

Increased release of matrix metalloproteinase-9 in the plasma of acute severe asthmatic patients

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Summary

Background Matrix metalloproteinases (MMPs) are likely to be relevant mediators of the extracellular matrix (ECM) degradation and airway remodelling.

Objective We have compared the levels of MMPs, eotaxin and soluble interleukin 2 receptor (IL-2R) in the plasma of healthy subjects, atopic patients and asthmatic patients.

Methods The asthmatic patients were separated into two groups, either well controlled on inhaled therapy or acute severe asthma. Patients with acute severe disease had all received systemic corticosteroids from 12 to 48 h before the blood was taken. Blood was recovered in EDTA tubes, incubated with either fMLP, PMA or vehicle for 10 min and centrifuged. MMP-9, TIMP-1, IL-2R and eotaxin levels were measured in the plasma by ELISA. Moreover, the activity of MMPs was also evaluated by zymography.

Results An increased basal level of MMP-9 and IL-2R was observed in acute severe asthma. Following stimulation with fMLP and PMA there was an enhanced production of MMP-9 in the plasma of all groups of patients. However, the MMP-9 level was significantly enhanced in acute severe asthma, compared with the others. No difference was found for the TIMP-1 level between the patients. The eotaxin level in plasma was found to be significantly lower in acute severe asthmatics compared with the others groups. Zymography technique showed a significant increased activity of MMP-9 (92 kDa) but not MMP-2 (66 kDa) in the plasma of patients with acute asthma.

Conclusion The increased in MMP-9 production and activity observed in the present study suggests a process of extracellular matrix degradation in acute severe asthmatic patients and proposes MMP-9 as a non-invasive systemic marker of inflammation and airway remodelling in asthma.

Keywords matrix metalloproteinase, eotaxin, soluble interleukin 2 receptor, acute asthma, airway remodelling

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Introduction

Structural bronchial alterations are often observed in asthma, with thickening of the lamina reticularis caused by an increased collagen deposition, smooth muscle hypertrophy/hyperplasia, and airway inflammatory cell infiltration. These structural changes, designed as airway remodelling, are often present in biopsy specimens of asthmatic subjects, even at the beginning of the disease [1].

Airway remodelling is partly due to an excess of extracellular matrix (ECM) deposition in the airway wall. Matrix metalloproteinases (MMPs) are major proteolytic enzymes that are involved in the normal ECM turnover. Several subclasses of MMPs have been identified according to their substrate specificities, including collagenases, gelatinases, stromelysins and membrane-type MMPs (MT-MMPs) [2]. MMPs are secreted

in a latent form as inactive pro-enzymes. The loss of their pro-peptide leads them to their activated state. Among MMPs, MMP-9 (92 kDa gelatinase or gelatinase B) is of particular interest as it especially cleaves collagen IV and gelatin [3].

Inflammatory cells involved in asthma, such as macrophages, lymphocytes, mast cells or eosinophils, are able to express MMP-9 [3]. Tissue inhibitors of metalloproteinases (TIMPs) specifically control the proteolytic activity of MMPs and four of these inhibitors have been characterized. Among them, the principal member TIMP-1 inhibits the activity of almost all MMPs except MT-MMPs.

Imbalance between MMP and TIMP activities has been observed in pathological disorders, including inflammatory diseases such as asthma, chronic degenerative disease and tumour invasion [3,4]. The possible implication of MMPs in asthma is suggested by morphologic studies where matrix remodelling, lung tissue damage, bronchial and alveolar structure alteration or abnormal repair have been described [5]. Moreover, increased levels of MMP-9 have been found in bronchoalveolar lavage (BAL) fluids, in bronchial biopsies from asthmatic patients and in alveolar macrophages from asthmatic patients [6–8].

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Whether or not matrix metalloproteinases, mainly gelatinases, can be used as biomarkers of airway remodelling is of interest. Therefore, the aim of the study was to investigate the levels of gelatinases in the plasma of asthmatic patients. Asthmatic patients were separated into two groups according to the gravity of the disease and compared with atopic non-asthmatic patients and healthy controls. In order to evaluate the inflammatory degrees of the patients we also evaluated the plasmatic level of soluble interleukin 2 receptor (IL-2R), which is a major parameter of T-cell activation and of eotaxin, a CC chemokine inducing chemotaxis of eosinophils.

