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Human eosinophils constitutively express a unique serine protease, PRSS33

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Abbreviations:
Ab, antibody; ADAM, a disintegrin and metalloprotease; CCR, CC chemokine receptor; COL, collagen; DAPI, 4,6-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; ECP, eosinophil cationic protein; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; GM-CSF, granulocyte macrophage colony-stimulating factor; HSA, human serum albumin; IFN-γ, interferon-γ; Ig, immunoglobulin; IL-, interleukin-; IMEM, Iscove's minimum essential medium; MMP, metalloproteinase; PAR-2, protease-activated receptor-2; PBMCs, peripheral blood mononuclear cells; PIC, protease inhibitor cocktail; PRSS33, serine protease 33; qPCR, quantitative polymerase chain reaction; sIgA, secretory IgA; TGF-β1, transforming growth factor-β1

ABSTRACT

Background: Eosinophils play important roles in asthma, especially airway remodeling, by producing various granule proteins, chemical mediators, cytokines, chemokines and proteases. However, protease production by eosinophils is not fully understood. In the present study, we investigated the production of eosinophil-specific proteases/proteinases by transcriptome analysis.
Methods: Human eosinophils and other cells were purified from peripheral blood by density gradient sedimentation and negative/positive selections using immunomagnetic beads. Protease/proteinase expression in eosinophils and release into the supernatant were evaluated by microarray analysis, qPCR, ELISA, flow cytometry and immunofluorescence staining before and after stimulation with eosinophil-activating cytokines and secretagogues. mRNAs for extracellular matrix proteins in human normal fibroblasts were measured by qPCR after exposure to recombinant protease serine 33 (PRSS33) protein (rPRSS33), created with a baculovirus system.
Results: Human eosinophils expressed relatively high levels of mRNA for metalloproteinase 25 (MMP25), a disintegrin and metalloprotease 8 (ADAM8), ADAM10, ADAM19 and PRSS33. Expression of PRSS33 was the highest and eosinophil-specific. PRSS33 mRNA expression was not affected by eosinophil-activating cytokines. Immunofluorescence staining showed that PRSS33 was co-localized with an eosinophil granule protein. PRSS33 was not detected in the culture supernatant of eosinophils even after stimulation with secretagogues, but its cell surface expression was increased. rPRSS33 stimulation of human fibroblasts increased expression of collagen and fibronectin mRNAs, at least in part via protease-activated receptor-2 activation.
Conclusions: Activated eosinophils may induce fibroblast extracellular matrix protein synthesis via cell surface expression of PRSS33, which would at least partly explain eosinophils' role(s) in airway remodeling.

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Introduction

The pathogenesis of asthma is characterized by repeated exacerbation of type 2 inflammation due to exposure to allergens, viral infection and so on. Recurrent and/or chronic type 2 inflammation reportedly induces structural changes in the lung (so-called airway remodeling), including goblet cell hyperplasia, basement membrane thickening, smooth muscle hypertrophy/hyperplasia, tissue fibrosis and hypervascularity.[1–4] At least some components of this airway remodeling are steroid-insensitive,[5] and airway remodeling causes early lung function decline. Prevention of airway remodeling is one of the major unmet needs in current asthma practice.[6]

Both leukocytes and tissue resident cells are involved in airway remodeling through complicated interactions among cytokines, chemokines, chemical mediators and proteases/proteinases. Eosinophils are known to play important roles in the pathogenesis of asthma, especially in airway remodeling.[6–8] Eosinophils produce and release several growth factors, including vascular endothelial growth factor (VEGF),[9] transforming growth factor-β1 (TGF-β1)[10] and amphiregulin,[11] and some proteases.

Proteases/proteinases not only facilitate replacement of soft tissue extracellular matrix proteins (EMP) with hard EMP, but also directly activate protease-activated receptor-2 (PAR-2) to trigger proliferation of airway smooth muscle cells.[12,13] To date, various proteases/proteinases have been reported, but only a few—such as matrix metalloproteinase-9 (MMP-9)[14] and MMP17[15]—have been reported to be produced by human eosinophils. Recently, eosinophil-targeted intervention therapy for bronchial asthma using anti-IL-5 or anti-IL-5R mAbs was approved. In that context, there is a need for a better understanding of the proteases produced specifically by eosinophils.

In the present study, we investigated the mRNA expression profiles of all proteases/proteinases in human eosinophils and other leukocytes by transcriptome analysis.

Methods

Reagents

All culture reagents were purchased from Life Technologies (Grand Island, NY) unless otherwise noted. Recombinant human IL-5 was purchased from R&D Systems (Minneapolis, MN). Recombinant human granulocyte-colony stimulating factor (GM-CSF), IFN-γ and macrophage-colony stimulating factor (M-CSF) were purchased from PeproTech (Rocky Hill, NJ).

