IL-17A acts via p38 MAPK to increase stability of TNF-α-induced IL-8 mRNA in human ASM

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ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) are chronic disorders of the airways affecting millions of people worldwide. Airways are remodeled, or thickened, resulting in airway obstruction and a decline in lung function. Structural cells, such as epithelial cells, fibroblasts, and airway smooth muscle (ASM), previously thought to be passively maintaining the airway wall integrity, are now recognized to play a critical active role in the pathogenesis of asthma and COPD. Structural cells are immunomodulatory; they synthesize and secrete inflammatory mediators and thus actively contribute to the perpetuation and amplification of airway inflammation. A complex interplay exists between diverse airway cells, that is, inflammatory cytokines secreted from infiltrating cells, recruited from the circulation and activated within the airway mucosa, can profoundly influence the synthetic function of resident structural cells and thus lead to a more pronounced inflammatory milieu. Interleukin (IL)−17A, the founding member of the IL-17 family (includes IL-17A−IL-17F), is one such cytokine, as it may act to orchestrate the inflammatory response of resident structural cells, serving to amplify and perpetuate inflammation in the lung (reviewed in Refs. 13, 18, 19).

IL-17A, released from CD4+ (35) and CD8+ (27) memory T lymphocytes, is found elevated in asthma (20) and severe airway inflammation (15). In humans, an increase in IL-17A levels in the bronchoalveolar space parallels local accumulation of inflammatory cells, including neutrophils (15). Studies in rodents have linked airway neutrophilia with IL-17A (14, 36). As IL-17A does not directly induce neutrophil chemotaxis (14), the activating effects observed with IL-17A in vivo are considered mediated via indirect mechanisms, such as the potentiation of the local release of the CXC chemokine IL-8 (14), a potent neutrophil chemoattractant.

Therefore, structural cells may play a critical role in the development of airway neutrophilia as they are well positioned to release neutrophil chemotactic signals to directly contribute to extravasation of neutrophils into the bronchial submucosa. In support, accumulating evidence demonstrates that IL-17A can enhance the production of the neutrophil-mobilizing chemokine IL-8 by airway epithelial cells (11, 12, 25, 29) and fibroblasts (20, 29). A number of recent studies have examined the effect of IL-17A on IL-8 secretion by ASM cells (26, 30, 33, 34); however, the potentiating effects of IL-17A and the underlying mechanisms are yet to be fully explored. As the 3′-untranslated region (3′-UTR) of IL-8 mRNA is known to contain adenylate + uridylate-rich elements (ARE), IL-8 can undergo mRNA decay. Thus, in this study we focus on investigating the posttranscriptional regulation of the IL-8 gene by IL-17A in ASM cells. We demonstrate that IL-17A acts to amplify the synthetic function of ASM cells, that is, IL-17A alone does not induce IL-8, but IL-17A potentiates IL-8 secretion in response to TNF-α. Interestingly, IL-8 gene expression can be regulated at multiple levels; we find that TNF-α acts at the transcriptional level via NF-κB, whereas IL-17A acts via a parallel p38 MAPK-mediated pathway to stabilize the TNF-α-induced IL-8 transcript. Together these stimuli act in concert to...
RAPIDLY ENHANCE THE PRODUCTION OF THE CHEMOKINE IL-8 AND ENHANCE THE NEUTROPHIL-CHEMOAATTACTANT FUNCTION OF ASM.

MATERIALS AND METHODS

ASM cell culture. Human bronchi were obtained from patients undergoing surgical resection for carcinoma or lung transplant donors in accordance with procedures approved by the Central Area Health Service and the Human Ethics Committee of the University of Sydney. ASM cells were dissected, purified, and cultured as previously described by Johnson et al. (10). A minimum of three different primary cell lines were used for each experiment.

Unless otherwise specified, all chemicals used in this study were purchased from the Sigma Chemical (St. Louis, MO).

