

Original article

Matrix metalloproteinases-7, -8, -9 and TIMP-1 in the follow-up of diisocyanate-induced asthma

Background: Diisocyanate-induced asthma (DIA) is known to be associated with poor prognosis. We wished to clarify if matrix metalloproteinases (MMP)-7, -8 or -9 or tissue inhibitor of matrix metalloproteinases (TIMP-1) are associated with the functional or inflammatory outcome in DIA patients.

Methods: This is a longitudinal study where 17 patients with DIA diagnosed by a specific challenge test to diisocyanates were monitored. Exposure to diisocyanates was terminated seven (mean) months before the challenge test. The studies included spirometry, histamine challenge test and bronchoscopy. MMP-7, MMP-8, TIMP-1 [Enzyme-linked immunosorbent assay (ELISA)- and immunofluorometric assay-methods], MMP-9 (ELISA and zymography), interferon-gamma, tumour necrosis factor-alpha, interleukin-6, -8, -15, -17, CXCL-5/ENA-78, monocyte chemoattractant protein-1 and macrophage inhibitory factor (MIF) (ELISA) were assayed from bronchoalveolar lavage (BAL) fluid. Inhaled steroid therapy was initiated after the examinations, which were repeated at 6 months and at 3 years during the treatment. The results were compared with those of 15 healthy controls.

Results: Inhaled steroid medication increased BAL levels of MMP-9 and MMP-9/TIMP-1 and decreased MMP-7 and MMP-7/TIMP-1. The increase in MMP-9 levels was associated with a decline in the TH-2 type inflammation.

Conclusions: Our data suggest that reduced TH-2 type inflammation in DIA after inhaled steroid medication is reflected as elevated MMP-9 and MMP-9/TIMP-1 levels in BAL. MIF may be the inducer of MMP-9. This might point to some protective role for MMP-9 in DIA.

P. Piirilä¹, A. Lauhio², M.-L. Majuri³,
A. Meuronen⁴, M. Myllärniemi⁵,
T. Tervahartiala⁶, K. Vuorinen⁵,
A. Laitinen⁴, H. Alenius³,
V. L. Kinnula⁵, T. Sorsa^{6,7}

¹Laboratory of Clinical Physiology, Helsinki University Hospital; ²Department of Infectious Diseases, Helsinki University Hospital; ³Unit of Excellence in Immunotoxicology, Finnish Institute of Occupational Health; ⁴Department of Anatomy, University of Helsinki; ⁵Department of Pulmonary Medicine, Helsinki University Hospital; ⁶Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital; ⁷Institute of Dentistry, University of Helsinki, Helsinki, Finland

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Päivi Piirilä, MD
Laboratory of Clinical Physiology
HUSLAB
P.O.Box 340
00029 HUS
Finland

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Matrix metalloproteinases (MMPs) represent a protease family having important physiological functions such as remodelling of the extracellular matrix. MMPs also modulate cell migration, cell signalling and immune responses. Genetically distinct but structurally related MMPs can further protect the host against infective processes by modulating anti-inflammatory defensins, cytokines and chemokines (1). In interstitial lung diseases, MMPs have been regarded as potential biomarkers of disease activity (1, 2). In asthma, elevation in the levels of MMP-9 in mucosal biopsies (3, 4), bronchoalveolar lavage (BAL) fluid (5) and in induced sputum specimens has been found (6, 7). MMP-9 levels have been found to increase in allergen challenge (3), and MMP-9 has been found to correlate with the degree of airflow obstruction in asthma and chronic bronchitis (7). Tissue inhibitor of matrix metalloproteinase (TIMP)-1 is the major

endogenous MMP inhibitor, and an imbalance between MMPs and TIMPs results in the pathological progression of allergen-induced inflammation (1, 7). Inhaled steroid treatment has been found to decrease MMP-9 activity in some (5, 8), but not in all reports (3).

