

Chemokine production by the BEAS-2B human bronchial epithelial cells: Differential regulation of eotaxin, IL-8, and RANTES by T_H2- and T_H1-derived cytokines

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Background: Bronchial epithelial cells produce many types of chemokines and may contribute to lung inflammation by recruiting inflammatory cells. The CC chemokine eotaxin is a potent, eosinophil-specific chemoattractant that has been detected in the bronchial epithelium of patients with asthma. **Objectives:** The aim of this study was to investigate the regulatory mechanisms of chemokine production from bronchial epithelium by inflammatory cytokines, especially T_H2- and T_H1-derived cytokines, in bronchial asthma. **Methods:** BEAS-2B human bronchial epithelial cells were cultured with TNF- α , IL-4, IL-13, and IFN- γ alone or in combination, after which supernatants were assayed for eotaxin, IL-8, and RANTES proteins with ELISA. Reverse transcription-PCR was also performed.

Results: TNF- α induced production of eotaxin, IL-8, and RANTES in a concentration-dependent manner. Both IL-4 and IL-13 synergistically enhanced TNF- α -induced eotaxin production, whereas IL-8 production induced by TNF- α was significantly down-regulated by the T_H2-derived cytokines. IFN- γ , a T_H1 cytokine, counteracted the enhancing effects of IL-4 and IL-13 on eotaxin production. RANTES production by TNF- α was not affected by IL-4 and IL-13 but was markedly enhanced by IFN- γ .

Conclusions: These results suggest that T_H2 cytokines are involved in preferential recruitment of eosinophils in bronchial asthma by enhancing eotaxin and reducing IL-8 production from bronchial epithelial cells and that T_H1 cytokines counteract the effects of T_H2 cytokines by reducing eotaxin production. (*J Allergy Clin Immunol* 2000;105:126-33.)

Key words: Chemokines, bronchial epithelial cells, eotaxin, RANTES, IFN- γ , IL-4, IL-13, IL-8

Eosinophil infiltration is a prominent feature of bronchial asthma and other allergic diseases. Several molecules are involved in preferential recruitment of

Abbreviations used

cDNA:	Complementary DNA
DMEM:	Dulbecco's modified Eagle's medium
F12:	Ham's F12 medium
MCP:	Monocyte chemotactic protein
mRNA:	Messenger RNA
RT-PCR:	Reverse transcription-PCR
T _H 1:	Helper T type 1
T _H 2:	Helper T type 2

eosinophils into the tissue of allergic inflammation. Both adhesion molecules, such as vascular cell adhesion molecule-1, on vascular endothelial cells, and counterligands, such as β 1 integrins, on eosinophils play pivotal roles. Cytokines that activate eosinophils and vascular endothelial cells also promote selective interaction, leading to preferential recruitment. Recently, the multiple roles of chemokines have been increasingly appreciated. The more than 40 chemokine molecules and 17 chemokine receptor molecules identified offer cell-type selectivity in recruitment.¹ For eosinophils, CC chemokines that bind to CCR3 are essential; of these chemokines, eotaxin is the most potent and most specific chemoattractant for eosinophils. In fact, expression of eotaxin messenger RNA (mRNA) and protein is increased in lung tissue and bronchoalveolar lavage fluid from patients with asthma.^{2,3} In guinea pigs and rats constitutive expression of eotaxin mRNA in the airways has been shown to increase markedly after allergen or ozone challenge.⁴⁻⁶ In the lung tissue, important sources of eotaxin are bronchial epithelial cells, alveolar macrophages, and lung fibroblasts.^{2-4,7,8}

Although eotaxin is constitutively expressed in the airways of humans and animals, expression is significantly enhanced in pathologic conditions and is positively correlated with eosinophil infiltration and tissue damage.^{3,7,9} Thus factors that induce eotaxin may be important therapeutic targets. IL-4 and TNF- α induce eotaxin production by human skin fibroblasts in vitro.¹⁰ TNF- α and IL-1 β induce eotaxin expression in human bronchial epithelial cell lines A549 and BEAS-2B.¹¹ Adoptively transferred T_H2 cells producing IL-4 and IL-5 induce

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eotaxin expression and eosinophilia in mouse lung.¹² In addition, the targeted pulmonary expression of IL-13 in mice induces eosinophilic inflammatory response and eotaxin production.¹³ These findings suggest that proinflammatory cytokines and T helper type 2 (T_H2) cytokines are important in inducing eotaxin expression. However, the effects of the eotaxin-inducing cytokines on the production of other sets of chemokines that recruit neutrophils and mononuclear cells have not been well studied. TNF- α is a rather nonspecific cytokine that induces various chemokines. Therefore we hypothesized that a T_H2 cytokine may play an important role in selective induction of chemokines of eosinophil preference and that helper T type 1 (T_H1) cytokines may counteract the effects of T_H2 cytokines on chemokine production. In the current study we investigated the effects of IL-4, IL-13, TNF- α , and IFN- γ on the epithelial production of the eosinophil-specific chemoattractant eotaxin, of the neutrophil chemoattractant IL-8, and of the mononuclear cell and eosinophil chemoattractant RANTES.

