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Dual Modulation of Airway Smooth Muscle Contraction by Th2 Cytokines via Matrix Metalloproteinase-1 Production

Yoshinori Ohta,*† Masayuki Hayashi,* Takaaki Kanemaru,‡ Kihachiro Abe,† Yushi Ito,* and Masahiro Oike2*†

Altered contractility of airway smooth muscle (SM) is one of the main causes of allergic asthma, in which the predominance of Th2 over Th1 cytokines plays a central role. In the present study, we examine the effects of Th2 cytokines on airway SM contraction. Treatment with a low concentration of IL-4 (0.2 ng/ml) for 6 h augmented, whereas higher concentrations (2–20 ng/ml) inhibited, agonist-induced contractions of collagen gels containing bovine tracheal SM cells. Another Th2 cytokine (IL-13) showed an augmentation of gel contraction in the concentration range of 20–200 ng/ml. IL-4 and IL-13 increased mRNA expression and protein secretion of matrix metalloproteinase (MMP)-1, but these cytokines did not affect Ca2+-mobilizing properties and phosphorylation levels of myosin L chain in bovine tracheal SM cells. These changes were sensitive to wortmannin, an inhibitor of PI3K, but not to leflunomide, an inhibitor of STAT6. Scanning electron microscope observation revealed that collagen fibers twining around SM cells were completely dissolved in 20 ng/ml IL-4-treated gels and reorganized into basket-like structure in 20 ng/ml IL-13-treated gels. Exogenous application of high and low concentrations of MMP-1 also induced the inhibition and augmentation of gel contraction, respectively. Furthermore, nonselective MMP inhibitor galardin suppressed the effects of IL-4 and IL-13 on gel contraction, and MMP-1-targeted small-interfering RNA reversed the inhibitory effects of IL-4 on gel contraction to the augmentation. This indicates that Th2 cytokines modulate airway contraction without affecting cellular contractility but by secreting MMP-1 from the SM cells via PI3K activation and changing cell-to-matrix interactions. The Journal of Immunology, 2008, 180: 4191–4199.

Materials and Methods

Cell culture and tissue preparation

Tracheas of 1-year-old calves were obtained from a local slaughterhouse, and SM cells were cultured in DMEM supplemented with 10% FBS by the explant method (17). Briefly, tracheal SM tissues were cut with scalpels into 1–2 mm³ pieces, attached to the bottom of culture dishes (100 × 20 mm), and cultured with 8 ml of culture medium at 37°C in 5% CO₂ air. BTSMCs that were migrated out of the tissues were harvested after 2 wk by trypsin digestion and stored at −80°C after two-step subculture. The present study was performed using BTSMC obtained from 12 tracheas.

Bovine tracheal tissue extract was prepared by homogenizing tracheal SM tissues with Polytron (Kinematica) according to a previously described method (18), and used as a control for Western blot analysis of SM marker...
Table I. Primers used for qualitative RT-PCR and quantitative real-time PCR

<table>
<thead>
<tr>
<th>Primer Application</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<td>RT-PCR</td>
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<td>5’-tgctgttaaaaagctagagca-3’</td>
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<tr>
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<td>5’-tggcccccctctagaagtga-3’</td>
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<tr>
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</tr>
<tr>
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<td>5’-gaaccgcagtgaggagtttc-3’</td>
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<td>5’-acatacgaccagccacacatc-3’</td>
</tr>
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Proteins. Bovine aortal endothelial cells (BAECs) were prepared as described previously (19), and used as a negative control for the same experiment.

**Gel contraction assay**

Contractility of cultured BTSMCs was examined with a gel contraction assay (20). Collagen solution was prepared by mixing 7 volumes of ice-cold type IA collagen (3 mg/ml) with 2 volumes of 5% concentrated DMEM and 1 volume of 200 mM HEPES buffer (resulting in 20 mM HEPES buffer), and pH was adjusted to 7.4 with NaOH. BTSMCs suspension per well was poured into a 24-well culture plate. The plate was kept at 37°C for 10 min to form a gel, and 1 ml of culture medium was added. The gels were cultured for 3 days and used for the contraction assay after each pretreatment. The lateral surface of the gel was carefully detached from the culture well with a fine needle. The culture plate was then placed on a hotplate (HP-19U300; KPI) and kept at 37°C. The gel surface images were captured with a digital camera (QV-800SX; Casio) every 1 min throughout the experiment. Contraction of the gel was then evaluated by measuring its surface area with image analysis software (Adobe Photoshop; Adobe Systems). Because the degree of contraction in control gels varied between batches, data after each treatment were compared with the control data obtained in the same batch.