Diisocyanates, most often toluene diisocyanate (TDI), diphenylmethane diisocyanate (MDI), and hexamethylene diisocyanate (HDI) are chemicals frequently used in the production of plastics and foams. Isocyanates are the most common chemicals evoking occupational asthma (9). Diisocyanate-induced asthma (DIA) is associated with airway inflammation with lymphocyte, eosinophil and neutrophil infiltration (10, 11). Asthma symptoms, nonspecific bronchial hyperreactivity (NSBH) and bronchial inflammation persist in a considerable proportion of patients with DIA even after cessation of diisocyanate exposure (12, 13).

We recently followed up 17 patients with DIA for 3 years (14). Diisocyanate exposure was interrupted and the patients were treated with regular inhaled steroid medication. Lung function and the inflammation status were assessed with histological and cytological samples, immunohistochemical studies and analysis of chemokines and cytokines from BAL cells. The level of markers of TH-2 type inflammation decreased as did the level of NSBH. However, some patients showed persistent NSBH at the end of the follow-up and in their BAL cells, levels of interleukin (IL)-6, IL-15 and tumour necrosis factor- α (TNF- α) mRNA levels were increased when compared with the healthy controls.

Earlier studies have indicated that MMPs are involved in bronchial inflammation of DIA (6, 15). Our aim was to study the levels of MMP-7, -8, -9 and TIMP-1 from the previous (14) patient material with DIA followed up for about 3 years after cessation of exposure and during inhaled steroid treatment. We hypothesized that MMPs or TIMP-1 may be associated with the prognosis of DIA. Thus, the MMP and TIMP-1 levels in BAL were measured throughout the follow-up and also compared with the values from the healthy controls. MMPs and TIMP-1 levels were also compared with the concentrations of inflammatory markers in both bronchial mucosa and BAL fluid.

Methods

Study design

The patient selection has been reported earlier (14). Seventeen consecutive patients with new DIA diagnosed at the Finnish Institute of Occupational Health (FIOH) during 1995–2001 volunteered for this study. Fiberoptic bronchoscopy and BAL were performed 48 h after a positive inhalation challenge test to diisocyanates. After bronchoscopy, treatment with budesonide (AstraZeneca, Södertälje, Sweden) 1600 μ g a day was initiated, and the patients used the standard medication for 1 year, after which changes in medication were allowed based on symptoms and peak flow measurements. Further exposure to diisocyanates was not permitted. The studies were repeated at 6 months (mean 7.9 months) and at 3 years (mean 2.8 years) after the diagnosis and the patients used regular inhaled steroids throughout the study. The inflammatory findings of the patients were compared with those of 15 healthy controls. Informed consent was obtained from all participants and the study protocol was approved by the Ethics Committee of FIOH.

Participants

Patients comprised 12 men and 5 women, who had been exposed to HDI ($n = 7$), MDI ($n = 7$), TDI ($n = 2$) or a combination of HDI and MDI ($n = 1$). Their anthropometric data are shown in Table 1. DIA diagnosis was based on specific inhalation challenge testing, as described (16). Bronchoscopic technique, methods for cytological analysis of BAL cells and immunohistochemical methods have been described previously (14). Bronchoscopy was also performed on eight nonsmoking, two ex-smoking and five smoking healthy control subjects (Table 1) (14).

Table 1. Data on age, gender and smoking habits of the patients and controls. Exposure and challenge data are provided for the patients

Parameter	Patients ($n = 17$)	Controls ($n = 15$)
Gender		
Male	12	5
Female	5	10
Age (years) (mean $\pm$ SD)	42.8 $\pm$ 12.7	35.5 $\pm$ 10.9
Smoking habits		
Nonsmokers	6	8
Ex-smokers	7	2
Smokers	4	5
Pack years		
(quitting, years) (mean $\pm$ SD)		
Ex-smokers	20.4 $\pm$ 16.5 (9.1 $\pm$ 11.1)	5.9 $\pm$ 4.1 (5 $\pm$ 1.4)
Smokers	16.8 $\pm$ 13.1	25.1 $\pm$ 11.8
Sensitizing agent (n)		No diisocyanate exposure
HDI	7	
MDI	7	
TDI	2	
HDI and MDI	1	
FEV ₁ (l) $\pm$ SD (% of predicted)	3.68 $\pm$ 0.79 (93.5 $\pm$ 14.6)	3.79 $\pm$ 0.86 (97.4 $\pm$ 9.7)
FVC (l) $\pm$ SD (% of predicted)	4.9 $\pm$ 0.98 (102.1 $\pm$ 13.0)	4.5 $\pm$ 1.0 (97.9 $\pm$ 8.8)
FEV ₁ reduction in specific challenge test (l) (mean $\pm$ SD)	22.7 $\pm$ 10.0	

