

IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines

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Background: IL-17 is a cytokine that has been reported to be produced by T lymphocytes. In vitro, IL-17 activates fibroblasts and macrophages for the secretion of GM-CSF, TNF- α , IL-1 β , and IL-6. A number of these cytokines are involved in the airway remodeling that is observed within the lungs of asthmatic individuals.

Objective: In this study, we investigated the expression of IL-17 in sputum and bronchoalveolar lavage specimens obtained from asthmatic subjects and from nonasthmatic control subjects.

Methods: IL-17 was detected through use of immunocytochemistry, in situ hybridization, and Western blot. Bronchial fibroblasts were stimulated with IL-17, and cytokine production and chemokine production were detected through use of ELISA and RT-PCR.

Results: Using immunocytochemistry, we demonstrated that the numbers of cells positive for IL-17 are significantly increased in sputum and bronchoalveolar lavage fluids of subjects with asthma in comparison with control subjects ($P < .001$ and $P < .005$, respectively). We demonstrated that in addition to T cells, eosinophils in sputum and bronchoalveolar lavage fluids expressed IL-17. Peripheral blood eosinophils were also positive for IL-17, and the level of IL-17 in eosinophils purified from peripheral blood was significantly higher in subjects with asthma than in controls ($P < .01$). To further investigate the mechanism of action of IL-17 in vivo, we examined the effect of this cytokine on fibroblasts isolated from bronchial biopsies of asthmatic and nonasthmatic subjects. IL-17 did enhance the production of pro-fibrotic cytokines (IL-6 and IL-11) by fibroblasts, and this was inhibited by dexamethasone. Similarly, IL-17 increased the level of other fibroblast-derived inflammatory mediators, such as the α -chemokines, IL-8, and growth-related oncogene- α .

Conclusion: Our results, which demonstrate for the first time that eosinophils are a potential source of IL-17 within asth-

matic airways, suggest that IL-17 might have the potential to amplify inflammatory responses through the release of proinflammatory mediators such as α -chemokines. (*J Allergy Clin Immunol* 2001;108:430-8.)

Key words: Asthma, cytokines, fibroblasts, eosinophils, remodeling

IL-17 is a newly described cytokine that was first identified as the human homolog of cytotoxic T lymphocyte-associated antigen 8.^{1,2} In mice, the $\alpha\beta$ TCR⁺ CD4⁺CD8⁻ cells appeared to be the only source of IL-17.³ Studies in human beings demonstrated that detectable levels of mRNA encoding IL-17 were found in peripheral blood T cells and were increased on activation.⁴ CD4⁺ T cells produced most of the IL-17. In agreement with this study, Fossiez et al⁵ showed that IL-17 expression was restricted to the CD4⁺CD45RO⁺ memory cell subset. In contrast to the apparently restricted expression of IL-17, the receptors for IL-17 show an almost ubiquitous cellular distribution.⁶

The major effect of IL-17 reported to date is the stimulation of stromal cells to secrete IL-6, IL-8, granulocyte colony-stimulating factor, prostaglandin E₂, and nitric oxide.^{5,7-10} Recently, IL-17 was demonstrated to upregulate the release of IL-1 β , TNF- α , IL-6, IL-10, IL-12, IL-1Ra, prostaglandin E₂, and stromelysin by human macrophages.¹¹ In contrast, IL-17 was reported not to have any major effect on cytokine secretion by T cells.⁵ The addition of IL-17 to fibroblasts enhanced their ability to sustain the growth of CD34⁺ hematopoietic progenitors and directed their preferential maturation into neutrophils.⁵

To date, IL-17 has been studied principally in allograft rejection¹²⁻¹⁴ and rheumatoid arthritis.^{5,15,16} Because IL-17 is produced mainly by CD4⁺ T cells and stimulates the release of fibroblast-derived mediators, we hypothesized in this study that IL-17 has a potential role in asthma, in which T cells and stromal cells are thought to play a key role in the progression of the disease.

The aim of this study was to analyze the presence of IL-17 in asthmatic airways by noninvasive (sputum) and invasive (bronchoalveolar lavage [BAL]) methods and to investigate the effect of IL-17 in vitro on bronchial fibroblasts for the release of cytokines such as IL-6 and IL-11 and chemokines such as IL-8 and growth-related oncogene- α (Gro- α). Our data demonstrate that IL-17 might be a potential player in the cytokine network involved in asthma, and they suggest a possible indirect effect of IL-17 on airway remodeling.

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Supported in part by Medical Research Council Canada (MT-13273), Astra Canada, and the Chair de Pneumologie de la Fondation J. Bégin. S. Molet is the recipient of a Canadian Lung Association/Canadian Thoracic Society and Merck-Frosst fellowship. Q. Hamid and J. Chakir are the recipients of a Chercheur-Boursier award from the Fonds de la Recherche en Santé du Québec.

Received for publication April 6, 2001; revised June 11, 2001; accepted for publication June 14, 2001.

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0091-6749/2001 \$35.00 + 0 1/83/117929

doi:10.1067/mai.2001.117929

Abbreviations used

BAF:	Bronchial fibroblasts isolated from asthmatic subjects
BAL:	Bronchoalveolar lavage
BNF:	Bronchial fibroblasts isolated from normal subjects
Dex:	Dexamethasone
ECM:	Extracellular matrix
Gro- α :	Growth-related oncogene- α
ICC:	Immunocytochemistry
MBP:	Major basic protein
TBS:	TRIS-buffered saline solution
TGF:	Transforming growth factor

MATERIALS AND METHODS

Subjects

Eleven asthmatic subjects fulfilling the American Thoracic Society criteria for asthma¹⁷ were recruited (Table I). Of these, 6 patients agreed to undergo BAL and sputum induction. Sputum induction was done first; BAL was done 1 week later. The symptoms of these patients were controlled by regular use of inhaled selective β_2 -agonists only. Six of the patients with mild asthma were atopic, inasmuch as they had positive skin reactions to at least 1 common allergen. All subjects were nonsmokers or were ex-smokers (<5 pack-years) who had stopped smoking for at least 12 months. Subjects who had used corticosteroids, theophylline, long-acting β_2 -agonists, leukotriene antagonists, or antihistamines within the 3 months before the study were excluded, as were subjects who had histories of respiratory tract infection within the previous 6 weeks or immunotherapy within the previous 12 months.