Material and methods

Characteristics of patients

Patients were recruited among subjects admitted to the Pneumology Unit at the Hôpital Pontchaillou (CHU de Rennes, France) and the patients seen at the clinic. Four groups of patients were defined. The first one (group 1) was a healthy control group in which the subjects were non-smokers and were not taking any treatment. Subjects in the second group were atopic non-asthmatic patients suffering from allergic rhinitis (out of seasonal allergen period) or atopic dermatitis who demonstrated positive skin prick tests for at least two common allergens. Groups 3 and 4 included the asthmatic patients who presented with the criteria of asthma as defined by the American Thoracic Society (ATS) [9]. All these patients were characterized by a reversible airway obstruction (characterized by a 20% increase in forced expiratory volume in one second, FEV₁, after the inhalation of salbutamol) and a bronchial hyper-reactivity to metacholine. Exclusion criteria were other respiratory or chronic diseases and smoking habits.

Asthmatic patients were divided into two groups according to the gravity of the disease. Thus, patients who were hospitalized for an exacerbation of their asthma, with clinical symptoms such as dyspnoea and cough, were classified into the 'acute severe asthma' group or group 3. Patients who were well-controlled by their daily treatment constituted the 'well-controlled asthma' group or group 4. Among these two later groups, asthmatic patients were separated into four steps in relation to the severity of the disease, according to international recommendations (WHO/NHLBI 1995) [10] (step 1,

intermittent asthma; step 2, mild persistent asthma; step 3, moderate persistent asthma; and step 4, severe persistent asthma). All the subjects included in our study were non-smokers.

Thirty-four patients were included in our study and all the clinical characteristics are summarized in Table 1. Patients hospitalized for acute asthma presented with clinical symptoms such as dyspnoea, tachycardia and cough. They all received systemic corticosteroids (methylprednisolone 180 mg/j) for 12–48 h before the blood was taken, nebulized salbutamol (2.5 mg/mL, six times a day) and oxygen therapy. None of them required assisted ventilation. Well-controlled asthmatic patients received inhaled corticosteroid treatment such as beclomethasone dipropionate (500–1000 µg/day) for four patients, budesonide (800 µg/day) for one patient and fluticasone (500–1000 µg/day) for three patients. Among them, two were corticosteroid-dependent (methylprednisolone 4 mg/day), six received long-acting beta-2 agonists (bambuterol or salmeterol) and two were given antileukotrienes (montelukast). Concerning patients with exacerbated asthma, eight were treated with inhaled corticosteroids (fluticasone, 500–1000 µg/day, *n* = 5), budesonide (800 µg/day, *n* = 1), beclomethasone (500–1000 µg/day, *n* = 2) and five with long-acting beta-2 agonists. None of these patients had a long-course oral corticosteroid treatment.

Plasma collection

Twenty-one millilitres of peripheral blood were recovered by veinopuncture in EDTA tubes. Then the whole blood was incubated with various stimulatory agents. Briefly, 2.7 mL of whole blood were incubated with 300 µL of either fMLP (10^{-6} M) or PMA (10^{-6} M) or PBS (as control) during 10 min at 37 °C. At the end of incubation blood was centrifuged and plasma aliquoted and stored at –80 °C until use. Blood cell counts and IgE levels were also evaluated.

MMP-9 and TIMP-1 quantification

The respective amounts of MMP-9 and TIMP-1 in plasma were evaluated by immuno-enzymatic method (ELISA). A Quantikine kit (R & D Systems, Minneapolis, MN, USA) was used to determine the total amount (both active and latent forms) of human MMP-9 in plasma. The detection limit of this assay was 0.156 ng/mL. Blood samples that have been incubated with fMLP or PMA were 1:40 and 1:20 diluted, respectively. The results were expressed in ng/mL.

Table 1. Clinical characteristics of subjects

	Groups			
	1 Healthy	2 Atopic non-asthmatics	3 Acute severe asthma	4 Well-controlled asthma
<i>n</i>	6	5	12	11
Mean age (years)	39.6 (31–47)	28.8 (23–40)	32.2 (21–52)	35.4 (19–72)
Sex ratio (F/M)	4/2	3/2	9/3	6/5
FEV ₁ /FVC (% predicted)			94 (38–117)	88 (68–109)
Severity				
Step I			<i>n</i> = 4	<i>n</i> = 3
Step II			<i>n</i> = 5	<i>n</i> = 4
Step III			<i>n</i> = 3	<i>n</i> = 2
Step IV				<i>n</i> = 2

Table 2. Serum IgE and blood cell numbers of the subjects

	Groups			
	1 Healthy	2 Atopic non-asthmatics	3 Acute severe asthma	4 Well-controlled asthma
<i>n</i>	6	5	12	11
IgE (IU/mL)	60.9 ± 270	558.0 ± 246	769.3 ± 341	808.4 ± 417
Leucocytes (Giga/L)	7118.1 ± 604	6216.0 ± 404	12409.0 ± 1436**	7800.0 ± 712
Neutrophils (Giga/L)	4354.5 ± 459	3116.6 ± 272	10454.0 ± 1571**	4941.6 ± 733
Eosinophils (Giga/L)	163.6 ± 31	383.3 ± 830	100.0 ± 50#	358.3 ± 73
Lymphocytes (Giga/L)	1700 ± 256	1800 ± 322	1200 ± 250	1700 ± 220

***P* < 0.01 in comparison with the other groups; #*P* < 0.05 in comparison with 'well-controlled asthma' and with 'atopic non-asthmatic patients'.

