

Chronic cough resembles asthma with IL-5 and granulocyte-macrophage colony-stimulating factor gene expression in bronchoalveolar cells

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Background: Chronic cough is a multifactorial condition, which, like asthma, can be associated with eosinophilic airway inflammation. In asthma, airway eosinophilia is believed to be mediated by cytokines such as interleukin-5 and granulocyte-macrophage colony stimulating factor (GM-CSF). The role of these cytokines in chronic cough is unclear.

Objective: The aim of this study was to examine gene expression for IL-5 and GM-CSF in chronic cough and compare the results with those found in asthma.

Methods: We studied adults with asthma ($n = 12$), chronic cough responsive to inhaled corticosteroid (ICS-responsive cough) ($n = 9$), and chronic cough not responsive to inhaled corticosteroid (non-ICS-responsive cough) ($n = 4$). Bronchoalveolar lavage (BAL) was performed, and cytokine gene expression was assessed by using a semiquantitative reverse transcriptase polymerase chain reaction.

Results: IL-5 mRNA was expressed by BAL cells from nine of 12 asthmatic subjects and six of nine subjects with ICS-responsive chronic cough. IL-5 mRNA was not detected in subjects with non-ICS-responsive chronic cough (zero of four subjects, $p < 0.05$). GM-CSF mRNA was expressed in BAL cells from seven of 12 asthmatic subjects and six of nine subjects with ICS-responsive cough. GM-CSF mRNA was not detected in non-ICS responsive cough subjects (zero of four subjects, $p < 0.05$). GM-CSF gene expression was related to the degree of methacholine airway responsiveness in asthmatic subjects ($r = -0.59$).

Conclusion: We conclude that chronic cough, like asthma, is associated with airway inflammation and gene expression for IL-5 and GM-CSF. Ongoing expression of these cytokines is likely to be related to the persistence of airway inflammation and chronic cough. (*J Allergy Clin Immunol* 1998;101:320-6.)

Key words: Asthma, cough, cytokine, IL-5, GM-CSF, PCR, eosinophil airway responsiveness

Abbreviations used

BAL:	Bronchoalveolar lavage
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
ICS:	Inhaled corticosteroid
RT-PCR:	Reverse transcriptase polymerase chain reaction

Chronic cough is a common problem that is associated with several different etiologic factors.^{1, 2} Clinical studies have identified that some patients with chronic cough respond well to corticosteroid therapy,^{1, 2-4} suggesting the presence of underlying airway inflammation. Recently, this has been confirmed by finding eosinophilic bronchitis in sputum and bronchial biopsy specimens from patients with chronic cough.^{3, 5-8} The role of cytokines as mediators of the airway inflammatory response in corticosteroid-responsive chronic cough has not been defined.

Asthma is characterized by airway inflammation with eosinophils and mast cells and by airway hyperresponsiveness. The eosinophilic bronchitis in asthma is thought to be mediated by cytokines such as IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which are produced by T_{H2} lymphocytes.⁹⁻¹³ These cytokines have important effects on the growth and differentiation of eosinophils and mediate the eosinophilia in other diseases. They may therefore be important in the eosinophilic bronchitis of corticosteroid-responsive chronic cough. In asthma, airway inflammation is also closely linked to the presence and severity of airway hyperresponsiveness. It is therefore somewhat anomalous that patients with chronic cough can have airway inflammation without airway hyperresponsiveness.^{2, 5, 7} This could occur if there were different mediators of eosinophilic bronchitis in chronic cough. Alternatively, airway inflammation and airway hyperresponsiveness may be separate traits in asthma, as recent genetic studies suggest.¹⁴ A study of the cytokines mediating inflammation in chronic cough is one way to investigate these issues.

Therefore this study was performed to compare the expression of GM-CSF and IL-5 in airway cells from subjects with chronic cough and to compare these results

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TABLE I. Subject characteristics (mean [SEM])

	Asthma	Chronic cough (corticosteroid- responsive)	Chronic cough (nonresponsive)
Number of subjects	12	9	4
Age (yrs)	43 (4)	38 (5)	48 (6)
Sex (M/F)	4/8	3/6	1/3
Allergy (%)	9 (75)	6 (60)	4 (100)
FEV ₁ (% predicted)	86 (5)	106 (4)	101 (7)
PD ₂₀ (μmol)	2.14 (0.58)	NR	NR
DRS	24.80 (8.56)*	1.45 (0.29)	1.92 (0.80)
Therapy			
B ₂ -agonist	12	0	0
ICS	10	0	0

NR, A 20% fall in FEV₁ was not reached during bronchial provocation challenge of cough subjects; DRS, dose-response as percent fall/μmol during bronchial provocation challenge.