Seven nonasthmatic volunteer subjects underwent sputum induction, and 6 of these underwent BAL; none were smokers or showed any evidence of a recent infection of the respiratory tract. They had no histories of allergy or asthma (Table I).

The Ethics Committee of the Montreal Chest Hospital and Laval Hospital approved the study, and each subject gave written informed consent.

Sputum

Sputum induction was performed as described elsewhere.¹⁸ Briefly, normal (n = 7) and asthmatic (n = 6) individuals received aerosols of increasing concentrations of hypertonic saline solution (3%, 4%, and 5%). The procedure was interrupted every 2 minutes for measurement of peak expiratory flow. Subjects coughed sputum into a sterile container when they felt that sputum might be present. These procedures were repeated sequentially for 8-minute periods at each concentration unless a fall in peak expiratory flow of greater than 10% occurred, in which case the procedure was stopped.

Portions of sputum that were free of salivary contamination were selected and processed.¹⁸ The final cell pellet was resuspended in PBS, and a total cell count of leucocytes and cell viability were determined. For immunocytochemistry (ICC), the cells were cytospun; cytopspins were fixed in acetone:methanol (60:40) for 7 minutes, air-dried, and kept at -20°C until further analysis.

Bronchoalveolar lavage

The technique of fiberoptic bronchoscopy with BAL in asthmatic and normal control subjects has been described elsewhere.¹⁸ Asthmatic (n = 6) and normal control (n = 6) subjects received nebulized salbutamol 15 minutes before the procedure. BAL was performed in the right middle lobe by rapidly instilling 50 mL of saline solution into a single subsegment and then aspirating a mean volume of 30 mL. The BAL fluid was transferred into sterile polypropylene tubes

and centrifuged (300g, 7 minutes, 4°C). The final cell pellet was resuspended in RPMI1640 medium (1×10^6 cells/mL) and cytopspins were made. After a brief fixation in acetone:methanol (60:40), cytopspins were stored at -20°C until they were used for ICC.

Immunocytochemistry for IL-17

ICC slides were immunostained with a goat antihuman IL-17 polyclonal antibody (R&D Systems, Minneapolis, Minn) at a concentration of 1 $\mu\text{g/mL}$. Incubation with the first antibody was followed by incubations with a biotinylated rabbit antigoat antibody (1/500; Sigma Chemical, St Louis, Mo) and then with an alkaline phosphatase-conjugated streptavidin solution (1/100; Sigma Chemical). The slides were developed in Fast Red (1 mg/mL; Sigma Chemical) in the presence of levamisole, as previously described.¹⁹ For negative control preparations, the primary antibody was replaced by TBS or nonimmune goat serum.

In situ hybridization

To confirm that IL-17 immunoreactivity is due to synthesis of this cytokine by eosinophils, we performed in situ hybridization of a purified eosinophil preparation collected from peripheral blood of asthmatic and normal individuals. Radiolabeled cRNA probe coding for IL-17 mRNA was used on a cytospin preparation of purified eosinophils. Hybridization was performed under high-stringency conditions, and the mRNA signal was visualized by means of autoradiography, as previously described.²⁰ A sense probe corresponding to an IL-17 mRNA identical sequence was used as negative control.

Peripheral blood eosinophil purification

Peripheral blood of asthmatic (n = 6) and normal control (n = 6) subjects was centrifuged (400g, 30 minutes) on a gradient of Ficoll-Paque (Pharmacia-Upjohn, Uppsala, Sweden). Granulocytes were obtained by dextran sedimentation. Human eosinophils were further purified (as described elsewhere) by negative selection with anti-CD16²¹ and anti-CD3-coated immunomagnetic microbeads through use of a Magnetic Cell Sorting system (Miltenyi Biotec, Bergisch-Gladbach, Germany) at 4°C . The degree of purity of the eosinophil populations, estimated after staining with May Grünwald-Giemsa, was greater than 95%. The cells were then cytopspun on slides, fixed in acetone:methanol (60:40), and stored at -20°C until ICC was performed.

Western blot analysis for intracellular IL-17

Western blot analysis was performed as described elsewhere, with some modification.²² The same number (1×10^6) of purified eosinophils (>95%) obtained from asthmatic and control subjects were lysed in the Tris solution with 2 mmol/L EDTA, 2% Triton X-100, and an antiprotease mix. Lysates were boiled in sample buffer (200 mmol/L Tris [pH6.8], 20% glycerol, 2% SDS, 0.1% bromophenol blue, and 10% β -ME), and the same amount of total protein was loaded onto a 10% SDS-PAGE. After electrophoretic separation, proteins were transferred onto nylon PVDF membrane (Millipore, Bedford, Mass) and blocked overnight with TBS-T (100 mmol/L Tris pH7.5, 0.9% NaCl, 0.1% Tween-20) complemented with 4% nonfat dry milk. Thereafter, the membrane was incubated for 1 hour at room temperature with the polyclonal goat antihuman IL-17 antibody (diluted 1/1000). The membrane was washed and incubated for 1 hour at room temperature with a horse-peroxidase-labeled swine anti-goat IgG (Cedarlane, Ontario, Canada). Blots were developed through use of an ECL kit (Amersham, United Kingdom).

