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Platelet-activating factor increases the expression of metalloproteinase-9 in human bronchial epithelial cells

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Abstract

Platelet-activating factor (PAF) plays an important role in the pathogenesis of bronchial asthma. To investigate the role of PAF in the maintenance and remodeling of the extracellular matrix, we evaluated the expression of matrix metalloproteinase-2 and matrix metalloproteinase-9 from human bronchial epithelial cells after PAF treatment. Gelatin zymography of human bronchial epithelial cell-conditioned media showed major pro-matrix metalloproteinase-9 and minor pro-matrix metalloproteinase-2 expression and these expressions were totally inhibited by the metalloproteinase inhibitor EDTA. The identification of matrix metalloproteinase-9 was confirmed by Western blot analysis. Northern blotting and zymography demonstrated that PAF induced the mRNA of matrix metalloproteinase-9 from human bronchial epithelial cells and an increase in the gelatinolytic activity of pro-matrix metalloproteinase-9 but not in that of pro-matrix metalloproteinase-2. Lyso-PAF did not induce matrix metalloproteinase-9 mRNA or the gelatinolytic activity of pro-matrix metalloproteinase-9. CV6209, an receptor antagonist of PAF, reduced the increases of pro-matrix metalloproteinase-9 mRNA and gelatinolytic activity induced by PAF. Another receptor antagonist of PAF, hexanolamine PAF, did not inhibit the increases in the synthesis or release of pro-matrix metalloproteinase induced by PAF. Based on these results, we propose that matrix metalloproteinase-9 may be actively involved in the PAF-induced physiopathological remodeling in human bronchial epithelial cells.

Keywords: Bronchial epithelial cell, human; Matrix metalloproteinase-2; Matrix metalloproteinase-9; PAF (platelet-activating factor); PAF receptor antagonist

1. Introduction

Platelet-activating factor (PAF) is the term first applied by Benveniste et al. (1972) to describe the platelet-aggregating activity released from anti-Immunoglobulin E stimulated rabbit basophils. Several groups of investigators later physiochemically defined this activity as that of 1-alkyl-2-acetyl-glycero-3-phosphocholine (Demopoulos et al., 1979). PAF has a wide tissue distribution and may function in normal physiological processes such as inflammation, neural activity, and reproduction. It may also have a role as a mediator in pathological states such as asthma, ischemia, gastric ulceration, hypertension, atherosclerosis and shock, among others. The biologic properties of PAF in the lungs include the chemo-attraction of eosinophils, the induction of an airway inflammatory response, augmentation of airway vascular permeability, mucus secretion, the elicitation of airway smooth muscle contraction, and a sustained increase in airway responsiveness (Wardlaw et al., 1986). It was recently shown that PAF can enhance the release of matrix metalloproteinase-9 from eosinophils during the migration of eosinophils to the basement membrane (Okada et al., 1997). Inhaled PAF can also induce epithelial shedding in guinea pigs (Lellouch-Tubiana et al., 1988).

The matrix metalloproteinases are a large family of zinc- and calcium-dependent endopeptidases that have the combined ability to degrade the various components of connective tissue matrices (Docherty and Murphy, 1990). They are synthesized and secreted by connective tissue cells and some hematopoietic cells, and are known to be important in both the repairing processes (Brown et al., 1989) and in the accelerated destruction occurring in many diseases. Matrix metalloproteinase-2 (gelatinase A/72-kDa gelatinase) and matrix metalloproteinase-9 (gelatinase B/92-kDa gelatinase) are two members of the matrix metalloproteinase family. In addition to expressing catalytic activity towards denatured fibrillar collagen, these

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two enzymes also degrade native type IV collagen and other structural proteins found in, or associated with, basement membranes that lie beneath epithelial surfaces (Emonard and Grimaud, 1990). Although they share certain substrate preferences, the proteins are products of the surfaces of separate genes (Murphy et al., 1989; Hipps et al., 1991) and the expression from these cells is under different regulation (Murphy et al., 1982, 1989; Hipps et al., 1991). Both enzymes are initially synthesized by cells in catalytically inactive proenzyme forms. It is believed that one mechanism for the activation of these enzymes is through the sequential proteolytic cleavage of the propieces causing destabilization of the coordinate interaction with the Zn^{2+} (Van-Wart and Birkedal-Hansen, 1990). In support of this, various proteolytic mechanisms underlying the activation of these enzymes have been identified (Wong et al., 1992; Crabbe et al., 1994a,b; Sato et al., 1994).

Human bronchial epithelial cells may play an important role in normal growth and development as well as in normal extracellular matrix turnover, thereby contributing to the maintenance of the structural and functional integrity of the lungs (Stoner et al., 1981; Dufour et al., 1986; Shoji et al., 1989). A disruption of the ordered architecture of airway epithelium is a hallmark feature of many lung diseases at autopsy (Montefort et al., 1992). Graded forms of injury to the airway epithelium are also present in less severe forms of airway infection and diseases. Significant disease-related elevations in the number of exfoliated epithelial cells have been reported in bronchial asthma (Beasley et al., 1989). matrix metalloproteinases are expressed in cultured human bronchial epithelial cells (Yao et al., 1996). The findings of matrix metalloproteinase-9 and -2 expressions in human bronchial epithelial cells strongly suggest that these enzymes are involved in the turnover and degradation of the subepithelial basement membrane as well as in epithelial cell–cell interactions.

