Mast cell chymase affects the functional properties of primary human airway fibroblasts: Implications for asthma

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Background: Mast cells (MCs) have a profound impact on allergic asthma. Under such conditions, MCs undergo degranulation, resulting in the release of exceptionally large amounts of MC-restricted proteases. However, the role of these proteases in asthma is only partially understood.
Objectives: We sought to test our hypothesis that MC proteases can influence the functionality of human lung fibroblasts (HLFs).
Methods: Primary HLFs were treated with MC chymase or tryptase, followed by assessment of parameters related to fibroblast function.
Results: HLFs underwent major morphologic changes in response to chymase, showing signs of cellular contraction, but were refractory to tryptase. However, no effects of chymase on HLF viability or proliferation were seen. Chymase, but not tryptase, had a major impact on the output of extracellular matrix–associated compounds from the HLFs, including degradation of fibronectin and collagen-1, and activation of pro–matrix metalloprotease 2. Further, chymase induced the release of various chemotactic factors from HLFs. In line with this, conditioned medium from chymase-treated HLFs showed chemotactic activity on neutrophils. Transcriptome analysis revealed that chymase induced a proinflammatory gene transcription profile in HLFs, whereas tryptase had minimal effects.
Conclusions: Chymase, but not tryptase, has a major impact on the phenotype of primary airway fibroblasts by modifying their output of extracellular matrix components and by inducing a proinflammatory phenotype. (J Allergy Clin Immunol 2022;149:718-27.)

Key words: *Mast cells, chymase, tryptase, fibroblasts, asthma*

Abbreviations used

CPA3:	Carboxypeptidase A3
CXCL:	C-X-C motif chemokine ligand
ECM:	Extracellular matrix
EdU:	5-Ethynyl-2′-deoxyuridine
ELISA:	Enzyme-linked immunosorbent assay
HGF:	Hepatocyte growth factor
HLF:	Human lung fibroblast
IGFBP:	Insulin-like growth factor-binding protein
MC:	Mast cell
MMP:	Matrix metalloprotease
SMC:	Smooth muscle cell
uPAR:	Urokinase-type plasminogen activator receptor

Asthma is a devastating and common disease affecting as many as 300 million people globally, with prevalence still rising.[1-3] Asthma represents a chronic inflammatory disorder associated with airway hyperresponsiveness, persistent airway inflammation, airflow obstruction, and airway remodeling. One hallmark feature of the asthmatic lung is airway remodeling, with chronic and irreversible structural changes of the bronchial wall during disease progression. This will subsequently lead to thickening of the airway basement membranes, subepithelial fibrosis, increased vascularity, and distorted deposition of extracellular matrix (ECM) components.[4] Previous investigations have established that the degree of airway wall thickening may correlate with disease severity.[5]

The airway wall is a heterogeneous 3-layered structure, from the airway lumen progressing to the connective tissue. It is composed of epithelium and of submucosal and smooth muscle layers. The regulatory mechanisms concerning airway remodeling during asthmatic conditions can involve dysregulated processes in all 3 layers of the airway wall.[6] In particular, numerous studies have identified a major effector role for the smooth muscle cells (SMCs) during asthma exacerbations with excessive airway narrowing. However, asthmatic responses can also be related to events occurring in the airway submucosa. This layer is predominately composed of vessels, immune cells, and mesenchymal cells, in particular fibroblasts.[4] Of relevance for asthma, lung fibroblasts have the versatility to differentiate into myofibroblasts.[3,7] Moreover, lung fibroblasts have the capacity to produce chemokines and ECM components; all together, fibroblasts can thereby promote progression from an acute resolving inflammation to a chronic persistent inflammatory condition.[8]

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It is now widely recognized that mast cells (MCs) can have a major detrimental impact on allergic asthma, as evidenced by both clinical studies and by experimental studies utilizing various asthma models.[9-11] MCs can initiate allergic inflammation through IgE-mediated MC degranulation, in which the preformed compounds stored in the MC granules will be released. The preformed mediators include histamine, cytokines, growth factors, and exceptionally large amounts of various MC-restricted proteases, the latter encompassing chymase, tryptase, and carboxypeptidase A3 (CPA3).[12,13] Two major subtypes of MCs have been recognized, with the MC_T type expressing tryptase only whereas the MC_{TC} subtype expresses tryptase, chymase, and CPA3.[14] The exact roles of the various MC proteases in asthma remain uncertain.[13] However, there is a wealth of evidence supporting a profound expansion of the MC_{TC} subtype during asthma, suggesting a role of chymase under such conditions.[15-18] Further, there are both clinical and animal experimental studies suggesting that the MC proteases have complex regulatory roles in asthma.[9-11]

Importantly, although the MC proteases are strongly implicated in asthmatic responses, their precise mechanisms of action in asthma are not known. One plausible scenario would be that the MC proteases, after their release following MC degranulation, can influence other airway cells, such as epithelial cells, SMCs, or fibroblasts. We addressed this issue by examining the effect of the MC proteases on primary human lung fibroblasts (HLFs). We found that MC chymase has a major functional impact on primary HLFs by inducing a proinflammatory phenotype and affecting processes related to ECM remodeling.

METHODS

Experimental details are provided in the Methods in this article's Online Repository at www.jacionline.org.

Reagents

Recombinant human β-tryptase was generated as previously described.[19] Recombinant human chymase (C8118-50UG) was purchased from Sigma-Aldrich (St Louis, Mo). Tryptase and chymase were used at 5 nmol (tryptase concentration based on size of tryptase tetramers[20]).