HDI, hexamethylene diisocyanate; MDI, diphenyl methane diisocyanate; TDI, toluene diisocyanate; FEV₁, forced expiratory volume in 1 s.

Lung function methods

Lung function methods have been described in detail earlier (14). In histamine challenge test, provocative dose causing a 15% reduction (PD15) in forced expiratory volume in 1 s (FEV₁) was calculated. Bronchial hyperreactivity was graded as follows: strong PD15 < 0.1 mg, moderate PD15 0.1–0.4 mg, slight PD15 0.4–1.6 mg and no hyperreactivity PD15 > 1.6 mg (17). For statistical calculations, a PD15 value was graphically extrapolated for those with PD15 > 1.6 mg.

Analysis of cytokines and chemokines

IL-6, IL-15 and TNF- α mRNA levels analysed in the previous study (14) were compared with the MMP protein levels in this study. The protein analysis of interferon- γ (IFN- γ), IL-17, IL-8 [Chemokine (C-X-C motif) ligand 8, CXCL-8], Chemokine (C-X-C motif) ligand 5, CXCL-5 (ENA-78), monocyte chemotactic protein-1 (MCP-1) and macrophage migration inhibitory factor (MIF) was performed with the Luminex bead system (Bio-Plex 200 System, Bio-Rad Laboratories, Hercules, CA, USA) by labelled cytokine capture antibody pairs: IL-8 (CXCL-8), IL-17, IFN- γ , MCP-1, TNF- α and MIF Luminex kits were purchased from Bio-Rad Laboratories and CXCL-5 from R&D Systems (Minneapolis, MN, USA).

Analysis of MMPs and TIMP-1

MMP-8 concentrations were analysed using a time-resolved immunofluorescence assay (IFMA), as described earlier (18). The

monoclonal MMP-8 specific antibodies 8708 and 8706 (Medix Biochemica, Kauniainen, Finland) were used as the catching and tracer antibody respectively. Europium-chelate was used to label the tracer antibody. The samples were diluted in assay buffer, incubated for 1 h, followed by incubation for 1 h with the tracer antibody. Enhancement solution was added, and the fluorescence was measured after 5 min using 1234 Delfia Research Fluoremeter (Wallac, Turku, Finland). The interassay coefficient of variation (CV)% was 7.3% ($n = 28$) and detection limit for the assay was 0.08 $\mu\text{g/l}$ (19).

MMP-7, MMP-9 and TIMP-1 concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits. The R & D and Biotrak ELISA systems (Amersham Biosciences UK Ltd, Buckinghamshire, UK) were used according to the manufacturers' protocol. All samples were analysed in duplicate. The secondary antibody in each kit was conjugated with horseradish peroxidase and tetramethylbenzidine was used as a substrate. The absorbance was measured at 450 nm using Labsystems Multiskan RC (Thermo Bioanalysis Corporation, Santa Fe, NM, USA). Detection limits for MMP-7 (CR & D Systems), -9 (Amersham) and TIMP-1 (Amersham) assays were 0.016, 0.6 and 1.25 ng/ml, respectively.