**p* < 0.05 asthma versus chronic cough.

with those found in asthma. We also sought to examine the relationship between cytokine gene expression and airway responsiveness in these conditions. We have used a semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) to detect cytokine gene expression.¹⁵ The method is very specific for the cytokines in question, and the semiquantitative nature allows comparison between clinical groups. We tested the hypotheses that chronic cough responsive to inhaled corticosteroids (ICS-responsive cough) is similar to asthma in gene expression for IL-5 and GM-CSF by airway cells and that the expression of these cytokines differs in subjects with and without airway hyperresponsiveness.

METHODS

Subjects

Adult nonsmokers (*n* = 25) were recruited from patients attending the Respiratory Medicine Unit at John Hunter Hospital, and their clinical characteristics are shown in Table I. Asthmatic subjects (*n* = 12) gave a history of episodic cough, wheeze, or breathlessness and had objective evidence of methacholine airway hyperresponsiveness (PD₂₀ < 4 μmol) or FEV₁ bronchodilator responsiveness of greater than 20%. The asthmatic subjects had controlled asthma on the basis of stable symptoms and medication requirements and most were using ICS. Subjects with chronic cough (*n* = 13) had normal spirometry and normal airway responsiveness, with no identifiable cause of cough from clinical assessment, chest radiography, or bronchoscopy. After bronchoscopy and sample collection, these subjects underwent a therapeutic trial of corticosteroids. The trial involved 2 weeks of high-dose inhaled beclomethasone (1 mg twice daily) delivered through a pressurized metered-dose inhaler and large-volume valved holding chamber (Volumatic), with assessment of symptom response as described.^{5,7} Nine subjects responded to ICS therapy and were defined as having chronic cough responsive to ICS (ICS-responsive chronic cough). We have previously described an association between airway eosinophilia and ICS-responsive chronic cough.^{2,5,7} Four subjects with chronic cough experienced no response to

TABLE II. Sequences of primers used in RT-PCR

Gene	Primer sequence
IL-5 P1	5' GCTTCTGCATTTGAGTTTGCTAGCT 3'
IL-5 P2	5' TGGCCGTCAATGTATTTCTTTATTAAG 3'
IL-5 Hyb	5' ATTTTATGTACAGGAACAGGAATCCTCAG 3'
GM-CSF P1	5' CTGCTGCTGAGATGAATGAAAC 3'
GM-CSF P2	5' GGATATCAGTCAGAAAGTTT 3'
GM-CSF Hyb	5' GCTCGAATATCTTCAGGCGG 3'
β-Actin P1	5' TCACCAACTGGGACGACATG 3'
β-Actin P2	5' GTACAGGGATAGCACAGCCT 3'
β-Actin Hyb	5' CAGCCATGTACGTTGCTATC 3'

cDNA was amplified with the relevant primers P1 and P2. PCR products were probed with the relevant Hyb primer.

ICS. These subjects were defined as having non-ICS-responsive chronic cough. Each subject gave written informed consent for this study, which was approved by the Institutional Research Ethics Committee.

Design

At the first visit, a clinical history was obtained by physician-administered questionnaire followed by allergy skin tests, spirometry, and assessment of methacholine airway responsiveness. Within 1 week the subjects returned for bronchoscopy with bronchoalveolar lavage (BAL) and bronchial brushings. Asthmatic subjects continued their regular therapy (Table I) before bronchoscopy. In the chronic cough subjects, bronchoscopy was performed before any therapy. After bronchoscopy, subjects with chronic cough underwent a corticosteroid trial and returned 2 weeks later for repeat clinical assessment, which was performed in blinded fashion with regard to the bronchoscopy results.

Allergy tests

Allergy skin tests were performed by the modified prick technique by using 14 common allergen extracts. Histamine and buffered saline acted as positive and negative controls, respectively. A 15-minute wheal greater than 3 mm larger than the negative control test was considered a positive skin prick test result. Subjects with greater than 1 positive test were considered allergic.

