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*J Immunol* 2009; 183:1739-1750; Prepublished online 10 July 2009; doi: 10.4049/jimmunol.0803951

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Mast Cell-Dependent Contraction of Human Airway Smooth Muscle Cell-Containing Collagen Gels: Influence of Cytokines, Matrix Metalloproteases, and Serine Proteases

Alexander Margulis,1 Karl H. Nocka, Agnes M. Brennan, Bijia Deng, Margaret Fleming, Samuel J. Goldman, and Marion T. Kasaian2

In asthma, mast cells infiltrate the airway smooth muscle cell layer and secrete proinflammatory and profibrotic agents that contribute to airway remodeling. To study the effects of mast cell activation on smooth muscle cell-dependent matrix contraction, we developed coculture systems of human airway smooth muscle cells (HASM) with primary human mast cells derived from circulating progenitors or with the HMC-1 human mast cell line. Activation of primary human mast cells by IgE receptor cross-linking or activation of HMC-1 cells with C5a stimulated contraction of HASM-embedded collagen gels. Contractile activity could be transferred withconditioned medium from activated mast cells, implicating involvement of soluble factors. Cytokines and proteases are among the agents released by activated mast cells that may promote a contractile response. Both IL-13 and IL-6 enhanced contraction in this model and the activity of IL-13 was ablated under conditions leading to expression of the inhibitory receptor IL-13Rα2 on HASM. In addition to cytokines, matrix metalloproteinases (MMPs), and serine proteases induced matrix contraction. Inhibitor studies suggested that, although IL-13 could contribute to contraction driven by mast cell activation, MMPs were critical mediators of the response. Both MMP-1 and MMP-2 were strongly expressed in this system. Serine proteases also contributed to contraction induced by mast cell-activating agents and IL-13, most likely by mediating the proteolytic activation of MMPs. Hypercontractility is a hallmark of smooth muscle cells in the asthmatic lung. Our findings define novel mechanisms whereby mast cells may modulate HASM-driven contractile responses. The Journal of Immunology, 2009, 183: 1739–1750.

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irway smooth muscle cell (ASM)3 hyperplasia and hypercontractility are hallmarks of asthma and contribute to airway remodeling (1). Infiltration of the smooth muscle layer by mast cells is a characteristic feature of asthma, which does not occur in related lung diseases (2, 3). Once present in the smooth muscle cell layer, activated mast cells may contribute to the hypercontractility of airway smooth muscle (4) and could promote chronic inflammatory and remodeling changes, such as fibrosis and airway narrowing, in the asthmatic airway (5). Numerous mast cell-derived mediators have the potential to contribute to ASM contractility, including cytokines, chemokines, and inflammatory mediators such as histamine, leukotrienes, and PGs (8–10). Mast cell-derived proteases, including the serine protease tryptase (11), and matrix metalloproteases (MMPs) (12) may also contribute to escalation of inflammatory responses and airway remodeling in asthma. In turn, airway smooth muscle-derived mediators, including TGF-β, PGE2, and soluble and membrane-bound stem cell factor (SCF) (5, 7) can modulate the activation state of infiltrating mast cells.

A system of smooth muscle cells embedded in a collagen gel matrix has been used to model the rapid contraction associated with bronchospasm. This response can be triggered by exposure of ASM-embedded gels to methacholine, ATP, or histamine and occurs within minutes (9, 13, 14). The rapidity of this response contrasts with the more gradual, but ultimately more pronounced, collagen gel contraction induced by the major mast cell serine protease tryptase and by MMPs. This response occurs over several days and models matrix contraction as may occur in processes such as wound healing or tissue remodeling (12, 15, 16). This smooth muscle-driven matrix contraction may involve smooth muscle cell differentiation to a more contractile phenotype, increased cell-adhesive interactions, or reorganization of the collagen matrix itself, but is not a direct measure of smooth muscle cell contraction (15–17).

We have recently used a contraction model with collagen gels coembedded with human lung fibroblasts and mast cells to study cellular interactions contributing to fibrosis (18). We now adapt this system to model mast cell interactions with human airway smooth muscle cells (HASM) and explore the consequences of human mast cell activation in coculture with smooth muscle cells in the collagen gel matrix. The human mast cell line HMC-1 was used to define the system. HMC-1 can be activated by exposure to the anaphylatoxin C5a to undergo calcium mobilization (19) and chemotaxis (20), but lack appreciable expression of FceRI. To confirm our observations in a primary system and to extend them to include consequences of IgE-mediated mast cell activation, all

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2 Current address: Genzyme Corporation, Framingham, MA.
3 Abbreviations used in this paper: ASM, airway smooth muscle cell; MMP, matrix metalloproteinase; HASM, human airway smooth muscle cell; hHMC, primary human mast cell; sIL-13Rα2-Fc; soluble IL-13Rα2-Fc; SCF, stem cell factor.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.0803951

Received for publication November 25, 2008. Accepted for publication May 27, 2009.