Gelatin-zymography analysis

Gelatinolytic activity was analysed by zymography using 1 mg/ml gelatine as substrate. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the gels were washed in 50 mM Tris-HCl, 2.5% Tween 80 and 0.02% (w/v) $\text{Na}_2\text{S}_2\text{O}_5$ for 30 min, followed by the same buffer supplement with 1 μM ZnCl_2 , 5 mM CaCl_2 for 30 min and finally incubated in 50 mM Tris-HCl, 5 mM CaCl_2 , 1 μM ZnCl_2 and 0.02% (w/v) $\text{Na}_2\text{S}_2\text{O}_5$ at 37°C for 24 h. The reaction was interrupted by staining the gels with 0.1% Coomassie Brilliant Blue R250 and then destained in 10% acetic acid, methanol solution. The gelatinolytic activity was visualized as clear bands against a blue background and the band intensity was quantified with a Bio-Rad Model GS-700 Imaging Densitometer using analysis program Bio-Rad. Densitometric units were measured in linear range of gelatinolysis for each gelatinase (20, 21). The 120 kDa gelatinase

complex, 92 kDa gelatinase and 72 kDa gelatinase were identified using Western-immunoblot and specific antibodies (21, 22) to be MMP-9-neutrophil gelatinase associated lipocalin, gelatinase B (MMP-9) and gelatinase A (MMP-2), respectively.

Statistical methods

Some of the parameters were not normally distributed. Therefore, for uniformity reasons, nonparametric tests were applied in all comparisons. Wilcoxon one-sample rank test was used when comparing results of patients' cytokine, chemokine, etc. results between examination phases. The results of patients at the different follow-up points were compared with those of controls using the Wilcoxon test for two independent samples and Bonferroni correction for the P -values. Spearman's correlation analysis was used with all analyses being carried out with spss for Windows release 16.0.

Results

Follow-up of MMPs and TIMP-1

The MMP-9 level was significantly lower than that in the controls in the first visit (Table 2; Fig. 1). However, MMP-9 level at the third visit was higher than that of controls and higher than MMP-9 in the first and second examinations (Fig. 1).

According to another independent MMP-9 analysis, i.e. gelatin-zymography (20, 21), the 120 kDa MMP-9 complex level was significantly lower in the first examination than in controls (Table 2) but its level increased in the follow-up, such that by the third examination it displayed tendency to be higher than that of controls. The major gelatinases in BAL samples were the 120 kDa complex, 92 kDa MMP-9 (proMMP-9), and 82 kDa active forms (actMMP-9) (21). The 120 kDa MMP-9

Table 2. MMP-7, MMP-8, MMP-9, their ratios to TIMP-1 and the levels of various cyto- and chemokines during the follow-up period

	First (Baseline) ($n = 16$)	Second ($n = 15$)	Third ($n = 12$)	Controls ($n = 14$)	Significance of comparisons
MMP-7	0.106 (0–1.5)	0.156 (0–0.78)	0.093 (0–0.52)	0.022 (0–0.217)	II/C, III/C
MMP-8	0 (0–0.32)	0 (0–0.13)	0.005 (0–0.14)	0.022 (1–0.03)	NS
MMP-9	0.507 (0.44–0.64)	0.516 (0.41–0.92)	0.854 (0.41–1.47)	0.595 (0.47–0.7)	I/III, II/III, I/C
120 kDa MMP-9 complex	0 (0–0.55)	0 (0–0.76)	0.231 (0–0.47)	0.12 (0–0.30)	I/C
ProMMP-9 (92 kDa)	0.07 (0–1.04)	0.138 (0–0.6)	0.244 (0–0.5)	0.115 (0–0.089)	NS
ActMMP-9 (82 kDa)	0 (0–0.19)	0 (0–0.82)	0.068 (0–0.29)	0 (0–0.09)	NS
TIMP-1	1.59 (0–4.07)	2.6 (0–6.65)	3.18 (0–5.98)	1.96 (0.35–8.39)	NS
MMP-7/TIMP-1	0.05 (0–0.57)	0.062 (0–0.42)	0.033 (0.01–0.16)	0.008 (0–0.21)	NS
MMP-8/TIMP-1	0 (0–0.24)	0 (0–0.0)	0 (0–0.07)	0 (0–0.04)	NS
MMP-9/TIMP-1	0.151 (0–8.67)	0.523 (0.28–4.84)	0.805 (0.35–3.93)	1.09 (0.21–5.91)	I/C
IFN- γ	0.54 (0–4.5)	0.54 (0–6.0)	0.54 (0–3.0)	1.0 (0–15.5)	NS
IL-17	0	0	0	0 (0–3.6)	NS
IL-8	4.6 (1.97–32.79)	4.6 (0.45–88.9)	6.8 (1.3–51.6)	7.8 (2.4–33.2)	NS
CXCL5	0 (0–76.2)	0 (0–18.7)	3.1 (0–23.7)	0 (0–10)	NS
MCP1	2.9 (0.33–11.9)	2.9 (0–9.4)	2.1 (0–13.11)	4.9 (1.1–24.1)	NS
MIF	12.1 (9.2–306.5)	12.1 (2.4–445.0)	42.4 (3.3–382.2)	100.1 (20.5–437.6)	I/C, II/C, III/C

