

## Children with stable asthma have reduced airway matrix metalloproteinase-9 and matrix metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio

G. M. Doherty\*†, S. V. Kamath†, F. de Courcey\*, S. N. Christie†, A. Chisakuta†, J. D. Lyons†, L. G. Heaney\*, M. Ennis\* and M. D. Shields\*†

\*Respiratory Research Group, Queen's University, Belfast, Northern Ireland and †Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland

### Summary

**Background** Childhood asthma is characterized by inflammation of the airways. Structural changes of the airway wall may also be seen in some children early in the course of the disease. Matrix metalloproteinases (MMPs) are key mediators in the metabolism of the extracellular matrix (ECM).

**Objective** To investigate the balance of MMP-8, MMP-9 and tissue inhibitor of metalloproteinases (TIMP)-1 in the airways of children with asthma.

**Methods** One hundred and twenty-four children undergoing elective surgical procedures also underwent non-bronchoscopic bronchoalveolar lavage (BAL). MMP-8, MMP-9 and TIMP-1 were measured by ELISA.

**Results** There was a significant reduction in MMP-9 in atopic asthmatic children ( $n = 31$ ) compared with normal children ( $n = 30$ ) [median difference: 0.57 ng/mL (95% confidence interval: 0.18–1.1 ng/mL)]. The ratio of MMP-9 to TIMP-1 was also reduced in asthmatic children. Levels of all three proteins were significantly correlated to each other and to the relative proportions of particular inflammatory cells in BAL fluid (BALF). Both MMP-8 and MMP-9 were moderately strongly correlated to the percentage neutrophil count ( $r = 0.40$  and  $0.47$ , respectively,  $P < 0.001$ ).

**Conclusions** An imbalance of MMPs and their inhibitors occurs in children with well-controlled asthma, which may indicate early derangement of the metabolism of the ECM.

**Keywords** asthma, child, matrix metalloproteinases, remodelling, tissue inhibitor of metalloproteinases-1

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### Introduction

Airways remodelling is characteristic of asthma [1]. It includes glandular hyperplasia, hypertrophy of the smooth muscle layer and thickening of the reticular basement membrane (also known as sub-basement membrane thickening) [2–4]. Collagen types I and III are deposited below the 'true' basement membrane on which the epithelial cells rest, which is constituted primarily of collagen type IV. These changes suggest an imbalance in enzymes such as matrix metalloproteinases (MMPs), which are secreted locally by cells in the airways to regulate the turnover of the extracellular matrix (ECM) [5].

Remodelling of the airways is seen early in the course of childhood asthma [6]. There has been considerable debate as to when the process begins [7, 8]. It seems to be present in early childhood, although possibly not in infancy [9, 10]. The discovery of physiological differences in both bronchial epithelial cells [11–13] and airway smooth muscle [2] derived from adult patients with asthma suggests that airways remodelling may be driven by these cells rather than by traditional inflammatory cells [14].

The functional consequences of airways remodelling are unclear. Mathematical modelling of the mechanical properties of the airways suggests that airway narrowing associated with remodelling may contribute to both bronchial hyper-reactivity and fixed airflow obstruction [15]. Some clinical studies appear to corroborate this [16–19]. However, other studies have suggested that some features of remodelling, such as thickening of the reticular basement membrane, may be protective [20].

MMPs are secreted as pro-enzymes that require activation by other proteases (such as trypsin-2) [21]. Their activity can be inhibited by binding to non-specific inhibitors (such as  $\alpha$ -globulin) or specific inhibitors including the tissue inhibitors of metalloproteinases (TIMPs). The net effect of MMPs on their collagen substrates depends on the balance between MMPs, their activators and their inhibitors.

Different MMPs have different but overlapping specificities [22]. MMP-9 (also known as gelatinase B) breaks down collagen type IV, but also degrades elastin and other structural proteins, and may be involved in the processing of some cytokines [23]. MMP-9 is produced by many cell types in the airways but is particularly associated with the neutrophil. MMP-8 (also known as neutrophil collagenase) is an 'interstitial collagenase' that acts on collagens types I, II and III and is also produced by neutrophils [22].