Measurement of IL-8 secretion. Confluent ASM were growth-arrested for 48 h with DMEM and 0.1% BSA. To observe whether IL-17A induces IL-8 secretion, cells were treated with vehicle (DMEM with 0.1% BSA) or increasing concentrations of IL-17A (0.1–10 ng/ml). To examine whether IL-17A acts to potentiate TNF-α-induced IL-8 secretion, ASM were treated with TNF-α (10 ng/ml; R&D Systems, Minneapolis, MN) or vehicle in the absence or presence of IL-17A (10 ng/ml). To observe the synergy between IL-17A and TNF-α, we assessed the effect of increasing concentrations of IL-17A (0.1–100 ng/ml) on IL-8 secretion induced by 10 ng/ml TNF-α. To investigate the effect of inhibition of p38 MAPK on the potentiation of TNF-α-induced IL-8 secretion by IL-17A, we pretreated ASM cells with 30 min with either the p38 MAPK inhibitor SB-203580, or the negative congener SB-202474 (both used at 1 μM; Calbiochem, San Diego, CA), before stimulation with TNF-α and IL-17A in combination (both 10 ng/ml), for 24 h. After 24 h of incubation at 37°C in 5% CO₂, cell supernatants were removed and frozen at −20°C for later analysis by ELISA. IL-8 (BD Biosciences PharMingen, Palo Alto, CA) ELISAs were performed according to the manufacturer’s instructions.

Flow cytometric analysis of IL-17R. To confirm the presence of the IL-17 receptor (IL-17R) on ASM we used a slight modification of the method published by Rahman et al. (26). Briefly, growth-arrested ASM cells were left unstimulated or treated with 5% FBS, TNF-α (10 ng/ml), IL-17A (10 ng/ml), or both TNF-α and IL-17A in combination (both 10 ng/ml). After 24 h of treatment at 37°C in 5% CO₂, cells were harvested with trypsin-EDTA, fixed, and stained using either a monoclonal antibody specific for IL-17R (clone 133617) or IgG1 isotype control (both 10 μg/ml, R&D), followed by a phycoerythrin-conjugated goat anti-mouse IgG (BD Biosciences PharMingen). Samples were then analyzed with Cell Quest software and a FACSCalibur Sort flow cytometer (BD Biosciences). IL-17R expression was assessed as the increase in mean fluorescence intensity over the isotype-matched control (MIIF) for each treatment.

Northern blot analysis of IL-8 gene expression. To examine the time course of IL-8 mRNA expression induced by TNF-α and its potentiation by IL-17A, we treated growth-arrested ASM cells with TNF-α (10 ng/ml), in the absence or presence of IL-17A (10 ng/ml), for 0, 3, 6, 9, 12, and 24 h. IL-8 mRNA expression was quantified by Northern blot (using GAPDH as a housekeeping gene). Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). Northern blotting was performed with 5 μg of RNA. We also assessed the kinetics of protein secretion by measuring IL-8 by ELISA in the cell supernatants.

The human IL-8 cDNA probe was prepared by RT-PCR, using the Access RT-PCR system (Promega, Madison, WI), with RNA extracted from human ASM cells stimulated with 10 ng/ml IL-1β (for 16 h) to induce maximal IL-8 gene expression. The sequences for the PCR primers were: forward, 5'-GCTGCTAGCCAGAGTTAAC-3'; reverse, 5'-ACACAGCTGGCAATGACAAG-3'. These correspond to bases 873–892 and 1351–1370, respectively, of the human IL-8 sequence (GenBank accession number NM_000584). We performed hybridization using [32P]dCTP-labeled probes generated using a random primed DNA labeling kit (Roche Diagnostics, Sydney, NSW, Australia). GAPDH expression was used to normalize for RNA loading, using primers spanning bases 841–1038 of the human GAPDH sequence (GenBank accession number NM_002046). Visualization of the hybridized bands was accomplished by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA), and quantitation of relative mRNA levels was performed by densitometry using Molecular Analyst Software (Bio-Rad, Hercules, CA).

Neutrophil chemotaxis. Neutrophils were purified from anticoagulated venous blood of a healthy volunteer by discontinuous density gradient centrifugation with monoply resolving medium (ICN Biomedicals, Aurora, OH) as previously described (28). To measure chemotaxis, we placed conditioned media from stimulated ASM cells (35 μl) in the lower wells of a 96-well microchemotaxis chamber (Neuroprobe, Cabin John, MD). The lower wells were covered with a 5-μm pore-size polycarbonate filter, and then 200 μl of neutrophil suspension (2 × 10⁵ cells/ml) were added to the upper wells. After incubation for 60 min at 37°C in 5% CO₂, the cells remaining on the upper side of the filter were removed and the filter fixed in 70% methanol, allowed to air-dry, and then stained with Diff-Quik (Lab Aids, Sydney, NSW, Australia). We quantified neutrophil chemotaxis microscopically by counting cells on the bottom side of the filter in a high-power (×400) field (results are expressed as number of cells/high-power field). In parallel experiments we determined the degree of chemokinesis (random migration) by setting up chemokinetic control wells in which conditioned media from ASM cells stimulated with TNF-α and IL-17A were placed above and below the polycarbonate filter. As there was no concentration gradient between the top and bottom surfaces of the filter, the number of migrated cells shows the extent of nonchemoatcic chemokinetic migration.

