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# Induction of eotaxin expression and release from human airway smooth muscle cells by IL-1$\beta$ and TNF$\alpha$: effects of IL-10 and corticosteroids

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**1** Eotaxin is a novel C-C chemokine with selective chemoattractant activity for eosinophils. We determined whether eotaxin could be produced by human airway smooth muscle (HASM) cells in culture and examined its regulation by interleukin-10 (IL-10) and the corticosteroid, dexamethasone.

**2** Stimulation of the cells with interleukin-1$\beta$ (IL-1$\beta$) or tumour necrosis factor (TNF$\alpha$) each at 10 ng ml$^{-1}$ induced the release of eotaxin protein with maximal accumulation by 24 h. Interferon-$\gamma$ (IFN$\gamma$) alone at 10 ng ml$^{-1}$ had no effect and there was no synergy between these cytokines on the release of eotaxin.

**3** Reverse phase high performance liquid chromatographic (HPLC) analysis of supernatents from cells treated with TNF$\alpha$ (10 ng ml$^{-1}$ for 96 h showed immunoreactivity to eotaxin which eluted with the expected retention time of 34.5–35 min.

**4** Both IL-1$\beta$ and TNF$\alpha$-induced release of eotaxin was not inhibited by dexamethasone (1 $\mu$M), however IL-10 (10 ng ml$^{-1}$) had a significant inhibitory effect. Dexamethasone and IL-10 did not inhibit the induction of eotaxin mRNA induced by IL-1$\beta$ or TNF$\alpha$.

**5** Thus, human airway smooth muscle cells can release eotaxin and could be an important source of chemokine production during airway inflammatory events.

**Keywords:** Eotaxin; airway smooth muscle cells; interleukin-10; interleukin-1$\beta$; interferon-$\gamma$; tumour necrosis factor-$\alpha$

**Abbreviations:** DMEM, Dulbecco's modified Eagle's medium; HBSS, Hanks' balanced salt solution; HASM, human airway smooth muscle; HPLC, high performance liquid chromatography; IFN$\gamma$, interferon-$\gamma$; IL-1$\beta$, interleukin-1$\beta$; IL-10, interleukin-10; RT–PCR, reverse transcription-polymerase chain reaction; TFA, trifluoroacetic acid; TNF$\alpha$, tumour necrosis factor $\alpha$

## Introduction

Asthma is characterized by reversible airway narrowing and inflammation of the airway wall leading to epithelial cell damage, mucus plugging, stimulation of neural reflexes and infiltration of eosinophils, macrophages and lymphocytes (Djukanovic *et al*., 1990; Azzawi *et al*., 1990; Bousquet *et al*., 1990). Structural changes in the airway wall such as increased smooth muscle content and subepithelial fibrosis associated with matrix deposition, are also present (Ebina *et al*., 1993; Roche *et al*., 1989) and may contribute to persistent airways obstruction and bronchial hyperresponsiveness (James *et al*., 1989). Until recently, airway smooth muscle was regarded to be solely contractile because its ability to shorten in response to many inflammatory mediators leads to a reduction in airway calibre. However, it is now known that airway smooth muscle cells are also capable of responding to cytokines to release other inflammatory mediators, in particular chemokines such as RANTES and interleukin-8 (IL-8; John *et al*., 1997; 1998), Airway smooth muscle may therefore act as an effector cell in perpetuating airway inflammation by releasing chemoattractants for various inflammatory cells.

Eotaxin is a newly described CC chemokine that was first isolated from lung lavage fluid of sensitized guinea-pigs following allergen exposure (Jose *et al*., 1994). Amongst eosinophil-active chemoattractants, eotaxin specifically attracts and activates eosinophils as demonstrated *in vitro* for both mouse and human eotaxin using assays for chemotaxis and calcium release (Rothenberg *et al*., 1996; Forssmann *et al*., 1997). Eotaxin has also been demonstrated to selectively induce eosinophil recruitment to the airways and to the skin *in vivo* (Rothenberg *et al*., 1995; Jose *et al*., 1994; Collins *et al*., 1995). Eotaxin is partly important in recruiting eosinophils to the airways following allergen challenge, and the time-course of its appearance coincides with that of eosinophil recruitment to the airways (Rothenberg *et al*., 1997). However, in mutant mice in which the eotaxin gene has been disrupted, lung eosinophilia induced by sephadex beads or by ovalbumin aerosol exposure was not affected (Yang *et al*., 1998). Eotaxin is expressed by several cell types in the inflamed airways of asthmatic patients including epithelial and endothelial cells, T-lymphocytes, macrophages and eosinophils (Ying *et al*., 1997; Lamkhioued *et al*., 1997; Mattoli *et al*., 1997). In the present study, we determined whether primary cultures of human airway smooth muscle (HASM) cells have the capacity to express eotaxin since they can also produce other chemokines (John *et al*., 1997; 1998). We therefore examined whether the pro-inflammatory cytokines interleukin-1$\beta$ (IL-1$\beta$), tumour necrosis factor $\alpha$ (TNF$\alpha$) and interferon-$\gamma$ (IFN$\gamma$) could induce eotaxin mRNA expression and release from airway smooth muscle cells *in vitro*, and we determined the modulatory effects

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of the anti-inflammatory cytokine, interleukin 10 (IL-10), and of the corticosteroid, dexamethasone.