Airway responsiveness

Spirometry was performed with a Vitalograph Compact (No. 4200; Vitalograph Ltd., Buckingham, England) electronic spirometer. Before methacholine inhalation challenge testing, subjects withheld bronchodilators for their duration of action. Methacholine airway responsiveness was assessed with the rapid method.¹⁶ The results were expressed as the PD₂₀ and as the dose-response slope. A PD₂₀ value of less than 4 μmol was regarded as indicative of airway hyperresponsiveness.

Fiberoptic bronchoscopy

Bronchoscopy was performed as previously described¹⁷ by using atropine (0.6 mg by subcutaneous injection) and 0.2 mg inhaled salbutamol as premedication and lignocaine as topical anesthetic. Electrocardiography and pulse oximetry were monitored throughout the procedure, and supplemental oxygen was delivered. The bronchoscope (Pentax FB-15X, Pentax Instruments) was wedged into a subsegmental bronchus of the middle lobe, and BAL was performed with 200 ml of prewarmed sterile 0.9% saline delivered in 20 ml aliquots and aspirated during

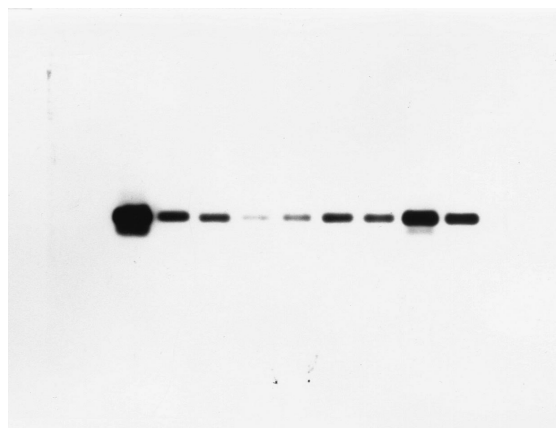


FIG 1. IL-5 gene expression in asthma. IL-5 PCR product detected with enhanced chemiluminescence (see Methods section). *Lane 1*, negative control; *lane 2*, positive control; *lanes 3 to 10*, samples from subjects with asthma.

gentle (<100 mmHg) wall suction. The fluid was collected into sterile polypropylene centrifuge tubes and held at 4° C. The bronchoscope was then placed in the lingula bronchus for the performance of standardized bronchial brushing.¹⁷ A sterile single sheathed nylon brush was passed by the suction channel into a segmental bronchus. The brush was advanced, and brushings were performed in three positions: 3 o'clock, 12 o'clock, and 9 o'clock. The brush was then retracted into the plastic sheath and withdrawn from the bronchoscope. The brush was directly smeared onto glass slides. In asthmatic subjects, a second brushing was taken from a different segment and placed immediately into lysis buffer for RNA extraction.

BAL processing

The lavage fluid volume was recorded, and cells were resuspended by gentle pipetting. An aliquot was removed for cell counts and the remaining cells were washed and placed in lysis buffer for RNA extraction. A total cell count was performed in duplicate on neat samples with a hemocytometer. Viability was determined by trypan blue exclusion. Cytocentrifuge preparations were made with 100 μ l aliquots of neat BAL fluid and a Shandon Cytospin 2 (Shandon Corporation, Sewickley, Pa.).

Cytochemistry

Air-dried alcohol-fixed cytocentrifuge preparations (BAL) and direct smears (brushings) were stained with May-Grünwald Giemsa, and a cell differential was performed by counting 400 cells in random fields. Metachromatic cells were stained with 0.5% toluidine blue at pH 0.1 after fixation in Carnoy's fluid. Metachromatic cells in bronchial brushings and BAL fluid have previously been characterized as formalin-sensitive and tryptase-positive mast cells.¹⁷ In bronchial brushings, where there was a high frequency of metachromatic cells, 1500 nucleated cells were counted in each of two slides. Metachromatic cells in BAL slides were counted as described previously.¹⁷

Processing of blood cells

Peripheral blood mononuclear cells were isolated from normal volunteers by dextran sedimentation (Dextran T-500; Pharmacia, Uppsala, Sweden) and density gradient centrifugation at 450 g on Lymphoprep (Nycomed, Oslo, Norway). The cells

were then cultured at 1×10^6 /ml in RPMI-1640 (GIBCO, Grand Island, N.Y.), 10% heat-inactivated fetal calf serum, 2 mmol/L L-glutamine, 25 mm HEPES buffer, and 5 μ g/ml phytohemagglutinin (Burroughs-Wellcome) at 37° C. After 21 hours, these cells were harvested and lysed immediately for RNA extraction as described below.