The quantification of TIMP-1 in the plasma was also performed after dilution to 1:4 (Biotrak from Amersham Pharmacia Biotech (Little Chalfont, UK), results expressed in ng/mL, detection limit 1.25 ng/mL).

Evaluation of gelatinases (MMP-9 and MMP-2) activities

MMP-9 and MMP-2 activities were also evaluated by zymography. This method allows the determination of various types of MMPs on the basis of their molecular weight, and allows determination of their state of activation (latent and/or active forms).

Plasma aliquots were subjected to electrophoresis on a 4.5% acrylamide stacking gel/7% acrylamide separating gel containing 1 mg/mL gelatin, in the presence of sodium dodecyl sulphate (SDS), under non-reducing conditions, as previously described [11]. After electrophoresis, gels were washed twice in 2.5% Triton X 100 for 10 min, rinsed abundantly with water and then incubated at 37 °C in 'reaction' buffer (49 mM Tris, 5 mM CaCl₂, 2 mM ZnCl₂, pH 8). After staining with Coomassie Brilliant Blue, molecular weights of gelatinolytic bands were estimated using recombinant protein molecular weight markers (10 000–225 000) (Amersham, Little Chalfont, UK). Enzyme amount was quantified by measuring the intensity of the negative bands using a densitometric analyser with «Densylab» software (Bioprobe Systems, Les Ulis, France). Results were expressed as a percentage of a band of migration of one sample loaded onto each gel. This sample was used as an internal standard to allow comparison between gels.

Evaluation of IL-2R and eotaxin

Soluble IL-2R and eotaxin were quantified in plasma by ELISA technique (Quantikine kit, R & D Systems). The detection limit of the assays was 5 pg/mL. Samples were used without any dilution.

Materials

Gelatin, ethylene diamine tetraacetic acid (EDTA), Triton X-100, N-formyl-Met-Leu-Phe (fMLP), phorbol myristate acetate (PMA) were purchased from Sigma (St Louis, MO, USA). Coomassie blue was from Biorad (München, Germany). Sodium dodecyl sulphate (SDS) was from Eurobio (Les Ulis, France).

Statistical analysis

Results were expressed as mean ± SEM. A variance analysis was carried out using the ANOVA test, then data were compared

using Scheffe and Bonferroni tests (software SPSS). *P*-values less than 0.05 were considered as significant. Correlation analyses were carried out using the Spearman's rank test.

Results

No differences in IgE levels and FEV₁ were found between the two groups of asthmatic patients (groups 3 and 4) and the group of atopic patients (group 2) (Table 2). The levels of IgE in the plasma of these groups are markedly increased in comparison with the level observed for the healthy subjects (group 1). Moreover, the number of blood total leucocytes was significantly increased in group 3 (acute severe). The significant increase in the number of polymorphonuclear neutrophils in this group may account for the enhancement of total blood leucocytes (Table 2). Interestingly, the number of eosinophils was significantly decreased in the acute severe group in comparison with the well-controlled asthmatic subjects. Although the FEV₁ values for acute severe asthmatics appeared higher than that noted for well-controlled patients, there is no statistical difference in Th FEV₁/FVC values between the two groups (Table 2).

Quantification of MMP-9 and TIMP-1 in plasma

Figure 1(a) represents the basal expression of MMP-9 in the plasma of healthy subjects, non-asthmatic atopic patients, acute severe subjects and well-controlled asthmatic patients. MMP-9 expression was significantly higher in all asthmatic groups (*P* < 0.01 for acute severe subjects and *P* < 0.05 for well-controlled asthmatics) compared with the control group.

The effects of fMLP or PMA stimulation on MMP-9 expression in the plasma of the same subjects as above are shown in Fig. 1(b and c), respectively. After blood stimulation, an enhanced production of MMP-9 in the plasma of various groups of patients was noted. However, the increased MMP-9 level was significantly higher in acute severe asthma, with a significant difference between this group and the other groups for the PMA and between the asthmatics with acute severe disease and the well-controlled ones with the fMLP.

A positive significant correlation could be highlighted between the rate of MMP-9 and the number of neutrophils (*r* = 0.699, *P* < 0.001) (data not shown).