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key findings were reproduced with primary human mast cells (pHMC) derived from circulating progenitors. pHMC undergo a relatively weak response to C5a (21), but exhibit strong IgE-mediated activation and induced potent contraction in the HASM coculture model.

This model was used to examine the role of serine proteases, MMPs, and cytokines in the collagen gel contraction response and to address potential cooperative interactions between these mediators. Both activated mast cells (22, 23) and smooth muscle cells (14, 24–26) can produce MMPs, which are released from the cell in an active form and activated by proteolytic processing. Mast cell-derived tryptase has been implicated in the proteolytic activation of MMPs (27–29), and mast cell- or smooth muscle cell-derived cytokines, including IL-6 and IL-13, can drive MMP expression (14, 30). Therefore, we propose that cytokines, serine proteases, and MMPs should all contribute to the contraction of HASM-containing collagen gels following activation of coembedded mast cells, with cytokines driving MMP generation, serine proteases promoting MMP activation, and MMPs ultimately driving the contraction response.

Materials and Methods

Cell culture

HASM were purchased from ScienCell Research Laboratories and maintained in a defined smooth muscle growth medium (Lonza). The human ASM cell line HMC-1 (gift from Dr. J. Butterfield, Mayo Clinic, Rochester, MN) was cultured in IMEM containing L-glutamine (2 mM), 30% charcoal-treated FBS (Sigma-Aldrich), and 1.2 mM CaCl2. Cells were seeded in IMEM containing 0.5% FCS (2 mM, 30% charcoal-treated FBS (Sigma-Aldrich), 50 µg/ml ir-saturating holotransferrin (Sigma-Aldrich), 1X penicillin-streptomycin (Sigma-Aldrich), 10–8 M 2-ME (Sigma-Aldrich), 100 ng/ml SCF (Invitrogen), 3% 20% concentrated conditioned medium generated from the HCC2157 BL lymphoblastoid cell line (American Type Culture Collection), and 10 ng/ml GM-CSF (R&D Systems) at 106 cells/ml. For the first 2 wk of culture, cells were maintained at a density of 1.5 × 105 cells/ml, and one-half of the medium was replaced weekly. At 6 wk, the culture was depleted of macrophages by magnetic bead separation using CD14 Ab (Invitrogen). Mast cell phenotype and purity were evaluated by Wright stain and by flow cytometry using Abs to CD14 and CD117 (BD Pharmingen). Cells were analyzed on a FACS-Calibur flow cytometer with CellQuest software (BD Biosciences), with mast cells identified as CD117+CD14+ . Cultures were used for assays at 7–9 wk and were >95% mast cells.

Type 1 collagen gel contraction

Collagen lattices were prepared by mixing neutralized bovine type I collagen (Organogenesis) with HASM at 2.5 × 105 cells/ml in 24-well plates. HMC-1 (2.5 × 105 cells/ml or pHMC (1 × 105 cells/ml) were added to the mixture, as indicated, and gels were solidified overnight at 37°C. Prior to culture with primary mast cells, the gel medium contained 50 ng/ml SCF (R&D Systems). Polymerized gels were gently released into a 6-well plate that contained 3 ml of serum-free DMEM and treated with the following agents, as indicated: 10 nM mast cell-derived tryptase (BIOMOL), or 100 µg/ml human α1-antitrypsin (Roche). Approximately 40% of the original area was found to correspond to the maximum contraction induced under these experimental conditions with TGF-β treatment. Therefore, data are shown on a scale of 40–100%.

In some cases, collagen gels were incubated with conditioned medium from activated mast cells. To generate conditioned medium, HMC-1 cells were seeded in 24-well plates at 1 × 106 cells/well in 1 ml of serum-free IMEM containing α-thioglycollate, and treated for 24 h as indicated. pHMC were seeded in a round-bottom 96-well plate at 5 × 106 cells/well in 200 µl of serum-free IMEM containing 50 ng/ml SCF and treated for 24 h as indicated. For each gel, 500 µl (HMC-1) or 200 µl (pHMC) of the resulting supernatant was added to the incubation medium.