Cell culture, cell viability, proliferation, Western blot analysis, and gelatin zymography

Primary HLFs (PCS-201-013) were from American Type Culture Collection (ATCC, Manassas, Va) (3 different donors; similar results were obtained for the individual donors). Cell viability was assessed by annexin V/DRAQ7 staining and flow cytometry analysis. Proliferation was assessed by 5-ethynyl-2′-deoxyuridine (EdU) staining.[21] Western blot analysis was performed as described elsewhere.[21] Gelatin zymography analysis was performed using 10% Zymogram Plus (Gelatin) Protein Gels (Invitrogen; Thermo Fisher Scientific, Waltham, Mass).

Antibody arrays

A Human Cytokine Array C1000 (RayBiotech, Peachtree Corners, Ga) was used.

Enzyme-linked immunosorbent assay

The following enzyme-linked immunosorbent assay (ELISA) kits from Invitrogen were used: uPAR (PLAUR) Human ELISA Kit, HGF Human ELISA Kit, and GCP-2/CXCL6 Human ELISA Kit. All the steps were performed according to the manufacturer's recommendations.

Granulocyte purification and chemotaxis assays

Granulocytes were purified from human peripheral blood using Percoll (Sigma-Aldrich) gradient centrifugation. Granulocyte chemotaxis was measured by a modified Boyden chamber technique.[22]

Transcriptome analysis

Transcriptome analysis was performed using the Ion AmpliSeq Transcriptome Human Gene Expression Kit (Life Technologies, Carlsbad, Calif), followed by analysis as described.[23]

Statistical analysis

All calculations were performed by Excel software (Microsoft, Redmond, Wash). Image editing and the array signal pixel density assessment were conducted by ImageJ software (https://imagej.nih.gov/ij/). Flow cytometry results were analyzed by FlowJo v10.6.1 software (Treestar, Ashland, Ore). Statistical differences between groups were assessed by multiple comparisons of ordinary 1-way ANOVA. Adjusted P values of $\leq .05$ were considered statistically significant. All figures were prepared by GraphPad Prism 8.0 software (GraphPad Software, La Jolla, Calif), with data presented as means ± SEMs. The results shown are from individual experiments that are representative of at least 3 experiments.

RESULTS

MC chymase induces morphologic changes in human airway fibroblasts without causing apoptosis or affecting proliferation

To assess whether MC proteases have an impact on fibroblasts of the airways, we incubated primary HLFs with chymase or tryptase. As seen in Fig 1, *A* and *B,* and in Fig E1 in this article's Online Repository at www.jacionline.org (dose response), and also as shown by time-lapse microscopy (see Videos E1, E2, and E3 in this article's Online Repository at www.jacionline.org), chymase induced major morphologic changes in the HLFs, with the chymase-treated cells being more contracted compared to nontreated cells; cell detachment was not noted, however. This effect was seen starting from 2 hours and lasted up to at least 24 hours. In contrast, tryptase had no apparent impact on HLF morphology (Fig 1, *A* and *B*).

Next, we determined whether the morphologic effects of chymase on HLFs were accompanied by effects on viability. However, neither chymase nor tryptase induced apoptosis in HLFs, as judged by annexin V/DRAQ7 staining (Fig 1, *C* and *D*). We also investigated whether any of the proteases could affect the proliferation rate of the HLFs. As seen in Fig 1, *E,* neither of the proteases affected HLF proliferation, as determined by EdU incorporation (Fig 1, *E* and *F*). As a positive control, transformed MCs (HMC-1 and LUVA) showed highly elevated incorporation of EdU compared to the primary HLFs (Fig 1, *E* and *F*).

Chymase degrades fibronectin and collagen-1 produced by primary HLFs and activates MMP-2 secreted from primary HLFs

We next investigated the possible effects of tryptase and chymase treatment on secreted ECM-related compounds, based on many previous studies indicating that the MC proteases could have a major role in ECM turnover.[13,24,25] We first used the Western blot technique to analyze effects on fibronectin, a major ECM protein that is a known substrate for chymase[26-28] and tryptase.[29] As seen in Fig 2, *A,* fibronectin was clearly detected in

