

Interleukin-17F Induces Pulmonary Neutrophilia and Amplifies Antigen-induced Allergic Response

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Interleukin (IL)-17F is a recently described human cytokine belonging to the IL-17 gene family, but its *in vivo* function remains to be determined. To this end, a full-length mouse IL-17F cDNA sequence with a 483-bp coding region sequence was first identified. Pulmonary gene transfer of an IL-17F expression construct (pcDNA_{IL-17F}) in mice was used to investigate its regulatory role. The results showed first that a significant increase in the number of neutrophils was seen in the bronchoalveolar lavage fluids of IL-17F-transduced mice, concomitant with increased expression of genes encoding C-X-C chemokines and inflammatory cytokines when compared with mock and phosphate-buffered saline control animals. Mucosal transfer of the IL-17F gene in ovalbumin (OVA)-sensitized mice before antigen (Ag) challenge enhanced the levels of Ag-induced pulmonary neutrophilia, but not eosinophilia, goblet cell hyperplasia, and mucin gene expression. However, no significant change in the levels of Th2 cytokine expression was noted. A significant enhancement of ventilatory timing in response to inhaled methacholine was also seen in IL-17F-transduced, Ag-sensitized mice, whereas a small but significant increase was found in IL-17F-transduced, naive mice. These results suggest a role for IL-17F in the induction of neutrophilia in the lungs and in the exacerbation of Ag-induced pulmonary inflammation.

Keywords: cytokine; interleukin-17F; pulmonary inflammation

Recent advances in human and other vertebrate genome sequencing have facilitated novel gene discovery. Several members of the interleukin (IL)-17 (IL-17A) gene family have been identified by database searches and degenerative reverse transcription-polymerase chain reaction, including IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F (1–3). Based on an amino acid alignment of IL-17 family members, they have well-conserved cysteines, which may be involved in intradisulfide and interdisulfide bonds as a common structural and functional feature (4). This cysteine knot formation is similar to a structural motif found in some growth factors. IL-17A is a CD4⁺ T-cell-derived proinflammatory cytokine that stimulates the production of inflammatory cytokines, chemokines, and hematopoietic factors. In contrast, IL-17B, IL-17C, IL-17D, and IL-17E are expressed in a wide variety of tissues. Although their functions partially overlap those of IL-17A, each IL-17 family member might have distinct biological roles (reviewed in References 1 and 2).

We and others have recently reported the discovery of an additional IL-17 gene family, ML-1 (3) or IL-17F (4, 5). IL-17F (ML-1) is produced by activated CD4⁺ T cells, basophils, and

monocytes (3, 5), and is able to induce *in vitro* IL-6 and several C-X-C chemokines, such as IL-8, growth-related oncogene- α (CXCL1), and epithelial cell-derived neutrophil-activating protein (CXCL5) via activation of the extracellular regulated kinases 1 and 2, but not p38 or the c-Jun NH2-terminal kinases, in normal human bronchial epithelial cells and human umbilical vein endothelial cells (3, 6–8). However, the murine homolog of IL-17F and its regulatory role *in vivo* have not been described.

We have previously shown that the expression of IL-17F is upregulated at sites of segmental allergen challenge in patients with asthma (3). This finding, together with findings from our *in vitro* studies (3, 6–8), suggests that IL-17F might be involved in the expression of pulmonary inflammation. To test this hypothesis, a mucosal (pulmonary) gene transfer strategy was used to overexpress the IL-17F gene *in vivo*. This expression system and protocol have previously been shown to result in sustained and effective cytokine production *in vivo*, resulting in a definitive phenotype (9–11). In this study, we report the identification of a full-length gene encoding mouse IL-17F (mIL-17F) and the investigation of its *in vivo* function using a pulmonary gene transfer strategy in both naive and antigen (Ag)-sensitized and Ag-challenged mice. Some of the results of these studies have been previously reported in the form of an abstract (12).

METHODS

Detailed methods are described in online supplement.

Animals

BALB/c mice (female, 6–8 weeks old) were obtained from the Jackson Laboratories (Bar Harbor, ME).

Full-length mIL-17F cDNA Cloning and mIL-17F Gene Expression Construct

A full-length cDNA sequence was identified using both 5'- and 3'-rapid amplification of cDNA ends (RACE) with cDNA from ovalbumin (OVA)-challenged mouse total lungs. Both 5'- and 3'-RACE-Ready cDNAs were generated by SMART RACE cDNA Amplification Kit (BD Biosciences, Palo Alto, CA), and polymerase chain reaction was performed using universal primers and primers based on the predicted exon sequences. Polymerase chain reaction products were subcloned into a cloning vector and sequenced. The mIL-17F coding region sequence was amplified from OVA-challenged mouse lungs by polymerase chain reaction and cloned into a pcDNA3.1. The resulting expression construct was propagated and purified.

Study Design

For mucosal gene transfer, groups of mice were administered phosphate-buffered saline (PBS), mock control, or pcDNA_{IL-17F} by intratracheal route at the various time points. In another set of experiments, groups of mice were sensitized and challenged with OVA as previously described (13). Mice were treated by intratracheal route with PBS, mock control, or pcDNA_{IL-17F} before and after OVA challenge. At 48 hours after OVA challenge, bronchoalveolar lavage fluids (BALFs) and lung tissues were collected. BALF cell counts were performed as previously described (13). After performing BAL, lungs were inflated and fixed. Lung tissues were embedded in paraffin and stained with periodic acid-Schiff (PAS). The percentage of PAS-positive cells was calculated by dividing PAS-positive cells over total bronchial

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epithelial cells. Total RNAs from each mouse in each group were subjected for analysis of cytokine and mucin gene expression using reverse transcription-polymerase chain reaction as previously described (13).