Mast cell activation

HMC-1 cells were incubated for 24 h at 37°C in medium containing 10 nM C5a (Sigma-Aldrich). Supernatants were assayed for IL-3 content by ELISA (BioSource International). For pHMC activation studies, cells were incubated overnight with 0.1 µg/ml human IgE (Millipore), then treated with 10 µg/ml anti-human IgE (Kirkegaard & Perry Laboratories). Supernatants were collected after 30 min. Degranulation was quantified as histamine, assayed by ELISA (Immunotech), or β-hexosaminidase release, assayed by mixing with an equal volume of 1.3 mg/ml p-nitrophenyl-N-acetyl-β-D glucosaminide (Sigma-Aldrich) in 0.08 M sodium citrate (pH 4.5), with mast cell supernatants, incubating overnight at 37°C, and reading absorbance at 405 nM. Tryptase activity was assayed using a kit from Millipore. Leukotriene C4/D4/E4 was quantified in mast cell supernatants by CAST ELISA (Buhlmann Labs, ALPCO Diagnostics). Supernatants were collected after 24 h for quantification of IL-13 by ELISA (R&D Systems).

Real-time PCR

Total RNA was extracted from cells using a Qiagen RNeasy mini kit per the manufacturer’s protocol. For each RT-PCR, 250 ng of total RNA in a 50-µl reaction mix was used according to the manufacturer’s instructions, with a one-step protocol using Reverse Transcriptase qPCR Master Mix (Eurogentec). Conditions were as follows: 48°C for 30 min (reverse transcription); 95°C for 10 min (hot starting); followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Cycle number at threshold was normalized to the threshold cycle value for the GAPDH gene. All TaqMan assays were performed on an Applied Biosystems Prism 7700 sequence detector. Primers and probes were obtained from Eurogentec, with sequences as follows: IL-13Rα forward, 5'-AGGCGGAATTTCCACACTCTACATAA-3' and IL-13Rα reverse, 5'-GGTAAAGAAGGAGTACTATGATGTCGA-3'; and IL-13Rβ probe (FAM/TAMRA), 5'-TGTTAATGCTACTGACCATCTCTGAA-3', and IL-13Rβ reverse (FAM/TAMRA), 5'-ATGCGAACCCCTACACTCTGAGGA-3'; IL-4Rα forward, 5'-TAAAGGAAAGATGGGTGGGATCAGAT-3' and IL-4Rα reverse, 5'-CCCTGACATTCCCTGATTTATAG-3'; and IL-4Rα probe (FAM/TAMRA), 5'-AGGCCGGACGCGCCTCGGT-3'; and GAPDH forward 5'-CCACATTGGCTCAGACACAT-3' and GAPDH reverse 5'-GGGCCCATATGACCAAAA-3' and GAPDH probe (FAM/TAMRA), 5'-CGTGGACACCCGTCTCCACCTCCC-3'.
EMD Biosciences) diluted 1/1000 in blocking buffer for 2 h at room temperature. Immunoreactive proteins were visualized by incubation with HRP-linked anti-rabbit secondary Ab (Jackson ImmunoResearch Laboratories) diluted 1/10,000 in blocking buffer for 20 min at room temperature. The blots were developed for 5 min in ECL Plus (GE Healthcare) and visualized by autoradiography.

**FIGURE 1.** HMC-1 cells induce HASM contraction following activation with C5a. a, HMC-1 cells were incubated overnight in the presence or absence of C5a (10 nM) and supernatants were assayed for IL-3 by ELISA. b, Collagen lattices were seeded with HASM in the absence or presence of HMC-1 cells and treated with medium or C5a for 48 h at 37°C. c, Conditioned medium (c.m.) HMC-1 cells treated for 24 h with C5a was harvested and added to collagen lattices seeded with HASM. Gels were incubated for 48 h at 37°C. Gel surface area was quantitated and expressed as a percentage of the area of a lattice that contained no cells. ^, p < 0.01 compared with HASM alone; *, p < 0.005 for treatment with C5a compared with medium.