**Measurement of intracellular calcium concentrations ([Ca2+]i)**

[Ca2+]i was measured in confluent BTSMCs with fura-2 by using an Attolux digital fluorescence microscopy system (Atto Instruments), as previously described (21). For the statistical analysis of [Ca2+]i, results from 20 to 30 cells on a coverslip were averaged and treated as one data point.

**Western blotting**

Expressions of α-actin, SM myosin, calponin, myosin L chain (MLC), phosphorylated MLC (p-MLC), RhoA, STAT6, phosphorylated STAT6 (p-STAT6), and MMP-1 were assessed with ECL Western blotting. Whole cell lysates were prepared after each pretreatment and separated by electrophoresis except for the analysis of RhoA and MMP-1. For the assessment of the membrane translocation of RhoA activation, cell lysate was centrifuged for 1 h at 100,000 X g, and the pellet was harvested as membrane fraction. Extracellular secretion of MMP-1 was examined using a thermal cycler (PC-8000; Astec) by using a commercial kit (Ready-To-Go RT-PCR beads; GE Healthcare Life Sciences). The resulting PCR products were analyzed with agarose gel electrophoresis, after which the cDNA bands were excised and extracted with a spin column (Quantum Prep; Bio-Rad) as standards for real-time PCR.

Real-time PCR was performed for a quantitative analysis of MMP-1, -2, -3, and TIMP-1 mRNA expression. First-strand cDNA was mixed with primers and a reaction reagent (Fuller SYBER Green PCR Master Mix; Stratagene), and real-time PCR was performed with MX3000P (Stratagen) to obtain the threshold cycle numbers at which the amplified fluorescent PCR products become detectable. The threshold cycle values were then converted to the equivalent amount of template mRNA using standard curves obtained with extracted RT-PCR bands. Data were expressed as relative to the amount of GAPDH in the same volume of first-strand cDNA. The primers used for these measurements are listed in Table I.

**Scanning electron microscopy**

Microstructure of collagen fibers and embedded SM cells was observed with scanning electron microscope. Collagen gels were fixed with 2.5% glutaraldehyde and 2% paraformaldehyde, and freeze-dried. The samples were cut with a fine razor and the section was coated with osmium and observed with scanning electron microscope (JXA-8600MX; JEOL).

**Gene silencing with small-interfering RNA (siRNA)**

The following siRNA sequences were used to target MMP-1: 5’-CAG CAAUUUCACCAUUAACUTT-3’ (sense) and 5’-AGUUGCUCUUAACACUTT-3’ (antisense). BTSMCs (3 x 10^6 cells in 600 ul) were transfected with siRNAs (final concentration 30 nM) by electroporation (350V, 975µF) using Gene Pulser XCell (Bio-Rad). Control cells were also electroporated without siRNAs. Cells were then cultured on plates for 2 days for real-time PCR or embedded into collagen gels for 3 days for gel contraction assay.

**Solution**

Kreb’s solution was used in the gel contraction assay and Ca2+ measurements contained (in millimoles): NaCl 132.4, KCl 5.9, CaCl2 1.5, MgCl2 1.2, glucose 11.5, HEPES 11.5, and pH was adjusted to 7.4 by NaOH.

**Materials**

Anti-MCL (clone MY-21), anti-β-actin (clone AC-15), anti-calponin (clone hCP), anti-SM myosin (clone HSM-V), and anti-MMP-1 Abs were purchased from Sigma-Aldrich. Anti-α-actin Ab (clone 1A4) was purchased from DakoCytomation. Anti-p-MLC (Thr19/Ser19), anti-STAT6, and anti-p-STAT6 (Tyr641) Abs were purchased from Cell Signaling Technology. Anti-RhoA Ab was purchased from Cytoskeleton. MMP-1 was purchased from Bionord International. Collagen type IA was purchased from Nitta Gelatin. Fura-2/AM was purchased from Wako Pure Chemicals. Human recombinant IL-4 and IL-13, and all other reagents were purchased from Sigma-Aldrich.
Statistics

Data were expressed as mean ± SEM values. Statistical significance in gel contraction assay was examined with repeated measures ANOVA with the Bonferroni post-hoc test, using data points from 10 to 70 min, and that in [Ca2+]i assay, real-time PCR, and Western blotting with the Student unpaired t test, by using StatView (SAS Institute) for both analyses. Probability below 0.05 (p < 0.05) was considered as a significant difference.

