

IL-17E upregulates the expression of proinflammatory cytokines in lung fibroblasts

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Background: IL-17E is a new T_H2 cytokine that promotes airway eosinophilia in mice. IL-17E proinflammatory activity has been proposed to involve induction of cytokine and chemokine production. Recruitment of inflammatory cells may be mediated by tissue-resident cells.

Objective: This study aimed to evaluate whether fibroblasts represent a target of IL-17E for the production of eosinophil active mediators in the lung.

Methods: Expression of IL-17B receptor (IL-17BR), a receptor for IL-17E, was evaluated by immunofluorescent staining, Western blot, and real-time PCR in human primary lung fibroblasts. Mediator production was analyzed by using real-time PCR and ELISA after stimulation of fibroblasts with IL-17E alone or in combination with TNF-   and TGF-  ₁. Expression of IL-17E and of eosinophil major basic protein was evaluated by immunohistochemistry in bronchial biopsies from subjects with asthma.

Results: Human primary lung fibroblasts constitutively expressed IL-17BR. IL-17BR mRNA levels were increased in cells stimulated with TNF-   and decreased with TGF-  ₁. IL-17E slightly upregulated CC chemokine ligand (CCL)–5, CCL-11, GM-CSF, and CXC chemokine ligand (CXCL)–8 mRNA in fibroblasts. Moreover, IL-17E and TNF-   synergistically induced GM-CSF and CXCL-8 mRNA. IL-17E also potentiated the upregulation of CXCL-8 transcripts observed with TGF-  ₁. In contrast, TGF-  ₁ decreased IL-17E–induced CCL-11 mRNA. The capacity of IL-17E to enhance GM-CSF and CXCL-8 responses to TNF-   was accompanied by production and secretion of both proteins by lung fibroblasts. Finally, IL-17E was detected in asthma in eosinophil-infiltrated bronchial submucosa.

Conclusion: IL-17E may contribute to the induction and maintenance of eosinophilic inflammation in the airways by

acting on lung fibroblasts. This study supports a role for IL-17E in asthma pathophysiology. (*J Allergy Clin Immunol* 2006;117:590-6.)

Key words: Allergic inflammation, asthma, chemokines, cytokines, cytokine receptors, IL-17E, lung fibroblasts, TGF-  ₁, TNF-  

Asthma is a chronic inflammatory disease of the airways, characterized by upregulation of T_H2-derived cytokines and eosinophil infiltration into the bronchial submucosa.^{1,2} Structural changes are observed in bronchial biopsies of subjects with asthma that include subepithelial fibrosis, increased smooth muscle mass, and goblet cell hyperplasia. Chronic inflammation and remodeling of the airways are believed to result from repetitive injury and subsequent repair processes in the lung. Accordingly, increased production of the proinflammatory cytokine TNF-   as well as of TGF-  ₁, a cytokine involved in wound healing and fibrosis, is detected in the airways of subjects with asthma.¹⁻⁴ Eosinophils are an important source of these cytokines in asthma and were recently suggested to play an important role in airway remodeling.^{2,4-6}

Eosinophil accumulation in the airways is regulated by local production of proinflammatory mediators, leading to their recruitment from peripheral blood and their retention in the tissue. Several eosinophil-associated cytokines and chemokines are upregulated in the airways of subjects with asthma. These include CC chemokine ligand (CCL)–11/eotaxin-1, CCL-5/RANTES, CXC chemokine ligand (CXCL)–8/IL-8, and the hematopoietic factor GM-CSF.^{1,7-10} In addition to producing extracellular matrix proteins, airway fibroblasts are capable of releasing several proinflammatory cytokines and chemokines in response to inflammatory or mechanical stimuli.¹¹

Several features of asthma can be reproduced in mice that overexpress, or are exposed to, IL-17E. This recently described T_H2 mediator is a member of the IL-17 family of cytokines and is also known as IL-25. IL-17E induces a T_H2-type inflammatory reaction in the airways, characterized by local upregulation of IL-4, IL-5, and IL-13, as well as increased expression of eotaxin and tissue eosinophilia.¹²⁻¹⁵ Importantly, the lungs of these mice also display features of tissue remodeling, in particular perivascular fibrosis.

