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Matrix metalloproteinase 8 contributes to solubilization of IL-13 receptor $\alpha 2$ *in vivo*

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Abstract

Background—IL-13 receptor $\alpha 2$ (IL-13R $\alpha 2$) is a high-affinity receptor for IL-13, a central mediator of allergic asthma. It acts predominantly as a decoy receptor but can also contribute to IL-13 responses under certain conditions. IL-13R $\alpha 2$ exists in soluble and membrane forms, which can both bind IL-13 and modulate its activity. Yet the proteolytic processes that contribute to the generation of soluble IL-13R $\alpha 2$ are largely unknown.

Objective—We sought to investigate the role of matrix metalloproteinases (MMPs) in the generation of soluble IL-13R $\alpha 2$.

Methods—Acellular cleavage assays by MMPs were performed by using glutathione-S-transferase fusion proteins of murine or human IL-13R $\alpha 2$. IL-13R $\alpha 2$ stable-transfected cells were used for analysis of surface expression and release of soluble IL-13R $\alpha 2$. Wild-type and MMP-8-deficient mice were used for analysis of allergen-induced airway hyperresponsiveness and solubilization of IL-13R $\alpha 2$.

Results—Among several MMPs tested, only MMP-8 cleaved IL-13R $\alpha 2$. Treatment of transfected human or murine cells expressing high levels of surface IL-13R $\alpha 2$ with MMP-8 resulted in release of soluble IL-13R $\alpha 2$ into the supernatants, with a concomitant decrease in surface IL-13R $\alpha 2$ levels. The IL-13R $\alpha 2$ solubilized by MMP-8 retained IL-13 binding activity. In an asthma model MMP-8-deficient mice displayed increased airway hyperresponsiveness and decreased soluble IL-13R $\alpha 2$ protein levels in bronchoalveolar lavage fluid compared with those seen in wild-type mice after house dust mite challenge.

Conclusion—MMP-8 cleaves IL-13R $\alpha 2$ *in vitro* and contributes to the solubilization of IL-13R $\alpha 2$ *in vivo*.

Keywords

Matrix metalloproteinase 8; IL-13; IL-13 receptor $\alpha 2$

IL-13 is an immunoregulatory cytokine secreted predominantly by activated T_H2 cells^{1,2} that has been implicated in the pathogenesis of asthma in multiple human and animal

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studies.^{3–5} IL-13 mediates its effects through specific interactions with a complex receptor system, including at least 3 receptor chains.⁶ IL-13 receptor α 1 and IL-4 receptor α 2 form a high-affinity signaling heterodimeric receptor for IL-13.⁶ A second IL-13 receptor, IL-13R α 2, binds IL-13 with high affinity and has been shown to act as a decoy receptor and exert an inhibitory influence on IL-13 signal transduction and responses.⁷ Administration of soluble IL-13R α 2 to the airways significantly reduced allergen-induced airway hyperresponsiveness (AHR) and inflammation.^{8,9} A role for IL-13R α 2 as a key modulator of allergic inflammation and asthma was suggested by the demonstration that overexpression of IL-13R α 2 in bronchially derived cells decreases IL-13-dependent signal transducer and activator of transcription 6 phosphorylation.¹⁰ This was confirmed by means of characterization of the IL-13R α 2-deficient mice, whereby mice infected with *Schistosoma mansoni* displayed enhanced IL-13 effects.^{11,12} A recent study revealed a role for IL-13R α 2 in IL-13-mediated TGF- β 1 production in lung fibrosis, supporting the hypothesis that IL-13R α 2 can also contribute to allergic inflammation under some conditions.¹³