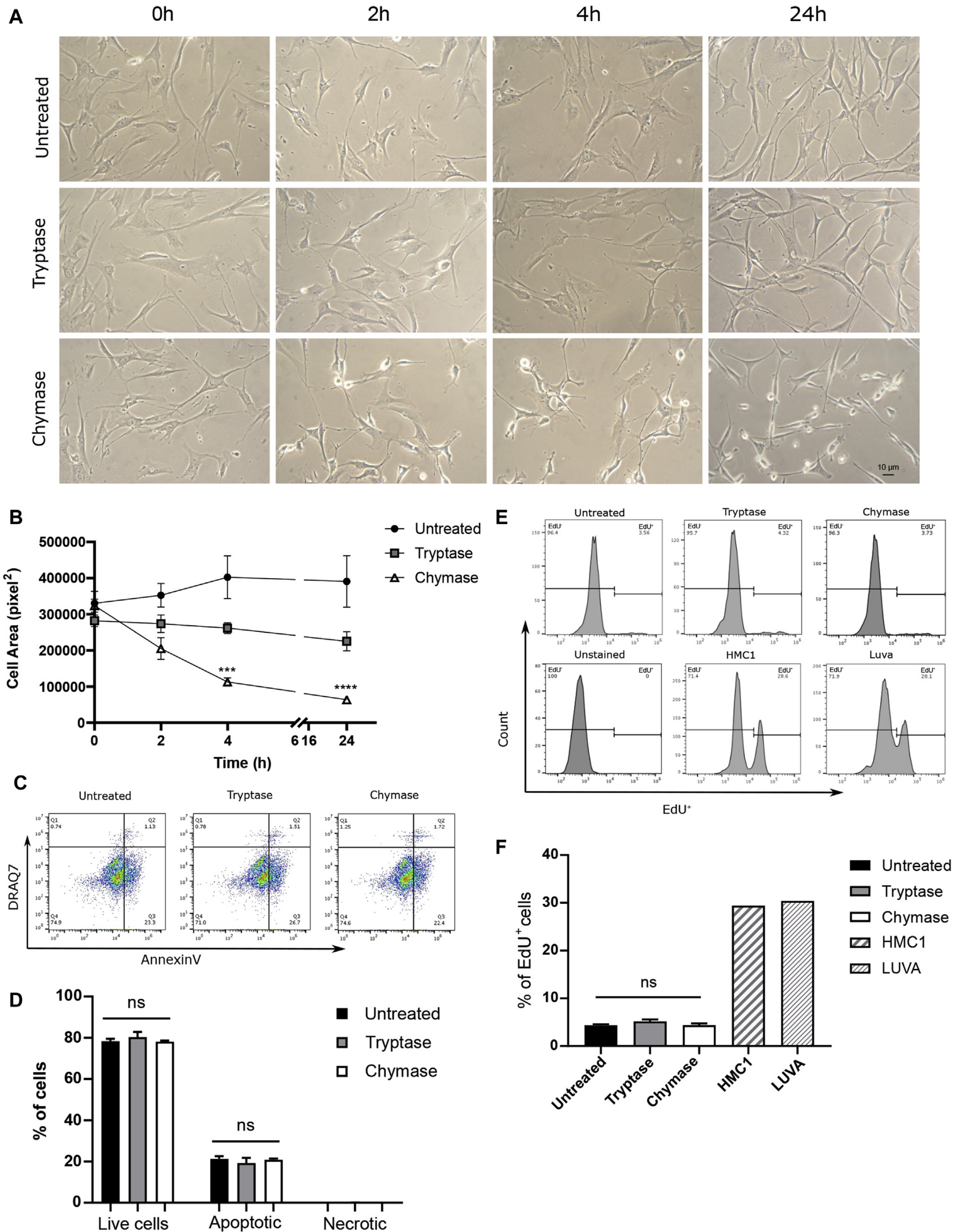

FIG 1. Chymase induces morphologic changes in primary HLFs but does not induce apoptosis or proliferation. **A,** HLFs were treated with chymase or tryptase for the time periods indicated, followed by phase contrast microscope assessment. **B,** Quantification of cells is made after the corresponding treatments. Data represent means of multiple determinations (n = 10-12) ± SEMs. $***P < .001$, $****P < .0001$.

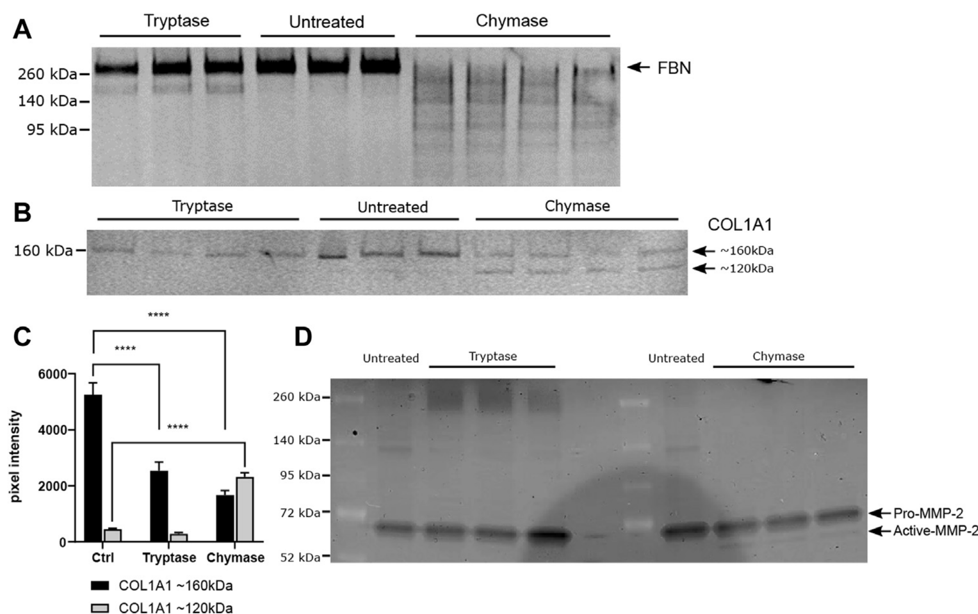

FIG 2. Effects of MC proteases on fibronectin, collagen-1, MMP-2, and uPAR in primary HLFs. HLFs were treated for 24 hours with either chymase or tryptase. Conditioned media were collected and analyzed by Western blot for fibronectin (FBN) (**A**) and collagen-1 (COL1A1) (**B**). **C,** Quantification of collagen band intensities. Data represent means of triplicate determinations ± SEMs. $^{****}P < .0001$. **D,** Conditioned media were analyzed by gelatin zymography. Bands corresponding to pro–MMP-2 and active MMP-2 are indicated. Note also the gelatin clearance zone at ~200 kDa after treatment with tryptase.