ASM cell transfection. The NF-κB reporter vector, pNF-κB-Luc, was purchased from Clontech (BD Biosciences). Transient transfection of ASM cells was performed using Lipofectamine 2000 (Invitrogen). In brief, ASM cells were plated onto six-well plates at a density of ~2.5 × 10⁵ cells/well for 24 h and then transfected with 2.4 μg of pNF-κB-Luc as well as 1.4 μg of pSV-β-galactosidase control vector (Promega) to normalize transfection efficiencies. After transfection, cells were cultured for 8 h and then grown-arrested for 16 h in DMEM supplemented with 0.1% BSA, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Invitrogen). Cells were then treated with vehicle or TNF-α (10 ng/ml), in the absence or presence of IL-17A (10 ng/ml), for 6 h. Cells were then harvested, and luciferase and β-galactosidase activities were assessed according to manufacturer’s instructions (Promega). Data represent normalized luciferase activity, relative to vehicle-treated cells (expressed as fold change).

The MAPK kinase 6 (MKK6) expression vector was a gift from Jiuhai Han (Scripps Research Institute, La Jolla, CA) (6). ASM cells (1.5 × 10⁵ cells/well) were transfected with 100 ng of MKK6 or empty vector control. After 24 h, the supernatants were removed, and IL-8 protein was measured by ELISA. Results were expressed as fold difference (normalized to empty vector control).

mRNA stability. To measure IL-8 mRNA stability, we treated growth-arrested ASM cells with TNF-α (10 ng/ml), in the absence or presence of IL-17A (10 ng/ml), for 9 h. In parallel experiments, we assessed the effect of p38 MAPK inhibition by pretreating the cells for 30 min with vehicle or 1 μM SB-203580 before stimulation with TNF-α and IL-17A (both at 10 ng/ml) for 9 h. Cells were then washed and incubated with actinomycin D (5 μg/ml) to inhibit further transcription. Total RNA was extracted following 0, 3, 6, 9, and 12, and 24 h incubation with actinomycin D, and IL-8 mRNA quantification was performed by real-time PCR. Reverse transcription of 0.5 μg of RNA was performed using the RevertAid First strand cDNA Synthesis Kit (Fermentas Life Sciences, Hanover, CA) as per the manufacturer’s protocol for the use of random hexamer primers. Two microcopies of the resulting cDNA were then amplified in duplicate using a 7500 real-time PCR Instrument (Applied Biosystems, Foster City, CA) and
an IL-8 primer set (Assays on Demand, human IL-8, Hs00174131_m1, Applied Biosystems). Samples were multiplexed with a eukaryotic 18S rRNA endogenous control probe (Applied Biosystems) and subjected to the following cycle parameters: 50°C for 2 min, 1 cycle; 95°C for 10 min, 1 cycle; 95°C for 15 s, 60°C for 1 min, 40 cycles. Results are presented as % mRNA remaining (i.e., compared with steady-state levels of mRNA expression following 9 h of cytokine treatment). First-order decay constants (k) were solved by nonlinear regression of the % mRNA remaining vs. time after actinomycin D (31).

Statistical analysis. Statistical analysis was performed by either one-way or two-way ANOVA and then Fisher’s post hoc multiple-comparison test, or Student’s unpaired t-test. P values <0.05 were sufficient to reject the null hypothesis for all analyses. Data represent means ± SE.