The plasmatic detection of TIMP-1 was carried out for five healthy control subjects and for 10 asthmatics (well-controlled, *n* = 5, and acute-severe asthmatics, *n* = 5). No difference could

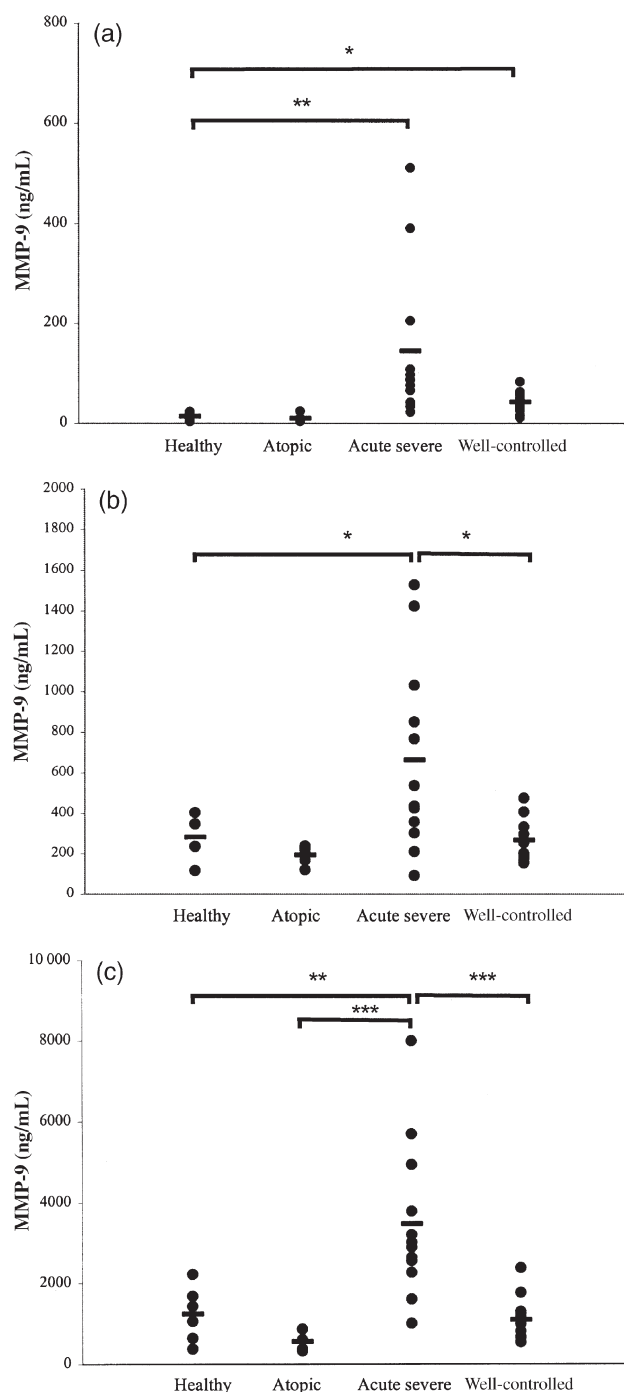


Fig. 1. MMP-9 levels in the plasma of healthy subjects (group 1), atopic subjects (group 2), subjects with acute severe asthma (group 3) or well-controlled asthmatic patients (group 4) without (a) or after *in vitro* stimulation of the blood with fMLP (b) or PMA (c). Each point represents a subject and the mean appears as a horizontal bar. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

be highlighted according to the groups of patients (data not shown).

Evaluation of MMPs activity by zymography

All plasma samples were analysed by zymography technique. On the zymograms, the highest level of gelatinase activity was

the 92 kDa gelatinase, which corresponds to the pro-MMP-9. The active form of MMP-9 (88 kDa) was inconstantly found and generally non-quantifiable. Moreover, gelatinolytic activity at 66 kDa was detected in all samples and corresponds to an intermediary form of MMP-2 (Fig. 2). After densitometric analysis of zymograms we have observed a significant increase ($P < 0.05$) of activity of pro-MMP-9 in the plasma of asthmatic patients during acute asthma compared with the control group (Fig. 3). Concerning MMP-2, no significant difference between the groups of patients was noted (Fig. 3).

Eotaxin and soluble IL-2R plasmatic levels

In order to evaluate the degree of inflammation in the various groups of patients, we determined the level of a cc-chemokine inducing chemotaxis of eosinophils, eotaxin and a marker of T cell activation, soluble IL-2R. Eotaxin level in plasma was found to be significantly lower in acute severe asthmatics compared with well-controlled asthmatics (group 4, $P < 0.05$) and healthy subjects (group 1, $P < 0.01$) (Fig. 4). However, we did not find any correlation between eotaxin level and eosinophil count.

On the other hand, soluble IL-2R was significantly higher in acute severe asthmatics (group 3) in comparison with healthy subjects (group 1), confirming the activation of lymphocytes during severe asthma (Fig. 5).