conditioned medium from the HLFs, confirming that the HLFs produce this protein. After incubation with chymase, complete degradation of the secreted fibronectin was seen, whereas tryptase only caused partial degradation of fibronectin into a 140 kDa product (Fig 2, *A*). We next assessed if any of the MC proteases could affect collagen-1, another major ECM component. As expected, collagen-1 was clearly detected in the conditioned medium from HLFs, confirming that this protein is a major product of the HLFs (Fig 2, *B*). When the cells were treated with tryptase, distinct collagen degradation products were not observed. However, a reduction in the intensity of the band corresponding to intact collagen was noted, suggesting that tryptase caused degradation of collagen to products that were not recognized by the antibody used for Western blot analysis (Fig 2, *B* and *C*). In contrast, chymase caused extensive degradation of collagen into distinct proteolytic fragments (Fig 2, *B* and *C*). The latter suggests that chymase either can have direct collagen-degrading activity or that chymase could activate other enzymes with collagenolytic activity. With respect to the latter possibility, previous studies have indicated that MC proteases can activate various pro–matrix metalloproteases (MMPs),[27,30-33] including the progelatinases pro–MMP-2 and pro-MMP9. To address whether the MC proteases could exert such activities in the HLF context, we adopted gelatin zymography analysis. This analysis revealed the presence of a band corresponding to pro–MMP-2 in conditioned medium from the HLFs, whereas pro-MMP9 was not detected (Fig 2, *D*). Further, a faint but clearly distinguishable band corresponding to active MMP-2 was seen when the cells had been treated with chymase, whereas pro–MMP-2 activation was not induced by tryptase (Fig 2, *D*). However, a gelatin clearance zone at ~200 kDa was seen when analyzing tryptase-treated conditioned medium. The latter is in agreement with a direct gelatin-degrading activity of tryptase.[34] As a control, the gelatin-degrading activity of tryptase was abolished in the presence of a general serine protease inhibitor (Pefabloc SC; data not shown).

MC proteases affect the output of growth factors and cytokines from HLFs

To further explore the impact of the MC proteases on the functional properties of primary HLFs, we assessed whether tryptase or chymase could affect the output of inflammatory compounds, as determined by a cytokine array approach (120 human cytokines; this array's setup is shown in Fig E2 in this article's Online Repository at www.jacionline.org). Taking into account that chymase and tryptase are coreleased after MC degranulation,[12] we also investigated whether the combined treatment with these major MC proteases could induce effects beyond

C, D, After treatment with chymase or tryptase (24 hours), cells were stained with annexin V and DRAQ7 (*C*), followed by flow cytometry analysis, with (*D*) quantification of viable (annexin V^-/$DRAQ7^-$), apoptotic (annexin V^+/$DRAQ7^-$), and necrotic (annexin V^+/$DRAQ7^+$) cells. **E,** Histograms showing EdU staining of untreated, tryptase-treated, chymase-treated (24 hours), unstained group, and positive control groups (highly proliferating HMC-1 and LUVA cells). EdU^+ populations represent proliferating cells, whereas EdU^- populations represent nonproliferating cells. **F,** Quantification of percentages of proliferating cells (EdU^+) in untreated HLFs, tryptase-treated HLFs, chymase-treated HLFs, and positive control cells. Data represent means of triplicate determinations ± SEMs. *ns*, Not significant.

