IL-17A Induces Eotaxin-1/CC Chemokine Ligand 11 Expression in Human Airway Smooth Muscle Cells: Role of MAPK (Erk1/2, JNK, and p38) Pathways

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IL-17A Induces Eotaxin-1/CC Chemokine Ligand 11 Expression in Human Airway Smooth Muscle Cells: Role of MAPK (Erk1/2, JNK, and p38) Pathways

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Recently, IL-17A has been shown to be expressed in higher levels in respiratory secretions from asthmatics and correlated with airway hyperresponsiveness. Although these studies raise the possibility that IL-17A may influence allergic disease, the mechanisms remain unknown. In this study, we investigated the molecular mechanisms involved in IL-17A-mediated CC chemokine (eotaxin-1/CCL11) production from human airway smooth muscle (ASM) cells. We found that incubation of human ASM cells with rIL-17A resulted in a significant increase of eotaxin-1/CCL11 release from ASM cells that was reduced by neutralizing anti-IL-17A mAb. Moreover, IL-17A significantly induced eotaxin-1/CCL11 release and mRNA expression, an effect that was abrogated with cycloheximide and actinomycin D treatment. Furthermore, transfection studies using a luciferase-driven reporter construct containing eotaxin-1/CCL11 proximal promoter showed that IL-17A induced eotaxin-1/CCL11 at the transcriptional level. IL-17A also enhanced significantly IL-1β-mediated eotaxin-1/CCL11 mRNA, protein release, and promoter activity in ASM cells. Primary human ASM cells pretreated with inhibitors of MAPK p38, p42/p44 ERK, JNK, or JAK but not PI3K, showed a significant decrease in eotaxin-1/CCL11 release upon IL-17A treatment. In addition, IL-17A mediated rapid phosphorylation of MAPK (p38, JNK, and p42/p44 ERK) and STAT-3 but not STAT-6 or STAT-5 in ASM cells. Taken together, our data provide the first evidence of IL-17A-induced eotaxin-1/CCL11 expression in ASM cells via MAPK (p38, p42/p44 ERK, JNK) signaling pathways. Our results raise the possibility that IL-17A may play a role in allergic asthma by inducing eotaxin-1/CCL11 production. The Journal of Immunology, 2006, 177: 4064–4071.

A irway smooth muscle (ASM)3 cells are key determinants of asthma due to their ability to contract in response to inflammatory cell products (1, 2). Due to intrinsic phenotype plasticity, airway myocytes exhibit a capacity for multifunctional behavior and contribute to local inflammation and fibrosis (3). Emerging evidence suggests that ASM cells can contribute directly to the pathogenesis of asthma by expressing adhesion and costimulatory molecules, and by secreting multiple proinflammatory cytokines and chemokines (4, 5).

Eotaxin-1/CCL11 is a CC chemokine that was first identified as an important chemoattractant for eosinophils in lungs from Ag-sensitized and -challenged guinea pigs (6). Increased eotaxin-1/ CCL11 mRNA expression has been found both in bronchial biopsies and in cells from bronchoalveolar lavage of mild human asthmatics (7–9). Moreover, measured concentrations of this chemokine appear to correlate well with the severity of the disease (10). Other studies have shown that eotaxin-1/CCL11 neutralization in mice substantially reduced eosinophil recruitment after Ag challenge, thus indicating an important functional role in promoting airway inflammation in asthma (11, 12). Studies using asthmatic airways and isolated primary cells indicate ASM cells are a significant source for eotaxin-1/CCL11 production both in vivo (4) and in vitro (13, 14).

Human IL-17A is the founding member of an emerging cytokine family, with pleiotropic biological activities and is released by activated CD4+ T cells (15). Exaggerated levels of IL-17A are observed in chronic inflammatory disorders (16, 17), suggesting a potential role of this cytokine in the initiation or maintenance of inflammatory responses. Although IL-17A has been shown to be present in higher levels in respiratory secretions from asthmatics (18) and increased concentrations of IL-17A correlate with airway hyperresponsiveness (19), the mechanism by which this cytokine may influence allergic disease processes remains unknown.

