Increased circulating 92 kDa matrix metalloproteinase (MMP-9) activity in exacerbations of asthma

Y Oshita, T Koga, T Kamimura, K Matsuo, T Rikimaru, H Aizawa

Background: The 72 kDa matrix metalloproteinase 2 (MMP-2) and the 92 kDa matrix metalloproteinase 9 (MMP-9) are type IV collagenases implicated in various aspects of inflammation including accumulation of inflammatory cells, tissue injury, and development of remodelling. The role of these enzymes in the pathogenesis of asthma exacerbations is unknown.

Methods: Circulating levels of MMP-2 and MMP-9 proteins and the expression of their inhibitor, tissue inhibitor of metalloproteinase 1 (TIMP-1), were measured in 21 patients experiencing an asthma exacerbation and 21 age matched patients with stable asthma. Circulating gelatinoytic activity was compared during the asthma exacerbation and during subsequent convalescence by gelatin zymography in the same individuals. In addition, MMP-9 specific activity was quantified with a colorimetric assay which uses an artificial proenzyme containing a specific domain recognised by MMP-9 in the same paired samples.

Results: A significant increase in the circulating level of MMP-9 was seen in patients with an asthma exacerbation compared with patients with stable asthma (202.9 (22.0) v 107.7 (9.9) ng/ml, p=0.0003). There were no significant differences in the circulating levels of MMP-2 or TIMP-1. Gelatin zymography identified two major circulating gelatinoytic activities corresponding to MMP-2 and MMP-9, and showed that asthma exacerbations are characterised by markedly increased MMP-9 activity with no significant change in MMP-2 activity compared with the activities during convalescence in the same individuals. Direct measurement showed that MMP-9 specific activity is significantly increased during asthma exacerbations compared with subsequent convalescence (269.6 (31.7) v 170.4 (12.6) ng/ml, p=0.0099).

Conclusions: Asthma exacerbations are characterised by increased circulating MMP-9 activity. This increased activity may be related to exaggerated airway inflammation and airway remodelling.
one patient was recruited during a moderate to severe exacerbation, either on their scheduled visit to the clinic or during an emergency visit. The patients underwent complete clinical examination and chest radiography, if required, to exclude the presence of concomitant acute illnesses such as pneumonia. The minimum criteria for the diagnosis of an asthma exacerbation included intense subjective breathlessness, audible wheezing on auscultation, and a morning peak expiratory flow (PEF) <70% of the personal best value in the previous 3 months. Patients were monitored to the subsequent convalescence which was defined by the continuous resolution of subjective symptoms and physical findings for at least 2 weeks, with a morning PEF or forced expiratory volume in 1 second (FEV₁) >80% of the personal best value. Twenty one age matched patients with stable asthma were recruited on their scheduled visit if the symptoms and PEF were stable with no change in treatment for at least 1 month. Table 1 summarises the clinical characteristics and morning PEF (expressed as a percentage of the personal best) of the patients on recruitment to the study. Serum samples were collected on the visits, aliquotted, and stored at −80°C until the time of assay.

**Table 1** Clinical characteristics of patients with asthma

<table>
<thead>
<tr>
<th></th>
<th>Exacerbation (n=21)</th>
<th>Stable (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>11/10</td>
<td>9/12</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>41.3 (4.3)</td>
<td>38.8 (3.9)</td>
</tr>
<tr>
<td>Asthma duration (years)*</td>
<td>12.4 (2.7)</td>
<td>14.1 (2.7)</td>
</tr>
<tr>
<td>Ateopic/non-ateopic</td>
<td>16/5</td>
<td>16/5</td>
</tr>
<tr>
<td>Step 1/2/3†‡</td>
<td>7/8/6</td>
<td>4/9/8</td>
</tr>
<tr>
<td>Morning PEF (% personal best)*</td>
<td>55.9 (2.1)</td>
<td>87.2 (1.8)</td>
</tr>
</tbody>
</table>

PEF = peak expiratory flow.
*Data are presented as mean (SD).
†Classification of the severity of asthma based on GINA guidelines: step 1-intermittent; step 2-mild persistent; step 3-moderate persistent.
‡p<0.001 v patients with stable asthma (Mann-Whitney U non-parametric test).

### Determination of MMP-2, MMP-9, and TIMP-1 protein concentrations

The concentrations of MMP-2, MMP-9, and TIMP-1 were determined using commercially available ELISA based (for MMP-2 and MMP-9) and EIA based (for TIMP-1) assay systems (all from Fuji Chemical Industries, Takaoka, Japan). Assays were performed using the protocols recommended by the manufacturer. The sensitivities of each assay were 0.37 ng/ml for MMP-2, 1 ng/ml for MMP-9, and 1.25 ng/ml for TIMP-1. The assay for MMP-2 does not crossreact with MMP-1, -3, -7, -8, -9, or MT1-MMP. The assay for MMP-9 detects proMMP-9 and proMMP-9-TIMP-1 complexes and does not crossreact with proMMP-1, proMMP-2, or proMMP-3. The assay for TIMP-1 does not crossreact with TIMP-2.