## Methods

### *Human airway smooth muscle cell culture*

HASM cells were obtained from tracheas or main bronchi of healthy donors from either heart/heart and lung transplantation donors (four male, two female, aged 11–47 years). The smooth muscle was dissected out under sterile conditions and placed in culture as previously described (Belvisi *et al*., 1997; Hirst *et al*., 1992). Once in culture, the cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with foetal calf serum (FCS, 10% $v^{-1}v$), sodium pyruvate (1 mM), L-glutamine (2 mM), non essential amino acid mixture (1 ×), and antimicrobial agents as previously described. All cultures were maintained in a humidified atmosphere at 37°C in air/$CO_2$ (95:5% $v^{-1}v$). Fresh medium was replaced every 72 h. Using immunofluorescence techniques for both smooth muscle actin and myosin, more than 95% of the cells displayed the characteristics of smooth muscle cells in culture.

### *Cell stimulation*

Cells were plated onto either 24 well plates with an initial seeding density of 8000 cells/well and 6 well plates (Costar U.K. Ltd, High Wycombe, U.K.) with an initial seeding density of 30 000 cells/well for eotaxin release experiments and Northern blot analysis respectively. Sub-confluent human airway smooth muscle cells (passage 3–8) were growth arrested by being placed in supplemented DMEM void of FCS for 24 h. Cells were stimulated in fresh FCS-free medium containing TNF$\alpha$, IL-1$\beta$ or IFN$\gamma$ in a concentration- and time-dependent manner. The effect of combinations of these pro-inflammatory cytokines was also investigated. Furthermore, the ability of IL-10 (10 ng $ml^{-1}$) or dexamethasone (1 $\mu$M) to inhibit any effects produced by these cytokines was examined. In these experiments, dexamethasone and IL-10 were preincubated for 2 h prior to the addition of TNF$\alpha$, IL-1$\beta$ or IFN$\gamma$. Following the treatment period, the supernatants were harvested, clarified by centrifugation and stored at −20°C until assayed. The cells were lysed by freeze-thawing once in 1 ml of 0.08% trifluoroacetic acid. After centrifugation of the lysate, the soluble fraction was freeze-dried, resuspended in 500 $\mu$l of assay buffer and adjusted to pH 7.20 for measurement of eotaxin protein by a sandwich ELISA.

### *Preparation of eotaxin cDNA probe*

The eotaxin probe was generated by reverse transcription polymerase chain reaction (RT–PCR). Total cellular RNA was extracted from airway smooth muscle cells stimulated with TNF$\alpha$ using a modification of the method of Chomczynski & Sacchi (1987). Following two phenolchloroform extractions and isopropanol precipitation, RNA samples were precipitated overnight and washed twice with 75% ethanol and dissolved in RNAse-free water. Reverse transcription was performed as follows: 1 $\mu$g of the RNA and oligo $dT_{15}$ primer (0.4 $\mu$g) were incubated at 65°C for 10 min then placed on ice for 5 min. AMV-reverse transcriptase 15 u, 1 mM of dATP, dCTP, dGTP and dTTP, RNAse inhibitor 30 u, $MgCl_2$ 5 mM, KCl 50 mM, Tris-HCl (pH 9.0) 10 mM and 0.1% Triton X-100 were added in a total volume of 40 $\mu$l to the samples and incubated at 42°C for 60 min followed by 10 min at 85°C. The cDNA was subsequently diluted to a final volume of 400 $\mu$l in nuclease-free water. PCR was performed with 10 $\mu$l of the cDNA solution using 7.5 pM of forward and reverse primers, dATP, dGTP, dTTP, dCTP at a final concentration of 0.2 mM each, Taq polymerase 1.5 U, $MgCl_2$ 1.5 mM, KCl 50 mM, Tris-HCl (pH 9.0) mM and 0.1% Triton X-100 in a final volume of 30 $\mu$l. The primers for eotaxin were: 5′-CTCGCTGGGCCA-GCTTCTGTC-3′ and 5′-GGCTTTGGAGTTGGAGAT-TTTTGG-3′ giving a product of 227 base-pairs. PCR was carried out in a Techne multiwell thermocycler (Techne, Cambridge, U.K.) at 95°C for an initial 5 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 60 s. Final extension was 10 min at 72°C. The product was sequenced and confirmed to be human eotaxin. Following phenol/chloroform/isoamyl alcohol (50 : 49 : 1) extraction and ethanol precipitation, the product (10 ng $ml^{-1}$) was labelled using [$^{32}$P]dCTP using a DNA labelling kit according to the manufacturer's instructions (Pharmacia, St Albans, Herts, U.K.).

### *Northern blot analysis*

After a 24 h treatment period total cellular RNA was extracted from adherent cells using a modification of the method of Chomczynski & Sacchi (1987). Briefly, cell monolayers (approximately $1 \times 10^6$/extraction) were washed twice with ice cold Hanks' balanced salt solution (HBSS) before addition of solution D (4 M guanidinium thiocyanate, 2-mercaptoethanol, 0.5% sodium sarcosyl) directly to each well. RNA was then isolated with a phenol:chloroform; isoamyl alcohol (50 : 49 : 1) extraction in the presence of 0.4 M sodium acretate (pH 4.0). RNA was precipitated in an equal volume of propan-2-ol and pelletted by centrifugation at full speed in a microcentrifuge for 15 min at 4°C. Residual salt was removed by a further wash in 75% ethanol. Denatured RNAs (20 $\mu$g) were size-fractionated by gel electrophoresis on 1% agarose/formaldehyde gels containing 20 mM morpholinosulphonic acid (MOPS), 5 mM sodium acetate and 1 mM EDTA (ph 7.0) before Northern blotting to 'Magna' nylon membranes (msi, Westborough, U.S.A.) by capillary action.