Expression of IL-13R α 2 varies across cell types and can be induced by allergic inflammation, parasitic infection, and cytokines, including IL-4 and IL-13.^{14–16} Thus its expression will vary in cells, depending on the inflammatory state. IL-13R α 2 exists largely intracellularly and can be mobilized to the cell surface after cytokine exposure.¹⁷ IL-13R α 2 also exists in a soluble form,¹⁸ which has been shown to inhibit IL-13-mediated airway inflammation in murine models.^{8,9} Interestingly, IL-13R α 2-deficient mice have greatly reduced levels of IL-13 in the serum but significantly greater tissue levels of IL-13 when compared with wild-type mice. Thus IL-13R α 2 regulates serum and tissue levels of IL-13.¹¹ This was further supported by a report that treatment of IL-13R α 2-deficient mice with soluble IL-13R α 2-Fc recombinant protein increases circulating IL-13 levels.¹² IL-13 has been shown to induce IL-13R α 2 expression, demonstrating a complex feedback loop between IL-13 and IL-13R α 2 whereby each modulates the level of the other.^{10–12} Regulation of the level of expression of IL-13R α 2 and its relative distribution among the membrane and soluble compartments likely affect IL-13 responses.¹⁹

There are mainly 2 mechanisms to generate soluble receptors: proteolytic cleavage to release the extracellular domain from the surface membrane-bound receptor and pre-mRNA alternative splicing to remove the transmembrane domain from the receptor.²⁰ Our laboratory has identified an alternatively spliced isoform of murine IL-13R α 2 that encodes a soluble form of IL-13R α 2.²¹ Often both mechanisms contribute to the generation of soluble receptors, such as is the case with soluble IL-1 receptor and soluble IL-6 receptor.^{22–25} Matrix metalloproteinases (MMPs) play a critical role in lung development and remodeling,²⁶ and this family of enzymes has been shown to cleave several surface receptors and signaling molecules, including fibroblast growth factor receptor 1,²⁷ CD44,²⁸ IL-5 receptor α ,²² and IL-2 receptor α ,²⁹ with biologically relevant results. For example, cleaved fibroblast growth factor receptor 1 maintains its fibroblast growth factor binding capacity,²⁷ and the cleavage of CD44 stimulates cell mobility.²⁸ Similarly, MMP-9, which is strongly induced in the airways of patients with asthma, contributes to inflammation and lung remodeling by means of modification of cellular functions through regulation of cytokines and growth factors, such as TGF- β 1 and vascular endothelial growth factor.³⁰ Herein we studied the role of MMPs in the generation of soluble IL-13R α 2 and identified that MMP-8 specifically cleaves the membrane form of IL-13R α 2 to yield soluble IL-13R α 2 in bronchoalveolar lavage fluid (BALF) in house dust mite (HDM)-treated C57BL/6 wild-type mice *in vivo*. This correlates with decreased AHR in HDM-treated C57BL/6 wild-type mice.

METHODS

Animals

Animals were maintained and handled under institutional animal care and use committee–approved procedures. C57BL/6 MMP-8–deficient mice were kindly provided by Dr Steven D. Shapiro (University of Pittsburgh School of Medicine, Pittsburgh, Pa),³¹ and the wild-type C57BL/6 and FVB/N mice were purchased from Jackson Laboratories (Bar Harbor, Me). HDM (*Dermatophagoides pteronyssinus*) was purchased from Greer Laboratories (Lenoir, NC). The mice were immunized as previously described.²¹ Two days after the last challenge, the AHR in response to acetylcholine (50 µg/kg) was measured as the airway pressure–time index (APTI), as previously described.³² After APTI measurement, blood, bronchoalveolar lavage (BAL) samples, and lung tissues were harvested. The BAL total and differential cell counting and hematoxylin and eosin or periodic acid–Schiff staining of lung tissue sections were done as previously described.³³

Multiprobe RNase protection assay

Total RNA was isolated from murine lungs by using Trizol (Invitrogen Corp, Carlsbad, Calif), digested with RNase-free DNase, and purified with the RNeasy kit from Qiagen, Inc (Valencia, Calif). The mRNA expression of MMPs was determined by using the Multi-Probe RNase Protection Assay System and Template Set mMMP-2 (BD Biosciences, Mississauga, Ontario, Canada) with 20 µg of sample RNA, as described by the manufacturer.