Values are presented as medians and ranges (in parenthesis).

After Bonferroni correction, the border for significance, $P = 0.008$.

MMP, matrix metalloproteinases; TIMP, tissue inhibited matrix metalloproteinases; IFN, interferon; IL, interleukin; MCP, monocyte chemotactic protein; MIF, macrophage inhibitory factor; NS, not significant.

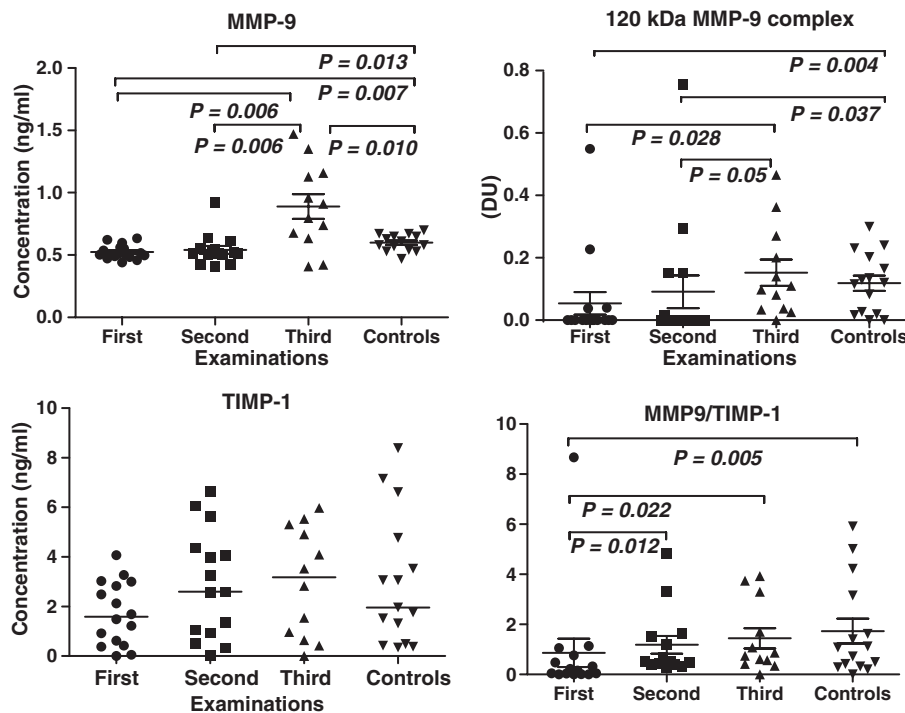


Figure 1. Matrix metalloproteinases (MMP)-9, 120 kDa MMP-9 complex, tissue inhibited matrix metalloproteinases (TIMP)-1 and MMP-9/TIMP-1 during the follow-up. First examination is the baseline, second examination at 6 months and the third about 3 years after diagnosis. Level of significance after Bonferroni correction is $P = 0.008$. DU, densitometric units.

form represents MMP-9 complexed with lipocalin (22). Overall MMP-9 and zymographic immunoreactive analyses correlated well. ProMMP-9 levels slightly, but nonsignificantly increased in the follow-up as did that of actMMP-9 (Table 2). 72 kDa proMMP-2 levels were almost undetectable (not shown).