In the current study, we show for the first time that IL-17A alone can induce eotaxin-1/CCL11 mRNA expression and protein release from ASM cells. This effect of IL-17A alone, or in combination with IL-1β, is dependent on de novo protein and mRNA synthesis. Transfection studies using eotaxin-1/CCL11 promoter luciferase construct showed that IL-17A induced eotaxin-1/CCL11 transcription in ASM cells. IL-17A signaling via MAPK (p38, p42/p44 ERK, JNK), and probably STAT-3, appeared to be required for the induction of eotaxin-1/CCL11 synthesis and release in ASM cells.

Materials and Methods

Reagents

Recombinant human IL-17A, IL-1β, IL-4, neutralizing mouse anti-human IL-17A mAb, mouse anti-human p38 α mAb, mouse anti-human ERK1/2

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Isolation and culture of human ASM cells

Human bronchial smooth muscle cells were obtained from macroscopically healthy segments of second to fourth generation lobar or main bronchi of patients undergoing surgery for lung carcinoma in accordance with the procedures approved by the Ethics Committee of the University of Manitoba (Winnipeg, Canada). Informed consent for ASM harvesting was obtained from all patients. Primary ASM cells were isolated from explants as described previously (20). Cells were cultured in DMEM supplemented with 10% FBS, l-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C with 5% CO2. At confluence, primary human ASM cells exhibited spindle morphology and a hill-and-valley pattern that is characteristic of smooth muscle in culture. Moreover, ASM cells at confluence retain smooth muscle-specific actin, SM22, and calponin protein expression, and mobilize intracellular Ca2+ in response to acetylcholine, a physiologically relevant contractile agonist (20).

Cell culture and ELISA analysis of chemokine release in cell supernatants

Semiconfluent ASM cells (75%; passages 2–5) were growth arrested for 48 h in serum-free medium as described above. Cells were then stimulated in fresh FBS-free medium with IL-17A (10 ng/ml), or medium alone. At selected time points, the cells were washed once with cold PBS, and total proteins were extracted with lysis buffer (1% Nonidet P-40, PMSF, 2 mM sodium vanadate, 0.1% sodium deoxycholate, and protease inhibitor mixture (Roche)). Har-vested lysates were centrifuged for 10 min at 4°C to pellet cellular debris. The supernatants were removed and stored at −70°C. Protein lysate (10 μg) was loaded on 10% SDS-PAGE, followed by transfer to nitrocellulose membranes (Invitrogen Life Technologies). The blots were then blocked with 5% nonfat dry milk in TBS/0.1% Tween (TBS-T) for 1 h at room temperature, and then incubated overnight at 4°C with Abs specific for phosphorylated ERK1/2 (T202/Y204), p38 MAPK (T180/Y182), JNK (T183/Y185), STAT-3 (Y705), STAT-5 (Y694), and STAT-6 (Y641). After washing with TBS-T, the blots were incubated with goat anti-mouse or goat anti-rabbit HRP-conjugated secondary Abs and bands were revealed with ECL reagents (Amersham Biosciences). After stripping, total anti-ERK, p38 MAPK, STAT-3, STAT-5, STAT-6, and β-actin were used as loading control.