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Abbreviations used

CCL: CC chemokine ligand
CXCL: CXC chemokine ligand
IL-17BR: IL-17B receptor
MBP: Major basic protein

IL-17E mRNA expression was first described in T_H2 cells.^{12,13} It was subsequently shown to be expressed by murine mast cells, where it is increased upon IgE exposure.¹⁶ IL-17E is also upregulated in macrophages in a model of particle-induced airway inflammation in rats.¹⁷ Two studies have recently reported the presence of this cytokine in humans. Casado et al¹⁸ detected it in the nasal fluid of subjects with sinusitis, and Hwang and Kim¹⁹ demonstrated its expression in synovial fluid mononuclear cells from patients with rheumatoid arthritis.

Little is known about the mechanisms of action of IL-17E. IL-17B receptor (IL-17BR)/IL-17 receptor homolog 1/EVI27, a receptor for IL-17E, is present in several mucosal tissues in humans, including the airways.^{20,21} Target cells for IL-17E in these tissues may include macrophages and dendritic cells, which express IL-17BR *in vitro*.²² IL-17E promotes the expression of the proinflammatory cytokines CXCL-8, IL-6, and G-CSF in fibroblast and renal cell lines and cultured chondrocytes.^{13,21,23} It can also directly target inflammatory cells since it was recently shown to enhance the production of monocyte chemoattractant protein-1, macrophage inflammatory protein-1 α , IL-6, and CXCL-8 in human peripheral blood eosinophils.²⁴ IL-17E intracellular signal can involve the activation of kinases of the mitogen-activated protein kinase family as well as the transcription factor nuclear factor- κ B, findings that further support a proinflammatory role for this cytokine.^{21,24}

In this study, we investigated the effects of IL-17E on the production of mediators associated with allergic inflammation in lung fibroblasts. We demonstrated that IL-17BR is expressed in cultured primary fibroblasts and that IL-17E stimulates the production of the eosinophil-associated mediators CCL-5, CCL-11, GM-CSF, and CXCL-8 in these cells. Furthermore, we showed that IL-17E is expressed in the bronchial submucosa in asthma, and that 2 cytokines involved in the pathogenesis of this disease, TNF- α and TGF- β ₁, have the ability to modulate the fibroblast response to IL-17E. These results suggest that exposure of lung fibroblasts to IL-17E may contribute to the recruitment and enhanced survival of inflammatory cells, in particular eosinophils, and thus participate in the maintenance of airway inflammation in asthma.

METHODS

Cell culture

The human normal lung fibroblasts CCD-8Lu were purchased from American Type Culture Collection (Rockville, Md) and studied at passages 4–7. Cells were cultured in Dulbecco's modified Eagle

Medium containing 100 U/mL penicillin, 100 μ g/mL streptomycin (GIBCO, Grand Island, NY), and 10% FCS (HyClone Laboratories, Logan, Utah). Unless otherwise specified, fibroblasts were seeded in 6-well plates and grown to confluence. Cells were washed in PBS and serum-deprived for 24 hours before stimulation with the recombinant human cytokines IL-17E (50 ng/mL), TNF- α , and TGF- β ₁ (both at 10 ng/mL; R&D Systems, Minneapolis, Minn), or their respective vehicles. These conditions were selected based on the results obtained from preliminary experiments.

IL-17BR immunofluorescent staining

Fibroblasts were grown to 50% to 60% confluence on Lab-Tek Chamber slides (Nalge Nunc International, Naperville, Ill). Cells were washed in PBS, fixed in a 4% paraformaldehyde solution, washed again, and stored dry at -80°C until use. After rehydration, endogenous fluorescence was quenched with a solution of 50 mmol/L NH₄Cl, and nonspecific binding of the antibody was prevented by incubating the slides for 1 hour with 20% normal goat serum. Cells were stained with 5 μ g/mL mouse IgG_{2B} antihuman IL-17BR or isotype-matched control antibodies (R&D Systems) and extensively washed before incubation with a 1/750 dilution of Alexa Fluor 555 labeled goat antimouse IgG (Molecular Probes, Eugene, Ore). Nuclei were visualized by staining with 2 μ g/mL Hoechst 33342 (Molecular Probes), and slides were examined with a BX51 Olympus epifluorescence microscope attached to a CoolSNAP-Pro color digital camera (Carsen, Markham, Ontario, Canada).