There are conflicting reports on the balance of metalloproteinases and their inhibitors in asthma. MMP-9 may be elevated

Correspondence: Dr M. D. Shields, Department of Child Health, Institute of Clinical Science, Queens University, Grosvenor Road, Belfast BT12 6BJ, UK.

E-mail: m.shields@qub.ac.uk

ed in acute exacerbations of asthma in blood [24, 25], sputum [26] or bronchoalveolar lavage fluid (BALF) [27]. Rises in MMP-9 also appear to be provoked by allergen challenge [28, 29]. However, in chronic stable asthma, treated with inhaled steroids, TIMP-1 may predominate with MMP-9 levels not significantly different from normal controls [30, 31]. In theory, increased metalloproteinases should lead to collagen breakdown; increased TIMPs should lead to collagen deposition.

The aim of this study was to investigate the levels of MMP-8, MMP-9 and TIMP-1 in BALF in children with stable asthma. For comparison, we also assessed normal children, atopic children without asthma and children who had outgrown their asthmatic symptoms. The latter have been shown to have evidence of airway inflammation without symptoms [32].

## Materials and methods

### Subjects

Children attending for elective surgery between 1994 and 2003 were recruited, with written informed parental consent and the approval of the local research ethics committee, as described previously [33]. Most children were attending for correction of prominent ears, strabismus or ptosis.

For the purposes of our study, children with gastro-oesophageal reflux, lung pathology other than asthma or inflammatory conditions were excluded. A physician completed a questionnaire and physical examination of the child. Children in the following groups were included: atopic asthmatic (AA), non-asthmatic atopic (NA), non-asthmatic non-atopic (N) and asthma outgrown (AO). Asthma was considered outgrown (AO) where symptoms had not occurred for more than 1 year. In all groups, 'evidence of atopy' was taken as either a personal or a family history of atopic disease or an elevated total serum IgE or both. Age-related reference intervals for total serum IgE were used. Children who demonstrated a pattern of viral-associated wheeze (wheeze precipitated only by an upper respiratory tract infection and no evidence of atopy) or cough-variant asthma (persistent cough without wheeze in an atopic individual) were excluded from the analysis.

### Sampling

Children underwent endotracheal intubation as part of the standard anaesthetic management of their elective surgery. Following intubation and pre-oxygenation, children underwent non-bronchoscopic BAL as described previously [33]. Briefly, a 20 mL aliquot of normal saline was instilled and then aspirated with an 8Fr gauge suction catheter via the endotracheal tube. The child's vital signs (including oxygen saturations) were monitored throughout. A blood sample for IgE measurement was obtained.

### Storage and preparation of samples

Cytospin preparations were made by centrifuging BALF at 80g onto 13 mm glass coverslips and stained using Speedy Diff (Clin-Tech, Clacton-on-Sea, UK) for differential cell counts, and toluidine blue for mast cell counts [34]. The cell-free supernatant was frozen at  $-80^{\circ}\text{C}$ .

### Measurement of matrix metalloproteinases and tissue inhibitor of metalloproteinase-1

Measurements of total MMP-8, MMP-9 and TIMP-1 were performed on undiluted BALF supernatant using commercially available ELISAs (RnD Systems, Minneapolis, MN, USA). The limits of detection (LoD) for the MMP-8, MMP-9 and TIMP-1 assays were 0.02, 0.156 and 0.08 ng/mL, respectively. The lowest standard (and presumed limit of quantification) for the MMP-8, MMP-9 and TIMP-1 assays were 0.156, 0.312 and 0.156 ng/mL, respectively. Molar ratios were calculated assuming relative molecular weights for MMP-8, MMP-9 and TIMP-1 of approximately 65, 92 and 28 kDa, respectively [31, 35]. A commercial assay (RnD Systems) was used to measure the amount of active MMP-9 present in a subset of patients ( $n = 27$ ). This assay is based on capture of MMP-9 with a monoclonal antibody and subsequent cleavage of a peptide with the release of a fluorophore.