Luciferase reporter constructs and cell transfection

Eotaxin-1/CCL11 promoter fragment was amplified from human cell genomic DNA using PCR with specific primers (23). Briefly, a 2.2-kb amplified fragment was inserted into pGL3-Basic vector (Promega) yielding the reporter construct pGL3-EO2. Constructs used for transient transfection were purified by cesium chloride density gradients. ASM cells (4 x 104) were seeded into 24-well culture plates in fresh complete DMEM. After 24 h at 70% confluency, cells were transfected with wild-type plasmids by the Dual-Luciferase Assay System Kit (Promega) using a luminometer (model LB9501; Berthold Lumat). Briefly, 20 μl of cell lystate was mixed with 100 μl of Luciferase Assay Reagent II (Promega) and briefly lucif-erase activity was first recorded. Then, 100 μl of Stop-and-Glo Reagent (Promega) was added, and Renilla luciferase activity was measured. All values are normalized to Renilla luciferase activity and expressed relative to the control transfected nonstimulated cells.
**Statistical analysis**

Results are expressed as means ± SD. Differences between the groups were analyzed using Kruskal-Wallis with Dunne test. Differences between pairs were assessed by Mann-Whitney U test. Values of $p < 0.05$ were considered statistically significant.

**Results**

**rIL-17A induces eotaxin-1/CCL11 protein release from ASM cells**

To investigate whether IL-17A influences eotaxin-1/CCL11 release from ASM cells, serum-deprived ASM cells were stimulated with a wide range of IL-17A concentrations (0.1–100 ng/ml). Stimulation with IL-17A induced the release of eotaxin-1/CCL11 in a dose-dependent manner with a maximum induction at 48 h (Fig. 1A). At both 24- and 48-h time points, a statistically significant increase in eotaxin-1/CCL11 release from ASM cells occurred with 1, 10, and 100 ng/ml IL-17A ($p < 0.05$; Fig. 1A).

Furthermore, analysis of the same supernatants revealed a normal level of IL-6 (Fig. 1B) and CXCL-8/IL-8 (data not shown) as we reported previously (24). Although at 100 ng/ml IL-17A seems to induce more eotaxin-1/CCL11 release (Fig 1A), analysis of data from five ASM cell lines showed no statistical significance in eotaxin-1/CCL11 release of cells stimulated with 10 or 100 ng/ml (fold increase, 2.5 ± 0.7 vs 3.3 ± 1.2, respectively, at 48 h; $p = 0.67$).

Coincubation with an anti-IL-17A neutralizing mAb prevented IL-17A-mediated eotaxin-1/CCL11 release ($p < 0.01$) (Fig. 1C). In contrast, mouse IgG isotype control, had no effect on IL-17A-induced eotaxin-1/CCL11 release by ASM cells. Taken together, these results suggest that eotaxin-1/CCL11 release in primary ASM cells is selectively mediated by IL-17A.

**IL-17A enhances IL-1β-mediated eotaxin-1/CCL11 expression in ASM cells by de novo mRNA and protein synthesis**

Because cells are exposed to multiple proinflammatory cytokines in the microenvironment of inflammatory diseases, we next investigated the combined effect of IL-17A and IL-1β stimulation on the induction of eotaxin-1/CCL11 release from ASM cells. As shown in Fig. 2A, IL-17A or IL-1β alone (0.1, 1, 10, and 100 ng/ml) induced a significant increase in eotaxin-1/CCL11 release compared with unstimulated ASM cells ($p < 0.01$). Addition of IL-17A (1 ng or 100 ng/ml) to a suboptimal dose of IL-1β (0.1 ng/ml) induced a significant increase of eotaxin-1/CCL11 release in ASM cells ($p < 0.05$; Fig. 2A). Interestingly, IL-17A at 1, 10, or 100 ng/ml combined with IL-1β at 1 or 10 ng/ml, but not at 100 ng/ml, resulted in enhanced eotaxin-1/CCL11 release from ASM cells ($p < 0.05$; Fig 2A).