Acellular cleavage assay

The coding region of the mature murine or human IL-13R 2 cDNA (mIL-13R 2 or hIL-13R 2) was amplified with a FLAG tag added at the C-terminus and cloned into *Sma*I and *Xho*I sites of the pGEX-KG vector to express glutathione-S-transferase (GST)–IL-13R 2–FLAG fusion protein.³⁴ The GST fusion protein was purified as previously described.³⁵ For cleavage assay by MMPs, in 20 µL of reactions, 500 ng of GST-fusion protein was incubated with 0.5 µg/mL MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9 (EMD Biosciences, Inc, San Diego, Calif), or MMP-12 (Sigma-Aldrich Corp, St Louis, Mo) at 37°C for 1 hour. For the time-course study, the GST fusion protein was incubated with MMP-8 for 0.5 to 24 hours. For the MMP-8 inhibitor assay, the MMP-8 inhibitor or MMP-8 inhibitor negative control (5, 50, or 500 nmol/L; EMD Biosciences) was used. Western blot analysis was performed by using anti-FLAG, anti-GST (Sigma-Aldrich), or anti-IL-13R 2 (R&D Systems, Inc, Minneapolis, Minn) antibodies. The proteins in SDS-PAGE gel were visualized by staining with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Hercules, Calif). Human collagen type I dialyzed against PBS and anti-human collagen type I antibody (Millipore Corp, Billerica, Mass) were used as a positive control for the MMP-8 inhibitor assay.

Cells

Cells overexpressing murine or human IL-13R 2 were previously described.^{21,36}

MMP-8 treatment

Untransfected WEHI cells, mIL-13R 2–transfected WEHI cells, U937 cells, and hIL-13R 2–transfected U937 cells were cultured in 10% FBS Dulbecco's modified Eagle's medium or RPMI-1640 medium at 37°C for 1 hour in the absence or presence of MMP-8 (0.2 µg/mL). This treatment did not affect the cell viability, as determined by means of trypan blue staining. The culture medium was analyzed by means of ELISA for soluble

IL-13R α 2 or IL-13 binding, and the cells were analyzed by means of flow cytometry for surface expression of IL-13R α 2.

ELISA

All antibodies were purchased from R&D Systems. Soluble IL-13R α 2 levels were determined by means of ELISA, as previously described.^{21,36} For IL-13 binding analysis, the plate was coated with 1 μ g/mL anti-mouse IL-13R α 2 polyclonal antibody, and the standards and samples were preincubated with 10 ng/mL mouse IL-13 (PeproTech, Rocky Hill, NJ) at 37°C for 1 hour. Biotinylated anti-mouse IL-13 polyclonal antibody (0.2 μ g/mL) was used as the detection antibody. For free and IL-13R α 2-bound IL-13 assay, the standards and samples were preincubated with 10 ng/mL murine IL-13R α 2-Fc (R&D Systems) at 37°C for 1 hour. Anti-mouse IL-13 polyclonal antibody (1 μ g/mL) and biotinylated anti-mouse IL-13R α 2 polyclonal antibody (0.5 μ g/mL) were used for capture and detection, respectively.

Flow cytometry

Surface IL-13R α 2 expression was assessed by means of flow cytometry, as previously described.¹⁷ The cells were incubated with goat anti-mouse IL-13R α 2 antibody or anti-human IL-13R α 2 antibody (R&D Systems) or normal goat IgG, detected with fluorescein isothiocyanate-conjugated rabbit anti-goat antibody (Santa Cruz Biotechnology) and analyzed in a FACSCalibur (BD Biosciences).

Statistical analysis

All values are expressed as the mean $\pm$ SD, and the data were analyzed with an unpaired 2-tailed Student *t* test with the Welch correction by using Microsoft Excel software (Microsoft Corp, Redmond, Wash). A *P* value of less than .05 was considered statistically significant.