Discussion

In the present study, MMP-9 was found to be increased at the basal level in the plasma from asthmatic patients, in comparison with control healthy subjects. Moreover, stimulation of the blood by fMLP and PMA elicited an enhanced activity of MMP-9 in the acute severe asthma group compared with all the others, even with the group of asthmatic patients well controlled with inhaled corticosteroids. This quantitative increase is associated with an enhancement of the 92 kDa gelatinase activity (MMP-9) in the blood of acute severe patients compared with the controls. Although it is known that treatment with corticosteroids markedly reduces the release of MMPs *in vivo* [11,12], our results clearly demonstrated that the MMP-9 levels (measured by immunoassay) and MMP-9 activity (determined by zymography technique) were significantly increased in the blood of acute severe patients.

In order to assess the inflammatory status of our patients, we tried to quantify inflammatory markers in the circulating blood. Soluble IL-2R, a subunit of the IL-2 cell surface receptor, is secreted from cell membranes during T cell activation. As previously reported [13], an increase of soluble IL-2R in acute severe asthmatic patients confirms the activation of lymphocytes during exacerbations of the disease. In contrast, the lower level of eotaxin can be explained by the effect of systemic corticosteroids received by the patients in acute asthma, several hours before the blood was taken [14].

An excess of MMP-9 in BAL fluids from patients with uncontrolled asthma or during a severe exacerbation has previously been reported [6,8]. The same increase was observed after local challenge [15]. In sputum, the TIMP-1:MMP-9 molar ratio was also higher in untreated stable asthmatics than in control subjects [4,5]. This ratio was negatively correlated with FEV₁ [5]. Moreover, in a previous study, Bossé et al.

Fig. 2. Example of gelatin zymogram performed with plasma of (b) healthy subjects, (c) well-controlled asthmatic patients and (d) acute severe asthmatic patients. (a) Molecular weight marker.

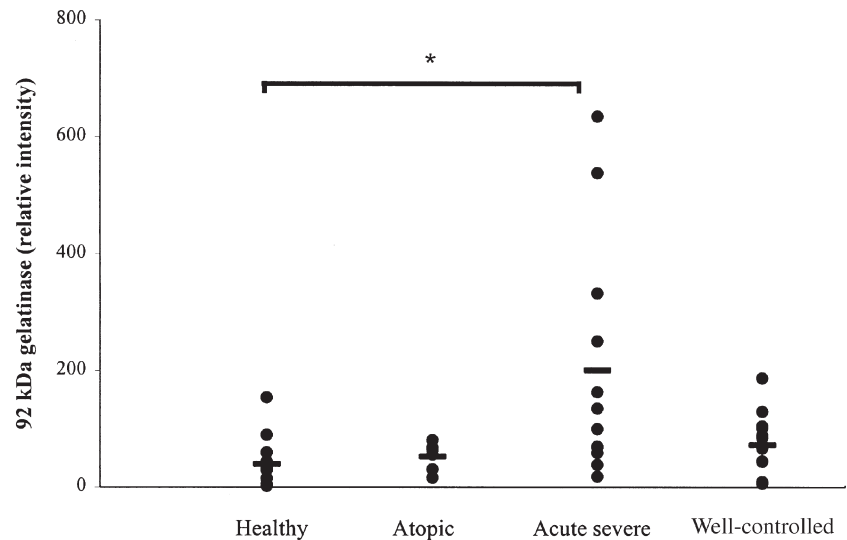
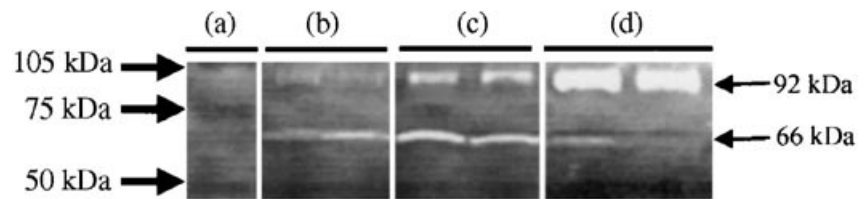
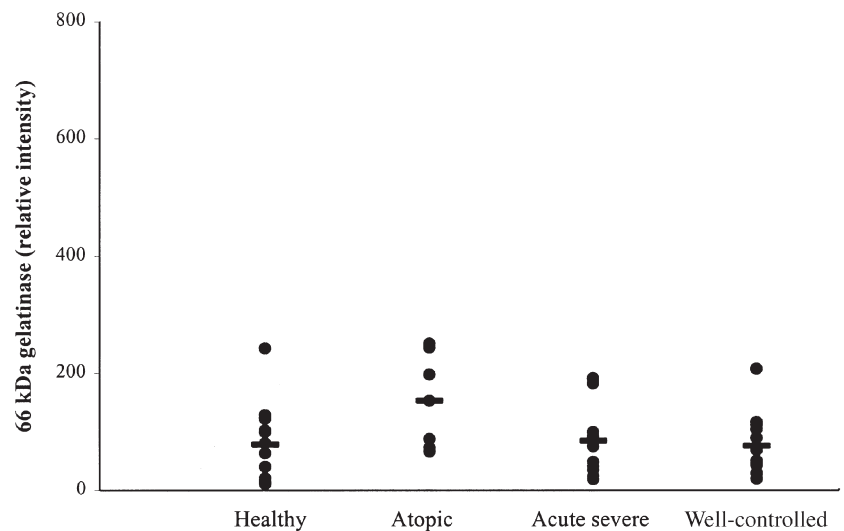


