\pm$ SD		
	after 10 min of incubation	after 20 min of incubation	after 30 min of incubation
Medium	100.0 $\pm$ 10.4	100.0 $\pm$ 5.9	100.0 $\pm$ 4.9
TNF- α			
5 ng/ml	108.4 $\pm$ 13.5	105.4 $\pm$ 10.4	110.2 $\pm$ 2.1*
0.5 ng/ml	104.3 $\pm$ 13.2	103.4 $\pm$ 8.4	104.2 $\pm$ 6.8
0.05 ng/ml	102.2 $\pm$ 10.8	100.6 $\pm$ 6.5	103.6 $\pm$ 4.7
IL-1 β			
1 ng/ml	100.6 $\pm$ 9.6	99.0 $\pm$ 6.1	101.6 $\pm$ 3.2
0.1 ng/ml	102.7 $\pm$ 9.8	99.1 $\pm$ 5.3	101.0 $\pm$ 5.0
0.01 ng/ml	102.8 $\pm$ 10.0	99.5 $\pm$ 5.2	101.9 $\pm$ 3.9
IL-6			
100 ng/ml	102.6 $\pm$ 9.8	102.8 $\pm$ 6.9	107.5 $\pm$ 3.7*
10 ng/ml	102.9 $\pm$ 10.1	101.9 $\pm$ 4.8	107.9 $\pm$ 5.3*
1 ng/ml	102.8 $\pm$ 9.9	101.3 $\pm$ 2.6	105.4 $\pm$ 6.7
IL-10			
10 ng/ml	100.9 $\pm$ 8.1	100.8 $\pm$ 1.4	98.5 $\pm$ 1.4
1 ng/ml	100.7 $\pm$ 3.8	101.3 $\pm$ 0.6	99.2 $\pm$ 2.2
0.1 ng/ml	100.9 $\pm$ 5.5	102.8 $\pm$ 1.1	100.5 $\pm$ 0.2
IFN- γ			
2,000 U/ml	99.2 $\pm$ 11.4	97.6 $\pm$ 5.6	92.2 $\pm$ 2.0*
200 U/ml	98.8 $\pm$ 10.4	95.9 $\pm$ 4.6*	94.6 $\pm$ 2.2*
IL-4			
10 ng/ml	102.7 $\pm$ 5.8	98.7 $\pm$ 4.5	97.4 $\pm$ 1.8
1 ng/ml	100.0 $\pm$ 6.7	102.1 $\pm$ 2.7	97.3 $\pm$ 0.7
0.1 ng/ml	100.4 $\pm$ 1.4	103.7 $\pm$ 2.3	98.2 $\pm$ 0.3
IL-13			
10 ng/ml	102.0 $\pm$ 5.5	101.0 $\pm$ 1.3	95.7 $\pm$ 1.7
1 ng/ml	101.7 $\pm$ 3.4	100.4 $\pm$ 1.7	97.1 $\pm$ 1.5
0.1 ng/ml	98.3 $\pm$ 2.4	100.1 $\pm$ 0.1	97.5 $\pm$ 2.8

* p < 0.05.

Mass., USA). Equal loading of proteins was confirmed by reprobing with anti-p44/42 MAP kinase antibody (1:1,000 dilution) (Cell Signaling Technology). After washing three times with TBST for 15 min, the membranes were incubated with an anti-rabbit IgG, HRP-linked antibody (1:4,000 dilution) (Cell Signaling Technology), and developed by enhanced chemiluminescence (ECL) (Amersham, Pharmacia Biotech, Piscataway, N.J., USA).

Chemotaxis Assay

All migration assays were performed in 48-well Boyden chambers (Neuro Probe, Gaithersburg, Md., USA). Purified eosinophils were resuspended in RPMI-1640 with 10% FBS at 2×10^6 cells/ml. Twenty-seven microliters of IL-5 was added to the lower chamber. A 5- μ m pore size PVDF membrane (Osmonics, Minnetonka, Minn., USA) was overlaid, and 1×10^5 eosinophils that had been preincubated with medium, IFN- γ or CHX for 60 min at 37°C were added to the upper chamber. The chambers were subsequently incubated for 3 h at 37°C. The filter was removed, and the migrating cells in the lower chamber were microscopically counted.