### Statistical analysis

Normally distributed variables were described using mean (standard deviation) and other variables using median (interquartile range (IQR)). In ELISAs, measurements below the LoD were given a substitute value of LoD/2. Measurements that fell above the LoD of an assay but below the lowest standard were assigned a value mid-way between the two [36–38]. Measurements that fell above the highest standard of the assay were assigned a value marginally above this.

For between-group comparisons, differences were established by the Kruskal–Wallis analysis of variance test. A  $P$ -value of less than 0.05 was assumed to be statistically significant. *Post hoc* testing using the Mann–Whitney test with a Bonferroni's correction was performed. With four subgroups (N, NA, AA and AO), up to six pair-wise comparisons are possible. Therefore, a  $P$ -value less than 0.0083 (i.e. 0.05/6) was assumed to be significantly different for *post hoc* testing. Ninety-five percent confidence intervals for the median difference were also calculated to establish the approximate magnitude of any statistically significant difference.

Correlations were examined using Spearman's correlation coefficients ( $\rho$ ). A  $P$ -value of less than 0.05 was assumed to be statistically significant. Tests were performed using SPSS v11.5 (Chicago, IL, USA) and StatsDirect v2.3.7 (Cheshire, UK).

## Results

One hundred and twenty-four children (70 males) were included in the study [mean age (range): 7.1 (0.8–15.1) years]. For the group as a whole, the median concentration (IQR) of MMP-8 was 0.24 ng/mL (0.09–0.66 ng/mL,  $n = 103$ ); the median concentration (IQR) of MMP-9 was 0.50 ng/mL (0.23–1.73 ng/mL,  $n = 106$ ); and the median concentration (IQR) of TIMP-1 was 1.07 ng/mL (0.63–2.78 ng/mL,  $n = 117$ ). Not all measurements could be made in all children because of limited sample volumes.

Nineteen of 27 measurements of active MMP-9 showed a level of activity above the LoD (0.02 ng/mL) but below the lowest standard (0.25 ng/mL); three values ranged from 0.51 to 0.75 ng/mL, and five were undetectable.

**Table 1.** Differential cell counts and BAL protein measurements in groups

|                                    | Normal (N)               | Atopic (NA)               | Asthmatic (AA)            | Asthma outgrown (AO)       |
|------------------------------------|--------------------------|---------------------------|---------------------------|----------------------------|
| Number                             | 35                       | 33                        | 34                        | 22                         |
| Age, mean (SD)                     | 7.0 (3.7)                | 6.9 (3.1)                 | 6.7 (3.1)                 | 8.3 (2.8)                  |
| Sex, no. male/female               | 19/16                    | 15/18                     | 21/13                     | 15/7                       |
| Differential cell count in BAL     | N = 35                   | N = 32                    | N = 33                    | N = 21                     |
| Macrophages (%), median (IQR)      | 74.4 (50.7–89.9)         | 75.2 (55.3–89.4)          | 62.2 (44.0–88.2)          | 79.8 (56.6–95.2)           |
| Epithelial cells (%), median (IQR) | 13.0 (5.7–32.5)          | 13.4 (2.7–34.7)           | 29.6 (2.2–47.6)           | 7.3 (0.6–27.2)             |
| Neutrophils (%), median (IQR)      | 2.2 (1.0–7.0)            | 3.2 (0.8–7.7)             | 1.8 (1.2–4.6)             | 2.6 (1.2–9.5)              |
| Eosinophils (%), median (IQR)      | 0.19 (0–0.4)             | 0.2 (0.03–0.6)            | 0.21 (0.08–1.25)          | 0.3 (0.05–0.62)            |
| Lymphocytes (%), median (IQR)      | 0.1* (0–0.4)             | 0.25 (0.06–0.5)           | 0.23 (0.09–0.60)          | 0.46 (0.1–1.58)            |
| Mast cells (%), median (IQR)       | 0.29 (0.1–0.44) [n = 31] | 0.26 (0.19–0.36) [n = 26] | 0.24 (0.13–0.34) [n = 31] | 0.08* (0.03–0.25) [n = 20] |
| Protein measurements               |                          |                           |                           |                            |
| Total protein, median (IQR)        | 59.7 (39.6–84.7), N = 27 | 49.2 (33.9–95.3), N = 26  | 58.5 (31.7–75.6), N = 30  | 50.8 (14.2–70.6), N = 21   |
| MMP-8 (ng/mL), median (IQR)        | 0.36 (0.18–0.89), N = 34 | 0.21 (0.09–0.51), N = 24  | 0.13 (0.09–0.50), N = 28  | 0.21 (0.09–1.04), N = 17   |
| MMP-9 (ng/mL), median (IQR)        | 1.22 (0.40–2.85), N = 31 | 0.37* (0.23–1.83), N = 25 | 0.40 (0.23–0.74), N = 30  | 0.46 (0.23–2.36), N = 20   |
| TIMP-1 (ng/mL), median (IQR)       | 1.51 (0.73–2.67), N = 34 | 0.98 (0.41–2.61), N = 28  | 1.12 (0.68–3.35), N = 34  | 0.85 (0.58–2.61), N = 21   |