Prehybridization and hybridization were carried out at 42°C with [$^{32}$P]dCTP-labelled probes (approximately $1.5 \times 10^6$ $cpm^{-1}ml$) in a buffer containing 50% formamide, 50 mM Tris-HCl (pH 7.50), 5 × Denhardts solution, 0.1% sodium dodecyl sulphate (SDS), 5 mM EDTA and 250 $\mu g^{-1}ml$ denatured salmon sperm DNA. Following hybridization, the blots were washed to stringency of 0.2 × SSC, 0.1% SDS at 60° before exposure to Kodak X-OMAT film. After suitable exposure times, autoradiographs were analysed by laser densitometry (Protein & DNA Imageware System, Discovery Series, New York, NY, U.S.A.). Probes were stripped by incubating the blot in a 50% formamide solution at 70°C for 2 h before hybridization with a [$^{32}$P]-labelled 1272-base pair rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe. Specific RNA levels were calculated as a ratio of eotaxin to GAPDH mRNA then expressed as a percentage of the control response.

### *Eotaxin immunoassay*

Eotaxin was measured by a sandwich ELISA using monoclonal capture and polyclonal detector antibodies as described previously (Evans *et al*., 1998). The limit of sensitivity was 2 pM.

### *Reversed phase HPLC separation of eotaxin*

The culture supernatant of human airway smooth muscle cells was collected after 96 h stimulation with TNFα (10 ng $ml^{-1}$). Conditioned medium (22 ml) was acidified to pH 2.0 with trifluoroacetic acid (TFA), filtered (0.45 μm) and applied to $C_{18}$ reversed phase SepPaks (Waters: $2 \times 840$ mg cartridges in series, pre-wetted with 0.1% TFA/acetonitrile and equilibrated in 0.1% TFA/water). After washing the cartridges with 0.1% TFA/water, the bound material was eluted with 0.1% TFA/acetonitrile and the solvents removed using a Savant SpeedVac. The sample was applied to a 300Å $C_{18}$ HPLC column (Vydac: $4.6 \times 250$ mm fitted with guard column $4.6 \times 20$ mm) in 0.08% TFA and eluted at 1 ml $min^{-1}$ with acetonitrile gradients in 0.08% TFA whilst collecting 0.5 min fractions (0–50% acetonitrile over 50 min followed by 50–80% acetonitrile over 6 min).

### *Materials*

All cytokines were purchased from R&D Laboratories (Oxford, U.K.). Phenol was obtained from Rathburn Chemicals (Perthshire, U.K.). Ethanol and propan-2-ol was from BDH (Poole, U.K.) and [$^{32}$P]dCTP was purchased from Amersham International (Amersham, Bucks, U.K.). AMV-reverse transciptase, dATP, dCTP, dGTP, dTTP, RNAse inhibitor, $MgCl_2$, KCl, Tris-HCl Triton X-100 was purchased from Promega (Southampton, U.K.). All other reagents were obtained from Sigma Chemical Company (Poole, U.K.).

### *Data analysis*

All data are reported as s.e.mean of $n$ determinations from HASM cells obtained from at least three patients. Data generated from Northern blot analysis was calculated as a ratio of eotaxin mRNA to GAPDH mRNA and then expressed as a percentage of the control response. Comparison between groups was performed using the non-parametric one-way analysis of variance (ANOVA) Kruskall-Wallis test followed by the Dunn's multiple comparison test. A $P$ value of $<0.05$ was considered to be significant.

## Results

### *Release of eotaxin protein by TNFα, IL-1β & IFNγ*

HASM cells stimulated with TNFα, IL-1β or IFNγ (10 ng $ml^{-1}$ for each cytokine) did not cause significant release of eotaxin at 4 h. However, TNFα and IL-1β induced maximal release of eotaxin into the supernatants at 24 h ($210 \pm 22.1$, $n=5$, $P<0.05$ compared to time-point control and $151.7 \pm 37.7$ fmol per well, $n=5$, $P<0.01$ compared to time-point control respectively). There was no further increase at 48 h (Figure 1a). The levels of eotaxin in the cytosolic fraction of airway smooth muscle cells did not change significantly at 4 and 24 h after stimulation with either TNFα or IL-1β (TNFα: $14.2 \pm 5.0$ and $10.7 \pm 3.5$; IL-1β: $21.2 \pm 5.4$ and $15.4 \pm 2.6$ $fmol^{-1}$ well, respectively; $n=3$), when compared to control levels ($12.1 \pm 4.7$ and $22.7 \pm 5.0$). This indicates that newly-synthesized eotaxin was released into the supernatant fraction. There was no evidence of additive effects on eotaxin release when the cells were stimulated with TNFα and IL-1β, or with IFNγ and TNFα together (Figure 1b).