Since PAF can induce the damage of bronchial epithelial cells, our hypothesis is that PAF takes part in the regulation and release of matrix metalloproteinase-9 in human bronchial epithelial cells. This study was carried out to examine the expression of matrix metalloproteinase-9 mRNA by Northern blot analysis and to observe the gelatinolytic activities of matrix metalloproteinase-9 and matrix metalloproteinase-2 by zymography in cultured human bronchial epithelial cells with and without stimulation by PAF. The effects of lyso-PAF and PAF receptor antagonists on matrix metalloproteinase-9 and matrix metalloproteinase-2 were also investigated.

2. Methods

2.1. Cell culture

Primary cultures of normal human bronchial epithelial cells (Clonetics, San Diego, CA) isolated from the trachea and central airways were maintained in serum-free modified LHC-9 medium (Clonetics, San Diego, CA) containing epidermal growth factor (EGF) (0.5 ng/ml) and bovine pituitary extract (50 μg/ml) as supplements. These cells were used at their second and third passages. Twenty-four hours before the experiments reported herein, all of the supplements were removed from the culture media for human bronchial epithelial cells. Cells grown to confluence on 6 mm dishes were washed twice and then exposed to PAF or other compounds. The cultured human bronchial epithelial cells were stimulated with PAF and lyso-PAF for 24 h, or they were preincubated for 30 min in the presence of CV6209 before PAF was added, then they were incubated for 24 h again.

2.2. Extraction of RNA and Northern blot analysis

The RNA was extracted from human bronchial epithelial cells using isogen (Nippon Gene, Tokyo, Japan) according to the standard protocol. Total RNA was quantified at 260/280 nm, and 15 μg of total RNA was denatured at 65°C for 15 min and size-fractionated by electrophoresis on a 1% agarose gel and transferred in 10× saline sodium citrate (SSC) to nylon filters (Hybond-N, Amersham International, Buckinghamshire, UK) for Northern blot hybridization. The filters were allowed to dry, and the RNA was fixed under UV light for 1 min. The membranes were prehybridized for 2 h at 42°C followed by overnight hybridization in a shaker, using 100 μg/ml heat-denatured herring sperm DNA. High-specific-activity ^{32}P-labeled DNA probes (> 109 cpm/μg) were generated by a random priming DNA labeling method according to the manufacturer's instruction (Amersham International, Buckinghamshire, UK). Filters were washed with 0.2× SSC and 0.1% sodium dodecyl sulfate (SDS) at 65°C for 20 min, twice, then air-dried and exposed to an Imaging Plate (BAS II, Fuji Xerox, Tokyo) for 2 h at room temperature. The relative intensities of signals were determined by an Autoimage Analyzer (BAS 2000, Fuji Xerox, Tokyo). Fifteen micrograms of total RNA was loaded in each lane of the gels. The blots were probed with an matrix metalloproteinase-9 cDNA probe, and we used the 28 S ribosomal RNA (rRNA) as an internal control of the amount of RNA. Small differences in loading were normalized according to the density of the 28 S (rRNA) bands.

2.3. Extraction of matrix metalloproteinase-9 from conditioned medium by anti-matrix metalloproteinase-9 antibody

To immunoprecipitate matrix metalloproteinase-9 from the conditioned medium, the conditioned medium was incubated with 3.3 μg of anti-matrix metalloproteinase-9 antibody conjugated with protein G-Sepharose (Pharmacia, Biotech, Sweden) for 90 min at 4°C. Anti-matrix metalloproteinase-9 antibody-conjugated with protein G-Sep-

harose was prepared by incubating 60 μl of protein G-Sepharose with 10 μg of anti-matrix metalloproteinase-9 antibody, followed by extensive washing. The immune complexes were washed three times with PBS. After incubating 20 μl of the immune complexes with 400 μl of conditioned medium at 4°C for 60 min, the associated proteins were recovered by boiling for 5 min in sample buffer for SDS-polyacrylamide gel electrophoresis (PAGE). To completely immunodeplete matrix metalloproteinase-9 from the conditioned medium, the conditioned medium after the first immunoprecipitation was subjected to two additional sequential immunoprecipitations with the same antibody complex. Then, the resulting supernatant was collected and stored at −20°C for zymography.

2.4. Western blot analysis

The matrix metalloproteinase-9 was detected by Western blot analysis after 10% SDS-PAGE. Aliquots of partially purified and concentrated human bronchial epithelial cells conditioned media were separated by 10% SDS-PAGE and transferred to a PVDF filter (Amersham International, Buckinghamshire, UK). Nonspecific staining was blocked by incubating the transfers for 60 min in Tris-buffered saline (TBS) containing 5% nonfat dry milk. The transfers were then incubated overnight with anti-matrix metalloproteinase-9 antibody (diluted 1:250 in TBS). The blots were washed three times in TBS, 0.05% Tween 20 and incubated for 60 min with horseradish peroxidase-labeled donkey anti-mouse Immunoglobulin G (diluted 1:1000) as the secondary antibody. Antigen detection was performed with a chemiluminescent detection system per the manufacturer's instructions (ECL™, Amersham International, Buckinghamshire, UK).