RESULTS

IL-17A-treated ASM cells do not secrete IL-8, but IL-17A potentiates TNF-α-induced IL-8 secretion in a concentration-dependent manner. Although there have been reports of IL-17A inducing IL-8 secretion by ASM (26, 30, 33, 34), in this study (Fig. 1A) ASM cells treated with a range of concentrations of IL-17A did not secrete detectable levels of IL-8. As shown in Fig. 1B, IL-17A acted to amplify the synthetic function of ASM; in the presence of 10 ng/ml IL-17A, TNF-α-stimulated ASM cells secreted 2.7-fold greater amounts of IL-8 (32.6 ± 3.2 ng/ml) than cells stimulated with TNF-α alone (12.0 ± 1.1 ng/ml, P < 0.05). As shown in Fig. 1C, this potentiation occurred in a concentration-dependent manner (P < 0.05).

In confirmation of a previous report (26), we found ASM constitutively express IL-17R. The MFI under unstimulated basal conditions was 26.5 ± 2.5, and these levels were not significantly altered by 24-h treatment with 5% FBS (30.0 ± 0.5). Furthermore, there were no significant differences in the MFI following 24-h treatment with TNF-α (24.4 ± 4.2) nor IL-17A (23.5 ± 1.1), both alone and in combination with TNF-α (22.2 ± 1.1), compared with unstimulated control. Thus we have no evidence to support the enhanced secretion of IL-8 in the presence of IL-17A and TNF-α being dependent on changes in the level of IL-17R expression.

Kinetcs of IL-8 mRNA expression and protein secretion. We then examined the effect of IL-17A on the time course of TNF-α-induced IL-8 mRNA expression and protein secretion. As shown in Fig. 2A, TNF-α rapidly enhanced IL-8 mRNA expression, detectable levels being apparent as early as 3 h following stimulation (210.6 ± 35.1% increase in IL-8 mRNA at 3 h, compared with 0 h; P < 0.05) and seen to increase gradually over time. Importantly, in the presence of IL-17A, IL-8 mRNA expression induced by TNF-α is augmented at all time points examined, as demonstrated in Fig. 2B. These changes in IL-8 mRNA expression were reflected in corresponding increases in IL-8 protein secretion (Fig. 2C), where the presence of IL-17A significantly potentiated TNF-α-induced IL-8 secretion from ASM cells in a time-dependent manner (P < 0.05).

IL-17A enhances human neutrophil chemotaxis in vitro. IL-17A orchestrates neutrophilic influx. In Fig. 1, A and C, we showed that IL-17A acts to amplify the synthetic function of ASM and increase the levels of the potent neutrophil chemoattractant chemokine, IL-8. In Fig. 3A, we show that human neutrophils undergo significant migration toward conditioned media from ASM cells treated with TNF-α (129.8 ± 19.1
IL-8 secretion, compared with secretion from cells stimulated with vehicle alone.  

**Fig. 2.** Time course of IL-8 mRNA expression and protein secretion induced by TNF-α and its augmentation by IL-17A. Growth-arrested ASM cells were treated with TNF-α (10 ng/ml), in the absence (○) or presence (■) of IL-17A (10 ng/ml), for the indicated times. A and B: IL-8 mRNA expression quantified by Northern blot (using GAPDH as a housekeeping gene; representative results of n = 3 primary cell lines). C: protein secretion measured by ELISA. Statistical analysis was performed by 2-way ANOVA and then Fisher’s paired least significant difference multiple-comparison test. *Significant effect of IL-17A on TNF-α-induced IL-8 secretion; §significant effect of TNF-α on IL-8 secretion; †significant effect of IL-17A on TNF-α-induced IL-8 secretion, compared with secretion from cells stimulated with TNF-α alone (P < 0.05). Data represent means ± SE values from 8–9 replicates.

