Increased Expression of Matrix Metalloproteinase in Clara Cell-Ablated Mice Inhaling Crystalline Silica

Kazuhiro Yatera,1 Yasuo Morimoto,2 Heung-Nam Kim,3 Hiroshi Yamato,3 Isamu Tanaka,3 and Masamitsu Kido1

1Department of Respiratory Disease, 2Department of Occupational Pneumology, and 3Department of Environmental Health Engineering, University of Occupational and Environmental Health, Japan, Kitakyushu City, Fukuoka Prefecture, Japan

We investigated the function of Clara cells in vivo during exposure to inhaled crystalline silica by examining pulmonary matrix metalloproteinase (MMP)-2 and MMP-9 mRNA levels in mice. The Clara cells of male FVB/n mice (8–12 weeks old) were ablated by intraperitoneal administration of naphthalene (300 mg/kg) in a corn oil vehicle. The mice were then exposed to crystalline silica (Mn-U-Sil-5 silica, 97.1 ± 9.5 mg/m2, 6 hr/day, 5 days/week) for up to 2 weeks. Transcriptional levels of mRNA extracted from the lungs were assessed by reverse transcription-polymerase chain reaction. Gene expression of both MMP-2 and MMP-9 was significantly more marked in the Clara cell-ablated group than in the group with Clara cells, indicating that Clara cells inhibit MMP expression. Our findings suggest that Clara cells inhibit pulmonary inflammation induced by crystalline silica via MMPs in vivo. Key words: Clara cell, crystalline silica, matrix metalloproteinase, naphthalene, reverse transcription-polymerase chain reaction. Environ Health Perspect 109:795–799 (2001). [Online 3 August 2001] http://ehpnet1.niehs.nih.gov/docs/2001/109p795-799yatera/abstract.html

Clara cells are nonciliated, nonmucous secreting cells localized mostly in the bronchiolar surface epithelium, and they are one of the most multifunctional and heterogeneous cell types in the mammalian lung (1). Clara cells appear to play a role in pulmonary inflammation and fibrosis in the distal airways by serving as progenitors of bronchial epithelial cells (2–5). Clara cells are particularly likely to undergo apoptosis (6,7) when alveolar and bronchial epithelial cell apoptosis induces pulmonary fibrosis (8).

Clara cells are reported to have an inhibitory effect on pulmonary inflammation and fibrosis. For example, they secrete Clara cell secretory protein (CCSP) (9), which inhibits pulmonary inflammation (10); surfactant proteins, which prevent alveolar collapse (11–15); and protease inhibitors such as secretory leukocyte protease inhibitor (SLPI) (16–18) and elafin (17,18). Clara cells can also promote pulmonary inflammation and fibrosis. Of the different cell types identified in the lung, Clara cells appear to have the highest level of cytchrome P450 monooxygenases (19). Studies have also shown that the metabolic products of these monooxygenases can damage bronchial epithelial cells and that most bronchial epithelial cells produce various chemokines and proinflammatory cytokines during bronchial injury (20–23). Attention has therefore focused on the balance between the inhibitory and promotional effects of Clara cells in the process of pulmonary inflammation and fibrosis in vivo.

Plopper et al. (24–26) and many other researchers have created a mouse model in which Clara cells are selectively ablated with naphthalene, allowing the general role of Clara cells in pulmonary inflammation and fibrosis to be analyzed in vivo.

Various factors are involved in pulmonary inflammation and fibrosis. Like free radicals, matrix-degrading matrix metalloproteinase (MMP) enzymes directly cause airway and pulmonary injury and inflammation, but they also play an important role in repair (27). Increased expression of MMP-2 and MMP-9, enzymes that degrade type IV collagen and elastin, major structural components of the basement membrane, has been observed in the acute stages of pulmonary inflammation in animal studies (28). Expression of these MMPs is also increased in various types of inflammatory lung diseases in humans, such as bronchiolitis obliterans organizing pneumonia and idiopathic pulmonary fibrosis (29). These findings suggest that MMP-2 and MMP-9 are closely involved in inflammation and fibrosis.

In the present study, we investigated the role of Clara cells in inflammation and fibrosis in vivo by exposing Clara cell-ablated mice to crystalline silica, which is known to cause pulmonary fibrosis (30–33), and examined the gene expression of MMP-2 and MMP-9 in lung tissue using reverse transcription-polymerase chain reaction (RT-PCR).

Materials and Methods

Animals. Male FVB/n mice (8–12 weeks of age), as described previously (3,34), were used in the study. Age-matched mice were purchased from CLEA Japan, Inc. (Tokyo, Japan).