2.5. Zymography

The human bronchial epithelial cell culture medium was harvested and stored at −20°C until use. Aliquots of each sample were subjected to electrophoresis in 10% polyacrylamide gels containing 1 mg/ml gelatin for SDS-PAGE. The method of Laemmli (1970) was followed,

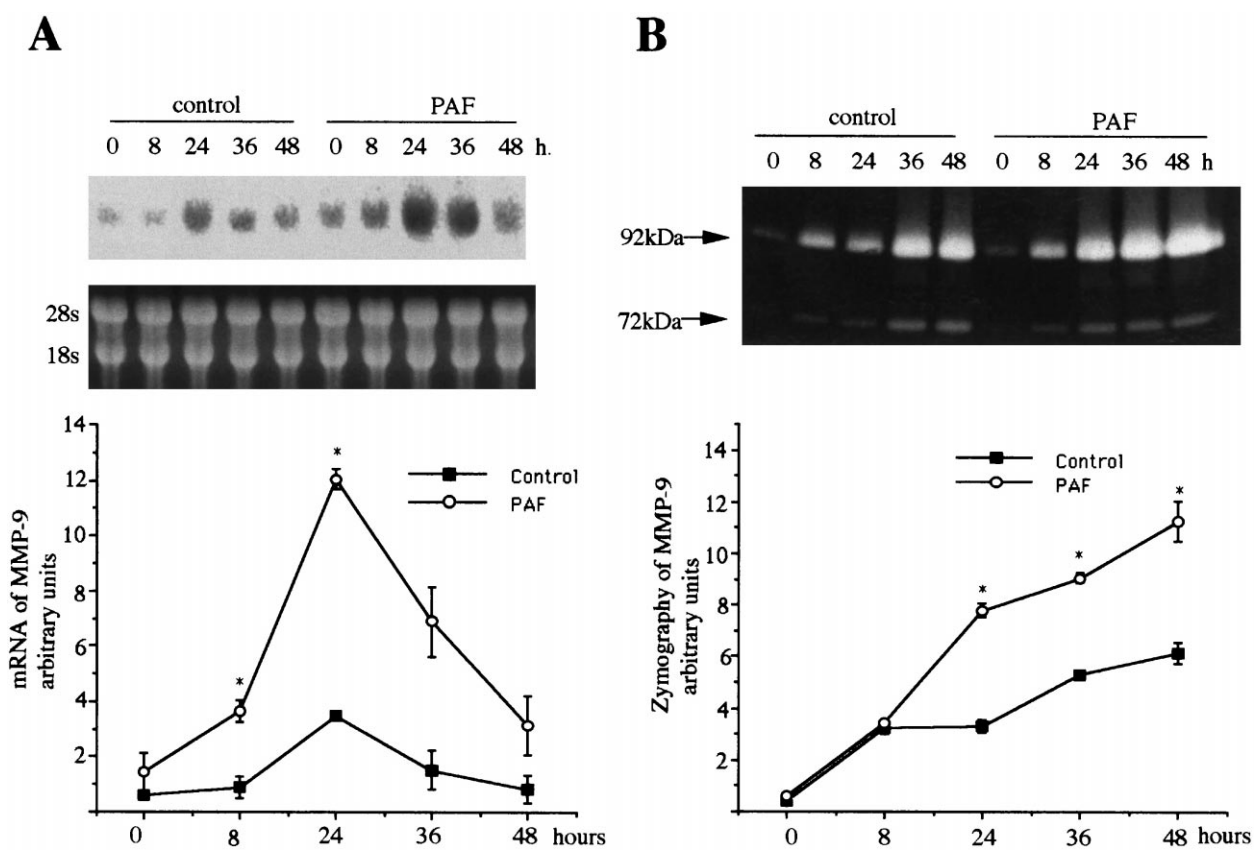

Fig. 1. Basal and PAF-stimulated matrix metalloproteinases secretion by cultured human bronchial epithelial cells. Evaluations were carried out by zymography and Northern blot. At confluence, cultured human bronchial epithelial cells were incubated for 24 h with primary medium and were treated with or without 100 nM PAF. (A) Northern blot analysis, (B) zymography for time course. (A) The mRNA of matrix metalloproteinase-9 increased from 8 h to a peak at 24 h after PAF stimulation. The blots were probed with an matrix metalloproteinase-9 cDNA probe, and we used the 28 S ribosomal RNA as an internal control for the amount of RNA. Values were quantified with an Autoimage Analyzer in arbitrary units. (B) The wide 92 kDa band and small 72 kDa band were found in both the control and PAF groups. The 92 kDa band increased with time in the PAF group, but the small 72 kDa band showed no simultaneous change. The values were quantified using a semiautomated image analysis program (NIH Image 1.55) and arbitrary units of band intensity. Values represent means ± S.E.M. of three experiments. $^{*}P < 0.01$, $^{**}P < 0.001$ vs. control.

excluding any reducing agents or boiling products. Samples were run at 20–30 mA, and washed for 20 min twice in 2.5% Triton X-100 at room temperature to remove the SDS. The gel was then incubated overnight at 37°C in reaction buffer (100 mM Tris–HCl, pH 7.5 10 mM $CaCI_2$, and 0.05% Brij 35). After staining with Coomassie Brilliant Blue R-250, the gelatinolytic activities were detected as clear bands against the blue background. Molecular weight standard proteins (Bio-Rad Laboratories, Hercules, CA) were run simultaneously. For the detection of zymogen, samples were incubated for 4 h at 37°C with 1 mM aminophenylmercuric acetate before undergoing SDS-PAGE. To determine the inhibition profile of the enzymatic activities observed in the gel containing gelatin, we also used incubation in reaction buffer containing 10 mM EDTA, a metalloproteinase inhibitor, and 2 mM phenylmethylsulfonyl fluoride, a serine proteinase inhibitor.