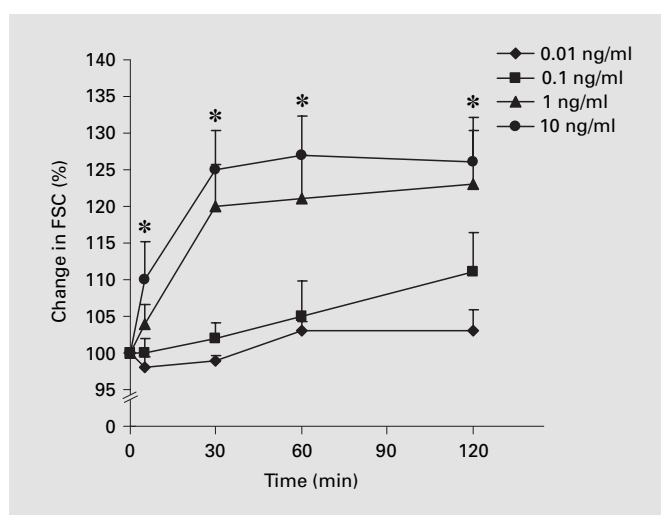


Fig. 1. Eosinophil shape change induced by IL-5. A total of 2×10^5 PMNL were incubated with varying concentrations of IL-5 for different time intervals. The percentage change in FSC = (the FSC from stimulated eosinophils + FSC from unstimulated eosinophils) $\times$ 100. The data are the average of three independent experiments (* p < 0.05).

Statistical Analysis

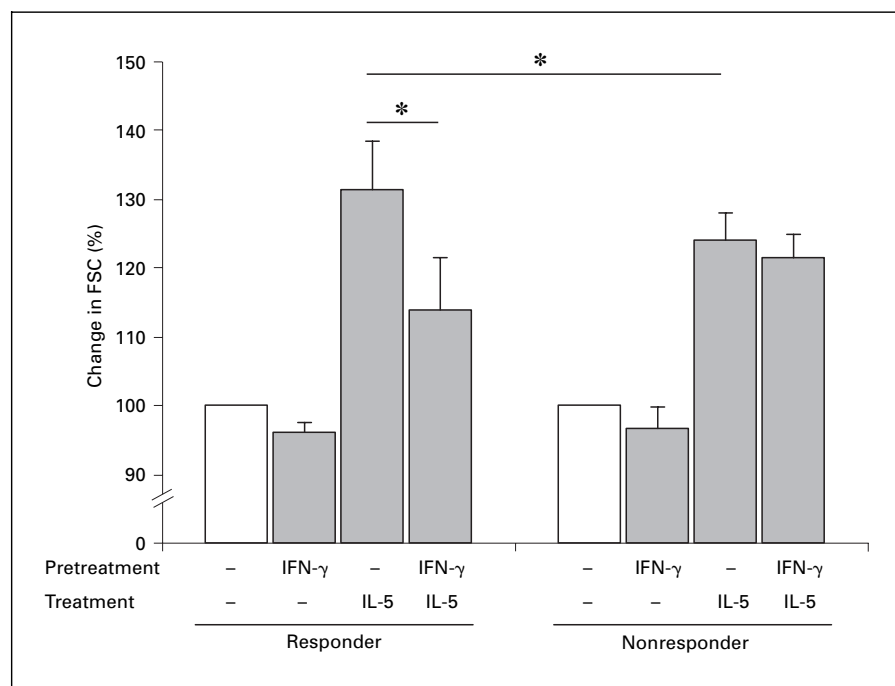
Analysis of data was performed by using SPSS, version 10.0 (Chicago, Ill., USA). Data were expressed as means $\pm$ SEM. Statistical differences were determined by means of ANOVA with Fisher protected least significant difference. To determine the inhibitory effect of IFN- γ , statistical significance was determined by Mann-Whitney's U test. A value of p < 0.05 was taken as significant.

Results

Eosinophil Shape Change by Cytokines

Gating of eosinophils by their characteristic autofluorescence and monitoring of the change in their FSC upon exposure to chemoattractant allow the assessment of eosinophil shape change without the separation of eosinophils from PMNLs, and this procedure – a GFAS assay – was further employed to estimate eosinophil activation regulated by agonists and antagonists [14, 16]. We examined the direct effect of a number of cytokines on eosinophil shape change. These included proinflammatory cytokines (IL-1 β , IL-6, and TNF), Th1 cytokine (IFN- γ), Th2 cytokines (IL-4, IL-5, and IL-13), and a cytokine (IL-10), which is generally recognized as an anti-inflammatory cytokine. IL-5, as expected, induced eosinophil shape change in a time- and dose-dependent manner. The shape change induced by this cytokine was evident as early as 5 min and reached a peak at 30–60 min (fig. 1). In con-