Determination of Ventilatory Timing in Response to Methacholine

"Ventilatory timing" (14) was assessed after challenge with aerosolized methacholine (Mch) in mice as a result of pulmonary Ag challenge and/or overexpression of IL-17F. This was measured using whole body plethysmography (Buxco Electronics, Inc., Sharon, CT). Mice were challenged with aerosolized 0.9% NaCl for the baseline measurement, followed by incremental doses of nebulized Mch for 2 minutes. Mean enhanced pause values representing ventilatory timing were assessed for 3 minutes after each nebulization.

Transduced Gene Expression

Transduced mIL-17F gene expression was assessed by reverse transcription-polymerase chain reaction from lung tissues by using gene specific primers for mIL-17F. The polymerase chain reaction products were analyzed on agarose gel and visualized after ethidium bromide staining. The level of gene expression was semiquantified by calculating the ratio of band intensities for mIL-17F and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Cytokine and Chemokine or Chemokine Receptor Analysis

The gene expression profiling of cytokine and chemokine or chemokine receptor genes was analyzed with GEArray Q Series cDNA array (SuperArray Inc., Frederick, MD). Total lung RNA was prepared from each pooled sample of three separate individual mice in each set of experiments. The data were represented as fold changes. Genes with more than twofold increase in signal intensity were shown. For validation, pooled RNA used in GEArray was assessed by reverse transcription-polymerase chain reaction using gene specific primers.

Statistical Analysis

The significance of difference between groups was calculated using analysis of variance with Bonferroni correction. Data are expressed as means $\pm$ SEM; a *p* value of less than 0.05 was considered significant.

RESULTS

Full-length mIL-17F Sequence

A full-length mIL-17F cDNA (GenBank accession number AY380822) of 1,178 bp was obtained using both 5'- and 3'-RACE from OVA-challenged mouse total lung RNAs. Within this sequence was an open reading frame encoding a protein of 161 amino acids, flanked by a predicted transcription start site at 71-bp upstream of the start codon and a polyadenylation signal sequence at 598-bp downstream of the stop codon (Figure 1A). A predicted signal peptide encompassing the first 28 amino acid residues was identified according to the Center for Biological Sequence Analysis signal peptide prediction server (www.cbs.dtu.dk/services/SignalP/).

Homology searches using the basic local alignment search tool (www.ncbi.nlm.nih.gov/BLAST/) program indicated a similarity in overall exon-intron organization between human and mIL-17F genes. The mIL-17F gene consists of two introns and three exons of 27, 222, and 237 bp, respectively. Also, this basic local alignment search tool search revealed that although the IL-17F coding region sequence is identical to a predicted sequence (AB116259), it differs from a previously reported sequence deposited in GenBank (AF458064). Unlike the sequence we have reported here, the previously reported sequence lacks the first exon. This sequence may represent a truncated form or, perhaps, a splice variant. Furthermore, when both sequences were aligned with a mouse BAC genomic contig (NT_039169), it became apparent that the start codon of the truncated form of IL-17F gene matches, in fact, the last codon in the first exon of the

sequence identified in this study (Figure 1B). Judging from the similar organization of the IL-17F gene reported in this study with human IL-17F gene and from the perfectly matched genomic sequence alignment, the sequence identified in this study was, therefore, named as mouse ortholog of the human IL-17F gene.

The alignment of amino acid sequences showed that mouse and rat IL-17F share the highest degree of homology (86.3% sequence identity), whereas mIL-17F shared 55.2% sequence homology to its human counterpart (data not shown; see sequence in GenBank, accession #AY380822). In addition, four cysteine residues predicted to be involved in intrachain and interchain disulfide bonds (4) were well conserved across species and other members of the IL-17 family.

mIL-17F Induces Airway Neutrophilia and Increases Ventilatory Timing to Inhaled Mch

To investigate the biologic effect of IL-17F *in vivo*, a mucosal Lipofectamine-mediated gene transfer approach was used. As seen in Figure 2A, the expression of IL-17F gene was verified in IL-17F-transduced mice 3 days after gene transfer. At this time, the total number of BALF cells was significantly increased in IL-17F-transduced mice ($10.62 \pm 0.25 \times 10^4$, *n* = 8) when compared with those seen in PBS-treated and mock-transduced mice ($4.90 \pm 0.20 \times 10^4$, $5.38 \pm 0.20 \times 10^4$, *n* = 7, *p* < 0.05, respectively) (Figure 2B). Cell differential analysis of BALF cells demonstrated significantly increased numbers of macrophages and neutrophils in IL-17F-transduced mice (macrophages, $7.12 \pm 0.45 \times 10^4$; neutrophils, $2.38 \pm 0.22 \times 10^4$, *n* = 8) when compared with those seen in PBS- and mock-treated mice (macrophages, $4.83 \pm 0.02 \times 10^4$, $4.45 \pm 0.07 \times 10^4$, *n* = 7, *p* < 0.05, respectively; neutrophils, $0.01 \pm 0.004 \times 10^4$, $0.09 \pm 0.003 \times 10^4$, *n* = 7, *p* < 0.05, respectively). It is noted, however, that mild lymphocytosis was seen in mock- and IL-17F-transduced mice (Figure 2C; $0.83 \pm 0.08 \times 10^4$, *n* = 7, $1.11 \pm 0.30 \times 10^4$, *n* = 8; *p* < 0.05, respectively). The total cell number and the number of macrophages and neutrophils did not significantly increase with time and were approximately the same at 72- and 96-hour time points (data not shown). In contrast, there were no significant differences in BALF cell counts among these three groups at 48-hour time point (data not shown). Therefore, the 72-hour time point was chosen for subsequent analysis.