RESULTS

Increased levels of soluble IL-13R α 2 in serum and BALF and MMP expression in lungs in a murine model of asthma

We characterized soluble IL-13R α 2 levels in the serum and BALF in mice sensitized and challenged with HDM. As shown in Fig 1, *A* and *B*, the level of soluble IL-13R α 2 was increased in both serum and BALF in HDM-treated mice compared with that seen in PBS-treated mice. To determine the expression of different MMPs in the murine lungs after HDM sensitization and challenge, we performed an RNase protection assay to examine mRNA expression for murine MMPs. As shown in Fig 1, *C*, HDM sensitization and challenge induced significant expression of MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-12 compared with PBS treatment.

Acellular cleavage of IL-13R α 2 protein by MMPs

Because soluble IL-13R α 2 levels were increased in both BALF and serum and MMPs were strongly induced in HDM-treated mice compared with PBS-treated mice, we tested whether MMPs could directly cleave full-length IL-13R α 2 by using GST-IL-13R α 2-FLAG fusion proteins. Among the panel of MMPs tested, only MMP-8 was able to cleave murine GST-IL-13R α 2-FLAG fusion protein, as determined by means of Western blotting and Coomassie blue staining (Fig 2, *A–C*). No cleavage of GST protein by MMP-8 was observed. To further determine the specificity of the MMP-8 cleavage, we used an MMP-8 inhibitor, as shown in Fig 2, *D*. The cleavage of IL-13R α 2 fusion protein by MMP-8 was inhibited by MMP-8 inhibitor (500 nmol/L), whereas there was no inhibition by a mock MMP-8 inhibitor control. Human collagen type I was included as a positive control because

MMP-8 is known to cleave human collagen type I. The MMP-8 inhibitor inhibited cleavage of the GST–IL-13R 2 fusion protein, as well as human collagen type I, although the inhibition efficiency of collagen cleavage was higher (Fig 2, *E*). MMP-8 was also able to cleave the human GST–IL-13R 2–FLAG fusion protein (Fig 2, *F*). We also used baculovirus-expressed proteins for the cleavage assays and found that only MMP-8 was able to cleave IL-13R 2 (data not shown).

Solubilization of cell-surface IL-13R α 2

To study the effect of MMP-8 on the solubilization of IL-13R 2 from the cell surface, we generated stably transfected cell lines expressing either murine or human FLAG–IL-13R 2, respectively. As shown in Fig 3, *A*, treatment of mIL-13R 2–transfected WEHI cells with MMP-8 resulted in an increased amount of soluble IL-13R 2 in the culture medium compared with that seen in the untreated cells. Untransfected cells had minimal soluble IL-13R 2 in their supernatants. Similarly, we observed solubilization of IL-13R 2 from hIL-13R 2–transfected U937 cells after MMP-8 treatment (Fig 3, *B*). Thus MMP-8 treatment resulted in release of soluble IL-13R 2 from the cell surface of both murine and human cells.

Because IL-13R 2 was released from cells after MMP-8 treatment, we next examined whether the surface level of IL-13R 2 was altered. Surface IL-13R 2 expression was decreased after MMP-8 treatment on mIL-13R 2–transfected WEHI cells (Fig 3, *C*) and hIL-13R 2–transfected U937 cells (Fig 3, *D*) compared with untreated cells, which was consistent with the observation that soluble IL-13R 2 levels are increased. Combined, these data demonstrate that MMP-8 cleaves both murine and human IL-13R 2 expressed on cell membrane.

IL-13 binding of soluble IL-13R α 2 released by MMP-8 treatment

We next determined whether soluble IL-13R 2 released from the cell surface after MMP-8 treatment retained IL-13 binding activity. Fig 4, *A*, shows that the IL-13 binding was increased in the culture medium from mIL-13R 2 transfectants after MMP-8 treatment, indicating the soluble IL-13R 2 generated by MMP-8 cleavage retained the IL-13 binding activity. However, we observed variation in this finding and observed no differences in IL-13 binding activity in some experiments. A kinetic analysis of MMP-8–mediated cleavage of murine IL-13R 2–GST fusion proteins revealed that the continued MMP-8 treatment resulted in further cleavage and degradation of the initially generated soluble IL-13R 2 (Fig 4, *B*). This might result in secondary loss of IL-13 binding from the MMP-8–generated soluble IL-13R 2 and explain the observed variation.