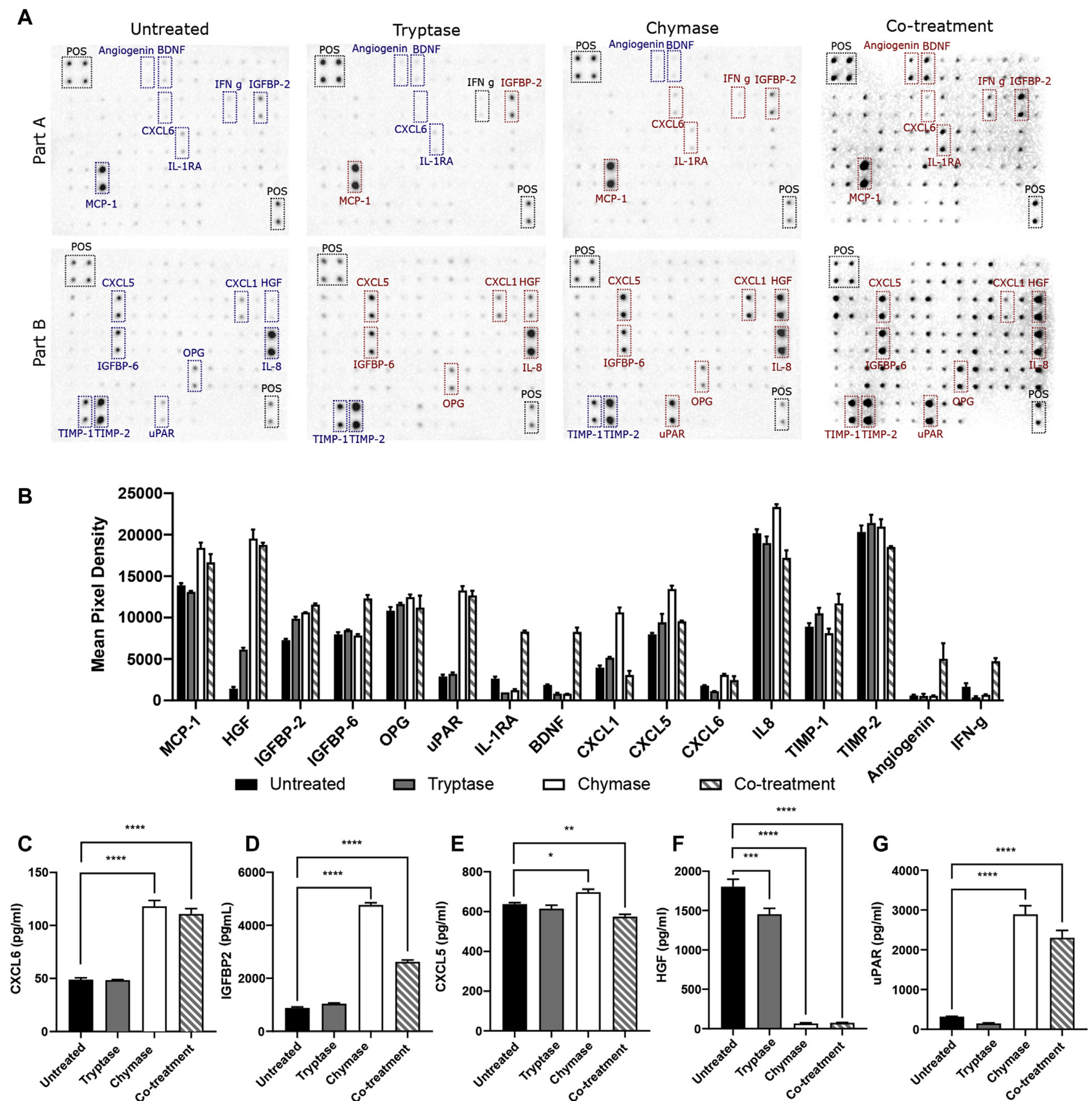

FIG 3. Chymase induces the release of proinflammatory compounds from primary HLFs. HLFs were treated for 24 hours with chymase, tryptase, or both. **A, B,** Conditioned media from the respective treatments were analyzed by a cytokine array approach. (*B*) Quantification of the data from (*A*). **C-G,** Quantification of the release of CXCL6, IGFBP2, CXCL5, HGF, and uPAR in response to chymase, tryptase, or both as determined by ELISA. Data represent means of quadruplicates ± SEMs. **P* < .05, ***P* < .01, ****P* < .001, *****P* < .0001.

those of the individual proteases alone. This analysis showed that chymase induced an upregulated output of several proinflammatory chemokines, including C-X-C motif chemokine ligand (CXCL) 1, CXCL5, MCP1/CCL2, insulin-like growth factor-binding protein (IGFBP) 2, and CXCL6 (Fig 3, *A* and *B*). In addition, a profound increase in secreted (soluble) urokinase-type plasminogen activator receptor (uPAR) and hepatocyte growth factor (HGF) was seen after treatment of the HLFs with chymase (Fig 3, *A* and *B*). In contrast, tryptase had minimal effects on the output on any of the cytokines, and the combination

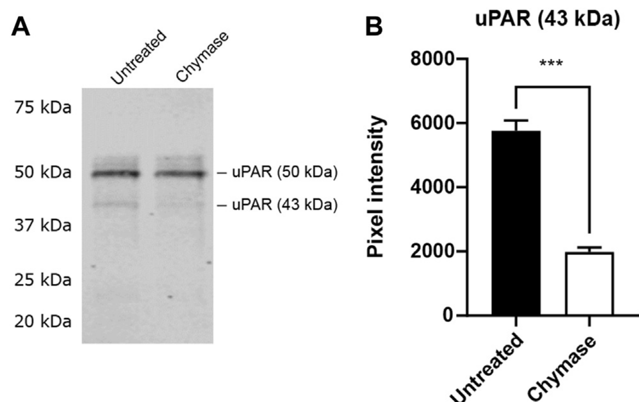

FIG 4. Effects of chymase on uPAR in primary HLFs. HLFs were treated for 24 hours with chymase. **A,** Cell pellets were recovered and analyzed by Western blot test for the presence of uPAR. **B,** Quantification of band intensities corresponding to the 43 kDa uPAR band. Data represent means of triplicates ± SEMs. ***$P < .001$.

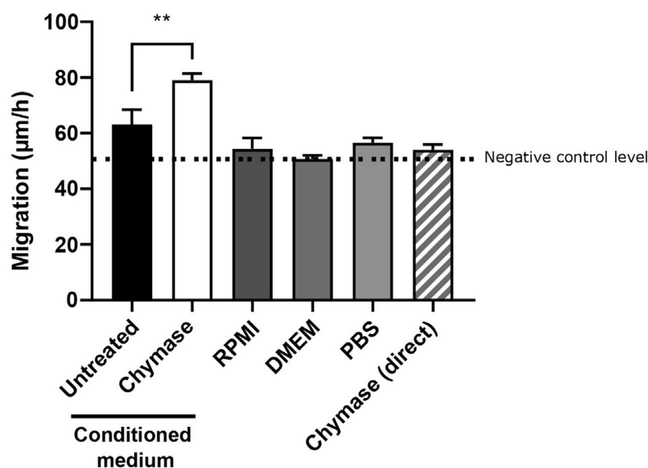

FIG 5. Chymase induces the release of neutrophil chemoattractants from primary HLFs. HLFs were incubated for 24 hours with chymase. Conditioned media from untreated and chymase-treated cells were collected and assessed for neutrophil-attracting activity using a Boyden chamber assay. Neutrophil migration in direct response to chymase and in the response to negative control media (RPMI 1640, Dulbecco modified Eagle medium [DMEM]) and phosphate-buffered saline (PBS) was also analyzed. Data represent means of quadruplicates ± SEMs. **$P < .01$.