The MMP-8 level was low and there were no significant changes between patients and controls at any of the time points (Table 2).

The MMP-7 level at the first examination did not differ from that of control subjects. MMP-7 level increased in the second examination, such that there was a significant difference between the patients and controls in the second and third examinations (Table 2), although MMP-7 decreased at the third examination.

Tissue inhibitor of matrix metalloproteinases-1 did not change during the follow-up (Fig. 1).

MMP-7/TIMP-1 ratio was higher in the first and second examinations compared with the controls ($P < 0.05$). The MMP-8/TIMP-1 ratio was low, although it slightly increased in the third examination. The MMP-9/TIMP-1 ratio in the first examination was significantly lower than in controls, although in the further follow-up its level increased (Fig. 1).

The level of MMPs or TIMP during the follow-up did not vary according to the diisocyanate the patient had been exposed to.

Associations of MMPs with inflammatory markers

In first examination, there were no significant correlations between the MMPs and cytokines or chemokines. However, there was a significant correlation between the MMP-9 concentration and the number of mast cells in bronchial mucosa ($\rho = 0.638$, $P = 0.035$) and total cell count in BAL fluid ($\rho = 0.517$, $P = 0.040$).

In second examination, proMMP-9 correlated significantly with IL-8 (CXCL-8) ($\rho = 0.656$, $P = 0.011$) and CXCL-5 (ENA-78) ($\rho = 0.782$, $P = 0.001$).

In third examination, 120 kDa MMP-9 complexes, proMMP-9 and actMMP-9 correlated inversely with a rather high correlation coefficient with several inflammatory markers (Table 3). MMP-9 correlated with MIF but CD4/CD8 ratio did not correlate with the amounts of MMPs in third examination.

Associations of MMPs with lung function

Matrix metalloproteinases neither displayed significant associations with the spirometric parameters during the follow-up, nor did they correlate with FEV₁ change. In the second examination, MMP-8 level correlated with PD15 ($\rho = 0.538$, $P = 0.047$), otherwise the MMPs did not correlate with bronchial hyperreactivity. In third examination, 120 kDa MMP-9 complex, proMMP-9 and

Table 3. Correlations between total MMP-9 and its different forms (pro- and active) or complexes with inflammatory cells or markers in third examination (after 3-year follow-up) during treatment with inhaled corticosteroids

MMP-9	Inflammatory marker or cell	Rho in Spearman's correlation test	Significance
120 kDa MMP-9 complex	CD4 cells (mucosa)	-0.786	0.036
	CD8 cells (mucosa)	0.881	0.004
	IL-6 (BAL fluid)	-0.564	0.071
	TNF- α (BAL fluid)	-0.573	0.066
	Activated eosinophils (mucosa)	-0.683	0.062
92 kDa proMMP-9	CD4 cells (mucosa)	-0.821	0.023
	CD8 cells (mucosa)	0.887	0.003
	TNF- α (BAL fluid)	-0.579	0.062
82 kDa active MMP-9	CD8 cells (mucosa)	-0.779	0.017
	Activated eosinophils (mucosa)	-0.676	0.066
MMP-9	MIF (BAL fluid)	0.753	0.012

MMP-9 forms and complexes were determined using gelatin-zymography as described in 'Methods'. Total MMP-9 represents sum of pro-, active and complex forms of MMP-9. MMP, matrix metalloproteinases; IL, interleukin; TNF, tumour necrosis factor; MIF, macrophage inhibitory factor; BAL, bronchoalveolar lavage.

actMMP-9 tended to be higher in those without bronchial hyperreactivity, whereas MMP-8 tended to be higher in those with bronchial hyperreactivity. CXCL5 (ENA-78) correlated with PD15 ($\rho = 0.626$, $P = 0.039$) in third examination.