### Gelatin zymography

Gelatin zymography was performed on paired serum samples obtained during exacerbations and subsequent convalescence to characterise the changes in circulating gelatinolytic activity. Each serum sample was diluted 1:20 in non-reducing sample buffer containing sodium dodecyl sulfate (SDS), glycerol, and bromophenol blue and subjected to electrophoresis on 8% (w/v) polyacrylamide SDS gels containing 1 mg/ml porcine skin gelatin (Sigma, St Louis, MO, USA). Protein standards (New England Biolabs, Beverly, MA, USA) were run on the same gel to estimate the molecular weight of the lytic bands. After electrophoresis the gels were washed in 2.5% Triton X-100 for 30 minute, rinsed briefly, and incubated at 37°C for 24 hours in a buffer containing 50 mM Tris HCl (pH 8.0) and 10 mM CaCl₂. Following incubation the gels were stained with Coomassie blue R-250 and destained in a solution of 5% acetic acid and 10% methanol. Gelatinolytic activity appeared as unstained zones against a blue background.

### Measurement of MMP-9 specific activity

Gelatin zymography revealed two major zones of gelatinolytic activity in the serum samples corresponding to MMP-2 and MMP-9 activity. The serum samples obtained during an asthma exacerbation had markedly higher MMP-9 activity than those obtained during convalescence in the same individuals. In contrast, the gelatinolytic activity corresponding to MMP-2 remained constant.

### Statistical analysis

Statistical analysis was performed using StatView (Version 4.5, Abacus Concepts, Berkeley, CA, USA). The Mann-Whitney U non-parametric test was used to calculate p values for unpaired comparisons. Comparisons for values in the same individual were performed using the Wilcoxon test. A p value of <0.05 was considered statistically significant.

### RESULTS

**MMPs and TIMP-1**

Patients with an asthma exacerbation had significantly higher circulating MMP-9 concentrations than patients with stable asthma. There was no significant difference in the circulating MMP-2 concentrations between the two groups, nor was there a significant difference in the circulating concentration of TIMP-1 which is an inhibitor of MMP-2 and MMP-9 (table 2). Coefficients of variation for MMP-2, MMP-9, and TIMP-1 were 0.196, 0.497, and 0.268 in the stable group, and 0.188, 0.420, and 0.216 in the exacerbation group, respectively.

### Gelatin zymography

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### MMP-9 specific activity

Although gelatin zymography is a sensitive method for detecting MMP-2 and MMP-9 activity, it is difficult to quantify. Furthermore, it is not suitable for the evaluation of a large number of samples. A colorimetric assay specific for MMP-9 activity was therefore used to quantify changes in the...
Asthma exacerbations and MMP-9 activity in the same individuals using gelatin zymography and a specific colorimetric activity assay and confirmed increased MMP-9 activity during exacerbations compared with subsequent convalescence. These biochemical changes may have significant implications in the pathophysiology of asthma exacerbations.

Current evidence suggests that MMP-9 mediates several important pathways responsible for asthma exacerbations including airflow obstruction, increased vascular permeability, and exaggerated airway hyperresponsiveness. MMP-9 has also been implicated in the development of tissue fibrosis. In a study conducted by Hoshino et al., inhaled corticosteroids reduced the amount of reticular basement membrane in association with decreased MMP-9 immunoreactivity in bronchial tissue, suggesting that enhanced MMP-9 activity promotes subepithelial fibrosis in asthmatic individuals. It is therefore likely that asthma exacerbations promote airway remodelling by altering MMP-9 mediated ECM homeostasis. Although many cell types can generate MMP-9, neutrophils are considered to be the major source of MMP-9 in the lower airways in status asthmaticus and in asthmatic individuals challenged with allergens. Circulating MMP-9 levels may therefore reflect a “spill over” of MMP-9 produced in the airways. As MMP-9 is a mediator of inflammation and tissue remodelling, an increase in its activity is likely to play a significant role in the pathophysiology of asthma exacerbations.

Inhaled or systemic corticosteroids are the mainstay of the current treatment for asthma, but they are not always effective in the treatment of asthma exacerbations. The most common trigger for an asthma exacerbation is known to be a respiratory tract infection, and the lower airway inflammation induced by experimental rhinovirus infection in asthmatic patients is not ameliorated by inhaled corticosteroids.

Recent investigations have shown that MMP-9 activity in the lower airways is enhanced by allergen challenge, and that inhaled corticosteroids have no effect on this enhancement. Better treatments for asthma exacerbations need to be established for the optimal management of the disease. In this context, MMP-9 may be a potential target for the management of exacerbations of asthma.

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REFERENCES

LUNG ALERT

Food allergy may predict life threatening asthma in children


In this case-control study, food allergy and degree of atopy were assessed as risk factors for life-threatening asthma in childhood. Currently, no objective clinical risk factors exist. Nineteen children aged 1–16 years with an exacerbation of asthma requiring ventilation were recruited and compared with 38 controls with a simple exacerbation of asthma. Controls were matched by age, sex, and ethnicity. Food allergy was diagnosed on a basis of history, positive skin prick tests, or serum specific IgE. Food challenge was performed in cases of clinical doubt. Children with a history of type 1 hypersensitivity symptoms in the 24 hours preceding the exacerbation were excluded, as those with evidence of a viral infection. 50% of cases had food allergy compared with 10% of controls. Multivariate analysis revealed that food allergy (odds ratio (OR) 5.89) and frequent admissions to hospital (OR 9.85) were independent risk factors for life threatening asthma, suggesting that more intensive treatment of this high risk group of children may improve outcome.

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