**IL-17A increases IL-8 mRNA stability**

IL-17A increases IL-8 mRNA stability via a p38 MAPK-dependent pathway. A recent study (33) demonstrated the activation of MAPK family members by IL-17A. We found that ASM cells transfected with a constitutively active form of MKK6, an upstream activator of p38 MAPK phosphorylation (6) secreted increased amounts of IL-8 (Fig. 6A). Moreover, as shown in Fig. 6B, the cell-permeable p38 MAPK inhibitor, SB-203580, significantly inhibited IL-8 secretion in response to both TNF-α and IL-17A by 31.1 ± 8.8% (P < 0.05). Thus it was of interest to investigate whether the p38 MAPK-dependent pathway underlies the enhanced stability of IL-8 mRNA induced by IL-17A. ASM cells pretreated with vehicle or the p38 MAPK inhibitor, SB-203580, were stimulated with TNF-α and IL-17A for 9 h, and IL-8 mRNA stability was assessed over time. As shown in Fig. 6C, we found that p38 MAPK inhibition significantly inhibited the stability of the IL-8 mRNA transcript induced in the presence of both TNF-α and IL-17A. Whereas the half-life of IL-8 transcript induced by TNF-α and IL-17A, in the presence of SB-203580 vehicle, was 67.3 h, pretreatment of cells with the p38 MAPK inhibitor SB-203580 significantly reduced the half-life of IL-8 mRNA to 12 h, with a significantly faster rate of decay (−0.05763 ± 0.01964), than in vehicle alone (−0.01030 ± 0.007963; P < 0.05). Because increased mRNA stability can lead to augmented accumulation of mRNA and the proteins they encode, we conclude that IL-17A significantly amplifies the synthetic function of ASM cells, acting via a p38 MAPK-dependent posttranscriptional pathway to augment...
DISCUSSION

There is increasing evidence to demonstrate that IL-17A enhances human neutrophil chemotaxis, but not chemokinesis, in vitro. A chemotaxis of human neutrophils toward supernatants from ASM cells treated with vehicle or TNF-α (10 ng/ml), in the absence (open bars) or presence (filled bars) of IL-17A (10 ng/ml), was measured using microchemotaxis chambers. B: to exclude chemokinesis, neutrophil migration toward conditioned medium from ASM cells stimulated with TNF-α and IL-17A placed above and below the polycarbonate filter (filled bar) was measured and compared with migration towards vehicle alone (open bar). Results are expressed as cells per high-power field. Statistical analysis was performed by Student’s unpaired t-test. *Significant effect of conditioned medium from ASM cells stimulated with TNF-α on neutrophil chemotaxis; $significant effect of conditioned media from ASM treated with TNF-α in the presence of IL-17A compared with TNF-α alone (P < 0.05). Data represent mean ± SE values obtained in neutrophils from a human donor stimulated in vitro with supernatants from n = 5 primary ASM cell lines.

Thus IL-17A acts via a posttranscriptional mechanism dependent on the p38 MAPK pathway to increase levels of the chemokine IL-8 and enhance the neutrophil-attractant function of ASM.

No longer simply being responsible for bronchomotor tone alone, ASM has emerged as playing an immunomodulatory role (17, 23). In response to inflammatory mediators such as TNF-α, ASM cells secrete a diverse repertoire of cytokines (8), including the CXC chemokine IL-8 (24), a potent neutrophil chemoattractant. We show, for the first time, that IL-17A acts to increase the IL-8 gene expression and levels of protein secreted by ASM in response to TNF-α. This is consistent with a recent report (29) in airway structural cells, primary bronchial epithelial cells and lung fibroblasts, where IL-17A was a TNF-α-induced secretion of the potent neutrophil chemoattractant IL-8 from ASM cells.

Fig. 3. Conditioned medium from ASM cells treated with TNF-α in the presence of IL-17A enhances human neutrophil chemotaxis, but not chemokinesis, in vitro. A: chemotaxis of human neutrophils toward supernatants from ASM cells treated with vehicle or TNF-α (10 ng/ml), in the absence (open bars) or presence (filled bars) of IL-17A (10 ng/ml), was measured using microchemotaxis chambers. B: to exclude chemokinesis, neutrophil migration toward conditioned medium from ASM cells stimulated with TNF-α and IL-17A placed above and below the polycarbonate filter (filled bar) was measured and compared with migration towards vehicle alone (open bar). Results are expressed as cells per high-power field. Statistical analysis was performed by Student’s unpaired t-test. *Significant effect of conditioned medium from ASM cells stimulated with TNF-α on neutrophil chemotaxis; $significant effect of conditioned media from ASM treated with TNF-α in the presence of IL-17A compared with TNF-α alone (P < 0.05). Data represent mean ± SE values obtained in neutrophils from a human donor stimulated in vitro with supernatants from n = 5 primary ASM cell lines.

Fig. 4. IL-17A does not activate NF-κB in ASM cells. ASM cells transfected with a NF-κB reporter vector, pNF-κB-Luc, were growth-arrested and then treated with vehicle or TNF-α (10 ng/ml), in the absence (open bars) or presence (filled bars) of IL-17A (10 ng/ml) for 6 h. Cells were then harvested, and luciferase and β-galactosidase activities were assessed. Data represent normalized luciferase activity, relative to vehicle-treated cells (expressed as fold difference). Statistical analysis was performed by Student’s unpaired t-test. §Significant effect of TNF-α on luciferase activity (∼P < 0.05). Data represent mean ± SE values from 6 –17 replicates.