**FIGURE 2.** Primary human mast cell activation responses. a, pHMC stained with Wright-Giemsa. b, pHMC were incubated overnight with 0.1 μg/ml IgE, challenged with anti-human IgE (10 μg/ml) for 24 h, and IL-13 content of supernatants was assayed by ELISA. c, pHMC were loaded with IgE and challenged with anti-human IgE for 30 min at 37°C. Supernatants were analyzed for histamine release and expressed as percent maximum release following lysis of cells with Triton X-100. d, Cells were challenged with anti-human IgE for 30 min and leukotriene release was quantitated by ELISA. e, Cells were challenged with anti-human IgE for 30 min and tryptase was quantitated in supernatants. f, Collagen lattices were seeded with HASM in the absence or presence of pHMC or pHMC conditioned medium (c.m.). Gels were treated with medium, IgE, or IgE + anti-IgE for 48 h at 37°C. ^, p < 0.001 compared with HASM alone; *, p < 0.005 for IgE cross-linked compared with medium control.
Gelatin zymography
Collagen gels containing HASM, HASM + HMC-1 (a), or HASM + pHMC (b) were treated for 48 h at 37°C with TGF-β (5 ng/ml) as positive control or with IL-6 (10 ng/ml), IL-13 (20 ng/ml), or mast cell-activating agents. Gel surface area was quantitated and expressed as a percentage of the area of a lattice that contained no cells. #, p < 0.05 for gels embedded with HASM and mast cells compared with HASM alone. ^, p < 0.05 or *, p < 0.0005 for treatment with cytokines compared with medium alone. Collagen gels seeded with HASM and HMC-1 (c) or pHMC (d) were treated for 48 h at 37°C with IL-6 (10 ng/ml), IL-13 (20 ng/ml), C5a (10 nM), or IgE (0.1 μg/ml) + anti-IgE (10 μg/ml), as indicated, in the presence or absence of batimastat or leupeptin. *, p < 0.005 compared with medium control. Gels seeded with HASM + pHMC (e) or HASM only (f) were treated for the indicated time periods with IgE + anti-IgE, TGF-β, cysteinyl leukotrienes (cysLT; 5 ng/ml), or histamine (100 μM). Gel surface area was quantitated and expressed as a percentage of the area of a lattice that contained no cells.

Results
Activation of HMC-1 induces contraction of HASM-embedded collagen gels
HASM cells were embedded in collagen gels in the presence or absence of mast cells. In asthma, mast cell infiltration has been quantitated at a mean of 5–10 mast cells/mm² of smooth muscle (range, 0–33) or approximately one mast cell per four smooth muscle cells (3, 31). In the collagen gels, HMC-1 and HASM were coembedded at a cell number ratio of 1:1, which would fall within the range of mast cell infiltration in ASM. The HMC-1 cell activation response to the anaphylatoxin C5a was confirmed by assaying production of IL-3 (Fig. 1a). When HASM were treated with C5a in the absence of mast cells, no significant collagen gel contraction was observed (Fig. 1b). When HMC-1 were embedded...
in the collagen gels along with HASM, a detectable contractile response was observed (Fig. 1b; p < 0.001). Significant contraction above this level was seen in response to mast cell activation with C5a (Fig. 1b; p < 0.005 for treatment with C5a compared with medium). Collagen gels embedded with mast cells alone, in the absence of HASM, did not undergo detectable contraction under these conditions.

To address whether this increased contraction upon mast cell activation involved generation of soluble mediators, HMC-1 cells were exposed to C5a for 24 h in the absence of HASM and the supernatant was collected. HASM embedded in collagen gels incubated with the supernatant of resting or C5a-activated HMC-1 cells. Increased contraction was observed upon exposure to supernatants of activated HMC-1 cells (Fig. 1c; p < 0.005 for supernatants of C5a-activated HMC-1 compared with control supernatants). These observations demonstrate that the contractile response could be at least partially transferred with agents secreted by activated mast cells.

**Activation of primary human mast cells induces contraction of HASM-embedded collagen gels**

A major mechanism for mast cell activation is cross-linking of the cell surface FceRI by IgE-bound allergen. HMC-1 do not express appreciable FceRI and lack this response. To confirm that IgE-mediated mast cell activation could influence contractility of HASM-embedded collagen gels, we investigated pHMC derived from progenitors in peripheral blood (Fig. 2a). pHMC responded to IgE receptor cross-linking with cytokine production (Fig. 2b), degranulation (Fig. 2c), leukotriene synthesis (Fig. 2d), and tryptase release (Fig. 2e). To investigate the effects on contraction, pHMC were coembedded with HASM in collagen gels. Because of their limited availability, the pHMC studies were done with a ratio of 1 pHMC per 2.5 HASM cells, which is lower than the 1:1 ratio used with HMC-1, but adequate to induce a robust signal. In the absence of pHMC, HASM embedded in collagen gels were exposed to IgE alone or to IgE plus anti-IgE, and no contractile response was observed.
In contrast, when pHMC were coembedded in the collagen gels, treatment with IgE plus anti-IgE or with the conditioned medium of IgE-activated pHMC resulted in increased gel contraction (Fig. 2f; p < 0.005 for IgE-activated compared with resting pHMC or conditioned medium). Although monomeric IgE has been reported to induce some activation responses in mast cells (32), cross-linking was required for induction of HASM contraction in this system (Fig. 2f).