Fig. 3. MMPs activity in plasma of healthy subjects, atopic subjects, subjects with acute severe asthma, or well-controlled asthmatic patients. The MMP-9 (92 kDa gelatinase) and MMP-2 (66 kDa gelatinase) have been evaluated using zymography. After scanning the gels, both surface and intensity of lysis bands were analysed by densitometry using special software. The results were expressed as a percentage (relative intensity) of an internal standard band of migration that was used on each gel.



[16] evaluated the relation of the MMP-9:TIMP-1 ratio and the response to corticosteroid in severe asthma. They concluded that serum MMP-9:TIMP-1 ratio could predict the response to oral corticosteroid. A low ratio was observed in subjects with little or no FEV₁ improvement, suggesting that in these patients bronchial fibrogenesis predominates over inflammation [16]. In our study, no difference was found in TIMP-1 level in plasma. However, since the level of MMP-9 was considerably enhanced in acute severe asthmatics (group 3), the MMP-9:TIMP-1 ratio in this group may be also higher than in other groups.

In the present study, we also determined the profile of various gelatinases using zymography techniques. Gelatinolytic activity in the plasma can be detected at 66 kDa, which is probably MMP-2 in a form of intermediate activation. Indeed, the final active form of MMP-2 has been described for 62 kDa [17,18]. However, we did not observe any difference in the MMP-2 activity between the groups of patients. In contrast, we were able to observe MMP-9 in the presence of the latent form (92 kDa), which was markedly enhanced in acute severe asthma, confirming the involvement of MMP-9 in airway remodelling during exacerbations of the disease. In contrast, the active form

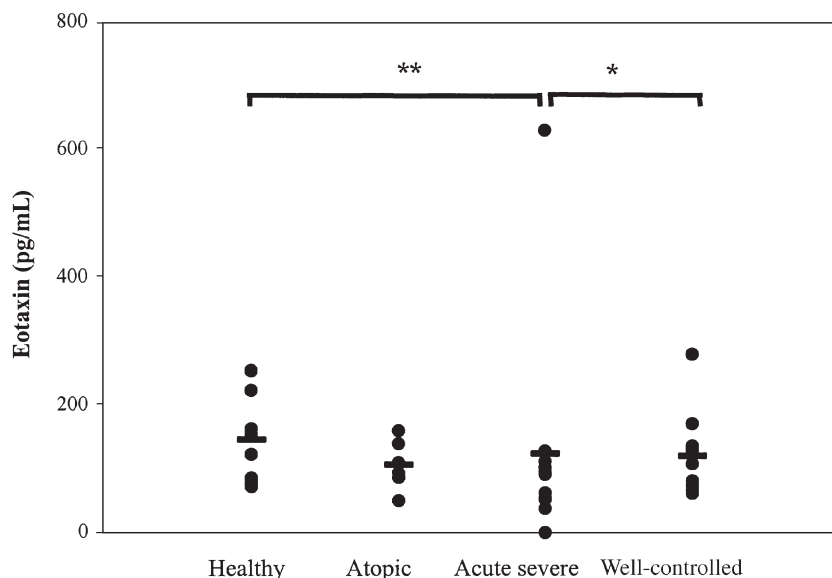


Fig. 4. Eotaxin levels in the plasma of healthy subjects (group 1), atopic subjects (group 2), subjects with acute severe asthma (group 3), or well-controlled asthmatic patients (group 4). Each point represents a subject and the mean appears as a horizontal bar. * $P < 0.05$; ** $P < 0.01$.