Previous studies have demonstrated that eotaxin-1/CCL11 expression is controlled at many points, particularly at the transcriptional and posttranscriptional levels (25). Thus, we next tested whether blocking mRNA and protein synthesis affected eotaxin-1/CCL11 production induced by IL-17A alone or in combination with IL-1β in ASM cells. Cells were pretreated with actinomycin D followed by stimulation with IL-17A, IL-1β, or both for 48 h. As observed in Fig. 2B, actinomycin D and cycloheximide abrogated eotaxin-1/CCL11 release from ASM cells treated with IL-17A, IL-1β, or both ($p < 0.05$). The inhibition of eotaxin-1/CCL11 mRNA expression by actinomycin D was also confirmed by quantitative real-time RT-PCR. ASM cells treated for 6 h with IL-17A, IL-1β, or both induced 1.8-, 9.4-, and 13.8-fold increase of eotaxin-1/CCL11 mRNA level, respectively, compared with unstimulated cells (Fig. 2C). Interestingly, this effect was significantly inhibited with actinomycin D treatment ($p < 0.05$; Fig. 2C).

**FIGURE 1.** Time course and concentration response of IL-17A-induced eotaxin-1/CCL11 (A) and IL-6 (B) release from ASM cells. Growth-arrested ASM cells were left unstimulated (medium alone) or treated with indicated concentrations of IL-17A for 24 and 48 h. Each point represents mean ± SD of triplicate values from one experiment and is representative of five experiments done with cells from five different donors, cell passages 3–5. * $p < 0.05$; ** $p < 0.001$ compared with unstimulated ASM cells. C. Effects of a monoclonal anti-IL-17A neutralizing Ab on release of eotaxin-1/CCL11 from ASM cells, as determined by ELISA. Growth-arrested cells were left unstimulated (medium alone) or treated with IL-17A for 48 h with or without pretreatment with anti-IL-17A Ab or isotype control IgG at indicated doses for 2 h. Each point represents mean ± SD of triplicate values from one experiment and is representative of five experiments done with cells from five different donors, cell passages 3–4. * $p < 0.01$ compared with IL-17A-stimulated ASM cells.

Similar results were observed at 24 h (data not shown). Actinomycin D and cycloheximide had no effect on eotaxin-1/CCL11 mRNA expression in unstimulated cells (data not shown).
IL-17A induces eotaxin-1/CCL11 promoter activity

To further investigate mechanisms by which IL-17A mediated eotaxin-1/CCL11 transcription, ASM cells were transiently transfected with a luciferase reporter construct driven by a 2.2-kb eotaxin-1/CCL11 proximal promoter (25). Primary human ASM cells transfected with CCL11 promoter construct showed a significant increase in luciferase activity in response to IL-17A and IL-1β (mean value of fold increase compared with baseline: 4.3 and 11.9, respectively; Fig. 3). As expected from previous studies (25), IL-4 proved to be a positive control for activation of the eotaxin-1/CCL11 promoter (Fig. 3). Furthermore, eotaxin-1/CCL11 promoter activity was further increased when ASM cells were stimulated with a combination of IL-1β and IL-17A (Fig. 3). Collectively, these data suggest that IL-17A induces eotaxin-1/CCL11 via at least a transcriptional mechanism in ASM cells.

IL-17A induces eotaxin-1/CCL11 release from ASM cells via MAPK (p38, JNK, and ERK1/2)

Previous studies suggest that IL-17A-induced signaling may differ between divergent resident lung cell populations (26). To characterize the signaling pathways involved in IL-17A-mediated eotaxin-1/CCL11 release from ASM cells, we first performed experiments using SB203580, U0126, or SP600125 specific and potent inhibitors of MAPK p38, p42/p44 ERK, and JNK, respectively. We also investigated the effects of wortmannin, a specific PI3K inhibitor. Treatment of ASM cells with SB203580, U0126, or SP600125 before IL-17A stimulation caused a significant and substantial inhibition of eotaxin-1/CCL11 release (p < 0.05; Fig. 4A). In contrast, inhibition of PI3K with wortmannin had little or no effect on IL-17A-induced eotaxin-1/CCL11 release by human airway myocytes (Fig. 4B). ASM cell pretreatment with U0126, SB203580, SP600125, or wortmannin profoundly reduced eotaxin-1/CCL11 release induced by IL-1β or IL-17A in combination with IL-1β suggesting that signaling via MAPK, JNK, and PI3K pathways are required for the induction of eotaxin-1/CCL11 by IL-1β (p < 0.01; Fig. 4, A and B). These results indicate that p38 MAPK, JNK, and p42/p44 ERK, but not PI3K, are essential for IL-17A-mediated release of eotaxin-1/CCL11 by human ASM cells.