Cytokines promote mast cell-dependent HASM gel contraction by a mechanism involving protease activation

In a coculture system of mast cells and smooth muscle cells, several agents have the potential to influence contractility. We first examined the contraction-inducing activity of cytokines. In the absence of mast cells, TGF-β directly induced contraction of HASM-containing gels over 48 h, but the cytokines IL-6 and IL-13 had no direct effect (Fig. 3a). In the presence of HMC-1 (Fig. 3a; p < 0.0005) or pHMC (Fig. 3b; p < 0.05), however, both IL-6 and IL-13 produced significant increases in gel contraction over 48 h. These findings show that, in the presence of mast cells, IL-6, IL-13, and mast cell-activating agents induced contraction of HASM-containing collagen gels.

Mast cell-mediated contraction of HASM-containing gels is MMP dependent

We next addressed the role of MMPs and serine proteases in promoting this mast cell-dependent response. Both the broad spectrum MMP inhibitor batimastat and the serine protease inhibitor leupeptin blocked HMC-1-dependent contraction of HASM-containing gels induced by IL-6, IL-13, or C5a (Fig. 3c) and blocked the pHMC-dependent contraction induced by IgE receptor cross-linking (Fig. 3d). The inhibitory effects of batimastat and leupeptin on contraction did not result from a shift in kinetics, as inhibition was seen at every time point measured for the contraction driven by pHMC activation (Fig. 3e). In contrast to cytokines, MMPs, and serine proteases, neither histamine nor cysteinyl leukotrienes promoted contraction of HASM-containing collagen gels under these experimental conditions (Fig. 3f).

MMPs and serine proteases induce contraction of HASM-containing gels

To further explore the ability of MMPs and serine proteases to contribute to the contraction response, collagen gels embedded with HASM in the presence or absence of HMC-1 were treated directly with catalytically active MMPs or with tryptase. MMPs 1, 2, 9, and 12 all directly induced contraction of collagen gels embedded with HASM, even in the absence of mast cells (Fig. 4, a–d; p < 0.002 for treatment of HASM-containing gels with MMP compared with medium). These responses were blocked by the MMP inhibitor batimastat, but not by the serine protease inhibitor leupeptin. When resting HMC-1 were coembedded with HASM, the gels underwent contraction resulting from the absence of HMC-1. There was a further response to MMPs 1, 2, 9, and 12 above this level, resulting in additive contractile effects due to the presence of HMC-1 and treatment with MMPs (Fig. 4, a–d; p < 0.002 for treatment of HASM plus HMC-1-containing gels with MMP compared with medium). As for HASM alone, these responses were blocked by batimastat, but not by leupeptin. These observations support direct contraction of HASM-containing gels in response to MMPs 1, 2, 9, and 12 and indicate that mast cells may enhance, but are not required for, HASM contractile responses to catalytically active MMPs.

Because HMC-1-dependent contraction responses to IL-6, IL-13, and C5a were blocked by leupeptin in addition to batimastat (Fig. 3c), we also examined the ability of the major mast cell serine protease tryptase to directly induce contraction of HASM-containing gels. Human lung tryptase promoted contraction of collagen gels embedded with HASM alone (Fig. 4e; p < 0.002). As expected, this response was blocked by the serine protease inhibitor leupeptin. Surprisingly, however, it was also blocked by the MMP inhibitor batimastat (Fig. 4e). The ability of batimastat to block this response is consistent with an MMP dependence of the contractile activity induced by tryptase.

As noted above, the presence of HMC-1 coembedded with HASM produced an appreciable gel contraction, even in the absence of additional stimulation. In contrast to observations with MMPs 1, 2, 9, and 12, tryptase did not produce any additional
contractile response above this level in gels coembedded with HMC-1 (Fig. 4e).

Tryptase activation of pro-MMPs induces contraction

In collagen gels embedded with HASM alone, tryptase-mediated contraction was inhibitable by batimastat (Fig. 4e), suggesting that tryptase activity involved MMP activation. To address whether serine protease activity could promote MMP-dependent gel contraction, HASM-embedded collagen gels were treated with the inactive precursor (pro) forms of MMP-1 and MMP-2. A partial contractile response was observed comparable to that produced by tryptase alone (Fig. 5a; \( p < 0.005 \) for pro-MMP compared with medium). Tryptase enhanced the contraction in response to either pro-MMP-1 or pro-MMP-2 (Fig. 5a; \( p < 0.005 \) for pro-MMP and tryptase compared with pro-MMP alone). In accordance with the increased contraction, the processed forms of MMP-1 and MMP-2 were detected following exposure of pro-MMP-1 or pro-MMP-2 to tryptase and were reduced by addition of leupeptin (Fig. 5b).