Fig. 2. Inhibitory effect of IFN- γ on IL-5-induced eosinophil shape change. PMNL prepared from 10 atopic individuals were preincubated with medium or IFN- γ (100 U/ml) for 60 min, and then treated with medium or IL-5 (10 ng/ml) for an additional 60 min. Varying degrees of responsiveness to IFN- γ were observed among individuals. Responders and nonresponders were grouped on the basis of responsiveness to IFN- γ ; half (n = 5) of the preparations tested, grouped with nonresponders, exhibited no or little inhibitory effect of IFN- γ on IL-5-induced eosinophil shape change, and the remaining half, grouped with responders, showed substantial inhibition by IFN- γ . In the latter group, the percentage change in FSC was decreased by IFN- γ pretreatment, ranging from 116 to 105%, as compared with 131.5% in the presence of IL-5 alone (* p < 0.05).



trast, other Th2 cytokines, IL-4 and IL-13 slightly inhibited eosinophil shape change without statistical significance. Both TNF and IL-6 significantly elicited eosinophil shape change, whereas IL-1 β and IL-10 had little effects. Among the cytokines tested, IFN- γ most significantly inhibited the shape change (table 1).

IFN- γ Inhibits IL-5-Induced Eosinophil Shape Change

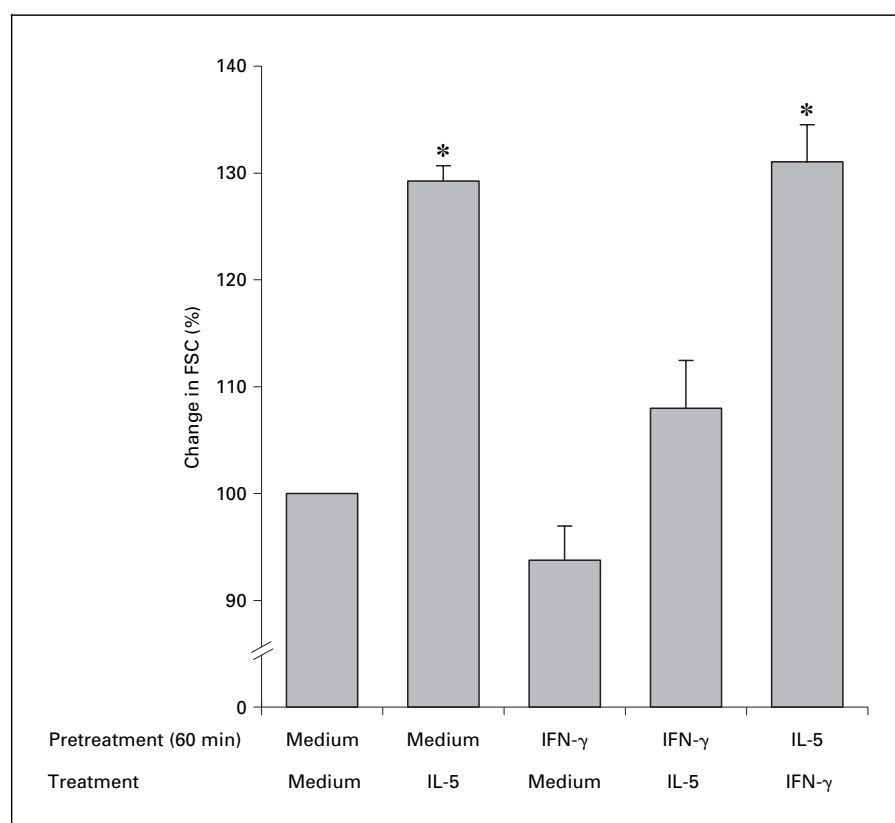
The fact that treatment with IFN- γ resulted in a decrease in the basal level of eosinophil shape change, of which induction is thought to be a consequence of Th2 immune responses, may provide an antagonism at a level of effector cells such as eosinophils, between Th1 and Th2 cytokines. Hence, we further investigated the inhibitory effect of IFN- γ on IL-5-induced eosinophil shape change. Interestingly, we observed a bimodal pattern of responsiveness to IFN- γ of eosinophils prepared from atopic donors having a 10–20% eosinophil count in their peripheral blood. Half of the eosinophil preparations exhibited the suppressed effect of IFN- γ on IL-5-induced eosinophil shape change (n = 5, from 131.5 $\pm$ 8.1 to 113.2 $\pm$ 8.3%) and were grouped as responders, whereas the remaining half did not (n = 5, from 121.0 $\pm$ 0.3 to 120.2 $\pm$ 2.7%) and were grouped as nonresponders. However, the inhibitory responsiveness of the preparations to IFN- γ alone demonstrated no significant difference. Rather, eosinophils from the responders tended to display a greater