MATERIAL AND METHODS

Cell culture

BEAS-2B cells (American Type Culture Collection, Rockville, Md), which are adenovirus 12-SV40 hybrid virus-transformed human bronchial epithelial cells, were cultured in 25-cm² tissue culture flasks and maintained in LHC-8 medium (Biofluids, Rockville, Md) containing penicillin (100 U/mL) and streptomycin (100 U/mL) (Sigma, St Louis, Mo). Cells were used between passages 39 and 46 and were plated on 24-well culture plates (Becton Dickinson, Franklin Lakes, NJ) in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 medium (F12) containing 5% heat-inactivated FCS (Gibco BRL, Gaithersburg, Md), penicillin (100 U/mL), and streptomycin (100 U/mL).

Cytokine stimulation of BEAS-2B cells

Cells were grown to 80% confluence in DMEM/F12 containing 5% FCS, after which the culture medium was replaced with DMEM/F12 without FCS. Cells were further cultured with TNF- α , IL-4, IL-13, and IFN- γ (Pepro Tech EC, London, United Kingdom) alone or in combination at various concentrations for 2 to 96 hours.

Detection of chemokine proteins

Culture supernatants of cytokine-stimulated BEAS-2B cells were harvested at various times and assayed for eotaxin, IL-8, and RANTES. Eotaxin was assayed with a double-ligand immunoassay with use of 2 different mouse mAbs against human eotaxin, as reported previously.¹⁴ Briefly, each well of a high-binding efficiency 96-well microtiter plate for ELISA (Nunc, Roskilde, Denmark) was coated with a mouse antihuman eotaxin mAb, designated 164.44, by a cross-linking reagent, disuccinimidyl suberate (Pierce, Rockford, Ill). Samples were added in duplicate to the coated wells, incubated overnight at 4°C, and washed. Subsequently, the horseradish peroxidase-conjugated antihuman eotaxin mAb 174.4 Fab' was added to each well. After incubation for 2 hours at room temperature, the plates were washed and developed with the TMB microwell peroxidase substrate system (Kirkengard and Perry, Gaithersburg, Md), and the reactions were stopped with 2N sulfuric acid. Absorbance was measured at 450 nm. RANTES and IL-8 proteins were measured with sandwich ELISA kits (BioSource International, Camarillo, Calif) according to manufacturer's instructions.

Reverse transcription-PCR analysis of chemokine mRNAs

Cytokine-stimulated BEAS-2B cells were washed twice with Ca⁺⁺- and Mg⁺⁺-free HBSS (Biofluids), treated for 1 minute with Trypsin Versene (Biofluids), and removed from plates by repeated pipetting with Ca⁺⁺- and Mg⁺⁺-free HBSS containing 10% FCS. The cells were counted and washed twice with PBS, after which total RNA was extracted from the cells with the SV Total RNA Isolation System (Promega, Madison, Wis). The RNA was then reverse transcribed with use of a deoxyribonucleotide mixture with a random sequence (Takara Shuzo, Otsu, Japan) as a primer and Moloney murine leukemia virus reverse transcriptase (Life Technologies, Paisley, United Kingdom). The eotaxin primers used were forward primer, 5'-CCCAACCACCTGCTTGCTTTAACCTG-3'; reverse primer, 5'-AAAAATGGTGATTATTTATGGC-3'; these primers produced a 226-bp product.

The RANTES primers were 5'-ATATTCCTCCTGGACACCACAC-3' for sense and 5'-CACTCCAGCCTGGGAAGG-3' for antisense, which produced a 370-bp product. The IL-8 primers were 5'-ATGACTTCCAAGCTGGCCGTGGCT-3' for sense and 5'-TCTCAGCCCTCTTCAAAAACCTTCTC-3' for antisense, which produced a 289-bp product. The β -actin primers were 5'-CTTC-TACAATGAGCTGCGTG-3' for sense and 5'-TCATGAGGTAGTCAGTCAGG-3' for antisense. Thermocycler settings for eotaxin complementary DNA (cDNA) were an initial step at 95°C for 9 minutes to denature and linearize cDNA, followed by 40 cycles of denaturing (94°C for 30 seconds), annealing (55°C for 30 seconds), polymerization (72°C for 30 seconds), and final polymerization (72°C for 5 minutes). Thermocycler settings for RANTES and IL-8 cDNA were the same as those for eotaxin except for an initial step at 95°C for 5 minutes. Amplified products were electrophoresed for 30 minutes on a 3% agarose gel, which was then stained with ethidium bromide.