The gelatinolytic activities in the gel slabs were quantified using a semiautomated image analysis (NIH Image 1.55), which quantifies both the surface and the intensity of lysis bands after scanning of the gels. The results are expressed as arbitrary units/24 h/10^3 cells. To check whether this method for measuring enzymatic activity on zymograms was linear over the range of gelatinolytic activities in unknown samples, we evaluated activities for increasing volumes of culture medium and found that the arbitrary units obtained with the image analysis system increased linearly with the volume of the samples ($r = 1.00$) (D'Ortho et al., 1994).

2.6. Materials

Gelatin, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor, PAF), lyso-PAF and CV6209

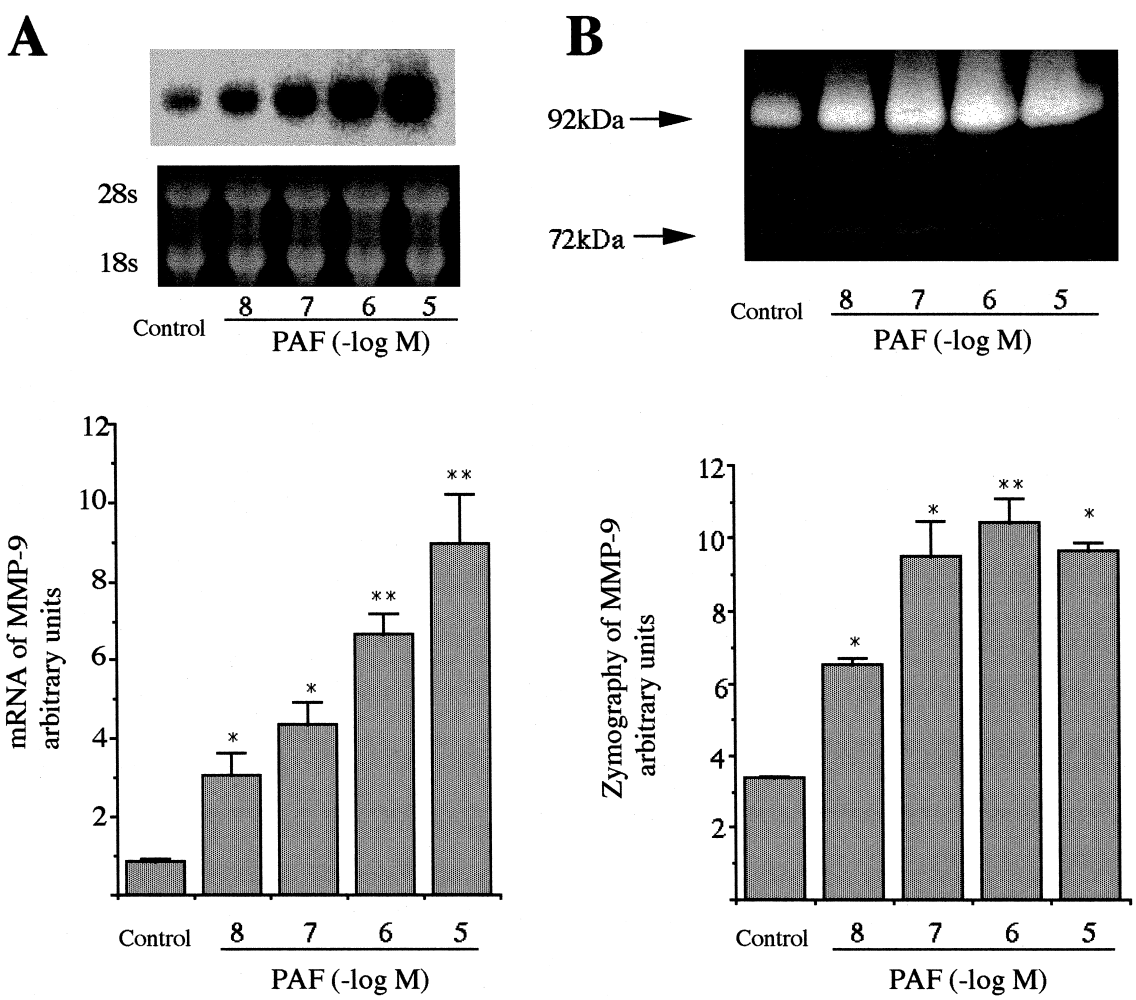

Fig. 2. The dose response of PAF in (A) Northern blot analysis and (B) zymography. Twenty-four hours after stimulation by PAF, the matrix metalloproteinase-9 mRNA and gelatinolytic activity of matrix metalloproteinase-9 in human bronchial epithelial cells were observed. The concentration of PAF was increased from 10 nM to 10 μM. Both the matrix metalloproteinase-9 mRNA and the 92 kDa band were increased from 10 nM to 10 μM of PAF. (A) The blots were probed with an matrix metalloproteinase-9 cDNA probe, and we used 28 S ribosomal RNA as an internal control for the amount of RNA. Values were quantified with an Autoimage Analyzer in arbitrary units. (B) The values were quantified using a semiautomated image analysis program (NIH Image 1.55) and arbitrary units of band intensity. Columns and bars represent the means ± S.E.M. of three experiments. $^{*}P < 0.01$, $^{**}P < 0.001$ vs. control. There was no significant difference in gelatinolytic activity between PAF 1 μM and PAF 10 μM.

were obtained from Sigma (St. Louis, MO). 0.05% Brij 35 was also obtained from Sigma. [*a*-32 *p*]dCTR was acquired from Amersham International (Buckinghamshire, UK). A monoclonal mouse anti-human matrix metalloproteinase-9 was acquired from Calbiochem (La Jolla, CA). The matrix metalloproteinase-9 cDNA probe was generously given by Karl Tryggvason, M.D. Stockholm, Sweden.