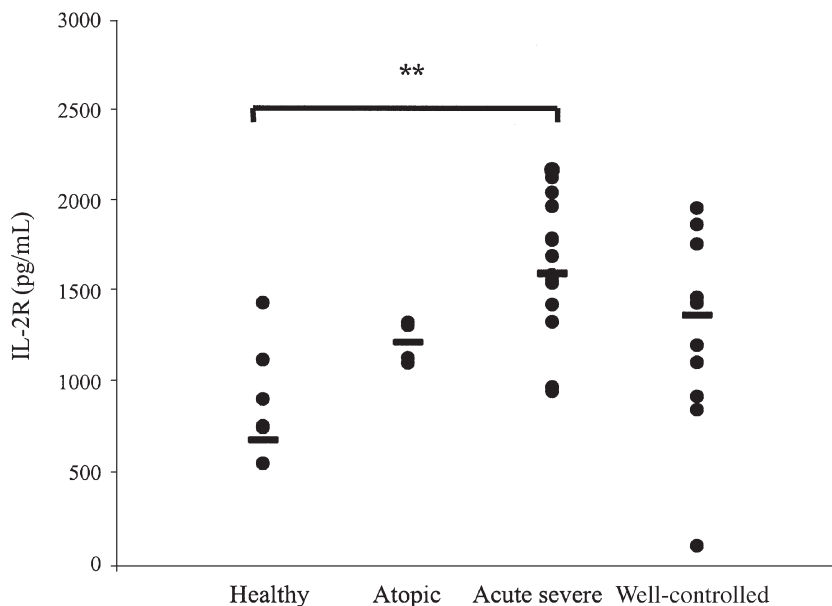


Fig. 5. Soluble interleukin 2 receptor (IL-2R) levels in the plasma of healthy subjects (group 1), atopic subjects (group 2), subjects with acute severe asthma (group 3), or well-controlled asthmatic patients (group 4). Each point represents a subject and the mean appears as a horizontal bar. * $P < 0.05$; ** $P < 0.01$.

of MMP-9 (88 kDa) was inconstantly found and generally non-quantifiable. Similar observations were made by Mautino et al. [19] in bronchoalveolar lavage from asthmatics. The authors explained the predominance of the latent form over the active form by an excess of TIMP-1, which was not confirmed in our study. However, such a discrepancy may be explained by the difference in the activation and release of MMP-9 and TIMP-1 in bronchoalveolar lavage and serum.

The release of MMP-9 may originate from neutrophils because we found a positive correlation between the number of neutrophils and MMP-9 ($r = 0.699$ and $P < 0.001$). However, other inflammatory cells, such as eosinophils and macrophages, can produce MMP-9 [19,20]. This result is supported by the study from Fujisawa et al. [21] reporting that circulating eosinophils have only small amounts of MMP-9 in comparison with neutrophils and mononuclear cells. Moreover, there was no

difference in MMP-9 content of eosinophils between normal donors and patients with asthma [21].

In contrast to MMP-9, MMP-2 is constitutively synthesized by various mesenchymal cells such as fibroblasts. In our hands, no difference in MMP-2 activity was observed between our groups of patients, suggesting that these cells are poorly implicated in the airway remodelling in asthma and recruitment of inflammatory cells in airways. It is clear that the presence of a 66 kDa MMP-2 is unexpected. However, endothelial cells are major sources of MMP-2 and this could explain the presence of MMP-2 in whole blood. The intermediary active form of MMP-2 could be the result of the interaction of the latent form with TIMP-2 and/or MT-MMP-1, which have been found to activate MMP-2 [17,18].

MMP-9, by degrading basement membrane and extracellular matrix, induces the migration of monocytes/macrophages or

eosinophils [22]. However, not all studies confirm this possibility and discordant results have been published concerning the migration of neutrophils [23–25]. The increase of MMP-9 in the plasma could consider these proteases as inflammatory markers obtained easily, associated with the degree of remodelling. It could be interesting for a future study to correlate the activity of MMP-9 in BAL and the same activity in plasma. Several studies confirm the role of MMPs during the remodelling found in asthma. Thickening of collagen III and V in subepithelial levels is associated with an increased expression of MMP-9 and TIMP-1 correlated with the number of eosinophils [7]. Moreover, the use of an immunostaining for MMP-9 on endobronchial biopsy specimens in asthmatic subjects can reveal the MMP-9 in extracellular matrix [26].

In conclusion, we clearly report that MMP-9 is largely increased in the whole blood of patients with acute severe asthma. The increase in MMP-9 production and activity observed in the present study suggests a process of extracellular matrix degradation in unstable asthmatic patients and proposes MMP-9 as a non-invasive systemic marker of inflammation and airway remodelling in asthma.