Competitive reverse transcription-PCR for eotaxin and IL-8

Semiquantitative PCR ELISA for eotaxin and IL-8 mRNA was performed as previously described¹⁴ with cDNA and varying amounts of competitor cDNA used as templates. Amplification was performed for 30 cycles, which did not yield plateau levels of PCR products (data not shown). The amplified PCR products were quantified with ELISA. The products were immobilized on carboxylated surface plates, and nonamplified DNA strands were removed by treating the plates with 0.1N sodium hydroxide. The resulting covalently bound single-stranded DNAs were then hybridized with digoxigenin-labeled oligonucleotide probes (5'-TACCCCTTCAGC-GACTAGAGA-3' for eotaxin, 5'-AAGTACCGTCGACGTCGGA-3' for eotaxin competitor, 5'-GATTGAGAGTGGACCACACTG-3' for IL-8, and 5'-ACGTACTCAGAACTGCTCTGA-3' for IL-8 competitor). The plates were then developed with peroxidase-conjugated antidigoxigenin antibodies (Boehringer Mannheim, Mannheim, Germany), after which signals were visualized with tetramethyl benzidine as the substrate. The optical absorbance data read at 450/630 nm were plotted on a logarithmic scale. Because the ratio of the target and competitive templates remained constant during amplification, the quantity of competitor DNA in the PCR templates, which yields an equal amount of the two PCR products, indicates the initial amount of the target gene.

Statistical analysis

Data are expressed as mean \pm SEM. The statistical significance of the differences was assessed with the Student *t* test (paired); differences with a *P* value less than .05 were considered significant.

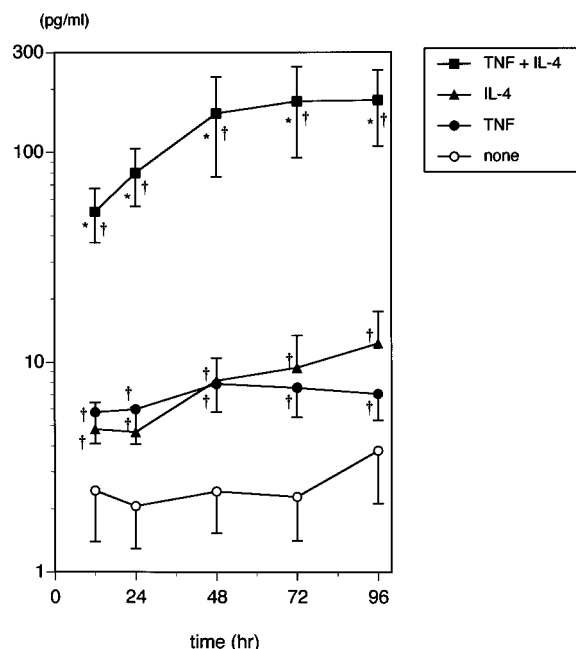


FIG 1. Time kinetics of eotaxin production by TNF- α and IL-4 from BEAS-2B cells. Cells grown to 80% confluence were cultured for up to 96 hours with TNF- α at 10 ng/mL, IL-4 at 10 ng/mL, or with combination of both cytokines. Culture supernatants were assayed for eotaxin with ELISA as described in Methods. Data are expressed as mean \pm SEM ($n = 5$). *Dagger*, $P < .05$, significantly different from eotaxin production in absence of cytokines; *asterisk*, $P < .05$; significantly different from eotaxin production induced by single cytokine, IL-4 or TNF- α .

RESULTS

Kinetics of eotaxin protein release from bronchial epithelial cells

First, we examined whether an inflammatory cytokine, TNF- α , and a T_H2 cytokine, IL-4, induces eotaxin production from BEAS-2B human bronchial epithelial cells. Both TNF- α at 10 ng/mL and IL-4 at 10 ng/mL induced release of small but significant amounts of eotaxin from 12 hours (Fig 1). Maximal eotaxin levels induced by TNF- α and IL-4 at 96 hours were 7.0 ± 1.8 and 12.2 ± 5.2 pg/mL, respectively, and were significantly higher than the level without cytokines, 3.8 ± 1.7 pg/mL ($P < .05$). Combinations of the 2 cytokines synergistically enhanced eotaxin production to levels 10- to 20-fold higher than with either cytokine alone. Eotaxin release with the combination reached a plateau at 48 hours (Fig 1).

Effects of IL-4 and TNF- α on chemokine release from bronchial epithelial cells

We further examined the effects of combinations of TNF- α and IL-4 at various concentrations on production of eotaxin, IL-8, and RANTES. Because TNF- α induces production of IL-8 and RANTES from BEAS-2B cells,^{15,16} the effects of IL-4 on TNF- α -induced release of eotaxin, IL-8, and RANTES from the cells after 48 hours of incubation were compared (Fig 2). Although

TNF- α induced only small amounts of eotaxin, IL-4 significantly enhanced eotaxin production in a concentration-dependent manner. For example, TNF- α at 100 ng/mL induced eotaxin release of 9.0 ± 2.0 pg/mL, and the release was augmented to 220.9 ± 53.1 pg/mL with combination of TNF- α at 100 ng/mL and IL-4 at 100 ng/mL. In contrast, TNF- α -induced IL-8 production was significantly suppressed by IL-4 (Fig 2). Maximal inhibition in this series of experiments was 65% when 9346 ± 2980 pg/mL of IL-8 production with TNF- α at 10 ng/mL was reduced to 3273 ± 648 pg/mL by IL-4 at 10 ng/mL. However, TNF- α -induced production of RANTES was not affected by IL-4 (Fig 2).