RNA extraction and cDNA synthesis

The BAL cell pellet was lysed in 2 to 4 ml denaturing solution containing 4 mol/L guanidinium thiocyanate (Fluka Chimica), 25 mmol/L sodium citrate (pH 7.0), 0.5% sarcosyl, and 0.1 mol/L 2-mercaptoethanol. RNA was then extracted by using the acid-guanidinium thiocyanate method.¹⁸ Gel electrophoresis was performed to ascertain that the RNA was intact by demonstration of 28S and 18S ribosomal bands. Intact total RNA was stored at -70° C in diethyl pyrocarbonate-treated (Calbiochem Corporation, La Jolla, Calif.) sterile water. A 1 μ g aliquot of total cellular RNA was reverse-transcribed to cDNA in a 20 μ l reaction mix containing 15 units AMV reverse transcriptase (Promega), 5 mmol/L $MgCl_2$, 10 mmol/L Tris-HCl (pH 8.8), 50 mmol/L KCl, 0.1% Triton X-100, 1 mmol/L of each dNTP, 20 U rRNasin (Promega), 10 μ g oligo (dT)₁₅, and DEPC-treated sterile water incubated at 42° C for 15 minutes and then at 95° C for 5 minutes to inactivate the reverse transcriptase. The cDNA was then stored at 4° C until used. RNA was extracted from bronchial brushings by using the same procedure, and all of the extracted RNA was used for preparation of cDNA.

PCR

cDNA equivalent to 0.25 μ g total cell RNA was amplified for all samples. The same amount of 21-hour phytohemagglutinin-stimulated peripheral blood mononuclear cell RNA was also included as a positive control, and sterile water was used as the negative control. The reaction mixture used for all amplifications contained 200 μ mol/L of each dNTP, 1 μ mol/L of each primer, 2.5 U Amplitaq DNA Polymerase (Perkin Elmer Cetus, Norwalk, Conn.), 1.5 mmol/L $MgCl_2$, 10 mmol/L Tris-HCl (pH 8.3), and 50 mmol/L KCl. Reactions were carried out in 50 μ l volumes overlaid with paraffin oil and amplified by repeated cycles of denaturation at 95° C for 1 minute, annealing at 60° C for 2 minutes, and extension at 72° C for 2 minutes with a Gene Machine GM10 (Innovonics, Victoria, Australia). The samples were allowed to cool to room temperature before the aqueous phase was extracted with 100 μ l of chloroform. PCR cycle numbers were chosen to allow a semiquantitative assessment of gene expression (see below). The PCR primers are listed in Table II. The IL-5 primers were purchased from Clontech (Palo Alto, Calif.). The β -actin primers were previously reported¹⁵ and the GM-CSF primers were synthesized on the basis of published sequences¹⁹ by using a Pharmacia LKB Gene Assembler Plus and then purified by standard methods.

Analysis of PCR products

Ten microliters of the total 50 μ l PCR reaction volume for each sample was electrophoresed in 2% 3:1 Nusieve (FMC, Rockland, Me.), agarose, and $1 \times$ TAE (40 mmol/L Tris-acetate, 2 mmol/L ethylenediamine tetraacetic acid) gels transferred to Hybond N⁺ nylon membrane (Amersham) in 0.4

mol/L NaOH for 4 hours, neutralized in $2 \times$ standard saline citrate, and stored in a sealed bag at 4°C until ready for hybridization. All blots were probed with internal primers (see Table II) designed to bind to a region of cDNA between the two amplification primers. These primers were tailed at the 3' end with fluorescein-dUTP and detected with an enhanced chemiluminescence 3' oligolabeling detection system (Amersham) as recommended by the manufacturer. The blots were then exposed to Hyperfilm MP (Amersham) for 5 and 10 minutes. The intensities of the bands were quantitated by a scanning densitometer (GS300; Hoeffer Scientific Instruments, San Francisco, Calif.). Each band on the film was converted to a curve by the densitometer, and the area under the curve was calculated by using Gaussian integration. The results are reported as the mean of duplicate values.