shape change than their counterparts in response to IL-5 alone (fig. 2). The reason for the differential responses is not clear, and thereafter samples from the nonresponder group were excluded from further studies. Pretreatment with IFN- γ augmented the inhibitory activity as compared with simultaneous treatment with IL-5 (data not shown), while IL-5 pretreatment followed by IFN- γ had no inhibitory effect (fig. 3), suggesting that a priming signal for inhibition is provided by IFN- γ . When eosinophils were pretreated with IFN- γ in the presence of CHX, the inhibitory activity disappeared (fig. 4a). CHX alone or in combination with IL-5 had no effect on eosinophil shape change. This result suggests the requirement of protein synthesis for the priming effect of IFN- γ . Further, we also tested the possibility that IFN- γ pretreatment resulted in the downregulation of IL-5R expression on eosinophils whereby eosinophils subsequently became unresponsive to IL-5. When eosinophils were treated with IFN- γ for 10–60 min, IL-5R α expression levels did not change during this period compared with the expression level of untreated eosinophils (data not shown).

IFN- γ Inhibited the Eosinophil Migration and ERK1/2 Phosphorylation Induced by IL-5

We tested whether IFN- γ inhibited migration of eosinophils by IL-5. This cytokine is shown to be able to induce chemokinesis of eosinophils rather than chemotaxis [17],

Fig. 3. Pretreatment effect of IFN- γ . PMNL were pretreated with medium, IFN- γ (100 U/ml) or IL-5 (10 ng/ml) for 60 min, and then stimulated with medium, IL-5 or IFN- γ , for an additional 60 min. These data were the average of at least three independent experiments. * $p < 0.05$ compared with medium only.



and the eosinophil migration is readily detected by a Boyden chamber assay [16]. As shown in figure 4b, eosinophil chemotaxis induced by IL-5 was inhibited by pretreatment with IFN- γ for 1 h, and the addition of CHX with IFN- γ -pretreated cells restored the eosinophil migration. Moreover, IFN- γ also strongly inhibited IL-5-induced phosphorylation of ERK1/2 (fig. 5).

Discussion

An array of cytokines interacts individually, synergistically, and antagonistically with infiltrating leukocytes and resident cells to modulate allergic inflammation such as asthma, reflecting the complex nature of the disease. Th2 cytokines act as proinflammatory mediators for the pathological development of such disease, and their action is counterbalanced by Th1 and anti-inflammatory cytokines. In this study, we investigated the direct effects of a variety of cytokines on shape change of eosinophils, the major effector cells for airway inflammation, in which eosinophil shape change is considered to reflect, at least in part, activation of the cell type. Our results reveal that

IFN- γ , a Th1 cytokine, suppresses both the basal level of eosinophil shape change and the IL-5-induced shape change and migration of eosinophils, with the requirement of protein synthesis for the latter case. This, to our knowledge, is the first report showing the direct suppressing effect of IFN- γ on in vitro eosinophil mobilization in the presence and absence of IL-5.

IL-5 strongly induces eosinophil shape change (fig. 1, table 1), and this result is consistent with our previous finding, in which IL-5 is indeed more potent than eotaxin in eliciting eosinophil shape change [14]. In contrast, other Th2 cytokines, IL-4 and IL-13, did not induce eosinophil shape change (table 1). Rather, they have a tendency to reduce the shape change even though the reduction is not statistically significant. This result is somewhat unexpected, since numerous reports show that both IL-4 and IL-13 mediate eosinophil recruitment in vivo. However, it is not clear whether the in vivo recruitment of eosinophils by these two cytokines is due to the direct enhancing effect of the cytokines on eosinophil migration. IL-4 achieves this function largely by inducing the expression of VCAM-1 on endothelial cells, by which it allows eosinophils to attach to and migrate across the endothelium