Effect of IL-13 and TNF- α on chemokine release from bronchial epithelial cells

We then examined the ability of another T_H2 cytokine, IL-13, to induce chemokine production (Table I). IL-13 induced eotaxin production at 1 and 10 ng/mL and synergistically enhanced TNF- α -induced production of eotaxin in a concentration-dependent manner. In contrast, IL-13 significantly inhibited TNF- α -induced IL-8 production. IL-13 at 10 ng/mL enhanced TNF- α -induced RANTES production to a small but significant degree.

Eotaxin, IL-8, and RANTES mRNA expression by bronchial epithelial cells

To determine whether production of eotaxin, IL-8, and RANTES is accompanied by transcription of the corresponding genes, we used reverse transcription (RT)-PCR to examine chemokine mRNA expression in BEAS-2B cells. BEAS-2B cells were stimulated for 8 hours with TNF- α at 100 ng/mL or IL-4 at 100 ng/mL or both (Fig 3). Eotaxin mRNA was not detected under basal conditions but was detected after stimulation with IL-4 or TNF- α . The combination of the 2 cytokines greatly enhanced expression of eotaxin mRNA. IL-8 mRNA was constitutively expressed without stimulation, but its expression was enhanced by TNF- α . IL-4 reduced constitutive and TNF- α -induced expression of IL-8 mRNA. RANTES mRNA was induced by TNF- α . IL-4 had no effect on expression of RANTES. Competitive RT-PCR was used to measure eotaxin and IL-8 mRNA expression (Fig 4) and confirmed the findings observed in the gel.

Effect of IFN- γ on chemokine production by bronchial epithelial cells

Because IL-4 and IL-13, principal T_H2 cytokines, induced eotaxin production, we examined whether IFN- γ , a T_H1 cytokine, inhibits eotaxin production. First, the effects of combinations of TNF- α and IFN- γ on production of eotaxin, IL-8, and RANTES were examined. When the cells were stimulated with TNF- α at 10 ng/mL and varying concentrations of IFN- γ for 48 hours, RANTES production was markedly enhanced by IFN- γ in a concentration-dependent manner, but production of eotaxin and IL-8 was not affected (Table II). Although production of IL-8 appeared to increase with combination of TNF- α and IFN- γ , the difference was not statisti-

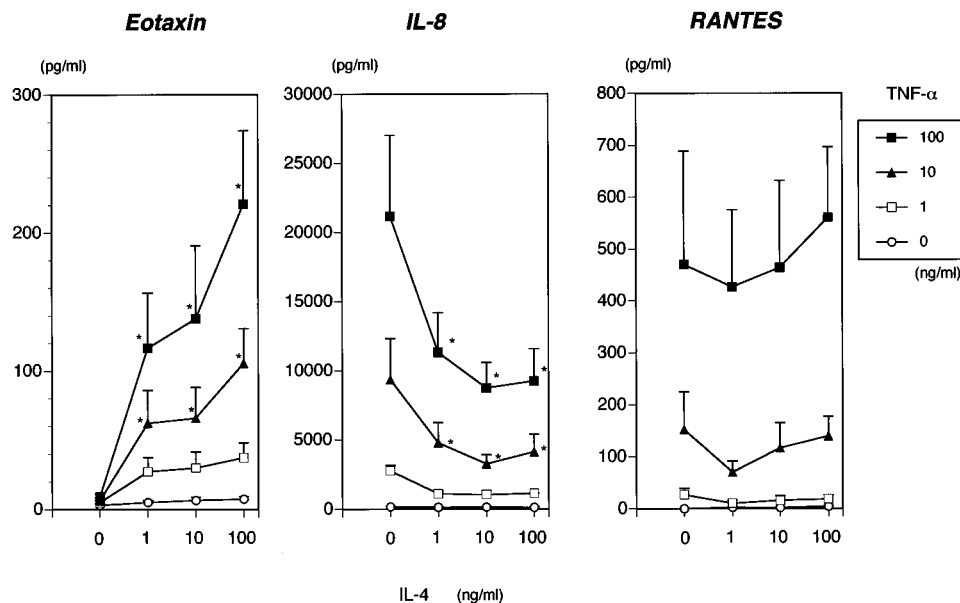


FIG 2. Effect of combinations of IL-4 and TNF- α on chemokine production by BEAS-2B cells. Cells were cultured for 48 hours with IL-4 and TNF- α at concentrations indicated. Culture supernatants were assayed for eotaxin, IL-8, and RANTES with ELISA. Data are expressed as mean \pm SEM (n = 5). Asterisk, $P < .05$, significantly different from control production without IL-4.