To investigate the concentration-dependent response of IL-1β, TNFα and IFNγ on eotaxin release, cells were stimulated

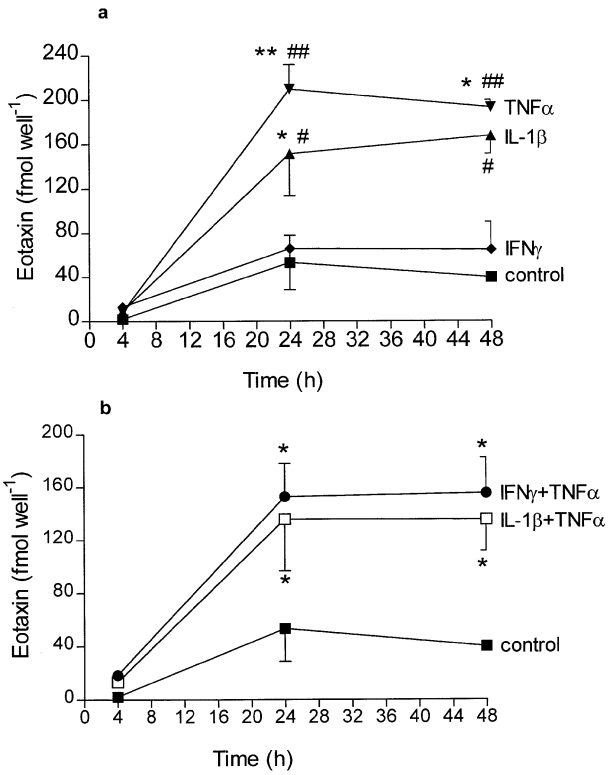


**Figure 1** Time-course of eotaxin release into the supernatants of airway smooth muscle cells in culture stimulated with either (a) TNFα, IL-1β or INFγ (10 ng $ml^{-1}$ each) alone or (b) IL-1β and TNFα together, and TNFα and INFγ together (all at 10 ng $ml^{-1}$). There was a significant release at 24 and 48 h following stimulation with either TNFα or IL-1β alone. Cells stimulated with the combination of cytokines did not release eotaxin in an additive or synergistic manner. Results shown as s.e.mean of smooth muscle cultures derived from three different donors. *$P<0.05$, **$P<0.01$ compared to control at similar time-points; #$P<0.05$, ##$P<0.01$ compared to the 4 h time-point.

with each of these cytokines at concentrations of 1, 3 and 10 ng $ml^{-1}$ for 24 h. IFNγ had no significant effect up to 10 ng $ml^{-1}$, while IL-1β and TNFα significantly induced the release of eotaxin at 10 ng $ml^{-1}$ ($151.7 \pm 37.8$, $n+5$, $P<0.05$ and $210.0 \pm 22.1$ fmol per well, $n=5$, $P<0.01$ respectively; Figure 2) compared to control unstimulated cells.

### *HPLC analysis of supernatants from HASM cells*

Immunoractivity to eotaxin eluted with the expected retention time of 34.5–35 min and a minor tailing peak at 36–36.5 min which was not further characterized (Figure 3). Both peaks eluted before RANTES (37–37.5 min), which is also produced by these cells (John *et al*., 1997).

### *Effects of IL-10 and dexamethasone on eotaxin release*

To investigate the effects of IL-10 and corticosteroids on eotaxin release and expression, human airway smooth muscle cells were stimulated for 24 h with either TNFα or IL-1β alone (10 ng $ml^{-1}$ each) in presence and absence of IL-10 (10 ng $ml^{-1}$) or dexamethasone (1 μM). Dexamethasone had no significant effect on the release of eotaxin, while IL-10 had a suppressive effect (Figure 4a). In order to ascertain that dexamethasone was having a suppressive effect on the release of other mediators, we measured the levels of the arachidonic acid metabolite, prostaglandin $E_2$ ($PGE_2$)

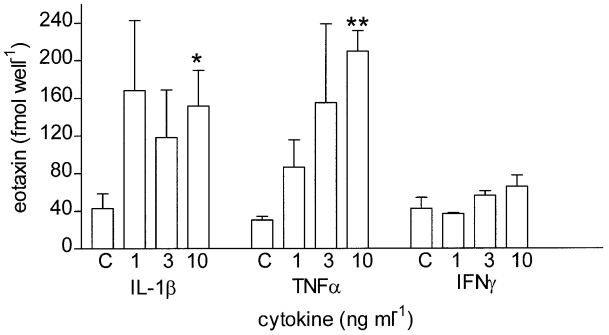


**Figure 2** Effect of increasing concentrations (1, 3 and 10 ng ml$^{-1}$) of IL-1$\beta$, TNF$\alpha$ and INF$\gamma$ on the release of eotaxin from cultured human airway smooth muscle cells measured 24 h after exposure. Both IL-1$\beta$ and TNF$\alpha$ caused a significant increase in eotaxin release at 10 ng $^{-1}$ but INF$\gamma$ had no effect. Data shown as s.e.mean of smooth muscle cultures derived from three different donors. *$P<0.05$, **$P<0.01$ compared to control values.