2.7. Statistical analysis

Data were expressed as mean ± S.E.M. Statistical evaluation of the data was performed by the Student's t test for unpaired observations using StatView 4.0 (Abacus Concepts, Berkeley, CA) for Macintosh.

3. Results

3.1. Time course of the matrix metalloproteinase-9 stimulated by PAF in cultured human bronchial epithelial cells

The matrix metalloproteinase-9 mRNA in human bronchial epithelial cells was increased 8 h after stimulation by 100 nM PAF, continued to increase to a peak at 24 h and then recovered to the initial state (Fig. 1A). Al-

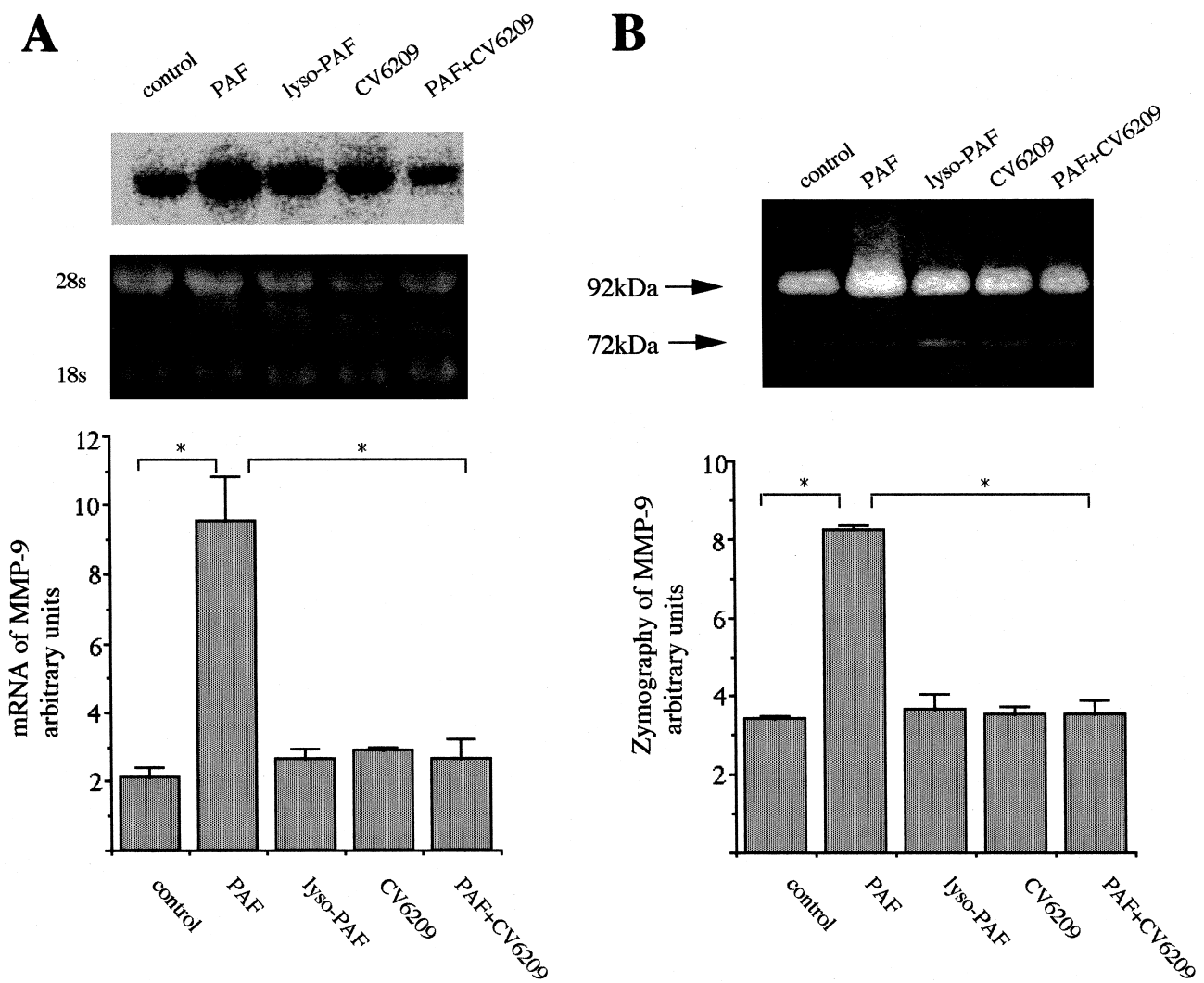

Fig. 3. The PAF receptor antagonist CV6209 attenuated the increase in the expression of matrix metalloproteinase-9. Cultured human bronchial epithelial cells were incubated for 24 h with primary medium. The cultured human bronchial epithelial cells were preincubated for 30 min in the presence of 10 nM of the receptor antagonist before PAF was added, then they were incubated for 24 h again. (A) 100 nM PAF augmented the expression of matrix metalloproteinase-9 mRNA in a Northern blot analysis. This expression of matrix metalloproteinase-9 mRNA was inhibited by CV6209 (10 nM). LysoPAF at 100 nM did not increase the expression of matrix metalloproteinase-9 mRNA. The blots were probed with an matrix metalloproteinase-9 cDNA probe, and we used 28 S ribosomal RNA as an internal control. Values were quantified with an Autoimage Analyzer in arbitrary units. (B) The gelatinolytic activity of matrix metalloproteinase-9 was investigated by zymography. The increase in the 92 kDa band by PAF was attenuated by CV6209. Lyso-PAF did not augment the 92 kDa band. The values were quantified using a semiautomated image analysis program (NIH Image 1.55) and arbitrary units of band intensity. Columns and bars represent the means ± S.E.M. of three experiments. $^{*}P < 0.01$.