All of the animals were maintained according to the Guidelines for Animal Experimentation of the University of Occupational and Environmental Health, Japan, and the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (Washington, D.C., USA).

Creation of Clara cell-ablated mice. Naphthalene (300 mg/kg body weight) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), dissolved in a corn oil vehicle, and injected intraperitoneally. Control mice were injected with vehicle only.

Inhalation of crystalline silica. Crystalline silica (Min-U-Sil-5 silica; U.S. Silica, Berkeley Springs, W.V., USA) was used in the study. The mice were housed in an exposure chamber and exposed to crystalline silica for 6 hr/day, 5 days/week for up to 2 weeks. The mass concentration of the crystalline silica was 97.1 ± 9.5 mg/m2 and was measured gravimetrically at 1-day intervals by the suction of air through a glass filter. Both the groups of wild-type mice and that of Clara cell-ablated mice were exposed to crystalline silica or fresh air. Each group, composed of five animals, was sacrificed after 1, 3, 7, and 14 days of exposure.

Preparation of RNA, cDNA synthesis, and polymerase chain reaction. RNA was extracted from the lung using guanidine thiocyanate-phenol-chloroform. Total RNA (0.5 μg) was used to synthesize single-strand cDNA with Moloney murine leukemia virus-derived reverse transcriptase (Perkin Elmer, Norwalk, C.T., USA). An equal amount of cDNA from each sample, standardized to give identical signals on the gel following amplification with β-actin primer, was amplified by specific primers for each gene (Table 1). The amplification was performed with a Thermocycler (Astech, Fukuoka, Japan) under the following conditions: denaturation at 94°C for 45 sec, annealing at 60°C for 45 sec, and extension at 72°C for 2 min. PCR products were analyzed by electrophoresis on a 2% agarose gel. The 26-bp product served as a control for cDNA synthesis.

Received 22 September 2000; accepted 15 February 2001.
min for MMP-2, MMP-9, and β-actin genes. β-actin was amplified as an internal standard in a quantitative polymerase chain reaction (PCR) amplification of mRNA.

The fragment amplified by PCR was detected by electrophoresis on a 2% agarose gel with DNA markers and was visualized by ethidium bromide staining. The gels were photographed with Polaroid Type 665 positive/negative film (Polaroid Corp., Cambridge, MA, USA) under ultraviolet light at identical exposure and development times. The bands of the positive film were scanned, and the density of each PCR product was measured using National Institutes of Health (NIH) Image 1.61 software (written by W. R. Rasband, National Institutes of Health, Bethesda, MD, USA). To quantify the transcriptional level of mRNA, the data were normalized to represent equivalent RNA loading based on the density of the β-actin product at the appropriate cycle of a given gene product.

Statistical analysis. Values are expressed as the mean ± standard error. We assessed the difference between values using the Mann-Whitney U test. p-Values < 0.05 were considered significant.

Histopathology and immunohistochemistry. The inflation-fixed lungs from the mice were washed in phosphate-buffered saline (PBS) three times and fixed in 10% buffered formalin. Paraffin-embedded specimens were sectioned at 5 µm and stained with hematoxylin and eosin for morphologic analysis by microscopy. For immunohistochemical staining for MMP-2 and MMP-9, the tissue sections were deparaffinized by washing in xylene four times for 10 min per wash, followed by dehydration by a series of 100% to 70% ethanol washes. The slides were placed in methanol containing 0.5% hydrogen peroxide to block endogenous peroxidase activity. Nonspecific binding was blocked by incubating the slides 0.1M-PBS for 1 hr at room temperature.

We incubated the lung sections overnight at 4°C with either affinity-purified goat polyclonal antibodies specific for MMP-2 and MMP-9 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; both at dilutions of 0.4 µg/mL) or rabbit anti-rat polyclonal antiserum specific for CCSP (courtesy of Gurmukh Singh, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; diluted 1:500–1:1000 before use). The sections were rinsed five times in 0.1 M PBS and incubated for 30 min at room temperature with biotinylated anti-goat secondary antibody made in rabbits (Vector Laboratories Inc., Burlingame, CA, USA) for MMP-2 and MMP-9, and anti-rabbit secondary antibody (DAKO JAPAN Co., Ltd., Kyoto, Japan) for CCSP, then incubated with avidin-biotin complex (DAKO JAPAN Co., Ltd.) for 30 min at room temperature. Sections were washed with PBS, rinsed briefly in 0.1 M acetate buffer (pH 6.0), incubated with diaminobenzidine for 3 min, and counterstained with hematoxylin. Incubations were carried out without the primary or secondary antibody as a labeling control.