Fibroblasts cell culture

Primary bronchial fibroblasts obtained from asthmatic subjects (BAF; n = 7) and primary bronchial fibroblasts obtained from nor-

TABLE I. Patient characteristics

	Nonasthmatic control subjects	Asthmatic subjects
Sex (M/F)	6/3	6/5
Age (y) (mean $\pm$ SEM)	29.6 $\pm$ 1.7	33.1 $\pm$ 3.0
Atopy (yes/no)	2 / 7	6 / 5
Inhaled steroids	No	No
FEV ₁ (% predicted) (mean $\pm$ SEM)	104.9 $\pm$ 2.2	95.9 $\pm$ 5.1
FEV ₁ /FVC (% predicted) (mean $\pm$ SEM)	83.3 $\pm$ 1.2	74.5 $\pm$ 2.7*

M, Male; F, female; FVC, forced vital capacity.

* $P < .05$ in comparison with nonasthmatic controls.

TABLE II. Sequences and predicted sizes of the primers used for PCR analysis

Cytokine/primer	Predicted size of amplicon (base pairs)
IL-6	
5' ATGTAGCCGCCCCACACAGA	187
3' CATCCATCTTTTCAGCCAT	
IL-11	
5' ACTGCTGCTGCTGAAGACTCGGCTGTGA	294
3' ATGGGGAAGAGCCAGGGCAGAAGTCTGT	
IL-8	
5' ATTTCTGCAGCTCTGTGTGAA	255
3' TGAATTCTCAGCCTCTTCAA	
Gro- α	
5' GGATCCAAGCAAATGGCCAATGAG	250
3' TGTCTAAGCCAGAAACACTG	
β -actin	
5' GTGGGGCGCCCCAGGCACCA	500
3' CTCCTTAATGTCACGCACGATTTC	

mal control subjects (BNF; $n = 5$) were used. The isolation, purity, and stability of these cells are described elsewhere.²³ Cells were maintained in Dulbecco modified Eagle medium (DMEM) in 75-cm² flasks and kept in an 8% CO₂/37°C atmosphere until they reached 80% confluence. Between passages 3 and 5, fibroblasts monolayers were dissociated with a 0.1% trypsin/EDTA solution (Sigma Chemical) and used as described below.

RT-PCR

Fibroblasts were seeded in 6-well plates, grown to confluence, and stimulated with recombinant human IL-17 (rhIL-17; 25 ng/mL) in the presence or absence of dexamethasone (Dex; 10⁻⁷ mol/L) for 24 hours. Optimal concentrations of Dex and IL-17 were selected according to a preliminary concentration-response curve. Cell viability was not affected by Dex as assessed by the Trypan blue exclusion method. Dex was used in co-incubation with rhIL-17 in culture medium on bronchial fibroblasts. Controls corresponding to the absence of Dex or its presence alone were also performed.

Total RNA was extracted through use of the Qiagen RNeasy Total RNA kit (Qiagen, Valencia, Calif) and quantified by fluorescence through use of Ribogreen (Molecular Probes, Inc, Eugene, Ore). Total RNA was reverse-transcribed into cDNA through use of mouse-Moloney leukemia virus reverse transcriptase (Canadian Life Technologies, Gaithersburg, Md) and random hexamers (Amersham Pharmacia Biotech, Baie d'Urfe, Quebec, Canada). From each cDNA product, 1 μ L was added to a 50- μ L PCR mixture containing

Taq polymerase (Qiagen) and forward and reverse primers (Keystone Laboratories, Camarillo, Calif). All reactions were performed in a Perkin-Elmer Cetus DNA thermal cycler (PerkinElmer Cetus, Norwalk, Conn) for 30 cycles at 95°C for 1 minute, 55°C for 30 seconds, and 72°C for 1 minute. The different sets of primers used to specifically amplify IL-6, IL-8, IL-11, Gro- α , and β -actin cDNAs are presented in Table II.²⁴⁻²⁶ After PCR, 10 μ L of the products were electrophoresed on a 2% agarose-ethidium bromide gel. A picture of the gels was taken, and the relative intensities of the bands were measured by digitalized image on a Macintosh computer through use of the public domain program NIH Image. β -actin was assayed on all cDNAs to test the efficiency of cDNA synthesis from all RNA extracts. Through DNA sequencing, we confirmed that the amplified sequences were the ones of interest.

Effect of rhIL-17 on fibroblast IL-6, IL-11, IL-8, and Gro- α release

Fibroblasts were seeded in 6-well plates at a density of 5×10^5 cells per well. Cells were stimulated for 24 hours with rhIL-17 at a 25-ng/mL concentration in the presence or absence of 10⁻⁷ mol/L Dex in DMEM supplemented with 10% FCS. At the end of incubation, supernatants were collected and cytokines concentrations were measured.

Cytokine detection

Measurement of hIL-6, hIL-8, hIL-11, and hGro- α were performed through use of ELISA kits (R&D Systems) in culture supernatants. IL-17 was measured by ELISA in unconcentrated BAL fluids obtained from normal and asthmatic subjects. The minimum detectable doses of IL-6, IL-11, IL-8, Gro- α , and IL-17 were less than 0.7 pg/mL, 8 pg/mL, 10 pg/mL, 0.08 ng/mL, and 5 pg/mL, respectively.

Statistical analysis

Data are expressed as means $\pm$ SEMs. Significant differences among groups and conditions for the ICC experiments were determined through use of nonpaired t testing corrected with Bonferroni testing (BAL, sputum) and nonparametric Kruskal-Wallis testing (eosinophils). In every case, a P value of less than .05 was considered statistically significant.