TABLE I. Effect of IL-13 on chemokine production by BEAS-2B cells

TNF- α	IL-13	Eotaxin	IL-8	RANTES
0	0	10.5 \pm 0.9	1870 \pm 339	14.5 \pm 4.3
	1	12.8 \pm 1.1*	1788 \pm 380	16.7 \pm 5.4
	10	29.8 \pm 4.3*	1570 \pm 444	25.1 \pm 5.9*
1	0	22.9 \pm 2.8	5842 \pm 1253	34.3 \pm 4.3
	1	37.5 \pm 9.0	4351 \pm 1018*	32.9 \pm 7.4
	10	108.1 \pm 27.1*	3741 \pm 1044*	41.4 \pm 6.6*
10	0	68.9 \pm 15.3	18600 \pm 2654	103.8 \pm 15.8
	1	135.3 \pm 31.2*	14467 \pm 1890*	90.6 \pm 13.0
	10	494.3 \pm 141.5*	13226 \pm 2152*	121.7 \pm 18.5*

BEAS-2B cells grown to subconfluence were cultured for 48 hours with combinations of TNF- α and IL-13 at indicated concentrations (nanograms per milliliter). Supernatants were collected and assayed for eotaxin, IL-8, and RANTES as described in Methods. Data represent mean \pm SEM from 5 experiments (picograms per milliliter).

* $P < .05$, significantly different from control production without of IL-13.

cally significant. We then examined the effects of IFN- γ on chemokine production induced by combinations of TNF- α and IL-4 or IL-13. We found that IFN- γ at 100 and 1000 U/mL significantly inhibited IL-4- or IL-13-enhanced eotaxin production by BEAS-2B cells (Fig 5). Small but significant enhancement of eotaxin production by IFN- γ at 10 U/mL was also observed. IL-8 production was not affected, and RANTES production was again significantly enhanced by IFN- γ .

DISCUSSION

Cumulative evidence shows that bronchial epithelial cells are the main target of inflammatory cells, particularly eosinophils, in asthma and result in functional impairment such as bronchial hyperresponsiveness. Besides being a target, airway epithelium also produces several

cytokines and chemokines that may directly contribute to the development of asthma symptoms. Expression of GM-CSF is up-regulated in asthmatic bronchial epithelium and is positively correlated with bronchial hyperresponsiveness.^{17,18} IL-6,¹⁹ IL-8,²⁰ monocyte chemoattractant protein (MCP)-1,²¹ MCP-4,²² RANTES,^{16,23} and eotaxin^{2,3,7} are also produced during airway inflammation in asthma. In particular, chemokines produced in epithelium strongly recruit inflammatory cells and cause pathologic changes in asthma.²⁴ CC chemokines such as eotaxin and RANTES recruit eosinophils, lymphocytes, and monocytes. In contrast, CXC chemokines such as IL-8 attract neutrophils. Among CC chemokines eotaxin is the most potent and specific chemoattractant for eosinophils and basophils,²⁵⁻²⁷ a fact that may explain why eosinophils predominate in allergic inflammation.

In the current study we investigated the effects of

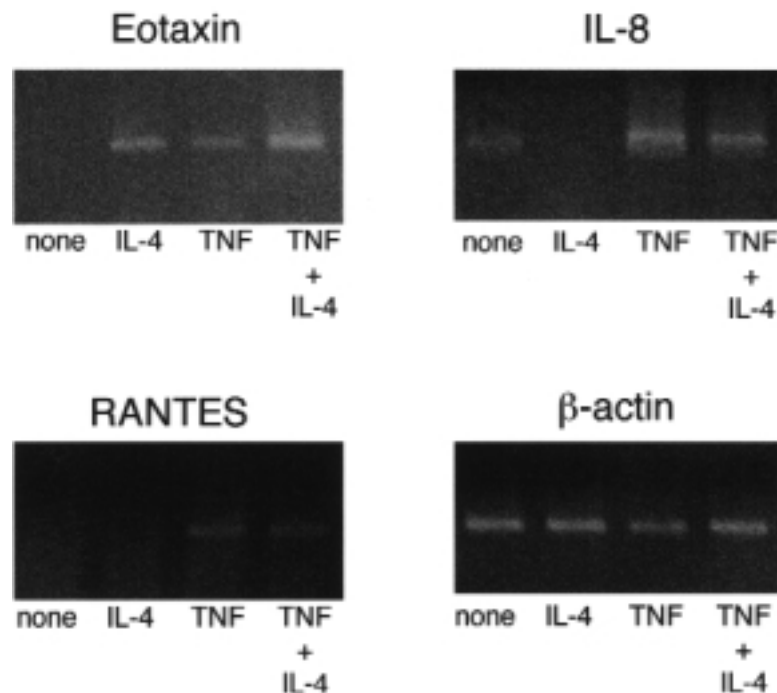


FIG 3. RT-PCR analysis of eotaxin, IL-8, and RANTES mRNA induced in BEAS-2B cells. Cells were cultured for 8 hours with no cytokines (none), IL-4 at 100 ng/mL, TNF- α at 100 ng/mL, and combination of the 2 cytokines. Total RNA was extracted and RT-PCR was performed as described in Methods. Representative data from 2 separate experiments are shown.