Results

Immunohistochemistry for CCSP. Figure 1 shows that immunostaining patterns for CCSP 14 days after the intraperitoneal injection of naphthalene (Figure 1B) or corn oil vehicle only (Figure 1A) to the silica nonexposed group. The Clara cells were most ablated 3 days after the exposure (data not shown), and Figure 1B shows that >70% of ablation continued 14 days after the exposure. Clara cells remained at the bifurcation of the bronchiole throughout the period.

MMP-2. The results for MMP-2 are shown in Figure 2. There was no difference in expression of MMP-2 between the unexposed groups of wild-type mice and that of Clara cell-ablated mice. MMP-2 expression was increased in both the Clara cell-ablated and nonablated groups exposed to crystalline silica, and comparison of these groups showed that the MMP-2 expression tended to be higher in the Clara cell-ablated group from the 3-day exposure and that the increase was statistically significant after 7 and 14 days of exposure.

MMP-9. The results for MMP-9 are shown in Figure 3. There was no difference in expression of MMP-9 between the unexposed groups of wild-type mice and that of Clara cell-ablated mice. In the two crystalline silica-exposed groups, the MMP-9 expression increased after 7 days of exposure and was statistically significantly higher in the Clara cell-ablated group than in the group of mice with Clara cells after 14 days of exposure.

Histopathology and immunohistochemistry. Histopathologic examination using hematoxylin and eosin staining revealed an increase in macrophage numbers after exposure in both the Clara cell-ablated and the nonablated groups. Of the two groups exposed, foamy macrophages were seen in the Clara cell-ablated group but not in the nonablated group (data not shown). No marked accumulation of crystalline silica was evident.

Table 1. Oligonucleotides of primers of target genes.

| mRNA species | mRNA Cycle bp |
|--------------|---------------|
| MMP-2 (sense) 5'-GAGATCTGCAAACAGGACAT 26 476 |
| (antisense) 5'-GGTTCTCCAGCTTCAGGTAA |
| MMP-9 (sense) 5'-CGACGAGTTGTGGTCGCTGG 33 624 |
| (antisense) 5'-GCACGCTGGAATGATCTGAG |
| β-actin (sense) 5'-ATCATGTTTGAGACCTTCAACACC 22 357 |
| (antisense) 5'-TAGCTTCTTCCAGGGGAGG |

Figure 1. Immunohistochemistry for CCSP. (A) Mouse with Clara cells 14 days after intraperitoneal injection of corn oil vehicle only. (B) Clara cell-ablated mouse 14 days after intraperitoneal injection of naphthalene. Bar = 50 µm.
Immunostaining revealed the expression of MMP-2 and MMP-9 in alveolar macrophages, bronchiolar epithelial cells, alveolar epithelial cells, and vascular endothelial cells in both the Clara cell-ablated and the nonablated groups. The alveolar macrophages, bronchiolar epithelial cells, and vascular endothelial cells were particularly strongly stained (Figure 4). However, no newly strongly stained cell types were found after both crystalline silica exposure or Clara cell ablation.

**Discussion**

We ablated the Clara cells in mice using the cytoselective toxicity of the metabolic products of naphthalene mediated by cytochrome P450 monooxygenase (3,24,25,34). In a morphologic study of murine lungs using transmission electron microscopy, Plopper and colleagues (24–26) reported that intraperitoneally administered naphthalene is specifically toxic to Clara cells and does not cause changes in other epithelial cells, indicating that the toxicity of naphthalene to Clara cells is cytoselective.

Stripp et al. (34) reported that treatment with naphthalene at a concentration of 300 mg/kg results in the ablation of Clara cells in the bronchiolar region for at least 20 days in FVB/n mice (34), which seem to be more susceptible to naphthalene than do other mouse strains (35). Using the same method, we also confirmed that Clara cells are removed 1 day after naphthalene treatment and remain absent for at least 2 weeks (Figure 1B). The Clara cell-ablated mice were therefore exposed to crystalline silica for 2 weeks after naphthalene treatment.