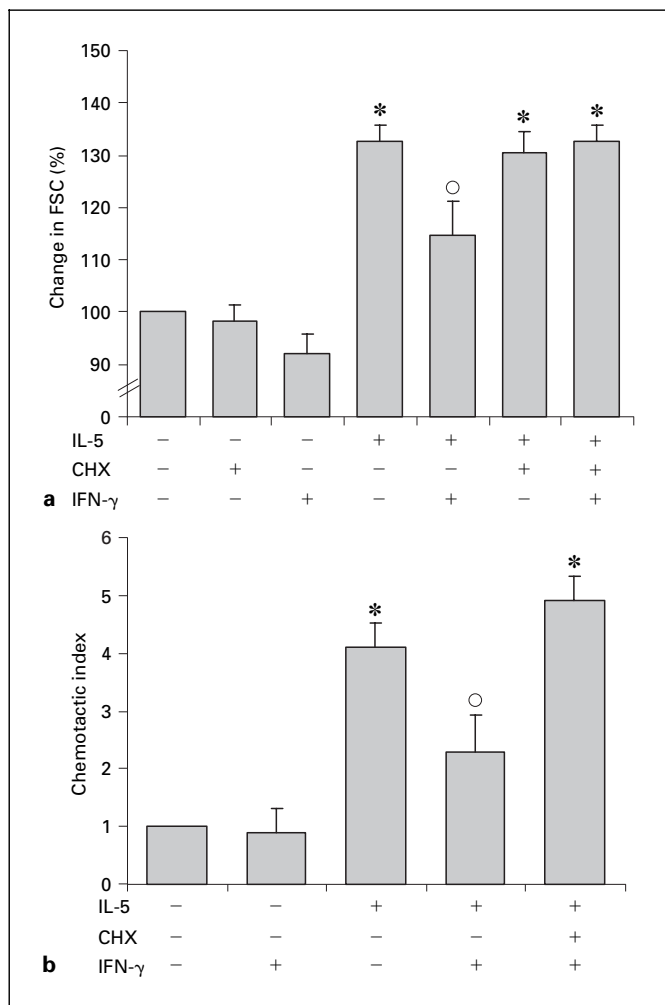
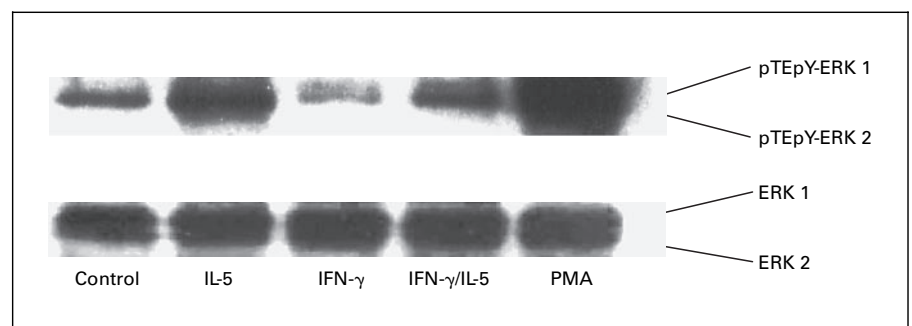


Fig. 4. CHX reverses the inhibition of IL-5-induced eosinophil shape change and chemotaxis by IFN- γ . ^o $p < 0.05$ compared with IL-5 only; * $p < 0.05$ compared with control. **a** For eosinophil shape change, PMNL or purified eosinophils were pretreated with IFN- γ (100 U/ml) in the presence or absence of CHX (5 μ g/ml) for 60 min, and then stimulated with IL-5 (10 ng/ml) for an additional 60 min ($n = 3-5$). **b** For chemotaxis, purified eosinophils were treated as for measurement of shape change, except that the incubation time with IL-5 was 3 h instead of 60 min ($n = 3$).

[18], and IL-13-induced airway eosinophilia is dependent on IL-5 and eotaxin [19]. However, we cannot rule out the possibility that because IL-13, but not IL-4, is known to directly induce eosinophil migration [20], it would activate eosinophil mobilization via a mechanism that is not detected by the method used in our study. We found that TNF and IL-6, but not IL-1 β , are the cytokines that induce significant eosinophil shape change. TNF prolongs eosinophil survival and induces eosinophil eotaxin production, and enhances eosinophil recruitment by induction of endothelial cell adhesiveness [21-23]. An increased level of TNF is also found in asthmatic airways [24]. IL-6 is shown to have an ability to augment Th2 tissue inflammation [25, 26], and exaggerated production of IL-6 is also documented in asthmatic tissues [27]. Therefore, the fact that TNF and IL-6 can promote eosinophil shape change suggests the possibility of its direct role in eosinophil recruitment. On the other hand, IL-10 is a plausible candidate as a suppressive cytokine due to a variety of inhibitory effects on T cell and eosinophil functions [5, 6]. However, our data reveal a minimal effect of IL-10 on eosinophil shape change (table 1).