Results

Expression of SM marker proteins in cultured BTSMCs

First, we examined the expression of SM marker proteins, i.e., α-SM actin, SM myosin, and calponin, in BTSMCs. Although lower than tissue extract, BTSMCs showed a significant amount of expression of these proteins compared with endothelial cells (Fig. 1). Thus, we consider that BTSMCs used in the present study sufficiently retained SM nature.

Effects of IL-4, IL-13, and IFN-γ on the contraction of BTSMCs

Next, we examined the effects of Th2 and Th1 cytokines on the contractility of BTSMCs. ATP (10 μM) induced a reversible contraction of untreated control collagen gels that contained BTSMCs (Fig. 2A). A treatment of the gels with IL-4 (20 ng/ml) for 6 h resulted in an inhibition, whereas a treatment with the same concentration of IL-13 (20 ng/ml) for 6 h induced a marked augmentation of the ATP-induced gel contraction. The Th1 cytokine IFN-γ (100 U/ml), however, did not affect the ATP-induced gel contraction. A similar degree of IL-4 (20 ng/ml)-induced inhibition of gel contraction was observed after 1 and 24 h of treatment (data not shown). In contrast, IL-13 (20 ng/ml) induced a less potent augmentation of gel contraction after 1 and 24 h of treatment (data not shown).

IL-4 showed a less potent inhibition of gel contraction with a lower concentration (2 ng/ml), whereas it augmented the ATP-induced gel contraction with a much lower concentration (0.2 ng/ml; Fig. 2B). In contrast, a lower concentration of IL-13 (2 ng/ml) did not alter ATP-induced gel contraction, and with a higher concentration (20 ng/ml), it induced a marked augmentation of gel contraction (Fig. 2C). A combination of IL-4 (20 ng/ml) and IL-13 (20 ng/ml) resulted in a marked augmentation of ATP-induced gel contraction (Fig. 2D).

FIGURE 1. Western blot analysis of SM marker proteins in BTSMCs. Cell lysates of BTSMCs and BAECs were electrophoresed with bovine tracheal tissue extract, and the expressions of calponin, α-SM actin, and SM myosin were analyzed using the corresponding Abs. Expression of housekeeping β-actin was also examined as an internal control. Representative band images are shown in the left panels. Band densities of SM markers were normalized to that of β-actin, and expressed as relative to tracheal tissue extract (n = 4, right panel).

FIGURE 2. Effects of Th1/2 cytokines on the contraction of airway SM-embedded collagen gels. A, BTSMCs were embedded into collagen gels and pretreated with IL-4 (20 ng/ml, n = 12), IL-13 (20 ng/ml, n = 12), or IFN-γ (100 U/ml, n = 10) for 6 h. Control gels were left untreated (n = 17). ATP (10 μM)-induced contraction of the gels was then examined. B, BTSMC-embedded gels were treated with 0.2, 2, and 20 ng/ml IL-4 for 6 h, and ATP (10 μM)-induced contraction was examined. Data were obtained from 10 repeated experiments. C, Effects of 2, 20, and 200 ng/ml IL-13 on gel contraction were examined. Data were obtained from 10 repeated experiments. D, BTSMC-embedded gels were simultaneously treated with IL-4 (20 ng/ml) and IL-13 (20 ng/ml) for 6 h, and ATP-induced contraction were examined. Data were obtained from eight measurements. In each panel, data are represented as mean ± SEM. *, p < 0.05, **, p < 0.01 vs control; †, p < 0.05. Because of the variability in the control gel contractions between different batches, we compared the data after each treatment to the control data in the same batch.
concentration (200 ng/ml) IL-13 augmented the contraction to a significantly lower degree than that with 20 ng/ml (Fig. 2C).

Simultaneous treatment with 20 ng/ml IL-4 and IL-13 for 6 h inhibited the ATP-induced gel contractions, and the degree of inhibition was to the same extent as in gels treated with 20 ng/ml IL-4 alone. In the following experiments, we treated BTSMCs and the gels for 6 h with 20 ng/ml IL-4 and 20 ng/ml IL-13 to explore the cellular mechanisms of the inhibition and the augmentation of gel contraction, respectively.