Soluble IL-13R α 2 levels in BALF were decreased in HDM-treated MMP-8–deficient mice

To elucidate the role of MMP-8 in the generation of soluble IL-13R 2 *in vivo*, we treated C57BL/6 wild-type and MMP-8–deficient mice with HDM and measured AHR, airway inflammation, and soluble IL-13R 2 levels in BALF. As shown in Fig 5, *A*, AHR was increased in HDM-treated MMP-8–deficient mice compared with that seen in the HDM-treated wild-type mice, as determined by means of APTI measurement. There was no difference in the total cell and eosinophil numbers between the HDM-treated MMP-8–deficient mice and the HDM-treated wild-type mice, but there was a decrease in neutrophils in BAL samples from HDM-treated MMP-8–deficient mice compared with that seen in HDM-treated wild-type mice (Fig 5, *B*). Airway inflammation and mucus production were unchanged in HDM-treated MMP-8–deficient mice compared with that seen in the wild-type mice, as assessed by means of hematoxylin and eosin and periodic acid–Schiff staining of lung tissue sections (data not shown). Next we determined the IL-13 binding activity of soluble IL-13R 2 in BALF and serum in these mice. As shown in Fig 5, *C*, the level of

soluble IL-13R 2 in BALF was decreased in HDM-treated MMP-8-deficient mice compared with that seen in HDM-treated wild-type mice. No significant difference was observed in serum IL-13R 2 levels (data not shown). The IL-13 binding activity of soluble IL-13R 2 in BALF was also decreased in HDM-treated MMP-8-deficient mice compared with that seen in HDM-treated wild-type mice (Fig 5, D). We also examined total and free IL-13 levels in the BALF to determine whether the change in IL-13R 2 level resulted in any change in detectable IL-13. We did not observe differences in the levels of total or free IL-13 in BALF from HDM-treated MMP-8-deficient mice versus those from HDM-treated wild-type mice (Fig 5, E).

DISCUSSION

Soluble cytokine receptors have important roles in the regulation of cell-signaling pathways. 37–39 Shedding of membrane-bound receptors has been shown as a mechanism to generate soluble receptors.^{24,25} In this article we demonstrated that soluble IL-13R 2 can be produced by means of direct cleavage by MMP-8 and that MMP-8 contributes to the solubilization of IL-13R 2 in BALF in HDM-treated mice.

MMPs have been shown to be important mediators of airway inflammation and remodeling. 40 Among them, MMP-8 has been found to be increased in BALF from patients with chronic obstructive pulmonary disease, asthma, or bronchiectasis.^{41–43} In a murine model of airway inflammation, we observed that the expression of several MMPs was increased. After HDM treatment, the most prominently increased MMP was MMP-12, whereas the expression of other MMPs, including MMP-1, MMP-7, MMP-8, and MMP-9, was also increased. Similar to MMPs, the level of soluble IL-13R 2 is also increased during allergic inflammation in the BALF and serum. We tested the ability of MMPs to directly cleave IL-13R 2. Only MMP-8 was able to cleave IL-13R 2. MMP-8 was able to cleave both murine and human mature full-length IL-13R 2. IL-13R 2 proteins expressed and purified from a baculovirus expression system were cleaved by MMP-8 in a manner similar to that for proteins expressed in bacteria, supporting that glycosylation did not affect the ability of MMP-8 to cleave IL-13R 2. However, because the baculovirus expression system is defective in some aspects of glycosylation, the effect of glycosylation on MMP-8 cleavage of IL-13R 2 warrants further investigation.^{44,45} We used an inhibitor of MMP-8 to further examine the specificity of the *in vitro* cleavage assay. The MMP-8 inhibitor inhibited cleavage of the GST-IL-13R 2 fusion protein, as well as human collagen type I. However, the inhibition efficiency of the cleavage of collagen was higher. MMP-8 (purchased from EMD Biosciences) is isolated from stimulated human neutrophils, and although it is 90% or more pure and does not have any detectable other MMP activity, we cannot rule out the possibility that there might be other contaminating proteases. Thus we cannot rule out that another protease might be contributing to the cleavage of IL-13R 2. Therefore our data demonstrate that MMP-8 contributes but is not solely responsible for IL-13R 2 cleavage. In fact, this is evident from the *in vivo* data.