To characterize further the phenotypic changes in IL-17F-transduced mice, ventilatory timing was measured using whole body plethysmography 72 hours after gene transfer. Figure 2D shows that mice given pcDNA_{mIL-17F} developed significant changes in ventilatory timing at the highest dose of Mch (30 mg/ml), whereas no significant change was seen in either PBS- and mock-treated groups.

mIL-17F Regulates Cytokine and Chemokine Gene Expression in the Lung

To provide molecular insight into the proinflammatory role of IL-17F, gene expression profiling for cytokines, chemokines, and chemokine receptors in IL-17F-transduced mice was performed. As shown in Figures 3A and 3B, IL-17F-transduced mouse lungs demonstrated 15 proinflammatory cytokine and chemokine genes with at least twofold increased levels compared with that seen in mock-transduced mice. The upregulated genes include those encoding IL-6 (2.06, fold induction), IL-1 β (3.11), interferon- γ (3.42), allograft inflammatory factor-1 (3.25), keratinocyte-derived chemokine (CXCL1, 11.05), macrophage inflammatory protein-2 (CXCL2, 2.18), interferon- γ -inducible protein (CXCL10, 3.97), macrophage inflammatory protein-1 α (CCL3, 4.67), macrophage inflammatory protein-1 β (CCL4,

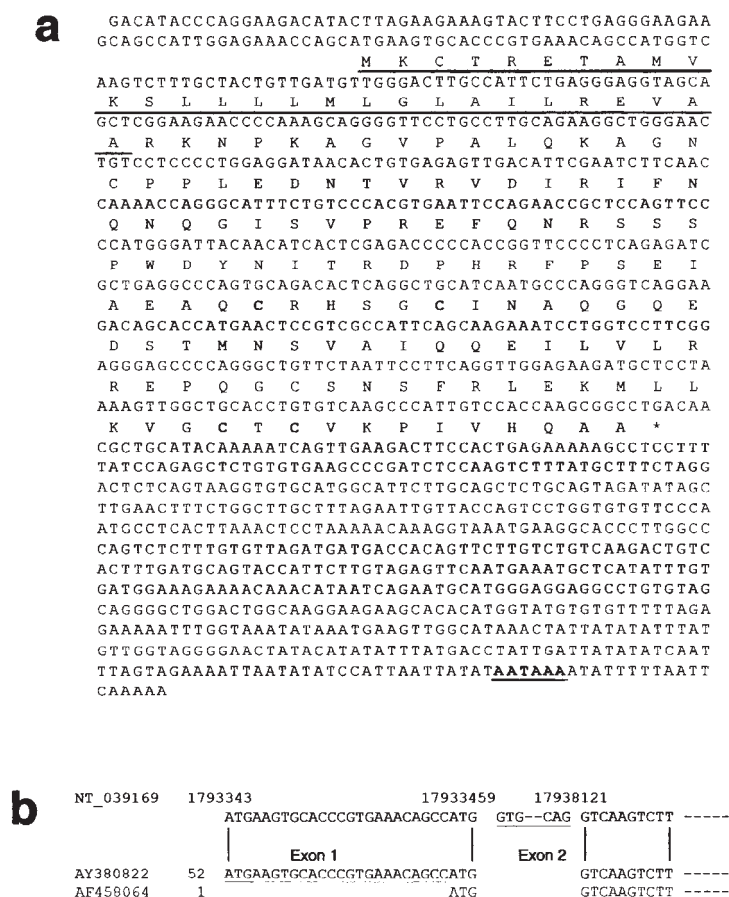


Figure 1. (A) Full-length cDNA and amino acid sequence of mouse interleukin-17F (mIL-17F). The 5'-leader sequence is *underlined*. The polyadenylation signal is in *bold and underlined*, and the conserved cysteines are in *bold*. (B) Alignment of a newly identified (this study, AY380822) and a previously reported IL-17F (AF458064) sequences with a mouse genomic contig (NT_039169) sequence. The first exon (coding) and the first 10 aa of the exon 2 of the AY380822 are shown, and the boundary sequence in the genomic contig is *underlined*. The number corresponds to the position in the respective sequence.

2.96), macrophage inflammatory protein-1 γ (CCL9, 2.54), macrophage chemoattractant protein-1 (CCL2, 2.57), and macrophage chemoattractant protein-2 (CCL8, 3.93) (Figures 3A and 3B).

It is of interest to note that the majority of the upregulated genes encode chemokines known to be involved in chemoattraction for neutrophils and macrophages (14–17). Other interesting chemokine and cytokine genes were also induced by mIL-17F,