To compare groups (normal control and asthmatic subjects) and conditions (cytokine and chemokine production on IL-17 stimulation) in the fibroblast experiments, a cross-nested design analysis was used. This design was used to compare IL-6, IL-11, IL-8, and Gro- α production after IL-17 stimulation within subjects and between groups. When interactions between groups and conditions were significant, each group was analyzed through use of a randomized block design. Posteriori multiple comparisons were performed through use of Tukey's procedure. Mean values $\pm$ SEMs of quantitative variables were used as representative measures. All reported P values were 2-sided and were declared significant at .05 levels.

RESULTS

Expression of IL-17 immunoreactivity in sputum and BAL fluid

Cells positive for IL-17 immunoreactivity were found in sputum and BAL fluid of both asthmatic and normal control subjects. The numbers of cells expressing IL-17 immunoreactive protein were significantly higher in sputum ($P < .001$) and BAL ($P < .005$) recovered from asthmatic subjects than in sputum and BAL recovered from nonasthmatic controls (Fig 1). The morphology of most of the IL-17-immunoreactive cells was consistent with eosinophils, as shown in Fig 2. Measurement of IL-17 by

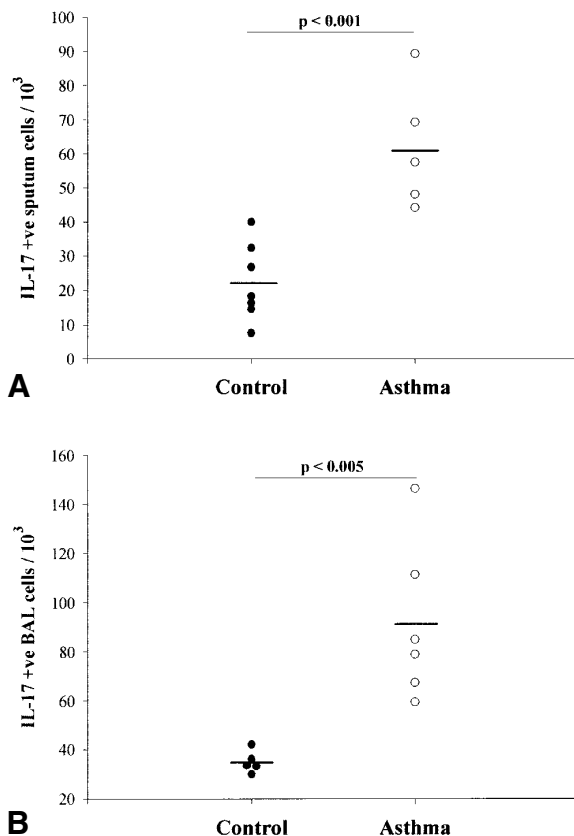


FIG 1. A, Expression of IL-17-immunoreactivity in sputum from normal (solid circles; $n = 7$) and asthmatic (open circles; $n = 6$) subjects. **B**, Expression of IL-17-immunoreactivity in BAL from normal (solid circles; $n = 6$) and asthmatic (open circles; $n = 6$) subjects. Results are shown as the numbers of IL-17⁺ cells per 10^3 counted cells for each individual. Black bars represent the average expression of IL-17 in each group.

ELISA in BAL fluid showed that there was an increased amount of IL-17 in patients with asthma in comparison with normal volunteers (15 ± 1 pg/mL and 9 ± 2 pg/mL, respectively; $P = .014$). Sputum and BAL fluid differential cell counts showed a significant increase in eosinophils in asthmatic subjects in comparison with normal controls (Table III).

Expression of IL-17 immunoreactivity in peripheral blood eosinophils

There were greater numbers of IL-17-immunoreactive eosinophils in samples obtained from asthmatic subjects than in samples obtained from normal subjects (486 ± 205 and 46 ± 13 positive cells per 10^3 counted cells, respectively; $P < .01$; Fig 3, A). Fig 2 a shows an example of asthmatic peripheral blood eosinophil immunostaining for IL-17. Western blot experiments confirmed that higher levels of intracellular IL-17 immunoreactive protein were recovered from the eosinophils purified from asthmatic individuals than from the eosinophils purified from normal subjects (Fig 3, B).

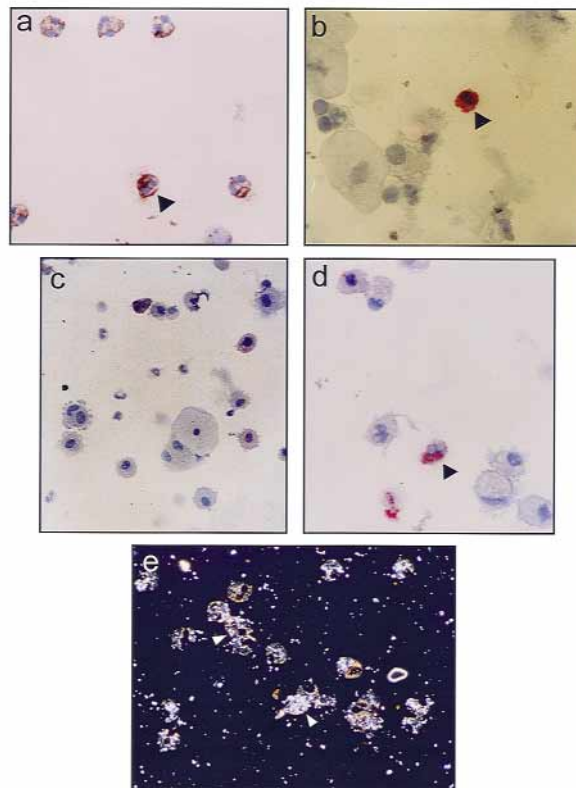


FIG 2. Photomicrographs show IL-17 protein and mRNA expression. Three of the panels present a representative example of IL-17 immunostaining in purified blood eosinophils (a), sputum (b), and BAL fluid (d) from an asthmatic subject; arrows indicate immunostained eosinophils. Also shown are a negative control (c) and a representative example of positive hybridization for IL-17 mRNA of purified blood eosinophils from an asthmatic subject (e) (magnification $\times 400$).