Induction of IL-13Ra2 blocks HASM contraction responses

In the presence of mast cells, IL-13-induced contraction of HASM was dependent on both MMPs and serine proteases (Fig. 3c). In the next series of experiments, we further explored the effects of IL-13 on mast cell-dependent contraction of HASM. IL-13 responses are mediated through a receptor consisting of IL-13Ra1/IL-4Ra chains. An additional binding chain, IL-13Ra2, is inducible on the surface of several cell types, including fibroblasts and smooth muscle cells, and antagonizes IL-13 responses on these cell types (33, 34). Recently, however, it has been proposed that, under certain conditions, IL-13Ra2 may act as an agonist, rather than antagonist, of IL-13 responses (35). To address whether IL-13Ra2 expression would be activating or inhibitory in this system, we treated HASM with a combination of IL-13 and TNF-\( \alpha \). Under these conditions, IL-13Ra2 could be detected on the cell surface by flow cytometry (Fig. 6a) and increased IL-13Ra2 transcript was found by real-time PCR (Fig. 6b). IL-13Ra1 transcript was induced by TNF-\( \alpha \), but was not further increased by the combination of IL-13 and TNF-\( \alpha \) (Fig. 6c), and there was constitutive expression of IL-4Ra under all treatment conditions (Fig. 6d). Under conditions associated with induction of IL-13Ra2 on the cell surface (IL-13 plus TNF-\( \alpha \)), HMC-1-dependent contraction of HASM-containing gels was significantly reduced compared with that seen in the presence of IL-13 alone (Fig. 6e; \( p < 0.001 \) for IL-13 plus TNF-\( \alpha \) compared with IL-13 alone). Thus, induction of IL-13Ra2 expression was associated with reduced IL-13-dependent HASM gel contractile responses in this system, consistent with antagonist function.

**FIGURE 6.** Induction of IL-13Ra2 is associated with reduced IL-13-stimulated HASM contraction. HASM were treated for 48 h with IL-13 (20 ng/ml) in the presence or absence of TNF-\( \alpha \) (1 ng/ml). a, Cell surface IL-13Ra2 was quantitated by flow cytometry. The percentage of cells staining with Ab to IL-13Ra2 was compared with that stained with control Ab to determine percent positive in each population. Expression of the transcript for IL-13Ra2 (b), IL-13Ra1 (c), or IL-4Ra (d) was determined by real-time PCR. *, \( p < 0.05 \). e, Collagen gels embedded with HASM only or HASM + HMC-1 were treated with IL-13 in the presence or absence of TNF-\( \alpha \). After 24 h at 37°C, gel surface area was quantitated and expressed as a percentage of the area of a lattice that contained no cells. *, \( p < 0.001 \) compared with medium control; \( ^{\wedge} \), \( p < 0.001 \) compared with IL-13 alone. f, Collagen gels embedded with HASM + HMC-1 were treated with C5a in the presence or absence of control human Ig or IL-13Ra2-Fc and the area was quantitated after 24 h. *, \( p < 0.0001 \) compared with medium control; \( ^{\wedge} \), \( p < 0.01 \) compared with C5a alone or C5a + control Ig.
Mast cells are a major source of IL-13. To determine whether IL-13 played a role in the contraction-inducing activity of C5a-activated HMC-1 in this system, sIL-13R/H92512-Fc was added to collagen gels coembedded with HASM and HMC-1. The potent contractile response to C5a was not affected by control human Ig, but was significantly antagonized by sIL-13R/H92512-Fc (Fig. 6f; \( p < 0.01 \) for C5a plus sIL-13R/H92512-Fc compared with C5a plus control Ig or C5a alone).

Cytokine effects on MMP content of HASM-HMC-1 cocultures

Both mast cells and smooth muscle cells can produce MMPs. To examine the range of MMPs produced in this system, gels were seeded with HASM in the presence or absence of HMC-1. The medium surrounding the gels was collected, concentrated 10-fold, and MMP content examined by the Fluorokine multiplex assay. High levels of MMP-1 and MMP-2 were seen, but no detectable MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, or MMP-13 (data not shown).

Gelatin zymography confirmed the strong expression of pro-MMP-2 in the HASM cultures. The MMP-2 content was marginally increased following IL-13 treatment of gels containing HASM and HMC-1 cells, but no bands corresponding to the low molecular weight processed forms of MMP-2 were seen (Fig. 7a). Strong expression of MMP-1 was confirmed by Western blot (Fig. 7b). Both the proform and the proteolytically processed form, indicative of MMP-1 activation, increased in gels embedded with HASM and HMC-1 upon treatment with IL-13, TNF-\( \alpha \), or both (Fig. 7, b and c).