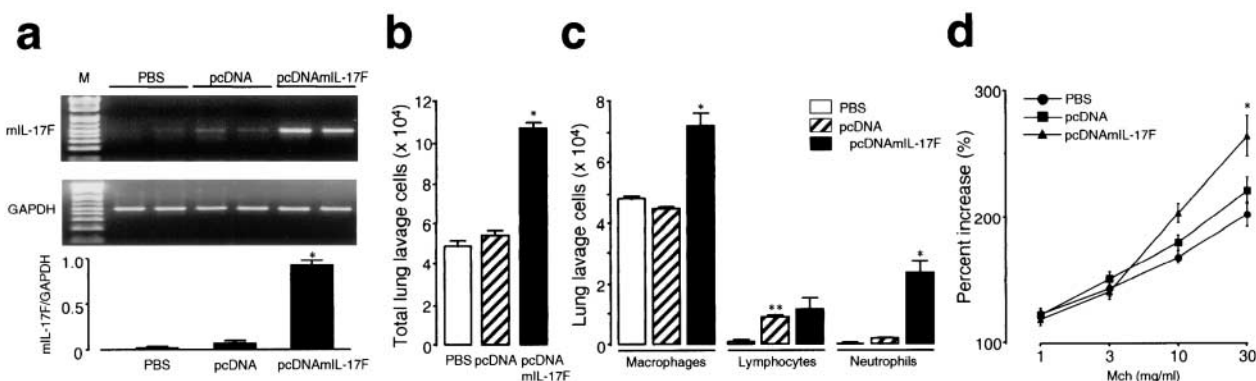


Figure 2. (A) Expression of transduced IL-17F gene by reverse transcription-polymerase chain reaction. Lung tissues were removed 72 hours after intratracheal administration of phosphate-buffered saline (PBS), pcDNA (mock control), or pcDNAmIL-17F. Purification of total lung RNA, cDNA synthesis, amplification of cDNA using gene specific primers and the calculation of ethidium bromide intensity were described in METHODS. One representative image of two individual mice per group is shown. * $p < 0.05$ versus PBS, pcDNA ($n = 7-8$). M = 1 kb plus DNA ladder. (B) Total cell counts and (C) cell differentials in bronchoalveolar lavage fluids (BALFs) of mice administered with PBS, pcDNA, or pcDNAmIL-17F. One representative datum out of two independent sets of experiments is shown. Data are expressed as mean $\pm$ SEM of counts. * $p < 0.05$ versus PBS, pcDNA; ** $p < 0.05$ versus PBS ($n = 7-8$). (D) Ventilatory timing to inhaled methacholine (Mch). Ventilatory timing to incremental dose of inhaled Mch was evaluated by measuring Penh values in mice that had received intratracheal PBS, pcDNA, or pcDNAmIL-17F as indicated. One representative datum out of two independent sets of experiments is shown. Results are measured as the percentage increase over baseline (after inhalation of 0.9% NaCl) Penh values, and data are expressed as mean $\pm$ SEM. M = DNA size marker. * $p < 0.05$ versus PBS or pcDNA ($n = 3-4$).

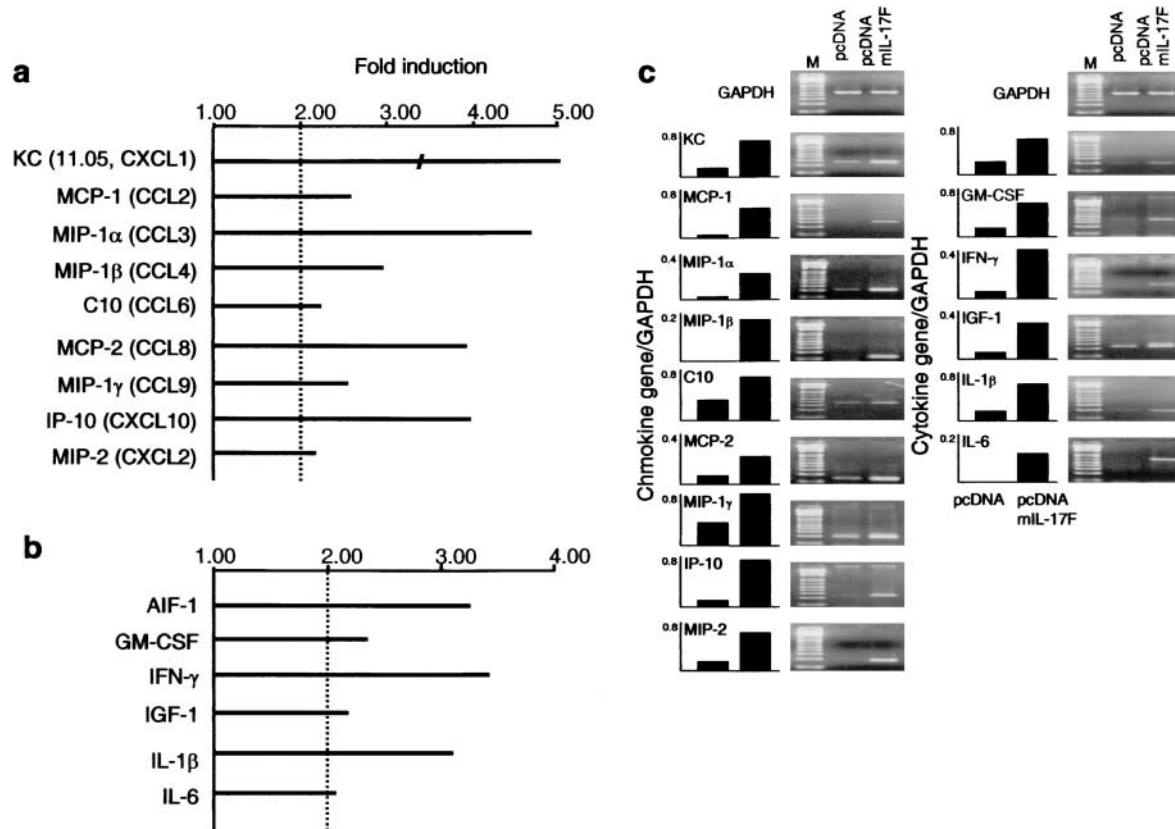


Figure 3. Profiling of gene expression for (A) 67 common chemokine and chemokine receptor genes and (B) 96 cytokine genes. The data are represented as fold changes of the normalized signal intensity of pcDNAmIL-17F versus pcDNA (mock control) group (pooled sample, $n = 3$). Genes with more than twofold increase in signal intensity are shown. (C) Validation by reverse transcription-polymerase chain reaction. GEArray results were confirmed by reverse transcription-polymerase chain reaction using gene specific primers as described in METHODS. M = 1 kb plus DNA ladder. GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