IL-17A mediates rapid phosphorylation of MAPK p38, JNK, and ERK1/2 in ASM cells

To further confirm the involvement of MAPK in IL-17A-mediated eotaxin-1/CCL11 expression in ASM cells, we performed Western blot analysis using specific Abs for the phosphorylated regulatory sites on both p38, JNK, and p42/p44 ERK, but not PI3K, are essential for IL-17A-mediated release of eotaxin-1/CCL11 by human ASM cells.
IL-17A causes rapid phosphorylation of STAT-3 but not STAT-6 or STAT-5

Eotaxin-1/CCL11 expression has been shown to be dependent on STAT-6 activation in various inflammatory and structural cells including ASM cells (25, 27). To determine STAT-6 activation in response to IL-17A in ASM cells, total cell protein was probed with an Ab specific for tyrosine-phosphorylated STAT-6 and total STAT-6. As shown in Fig. 6, IL-17A stimulation did not induce STAT-6 tyrosine phosphorylation over a 2-h time period in ASM cells.

A previous study in a monocytic cell line has shown that IL-17A receptor signaling can activate STAT-3 and STAT-5 (28). We then determined the tyrosine phosphorylation of both STAT-3 and STAT-5 in IL-17A-stimulated ASM cells. IL-17A induced 2- and 1.8-fold increases of STAT-3 phosphorylation in ASM cells at 5 and 10 min, respectively, reaching baseline levels at 20 min. However, IL-17A did not induce noticeable STAT-5 phosphorylation in ASM cells (normalized to total STAT-5). These data suggest that IL-17A may induce eotaxin-1/CCL11 release via STAT-3.

To further characterize the involvement of STAT-3 signaling pathways in IL-17A-mediated eotaxin-1/CCL11 release from ASM cells, we performed experiments using an universal JAK inhibitor (JAK inhibitor I). Treatment of ASM cells with a JAK inhibitor I before IL-17A stimulation caused a significant and substantial inhibition of eotaxin-1/CCL11 release (p < 0.001; Fig 6B). This result indicates that STAT pathway may participate in IL-17A-mediated release of eotaxin-1/CCL11 by human ASM cells.

Discussion

The current study was designed to explore the role of IL-17A and the signaling pathways by which it mediated eotaxin-1/CCL11 expression in ASM cells. Our data demonstrate for the first time that IL-17A, a cytokine involved primarily in inducing neutrophilic inflammatory responses, can induce the synthesis and release of eotaxin-1/CCL11 in human primary ASM cells. Furthermore, the induced expression of eotaxin-1/CCL11 by IL-17A in human ASM cells depended on de novo mRNA and protein synthesis, suggesting transcriptional and posttranscriptional regulatory mechanisms. Promoter luciferase reporter assay revealed that IL-17A significantly induced eotaxin-1/CCL11 promoter activity in ASM cells. Inhibitors of MAPK (p38, JNK, and ERK1/2), but not PI3K, prevented IL-17A-induced eotaxin-1/CCL11 production from ASM cells, and IL-17A induced rapid phosphorylation of MAPK (p38, JNK, ERK1/2). Finally, phosphorylation of STAT-3 but not STAT-6 or STAT-5 occurred upon IL-17A stimulation of ASM cells. This study suggests that IL-17A mediates eotaxin-1/CCL11 expression at least at a transcriptional level and involves MAPK pathways but not PI3K or STAT-6. The results of this study not only have functional implications for ASM cell-mediated inflammation, but also provide a novel mechanism by which IL-17A may contribute to the development of airway diseases such as asthma.