MMP-8 treatment resulted in increased IL-13R 2 levels in the supernatants and a corresponding decrease in IL-13R 2 levels on the cell membrane. The observed decrease in surface IL-13R 2 levels was modest, which is not surprising because we have observed that surface IL-13R 2 levels are maintained relatively constant as a result of mobilization of intracellular pools.³⁶ It has been reported that membrane-bound MMP-8 expressed on the cell surface is more catalytically stable than soluble MMP-8, which might be a more important form of MMP-8.⁴⁶ Also, there are many proteases that likely contribute to solubilization of IL-13R 2. We recently demonstrated that proteolytic allergens, including HDM, can directly cleave IL-13R 2.³⁵ It is likely that both HDM and MMP-8 contributed to the proteolytic cleavage of IL-13R 2 in HDM-treated mice. While this manuscript was in

review, Matsumura et al⁴⁷ reported that endogenous MMP(s) solubilize IL-13R₂ in airway epithelial cells. Thus there are several pathways that lead to IL-13R₂ solubilization. Our observation that soluble IL-13R₂ levels are decreased in MMP-8-deficient mice compared with wild-type mice supports that MMP-8 is an important endogenous regulator of IL-13R₂ *in vivo*.

The MMP-8-generated soluble IL-13R₂ retained IL-13 binding ability. It is likely that there are several proteolytic cleavage sites in IL-13R₂, and MMP-8 might have preference for multiple sites. The initially cleaved IL-13R₂ has intact IL-13 binding; however, continued MMP-8 exposure leads to additional cleavage and degradation of soluble IL-13R₂. The site of MMP-8 cleavage of IL-13R₂ is not yet known. We tested mutated human IL-13R₂ in the juxtatransmembrane region by means of deletion or replacement of 13 amino acid residues (WE-GEDLSKKTLLR), and the cleavage of the mutated receptor GST fusion protein *in vitro* by MMP-8 was unaffected (data not shown). Additional studies are needed to determine the cleavage site in IL-13R₂ for MMP-8.

To study the role of MMP-8 cleavage of IL-13R₂ *in vivo*, we used a murine model of asthma to determine the effect of MMP-8 deficiency on the solubilization of IL-13R₂. In this murine model the AHR was enhanced in MMP-8-deficient mice after HDM challenge, which is similar to a previous report in an ovalbumin-induced murine model.⁴⁸ We observed that the neutrophil infiltration in the airways was decreased in HDM-treated MMP-8-deficient mice compared with that seen in the HDM-treated wild-type mice. It has been reported that the neutrophil infiltration in murine wound healing is delayed in MMP-8-deficient mice.⁴⁹ Thus the relative level of neutrophils in the airways after HDM treatment would be determined by the time period after the treatment. We found that soluble IL-13R₂ levels in BALF were decreased in HDM-treated MMP-8-deficient mice compared with those in HDM-treated wild-type mice, suggesting that the HDM-induced MMP-8 contributes to the solubilization of IL-13R₂ in BALF. The fact that we did not observe a similar change in serum suggests that the majority of soluble IL-13R₂ in serum might be generated by the alternatively spliced isoform encoding the soluble IL-13R₂, which was induced by HDM treatment.²¹ IL-13 binding activity of soluble IL-13R₂ in BALF from HDM-treated MMP-8-deficient mice was also decreased compared with that seen in the HDM-treated wild-type mice, demonstrating that MMP-8 contributes to the generation of soluble IL-13R₂ with IL-13 binding activity. Not surprisingly, we did not observe any change in total IL-13 levels. We anticipated that we might observe an increase in free IL-13 levels in BALF from HDM-treated MMP-8-deficient mice compared with that seen in HDM-treated wild-type mice because soluble IL-13R₂ levels are decreased in the BALF. However, we did not observe a difference in free IL-13 levels. It is technically difficult to accurately assess free IL-13 levels because free IL-13 has a short half-life compared with bound IL-13. Thus we cannot reliably make a conclusion regarding free IL-13 in these experiments. In contrast to the mouse, no alternatively spliced isoform encoding soluble IL-13R₂ has been identified in human subjects. Whether MMP cleavage plays a role in the generation of soluble IL-13R₂ in the human subject remains to be determined.