such as granulocyte colony-stimulating factor (2.35), insulin-like growth factor-1 (2.18), and C10 (CCL6, 2.22) (Figures 3A and 3B). The increased expression of all 15 genes with at least twofold change by profiling was validated by reverse transcription-polymerase chain reaction analysis (Figure 3C). Varying degrees of enhancement were seen by semiquantitative analysis; no down-regulated (under 0.5-fold) genes were identified.

mIL-17F Enhances Ag-induced Pulmonary Allergic Responses in a Murine Model of Asthma

To examine a potential role for IL-17F in the modulation of Ag-induced pulmonary allergic inflammation, pulmonary IL-17F gene transfer was performed before Ag challenge in Ag-sensitized mice. These results are summarized in Figure 4. First, although a clear induction of endogenous expression of IL-17F was seen in sensitized mice after challenge with Ag in comparison to that seen PBS-challenged control mice, transfer of IL-17F gene in the lung significantly enhanced the level of IL-17F (Figure 4A). When the inflammatory cells were analyzed, the total number of BALF cells was significantly increased 72 hours after Ag challenge in mice transduced with pcDNAmIL-17F ($97.8 \pm 5.5 \times 10^4$, $n = 4$) when compared with the number of BALF cells obtained from PBS-treated or mock-transduced mice ($67.4 \pm 4.4 \times 10^4$, $71.0 \pm 5.8 \times 10^4$, $n = 3$; $p < 0.05$, respectively) (Figure 4B). Second, differential BALF cell counts demonstrated that although no neutrophils could be found in PBS-treated and mock-transduced mice, significantly increased numbers of neu-

trophils in IL-17F-transduced mice ($9.9 \pm 0.8 \times 10^4$, $n = 4$, $p < 0.05$) were noted. In contrast, no significant differences between groups were seen in the numbers of BALF eosinophils (Figure 4B).

Similar to our results in gene-transduced naïve mice, increased numbers of lymphocytes were found in Ag-sensitized and -challenged, mock-transduced mice ($10.0 \pm 0.9 \times 10^4$, $n = 3$; $p < 0.05$ vs. PBS group), and significantly higher numbers of lymphocytes were observed in the IL-17F-transduced mice ($20.5 \pm 3.3 \times 10^4$, $n = 4$; $p < 0.05$ vs. mock control) (Figure 4C). It is noted, however, that a significant increase in the number of lymphocytes was also seen in mock-transduced mice, which may be due to the use of Lipofectamine as we have previously observed (9). Of significance is the finding that when ventilatory timing is analyzed, transduction of IL-17F gene significantly enhanced ventilatory timing in response to inhaled Mch compared with that seen in PBS-treated and mock-transduced mice (Figure 4D). When the relative levels of Th2 cytokines for IL-4, IL-5, and IL-13 were analyzed, no significant upregulation could be seen in IL-17F-transduced, Ag-challenged mice when compared with those seen in mock control animals (data not shown). Also, no further increase in the level of pulmonary interferon- γ expression was found.

To examine whether overexpression of IL-17F in the lung plays a role in the regulation of mucus hypersecretion, histologic analysis of airway epithelium stained with PAS was performed. As shown in Figures 5A–5E, histologic examination of the lungs