In this study, the mice were exposed to a higher concentration of crystalline silica than in humans to investigate the role of Clara cells in the acute inflammatory response. We previously reported marked inflammatory cell accumulation and silica deposition in the lungs after intratracheal instillation of 2 mg crystalline silica in rats (36). The present histopathology findings using hematoxylin and eosin staining and phase-contrast microscopy of the lungs revealed an increase in inflammatory cell numbers in the alveoli, but showed no excessive deposition of crystalline silica in the lungs as was seen in our previous study. Although different animal species were used in the two studies, in the present study it was estimated that <2 mg of crystalline silica was deposited in the lungs after inhalation, suggesting that this dose might not be excessive. In long-term inhalation studies conducted to date, we found that the maximum amount of dust deposited in the lungs is approximately 2 mg regardless of particle type (37).

In the current study, gene expression of MMP-2 and MMP-9 in the lungs tended to increase after crystalline silica exposure. We are the first to investigate MMPs induced by inhaled crystalline silica. Increased MMP-2 and MMP-9 expression was observed in...
cytokines (MMP-9) inhibit gene expression of MMP-2 and present study. In addition to producing factors thatducted (40,41), proinflammatory cytokines (40,41), and chemokines (42,43), bronchial epithelial cells, including Clara cells, also produce factors that inhibit MMPs, although it is unclear which factors confirm that Clara cells generally inhibit expression, such as CCSP, SLPI (16–18), which the Clara cells had been ablated with naphthalene. Gene expression of MMP-2 and MMP-9 was significantly more marked in the Clara cell-ablated group than in the mice with Clara cells, indicating that Clara cells inhibited MMP-2 and MMP-9 expression. Our findings suggest that Clara cells inhibit pulmonary inflammation and fibrosis via MMPs in vivo.

In summary, we investigated gene expression of MMP-2 and MMP-9 in the lungs after inhalation of crystalline silica in mice in which the Clara cells had been ablated with naphthalene. Gene expression of MMP-2 and MMP-9 was significantly more marked in the Clara cell-ablated group than in the mice with Clara cells, indicating that Clara cells inhibited MMP-2 and MMP-9 expression. Our findings suggest that Clara cells inhibit pulmonary inflammation and fibrosis via MMPs in vivo.