IL-4 and IL-13 do not affect Ca^{2+}-mobilizing properties in BTSMCs

Because muscle contraction is triggered by an elevation of \([\text{Ca}^{2+}]_i\), we then examined the effects of IL-4, IL-13, and IFN-\(\gamma\) on ATP-induced \(\text{Ca}^{2+}\) transients. Treatment of BTSMCs with IL-4 (20 ng/ml), IL-13 (20 ng/ml), and IFN-\(\gamma\) (100 U/ml) for 6 h did not alter their basal level of \([\text{Ca}^{2+}]_i\) (Fig. 3Aa). Furthermore, the peak increase of \([\text{Ca}^{2+}]_i\) during ATP-induced \(\text{Ca}^{2+}\) transients was not significantly different between control and IL-4 (20 ng/ml)-, IL-13 (20 ng/ml)-, or IFN-\(\gamma\) (100 U/ml)-treated cells (Fig. 3Ab). Also, the time course and shape of the \(\text{Ca}^{2+}\) traces induced by 10 \(\mu\)M ATP in IL-4- and IL-13-treated BTSMCs were comparable to those in control cells (Fig. 3A, c–e).

IL-4 and IL-13 do not affect MLC phosphorylation and RhoA activation in BTSMCs

We then examined the effects of 20 ng/ml IL-4 and 20 ng/ml IL-13 on MLC phosphorylation, which leads to contraction of SM cells (22). ATP increased the amount of p-MLC not only in control BTSMCs, but also in IL-4- and IL-13-treated cells, and the time course and maximal level of phosphorylation under these conditions were not significantly different from control (Fig. 3B).

Furthermore, activation of the RhoA/Rho-kinase pathway, which increases the \(\text{Ca}^{2+}\) sensitivity of the contractile proteins in airway SM cells (16), is unlikely because neither IL-13 nor IL-4 affected the membrane translocation of RhoA, which is considered as a hallmark of RhoA activation (23) (Fig. 3C).

Wortmannin, but not leflunomide, suppressed IL-4- and IL-13-induced alteration of gel contraction

Next, we examined the effects of the inhibitors of the intracellular signals that are activated by IL-4 and IL-13 on gel contraction. Pretreatment of the BTSMC-embedded gels with wortmannin (1 \(\mu\)M), a PI3K inhibitor (24), did not affect the control gel contraction, but reduced the IL-4-induced decrease and the IL-13-induced increase in gel contractions in a concentration-dependent manner (Fig. 4A).
IL-4 and IL-13 induced phosphorylation of STAT6 in BTSMCs, and there was no difference in the phosphorylation levels between these two conditions (Fig. 4B). Leflunomide, a STAT6 inhibitor (25), did not affect the ATP-induced contractions of control, IL-4-, and IL-13-treated gels (Fig. 4C).

**FIGURE 4.** Effects of the inhibitors of PI3K and STAT6 on IL-4- and IL-13-induced alteration of gel contraction. A, Gels were pretreated with wortmannin (100 nM and 1 μM), an inhibitor of PI3K, for 6 h with or without IL-4 (a, 20 ng/ml) and IL-13 (b, 20 ng/ml), and ATP (10 μM)-induced contraction was examined (n = 8 for each experimental condition). *, p < 0.05, **, p < 0.01 vs control. B, Effects of Th1/2 cytokines on STAT6 phosphorylation in BTSMCs. Cells were grown on culture plates and treated with IL-4 (20 ng/ml), IL-13 (20 ng/ml), or IFN-γ (100 U/ml) for 6 h or left untreated (control). Western blot analysis of cell lysates was performed for phosphorylated STAT6 and total STAT6 protein. Representative band images are shown in the upper panels. Band density of phosphorylated STAT6 was normalized to that of total cellular STAT6 protein, and expressed as relative to untreated control (n = 4, lower panel). Note that IL-4- and IL-13-induced similar levels of STAT6 phosphorylation. n.s., p > 0.05. C, Effects of leflunomide (100 μM), an inhibitor of STAT6, on IL-4 (a, 20 ng/ml)- and IL-13 (b, 20 ng/ml)-induced alteration of gel contraction were examined (n = 8 for each experimental condition). *, p < 0.05, **, p < 0.01 vs control.