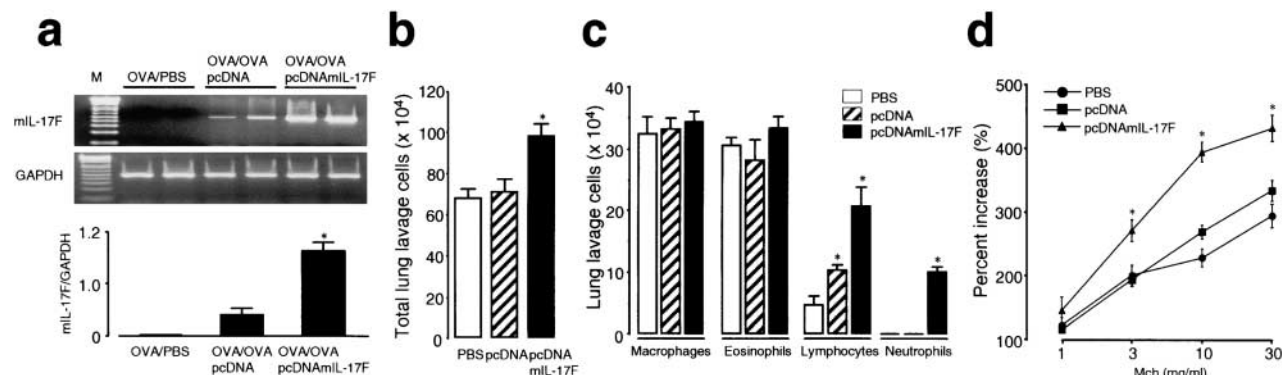


Figure 4. (A) Reverse transcription-polymerase chain reaction analysis of IL-17F gene expression in Ag-sensitized and PBS-challenged mice (ovalbumin [OVA]/PBS) and in Ag-sensitized and -challenged mice (OVA/OVA) treated with pcDNA (mock control) or pcDNAmIL-17F. Purification of total lung RNA, cDNA synthesis, amplification of cDNA using gene specific primers, and the calculation of ethidium bromide intensity were described in METHODS. One representative image of two individual mice per group is shown. * $p < 0.05$ versus OVA/PBS, OVA/OVA pcDNA ($n = 3-4$). (B) Total cell counts and (C) cell differentials in BALFs of Ag-sensitized and -challenged mice transduced with PBS, pcDNA, or pcDNAmIL-17F. One representative datum of three independent sets of experiments is shown. Data are expressed as mean $\pm$ SEM of counts. * $p < 0.05$, pcDNA versus PBS; * $p < 0.05$, pcDNAmIL-17F versus PBS or pcDNA ($n = 3-4$). (D) Ventilatory timing to inhaled Mch. Ventilatory timing to incremental doses of inhaled Mch was evaluated by measuring Penh values in Ag-sensitized and -challenged mice that had received intratracheal PBS, pcDNA, or pcDNAmIL-17F as indicated. One representative datum out of three independent sets of experiments is shown. Results are measured as the percentage increase over baseline (after inhalation of 0.9% NaCl) Penh values, and data are expressed as mean $\pm$ SEM. * $p < 0.05$ versus PBS or pcDNA ($n = 3-4$).

revealed distinct differences between different groups of mice. Although Ag-sensitized and Ag-challenged mice exhibited PAS-positive mucous cells in both the large and small airways, a significant enhancement in the number of PAS-positive cells was found in IL-17F-transduced mice (Figure 5D), but the difference

was seen only in the small airway (Figures 5B, 5D, and 5E) when compared with sections from PBS-treated or mock-transduced group. In contrast, no PAS-positive cells were seen in IL-17F-transduced mice without Ag sensitization and challenge (Figure 5C). Furthermore, the increased level of mucin 5ac gene

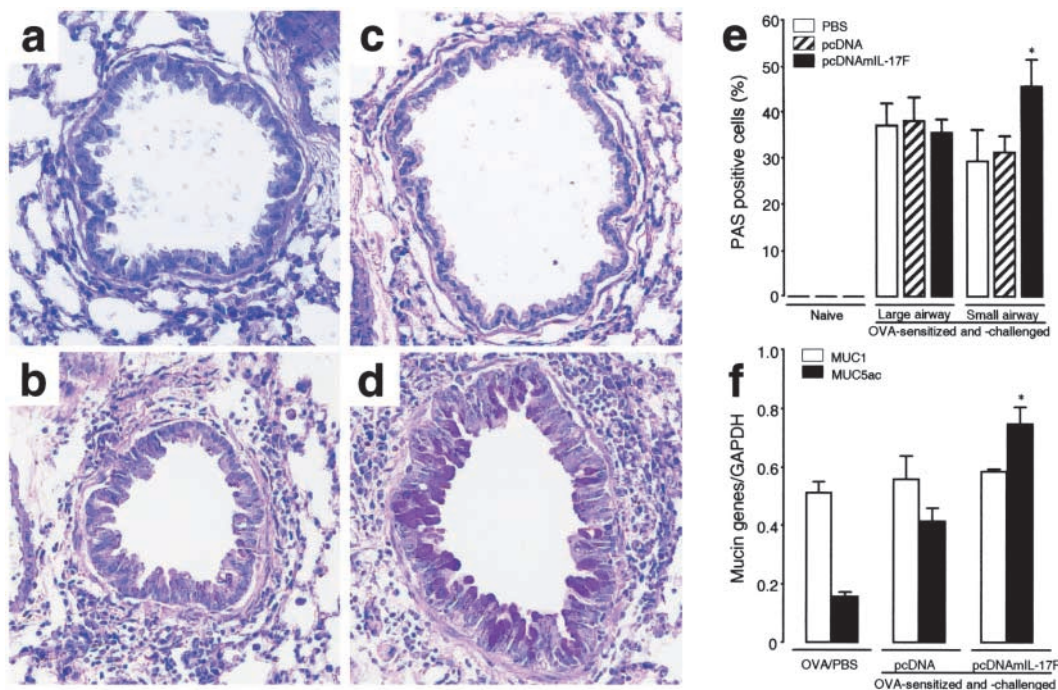


Figure 5. Goblet cell hyperplasia in large and small airways of naïve or Ag-sensitized and -challenged mice. Representative sections are shown in naïve mice receiving (A) pcDNA (mock control) or (C) pcDNAmIL-17F. Ag-sensitized and -challenged (OVA/OVA) mice treated with (B) pcDNA, or (D) pcDNAmIL-17F. Original magnification, A–D: $\times 200$. (E) The percentage of periodic acid-Schiff (PAS) stain-positive cells. One representative datum out of two independent sets of experiments is shown. Results are expressed as mean $\pm$ SEM of PAS-positive cells (%). * $p < 0.05$ versus OVA/OVA PBS, OVA/OVA pcDNA ($n = 3-4$). (F) Reverse transcription-polymerase chain reaction analysis of mucin gene expression. One representative datum of two independent sets of experiments is shown. The results are expressed as mean $\pm$ SEM of semiquantitative intensity. * $p < 0.05$ versus OVA/PBS, OVA/OVA pcDNA ($n = 3-4$).

expression was also noted in Ag-challenged, IL-17F-transduced mice when compared with the mock control animals (Figure 5F).