of tryptase and chymase had similar effects as those of chymase only (Fig 3, *A* and *B*). However, we noted that angiogenin, IL-1RA, and brain-derived neurotrophic factor were markedly induced by the combination of chymase and tryptase (Fig 3, *A* and *B*). To confirm these findings by an independent method, we looked for selected compounds by ELISA. As shown in Fig 3, *C-E,* the upregulated output of CXCL6, IGFBP2, and CXCL5 in response to chymase was confirmed. However, although the cytokine array approach indicated a robust increase in HGF release in response to chymase, the ELISA measurements indicated the opposite—that is, chymase caused a reduction of HGF levels (Fig 3, *F*). One possible explanation for this apparent discrepancy could be that the HGF antibodies included in the cytokine array and in the HGF ELISA are toward distinct epitopes. Hence, epitopes detected through the array approach may be resistant to chymase, whereas the ELISA approach may detect HGF epitopes that are subject to degradation by chymase. Indeed, previous studies have shown the ability of chymase to degrade HGF (cleavage at Leu480).[35]

Chymase induces release of uPAR from HLFs

A profound increase in the output of soluble uPAR was also confirmed by ELISA analysis (Fig 3, *G*). uPAR is a cell membrane–bound receptor for uPA and is known to have a complex association with lung pathologies, including asthma.[36-40] For example, uPAR release by inflammatory cells of the lamina propria is elevated in asthmatic conditions.[40] To provide further insight into the mechanism of uPAR release in response to chymase, we performed Western blot analysis. This showed that cell-associated uPAR migrated as 2 bands of ~50 and ~43 kDa, respectively, and that chymase caused a significant reduction of the 43 kDa band (Fig 4). This suggests that chymase induces the release of preformed 43 kDa uPAR from the cells.

Chymase induces the release of neutrophil chemoattractants from HLFs

Soluble uPAR is known to have chemotactic activity on various cell types, including leukocytes,[41,42] and it is also notable that the data above indicated that chymase treatment of HLFs resulted in the release of neutrophil-attracting CXCL-type chemokines. Taken together, this suggests that the releasate from chymase-treated HLFs can have chemotactic activity on leukocytes. To address this possibility, we assessed whether conditioned medium from chymase-treated HLFs had chemotactic activity for human neutrophils. As shown in Fig 5 as well as in Fig E3 in this article's Online Repository at www.jacionline.org (showing dose responses), the conditioned medium collected from chymase-treated primary HLFs had a significantly higher chemoattractant effect on human neutrophils than had conditioned medium from nontreated HLFs. Purified chymase itself had no significant direct chemoattractant effect on the neutrophils.

MC proteases affect the transcriptome of primary HLFs

To provide further insight into how the MC proteases functionally affect primary HLFs, we assessed their effects on the HLF transcriptome. For this purpose, HLFs were treated for 24 hours with chymase, tryptase, or a combination of both, followed by transcriptome analysis using AmpliSeq. As shown by multidimensional scaling plot analysis, the untreated samples clustered closely together (Fig 6, *A*). The samples from tryptase-treated cells also clustered separately, although relatively closely to the untreated samples. However, samples from the chymase-treated cells were clearly separated from both the untreated and tryptase-treated samples, suggesting more profound effects of chymase on the HLF transcriptome (Fig 6, *A,* and see Table E1 in this article's Online Repository at www.jacionline.org). The samples from the chymase + tryptase cotreated cells clustered closely to the chymase-treated samples. These findings were also reinforced by a heat map analysis depicting 286 differentially expressed genes in all groups, which revealed that samples from untreated and tryptase-treated cells clustered closely together,