Semiquantitation of the PCR

Experiments were conducted to assess accumulation of products after different numbers of PCR cycles for each cytokine and β -actin.¹⁵ Using total RNA extracted from the 21-hour phytohemagglutinin-stimulated peripheral blood mononuclear cells, equal quantities of cDNA (equivalent to 0.25 μg total RNA) were amplified in duplicate for 24, 26, 28, and 30 cycles in the case of IL-5 and GM-CSF or 14, 16, 18, and 20 cycles in the case of β -actin. The appropriate cycle number was then chosen for each product so that the PCR was terminated while products were still accumulating and before the plateau phase had been reached. The cycle number chosen for IL-5 and GM-CSF was 30, and for β -actin it was 18.

Analysis

The FEV₁ response to methacholine was expressed as the percentage fall from the mean baseline value and plotted against log-nebulized cumulative dose. The PD₂₀ was calculated by linear interpolation and used to characterize the position of the dose-response curves to methacholine. Methacholine dose-response curves were also characterized by their dose-response slope. Continuous variables for the groups were summarized as mean with standard error in parentheses and compared by using nonparametric methods (Kruskal-Wallis). Categorical variables were compared with Fisher's exact test. Associations between variables were examined by rank correlation. Significance was accepted at the 5% level.

RESULTS

Bronchoscopy and cell counts

Bronchoscopy was performed safely in each subject. BAL recovered 66% of the instilled fluid, which contained an average cell number 16 to 30×10^4 cells/ml in the three groups (Table III). The subjects with asthma and chronic ICS-responsive cough had evidence of ongoing airway inflammation (Table III). BAL eosinophils were elevated in subjects with asthma and ICS-responsive cough in comparison with non-ICS-responsive cough ($p < 0.05$). Intraepithelial mast cells were increased to 0.9% of cells in ICS-responsive cough ($p < 0.05$).

Cytokine gene expression

RT-PCR identified gene expression for IL-5 in nine of 12 asthmatic subjects and in six of nine subjects with ICS-responsive cough (Figs. 1 and 2). IL-5 gene expression was not detected in any of the subjects with

TABLE III. Cell counts in BAL and brushings (mean [SEM])

	Asthma	Chronic Cough (ICS-responsive)	Chronic cough (non-ICS-responsive)
BAL			
Percent return	69 (3)	68 (3)	63 (2)
Total cell count $\times 10^4/\text{ml}$	16 (2)	25 (3)	26 (7)
Macrophages (%)	84 (4)	87 (4)	88 (2)
Lymphocytes (%)	3.6 (1.2)	1.7 (0.7)	1.1 (0.8)
Neutrophils (%)	4.8 (2.8)	3.6 (2.1)	3.2 (1.9)
Eosinophils (%)	1.7 (0.5)*	3.8 (2.5)**	0.2 (0.1)
Mast cells (%)	0.29 (0.08)	0.21 (0.10)	0.15 (0.06)
Epithelial cells (%)	5.5 (2.5)	3.7 (2.3)	9.5 (1.7)
Brushings			
Mast cells (%)	0.53 (0.14)	0.91 (0.30)**	0.21 (0.15)

* $p < 0.05$, asthma versus chronic cough not responsive to inhaled corticosteroid.

† $p < 0.05$, ICS-responsive chronic cough versus non-ICS-responsive chronic cough.

non-ICS-responsive cough (asthma vs non-ICS-responsive cough, $p = 0.03$).

Asthma and ICS-responsive cough demonstrated a similar pattern of gene expression for GM-CSF (Fig. 3). GM-CSF mRNA transcripts were detected in seven of 12 asthmatic subjects and six of nine subjects with ICS-responsive cough (Fig. 3). GM-CSF mRNA was not detected in any of the subjects with non-ICS-responsive cough (asthma vs non-ICS-responsive chronic cough, $p = 0.06$). There was coexpression of GM-CSF and IL-5 mRNA in five of 12 asthmatic subjects and in four of nine subjects with chronic cough. Neither cytokine was expressed in two asthmatic subjects and one subject with ICS-responsive cough. Expression of GM-CSF and IL-5 message were not strongly correlated, suggesting that these two cytokines were not coregulated in asthma ($r = -0.185$, $p > 0.05$). We also examined GM-CSF gene expression in bronchial epithelial cells in asthma by using bronchial brushings in 10 asthmatic subjects. These samples harvest approximately 10^6 cells, of which 98% are bronchial epithelial cells,¹⁷ and GM-CSF mRNA was detected in seven of the 10 samples.