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Abbreviations used

AHR Airway hyperresponsiveness

APTI	Airway pressure–time index
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
GST	Glutathione-S-transferase
HDM	House dust mite
IL-13R 2	IL-13 receptor 2
MMP	Matrix metalloproteinase

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FIG 1.

Levels of soluble IL-13R α 2 in serum and BALF and the expression of MMPs in lungs from PBS- or HDM-treated FVB/N mice. **A** and **B**, Soluble IL-13R α 2 in serum and BALF. Data were shown as means $\pm$ SD (n = 4–5). * P < .05. The result is representative of 3 experiments. **C**, RNase protection assay of the RNA from the lungs of PBS- or HDM-treated mice (n = 4). *TIMP*, Tissue inhibitor of metalloproteinases; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

FIG 2.

Acellular cleavage of GST-IL-13R 2-FLAG fusion protein by MMPs. **A**, Cleavage of GST-mIL-13R 2-FLAG. **B** and **C**, Cleavage of GST or GST-mIL-13R 2-FLAG by MMP-8. **D** and **E**, Cleavage of GST-mIL-13R 2-FLAG or human collagen type I by MMP-8 in the presence or absence of an MMP-8 inhibitor or mock MMP-8 inhibitor control (5, 50, or 500 nmol/L). **F**, Cleavage of GST-hIL-13R 2-FLAG.

FIG 3.

Measurement of soluble IL-13R α2 in the culture medium and on the cell surface after MMP-8 treatment. Soluble IL-13R α2 levels are shown in panels **A** and **B**. IL-13R α2 surface expression is shown as mean channel fluorescence (*MCF*) in panels **C** and **D**. Data are shown as means ± SD ($n = 3-5$). * $P < .05$. The result is representative of 3 experiments.

FIG 4.

IL-13 binding of soluble IL-13R α 2 in culture medium from MMP-8-treated cells. The result is representative of 3 experiments. **A**, MMP-8 treatment of untransfected or mIL-13R α 2-transfected WEHI cells. The data are shown as means $\pm$ SD ($n = 5$). * $P < .05$. **B**, Western blot analysis of MMP-8 treatment of murine GST-IL-13R α 2-FLAG fusion protein for the indicated time (0.5–24 hours). U, Untreated.

FIG 5. AHR, airway inflammation, BALF soluble IL-13R 2 and its IL-13 binding activity, and BALF IL-13 of PBS- or HDM-treated wild-type and MMP-8-deficient mice. **A**, APTI (n = 6–8). **B**, BAL cell counting (n = 6–8). **C**, Soluble IL-13R 2 (n = 6–8). **D**, IL-13 binding activity of soluble IL-13R 2 (n = 12–17, pooled from 2 experiments). **E**, Free and IL-13R 2-bound IL-13 (n = 5). *WT*, Wild-type mice; *KO*, MMP-8-deficient mice. The data are shown as means ± SD. **P* < .05.