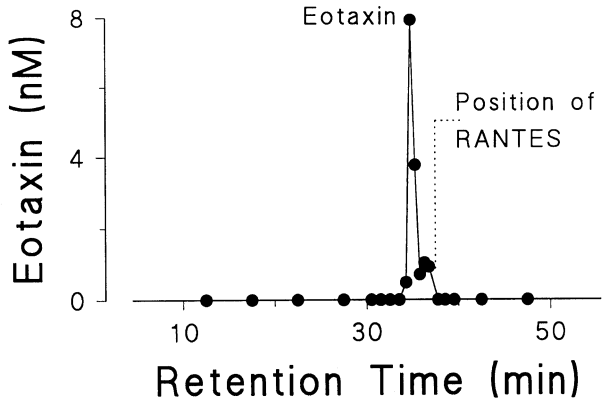


**Figure 3** Reverse-phase HPLC separation of eotaxin from supernatants of stimulated airway smooth muscle cells. The supernatant was separated by C18 HPLC and 0.5 min fractions were collected. Immunoreactive eotaxin was detected with a major peak at 34.5–35 min and a minor peak at 36–36.5 min. The position for RANTES is shown for comparison.

using a radioimmunoassay as previously described (Mitchell *et al*., 1994). IL-1$\beta$ induced significant release of $PGE_2$ from $0.7\pm0.6$ ng ml$^{-1}$ to $4.3\pm0.7$ ng ml$^{-1}$ ($P<0.05$). This effect was suppressed in the presence of dexamethasone (1 $\mu$M) to $0.23\pm0.01$ ng ml$^{-1}$ (Figure 4b).

### *Effects of IL-10 and dexamethasone on IL-1β and TNFα-induced eotaxin mRNA expression*

Preliminary experiments showed that eotaxin mRNA induction appeared by 4 h and was optimal after stimulation of HASM cells with IL-1$\beta$ or TNF$\alpha$ for 24 h at 10 ng ml$^{-1}$ each (data not shown). Hence these conditions were used for subsequent experiments. Stimulation of HASM cells with IL-1$\beta$ and TNF$\alpha$ (each at 10 ng ml$^{-1}$) for 24 h induced the expression of eotaxin mRNA ($198\pm31\%$, $n=3$, $P<0.01$ and $205\pm54\%$ of control, $n=4$, $P<0.05$ respectively). This was observed as a single, distinct band of approximately 800 base pairs by Northern blot analysis (Figure 5a and b). This transcript size is similar to that reported for eotaxin mRNA expressed in human lung epithelial cells (Lilly *et al*., 1997). Both dexamethasone (1 $\mu$M) and IL-10 (10 ng ml$^{-1}$) had no effect on eotaxin mRNA expression induced by IL-1$\beta$ or TNF$\alpha$ under similar conditions (Figure 5a and b).

## Discussion

We have shown that human airway smooth muscle cells in culture can be induced to express eotaxin mRNA and to release eotaxin protein when incubated with TNF$\alpha$ or IL-1$\beta$. IFN$\gamma$ however had no effect on the release of eotaxin from these cells and there was no additive effect of any of these cytokines on eotaxin release or expression. Eotaxin produced by the cells was mostly released into the supernatants. The appearance of eotaxin mRNA at 4 h after stimulation, prior to release of eotaxin into the supernatant, indicates that the protein release is dependent on transcription. IL-10 significantly inhibited eotaxin release while corticosteroids had no effect, but neither had an effect on the induction of eotaxin mRNA. Thus, airway smooth muscle may contribute directly

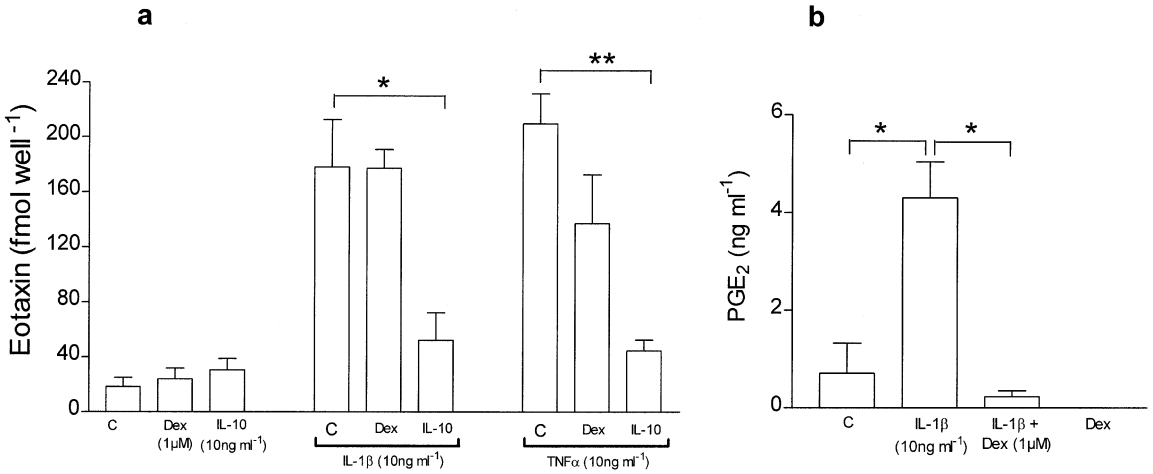


**Figure 4** (a) Effect of IL-10 (10 ng ml$^{-1}$) and dexamethasone (1 $\mu$M) on eotaxin release induced by IL-1$\beta$ (10 ng ml$^{-1}$) and TNF$\alpha$ (10 ng ml$^{-1}$). Dexamethasone did not inhibit eotaxin release while IL-10 suppressed both IL-1$\beta$- and TNF$\alpha$-induced release. However, dexamethasone significantly inhibited release of prostaglandin $E_2$ release induced by IL-1$\beta$ (b). Results shown as s.e.mean of 3–4 different donors. *$P<0.05$, **$P<0.001$.