β -Actin mRNA was similar in all four groups ($p > 0.05$). The presence of detectable β -actin in the samples without detectable cytokine mRNA attests to the integrity of the mRNA used for analysis.

Relation to airway responsiveness

Overall, there was no association between IL-5 and GM-CSF gene expression and the presence of airway hyperresponsiveness (chi-square = 0.430, $p =$ not significant) or its severity ($p = 0.366$) in the study population as a whole. When the subjects with asthma were considered as a separate group, methacholine airway responsiveness was related to GM-CSF gene expression. As the severity of methacholine hyperresponsiveness

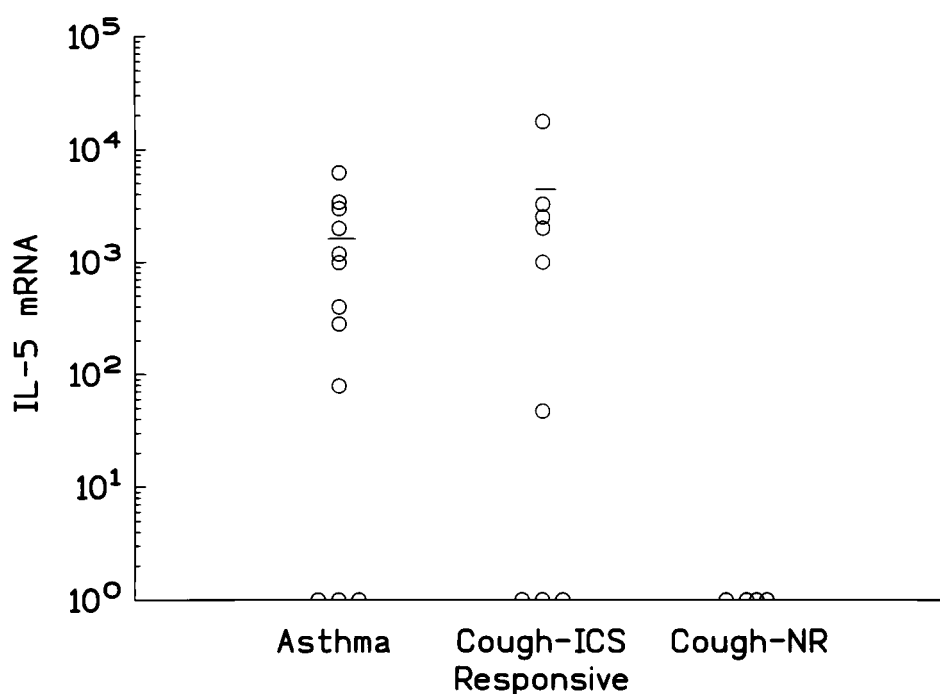


FIG. 2. Gene expression for IL-5 as mean area under curve from subjects with asthma, ICS-responsive chronic cough, and non-ICS-responsive chronic cough (*Cough-NR*).

increased, so did GM-CSF gene expression in BAL cells ($r = -0.591$, $p < 0.05$). Subjects with moderate airway hyperresponsiveness ($PD_{20} < 2 \mu\text{mol}$) had more GM-CSF mRNA (3897 relative units) in comparison with those with mild ($PD_{20} > 2 \mu\text{mol}$) airway hyperresponsiveness (164 units, $p = 0.009$; Fig. 4). There was no association with IL-5 gene expression and methacholine responsiveness ($r = -0.14$, $p > 0.05$). Similarly, we were unable to identify a significant association between BAL eosinophilia and cytokine gene expression.

We examined the relationship between atopy and gene expression and found that IL-5 and GM-CSF mRNA levels were similar in atopic and nonatopic subjects ($p > 0.05$). IL-5 mRNA was detected in 66% of atopic subjects and 80% of nonatopic subjects ($p > 0.05$). GM-CSF mRNA was detected in 50% of atopic subjects and 80% of nonatopic subjects ($p > 0.05$).