Recombinant human serine protease 33 (PRSS33) was synthesized at Sysmex Corporation (Kanagawa, Japan) using a baculovirus gene expression system based on the reference sequence (NM_152891.2).

Isolation of leukocytes

Each type of leukocyte was isolated from peripheral blood of both healthy and mildly allergic donors ($n = 10$) by density gradient sedimentation using Lymphocyte Separation Medium (Wako Pure Chemical Industries, Osaka, Japan) or Percoll (GE Healthcare, Piscataway, NJ), and also by positive and/or negative selection using immunomagnetic beads (Miltenyi Biotec, Bergisch-Gladbach, Germany), as described previously.[11,16] The purity of eosinophils based on light microscopic examination of cytocentrifuge preparations using Cytospin (Shandon, Pittsburgh, PA) and staining with Diff-Quik (American Scientific Products, McGraw Park, IL) always exceeded 98%. Eosinophil viability always exceeded 99% by trypan blue (Sigma) dye exclusion. The purity of other types of blood cells always exceeded 95%.

Culture of eosinophils

Purified eosinophils were suspended at a cell density of 1×10^6 cells/ml in Iscove's minimum essential medium (IMEM), supplemented with 10% heat-inactivated fetal calf serum (FCS; Equitech-Bio, Kerrville, TX), 5×10^{-5} M 2-mercaptoethanol and an antibiotics mixture (10 units/ml penicillin G and 10 μg/ml streptomycin; Nacalai Tesque, Kyoto, Japan). The cells were cultured in PBS at 4 °C overnight in 24-well flat-bottom plastic plates (IWAKI, Tokyo, Japan) pre-coated with 1% heat-denatured human serum albumin (HSA; Sigma–Aldrich, St. Louis, MO) to reduce non-specific adherence of eosinophils to the plates.[17] To examine the effects of various stimulants, the cells were cultured in the presence and absence of various concentrations of IL-5, 10 ng/ml GM-CSF or 10 ng/ml IFN-γ at 37 °C for 6 h. In some experiments, the cells were cultured at 4 °C overnight in 96-well flat-bottom plates (IWAKI) coated with 100 μg/ml secretory IgA (sIgA; ICN Biomedicals, Aurora, OH). After washing the wells, 0.2 ml of 1% heat-denatured HSA in PBS was added to each well, and the plates were incubated at 4 °C for at least 2 h before use.[11]

Preparation of monocyte-derived macrophages

Monocyte-derived macrophages were obtained as described previously.[18] Briefly, PBMCs were suspended at a density of 2×10^6 cells/ml in RPMI 1640 medium (Nacalai Tesque) supplemented with 10% FCS in T75 flasks (IWAKI) and incubated at 37 °C for 1 h. The adherent cells (mainly monocytes) were obtained after removal of non-adherent cells by gentle pipetting and washed once with PBS. To obtain macrophages, the adherent cells were then cultured in the presence of 10 ng/ml M-CSF in T75 flasks at 37 °C for 7 days.

Macrophage-like U-937 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA). The U-937 cells were subcultured twice per week in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 10 mM Hepes buffer, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 1.0 mM sodium pyruvate and the previously described antibiotics mixture at 37 °C in a 5% CO_2 incubator. For assay, these cells were treated with 160 nM phorbol 12-myristase 13-acetate (PMA; Sigma–Aldrich, St. Louis, MO) for 2 or 5 days, as described previously by others.[19]

Culture and stimulation of fibroblasts

Human nasal fibroblasts were obtained from normal mucosal membranes of the sphenoid sinus removed during surgery for pituitary adenoma as previously described,[20] with slight modification. In brief, extensively dissected nasal tissue pieces were cultured in Dulbecco's modified Eagle's medium/F-12 (DMEM/F12) medium supplemented with 10% FCS and the antibiotics mixture without digestive enzymes. Cultured cells were analyzed between the third and eighth passages. The fibroblasts (1×10^5 cells/ml) were cultured in DMEM/F12 supplemented with an antibiotics mixture, but without FCS, one day before planned stimulation. On the next day, the fibroblasts were cultured in the presence and absence of 25 ng/ml recombinant human PRSS33, a 0.1% protease inhibitor cocktail (PIC) (Sigma–Aldrich) or 10 μM FSLLRT-NH_2, a PAR-2 antagonist (Tocris, Ellisville, MO), at 37 °C for 24 h. The cells were then harvested, and the total RNA was extracted.

Microarray analysis

Transcriptome analysis using a microarray system was performed as described previously.[21,22] Briefly, total RNA from leukocytes, excluding eosinophils, was extracted and then digested using