\* $P < 0.05$ . Analysis of variance by Kruskal–Wallis test with *post hoc* testing by Mann–Whitney test.

BAL, bronchoalveolar lavage; IQR, interquartile range; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1; SD, standard deviation.

### Between-group differences

Children were classified into groups as described previously (N = 35, NA = 33, AA = 34, AO = 22). No significant differences in the levels of MMP-8 or TIMP-1 were found between the groups (Table 1). However, MMP-9 concentrations did differ significantly between groups (Fig. 1). MMP-9 was significantly lower in the asthmatic group than in normal children [median difference: 0.57 ng/mL (95% CI: 0.18–1.1 ng/mL),  $P = 0.003$ ]. Compared with normal children, MMP-9 was also lower in NA children [median difference: 0.35 ng/mL (95% CI: 0–0.1 ng/mL),  $P = 0.044$ ] and children with outgrown asthma [median difference: 0.44 ng/mL (95% CI: 0–1.1 ng/mL),  $P = 0.054$ ], although these differences were not significant.

The MMP-9/TIMP-1 ratio was significantly decreased in asthmatic patients compared with both normal children and atopic children; there was a similar non-significant trend for the MMP-8/TIMP-1 ratio (see Figs 2 and 3).

MMP-9 activity was assessed in only 27 patient samples (13 N, five NA, seven A and two AO) because of restricted sample volumes. All normal children had detectable levels of MMP-9 activity, whereas three of seven asthmatic children did not (Fisher's exact test,  $P = 0.03$ ), and two of five NA children did not (Fisher's exact test,  $P = 0.07$ ).

The neutrophil count in the children with asthma was slightly lower than in normals, but this difference was not significant [median difference 0.2% (95% CI: –0.8% to 1.2%,  $P = 0.7$ )]. In a multiple regression model including only asthmatics and normals, both log-transformed percentage neutrophil count ( $\beta$  coefficient = 0.68,  $P = 0.04$ ) and the presence of asthma ( $\beta$  coefficient = –1.02,  $P < 0.01$ ) remained significant and independent predictors of the MMP-9/TIMP-1 ratio.

### Correlations

The total levels of MMP-8 were strongly correlated with MMP-9 [ $\rho = 0.75$  (95% CI: 0.64–0.83,  $P < 0.0001$ ),  $n = 90$ ]. TIMP-1 levels were strongly correlated with MMP-8 [ $\rho = 0.60$  (95% CI: 0.45–0.71,  $P < 0.0001$ ),  $n = 99$ ] and MMP-9 [ $\rho = 0.61$  (95% CI: 0.47–0.71,  $P < 0.0001$ ),  $n = 103$ ].