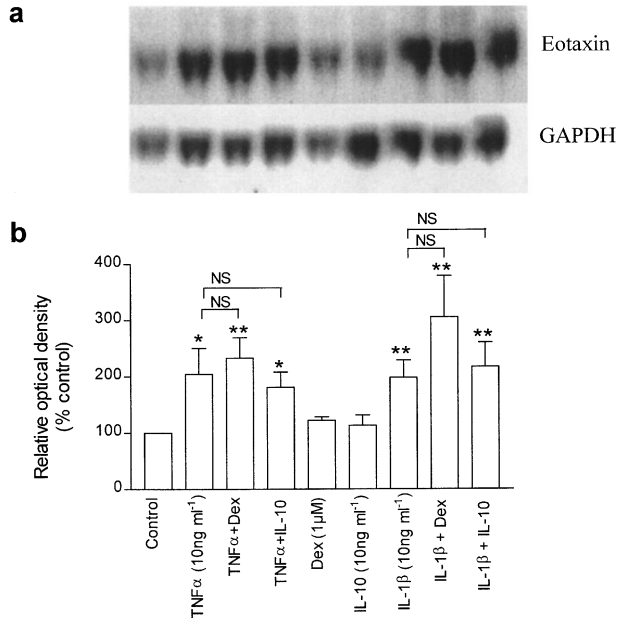


**Figure 5** (a) Representative Northern blot analysis of eotaxin mRNA with GAPDH mRNA from airway smooth muscle cells under control unstimulated conditions, and following exposure to IL-1$\beta$, TNF$\alpha$ and INF$\gamma$ each at 10 ng ml$^{-1}$ mRNA was extracted after a 24 h exposure period to these cytokines. The effects of dexamethasone (1 $\mu$M) and IL-10 (10 ng ml$^{-1}$) on the induction of eotaxin mRNA are also shown. (b) Mean densitometric measurements for eotaxin mRNA calculated as a ratio of GAPDH mRNA and expressed as percentage of control. Both IL-1$\beta$ and TNF$\alpha$ increased eotaxin mRNA expression. These effects were not significantly affected by dexamethasone or IL-10. Results shown are s.e.mean of cells from 3–4 different donors. *$P<0.05$, **$P<0.01$ compared to control.

to airway inflammation by interacting with IL-1$\beta$ and TNF$\alpha$ released during inflammation and by attracting eosinophils to the airway submucosa through the release of eotaxin and other chemoattractant cytokines.

The conditions under which release of eotaxin from human airway smooth muscle cells was observed were different from those of RANTES in many respects. Of the pro-inflammatory cytokines, TNF$\alpha$, IL-1$\beta$ and IFN$\gamma$, both TNF$\alpha$ and IL-1$\beta$ were effective stimulators of eotaxin release, while TNF$\alpha$ but not IL-1$\beta$ stimulated RANTES release (John *et al*., 1997). IFN$\gamma$ was a poor stimulator of both RANTES and eotaxin release, but synergized with TNF$\alpha$ to cause RANTES release. However, no such synergy was observed regarding eotaxin. We also found differences when compared to IL-8 expression and release from airway smooth muscle cells (John *et al*., 1998). Examining the profile of eotaxin protein release and mRNA expression, however, supported the notion that these chemokines were transcriptionally regulated by the pro-inflammatory cytokines, particularly TNF$\alpha$ and IL-1$\beta$. In the studies of eotaxin release, we found no significant changes in cytosolic eotaxin following cytokine stimulation indicating that most of the newly-synthesized eotaxin was released extracellularly. The differences in the pattern of chemokine release observed are likely to be due to differences in signalling pathways and effector elements for transcriptional activation of each of these chemokines. Analysis of the 5′ flanking regions of the human RANTES, eotaxin and IL-8 genes have revealed a number of positive and negative transcriptional regulatory elements, including AP-1 and NF-$\kappa$B elements (Garcia Zepeda *et al*., 1997; Hein *et al*., 1997). The presence of an NF-$\kappa$B binding site may underlie the observed regulation of eotaxin by the pro-inflammatory cytokines TNF$\alpha$ and IL-1$\beta$.

Corticosteroids had no effect on the release of eotaxin from airway smooth muscle cells, which is in contrast with the inhibitory effects of corticosteroids on the release and expression of IL-8 and RANTES (John *et al*., 1997; 1998). In the present study, the concentration of dexamethasone used should have been effective because the release of $PGE_2$ from stimulated cells were inhibited. The mechanism of the relative resistance of eotaxin mRNA expression to suppression by corticosteroids is unclear. This observation may however, be related to a specific property of airway smooth muscle cells since corticosteroids have been shown to be capable of inhibiting the induction of eotaxin mRNA from the epithelial cell line, A549 cells (Lilly *et al*., 1997). The presence of GRE sequences on the eotaxin promoter indicates that corticosteroids should inhibit transcription of eotaxin, since, for example, deletion analysis of the GRE from the IL-8 promoter revealed that this element participated in dexamethasone suppression of IL-8 (Mukaida *et al*., 1992). By contrast, IL-10 inhibited the stimulated release of IL-8, RANTES and eotaxin from airway smooth muscle cells (John *et al*., 1997; 1998). Although IL-10 also inhibited the enhanced expression of IL-8 and RANTES, it did not suppress the induction of eotaxin mRNA, indicating that IL-10 may interfere with post-transcriptional regulation of eotaxin.