MMP content of HASM-HMC-1 cocultures following mast cell activation

Following C5a activation of HMC-1, MMP-2 expression was increased in cocultures of HASM and HMC-1 and expression of MMP-9 was marginally detectable (Fig. 8a). The processed form of MMP-1 was readily detectable in medium surrounding collagen gels coembedded with HASM and HMC-1 (Fig. 8, b and c). Both the proform and processed MMP-1 were also found in extracts of the gels themselves (Fig. 8, b and c), indicating association with the collagen gel matrix. In gels seeded with HASM only, pro-MMP-1 could be found, but the processed form was not apparent (Fig. 8, b and c).

In cultures containing pHMC only, high levels of MMP-9 were found by zymography, but little MMP-2 was expressed (Fig. 9a). In contrast, cultures of HASM only contained high levels of MMP-2, but no detectable MMP-9. In medium surrounding collagen gels coembedded with HASM and pHMC, trace amounts of MMP-9 were seen, but pro-MMP-2 was abundantly expressed and the processed form was readily detectable (Fig. 9a). Neither MMP-2 nor MMP-9 expression was modulated by IgE receptor cross-linking. MMP-1 was also expressed in the cocultures of HASM and pHMC (Fig. 9b). The processed form of MMP-1 was readily detectable and extracts of gels coembedded with HASM and pHMC revealed processed MMP-1 as the predominant form (Fig. 9, b and c). In both the gels and surrounding medium, processed MMP-1 increased following IgE-dependent mast cell activation (Fig. 9, b and c).

Discussion

Mast cell infiltration of airway smooth muscle appears to be unique to asthma and does not occur in related respiratory conditions (2, 3, 5). Elaboration of chemokines by ASM likely initiates the process (36, 37), but the cellular interactions maintaining infiltration and promoting subsequent airway remodeling may involve activation of either cell type. We applied a model system of human mast cell/HASM coculture in a collagen gel matrix to study the consequences of mast cell activation on HASM contractility. Within collagen lattices, C5a activation of HMC-1 cells or IgE-dependent activation of pHMC triggered contraction of HASM-containing gels, which was dependent on the activity of MMPs. Cytokines contributed to MMP generation and serine proteases contributed to MMP activation, leading to mast cell dependence of HASM contractile responses within the gel matrix.

The matrix contraction model used in our study is driven by smooth muscle activation, but is distinct from the immediate-type smooth muscle contraction described by the classic Schultz-Dale...
collagen gel contraction were both increased following treatment in gels coembedded with HASM and HMC-1, MMP-2 content and produced by HASM and increased upon coculture with mast cells. Thus, MMP-2 directly responsible for HASM contraction in collagen gels. 

MMPs are key mediators of contractility in HASM-embedded collagen gels. Both activated mast cells (42, 43) and smooth muscle cells (14, 24–26) can produce MMPs, including MMP-1, MMP-2, MMP-9, and MMP-12, all of which are expressed in asthmatic airways (44). Although exogenously added, catalytically active MMPs 1, 2, 9, and 12 all triggered contraction of HASM-containing collagen gels, the collagenase MMP-1, and the gelatinase MMP-2 were the predominant forms endogenously produced by HASM in the collagen gel system.

Although activation of HMC-1 with C5a or activation of pHMC with anti-IgE induced muscle contraction of HASM in coculture with HASM, mast cells were not required for expression of MMP-1. In gels containing HASM only, the combination of IgE/anti-IgE induced low levels of MMP-1 expression and processing. HASM express FceRII (CD23) (45), which can stimulate production of IL-1β (46), an activator of MMP-1 expression (47, 48). HASM may also express FceRI, resulting in IgE-dependent production of IL-13 (49), an additional inducer of MMP-1 (14). Although MMP-1 expression was found in gels containing HASM only, no IgE-dependent contraction was observed in the absence of mast cells, indicating that MMP-1 expression was not sufficient for induction of HASM contraction. Similarly, although MMP-1 expression and activation were increased upon treatment of HASM-embedded collagen gels with TNF-α, this did not result in appreciable contraction. Thus, MMP-1 does not appear to be either sufficient or directly responsible for, HASM contraction in collagen gels.