Fig. 5. IL-17A enhances the stability of TNF-α-induced IL-8 mRNA. Growth-arrested ASM cells were treated with TNF-α (10 ng/ml), in the absence (□) or presence (●) of IL-17A (10 ng/ml), for 9 h. Cells were then washed and incubated with actinomycin D (5 µg/ml) to inhibit further transcription. Total RNA was extracted following 0, 3, 6, 9, 12, and 24 h. IL-8 mRNA expression was quantified by real-time PCR. Results are expressed as % mRNA remaining over time (where the lines represents nonlinear regression of the % mRNA remaining vs. time after actinomycin D).
IL-17A (both at 10 ng/ml) for 9 h. Stability of IL-8 mRNA transcripts was maintained with vehicle (expression induced by IL-17A, growth-arrested ASM cells pretreated for 30 min/H9251 MAPK inhibition on the enhanced stability of TNF-α induced IL-8). The authors also found that TNF-α, whereas IL-17A markedly enhanced synthesis of airway structural cells and by potentiating the release of neutrophil chemotactic signals, such as IL-8, may contribute to chemotaxis of neutrophils into the bronchial submucosa and play a critical role in the development of airway neutrophilia.

There have been four publications, to date, in ASM in which IL-17A has been reported to induce IL-8 secretion (26, 30, 33, 34); however, we have been unable to measure IL-8 secretion from ASM cells treated with a range of concentrations of IL-17A. The reasons underlying this discrepancy are currently unclear, but we speculate that disease state-dependent differences in IL-17A responsiveness (30, 33, 34), or differences in vitro culture conditions (26), may result in ASM that are more "primed" and thus able to secrete IL-8 in response to IL-17A. It is possible that differences in the disease state of lung donors or patient treatment regimens (such as immunosuppressive agents) may have resulted in ASM cells that have intrinsic differences in IL-17A responsiveness. These changes could be genomic or nongenomic; the underlying molecular mechanisms are unknown at this stage. We have confirmed, however, that the differences between our study and those published previously (26, 30, 33, 34) are IL-17R independent, as the levels of IL-17R on ASM corroborates those published previously by Rahman et al. (26) and there is no evidence of proteolytic cleavage of IL-17R during the isolation procedure, nor regeneration with TNF-α.

Although one of the limitations of this study is that we have not performed any in vivo experiments, we have exploited the in vitro system to address the molecular mechanisms underlying the enhancement of TNF-α-induced IL-8 by IL-17A. The expression of the IL-8 gene can be regulated at multiple levels (reviewed in Ref. 9). The 5′-promoter region of the IL-8 gene contains cis-acting binding elements for NF-κB (21), and NF-κB is a critical transcriptional regulator of IL-8 gene expression. Moreover, the transcript for IL-8 is very unstable; the 3′-UTR of the IL-8 mRNA contains ARES, a cis-acting determinant of posttranscriptional mRNA decay (4). Together, coordinated control of transcriptional and posttranscriptional gene regulation ensures that proinflammatory proteins such as IL-8 are maintained at low levels but can then be rapidly induced and degraded once their transient, but critical, roles have ceased. We have found that whereas TNF-α activates NF-κB, IL-17A was without effect. Rather than acting at the transcriptional level, IL-17A stabilizes the TNF-α-induced IL-8 transcript. These results add to the accumulating evidence supporting a role for IL-17A in the posttranscriptional regulation of genes, evidence that has been steadily accumulating since the original observation made by Cai et al. in 1998 (3) where IL-17A was first shown to increase mRNA stability and enhance granulocyte-colony stimulating factor secretion from 3T3 fibroblasts. Since that time, IL-17A has been shown to interact with a variety of proinflammatory cytokines, including TNF-α (5, 7, 11, 16, 22, 29), to enhance airway structural cell synthetic function (reviewed in Refs. 13, 18, 19). In ASM we show that the half-life of the IL-8 transcript induced by TNF-α was ~14.6 h. Treatment of ASM cells with TNF-α, in the presence of IL-17A, resulted in an IL-8 transcript with enhanced stability (t1/2 = 67.3 h). These results are in agreement with a previous study in primary bronchial epithelial cells and poor inducer of IL-8, whereas IL-17A markedly enhanced TNF-α-induced IL-8. The authors also found that TNF-α-induced IL-6 was potently upregulated by IL-17A, corroborating our earlier study in ASM (7). Importantly, the chemotaxis of human neutrophils to conditioned media from ASM cells stimulated with TNF-α was enhanced in the presence of IL-17A. Together, these observations serve to demonstrate that IL-17A can profoundly enhance the synthetic function of airway structural cells and by potentiating the release of neutrophil chemotactic signals, such as IL-8, may contribute to chemotaxis of neutrophils into the bronchial submucosa and play a critical role in the development of airway neutrophilia.