TABLE II. Effect of IFN- γ on chemokine production by BEAS-2B cells

TNF- α	IFN- γ	Eotaxin	IL-8	RANTES
0	0	2.5 \pm 1.2	217 \pm 73	1.0 \pm 1.0
	1	3.5 \pm 1.3	186 \pm 73	1.2 \pm 1.2
	10	2.9 \pm 1.1	177 \pm 91	1.0 \pm 1.0
	100	3.4 \pm 1.4	150 \pm 73	1.0 \pm 1.0
	1000	1.9 \pm 8.3	173 \pm 85	5.6 \pm 2.9
10	0	6.1 \pm 2.2	6959 \pm 739	29 \pm 17
	1	5.2 \pm 1.4	6058 \pm 2786	106 \pm 30
	10	7.4 \pm 2.1	9776 \pm 3432	1346 \pm 541*
	100	7.1 \pm 1.8	11218 \pm 2921	5278 \pm 2520*
	1000	8.3 \pm 2.0	6221 \pm 1913	9153 \pm 5525*

BEAS-2B cells grown to subconfluence were cultured for 48 hours with varying concentrations of IFN- γ (units per milliliter) with or without TNF- α (nanograms per milliliter). Supernatants were collected and assayed for eotaxin, IL-8, and RANTES as described in Methods. Data represent mean \pm SEM from 5 experiments (picograms per milliliter).

* $P < .05$, significantly different from control production without IFN- γ .

inflammatory cytokines on chemokine production by BEAS-2B human bronchial epithelial cells. The chemokines examined were eotaxin, an eosinophil-specific CC chemokine that binds to CCR3; IL-8, a neutrophil-specific CXC chemokine; and RANTES, a nonspecific CC chemokine that attracts eosinophils and mononuclear cells by binding to CCR1, CCR3, and CCR5. We found that, although TNF- α induced production of all 3

types of chemokines, IL-4 and IL-13 up-regulated eotaxin production and down-regulated IL-8 production. RANTES production was not affected by either IL-4 or IL-13 but was markedly enhanced by IFN- γ , as previously reported.¹⁶

IL-4 and IL-13 are important T_H2 cytokines that induce IgE synthesis.²⁸ These cytokines have been suggested to also be involved in selective tissue recruitment of eosinophils. Intradermal and intraperitoneal injection of IL-4 results in marked eosinophil infiltration in mice.²⁹ In a murine model of asthma with antigen-induced eosinophilic inflammation, IL-4 production was positively correlated with eosinophil recruitment.³⁰ In vitro experiments with human umbilical vein endothelial cells have shown that IL-4 promotes adhesion of eosinophil, not neutrophil, and promotes transmigration by inducing vascular cell adhesion molecule-1 expression on endothelial cells and $\beta 1$ integrin very late activation antigen-4 expression on eosinophils.^{31,32} IL-4 also acts directly on eosinophils and causes chemotaxis of eosinophils from atopic donors but not from normal donors.³³ Last, IL-4 has been shown to induce production of eotaxin mRNA and protein by human skin fibroblasts, a finding that may explain why eosinophilic inflammation occurs in T_H2 -mediated skin diseases such as atopic dermatitis.

In the current study, we have found that in bronchial epithelial cells IL-4 and IL-13 induce eotaxin production mostly with synergism with TNF- α . IL-4 and IL-13

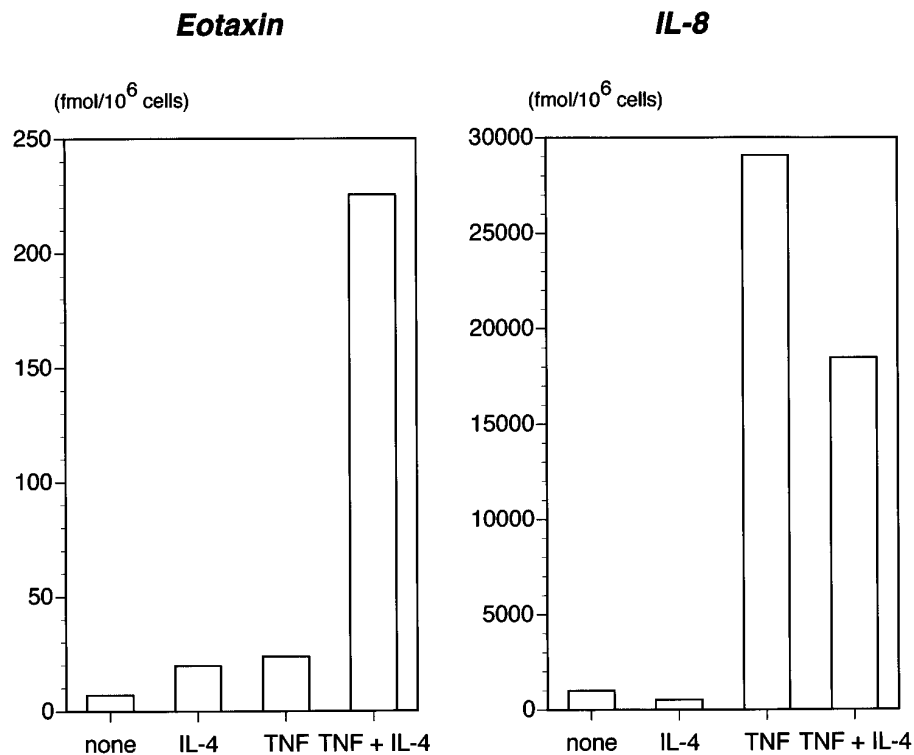


FIG 4. Semiquantitative determination of eotaxin and IL-8 mRNA by competitive RT-PCR. Cells were stimulated for 8 hours with IL-4 at 100 ng/mL, TNF- α at 100 ng/mL, and combination of the 2 cytokines and then competitive RT-PCR was performed as described in Methods. Amplified PCR products were quantified with ELISA. Results are from 1 of 2 similar experiments.