Discussion

In this prospective study on DIA, we found that after cessation of the exposure and treating the patients with inhaled steroid, the BAL fluid MMP-7 levels decreased, no change occurred in the level of MMP-8 whereas the MMP-9 levels increased significantly. Gelatin-zymography revealed that 120 kDa MMP-9 complex, 92 kDa proMMP-9 and 82 kDa active MMP-9 increased similarly to the MMP-9 immunoreactive levels. Inhaled steroid medication did not influence the TIMP-1 levels. The MMP-9 results were unexpected as the MMP-9 has been claimed to decline, at least in epithelial and mucosal cells (8), serum (23) or in BAL fluid (5), with inhaled steroid medication. One study (3) on severe nonoccupational asthma treated with high doses of inhaled steroids has reported opposite findings, i.e. higher MMP-9 levels in the patients than in the controls. In that study, no associations were calculated either between the MMPs and inflammatory markers, or with the functional status (3). As this is a longitudinal follow-up study evaluating several MMPs and potential cytokines, our results significantly expand the results obtained from earlier investigations.

In an earlier study on the present patients (14), the numbers of mast cells, lymphocytes, activated eosinophils and neutrophils in bronchial mucosa decreased but the numbers of macrophages increased after initiation of inhaled steroid treatment. The numbers of IL-4 cells remained elevated after 6-month treatment with inhaled steroids but tended to decrease after 3-year treatment. These phenomena are presented schematically in Fig. 2.

MMP-9 might eventually act differently in asthma without treatment or in the initial stages of steroid treatment compared with the situation with long-lasting steroid treatment. At the first examination (baseline), MMP-9 was significantly lower than that in the controls, and there was a significant association between its level and the numbers of mast cells and the total cell count in the native BAL sample. Thus, at the baseline, the results are in agreement with earlier studies pointing to an association between MMP-9 and eosinophilic inflammation (4). For example, mast cells could be potential MMP-9 producers.

In patients with nonoccupational asthma, MMP-9 has been reported to be higher than that in the healthy controls (7). In DIA patients, MMP-9 level has been claimed to be lower than that in healthy controls, while a specific diisocyanate exposure in challenge testing has increased the MMP-9 levels in induced sputum specimens

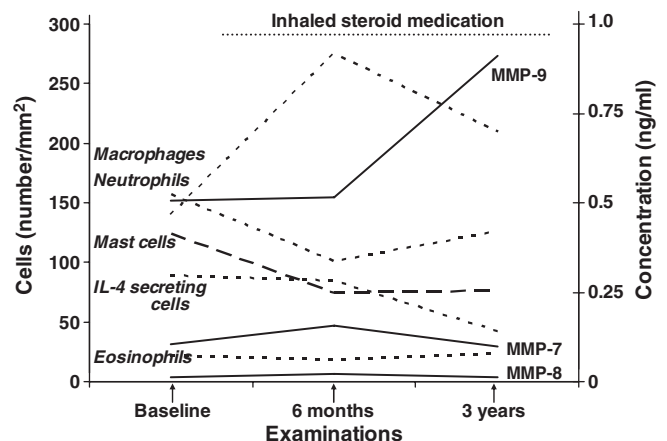


Figure 2. Schematic representation of cell counts in the histological samples of bronchial mucosa (broken lines) and the studied MMPs in the follow-up (solid lines).

(15). The present material does not represent patients with acute DIA because diisocyanate exposure had ended 7 months (mean) prior to the first examination. Thus, the situation of DIA without actual specific exposure to diisocyanates is not comparable with that encountered in untreated nonoccupational asthma.

In the second examination, a slight increase in TIMP-1 was found in comparison with the first examination; these results agree with other studies on asthma after 6-month treatment with inhaled steroids (8, 23). We also found a slight increase in MMP-7 but MMP-9 did not change, although a reduction of MMP-9 has been reported in a similar follow-up of cases of nonoccupational asthma (8, 23).