Fig. 6. IL-17A acts via p38 MAPK-mediated pathway to increases the stability of TNF-α-induced IL-8 mRNA. A: to demonstrate a role of p38 MAPK in IL-8 secretion, we transfected ASM cells with MKK6, an upstream regulator of p38 MAPK phosphorylation, or empty vector control. After 24-h expression IL-8 was measured by ELISA. Statistical analysis was performed by Student’s unpaired t-test. *Significant effect of MKK6 expression on IL-8 secretion compared with secretion from cells pretreated with the vector control (P < 0.05). Data represent mean ± SE values from 20–21 replicates. B: to investigate the effect of inhibition of p38 MAPK on the potentiation of TNF-α-induced IL-8 secretion by IL-17A, we stimulated growth-arrested ASM cells pretreated for 30 min with vehicle (open bar), a p38 MAPK inhibitor, 1 μM SB-203580 (filled bars), or its negative congener, 1 μM SB-202474 (striped bar), with TNF-α and IL-17A (both at 10 ng/ml) for 24 h. Secreted IL-8 was measured by ELISA. Statistical analysis was performed by Student’s unpaired t-test. *Significant effect of SB-203580 pretreatment compared with secretion from cells pretreated with vehicle (P < 0.05). Data represent mean ± SE values from 9 replicates. C: to examine the effect of p38 MAPK inhibition on the enhanced stability of TNF-α-induced IL-8 mRNA expression induced by IL-17A, growth-arrested ASM cells pretreated for 30 min with vehicle (●) or 1 μM SB-203580 (▲) were stimulated with TNF-α and IL-17A (both at 10 ng/ml) for 9 h. Stability of IL-8 mRNA transcripts was measured by an actinomycin D time course by real-time PCR.
lung fibroblasts (29), where the combination of IL-17A and TNF-α markedly augmented the stability of the IL-8 mRNA transcript.

Extracellular stimulation can antagonize ARE-dependent mRNA decay of IL-8 via activation of the phosphoprotein p38 MAPK (32). IL-17A has been shown to induce phosphorylation of MAPK family members in ASM, including p38 MAPK (33). In this study we directly link the activation of p38 MAPK to IL-8 secretion. ASM cells transfected with constitutively active MKK6, an upstream activator of p38 MAPK phosphorylation (6), secreted increased levels of IL-8 protein. Inhibition of the p38 MAPK pathway with the pharmacological inhibitor SB-203580 attenuates IL-8 secretion in response to both TNF-α and IL-17A. Finally, we found that p38 MAPK inhibition significantly decreased the stability of the IL-8 mRNA transcript induced in the presence of both TNF-α and IL-17A. Interestingly, the p38 MAPK pathway appears to be the predominant pathway underlying the augmentation of IL-8 stability by IL-17A, as inhibition by SB-203580 results in an IL-8 mRNA transcript with decay kinetics that are not significantly different from those obtained with TNF-α alone.

Through its role as a chemoattractant, IL-8 activates and recruits neutrophils; hence IL-8 may play a role in asthma and COPD. Our results show that TNF-α transcriptionally regulates IL-8 gene expression via NF-κB, and then IL-17A acts via a parallel p38 MAPK-mediated pathway to stabilize the TNF-α-induced IL-8 transcript. Together these stimuli act in concert to rapidly enhance the production of the chemokine IL-8 and enhance the neutrophil-chemoattractant function of ASM. Collectively, these studies suggest that IL-17A plays an important role in airway inflammation and serve to illustrate that IL-17A is a potential target for therapeutic intervention in asthma.

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