though the matrix metalloproteinase-9 mRNA was slightly increased at 24 and 36 h in the control group, the matrix metalloproteinase-9 mRNA was increased by more than two-fold in the PAF groups compared with the control group at the same timepoints. A wide 92 kDa band produced by the pro form of matrix metalloproteinase-9 and a small 72 kDa band produced by the pro form of matrix metalloproteinase-2 were found in the control and PAF-containing media by zymography (Fig. 1B). Twenty-four hours after the addition of 100 nM PAF, the 92 kDa band began increasing and continued to increase at 48 h.

3.2. Dose dependence of matrix metalloproteinase-9 stimulated by PAF in cultured human bronchial epithelial cells

The matrix metalloproteinase-9 mRNA in human bronchial epithelial cells and the gelatinolytic activity of matrix metalloproteinase-9 increased according to the dose of PAF (Fig. 2A, B). The matrix metalloproteinase-9 mRNA increased beginning at 10 nM PAF and continued to increase at 10 μM PAF. The zymography showed that the large 92 kDa band increased after stimulation by 10 nM PAF, and continued to increase until the concentration of PAF was 1 μM. Although it slightly decreased at 10 μM PAF, there was no significant difference in gelatinolytic activity between PAF 1 μM and PAF 10 μM. The small 72 kDa band did not change with the PAF concentration.

3.3. The secretion of matrix metalloproteinase-9 in cultured human bronchial epithelial cells by PAF was modulated by CV6209

Although PAF increased the expression of matrix metalloproteinase-9 mRNA and the gelatinolytic activity of pro-matrix metalloproteinase-9 after 24 h incubation, lyso-PAF (100 nM), which has similar structural and detergent properties, did not increase the expression of matrix metalloproteinase-9 or the gelatinolytic activity of pro-matrix metalloproteinase-9 at 24 h (Fig. 3). Ten nM CV6209, an receptor antagonist of PAF, attenuated the increase in the expression of matrix metalloproteinase-9 mRNA induced by PAF at 24 h. Ten nM of CV6209 without PAF treatment did not influence the expression of matrix metalloproteinase-9 mRNA. At the same concentration as that used for PAF, 100 nM, lyso-PAF did not affect the matrix metalloproteinase-9 mRNA level. The same culture media were examined by zymography (Fig. 3B). This result also demonstrated that the 92 kDa band which was increased by 100 nM PAF at 24 h decreased after the addition of 10 nM CV6209. The lyso-PAF did not affect the 92 kDa band at 24 h as shown by zymography. The CV6209 without PAF treatment also did not affect the release of the 92 kDa band. Another receptor antagonist of PAF, hexanolamine

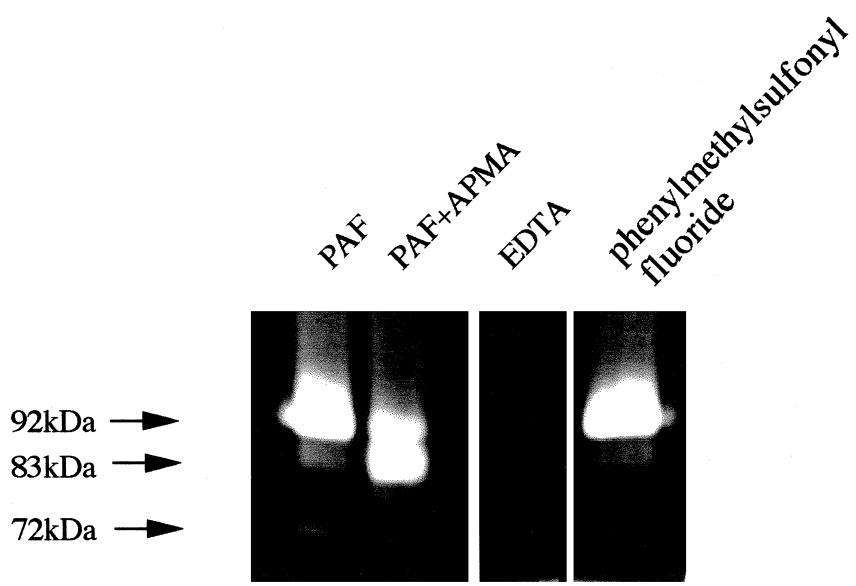

Fig. 4. Identification of expressed band as metalloproteinases. Twenty-four hours after the PAGE/gelatin electrophoresis of 100 nM PAF-stimulated medium from human bronchial epithelial cells, gelatin substrate gels were incubated in 100 mM Tris incubation buffer without inhibitor (control) or in the presence of 10 mM EDTA or 2 mM phenylmethylsulfonyl fluoride. All of the gelatinase, including the 92- and 72-kDa gelatinases, was inhibited by EDTA, but was not inhibited by phenylmethylsulfonyl fluoride. In addition, 24 h after being stimulated with PAF, media were incubated for 1 h at 37°C with 1 mM aminophenylmercuric acetate before undergoing PAGE/gelatin electrophoresis. After stimulation by aminophenylmercuric acetate, a small 92 kDa and large 83 kDa band were observed ($n = 3$).