DISCUSSION

In this report, we described the identification of a full-length mIL-17F gene with a 483-bp coding region sequence. Similar to its human counterpart, the mIL-17F gene consists of three exons separated by two introns. The sequence differs from a previously reported sequence (AF458064) that lacks the first exon, and no functional study of this truncated sequence was reported. We demonstrated herein that overexpression of IL-17F through intratracheal delivery of mIL-17F gene resulted in an increase in the number of neutrophils and macrophages in the airways. The increase in infiltrating neutrophils and macrophages was associated with upregulated expression of cytokine and chemokine genes, such as IL-1 β , IL-6, granulocyte colony-stimulating factor, keratinocyte-derived chemokine, macrophage inflammatory protein, and macrophage chemoattractant protein-1/2, all known to be involved in chemotaxis and activation for monocytes/macrophages and neutrophils (15–18). These results corroborate the recent findings of Kawaguchi and colleagues, who demonstrated that IL-17F (ML-1) was able to induce the production of IL-6, IL-8, and growth-related oncogene- α *in vitro* by primary bronchial epithelial cells (3, 7). Furthermore, a significant increase in ventilatory timing to inhaled Mch was seen in IL-17F-transduced mice, although the magnitude is significantly lower than that seen in the Ag-sensitized and Ag-challenged model. At present, the functional basis for the increased ventilatory timing is unclear. One plausible explanation could be a consequence of neutrophil activation and subsequent release of soluble mediators. Also, it is unclear at present as to whether the increased ventilatory timing or “mean enhanced pause” indicates an increased airway hyperreactivity, for which the relationship has been challenged (14, 19, 20). An additional invasive measurement would be required to derive a quantitative assessment.

In a murine model of asthma, we provided evidence that overexpression of IL-17F provided an additive effect on Ag-induced allergic inflammatory responses, characterized in part by an increase in the levels of neutrophilia. Furthermore, increased goblet cell hyperplasia of the small airways was prominent in this model. Eosinophil number was not significantly increased in this model, which is consistent with our previous *in vitro* findings that human IL-17F is incapable of inducing CC chemokines, such as CCL11 and CCL5, in primary bronchial epithelial cells (3). Also, no effect on the levels of Th2 cytokines, IL-4, IL-5, and IL-13 was noted. Small but significant increases in the number of BALF lymphocytes were also seen in mock-transduced mice compared with PBS-treated mice, suggesting a potential contribution from the use of Lipofectamine gene carrier as previously described (9). Taken together, these results suggest that IL-17F is involved in the regulation of pulmonary inflammation, in part through the induction of neutrophilia.

Although a comprehensive and comparative analysis of IL-17F and IL-17A has not been pursued, IL-17F appears to have biological actions similar to those of IL-17A both *in vitro* and *in vivo*. IL-17A is also able to induce pulmonary neutrophilia *in vivo* (21). However, as we demonstrated in this report, IL-17F is able to mediate an overlapping, but also distinct, panel of cytokine and chemokine expression *in vivo*, suggesting an incomplete redundancy in the functions of these two structurally similar cytokines. It is of interest to note that upregulation of insulin-like growth factor-1 and C10 gene expression is seen in IL-17F-transduced mice. These genes are known to be involved in lung fibrosis and airway remodeling (22, 23), suggesting a

potential role for IL-17F in tissue remodeling. As a corollary, Hurst and colleagues have also shown that adenoviral-mediated human IL-17F gene transfer induced pulmonary neutrophilia and a similar, but not identical, pattern of gene expression in mouse lungs (24). The differences in cytokine/chemokine gene expression may reflect species-specific differences related to the use of mouse versus human IL-17F. Alternatively, it may indicate a quantitative difference in the level of transduced gene expression or a nonspecific effect attributable to the use of an adenoviral vector. Further work is needed to characterize the regulatory effects of IL-17F and the potential role of IL-17F in the regulation of tissue remodeling processes.

It has been reported that IL-17A is able to induce mucin gene expression *in vitro* (25) and that an increased expression of IL-17A is associated with enhanced mucin gene expression *in vivo* (26). In our study, it is interesting that goblet cell hyperplasia is seen only in the small airways of IL-17F-transduced, Ag-challenged mice. It is noted also that IL-17F and IL-17A may use different cellular receptors (3) and that in a segmental allergen challenge model a prominent expression of IL-17F, but not IL-17A, at sites of allergen challenge is noted (3). Also, IL-17F expression has a wider tissue distribution than that seen for IL-17A, and IL-17F is produced by both Th1 and Th2 cells (3), whereas IL-17A is expressed primarily in Th1 cells. Also, activated human mast cells and basophils are also able to express IL-17F (3), suggesting a wider role of IL-17F in inflammatory responses.

Finally, several limitations in this report must be mentioned. Although a total of five related type I membrane proteins have been reported as potential receptors for IL-17 family cytokines, the ligand specificities of these receptors have not been established. In addition, we do not know, quantitatively, the level of IL-17F protein in the lungs of various groups of mice. This is one limitation of our current study, and the availability of recombinant mIL-17F would be ideal to establish a quantitative, dose-dependent relationship. The availability of anti-mIL-17F antibodies for specific blockade of biological function would be useful. Also, the use of IL-17F transgenic and knockout mice will be required to assess fully the regulatory and chronic effects of IL-17F *in vivo* and will provide further details of the mechanism of action of IL-17F. In conclusion, we have demonstrated that IL-17F induced significant pulmonary neutrophilia in mice, suggesting a role for this new cytokine in neutrophil-associated pulmonary inflammatory responses.