Western blot

Cells were washed in ice-cold PBS, and lysis buffer (10 mmol/L HEPES pH 8.0, 150 mmol/L NaCl, 500 mmol/L saccharose, 1 mmol/L Na₂EDTA, 1% Nonidet P40, 1% 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate) containing Complete Protease Inhibitor Cocktail (Roche Diagnostics, Indianapolis, Ind) was added to the wells and left for 30 minutes on ice. Cell debris was removed by centrifugation of the lysates at 14,000g for 15 minutes at 4 $^{\circ}\text{C}$, and protein concentration was determined by using the Bradford assay (Bio-Rad Laboratories, Hercules, Calif). Thirty micrograms of proteins was resolved by 10% SDS-PAGE and electrotransferred to Hybond polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NY). Membranes were washed with 0.05% Tween-20 in 10 mmol/L Tris-buffered saline, pH 7.5, saturated with 5% BSA in Tris-buffered saline, pH 7.5, for 2 hours at room temperature, and incubated with 1 μ g/mL mouse monoclonal anti-IL-17BR Ab (R&D Systems). Alternatively, anti-IL-17BR Ab was first exposed to a 10 molar excess of recombinant human IL-17BR/Fc chimera (R&D Systems) for 2 hours. After extensive washing, membranes were treated with horseradish peroxidase-conjugated anti-mouse secondary antibody and developed by using the ECL Plus system coupled to autoradiography (Amersham Pharmacia Biotech).

Quantitative RT-PCR

Total cellular RNA was isolated by using RNeasy mini kit extraction columns (Qiagen, Valencia, Calif) and reverse-transcribed by using oligo(dT)12–18 primer in the presence of RNAGuard (both from Amersham Pharmacia Biotech) and Superscript II reverse transcriptase (Invitrogen, San Diego, Calif). Quantification of mRNA levels was performed by real-time PCR using the Roche LightCycler PCR (Roche Diagnostics, Indianapolis, Ind) and QuantitTect SYBR-Green PCR mastermix (Qiagen). Primers were designed to span an intron and the sequences were as follows: IL-17BR (5') AAC AGG CGT CCC TTT CCC TCT GGA, (3') TTC TTG ATC CTT TCG TGC CTC CAC; CCL-11 (5') GGG CCA GCT TCT GTC CCA AC, (3') TTA TGG CTT TGG AGT TGG AGA TTT; GM-CSF (5') CCA GCC ACT ACA AGC AGC AC, (3') GGG ATG ACA

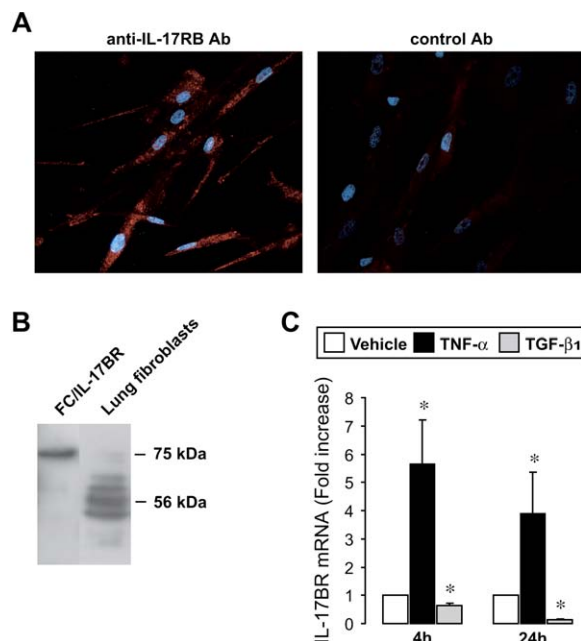


FIG 1. Expression of IL-17BR in human airway fibroblasts. **A**, IL-17BR immunofluorescent staining in cultured lung fibroblasts (*left panel*). Negative control experiment using an isotype-matched control primary antibody is shown in *right panel*. Magnification: 200 $\times$. **B**, Detection of IL-17BR expression by Western blot in fibroblast lysates (*lane 2*) in parallel with the purified lung fusion protein Fc/IL-17BR (*lane 1*). Results are representative of a minimum of 3 experiments. **C**, Expression of IL-17BR mRNA, as quantified by real-time PCR in fibroblasts stimulated with vehicle, TNF- α , or TGF- β 1. IL-17BR levels were normalized over those of the housekeeping gene S9, and values are expressed as a fold increase compared with vehicle-treated cells. Values are means $\pm$ SEMs of 3 to 5 experiments. * $P \leq .05$ compared with vehicle-treated fibroblasts at the same time point.