In addition to MMP-1, the gelatinase MMP-2 was constitutively produced by HASM and increased upon coculture with mast cells. In gels coembedded with HASM and HMC-1, MMP-2 content and collagen gel contraction were both increased following treatment with C5a or IL-13. In contrast, coembedding pHMC with HASM markedly induced the proteolytically processed form of MMP-2, even in the absence of IgE receptor cross-linking and mast cell-dependent contraction. Thus, MMP-2 may contribute to the low level contraction seen in the presence of resting mast cells (50), but expression of the processed form was not modulated upon IgE-dependent activation. As for MMP-1, the presence or activation of MMP-2 did not appear sufficient to account for contractile activity induced by mast cell activation. MMP-9 was produced by cultures of pHMC alone, but was greatly down-modulated under coculture conditions and therefore also not associated with the activation response. Additional proteases, cytokines, or other agents present in the coculture system may influence contractile responses to MMPs.

The major mast cell serine protease tryptase may modulate MMP activation. pHMC contained tryptase that was released upon activation. Although HMC-1 cells are sparsely granulated, they also contain tryptase (51), as was confirmed here (data not shown). Tryptase induces a range of responses in smooth muscle cells, including cytokine and chemokine synthesis (52), TGF-β production and activation (15, 52, 53), proliferation (54), direct contraction (15), hyperresponsiveness to histamine (11), and differentiation to myofibroblasts (15). In our system, tryptase triggered HASM contraction in collagen gels and this activity could be blocked by leupeptin, in agreement with published observations (15). Interestingly, however, this response was also blocked by batimastat, raising the possibility that endogenous MMP(s) mediate contraction in response to tryptase. MMPs are released as latent precursors and are activated extracellularly by proteolytic processing. In accordance with this, we observed direct proteolysis of both pro-MMP-1 and pro-MMP-2 by human lung tryptase, in agreement with observations in other systems (28, 29). Our findings suggest that tryptase-mediated MMP activation may contribute to mast cell-dependent HASM contraction.

In the presence of mast cells, contraction of HASM-containing collagen gels was stimulated by IL-6 or IL-13, both of which may directly induce expression of MMP-1 from smooth muscle cells (14, 30). IL-6, a product of activated HASM (55), drives human lung mast cell proliferation in coculture with HASM and may contribute to mast cell myositis in asthma (31). Because IL-13 is a
critical cytokine for generation of asthmatic responses (56), we explored the IL-13-induced contraction further. IL-13 has been localized to mast cells within the smooth muscle layer in asthmatic airways (57, 58). Smooth muscle cells express IL-13Rα1 and IL-4Rα (59) and respond to IL-13 with a range of activities, including proliferation (60), migration (61), chemokine release (62), and contraction (63). A second IL-13 binding chain, IL-13Rα2, has higher affinity than IL-13Rα1, but triggers no known signaling responses and is thought to act as a “decoy” to antagonize IL-13 activity (34). Recent studies, however, suggest that IL-13Rα2 may mediate profibrotic responses to IL-13 by an undefined mechanism (35). Expression of IL-13Rα2 can be inducibly regulated on the surface of various cell types including smooth muscle cells (33). Human cell lines overexpressing IL-13Rα2 have reduced signaling responses to IL-13 (64, 65), consistent with antagonist function, but there have been relatively few studies of IL-13Rα2 effects on IL-13 responses of primary human cells (33, 66, 67). We induced IL-13Rα2 expression on HASM by treatment with IL-13 in combination with TNF-α. Under these conditions, the mast cell-dependent HASM contractile activity of IL-13 was ablated, strongly suggesting that in this system, IL-13Rα2 antagonizes IL-13 functional activity. IL-13 may also promote contraction of HASM-containing gels following C5a-induced mast cell activation, as sIL-13Rα2-Fc, a potent IL-13 antagonist, significantly reduced contraction in response to C5a-activated HMC-1.

Numerous processes may contribute to hypercontractility of ASM in asthma, including increased responsiveness to activating agents or reduced responsiveness to negative regulators (68). In asthma, the airway smooth muscle is infiltrated by mast cells, which may show signs of activation and degranulation (2, 6) and contribute to airway remodeling. Our findings with IgE-dependent activation of primary mast cells, C5a activation of HMC-1, or cytokine treatment support a model by which mast cell-derived cytokines induce MMP generation and serine proteases mediate MMP activation, leading to HASM-dependent matrix contraction. Such interactions have the potential to exacerbate pathology in asthma and other lung disorders.

Disclosures
Karl H. Nocka, Agnes Brennan, Bijia Deng, Margaret Fleming, Samuel J. Goldman, and Marion T. Kasaian are current employees of Wyeth Research. Alexander Margulis is a past employee of Wyeth Research.

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