Our results indicate that the airway smooth muscle should not be regarded solely as a specialized cell involved in contractile responses. Pro-inflammatory cytokines and several growth factors are capable of modulating airway smooth muscle phenotype and mitogenesis (Hirst, 1996) and the resulting increase in airway smooth muscle mass may contribute to airways obstruction and bronchial hyperresponsiveness in asthma (James *et al*., 1989). The additional secretory potential of airway smooth muscle, particularly in terms of eotaxin release, adds another dimension to the putative role of airway smooth muscle in airway inflammation. Because eotaxin has selective chemoattractant effects on eosinophils, airway smooth muscle could contribute directly to the recruitment of eosinophils to the airways. Whether eotaxin produced by airway smooth muscle cells could also in turn contribute to altered smooth muscle function and airway remodelling is not known. Our observations support the notion that airway smooth muscle could be a major contributor to the inflammatory and pathophysiologic features of the airways in asthma.

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## References

AZZAWI, M., BRADLEY, B., JEFFERY, P.K., FREW, A.J. WARDLOW, A.J., KNOWLES, G., ASSOUFI, B., COLLINS, J.V., DURHAM, S. & KAY, A.B. (1990). Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am. Rev. Respir. Dis.,* **142,** 1407–1413.

BELVISI, M.G., SAUNDERS, M.A., HADDAD, E-B., HIRST, S.J., YACOUB, M.H., BARNES, P.J. & MITCHELL, J.A. (1997). Inductuin of cyclo-oxygenase-2 by cytokines in human cultured airway smooth muscle cells: novel inflammatory role of the cell type. *Br. J. Pharmacol.,* **142,** 910–916.

BOUSQUET, J., CHANEZ, P., LACOSTE, J.Y., BARNEON, G., GHAVANIAN, N., ENANDER, I., VENGE, P., AHLSTEDT, S., SIMONY-LAFONTAINE, J., GODARD, P., & MICHEL, F.B. (1990). Eosinophilic inflammation in asthma. *N. Engl. J. Med.,* **323,** 1033 – 1039.

CHOMCZYNSKI, P. & SACCHI, N. (1987). Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.,* **162,** 156 – 160.

COLLINS, P.D., MARLEAU, S., GRIFFITHS-JOHNSON, D.A., JOSE, P.J. & WILLIAMS, T.J. (1995). Co-operation between interleukin-5 and the chemokine, eotaxin, to induce eosinophil accumulation *in vivo*. *J. Exp. Med.,* **182,** 1169 – 1174.

DJUKANOVIC, R., WILSON, J.W., BRITTEN, K.M., WILSON, S.J., WALLS, A.F., ROCHE, W.R., HOWARTH, P.H. & HOLGATE, S.T. (1990). Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. *Am. Rev. Respir. Dis.,* **142,** 863 – 871.

EBINA, M., TAKAHASHI, T., CHIBA, T. & MOTOMIYA, M. (1993). Cellular hypertrophy and hyperplasia of airway smooth muscle underlying bronchial asthma. *Am. Rev. Resp. Dis.,* **148,** 720 – 726.

EVANS, C.A., GARCIA, H.H., HARTNEL, A., GILMAN, R.H., JOSE, P.J., MARTINEZ, M., REMICK, D.G., WILLIAMS, T.J. & FRIEDLAND, J.S. (1998). Elavated concentrations of eotaxin and interleukin-5 in human neurocysticercosis. *Infect. Immun.,* **66,** 4525.

FORSSMANN, U., UGUCCIONI, M., LOETSCHER, P., DAHINDEN, C.A., LANGEN, H., THELEN, M. & BAGGIOLINI, M. (1997). Eotaxin-2, a novel CC chemokine that is selective for the chemokine receptor CCR3, and acts like eotaxin on human eosinophil and basophil leukocytes. *J. Exp. Med.,* **185,** 2171 – 2176.

GARCIA ZEPEDA, E.A., ROTHENBERG, M.E., WEREMOWICZ, S., SARAFI, M.N., MORTON, C.C. & LUSTER, A.D. (1997). Genomic organization, complete sequence, and chromosomal location of the gene for human eotaxin (SCYA11), an eosinophil-specific CC chemokine. *Genomics,* **41,** 471 – 476.

HEIN, H., SCHLUTER, C., KULKE, R., CHRISTOPHERS, E., SCHRODER, J.M. & BARTELS, J. (1997). Genomic organization, sequence, and transcriptional regulation of the human eotaxin gene. *Biochem. Biophys. Res. Commun.,* **237,** 537 – 542.

HIRST, S.J. (1996). Airway smooth muscle cell culture: application to studies of airway wall remodelling and phenotype plasticity in asthma. *Eur. Resp. J.,* **9,** 908 – 918.