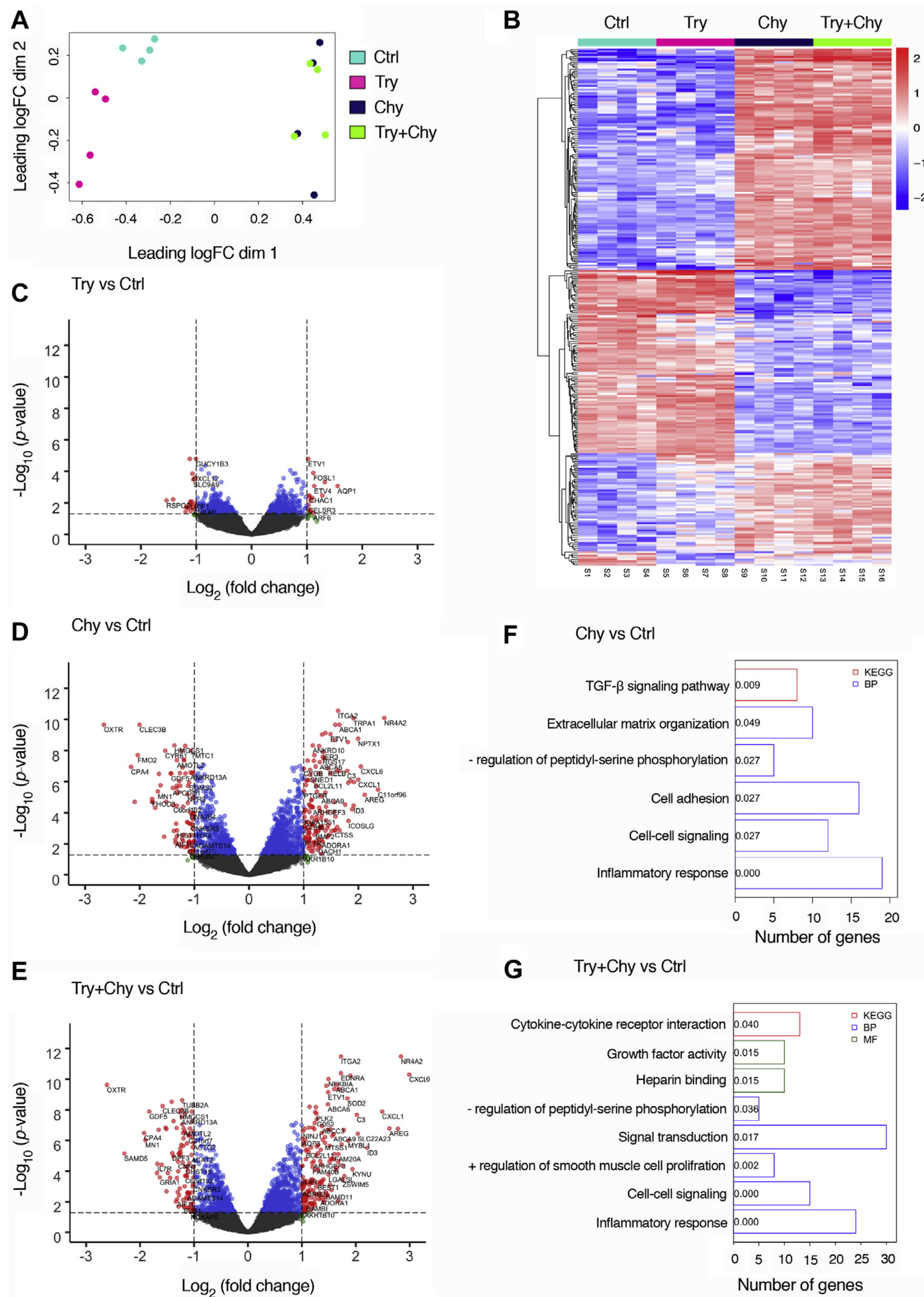

FIG 6. Effects of chymase and tryptase on the transcriptome of primary HLFs. HLFs were untreated (control, Ctrl) or treated with chymase, tryptase, or both, followed by transcriptome analysis. **A,** Multidimensional scaling plot displaying the clustering of samples based on their gene expression patterns. **B,** Heat map displaying hierarchical clustering of 286 differentially expressed genes (DEGs; *red* represents upregulation and *blue* downregulation). Original data were scaled to have 0 mean. **C-E,** Volcano plot of $\log_2$ fold changes (FC) of gene expression after treatment of HLFs with tryptase (*C*), chymase (*D*), or both (*E*) compared to untreated cells. DEGs with $P < .05$ and absolute FC > 1 are indicated in *red*. *Vertical dashed lines* represent the $\log_2$ fold change of −1 or 1; *horizontal dashed lines* denote *P* value cutoffs of .05. **F, G,** Gene Ontology (GO; http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg/) pathway enrichment analyses for DEGs in chymase-treated (*F*) and chymase + tryptase cotreated (*G*) versus untreated cells (*P* values are indicated within the bars). GO and KEGG pathway enrichment analyses indicate the number of genes that were significantly enriched in biological processes (BP) and molecular function (MF) categories. All *P* values were adjusted using the Benjamini-Hochberg procedure ($P < .05$).

with a clear separation from the samples corresponding to chymase-treated cells (Fig 6, *B*). Again, the heat map analysis supported the notion that the samples from the chymase-treated versus chymase + tryptase cotreated cells had a similar profile.

Effects on the HLF transcriptome were also evaluated through volcano plots, where individual genes affected by the MC proteases are visualized. As seen in Fig 6, *C,* and Table E1, tryptase had a significant impact (≥2-fold differential expression) on only 35 genes. In contrast, chymase caused a much more profound effect on gene expression, with 218 genes (133 genes upregulated and 85 genes downregulated) being differentially expressed (≥2-fold difference) compared to nontreated cells (Fig 6, *D*). Of the genes that were differently regulated in the presence of tryptase, 5 were affected similarly by chymase (*RSPO2, FMO4 GUCY1B3, ETV1, ID1*). A similar pattern as for chymase treatment was seen after combined chymase + tryptase treatment (253 differentially expressed genes, with 162 upregulated and 91 downregulated) (Fig 6, *E*). Notably, the genes that were upregulated by chymase or chymase + tryptase treatment included numerous genes representing proinflammatory compounds, such as CXCL chemokines, complement factors, and nuclear receptors. Importantly, several of the genes that were shown to be upregulated by chymase at the messenger RNA level (through the transcriptomic approach) corresponded to factors that were shown to be induced at the protein level by chymase (see Fig E4 in this article's Online Repository at www.jacionline.org).