AGC AGA AAG TCC, *CCL-5* (5') TCC AAC CCA GCA GTC GTC, (3') CAA GAG CAA GCA GAA ACA GG; *CXCL-8* (5') GCA GAG GGT TGT GGA GAA G, (3') TCT TGT ATT GCA TCT GGC AAC; and *S9* (5') TGC TGA CGC TTG ATG AGA AG, (3') CGC AGA GAG AAG TCG ATG TG (Invitrogen). Quantification of endogenous ribosomal protein S9 was performed as a control to correct for variations in cDNA content among samples. Of note, the expression of S9 remained stable under our experimental conditions. Results were analyzed with the LightCycler software version 3.5.3 (Roche Diagnostics), and melting curve and agarose gel electrophoresis analysis were used to check for the specificity of the amplification products. Quantification of the PCR products was achieved by using a standard curve obtained by simultaneously amplifying serial dilutions of the amplicon. Ratios of the values obtained for each cytokine versus values of the housekeeping gene S9 were calculated, and the results expressed as a fold increase, compared with the ratio obtained for the vehicle-treated cells.

Immunohistochemistry for IL-17E and major basic protein

Immunohistochemistry for IL-17E and major basic protein (MBP) was performed on bronchial biopsies obtained from 3 subjects with asthma. The tissues were obtained from the Montreal Chest Institute/Meakins-Christie Tissue Bank (McGill University). A clinical diagnosis of asthma was made based on the evaluation of the patient's

medical file by a respiratory physician. Diagnostic criteria included previous diagnosis and treatment for asthma, documented evidence of reversible airflow obstruction of $\geq 15\%$, and bronchial hyperresponsiveness. IL-17E immunoreactivity was detected by the avidin-biotin complex method, as previously described,²⁵ with a monoclonal anti-IL-17E Ab (a gift from DNAX Research Institute, Palo Alto, Calif). MBP staining was performed to detect the presence of eosinophils by using a monoclonal anti-MBP Ab (kindly provided by Dr R. Moqbel, University of Alberta, Canada). Slides were developed by using Fast Red (Sigma) and counterstained with Gill modified hematoxylin (Harleco; Fisher Scientific International, Hampton, NH).

Measurement of GM-CSF and CXCL-8 release

Cytokine release in cell supernatants was assessed by sandwich ELISA. After 48 hours of stimulation, fibroblast supernatants were collected and centrifuged to remove residual cells. Samples were stored frozen at -80°C until quantification by using commercially available ELISA kits specific for human GM-CSF and CXCL-8 (Biosource International, Camarillo, Calif). The detection limits were 3 pg/mL for GM-CSF and 5 pg/mL for CXCL-8.

Statistical analysis

The results are expressed as the means $\pm$ SEMs of the indicated number of experiments. Statistical significance of differences was determined with a paired Student *t* test. *P* values of .05 or less were considered significant.

RESULTS

Expression and regulation of a receptor for IL-17E, IL-17BR, in human lung fibroblasts

By using cultured fibroblasts obtained from human lung, we demonstrated a constitutive expression of IL-17BR, as shown by immunofluorescent staining using a specific mAb directed against the extracellular domain of the receptor (*Fig 1, A, left panel*). No staining was observed when an irrelevant isotype-matched antibody was used (*Fig 1, A, right panel*).

The presence of IL-17BR in fibroblasts was confirmed by Western blot analysis. The mAb detected a 56-kD protein corresponding to the full length receptor, containing both the transmembrane and cytoplasmic domains (*Fig 1, B, lane 2*). Several isoforms of the protein were also detected in cell lysates, which is consistent with previously described variants and glycosylated forms of the receptor. The chimera protein IL-17BR/Fc was used as an internal positive control and led to the detection of a band with the expected size of 75 kD (*Fig 1, B, lane 1*). Preadsorption of the antibody with the recombinant peptide abolished its binding, further demonstrating the specificity of the immunodetection (not shown).

The presence of IL-17BR in human lung fibroblasts was associated with the constitutive expression of its mRNA transcript as detected by quantitative real-time PCR (*Fig 1, C*). In resting fibroblasts, the PCR products started to become detectable after 30 cycles in our amplification conditions (not shown). To investigate whether IL-17BR expression could be modulated by inflammatory stimuli, fibroblasts were incubated with 10 ng/mL TNF- α or TGF- β 1, or their respective vehicles—that is, medium alone or 20 $\mu\text{mol/L}$ HCl—for 4 to 24 hours. The