IL-17 mRNA expression on eosinophils

We performed in situ hybridization to confirm the ability of eosinophils to express IL-17. Positive hybridization for IL-17 mRNA was demonstrated in all samples tested. The percent of eosinophils with a positive signal ranged between 10% and 42%, the median value being $20\% \pm 7.5\%$ (Fig 2, E). No positive hybridization was demonstrated through use of the sense probe; this confirms the specificity of the hybridization. The demonstration of a positive mRNA signal in eosinophils of relatively high purity in asthmatic individuals supports the ICC and Western blot results. This shows that eosinophils have the capacity to synthesize this cytokine.

Effect of rhIL-17 on IL-6 and IL-11 mRNA and protein release by bronchial fibroblasts

Through use of RT-PCR, mRNAs encoding IL-6 and IL-11 were detected at baseline in both BNF and BAF (Fig 4, A and C). The signals were significantly amplified on rhIL-17 stimulation, with the exception of IL-11

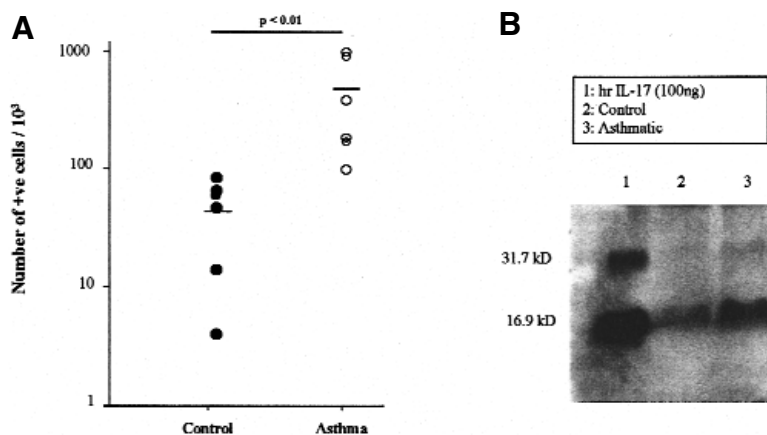


FIG 3. A, Expression of IL-17-immunoreactivity in peripheral blood eosinophils from normal (solid circles; $n = 6$) and asthmatic (open circles; $n = 6$) subjects. Results are shown as the numbers of IL-17⁺ cells per 10^3 counted cells for each individual. Black bars represent the average expression of IL-17 in each group. **B**, A representative example of IL-17 expression through use of Western blot in peripheral blood eosinophils from normal (lane 2) and asthmatic (lane 3) subjects; lane 1 represents the positive control with recombinant IL-17 loaded on the gel. IL-17 appears as a protein with an apparent molecular weight of 17 kD.

TABLE III. Differential cell counts in bronchoalveolar lavage fluid and sputum obtained from normal control and asthmatic subjects

	Bronchoalveolar lavage fluid		Sputum	
	Normal controls	Asthmatics	Normal controls	Asthmatics
Total cell counts (cells/mL $\times 10^{-3}$ or 10^6 cells/g mucus)	53 ± 2.4	68 ± 8.3	2.5 ± 0.5	2.6 ± 0.5
Macrophages (%)	86 ± 1	84 ± 2.4	72 ± 10	51 ± 4.3
Lymphocytes (%)	13 ± 1	9 ± 2	3 ± 1.5	1 ± 0.2
Eosinophils (%)	2 ± 0.2	$4.1 \pm 0.4^*$	0.2 ± 0.1	$3 \pm 1^*$
Neutrophils (%)	0	1.4 ± 0.3	25 ± 12	42 ± 5

* $P < .01$.

mRNA synthesis in BNF, which was unaffected by rhIL-17. Dex significantly decreased IL-6 and IL-11 mRNA and protein expression and brought IL-17-induced IL-6 (in both BNF and BAF) and IL-11 (in BAF) syntheses to at least their basal levels. With respect to protein release, at baseline there were detectable levels of IL-6 in BNF and BAF that were not significantly different (1.7 ± 0.3 and 1.5 ± 0.1 ng/mL, respectively; Fig 4, B). rhIL-17 induced a significant increase of the basal IL-6 release from both BNF (3.8 ± 0.7 ng/mL) and BAF (3.7 ± 0.3 ng/mL; $P < .0001$). This effect was significantly inhibited by Dex (1.4 ± 0.2 and 1.5 ± 0.1 ng/mL for BNF and BAF, respectively; $P < .0001$). A basal IL-11 release from both BNF and BAF was also demonstrated (1.1 ± 0.6 and 1.5 ± 0.3 ng/mL, respectively; Fig 4, D). In accord with its inability to promote an increase in IL-11 mRNA expression, IL-17 did not induce a significant increase in IL-11 protein release by BNF. In contrast, IL-17 significantly increased the release of IL-11 by BAF (1.9 ± 0.3 ng/mL; $P < .001$). Dex inhibited IL-17-induced IL-11 release and decreased it (0.8 ± 0.2 ng/mL).