IL-17A is a homodimeric protein, with pleiotropic biological activities, that can be released by activated CD4+ T cells (15, 29, 30). IL-17A has been shown to be capable of stimulating the production of various cytokines such as IL-6, IL-8, and GM-CSF from diverse cell types, such as fibroblasts, keratinocytes, and renal epithelial cells (17, 31–33). A potential role of IL-17A in the initiation or maintenance of inflammatory responses is suggested by elevated IL-17A expression in mononuclear cells from patients with multiple sclerosis (16), rheumatoid arthritis (34), or systemic lupus erythematosus (35). Furthermore, studies using a murine...
model have clearly demonstrated the role of IL-17A network in inducing neutrophil recruitment to inflammatory sites (36). In the current study, we show additional effects mediated by IL-17A. This cytokine induced eotaxin-1/CCL11 in ASM cells, and in combination with IL-1β, exceeding the maximal induction of eotaxin-1/CCL11 mRNA and protein caused by either cytokine alone. Our data are in agreement with previous studies where IL-17A alone, or in combination with proinflammatory cytokines, enhanced chemokine and cytokine expression (37). Similar to what we recently showed for chronic obstructive pulmonary disease patients (24), IL-17RA protein expression was detected in vivo within ASM area of an allergic bronchopulmonary aspergillosis patient airway (data not shown). This preliminary result suggests that ASM cells of allergic patients may be one of the cellular targets for IL-17A, contributing with IL-1β to eotaxin-1/CCL11 expression in the airways. Taken together, these findings suggest that IL-17A might play a general proinflammatory role through a combined effect with other cytokines such as IL-1β.

Eotaxin-1/CCL11 is a chemokine belonging to the CC family, and was first isolated from lung lavage fluid of a guinea pig model of allergic disease (6). It has been shown to be a potent chemotactant for eosinophils both in vitro and in vivo (11, 12, 38–40). Increased production of eotaxin-1/CCL11 has been associated with allergic diseases such as asthma (7–10, 41), allergic rhinitis (42), and atopic dermatitis (43). Previous studies have shown that Th2 cytokines, particularly IL-4 can induce eotaxin-1/CCL11 release in many structural cells including ASM cells (13, 14). Previously, it has been demonstrated that human ASM cells express eotaxin-1/CCL11 following TNF-α and/or IL-1β stimulation (4). The eotaxin-1/CCL11 produced and secreted by ASM cells may then amplify the chemokine signal generated by infiltrating inflammatory cells in the airway, thereby augmenting the recruitment of eosinophils, basophils, and Th2 lymphocytes to the airways. The accumulation of these inflammatory cells may subsequently contribute to the development of airway hyperresponsiveness, local inflammation, and tissue injury through the release of granular enzymes and other cytokines (44).

Our current study shows that IL-17A-dependent activation of ASM cells can also induce eotaxin-1/CCL11, thus revealing a new pathway for induction of eotaxin-1/CCL11 within the airways. Because an earlier study in primary bronchial epithelial cells did not report similar findings (45), the effect of IL-17A may be cell specific. Besides its role in attracting eosinophils, eotaxin-1/CCL11 has been shown to inhibit neutrophil recruitment in a mouse model of endoxemia (46) and down-regulate CXC chemokines particularly IL-8/CXCL8 in human dermal microvascular endothelial cells (47). In light of prior evidence and present data, it is tempting to speculate that IL-17A-mediated eotaxin-1/CCL11 release may down-regulate exaggerated neutrophilic inflammation by suppressing CXC chemokine release during acute inflammation, hence creating a negative feedback to establish tissue homeostasis. Interestingly, it is worth to mention that many prototypic proinflammatory cytokines such as TNF-α exhibit both immunosuppressive and immunoregulatory roles depending on the microenvironment and cellular targets (48). Further studies in vivo using animal models are needed to address this possibility.