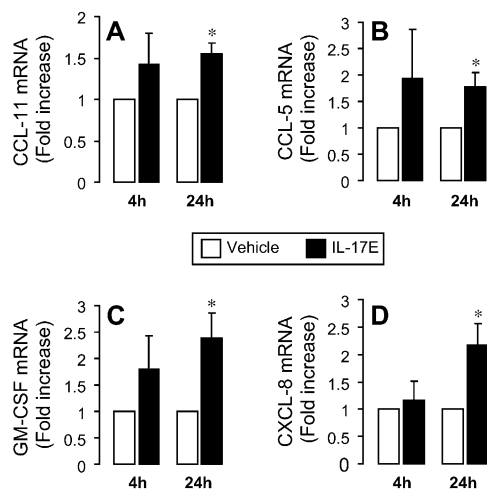


FIG 2. Effect of IL-17E on the expression of fibroblast-derived mediators. Serum-deprived lung fibroblasts were stimulated with IL-17E or its vehicle and CCL-11 (A), CCL-5 (B), GM-CSF (C), and CXCL-8 (D) mRNA levels were evaluated. Results are presented as a fold increase compared with vehicle-treated cells at the same time point. Values are means $\pm$ SEMs of 6 experiments. * $P \leq .05$ compared with vehicle-treated fibroblasts.

expression of IL-17BR mRNA was markedly upregulated by TNF- α both at 4 and 24 hours (Fig 1, C). In contrast, TGF- β_1 inhibited IL-17BR mRNA expression, in particular after 24 hours (Fig 1, C).

Effect of IL-17E on the expression of proinflammatory mediators in human lung fibroblasts

To test the hypothesis that IL-17E stimulates the synthesis of chemokines involved in allergic inflammation, we cultured lung fibroblasts in the presence of 50 ng/mL of the recombinant human protein or its vehicle—that is, 20 μ mol/L HCl. After 4 or 24 hours, cells were harvested for total RNA extraction to quantify the expression of CCL-11, CCL-5, GM-CSF, and CXCL-8 mRNA. Transcripts coding for all 4 cytokines were detected in unstimulated fibroblasts (Fig 2). IL-17E induced a modest increase in CCL-11, CCL-5, and GM-CSF mRNA expression in fibroblasts after as little as 4 hours, compared with vehicle (Fig 2, A-C). However, the difference in the expression of these cytokines after IL-17E stimulation compared with medium alone only reached statistical significance after 24 hours (1.5-fold to 2-fold increases; Fig 2, A-C). IL-17E also promoted higher expression of CXCL-8 mRNA, as detected after 24 hours of stimulation (Fig 2, D).

Effect of the combination of IL-17E and TNF- α on cytokine expression in fibroblasts

We next investigated whether the response of lung fibroblasts to IL-17E could be altered if cells were placed in an inflammatory milieu. The effect of the combination of TNF- α together with IL-17E was thus evaluated on the expression of CCL-11, CCL-5, GM-CSF, and CXCL-8

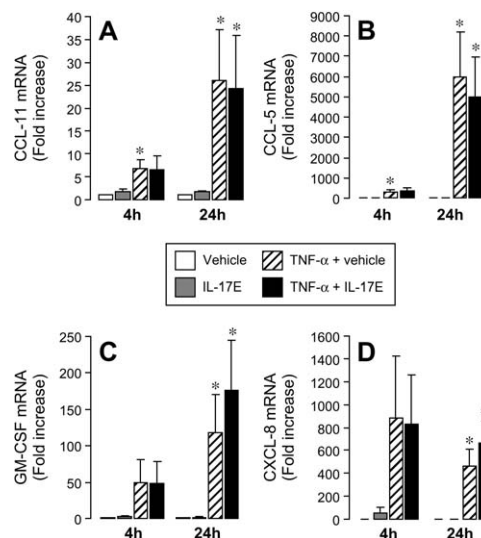


FIG 3. Synergistic effect of TNF- α and IL-17E on the expression of cytokines in lung fibroblasts. Fibroblasts were stimulated with IL-17E or its vehicle, alone or in combination with TNF- α , and CCL-11 (A), CCL-5 (B), GM-CSF (C), and CXCL-8 (D) mRNA levels were quantified. Results are expressed as a fold increase, compared with the value obtained in vehicle-treated cells at the same time point. Values are means $\pm$ SEMs of 3 to 7 experiments. * $P \leq .05$ compared with IL-17E-treated cells at the same time point.

mRNA in fibroblasts cultured for 4 and 24 hours. As shown in Fig 3, mRNA coding for all these mediators was strongly upregulated by TNF- α at both time points. IL-17E and TNF- α synergistically increased GM-CSF mRNA levels after a 24 hours stimulation of fibroblasts (from 2-fold and 118-fold increases with IL-17E or TNF- α alone to a 176-fold increase with the combination of cytokines; Fig 3, C). CXCL-8 mRNA was also increased to a greater extent with the combination of cytokines, compared with each cytokine alone (464-fold and 666-fold increases at 24 hours, in the presence of TNF- α and both cytokines, respectively; Fig 3, D). In contrast, no synergism was observed between IL-17E and TNF- α concerning CCL-11 and CCL-5 mRNA expression at either 4 or 24 hours (Fig 3, A and B).