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References

- Bousquet J, Chanez P, Lacoste JY et al. Asthma: a disease remodeling the airways. *Allergy* 1992; 47:3–11.
- Mautino G, Capony F, Bousquet J. Balance in asthma between matrix metalloproteinases and their inhibitors. *J Allergy Clin Immunol* 1999; 104:530–3.
- Shapiro S, Senior R. Matrix metalloproteinases. *Am J Respir Cell Mol Biol* 1999; 20:1100–2.
- Tanaka H, Miyazaki N, Oashi K et al. Sputum matrix metalloproteinase-9: tissue inhibitor of metalloproteinase-1 ratio in acute asthma. *J Allergy Clin Immunol* 2000; 105:900–5.
- Vignola A, Riccobono L, Mirabella A et al. Sputum metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio correlates with airway obstruction in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1998; 158:1945–50.
- Mautino G, Oliver N, Chanez P, Bousquet J, Capony F. Increased release of matrix metalloproteinase-9 in bronchoalveolar lavage fluid and by alveolar macrophages of asthmatics. *Am J Respir Cell Mol Biol* 1997; 17:583–91.
- Hoshino M, Nakamura Y, Sium J, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. *J Allergy Clin Immunol* 1998; 102:783–8.
- Lemjabbar H, Gosset P, Lamblin C et al. Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. *Am J Respir Crit Care Med* 1999; 159:1298–307.
- Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease and asthma. *Am Rev Respir Dis* 1987; 136:225–44.
- WHO/NHLBI. Workshop Report. Global Strategy for Asthma and Prevention. Bethesda, Maryland: National Institute of Health, National Heart, Lung and Blood Institute, 1995.
- Corbel M, Lagente V, Th  ret N et al. Comparative effects of betamethasone, cyclosporin and nedocromil sodium in acute pulmonary inflammation and metalloproteinase activities in bronchoalveolar lavage fluid from mice exposed to lipopolysaccharide. *Pulm, Pharmacol Therapeutics* 1999; 12:165–71.
- Hoshino M, Takahashi M, Takai Y, Sim J. Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma. *J Allergy Clin Immunol* 1999; 104:356–63.
- Matsumoto K, Taki F, Miura M, Matsuzaki M, Takagi K. Serum levels of soluble IL-2R, IL-4, and soluble Fc  RII in adult bronchial asthma. *Chest* 1994; 105:681–6.
- Taha R, Minshall E, Miotto D et al. Eotaxin and monocyte chemotactic protein-4 mRNA expression in small airways of asthmatic and non asthmatic individuals. *J Allergy Clin Immunol* 1999; 103:473–83.
- Warner J, Julius P, Luttmann W, Kroegel C. Matrix metalloproteinase in bronchoalveolar lavage fluid following antigen challenge. *Int Arch Allergy Immunol* 1997; 113:318–20.
- Boss   M, Chakir J, Rouabhi M et al. Serum matrix metalloproteinase-9: tissue inhibitor of metalloproteinase-1 ratio correlates with steroid responsiveness in moderate to severe asthma. *Am J Respir Crit Care Med* 1999; 159:596–602.
- Atkinson SJ, Crabbe T, Cowell S et al. Intermolecular autolytic cleavage can contribute to the activation of progelatinase A by cell membranes. *J Biol Chem* 1995; 270:30479–85.
- Zucker S, Drews M, Conner C et al. Tissue inhibitor of metalloproteinase-2 (TIMP-2) binds to the catalytic domain of the cell surface receptor, membrane type 1-matrix metalloproteinase (MT1-MMP). *J Biol Chem* 1998; 273:1216–22.
- Mautino G, Henriquet C, Gougat C et al. Increased expression of tissue inhibitor of metalloproteinase-1 and loss of correlation with matrix metalloproteinase-9 by macrophages in asthma. *Lab Invest* 1999; 79:39–41.
- Ohno I, Ohtani H, Nitta Y et al. Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1997; 16:212–9.
- Fujisawa T, Kato Y, Terada A, Iguchi K, Kamiya H. Matrix metalloproteinase-9 in peripheral blood eosinophils. *Int Arch Allergy Immunol* 1999; 120:65–9.
- Okada S, Kita H, George T, Gleich G, Leiferman K. Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9. *Am J Respir Cell Mol Biol* 1997; 17:519–28.
- Delclaux C, Delacourt C, D'Ortho MP et al. Role of gelatinase B and elastase in human polymorphonuclear neutrophil migration across basement membrane. *Am J Respir Cell Mol Biol* 1996; 14:288–95.
- Betsuyaku T, Shipley J, Liu Z, Senior R. Neutrophil emigration in the lungs, peritoneum, and skin does not require gelatinase B. *Am J Respir Cell Mol Biol* 1999; 20:1303–9.
- Mackarel A, Cottell D, Russell K, FitzGerald M, O'Connor C. Migration of neutrophils across human pulmonary endothelial cells is not blocked by matrix metalloproteinase or serine protease inhibitors. *Am J Respir Cell Mol Biol* 1999; 20:1209–19.
- Dahlen B, Shute J, Howarth P. Immunohistochemical localisation of the matrix metalloproteinases MMP-3 and MMP-9 within the airways in asthma. *Thorax* 1999; 54:590–6.