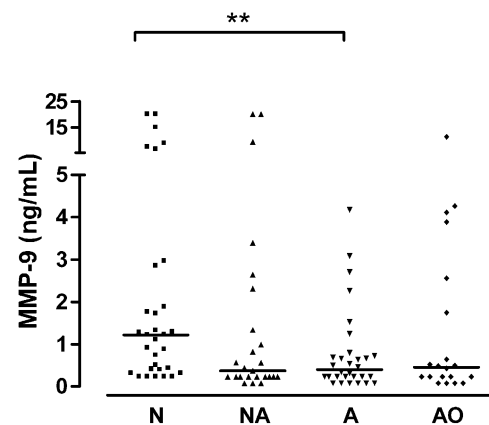


**Fig. 1.** Matrix metalloproteinase-9 (MMP-9) levels in bronchoalveolar lavage from different groups of children (N, normal; NA, non-asthmatic atopic; A, asthmatic; AO, asthma outgrown). \*\* $P < 0.05$  by Kruskal–Wallis analysis of variance;  $P < 0.0083$  by Mann–Whitney *post hoc* testing.

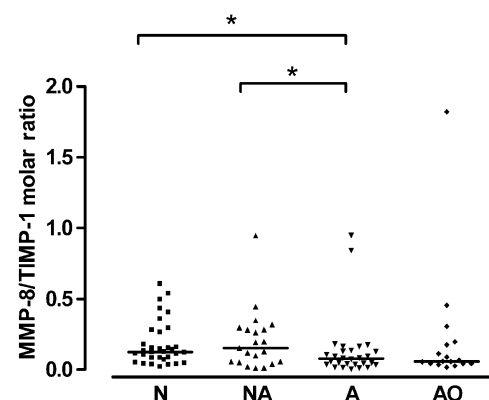


**Fig. 2.** Molar ratio of matrix metalloproteinase-8 (MMP-8) to tissue inhibitor of metalloproteinase-1 (TIMP-1) in bronchoalveolar lavage from different groups of children (N, normal; NA, non-asthmatic atopic; A, asthmatic; AO, asthma outgrown). \* $P < 0.1$  by Kruskal–Wallis analysis of variance;  $P < 0.05$  by Mann–Whitney *post hoc* testing.

Similar correlations were obtained if the values of the MMPs and TIMP-1 were corrected for the total amount of protein in the samples.

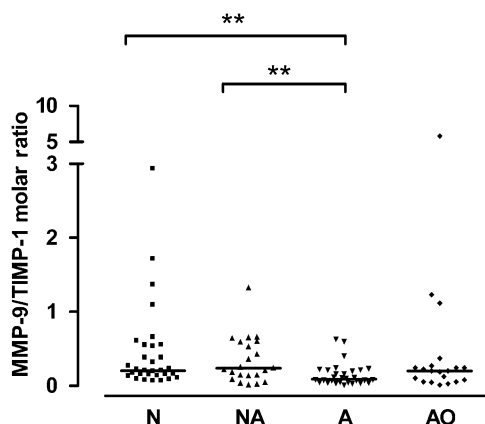


**Fig. 3.** Molar ratio of matrix metalloproteinase-9 (MMP-9) to tissue inhibitor of metalloproteinase-1 (TIMP-1) in bronchoalveolar lavage from different groups of children (N, normal; NA, non-asthmatic atopic; A, asthmatic; AO, asthma outgrown). \*\* $P < 0.05$  by Kruskal–Wallis analysis of variance;  $P < 0.0083$  by Mann–Whitney *post hoc* testing.