Interaction of IL-17E and TGF- β_1 in cytokine expression in fibroblasts

Contrary to TNF- α , fibroblast exposure to TGF- β_1 did not lead to any change in the levels of CCL-11 and CCL-5 mRNA during the whole time course of the experiment (Fig 4, A and B). However, TGF- β_1 slightly promoted GM-CSF mRNA expression after 24 hours (Fig 4, C) and was also a potent inducer of CXCL-8 mRNA at 4 and 24 hours (Fig 4, D). Concomitant stimulation of fibroblasts with IL-17E and TGF- β_1 did not further affect the expression of CCL-11 and CCL-5 (Fig 4, A and B). Furthermore, GM-CSF levels observed with IL-17E were transiently lowered by TGF- β_1 (4 hours; Fig 4, C). CCL-11 mRNA was also decreased by the presence of TGF- β_1 together with IL-17E, compared with IL-17E alone, after 24 hours (Fig 4, A). In contrast, IL-17E

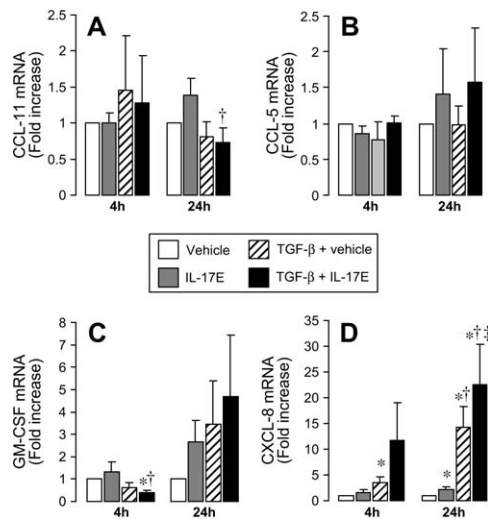


FIG 4. Effect of the combination of TGF- β_1 and IL-17E on the expression of cytokine expression in fibroblasts. Fibroblasts were stimulated with IL-17E or its vehicle, alone or in combination with TGF- β_1 , and mRNA levels of CCL-11 (A), CCL-5 (B), GM-CSF (C), and CXCL-8 (D) were quantified. Results are expressed as a percentage of the value obtained in vehicle-treated cells at the same time point. Values are means $\pm$ SEMs of 3 to 6 experiments. * $P \leq .05$ compared with IL-17E-stimulated cells and † $P \leq .05$ compared with TGF- β_1 -treated cells at the same time point.

potentiated TGF- β_1 -mediated upregulation of CXCL-8 transcript at both time points (Fig 4, D). This effect became statistically significant after 24 hours (from a 14.3-fold increase with TGF- β_1 alone to a 22.5-fold increase in combination with IL-17E; Fig 4, D).

Effect of IL-17E on the release of cytokines by fibroblasts

We then investigated whether IL-17E effects on cytokine mRNA levels were translated into mediator production and release by fibroblasts. Lung fibroblasts were stimulated for 48 hours with IL-17E alone or in combination with TNF- α , and supernatants were subsequently analyzed for cytokine detection by specific sandwich ELISA. Among the cytokines tested for mRNA modulation, GM-CSF and CXCL-8 were chosen to evaluate any synergism between IL-17E and TNF- α . Both GM-CSF and CXCL-8 were constitutively released at low levels by fibroblasts (Fig 5). Higher quantities of both cytokines were found in supernatants of cells treated with IL-17E compared with its vehicle, although the differences were not statistically significant ($P = .14$ and $.27$, respectively). TNF- α had a slight effect on GM-CSF release but potently increased CXCL-8 production by fibroblasts (Fig 5). The combination of IL-17E and TNF- α further upregulated both GM-CSF and CXCL-8 release in fibroblast supernatants (Fig 5).