DISCUSSION

This study confirms the presence of eosinophilic bronchitis in chronic cough that is responsive to ICS therapy, and has identified the novel finding of IL-5 and GM-CSF gene expression in this condition. Chronic cough and asthma appear to share a common pattern of inflammation in terms of both cellular infiltration and inflammatory cytokine gene expression. This profile can also persist in subjects with controlled asthma treated with ICS. In asthma, GM-CSF mRNA was related to the severity of airway responsiveness. The steroid use by most of the asthmatic subjects in this study may have

obscured a relationship between IL-5 gene expression and airway responsiveness.

Cytokine gene expression was assessed by using RT-PCR, which offers both high sensitivity and high specificity for the detection of cytokine mRNA. A semiquantitative PCR assay was performed by amplifying a similar baseline amount of mRNA and characterizing the relationship between PCR product and cycle number for each cytokine.¹⁵ This provided standardization for the high sensitivity of PCR and allowed a semiquantitative comparison to be performed. Cytokine protein levels in BAL fluid were not assayed. In recent studies, however, both GM-CSF and IL-5 have been detected in BAL fluid from asthmatic subjects⁹⁻¹² at the mRNA and protein levels, indicating that the cytokine mRNA we have described is likely to be translated into protein.

This study supports a role for IL-5 and GM-CSF in leading to the specific eosinophil infiltrate that characterizes asthma and corticosteroid-responsive cough. In subjects without BAL eosinophilia, no IL-5 or GM-CSF mRNA was detected even though these subjects had symptoms of airway disease (cough). In this respect ICS-responsive cough resembles asthma, and a trial of corticosteroid therapy should be considered to identify this condition in patients who are first seen with chronic cough. It will be interesting to further examine the relationship between chronic cough and asthma and to determine whether corticosteroid-responsive chronic cough may be an antecedent of asthma as some epi-

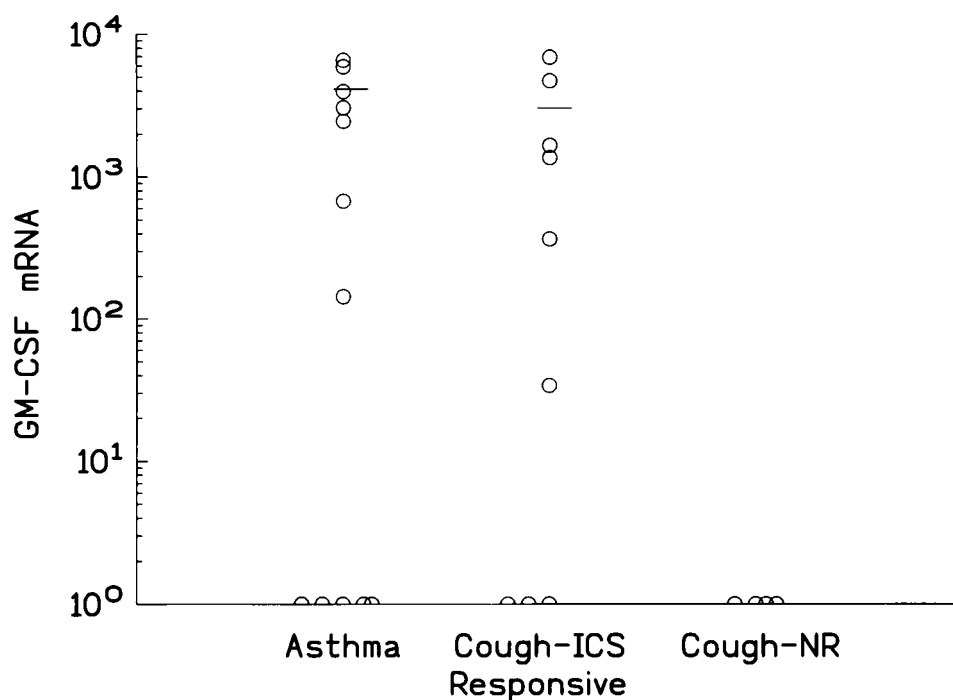


FIG. 3. Gene expression for GM-CSF in subjects with asthma, ICS-responsive chronic cough, and non-ICS-responsive chronic cough (*Cough-NR*).

miologic studies suggest.²⁰ GM-CSF can be produced by most of the cells present in the airway lumen, and it has potent proinflammatory effects on granulocytes, macrophages, and lymphocytes. Other groups have detected GM-CSF in asthmatic airways by using BAL and bronchial biopsies and by assaying at the mRNA and active protein levels.⁹⁻¹³ We have extended these observations by demonstrating GM-CSF gene expression by bronchial epithelial cells obtained in vivo with bronchial brushings.