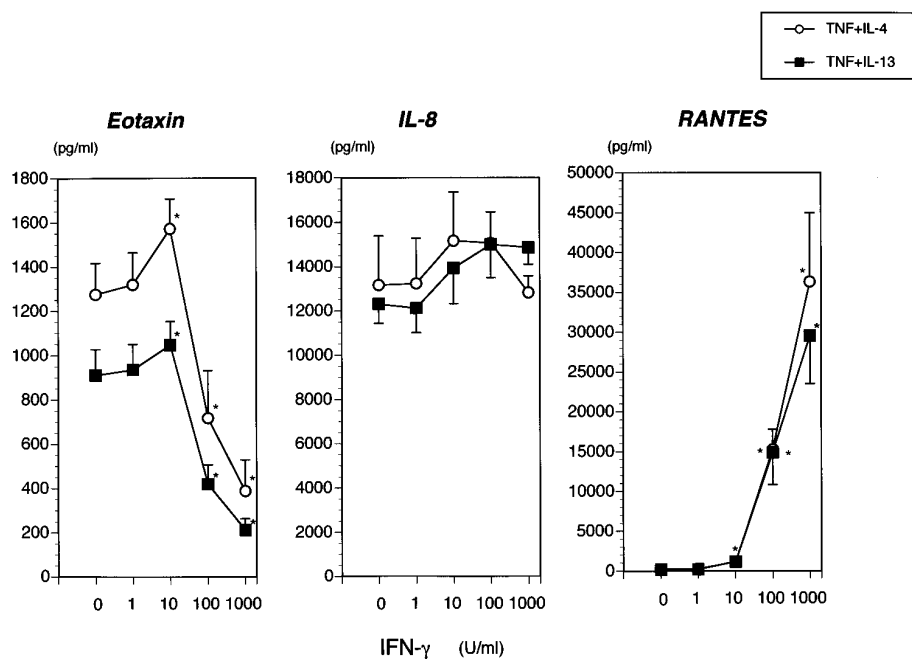


FIG 5. Effect of IFN- γ on eotaxin, IL-8, and RANTES production induced by both IL-4 and TNF- α or by both IL-13 and TNF- α . Cells were cultured for 48 hours with IFN- γ at various concentrations in the presence of TNF- α at 10 ng/mL and IL-4 at 10 ng/mL, or TNF- α at 10 ng/mL and IL-13 at 10 ng/mL. Supernatant was assayed for eotaxin, IL-8, and RANTES with ELISA. Data are expressed as mean \pm SEM (n = 4). Asterisk, $P < .05$, significantly different from control production without IFN- γ .