HIRST, S.J., BARNES, P.J. & TWORT, C.H.L. (1992). Quantifying proliferation of cultured human and rabbit airway smooth muscle cells in response to serum and platelet-derived growth factor. *Am. J. Respir. Cell Mol. Biol.,* **7,** 574 – 581.

JAMES, A.L., PARE, P.D. & HOGG, J.C. (1989). The mechanics of airway narrowing in asthma. *Am. Rev. Respir. Dis.,* **139,** 242 – 246.

JOHN, M., AU, B.T., JOSE, P.J., LIM, S., SAUNDERS, M., BARNES, P.J., MITCHELL, J.A., BELVISI, M.G. & CHUNG, K.F. (1998). Expression and release of interleukin-8 by human airway smooth muscle cells: inhibition by Th-2 cytokines and corticosteroids. *Am. J. Respir. Cell. Mol. Biol.,* **18,** 84 – 90.

JOHN, M., HIRST, S.J., JOSE, P.J., ROBICHAUD, A., BERKMAN, N., WITT, C., TWORT, C.H.C., BARNES, P.J. & CHUNG, K.F. (1997). Human airway smooth muscle cells express and release RANTES in response to Th-1 cytokines: regulation by Th-2 cytokines and corticosteroids. *J. Immunol.,* **158,** 1841 – 1847.

JOSE, P.J., GRIFFITHS-JOHNSON, D.A., COLLINS, P.D., WALSH, D.T., MOQBEL, R., TOTTY, N.F., TRUONG, O., HSUAN, J.J. & WILLIAMS, T.J. (1994). Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J. Exp. Med.,* **179,** 881 – 887.

LAMKHIOUED, B., RENZI, P.M., ABI YOUNES, S., GARCIA ZEPADA, E.A., ALLAKHVERDI, Z., GHAFFAR, O., ROTHENBERG, M.D., LUSTER, A.D. & HAMID, Q. (1997). Increased expression of eotaxin in bronchoalveolar lavage and airways of asthmatics contributes to the chemotaxis of eosinophils to the site of inflammation. *J. Immunol.* **159,** 4593 – 4601.

LILLY, C.M., NAKAMURA, H., KESSELMAN, H., NAGLER ANDERSON, C., ASANO, K., GARCIA ZEPEDA, E.A., ROTHENBERG, M.E., DRAZEN, J.M. & LUSTER, A.D. (1997). Expression of eotaxin by human lung epithelial cells: induction by cytokines and inhibition by glucocorticoids. *J. Clin. Invest.,* **99,** 1767 – 1773.

MATTOLI, S., STACEY, M.A., SUN, G., BELLINI, A. & MARINI, M. (1997). Eotaxin expression and eosinophilic inflammation in asthma. *Biochem. Biophys. Res. Commun.,* **236,** 299 – 301.

MITCHELL, J.A., BELVISI, M.G., AKARASEREENONT, P., ROBBINS, R.A., KWON, O-J., CROXTALL, J., BARNES, P.J. & VANE, J.R. (1994). Induction of cyclo-oxygenase-2 by cytokines in human pulmonary epithelial cells: regulation by dexamethasone. *Br. J. Pharmacol.,* **113,** 1008 – 1014.

MUKAIDA, N., GUSSELLA, G.L., KASAHARA, T., KO, Y., ZACHARIAE, C.O., KAWAI, T. & MATSUSHIMA, K. (1992). Molecular analysis of the inhibition of interleukin-8 production by dexamethasone in a human fibrosarcoma cell line. *Immunology,* **75,** 674 – 679.

ROCHE, W.R., BEASLEY, R., WILLIAMS, J.H. & HOLGATE, S.T. (1989). Subepithelial fibrosis in the airways of asthmatics. *Lancet,* 520 – 522.

ROTHENBERG, M.E., LUSTER, A.D., LILLY, C.M., DRAZEN, J.M. & LEDER, P. (1995). Constitutive and allergen-induced expression of eotaxin mRNA in the guinea pig lung. *J. Exp. Med.,* **181,** 1211 – 1216.

ROTHENBERG, M.E., MACLEAN, J.A., PEARLMAN, E., LUSTER, A.D. & LEDER, P. (1997). Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. *J. Exp. Med.,* **185,** 785 – 790.

ROTHENBERG, M.E., OWNBEY, R., MEHLHOP, P.D., LOISELLE, P.M., VAN DE RIJN, M., BONVENTRE, J.V., OETTGEN, H.C., LEDER, P. & LUSTER, A.D. (1996). Eotaxin triggers eosinophil-selective chemotaxis and calcium flux via a distinct receptor and induces pulmonary eosinophilia in the presence of interleukin 5 in mice. *Mol. Med.,* **2,** 334 – 348.

YANG, Y., LOY, J., RYSECK, R-P., CARRASCO, D. & BRAVO, R. (1998). Antigen-induced eosinophilic lung inflammation develops in mice deficient in chemokine eotaxin. *Blood,* **92,** 3912 – 3923.

YING, S., ROBINSON, D.S., MENG, Q., ROTTMAN, J., KENNEDY, R., RINGLER, D.J., MACKAY, C.R., DAUGHERTY, B.L., SPRINGER, M.S., DURHAM, S.R., WILLIAMS, T.J. & KAY, A.B. (1997). Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. *Eur. J. Immunol.,* **27,** 3507 – 3516.

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