IL-4 and IL-13 induced MMP-1 mRNA and protein expression in BTSMCs

Because IL-4 and IL-13 did not alter [Ca2+]i, and MLC phosphorylation of BTSMCs, next we examined the possible alteration of the relationship between SM cells and extracellular matrix.

RT-PCR revealed that BTSMCs expressed mRNAs of MMP-1, -2, -3, and TIMP-1 but not MMP-9 and MMP-13 (Fig. 5Aa). Absence of MMP-9 and MMP-13 mRNA expression was confirmed with two other sets of primers (data not shown), and the expression of these mRNAs was not evoked by IL-4 or IL-13 (Fig. 5Aa). A quantitative analysis of MMP-1, -2, -3, and TIMP-1 mRNAs with real-time PCR revealed that the expression of MMP-1 mRNA was significantly increased by a treatment for 6 h with IL-4 (20 ng/ml) and IL-13 (20 ng/ml) but not by IFN-γ (100 U/ml). IL-4 and IL-13, however, did not alter the expressions of MMP-2, -3, and TIMP-1 mRNA (Fig. 5Ab).

Because MMP-1 protein synthesized in the cells is secreted into the extracellular space (12), we also estimated the amount of MMP-1 protein secreted into the extracellular space (12), we also estimated the amount of

MMP-1 protein secreted into the culture medium in control, IL-4-, IL-13-, and IFN-γ-treated BTSMCs. As shown in Fig. 5B, MMP-1 protein in the culture medium was markedly increased by 20 ng/ml IL-4. IL-13 (20 ng/ml) also significantly increased the amount of MMP-1 in the culture medium, but to a lesser extent than in IL-4-treated medium. IFN-γ did not increase the amount of MMP-1 protein secretion. As in case of the gel contraction assay, 1 μM wortmannin, but not 100 μM leflunomide, reversed the IL-4- and IL-13-induced MMP-1 secretion (Fig. 5B).

Scanning electron microscopic observations of BTSMC-embedded collagen gels

Scanning electron microscopic observations of control gels containing BTSMCs revealed a dense network of collagen fibers twined around the SM cells (Fig. 6A). After a pretreatment with 20 ng/ml IL-4 for 6 h, these collagen fibers around the cells were almost completely removed (Fig. 6B). In the 20 ng/ml IL-13-treated gels, this meshwork was partially dissolved, and the collagen fibers became reorganized into basket-like structures surrounding the SM cells (Fig. 6C).

Effects of exogenous MMP-1 on collagen fibers and gel contraction

The results above indicate the central role of MMP-1 in the modulation of contraction of BTSMC-embedded gel. We then
examined the effects of exogenous MMP-1 on the ultrastructure of collagen fibers and gel contraction.

Scanning electron microscopic observation revealed that collagen fibers were fine in untreated gels, and were fused and reorganized into thick fibers in 10 ng/ml MMP-1-treated gels (Fig. 7A). Collagen fibers became further thicker and coarse after the treatment with 100 ng/ml MMP-1 (data not shown).

Pretreatment of the BTSMC-embedded gels with 1 ng/ml MMP-1 for 6 h did not affect the ATP-induced contraction. In contrast, 10 ng/ml MMP-1 augmented the gel contraction (Fig. 7B).

Effects of MMP inhibitor and MMP-1 gene silencing on gel contraction

We finally examined the effects of MMP inhibitor and MMP-1-targeted siRNA on IL-4- and IL-13-induced changes in gel contraction.

Galardin, a nonspecific MMP inhibitor (26), reversed the IL-4-induced inhibition and IL-13-induced augmentation of gel contraction, respectively, in a concentration-dependent manner (Fig. 8A). Galardin (10 nM), however, did not affect the control gel contraction (data not shown).

Transfection of MMP-1-targeted siRNA significantly inhibited the expression of MMP-1 mRNA in control, IL-4-treated and IL-13-treated BTSMCs (Fig. 8B). In IL-4-treated cells, siRNA inhibited MMP-1 mRNA to the similar level as that in IL-13-treated control cells. As expected from the amount of MMP-1 mRNA, the ATP-induced contraction of the gels containing siRNA-transfected BTSMCs was significantly augmented by IL-4, and was not affected significantly by IL-13 (Fig. 8B).