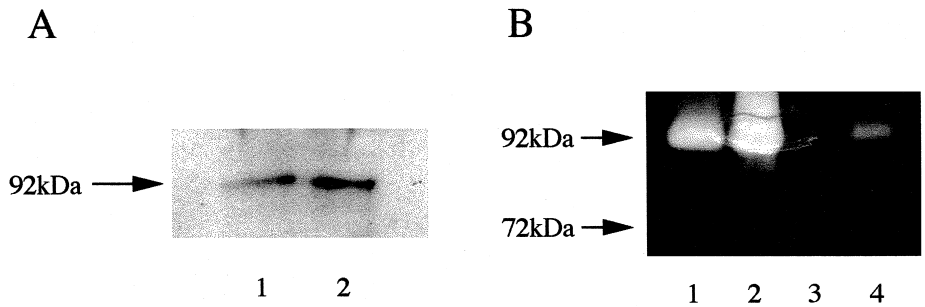

Fig. 5. Identification of pro-matrix metalloproteinase-9 binding protein. (A) Western blot detection of matrix metalloproteinase-9 in conditioned medium and PAF-stimulated medium by anti-matrix metalloproteinase-9 antibody. The conditioned and PAF-stimulated media were immunodepleted matrix metalloproteinase-9 by anti-matrix metalloproteinase-9 antibody-conjugated with protein G-Sepharose. The protocol is described in Section 2. Lane 1: conditioned medium. Lane 2: PAF-stimulated medium. (B) Zymography analysis. The same conditioned and PAF-stimulated media which completely immunodepleted matrix metalloproteinase-9 were used for zymography analysis. The protocol is described in the Methods. Lanes 1 and 2: original conditioned medium and PAF-stimulated medium immunoprecipitated matrix metalloproteinase-9. Lanes 3 and 4: conditioned medium and PAF-stimulated medium depleted by anti-matrix metalloproteinase-9 antibody.

PAF, did not block the increases of pro-matrix metalloproteinase induced by PAF at 24 h.

3.4. Increased 92- and 72-kDa gelatinases were confirmed to be matrix metalloproteinases

EDTA completely inhibited the activity of all of the matrix metalloproteinases, whereas phenylmethylsulfonyl fluoride did not (Fig. 4). After the PAGE/gelatin electrophoresis of PAF-stimulated medium from human bronchial epithelial cells, gelatin substrate gels were incubated in 100 mM Tris reaction buffer without inhibitor (control), or in the presence of 10 mM EDTA or 2 mM phenylmethylsulfonyl fluoride. All of the gelatinase, including the 92- and 72-kDa gelatinases, was inhibited by EDTA, but were resistant to inhibition by phenylmethylsulfonyl fluoride, a serine proteinase inhibitor. After treatment of 1 mM aminophenylmercuric acetate before undergoing PAGE/gelatin electrophoresis, the 92-kDa band was activated to the 83-kDa band.

The conditioned and PAF-stimulated media were depleted by anti-matrix metalloproteinase-9 antibody and then detected by both Western blotting and zymography. As shown in Fig. 5A, the 92 kDa bands were detected by anti-matrix metalloproteinase-9 antibody in Western blots. In Fig. 5B, the same medium in which the gelatinolytic activity was removed by anti-matrix metalloproteinase-9 antibody was electrophoresed with zymography. Compared with lanes 1 and 2, the 92 kDa band disappeared or was largely decreased in lanes 3 and 4, in which gelatinolytic activity was depleted from the medium.

4. Discussion

The results of this study demonstrated that matrix metalloproteinase-9 mRNA and synthesis and release of matrix metalloproteinase-9 in cultured human bronchial epithelial cells are significantly enhanced by treatment with PAF, and these enhancements are inhibited by CV6209, a PAF receptor antagonist. Lyso-PAF, which has same structure as PAF, did not increase the synthesis and release of matrix metalloproteinase-9. Our data also demonstrated that another PAF receptor antagonist, hexanolamine PAF, did not inhibit the increase of matrix metalloproteinase-9 mRNA expression or the matrix metalloproteinase-9 gelatinolytic activity induced by PAF.

Although the expression of matrix metalloproteinases has been reported in many cell lines and human bronchial epithelial cells (Yao et al., 1996), this is the first report of the expression and release of matrix metalloproteinase-9 in human bronchial epithelial cells stimulated by PAF, to our knowledge. Our findings demonstrated that 100 nM PAF induced a marked increase in matrix metalloproteinase-9 mRNA after 24 h, and the first increase in expression was found at 8 h. The greatest gelatinolytic activity was found at 48 h. These results suggest that the matrix metalloproteinase-9 was translated and synthesized in the cells and may be stored in the cells (Nguyen et al., 1998). In contrast, matrix metalloproteinase-2 did not change after stimulation with PAF. Buisson et al. (1996) indicated that matrix metalloproteinase-9, produced by human surface respiratory epithelial cells, actively contributes to the wound repair process of the respiratory epithelium, whereas matrix metalloproteinase-2 remains stable during the repair process. Yao et al. (1996) also found a slightly increase in the mRNA level and the gelatinolytic activities of matrix metalloproteinase-2 stimulated by lipopolysaccharides in human bronchial epithelial cells compared to that of matrix metalloproteinase-9. These findings demonstrated that although matrix metalloproteinase-2 is very similar to matrix metalloproteinase-9 in terms of structure, substrate specificity and properties, there may be marked differences regarding tissue specificity and regulation of expression. (Wilhelm et al., 1989)