Conflict of Interest Statement: N.O. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; P.B.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; D.M.E. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; B.A.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; A.C.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.-K. H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17 family and IL-17 receptors. *Cytokine Growth Factor Rev* 2003;14:155–174.
2. Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. *J Leukoc Biol* 2002;71:1–8.
3. Kawaguchi M, Onuchic LF, Li XD, Essayan DM, Schroeder J, Xiao HQ, Liu MC, Krishnaswamy G, Germino G, Huang SK. Identification of a novel cytokine, ML-1, and its expression in subjects with asthma. *J Immunol* 2001;167:4430–4435.

4. Hymowitz SG, Filvaroff EH, Yin JP, Lee J, Cai L, Risser P, Maruoka M, Mao W, Foster J, Kelley RF, *et al.* IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. *EMBO J* 2001;20:5332–5341.
5. Starnes T, Robertson MJ, Sledge G, Kelich S, Nakshatri H, Broxmeyer HE, Hromas R. IL-17F, a novel cytokine selectively expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. *J Immunol* 2001;167:4137–4140.
6. Kawaguchi M, Onuchic LF, Huang SK. Activation of extracellular signal-regulated kinase (ERK) 1/2, but not p38 and c-Jun N-terminal kinase, is involved in signaling of a novel cytokine, ML-1. *J Biol Chem* 2002;277:15229–15232.
7. Kawaguchi M, Kokubu F, Matsukura S, Ieki K, Odaka M, Watanabe S, Suzuki S, Adachi M, Huang SK. Induction of C-X-C chemokines, growth-related oncogene (GRO)[alpha] expression and ENA-78, by ML-1 (IL-17F) involves activation of Raf1-MEK-ERK1/2 pathway. *J Pharmacol Exp Ther* 2003;307:1213–1220.
8. Kawaguchi M, Adachi M, Huang SK. Structural and functional analysis of a new cytokine, ML-1 (interleukin-17F). *Allergol Int* 2003;52:117–122.
9. Li XM, Chopra RK, Chou TY, Schofield BH, Wills-Karp M, Huang SK. Mucosal IFN-gamma gene transfer inhibits pulmonary allergic responses in mice. *J Immunol* 1996;157:3216–3219.
10. Kuklin NA, Daheshia M, Chun S, Rouse BT. Immunomodulation by mucosal gene transfer using TGF-beta DNA. *J Clin Invest* 1998;102:438–444.
11. Huang SK, Li XM. Modulation of pulmonary allergic responses by mucosal cytokine-gene transfer. *Allergol Int* 1997;46:11–15.
12. Oda N, Essayan DM, Huang SK. Mucosal transfer of a new mouse IL-17F gene induces pulmonary neutrophilia [abstract]. *Am J Respir Crit Care Med* 2003;167:A360.
13. Chen LC, Zhang Z, Myers AC, Huang SK. Altered pulmonary eosinophilic inflammation in mice deficient for Clara cell secretory 10-kDa protein. *J Immunol* 2001;167:3025–3028.
14. Mitzner W, Tankersley C. Interpreting Penh in mice. *J Appl Physiol* 2003;94:828–831.
15. Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, Oliver P, Huang W, Zhang P, Zhang J, *et al.* Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J Exp Med* 2001;194:519–527.
16. Laan M, Cui ZH, Hoshino H, Lotvall J, Sjostrand M, Gruenert DC, Skoogh BE, Linden A. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J Immunol* 1999;162:2347–2352.
17. Leemans JC, Vervoordeldonk MJ, Florquin S, van Kessel KP, van der Poll T. Differential role of interleukin-6 in lung inflammation induced by lipoteichoic acid and peptidoglycan from *Staphylococcus aureus*. *Am J Respir Crit Care Med* 2002;165:1445–1450.
18. Drannik AG, Pouladi MA, Robbins CS, Goncharova SI, Kianpour S, Stampfli MR. Impact of cigarette smoke on clearance and inflammation following *Pseudomonas aeruginosa* infection. *Am J Respir Crit Care Med* 2004;170:1164–1171.
19. Adler A, Cieslewicz G, Irvin CG. Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. *J Appl Physiol* 2004;97:286–292.
20. Bates J, Irvin C, Brusasco V, Drazen J, Fredberg J, Loring S, Eidelman D, Ludwig M, Macklem P, Martin J, *et al.* The use and misuse of Penh in animal models of lung disease. *Am J Respir Cell Mol Biol* 2004;31:373–374.
21. Laan M, Prause O, Miyamoto M, Sjostrand M, Hytonen AM, Kaneko T, Lotvall J, Linden A. A role of GM-CSF in the accumulation of neutrophils in the airways caused by IL-17 and TNF-alpha. *Eur Respir J* 2003;21:387–393.
22. Krein PM, Sabatini PJ, Tinmouth W, Green FH, Winston BW. Localization of insulin-like growth factor-I in lung tissues of patients with fibroproliferative acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2003;167:83–90.
23. Belperio JA, Dy M, Burdick MD, Xue YY, Li K, Elias JA, Keane MP. Interaction of IL-13 and C10 in the pathogenesis of bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2002;27:419–427.
24. Hurst SD, Muchamuel T, Gorman DM, Gilbert JM, Clifford T, Kwan S, Menon S, Seymour B, Jackson C, Kung TT, *et al.* New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 2002;169:443–453.
25. Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem* 2003;278:17036–17043.
26. Hashimoto K, Graham BS, Ho SB, Adler KB, Collins RD, Olson SJ, Zhou W, Suzutani T, Jones PW, Goleniewska K, *et al.* Respiratory syncytial virus in allergic lung inflammation increases Muc5ac and gob-5. *Am J Respir Crit Care Med* 2004;170:306–312.