Effect of rhIL-17 on IL-8 and Gro- α mRNA and protein release by bronchial fibroblasts

Through use of RT-PCR, mRNAs encoding IL-8 and Gro- α were detected at baseline in both BNF and BAF

(Fig 5, A and C). rhIL-17 stimulation enhanced Gro- α mRNA levels in both BNF and BAF. However, this stimulation had no effect on IL-8 mRNA levels. Dex did not decrease the IL-17-induced IL-8 and Gro- α mRNA levels (Fig 5, A-C). With respect to protein release, unstimulated BNF and unstimulated BAF released similar amounts of IL-8 (0.17 ± 0.02 and 0.24 ± 0.09 ng/mL respectively; Fig 5, B). IL-17 induced a significant increase in IL-8 production by BNF (0.53 ± 0.08 ng/mL) and BAF (0.96 ± 0.22 ng/mL; $P < .0001$). Dex significantly inhibited the rhIL-17-induced increase in IL-8 for BNF and BAF (0.34 ± 0.10 and 0.50 ± 0.13 ng/mL, respectively; $P < .0001$). Similarly, rhIL-17 significantly increased the Gro- α release by both BNF (from 0.22 ± 0.09 to 3.00 ± 0.73 ng/mL) and BAF (from 0.43 ± 0.18 to 3.82 ± 0.80 ng/mL; $P < .0001$; Fig 5, D). Dex decreased the effect of IL-17 for BNF and BAF (0.89 ± 0.18 and 1.19 ± 0.58 ng/mL, respectively; $P < .0001$).

DISCUSSION

In the present study, we demonstrated that IL-17 is upregulated in asthma, that eosinophils also are cellular sources of its production, and that IL-17 increases synthesis of IL-6 and IL-11 by bronchial fibroblasts derived from bronchial biopsies of asthmatic subjects.

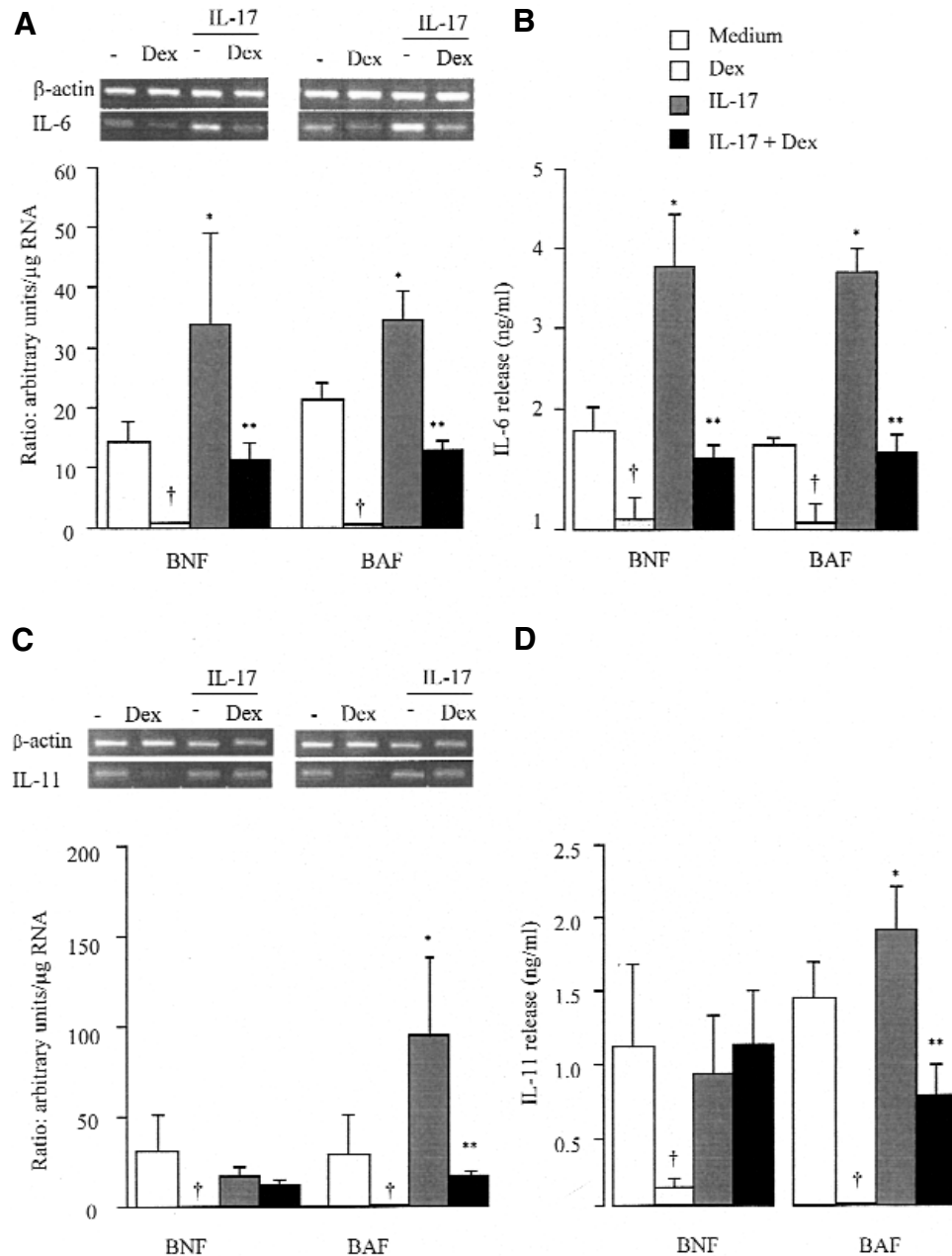


FIG 4. Measurement of IL-6 mRNA (A) and IL-11 mRNA synthesis (C) by RT-PCR and measurement of IL-6 (B) and IL-11 (D) release by ELISA by bronchial fibroblasts from normal (BNF; $n = 5$) and asthmatic (BAF; $n = 7$) subjects at baseline (open bars), in the presence of 10^{-7} mol/L Dex (dotted bars), or stimulated with 25 ng/mL rhIL-17 in the absence 10^{-7} mol/L Dex (hatched bars) or in the presence of 10^{-7} mol/L Dex (black bars). Each value represents the average $\pm$ SEM. Above panel A: picture of 1 representative RT-PCR experiment. Above panel B: the relative intensities of IL-6 mRNA/ β -actin mRNA ($n = 3$) and IL-11 mRNA/ β -actin mRNA ($n = 3$) bands. * $P < .001$ for IL-17 stimulation vs control. † $P < .0001$ for Dex stimulation vs control. ** $P < .0001$ for IL-17 + Dex stimulation vs IL-17 stimulation.