Discussion

We have shown in this study that Th2 cytokines show dual actions on the ATP-induced contraction of BTSMC-embedded collagen gels (Fig. 2A). IL-4 augmented the gel contraction at 0.2 ng/ml and inhibited it at 2 and 20 ng/ml (Fig. 2B). IL-13 only induced an augmentation of gel contractions (Fig. 2C), but the effects were less potent at higher concentrations (200 ng/ml). Furthermore, simultaneous application of IL-4 and IL-13 did not lead to the summation of their solo effects, but inhibited the gel contraction as in the case of IL-4 alone (Fig. 2D). It is known that the IL-4Rα chain binds IL-4 and dimerizes with IL-13Rα1 to form a type II IL-4R in nonhematopoietic cells (27). IL-13 binds to IL-13Rα1 and induces heterodimerization with IL-4Rα to form a complex identical with the type II IL-4R (28). IL-13 also binds to IL-13Rα2, a decoy
Ca²⁺, but we could not detect significant differences in the ATP-induced Ca²⁺ transients (Fig. 3A). Furthermore, the ATP-induced MLC phosphorylation receptor, with greater affinity than IL-13Rα1 but this does not induce a signal (29), and from this point of view quantitative rather than qualitative differences may cause the differing functional responses of IL-4 and IL-13. Therefore, we hypothesize that the limited activation of the IL-4/13 receptors on BTSMCs augments the gel contraction, and that the inhibition is caused by the more potent receptor activation.

Previous studies showed that IL-4 (50 ng/ml) inhibited (30) and IL-13 (50 ng/ml) augmented (31) agonist-induced Ca²⁺ transients, but we could not detect significant differences in the ATP-induced Ca²⁺ signals in control, IL-4-treated, and IL-13-treated BTSMCs (Fig. 3A). Furthermore, the ATP-induced MLC phosphorylation was not affected by IL-4 or IL-13 (Fig. 3B). Activation of RhoA, which would inhibit myosin phosphatase and thereby increase muscle contractility (32), is also unlikely, because we failed to detect an increased membrane translocation of RhoA by IL-4 and IL-13 (Fig. 3C). Therefore, the present results strongly indicate that inhibition and augmentation of BTSMC-gel contractions induced by IL-4 and IL-13 were not due to an altered intrinsic contractility of the embedded SM cells.

Binding of IL-4 and/or IL-13 to the type II IL-4R leads to the activation of PI3K and STAT6 as the two major intracellular immediate signals (9). STAT6 phosphorylation in IL-4-treated cells and surrounding collagen fibers are shown in each panel. Two different view fields are shown for each condition. Scales, 1 μm.

**FIGURE 6.** Scanning electron microscopic observation of BTSMCs embedded in collagen gels. Control (A), IL-4-treated (B, 20 ng/ml, 6 h), and IL-13-treated (C, 20 ng/ml, 6 h) BTSMCs embedded in collagen gels were observed with a scanning electron microscope (×10,000). Part of the single SM cell and surrounding collagen fibers are shown in each panel. Two different view fields are shown for each condition. Scales, 1 μm.

IL-13 on gel contractions (Fig. 4A). Several recent reports on the role of PI3K activation in migration and proliferation of stimulated airway SM cells (33, 34)—and also in Th2 cytokine secretion, airway inflammation, and airway hyperresponsiveness in asthma model mice (11, 35)—point to the possible involvement of PI3K in the pathogenesis of asthma. However, these studies did not provide more detailed cellular mechanisms of these PI3K-induced changes (33–35). IL-4 and IL-13 increased the expression level of mRNA and the protein secretion of MMP-1 in BTSMCs (Fig. 5). The increased secretion of MMP-1 protein was also inhibited by the PI3K inhibitor wortmannin, but not by leflunomide, which shows for the first time that MMP-1 secretion might be involved in the IL-4- and IL-13-induced and PI3K-mediated pathogenesis of airway SM cells. MMP-1 secretion induced by IL-4 was significantly larger than that by IL-13 (Fig. 5B), which correlates with the observation that IL-4 showed stronger effects on gel contraction than IL-13 (Fig. 2D). A similar increased expression level of MMP-1 by activation of PI3K has been observed in vascular endothelial cells and prostate cancer cells stimulated with FGF-1 (36) and integrin αvβ3 ligand (37), respectively.