In the second examination, we found a significant association between MMP-9 and CXCL8 (IL-8), which is a cytokine known to induce MMP-9 release from neutrophils (24). A second association found was the positive correlation between MMP-9 and CXCL-5 (ENA-78), another member of CXCL chemokine family. CXCL-5 is a chemokine with a neutrophil attraction function, e.g. secreted by macrophages and fibroblasts (25). The increase of CXCL-5 could be associated with the increase in the numbers of macrophages. However, the exact role of these chemokines in this phase of DIA remains unclear. Surprisingly, at 3 years, CXCL-5 was associated with decreasing bronchial hyperreactivity.

In the third examination, at the same time as there was an MMP-9 elevation, there were signs of improvement of lung function; decreased percentage and grade of NSBH. The studied MMPs did not associate with bronchial hyperreactivity, but 120 kDa MMP-9 complex, proMMP-9 or actMMP-9 tended to be higher in those individuals without bronchial hyperreactivity in comparison with those with persistent bronchial hyperreactivity. In addition, there were several inverse correlations between these complexes and activated eosinophils, IL-6 and TNF- α mRNA levels. The elevated levels of these cytokines have been described previously (14) to be associated with bronchial hyperreactivity in DIA.

The third examination was an important step in further evaluating the long-term behaviour of MIF. MIF is a cytokine produced mainly by macrophages, which has been found to be associated with allergic inflammation and NSBH without affecting the production of TH-2 cytokines of immunoglobulin (Ig)E (26). MIF can induce MMP-9 expression (27), which may explain the correlation noted at this time point.

MMP-9 itself may have a modulatory role in the traffic of leucocytes and the consequent inflammatory response. This has been found in sensitized MMP-9 knockout mice in which allergen challenge resulted in accentuation of airway inflammation compared with wild-type controls (28). There was no difference in the response to methacholine, but levels of TH-2 type

cytokines IL-4, IL-5 and IL-13, the TH-2 type cytokine IFN- γ and the chemokines eotaxin (CCL11) and MDC (CCL22) were significantly higher in the knockout mice. It was proposed that MMP-9 might be involved in the resolution of TH-2 type inflammation in the airways. Corresponding findings have been found in mouse studies on ozone induced airway inflammation (29). The mechanism for this may be associated with the capability of MMP-9 to reduce the recruitment of leucocytes to the inflammatory site, either by affecting the development of allergen-specific T cell responses or by processing of inflammatory mediators.

Some authors have found in TDI that sensitized rats MMP-9 inhibitor can reduce the signs of inflammation (30, 31). The overall results of these earlier studies in TDI sensitized rats (30, 31) might be attributable to nonspecific effects of MMP inhibitors on inflammatory cells and MMPs rather than any specific effect on MMP-9 alone. TIMP-1 has been found to inhibit most MMPs, and to account for about 50% inhibition of polymorphonuclear cells migration (32, 33).

MMP-7 in induced sputum has not been found to differ between patients with mild asthma and the healthy controls (34). In our study, the MMP-7 level initially slightly increased during the follow-up but decreased back to baseline levels at the end of the follow-up in conjunction with the decrease of markers of TH-2 type inflammation.

An MMP-8 deficiency has been associated with increased levels of IL-4 and increased allergic response (35), i.e. having protective properties. In the present material, the level of MMP-8 tended to associate with NSBH but it was very low during the follow-up, which may have influenced the results.

In conclusion, inhaled steroid medication in DIA was found to decrease BAL MMP-7 and MMP-7/TIMP-1 level but to increase MMP-9 and MMP-9/TIMP-1. Overall, our data suggest that the reduced TH-2 type inflammation in DIA after inhaled steroid medication is reflected in elevated MMP-9 and MMP-9/TIMP-1 levels in BAL. We suggest that MMP-9, in addition to its surrogate destructive actions (1) might exert even protective effects against the lung inflammation, a phenomenon reported previously in animal experiments (28, 29). This paper is the first study to describe this finding in a human material.

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