The persistence of IL-5 and GM-CSF gene expression in these asthmatic subjects receiving ICS is interesting and may suggest a population of airway cells that are resistant to this effect of ICS. Although corticosteroids reduce airway cytokine gene expression^{15, 21} and protein production,²² the reported posttreatment levels were still higher than those in control subjects. We have confirmed that there is incomplete suppression of IL-5 and GM-CSF gene expression by ICS in asthma and that the level of ongoing GM-CSF expression is related to the degree of methacholine airway hyperresponsiveness. Ongoing cytokine production may explain some features of asthma, such as airway hyperresponsiveness and eosinophilia, that persist despite high-dose ICS therapy.²³

Airway hyperresponsiveness in asthma is closely associated with eosinophil and mast cell infiltration and cytokine gene expression. In ICS-responsive cough this inflammatory response occurred without methacholine airway hyperresponsiveness. We have previously investigated patients with steroid-responsive cough and re-

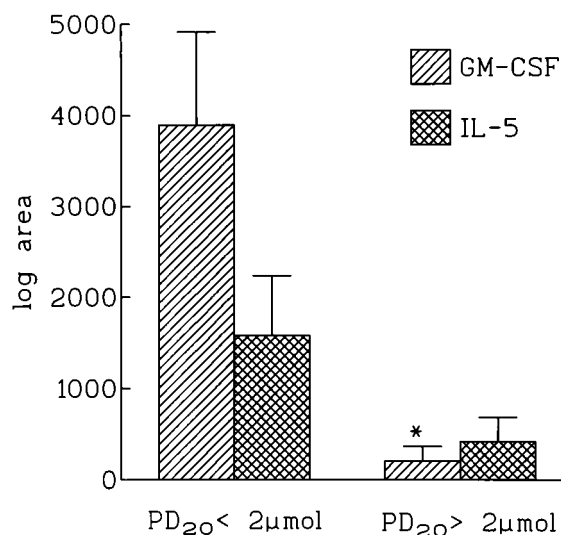


FIG. 4. Gene expression for GM-CSF (*hatched bars*) and IL-5 (*cross-hatched bars*) in asthmatic subjects with moderately severe methacholine airway hyperresponsiveness ($PD_{20} < 2 \mu\text{mol}$) and mild methacholine airway hyperresponsiveness ($PD_{20} > 2 \mu\text{mol}$). * $p < 0.05$.

ported no evidence for excessive airway narrowing or increased peak flow variability.⁷

In this study there was no overall relationship between cytokine gene expression and airway hyperresponsiveness. In particular, cytokine gene expression was not different in subjects with and without airway hyperresponsiveness. These results argue against airway hyper-

responsiveness as being solely mediated by airway inflammation and cytokine gene expression. The results are consistent with airway inflammation and airway hyperresponsiveness being distinct traits under separate genetic control.¹⁴ Coexpression of both traits gives rise to the clinical syndrome of asthma. Expression of one trait only could explain the disparate conditions of chronic cough with eosinophilic bronchitis and asymptomatic airway hyperresponsiveness without airway inflammation.²⁴

A limitation of our study was that most of the asthmatic subjects were taking ICS, which could have suppressed cytokine gene expression. We sought to overcome this by using a very sensitive assay system (RT-PCR). Furthermore, a relationship between GM-CSF mRNA and airway responsiveness was found, but only in the asthmatic subjects. If airway responsiveness was related to cytokine gene expression in all subjects, then inclusion of the subjects with steroid-responsive cough should have strengthened the relationship. Our results demonstrated the opposite. It is quite possible, however, that steroid use by the asthmatic subjects obscured a relationship between IL-5 cytokine gene expression to airway responsiveness in asthma and chronic cough before and after corticosteroid therapy.

In conclusion, this study has identified that, like asthma, IL-5 and GM-CSF form the characteristic cytokine profile in chronic cough that responds to ICS and is associated with eosinophilic bronchitis. These cytokines may be an important target for future therapy in this condition.

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