In accordance with a previous in situ hybridization study (Canete-Soler et al., 1994), the mRNA of matrix metalloproteinase-9 was expressed in human bronchial epithelial cells, and the specific activity of matrix metalloproteinase-2 against gelatin is 25 times lower than that of matrix metalloproteinase-9 (Yasumitsu et al., 1992). This may account for the weaker zymography signal of matrix metalloproteinase-2 compared with matrix metalloproteinase-9. Both matrix metalloproteinase-9 and matrix metalloproteinase-2 most specifically cleave collagen type IV and gelatin (Murphy and Docherty, 1992). Collagen type IV is an important constituent of the extracellular matrix proteins in the airways including basement membrane. An increased release of matrix metalloproteinases might facilitate inflammatory cell migration and induce extracellular matrix solubilization in guinea pigs (D'Ortho et al., 1994). Our present data also showed that cultured human bronchial epithelial cells can synthesize and release mainly pro-matrix metalloproteinase-9 rather than pro-matrix metalloproteinase-2. A recent study (Dunsmore et al., 1998) demonstrated that excluding matrix metalloproteinase-7 (matrilysin), matrix metalloproteinase-9 was not found in normal or diseased lung tissue samples. For diseased lung, this cystic fibrosis, organization pneumonia, adenocarcinoma and sarcoidosis were examined. Our study and other studies showed that matrix metalloproteinase-9 was mainly produced by inflammatory cells (Stahle-Backdahl et al., 1994) or by stimulation with lipopolysaccharide, or proinflammatory cytokines in human bronchial epithelial cells (Yao et al., 1996, 1997). Matrix metalloproteinase-9 may be modulated in response to inflammatory processes in human bronchial epithelial cells in vivo. A literature review suggested that various matrix metalloproteinases, including matrix metalloproteinase-9, are released as pro-enzymes and are activated extracellularly by other proteinases (Murphy et al., 1994). The 92 kDa matrix metalloproteinase-9 proenzyme is converted to the active form of 88 kDa matrix metalloproteinase-9 through the cleavage of an N-terminal peptide. We are now investigating the mechanism and the pathway of the activation of pro-matrix metalloproteinase.

Although matrix metalloproteinase-9 is produced by various inflammatory cells, little is known about the relationship between PAF, a potent lipid inflammatory mediator, and matrix metalloproteinase-9 in human bronchial epithelial cells. Okada et al. (1997) recently demonstrated that eosinophils migrate through basement membranes by releasing and activating matrix metalloproteinase-9 and by degrading basement membrane barriers when they are activated by PAF and interleukin (IL)-5. Tao et al. (1995) reported that PAF can induce the expression of matrix metalloproteinase-9 in the corneal epithelium. Our present study demonstrated that PAF alone strongly enhanced the expression and release of pro-matrix metalloproteinase-9 from human bronchial epithelial cells. The increased expression of matrix metalloproteinase-9 by PAF was attenuated by CV-6209, an receptor antagonist of PAF. CV6209, which inhibited the aggregation of rabbit and human platelets induced by PAF, was more potent in this regard than other PAF receptor antagonists (Terashita et al., 1987). CV6209 also potently protected mice and rats from the lethality induced by PAF. To demonstrate whether the regulation of matrix metalloproteinase-9 by PAF is specific to a PAF receptor, we used hexanolamine PAF (Tokumura et al., 1985) as a PAF receptor antagonist, because it is a close structural analog of C_{16}-PAF. Although hexanolamine PAF inhibited the increase in vascular permeability induced by PAF (Fujii et al., 1995), we found that hexanolamin PAF did not block the increases of pro-matrix metalloproteinase-9 stimulated by PAF in human bronchial epithelial cells. Hexanolamine PAF was demonstrated to be a partial agonist at PAF receptors in macrophages and platelets (Grigoriadis and Stewart, 1991). Furthermore, lyso-PAF (Prescott et al., 1990), an inactive metabolite of PAF, did not induce matrix metalloproteinase-9. Our data suggest that regulation of matrix metalloproteinase-9 by PAF in cultured human bronchial epithelial cells is mediated by specific PAF receptors.

The 92- and 72-kDa bands at 24 h after stimulation by PAF were inhibited by EDTA, but not inhibited by another proteinase inhibitor, phenylmethylsulfonyl fluoride. These findings suggested that the 92- and 72-kDa gelatinases belong to the matrix metalloproteinases family. Moreover, after depletion with anti-matrix metalloproteinase-9 antibody, the 92-kDa band was detected in the Western blot analysis and disappeared in the zymography. The pro-matrix metalloproteinase-9 protein released from human bronchial epithelial cells to medium can be demonstrated in vitro. The incubation of aliquots of human bronchial epithelial cells culture medium in the presence of aminophenylmercuric acetate organomercurially activated major 92-kDa gelatinase into smaller bands of 83 kDa.

In conclusion, our present findings demonstrated that PAF can enhance the expression of the mRNA of pro-matrix metalloproteinase-9 and the gelatinolytic activities of matrix metalloproteinase-9 in human bronchial epithelial cells. Based on these data, we propose that matrix metalloproteinase-9 may be actively involved in the physiopathological remodeling of human bronchial epithelial cells by PAF.

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