Presence of IL-17E in the airways in asthma

To examine the relevance of our observations in asthma physiopathology, we evaluated the expression of IL-17E

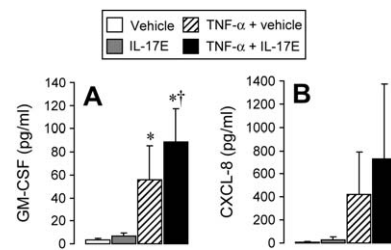


FIG 5. Synergistic effect of IL-17E and TNF- α on cytokine release by fibroblasts. Fibroblasts were stimulated for 48 hours with IL-17E or its vehicle, with or without TNF- α . GM-CSF (A) and CXCL-8 (B) were quantified in supernatants. Values are expressed as means $\pm$ SEMs of 3 to 6 experiments. * $P \leq .05$ compared with vehicle and † $P \leq .05$ compared with IL-17E-treated cells.

in bronchial biopsies obtained from asthmatic subjects by immunohistochemistry. As shown in Fig 6, IL-17E positive cells were detected in the bronchial submucosa. This expression was mainly associated with inflammatory cells, some of which had an eosinophil-like morphology. MBP staining revealed a marked infiltration of the tissue with eosinophils (Fig 6). As a negative control, no staining was observed when the first antibody was omitted (not shown).

DISCUSSION

The recently described cytokine IL-17E may play a role in allergic inflammation and remodeling of the airways. To date, however, very little is known about its involvement in human disease, particularly in asthma. This study demonstrates for the first time the constitutive expression of a receptor for IL-17E, IL-17BR, in human primary lung fibroblasts, and supports a role for IL-17E in allergic airway inflammation.

We show that IL-17E stimulates the production of several mediators known to favor tissue eosinophilia, namely CCL-11, CCL-5, GM-CSF, and CXCL-8, in lung fibroblasts. Indeed, CCL-11 and CCL-5 display selective chemotactic activity toward eosinophils but not neutrophils.^{26,27} This property may participate in the differences in biological activity reported for IL-17E, compared with other IL-17 family members, such as IL-17A and IL-17F, known to elicit T_H1 responses associated with neutrophil infiltration in murine airways.^{13,15} GM-CSF is one of the main cytokines involved in the proliferation of granulocyte progenitors, as well as their differentiation into mature eosinophils.²⁸ Moreover, GM-CSF is a potent activator of eosinophils and promotes their chemotaxis.^{28,29} Although CXCL-8 is classically seen as a strong activator and chemoattractant for neutrophils, it also induces the migration of primed eosinophils through induction of chemokinesis/chemotaxis.^{29,30} CXCL-8 also favors eosinophil adhesion to the activated endothelium.³¹ Through its ability to upregulate CCL-11, CCL-5, GM-CSF, and CXCL-8 expression, IL-17E may thus be involved in the initiation and maintenance of eosinophil infiltration during allergic airway inflammation.

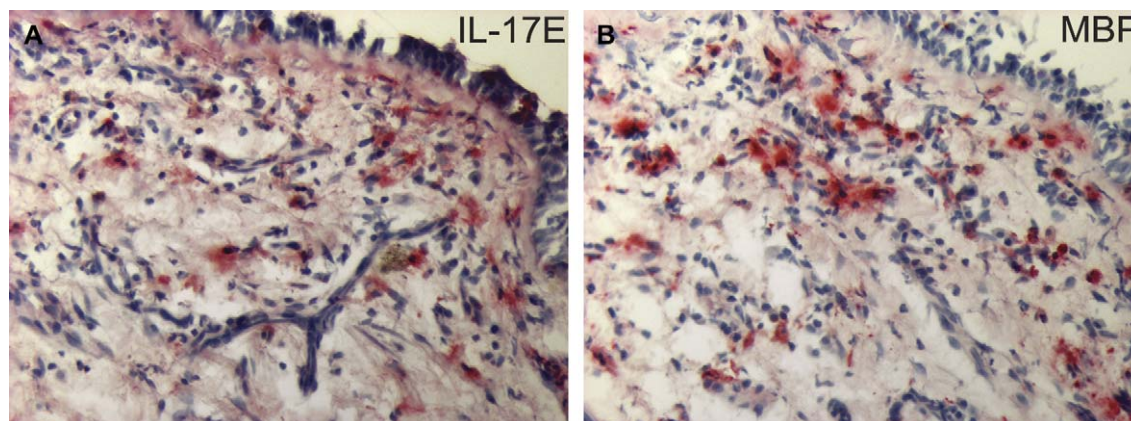


FIG 6. Expression of IL-17E in asthma. Immunohistochemistry for IL-17E (A) and MBP (B) was performed on bronchial biopsies of subjects with asthma. Representative staining experiments are shown.