REFERENCES AND NOTES

1. Massaro GD, Singh G, Mason R, Plopper CG, M akinson AM , Gail DB. Biology of the Clara cell. Am J Physiol 266(1 Pt 1):L101–106 (1994).
2. Barth PJ, Muler B. Effects of nitrogen dioxide exposure on Clara cell proliferation and morphology. Pathol Res Pract 199:487–493 (1999).
3. Reynolds SD, Giangreco A, Power JH, Stripp BR. Neuroepithelial bodies of pulmonary airways serve as a reservoir of progenitor cells capable of epithelial regeneration. Am J Pathol 156:289–278 (2000).
4. Plopper CG, Nishio SJ, Alley JL, Kass P, Hyde DM. The role of the nonciliated bronchiolar epithelial (Clara) cell as the progenitor cell during bronchiolar epithelial differentiation in the perfused rat rabbit lung. Am J Respir Cell Mol Biol 7:606–612 (1992).
5. Singh G, Katabi S, Clara cells and Clara cell 10 kD protein (CC10). Am J Respir Cell Mol Biol 17:141–143 (1997).
6. Schwarze PE, Jonsen NM, Samuels J, Thane EV, Lund K, Laj M, Refnes M, Kongerud J, Recher R, Boe J, et al. The use of isolated lung cells in vitro pulmonary toxicology: studies of DNA damage, apoptosis and alteration of gene expression. Cent Eur J Public Health 4 Suppl 10:18 (1996).
7. Gochulko BR, M iro N, Hessel EM, De Bie JJ, Van Oosterhout AJ, Cruikshank WW, Fine A. Airway epithelial Fas ligand expression: potential role in modulating bronchial inflammation. Am J Physiol 274(3 Pt 1):L444–L449 (1998).
8. Kuwano K, Hagimoto N, Tanaka T, Kawasaki M, Kunitake R, Miyajima H, Kaneko Y, Matsuba T, Maeyama T, Hara N. Expression of apoptosis-regulatory genes in epithelial cells in pulmonary fibrosis in mice. J Pathol 190:221–229 (2000).
9. Buisson D, Bernard AM, Begin RO. Clara cell protein (CC-16) and surfactant-associated protein A (SP-A) in asbestos-exposed workers. Chest 109:467–474 (1996).
10. Lesur O, Bernard A, Arsalaire K, Lauwers Y, Begin R, Cantin A, Lane D. Clara cell protein (CC-16) induces a phospholipase A2-mediated inhibition of fibroblast migration in vitro. Am J Respir Crit Care Med 152:290–297 (1995).
11. Kalina M, Mason RJ, Shannon JM. Surfactant protein C is expressed in alveolar type II cells but not in Clara cells of rat lung. Am J Respir Cell Mol Biol 6:594–600 (1992).
12. Cottrell RC, Foster J, Pelling D, Heroil IA, Lee VS, Purchase R, Bayley D, Miller K. The Clara cell and pulmonary surfactant: a study using selective chemical ablation. Cell Biochem Funct 2:201–207 (1984).
13. Horowitz TS, Watkins RM, Aulben RLJ, Mercier CE, Cheng ER. Differential accumulation of surfactant protein A, B, and C mRNAs in two epithelial cell types of hyperoxic lung. Am J Respir Cell Mol Biol 5:511–515 (1991).
14. Cruach E, Parah D, Kuan SF, Persson A. Surfactant protein D: subcellular localization in nonciliated bronchiolar epithelial cells. Am J Physiol 263(1 Pt 1):L60–L66 (1992).
15. Voorhout WF, Veenendaal T, Kurok Y, Ogawa Y, van Golde LM, Geuze M. Immunocytochemical localization of surfactant protein D (SP-D) in type II cells, Clara cells, and alveolar macrophages of rat lung. J Histochem Cytochem 40(10):1589–1597 (1992).
16. De Water R, Willems LN, van Milijen GN, Franke C, Fransen JA, Dijkstra H, Kramps JF, et al. Ultrastructural localization of bronchial antileukoprotease in central and peripheral human airways by a gold-labeling technique using monoclonal antibodies. Am Rev Respir Dis 133:882–890 (1986).
17. Sallenave J, Silva M, Marsden ME, Ryle AP. Secretion of mucus proteinase inhibitor and elafin by Clara cells and type II pneumocyte cells. Am J Respir Cell Mol Biol 2:101–111 (1993).
18. Sallenave J, Silva M, A. Characterization and gene sequence of the precursor of elafin, an elastase-specific inhibitor in bronchial secretions. Am J Respir Cell Mol Biol 8:439–445 (1993).
19. Devereux TR, Domon BA, Philpot RM. Xeno-mitobol metabolism by isolated pulmonary cells. Pharmaco Ther 41:243–256 (1999).
20. Mabuti S, Colotta F, Fancio G, Mazzetti M, Mantovani A, Patalano F, Fasoli A. Time course of IL1 and IL6 synthesis and release in human bronchial epithelial cell cultures exposed to tolune disocyanate. Toxicol Sci 50:64–71 (1999).
21. Khair OA, Dевяля J, Abadelilaz MM, Sapsford J, Tarraf H, Davies R. Effect of Haemophilus influenzae endotoxin on the synthesis of IL-6, IL-8, TNF-alpha and expression of ICAM-1 in cultured human bronchial epithelial cells. Eur Respir J 7:1210–1216 (1994).
22. Devalia J, Campbell AM, Sapsford J, Ruzskaian Z, Goddard P, Bousquet J, Davies R. Effect of nitrogen dioxide on synthesis of inflammatory cytokines expressed by human bronchial epithelial cells in vitro. Am J Respir Cell Mol Biol 18:895–902 (1993).
23. Takizawa H. Airway epithelial cells as regulators of airway inflammation. Int J Mol Med 1:367–378 (1998).
24. Plopper CG, Suverkropp C, Moen D, Nishio S, Buckpitt A. Relationship of cytomegaphor P-450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. J Pharmacol Exp Ther 261:353–363 (1993).
25. Plopper CG, Macklin J, Nishio SJ, Hyde DM, Buckpitt AR. Relationship of cytomegaphor P-450 activity to Clara cell cytotoxicity. III. Morphometric comparison of changes in
the epithelial populations of terminal bronchioles and lobar bronchi in mice, hamsters, and rats after parenteral administration of naphthalene. Lab Invest 67:553–565 (1992).

26. Cho M, Chichester C, Plopper C, Buckpitt A. Biochemical factors important in Clara cell selective toxicity in the lung. Drug Metab Rev 27:2:369–386 (1995).

27. Murphy G, Docherty AJ. The matrix metalloproteinases and their inhibitors. Am J Respir Cell Mol Biol 7:120–125 (1992).

28. Bakowska J, Adamson IY. Collagenase and gelatinase activities in bronchoalveolar lavage fluids during bleomycin-induced lung injury. J Pathol 185(3):319–323 (1998).