Several reports have demonstrated that IL-17 has the capacity to induce the production of other cytokines from structural cells and to enhance the surface expression of intracellular adhesion molecule-1.^{4,5} Chabaud et al¹⁵ reported that T_H2 -type cytokines, including IL-4 and IL-13, enhance IL-17-induced release of IL-6 from fibroblasts, suggesting that IL-17 might be involved in asth-

ma, which is a T_H2 -mediated disease. Our results of increased expression of IL-17 in asthmatic samples support the role of IL-17 in asthma but do not explain the mechanism of its action. To our knowledge, this is the first study that shows the expression of IL-17 in asthmatic lungs and demonstrates the capacity of eosinophils to produce this potentially pro-fibrotic cytokine. This

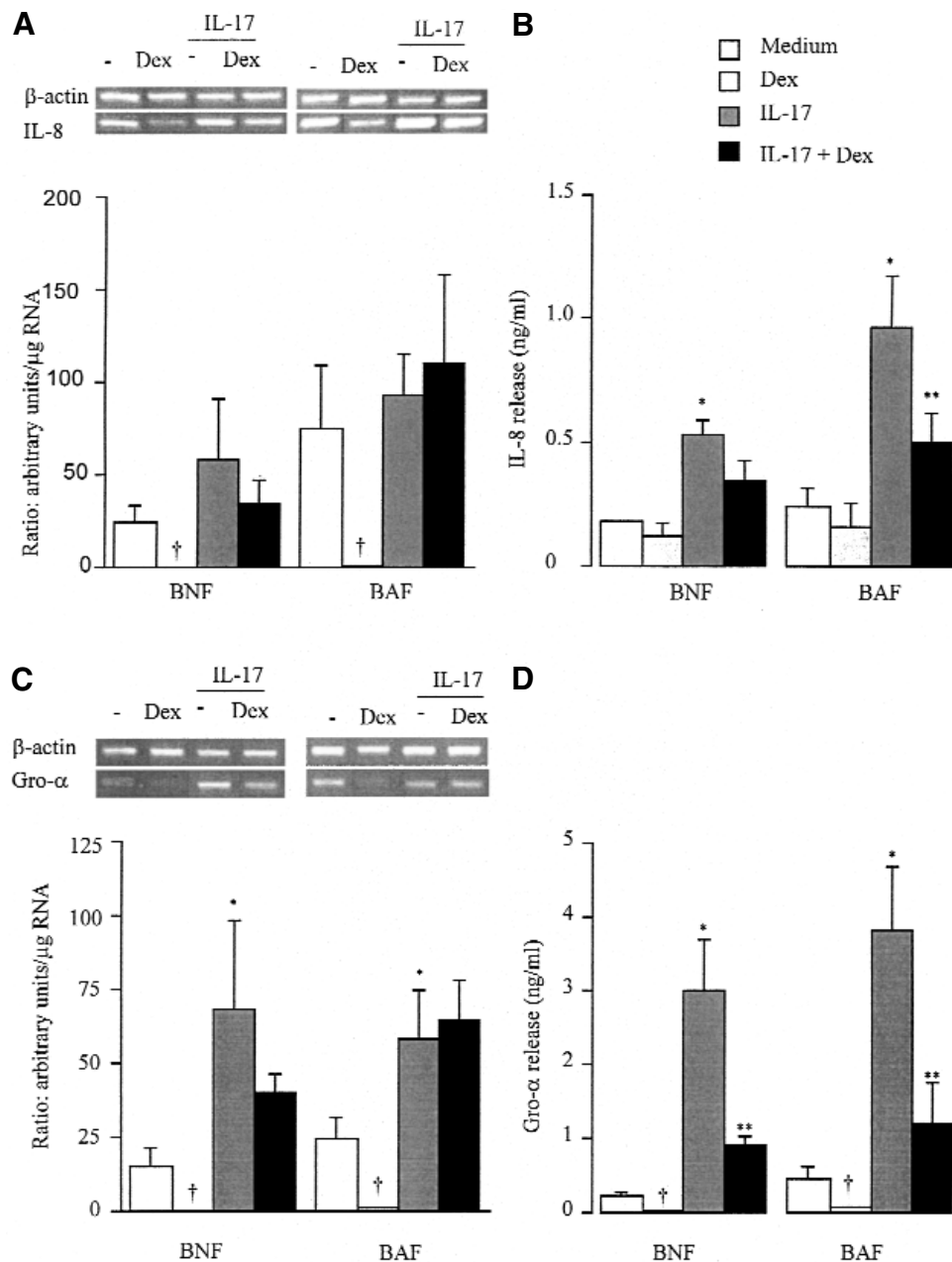


FIG 5. Measurement of IL-8 mRNA (A) and Gro- α mRNA synthesis (C) by RT-PCR and measurement of IL-8 (B) and Gro- α release (D) by ELISA by bronchial fibroblasts from normal (BNF; $n = 5$) and asthmatic (BAF; $n = 7$) subjects at baseline (open bars), in the presence of 10^{-7} mol/L Dex (dotted bars), or stimulated with 25 ng/mL rhIL-17 in the absence of 10^{-7} mol/L Dex (hatched bars) or in the presence (black bars) of 10^{-7} mol/L Dex. Each value represents the average $\pm$ SEM. Above panel A: picture of 1 representative RT-PCR experiment. Above panel B: the relative intensities of the IL-8 mRNA/ β -actin mRNA ($n = 3$) and Gro- α mRNA/ β -actin mRNA ($n = 3$) bands. * $P < .001$ for IL-17 stimulation vs control. † $P < .0001$ for Dex stimulation vs control. ** $P < .0001$ for IL-17 + Dex stimulation vs IL-17 stimulation.