Our results also suggest that fibroblast sensitivity to IL-17E is affected by the inflammatory milieu. Indeed, IL-17BR message was markedly upregulated by the pro-inflammatory cytokine TNF- α . This observation is consistent with *in vivo* data describing high levels of IL-17BR mRNA in a model of inflammatory bowel disease in rats.²⁰ In contrast with TNF- α , TGF- β_1 inhibited IL-17BR expression in fibroblasts. This phenomenon may contribute to the downregulation of tissue inflammation that is necessary for wound healing, and supports the anti-inflammatory role proposed for TGF- β_1 . Taken together, these observations suggest that mediators of the innate immune system and cytokines involved in adaptive immunity may interact in the regulation of cell sensitivity to IL-17E.

IL-17E appeared to be relatively weak, in comparison with TNF- α , in inducing mediator production by fibroblasts. IL-17E may, in fact, work by fine-tuning the cellular response under inflammatory conditions and favor an allergic profile for ongoing inflammation. Indeed, upregulation of GM-CSF and CXCL-8 expression was potentiated by the combination of IL-17E and TNF- α . IL-17E signal transduction has been shown potentially to involve nuclear factor- κ B.²¹ This transcription factor is also a main transducer of TNF- α intracellular signaling³² and may thus be involved in this synergistic effect. In addition, this phenomenon may be related to the ability of TNF- α to upregulate IL-17BR expression in fibroblasts, as discussed. However, TNF- α did not upregulate IL-17E-mediated CCL-11 and CCL-5 expression in a manner similar to GM-CSF and CXCL-8. This discrepancy between IL-17E activity and the levels of its receptor was also observed for TGF- β_1 . Indeed, TGF- β_1 -mediated decrease in IL-17BR mRNA expression in fibroblasts was concomitant with an inhibition of IL-17E-related CCL-11 levels. However, TGF- β_1 also synergized with IL-17E to upregulate CXCL-8 mRNA. These observations support the existence of distinct intracellular pathways for the regulation of chemokine expression in lung fibroblasts.

T_H2 cytokines, other than IL-17E, have been previously described to stimulate the production of eosinophil specific mediators in stromal cells. In particular, IL-4 and

IL-13 trigger CCL-11 release in airway fibroblasts.³²⁻³⁴

Similar to our observations, the inflammatory milieu has been shown to influence the cellular response to T_H2 cytokines. In particular, higher expression of CCL-11 was detected on stimulation of airway fibroblasts with a combination of TNF- α with either IL-4 or IL-13.^{33,34} However, TGF- β_1 and IL-13 were reported to act synergistically to upregulate CCL-11, but not CXCL-8, expression by airway fibroblasts.³⁵ Altogether, these findings further support the hypothesis that fibroblasts are capable of modulating immune cell function in inflamed tissues and may be critically involved in the initiation and propagation of inflammation mediated by both innate and adaptive immunity.

IL-17E-induced eosinophilia has been reported to be dependent on the production of IL-13 and IL-5 *in vivo*.¹⁵ Although the cells responding to IL-17E could not be identified, they were shown to belong to a non-B-cell, non-T-cell population in mouse models.^{12,15} Our observations suggest a direct effect of IL-17E on stromal cells favoring selective accumulation of eosinophils in tissues. Antigen-presenting cells have been shown to express IL-17BR in a T_H2 milieu.²² In our hands, IL-17E did not upregulate the IL-17BR message (data not shown), but it would be interesting to evaluate whether this receptor is modulated by other T_H2 cytokines in lung fibroblasts.

We describe here the expression of IL-17E in the airways of subjects with asthma, mainly associated with inflammatory cells. This was concomitant with eosinophil infiltration in the bronchial submucosa, an observation which further supports a role for IL-17E in allergic inflammation. T_H2 cells and macrophages represent potential sources of IL-17E in the airways, and we are currently studying this cytokine expression by eosinophils. In addition, by favoring GM-CSF and CXCL-8 production in fibroblasts, IL-17E may contribute to the enhanced neutrophilic inflammation that is observed in many subjects with asthma, in particular in subjects with more severe disease.³⁶ CXCL-8 is known as a potent angiogenic factor³⁷ and may thus be involved in the induction of airway structural changes reported for IL-17E in mice.

Eosinophils have very recently been shown to respond to IL-17E and may also be a major source of profibrotic mediators. Further studies will specifically aim at evaluating whether IL-17E is capable of directly inducing cell proliferation and production of extracellular matrix proteins in fibroblasts, 2 phenomena involved in tissue fibrosis observed in asthma.

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