29. Fukuda Y, Ishizaki M, Kudoh S, Kitaichi M, Yamanaka N. Localization of matrix metalloproteinases-1, -2, and -9 and tissue inhibitor of metalloproteinase-2 in interstitial lung diseases. Lab Invest 78(6):687–698 (1998).

30. Muhle H, Kittel B, Ernst H, Mohr U, Mermelstein R. Neoplastic lung lesions in rat after chronic exposure to crystalline silica. Scand J Work Environ Health 21(suppl 2):27–29 (1995).

31. Gift JS, Faust RA. Noncancer inhalation toxicity of crystalline silica: exposure-response assessment. J Expo Anal Environ Epidemiol 7:345–358 (1997).

32. Driscoll KE, Lindenschmidt RC, Mauer J K, Perkins L, Perkins M, Higgins J. Pulmonary response to inhaled silica or titanium dioxide. Toxicol Appl Pharmacol 122:201–210 (1991).

33. Fubini B, Giameo E, Volante M, Bolis V. Chemical functionalities at the silica surface determining its reactivity when inhaled. Formation and reactivity of surface radicals. Toxicol Ind Health 6:571–589 (1990).

34. Stripp BR, Maxon K, Mera R, Singh G. Plasticity of airway cell proliferation and gene expression after acute naphthalene injury. Am J Physiol 266(6 Pt 1):L791–799 (1999).

35. Van Winkle LS, Buckpitt AR, Nishio SJ, Isaac JM, Plopper CG. Cellular response in naphthalene-induced Clara cell injury and bronchiolar epithelial repair in mice. Am J Physiol 269(6 Pt 1):L800–818 (1995).

36. Morimoto Y, Tsuda T, Nakamura H, Hori H, Yamato M, Nagata N, Higashi T, Kido M, Tanaka I. Expression of matrix metalloproteinases, tissue inhibitors of metalloprotei- nases, and extracellular matrix mRNA following exposure to mineral fibers and cigarette smoke in vivo. Environ Health Perspect 105(suppl 5):1247–1253 (1997).

37. Oyabu T, Tanaka I, Ishimatsu S, Yamato M, Morimoto Y, Tsuda T, Hori H, Higashi H. Effects of exposure period and lung burden on clearance rate of inhaled aluminium-silicate ceramic fibre from rat lung. Ann Occup Hyg 41(suppl 1):210–212 (1997).

38. Pardo A, Perez-Ramos J, Segura-Valdez L, Ramirez R, Selman M. Expression and localization of TIMP-1, TIMP-2, MMP-3, and MPP-9 in early and advanced experimental lung silicosis. Ann NY Acad Sci 878:587–589 (1999).

39. Firth JD, Putnins EE, Lajivaja H, Ulto V. Bacterial phospholipase C upregulates matrix metalloproteinase expression by cultured epithelial cells. Infect Immun 65:4931–4936 (1997).

40. Kusano K, Miyaura C, Inada M, Tamura T, Ito A, Nagase H, Kamoi K, Suda T. Regulation of matrix metalloproteinases (MMP-2, -9, and -13) by interleukin-1 and interleukin-6 in mouse calvaria: association of MMP induction with bone resorption. Endocrinology 139:1338–1345 (1998).

41. Schwingshackl A, Duszyk M, Brown N, Mogbel R. Human eosinophils release matrix metalloproteinase-9 on stimulation with TNF-alpha. J Allergy Clin Immunol 104:983–989 (1999).

42. Stuve O, Chabot S, Jung SS, Williams G, Yong VW. Chemokine-enhanced migration of human peripheral blood mononuclear cells is antagonized by interferon beta-1b through an effect on matrix metalloproteinase-9. J Neurommunol 80:38–46 (1997).

43. Johnatty RN, Taub DD, Reeder SP, Turcovic-Corrales SM, Cotiam DW, Stephenson TJ, Rees RC. Cytokine and chemokine regulation of proMMP-9 and TIMP-1 production by human peripheral blood lymphocytes. J Immunol 159:2327–2333 (1997).

44. Szabo E, Goheer A, Witschi H, Linnolia RI. Overexpression of CC10 modifies neoplastic potential in lung cancer cells. Cell Growth Differ 9:475–485 (1998).

45. Gipson TS, Bless NM, Shanley TP, Crouch LD, Bleavins MR, Youskin EM, Sarma V, Gibbs DF, Tefera W, McConnell PC, et al. Regulatory effects of endogenous protease inhibitors in acute lung inflammatory injury. J Immunol 162:3653–3662 (1999).