An effect of chymase or chymase + tryptase on inflammatory pathways was also apparent after conducting pathway enrichment analysis with Gene Ontology (http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (https://www.genome.jp/kegg/) protocols. This analysis revealed that there was a significant association between chymase treatment and differential expression of genes involved in inflammatory responses. Further, chymase (and/or chymase + tryptase) had a significant impact on pathways related to ECM turnover, cell adhesion, and regulation of SMC growth. It was also noted that the chymase and/or chymase + tryptase treatment had significant effects on a large group of genes involved in signaling pathways, including genes involved in phosphorylation processes and cell–cell signaling as well as in growth factor pathways such as the TGF-β pathway.

DISCUSSION

It is now established that MCs have a major detrimental impact in allergic conditions, including asthma. However, the mechanisms by which MCs influence asthma pathology may be complex, considering that MCs can secrete a wide panel of potentially harmful compounds upon activation. These include bioactive amines such as histamine, a panel of proinflammatory cytokines, and growth factors capable of modulating the pathology.[12] However, in quantitative terms, the MC-restricted proteases (chymase, tryptase, and CPA3) represent the dominant products released by activated MCs, considering that these proteases may account for up to 50% of the total protein in MCs.[43,44] They are released in massive amounts after MC degranulation and are thus likely to be present in large amounts in the extracellular space under inflammatory conditions, such as asthma, where extensive MC activation takes place.

The MC proteases are relatively promiscuous in terms of cleavage specificity and are known to cleave a myriad of different substrates.[13] Hence, it is likely that the MC proteases can exert extensive proteolytic activity in the extracellular space in conditions where MCs have been activated, such as in asthma. However, although the MC proteases have been implicated in regulation of asthmatic responses,[13] the exact target or targets for the MC proteases under such conditions have not been firmly clarified.

A likely scenario would be that the MC proteases, after their release, could have an impact on other cell types present in the airways and could possibly affect their phenotype in such a way that the pathology may be influenced. Here we addressed this possibility by investigating whether MC proteases can affect the phenotype of airway fibroblasts. Our findings reveal that MC chymase has extensive effects on primary human airway fibroblasts, whereas tryptase was essentially without effects. These findings thus do not agree with previous findings in which both tryptase and chymase have been found to promote proliferation and collagen production in fibroblasts, and in which tryptase has been shown to cause chemokine secretion from fibroblasts.[13] We cannot yet explain these apparent discrepancies. However, a possible explanation could be that previous investigations were mainly performed by using various transformed fibroblast cell lines or on fibroblasts of nonairway origin, whereas this study was conducted on primary airway fibroblasts. Although tryptase had relatively little impact on the airway fibroblasts, we cannot exclude the notion that this protease may have more profound effects on other types of airway cells, such as epithelial cells or SMCs.

A major finding in this investigation was that chymase exerted effects on ECM-associated compounds released from the HLFs. First, fibronectin released by the HLFs was extensively degraded in the presence of chymase, a finding that is in good agreement with several previous studies, both *in vivo* and *in vitro,* showing that fibronectin is a major substrate for MC chymase.[26-28] Second, we noted that collagen-1 secreted by HLFs was degraded in the presence of chymase. This could potentially be explained by a direct collagen-cleaving activity of chymase. However, an alternative explanation is that chymase could proteolytically activate MMPs having collagenolytic activity—with this being in agreement with previous reports demonstrating that chymase has procollagenase activity.[45,46] Third, our data show that chymase activates pro–MMP-2 that is secreted by the airway fibroblasts. Again, this is in agreement with previous reports.[27,47] Taken together, these data suggest that chymase (but not tryptase) has the capacity to affect various ECM-related compounds, further suggesting that chymase could have an impact on the extensive ECM remodeling that is a hallmark of asthma.[48]

Another major finding in this investigation is that chymase had profound effects on the morphologic features, cytokine/chemokine output, and gene expression profile of airway fibroblasts. As a general pattern, chymase was shown to induce a more proinflammatory phenotype of the fibroblasts, as manifested by the expression/secretion of several chemotactic factors, including chemokines and soluble uPAR. This clearly could have an impact on the inflammatory component of asthma by recruiting various leukocytes to the injured tissue. Notably, however, chymase induces the release of factors capable of recruiting neutrophils/monocytes rather than eosinophils. Hence, chymase could potentially contribute to a shift from eosinophilic to neutrophilic/monocytic infiltration. We cannot yet explain the exact mechanism through which chymase exerts the effects observed in this study. A plausible scenario would be that chymase activates

any of the protease-activated receptor (PARs). However, agonists of PAR1-4 have no apparent effects on the HLFs, suggesting that other mechanisms might be operating (X.O.Z., A.P., and G.P., unpublished observations). On a different angle, a likely scenario could be that factors induced by chymase could act on the HLFs in an autocrine fashion. For example, we note that chymase induces the expression of BMP2, HGF, epiregulin, amphiregulin, and TNFSF10, which all have the potential to influence fibroblasts' properties.

In summary, this study reveals that MC chymase has a major impact on airway fibroblasts. Our data implicate chymase in modulating fibroblast-mediated inflammatory and tissue-remodeling events. These effects could possibly be at work in a human asthma context, so strategies to regulate chymase activity could be of clinical usefulness.

Clinical implications: MC chymase has a major impact on airway fibroblasts, thereby providing insight into how MCs can influence asthma's manifestations.

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