Because MMP-1 functions as a collagenase, the enhanced MMP-1 secretion in the presence of IL-4 or IL-13 probably accounts for the degradation of the dense collagen fiber networks twining around SM cells observed in control gels (Fig. 6). It is conceivable that removal of collagen fibers from the SM cell surface as observed in the presence of IL-4 might detach these SM cells from the extracellular collagen fibers, which would reduce the gel contraction even if the cellular contractility remains intact. This degradation of the collagen network might also provide space for the cells to migrate and/or proliferate, so that MMP-1 production...
Effects of MMP inhibitor and MMP-1-targeted siRNA on gel contraction. A. Gels were pretreated with nonselective MMP inhibitor galardin (1 or 10 nM) together with IL-4 (20 ng/ml, a) or IL-13 (20 ng/ml, b). Gels left untreated (control) or treated with IL-4 or IL-13 alone were also examined in the same experiments. *, p < 0.05, **, p < 0.01 vs control (n = 8). B. Gene silencing of MMP-1 with siRNA significantly suppressed MMP-1 mRNA expression in control, IL-4 (20 ng/ml)-treated, and IL-13 (20 ng/ml)-treated BTSMCs (a). Untransfected cells were also exposed to electroporation pulses without siRNA. The numbers in parentheses refer to the number of experiments. **, p < 0.01 vs untransfected control; †, p < 0.05 vs IL-4-treated, untransfected cells; ‡, p < 0.05 vs IL-13-treated, untransfected cells. ATP-induced contraction of gels containing siRNA-transfected BTSMCs were examined after the treatment with IL-4 (20 ng/ml) or IL-13 (20 ng/ml) for 6 h (b). Data are mean ± SEM values from six measurements. *, p < 0.05 vs control (siRNA alone).

May be partially responsible for the development of IL-4-induced airway remodeling (38). In contrast, the basket-like redistribution of collagen fibers surrounding the cells in IL-13-treated gels might induce a more efficient gel contraction than in the control gels with an intact collagen network. Basket-like structure in IL-13-treated gels probably resulted from the partial degradation and redistribution of collagen fibers, as was observed with the exogenous application of 10 ng/ml MMP-1 (Fig. 7A). Furthermore, exogenous MMP-1 (10 ng/ml) augmented the ATP-induced gel contraction as in the case of IL-13-treated gels, and a much higher concentration of MMP-1 (100 ng/ml) reduced the gel contraction (Fig. 7B). Inhibition of MMP with galardin (26) reversed the effects of IL-4 and IL-13 (Fig. 7A), and partial inhibition of MMP-1 mRNA expression with gene silencing reversed the IL-4-induced inhibition of gel contraction to the augmentation (Fig. 8B). Therefore, we have concluded that the inhibitory and augmentative effects of Th2 cytokines on the contraction of BTSMC-containing gels were due to MMP-1-induced removal and reorganization of extracellular collagen networks, respectively.

There are some marked histological differences between airway SM tissue and other SM tissues, such as blood vessels; i.e., airway SM cells are arranged into bundles separated by collagen and connective tissue cells, and are loosely connected to each other (39). These histological characteristics of airway SM are conserved in BTSMC-embedded collagen gels, i.e., randomly oriented and not connected to each other, as demonstrated previously (16). Recently, a role of the interrelationship between airway SM cells and extracellular matrix in airway diseases has been discussed (40). Our results with the model gel system are consistent with such a contention as they provide some evidence that MMP-1 production and the ensuing collagen redistribution represent a potential therapeutic target for bronchial asthma. The increased contractility induced by IL-13 has been reported previously as a main pathogenic factor of bronchial asthma (7, 41), and spontaneous contraction of airway mesenchymal cell-embedded gels as a model of airway remodeling was also increased by IL-4 and IL-13 (42). However, so far, no studies have suggested MMP-1 as a cause of hyperresponsiveness. MMPs have been proposed as a potential cause of airway remodeling (43), but the present study has clearly demonstrated that MMP-1 production might also contribute to altered contractions induced by Th2 cytokines. Thus, although Ca\textsuperscript{2+} mobilization and the phosphorylation state of MLC are the main determinants of the contractile state of SM cells (22, 44), it is likely that the interaction between SM cells and the extracellular collagen network is another important regulatory factor of the contractile response especially in allergic situations.

In conclusion, we have shown in the present study that IL-4 and IL-13 affect airway SM gel contraction via a PI3K-mediated endogenous production of MMP-1 that alters the interaction between SM cells and collagen fibers.

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Disclosures
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References