observation is consistent with recent reports from our laboratory and from other groups showing that eosinophils have the ability to produce other potent fibrogenic mediators—eg, TGF- β^{27-30} and IL-11³¹—that are upregulated in asthmatic airways in comparison with normal airways.

To gain further insights into the mechanisms by which IL-17 might be involved in the pathogenesis of asthma and to examine the interaction between IL-17 and fibroblasts, we studied the effect of this cytokine on some fibroblast functions. We were unable to show any significant pro-mitogenic activity of IL-17 on fibroblasts from

either asthmatic airways or nonasthmatic airways (data not shown), suggesting that this cytokine does not play a role in fibroblast proliferation within this tissue. To determine whether IL-17 has an effect on the secretion of collagens, we examined the release of type I and type III procollagens from bronchial fibroblasts in response to IL-17 (data not shown). Although we did not demonstrate a significant upregulation of these 2 types of collagen, we cannot exclude the possibility that other extracellular matrix (ECM) components (such as fibronectin, tenascin, and proteoglycans) might be upregulated on IL-17 stimulation. IL-17 might decrease the production of fibroblast-derived matrix metalloproteinases or increase the synthesis of the tissue inhibitors of metalloproteinases in such a way that among the net results would be an increased deposited ECM and collagen content without IL-17's affecting collagen production itself.

Although the major function of fibroblasts corresponds to ECM component production and maintenance of tissue integrity, it is increasingly apparent that resident cells could also be important in regulating immune and inflammatory responses. When we examined the effect of IL-17 on the production of some pro-fibrotic cytokines by fibroblasts, we were able to demonstrate an increased synthesis of IL-6 and IL-11 mRNA and immunoreactive proteins. This observation is consistent with previous reports on the effect of IL-17 on IL-6 synthesis⁵; however, this is the first demonstration of the effect of IL-17 on IL-11 production. Through the upregulation of IL-6 and IL-11 synthesis, IL-17 might exert an indirect role in airway remodeling, inasmuch as both IL-6 and IL-11 have been shown to enhance the accumulation of collagen and TIMP-1,³²⁻³⁵ thereby preventing ECM breakdown and favoring fibrosis. IL-6 might have an additional effect in asthmatic airways through its ability to stimulate the proliferation of T cells³⁶ and drive naive CD4⁺ T cells toward IL-4-producing cells.³⁷ Furthermore, we recently showed that IL-11 was highly expressed in asthmatic airways and that its expression was correlated with the severity of the disease.³¹ IL-11 might modulate the T_H2 response in asthma. It has been shown that IL-11 mediates T_H2 polarization directly—by stimulating CD4⁺ T cells to produce IL-4—or indirectly—by inhibiting IL-12 production by monocytes.³⁸

Recently, IL-17 has been reported to be involved in inflammatory cell recruitment into airways, particularly neutrophils, through IL-8 and MIP-2 upregulation.⁷ We showed that IL-17 increased IL-8 protein release from bronchial fibroblasts. This observation is consistent with previous reports on the effect of IL-17 on IL-8 synthesis⁷; however, this is the first demonstration of the effect of IL-17 on Gro- α production. Interestingly Gro- α has also been shown to be a potent chemoattractant for T lymphocytes, particularly the CD45RO⁺ memory CD4 subset,³⁹ a cell type described as a potent source of IL-17.⁵ Through selective modulation of chemokine release by fibroblasts, IL-17 might regulate the recruitment of distinct leucocytes and T-cell subsets and might ultimately direct the outcome of cell-mediated immune responses in the airways. When we looked at the effect of

IL-17 on cytokine release from asthmatic and nonasthmatic fibroblasts, no difference was seen for the release of IL-6 or Gro- α between the 2 groups. In contrast, the release of IL-8 was significantly increased on IL-17 stimulation only in BAF. This observation suggests that fibroblasts originating from asthmatic airways, where airway remodeling takes place, are more sensitive to IL-17 for the release of the α -chemokine IL-8.

Glucocorticoids are widely used in the treatment of inflammation, especially in asthma, in part because of their inhibitory effects on cytokine expression and the action of these mediators within the airways. We showed that some IL-17-inducible responses are decreased by Dex (IL-6 and IL-11 at mRNA and protein levels). This finding is in agreement with those of a prior study, which demonstrated that IL-17 activates NF- κ B (a transcription factor initiating cytokine and chemokine expression) in chondrocytes and that this activation was inhibited by Dex.¹⁰

Taken together, our results provide evidence that IL-17 might play an indirect role in airway remodeling in asthma. IL-17 was found to stimulate fibroblasts to produce IL-6, IL-11, IL-8, and Gro- α , which might contribute to the amplification, regulation, and/or perpetuation of local airway inflammation and remodeling. Further studies are required to clarify all of the potential IL-17-mediated effects in vivo in airway inflammation, and this might be achieved through the use of animal models of asthma.

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