The MAPK family is fundamental in mediating numerous changes in cell function such as cytokine expression, proliferation, and apoptosis (49, 50). The p38 MAPK, JNK, and ERK play a central role in these cell responses. Upon activation by upstream regulators, the MAP kinases translocate to the cell nucleus where they transform the action of nuclear transcription factors and kinases, which in turn cause changes in cell function such as release of cytokines (50). Using pharmacological inhibitors of MAPK and investigating the kinetics of phosphorylation of p38, ERK1/2, and JNK, we demonstrate that IL-17A-induced eotaxin-1/CCL11 release is in ASM cells involves MAPK p38, JNK, and p42/p44 ERK. These results are in agreement with previous studies in human bronchial epithelial cells, colonic myofibroblasts and chondrocytes (32, 51–54). Because the IL-17RA cytoplasmic domain encompasses >500 aa but lacks identifiable signaling motifs within this region, it is plausible that numerous signaling pathways can be activated by this receptor such as PI3K and STATs. Our data showed no substantial effect of PI3K inhibitor (wortmannin) on IL-17A-induced eotaxin-1/CCL11 release, suggesting that PI3K is not involved in IL-17A signaling in ASM cells, as observed in human bronchial epithelial cells (51). Furthermore, because IL-4R signaling involves PI3K (55), our results may also imply that IL-17A does not mediate its effect through IL-4 release and autocrine mechanism. Interestingly, we found that PI3K signal transduction is involved in IL-1β-induced eotaxin-1/CCL11 release from ASM. This difference in signaling pathways may explain the difference in patterns of eotaxin-1/CCL11 release observed with IL-17A or IL-1β stimulation. The potential synergy between IL-17A and IL-1β at the intracellular level is currently under investigation.
STAT-6 plays a pivotal role in IL-4-mediated eotaxin-1/CCL11 expression (27). In our study, a significant increase in STAT-3 but not STAT-6 or STAT-5 phosphorylation was detected following IL-17A stimulation, which may suggest that IL-17A used a different pathway than IL-4. These findings are in agreement with oncostatin-M-induced eotaxin-1/CCL11 expression in human ASM cells and mouse fibroblasts (56, 57). Transcriptional activation of cytokine-responsive genes requires coordinated cooperation between STATs and other sequence-specific transcription factors that recruit transcriptional coregulators and components of the basal transcription machinery to the promoter. Promoter of eotaxin-1/CCL11 contains response elements for NF-κB in addition to STAT-6 binding sites (25). Preliminary EMSA data using mouse nuclear protein from ASM cells of two subjects revealed that IL-17A induced STAT-6 and NF-κB binding to eotaxin-1/CCL11 promoter region (our unpublished data). Although IL-17A did not have any effect on STAT-6 phosphorylation, it is likely that IL-17A may affect STAT-6 cooperation with other transcription factors such as NF-κB or C/EBP (58–60), recruitment of transcriptional coactivators at the promoter region (61), or posttranslational modifications of STAT-6 by serine threonine kinases (62). A study on ASM cells isolated from STAT-6 knockout mice and transfected ASM cells with dominant-negative STAT-6 will be useful to address those possibilities.

In conclusion, this study is the first demonstration of the capacity for IL-17A to induce eotaxin-1/CCL11 expression by ASM cells. Induction occurs at least through a transcriptional mechanism and involves signaling pathways that include MAPK (p38, JNK, and p42/p44 ERK) and probably STAT-3. Our findings support the concept that IL-17A might act as an amplier of airway eosinophilic inflammation by enhancing eotaxin-1/CCL11 expression. Our results also provide a better understanding of IL-17A-ASM cell interactions and their role in airway inflammatory responses.

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Disclosure

The authors have no financial conflict of interest.

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