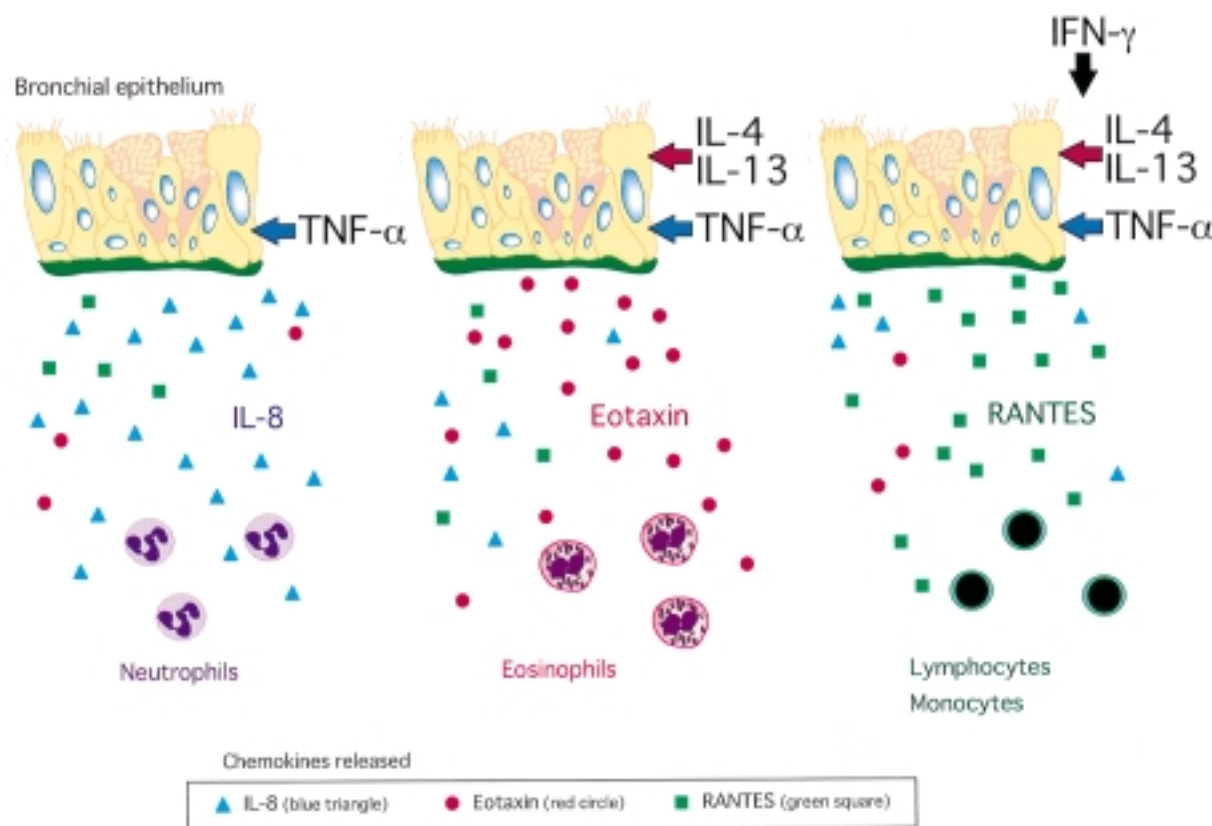


FIG 6. Illustrated summary of current study on regulation of chemokine production by bronchial epithelial cells. $\text{TNF-}\alpha$ induces IL-8 production from bronchial epithelial cells and may cause neutrophil recruitment. $\text{T}_\text{H}2$ cytokines, IL-4 and IL-13, alter the chemokine production pattern to that of eosinophil preference by enhancing eotaxin and reducing IL-8. $\text{T}_\text{H}1$ cytokine, $\text{IFN-}\gamma$, counteracts eotaxin-enhancing effects of $\text{T}_\text{H}2$ cytokines and induces RANTES, which may lead to preferential recruitment of lymphocytes and monocytes.

induce eotaxin production by bronchial epithelial cells and act synergistically with $\text{TNF-}\alpha$. It is noteworthy that IL-4 and IL-13 not only enhanced $\text{TNF-}\alpha$ -induced eotaxin production but also suppressed IL-8 production. In this experiment we first measured protein release from cells and then confirmed with RT-PCR that protein production is controlled at the transcriptional level. Although IL-8 protein was released in much greater quantities than was eotaxin, IL-8 production was significantly decreased by IL-4 and IL-13, perhaps to an extent that would inhibit neutrophil recruitment. These results suggest that the $\text{T}_\text{H}2$ cytokines IL-4 and IL-13 are involved in selective recruitment of eosinophils by up-regulating eotaxin and down-regulating IL-8.

We have hypothesized that $\text{IFN-}\gamma$, a $\text{T}_\text{H}1$ cytokine, inhibits eotaxin production by bronchial epithelial cells similarly to the way it inhibits IgE production by B cells. We have demonstrated that $\text{IFN-}\gamma$ counteracts the enhancing effect of IL-4 and IL-13 on eotaxin production. This result is consistent with our previous findings in human lung fibroblasts.³⁴ Although the mechanisms by which $\text{IFN-}\gamma$ inhibits IgE production are still poorly understood, a fact that inhibition occurred only in a T cell-dependent system suggests that $\text{IFN-}\gamma$ does not act directly on B

cells. In this experiment our observation that $\text{IFN-}\gamma$ inhibited eotaxin production only when epithelial cells were stimulated with both $\text{TNF-}\alpha$ and IL-4 or IL-13, not by $\text{TNF-}\alpha$ alone, suggests that $\text{IFN-}\gamma$ interferes with the signal transduction pathways of IL-4 and IL-13.

We have also found that $\text{TNF-}\alpha$ -induced RANTES production was markedly enhanced by $\text{IFN-}\gamma$. This result is consistent with findings of previous experiments.¹⁶ In the analysis of the response of human T cells to antigens, RANTES was produced by T cells and other mononuclear cells of the $\text{T}_\text{H}1$ -like phenotype that also produces $\text{IFN-}\gamma$ and IL-2. RANTES has binding specificity for CCR1, CCR3, and CCR5 and can cause chemotaxis of eosinophils, T cells, and monocytes. In contrast, eotaxin binds only to CCR3, which is expressed on eosinophils, basophils, and $\text{T}_\text{H}2$ cells. Our observation that $\text{TNF-}\alpha$ -induced RANTES production from BEAS-2B cells was not affected by $\text{T}_\text{H}2$ cytokine IL-4 but was markedly enhanced by $\text{T}_\text{H}1$ cytokine $\text{IFN-}\gamma$ may indicate the relative importance of RANTES in the $\text{T}_\text{H}1$ immune response. In the $\text{T}_\text{H}2$ immune response, in which IL-4 plays a major role, eotaxin may be an extremely important chemokine.

In summary, this study suggests that $\text{T}_\text{H}2$ cytokines act

on bronchial epithelial cells to shift proinflammatory cytokine-induced chemokine production pattern from neutrophil to eosinophil preference and T_H1 cytokines shift that to mononuclear cell preference (Fig 6).

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