Among the cytokines tested in our study, IFN- γ is the most potent in inhibiting the shape change of untreated and IL-5-treated eosinophils. IFN- γ is documented as a prime Th1 cytokine that reverses allergic airway inflammation in asthmatics and animal models. Aerosolized IFN- γ inhibits allergen-induced eosinophil infiltration in the lungs [11] and reduces the number of eosinophils in BAL fluid with asthma [28]. Moreover, mice lacking either IFN- γ or IFN- γ R genes show allergen-induced prolonged airway eosinophilia [11], and transfer of the IFN- γ gene to airways inhibits pulmonary allergic responses [29]. These roles of IFN- γ in airway inflammation presumably occur through various mechanisms: inhibition of Th2 cytokine production [30], promotion of differentiation toward the Th1 phenotype by inhibiting development of Th2 cells [9], decrease in IgE production [31] and

Fig. 5. Inhibition of IL-5-induced phosphorylation of extracellular signal-regulated kinases (ERK1/2) by IFN- γ . Purified eosinophils (2×10^6 cells) were preincubated with IFN- γ (100 U/ml) or medium for 60 min, then stimulated with IL-5 (10 ng/ml) for 10 min. The phosphorylation of ERK1/2 and equal loading of proteins was analyzed with anti-phospho-p44/42 MAP kinase and anti-p44/42 antibodies, respectively. This result is representative of two experiments.



attenuated eosinophil differentiation [32], decrease of eosinophil infiltration and survival [33, 34]. Therefore, our observation that IFN- γ inhibits eosinophil shape change is consistent with the effects of IFN- γ at multiple levels on Th2-cell-driven inflammations, as mentioned above. We postulate that the suppressive effect of endogenous and exogenous IFN- γ on eosinophil recruitment observed in allergen-induced asthma models may be attributed, in part, to the direct inhibitory effect of IFN- γ on eosinophil shape change and mobilization in vitro. However, eosinophils from half the atopic donors are refractory to IFN- γ for such inhibitory activity (fig. 2). The difference in responsiveness to IFN- γ between the responders and the nonresponders is unlikely to be due to the number and function of IFN- γ R on eosinophils, since eosinophils prepared from both normal individuals and asthmatics are shown to have comparable levels of functional IFN- γ R [35]. Moreover, as seen in figure 2, unstimulated eosinophils from either group do not exhibit any significant difference in responsiveness to IFN- γ itself. We do not know whether the eosinophil responsiveness to IFN- γ is relevant to disease severity and recovery and recurrence upon treatment, as we did not track the atopic individuals. The differential expression of surface IL-5 receptor (IL-5R), and the resulting difference in functional response to IL-5 observed on eosinophils in the circulation and BAL fluid from asthmatics is suggested to regulate eosinophil functions [36]. Supporting this possibility, there is considerable variation in the degree of surface IL-5R α expression on blood eosinophils in asthmatics [37]. When eosinophil responses to IL-5 alone are compared, the response of eosinophils from nonresponders tends to be lower than that from responders (fig. 2), and thus eosinophils from nonresponders are not activated to a level that is high enough to produce an observable reduction in eosinophil

shape change by IFN- γ . Whether the difference in the IL-5 responsiveness or IL-5R expression accounts for the differential responsiveness to IFN- γ of eosinophils in atopic individuals awaits further characterization. In this context, it is worth noting that IFN- γ pretreatment itself does not downregulate IL-5R surface expression or potentiate rapid clearance of surface IL-5R since eosinophils pretreated with IFN- γ exhibit the same level of surface IL-5R as untreated eosinophils.

Our results show that IFN- γ pretreatment results in more inhibition of the IL-5-induced eosinophil shape change and mobility than simultaneous treatment (data not shown), and that the inhibitory effect of IFN- γ on eosinophil shape change and chemotaxis disappears in the presence of CHX (fig. 4), suggesting that a priming signal is necessary and dependent on new protein synthesis. Since ERK1/2 activation is essential for eosinophil chemotaxis and activation by IL-5 [14, 16], treatment with IFN- γ is expected to inhibit the phosphorylation of ERK1/2. Our result reflects this possibility (fig. 5), although we did not prove that the generation of the priming signal is directly related to inactivation of ERK1/2 in this study. In summary, the inhibition of eosinophil mobilization by the presence of IFN- γ in inflamed airways may contribute to the reduction of eosinophilic recruitment during airway inflammation. Our findings also support the concept that treatment with IFN- γ may be useful in reversing eosinophilic inflammation.

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