**Table 2.** Correlations between MMP-8, MMP-9, TIMP-1 and BAL cells

| Protein                 | Cell                    | Spearman's $\rho$ (95% CI) | $P$       |
|-------------------------|-------------------------|----------------------------|-----------|
| MMP-8<br>( $n = 100$ )  | Macrophage              | −0.42 (−0.57 to −0.25)     | <0.0001   |
|                         | Epithelial cell         | 0.10 (−0.09 to 0.29)       | NS (0.30) |
|                         | Neutrophil              | 0.40 (0.22–0.56)           | <0.0001   |
|                         | Eosinophil              | −0.10 (−0.29 to 0.10)      | NS (0.34) |
|                         | Lymphocyte              | −0.24 (−0.42 to −0.05)     | 0.02      |
|                         | Mast cell ( $n = 91$ )  | −0.10 (−0.09 to 0.29)      | NS (0.30) |
| MMP-9<br>( $n = 104$ )  | Macrophage              | −0.50 (−0.63 to −0.34)     | <0.0001   |
|                         | Epithelial cell         | 0.30 (0.11 to 0.46)        | 0.002     |
|                         | Neutrophil              | 0.47 (0.36–0.63)           | <0.0001   |
|                         | Eosinophil              | −0.03 (−0.22 to 0.16)      | NS (0.77) |
|                         | Lymphocyte              | −0.15 (−0.34 to 0.04)      | NS (0.11) |
|                         | Mast cell ( $n = 93$ )  | 0.04 (−0.17 to 0.24)       | NS (0.73) |
| TIMP-1<br>( $n = 114$ ) | Macrophage              | −0.62 (−0.73 to −0.49)     | <0.0001   |
|                         | Epithelial cell         | 0.46 (0.30–0.59)           | <0.0001   |
|                         | Neutrophil              | 0.26 (0.08–0.43)           | 0.005     |
|                         | Eosinophil              | 0.18 (0.02–0.34)           | 0.03      |
|                         | Lymphocyte              | −0.28 (−0.44 to −0.10)     | 0.003     |
|                         | Mast cell ( $n = 100$ ) | 0.01 (−0.18 to 0.21)       | NS (0.90) |

MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1; BAL, bronchoalveolar lavage; NS, non-significant; CI, confidence interval.

Correlations between MMP-8, MMP-9 and TIMP-1 levels and the type of inflammatory cell present in the BALF are shown in Table 2. The MMP-9/TIMP-1 ratio was moderately correlated with the percentage neutrophil count [ $\rho = 0.45$  (95% CI: 0.28–0.60,  $P < 0.0001$ ),  $n = 100$ ]. The MMP-8/TIMP-1 ratio correlated weakly with the percentage neutrophil count [ $\rho = 0.32$  (95% CI: 0.13–0.49,  $P = 0.002$ ),  $n = 95$ ] and had a weak negative correlation with the epithelial cell count [ $\rho = -0.32$  (95% CI: −0.49 to −0.12,  $P = 0.002$ ),  $n = 95$ ]. No other cells correlated significantly with the MMP/TIMP ratios.

When the percentage cell counts were replaced with absolute cell counts, the highly significant correlations of MMP-8, MMP-9 and TIMP-1 to the neutrophil count persisted, whereas the highly significant inverse correlations to the macrophage count disappeared.

Age did not correlate significantly with the concentrations of MMP-8, MMP-9 or TIMP-1.

## Discussion

We have shown that MMP-8, MMP-9 and TIMP-1 are present in the airways of healthy children. We have also shown that MMP-9 levels are reduced in asthmatic children and in atopic children without asthma. In asthmatic children, the ratio of MMP-9 to TIMP-1 is reduced. This finding is corroborated by the reduced incidence of MMP-9 activity in asthmatic children. This is the largest study reporting levels of MMP-8, MMP-9 or TIMP-1 in paediatric BAL fluid [39].

The levels of MMP-8, MMP-9 and TIMP-1 in the airways are closely correlated with each other. This balance probably accounts for the low level of MMP-9 activity seen in BAL fluid. The levels of the MMPs and TIMPs measured also correlate closely with the relative proportions of inflammatory cells found in the airways.

### Matrix metalloproteinase and tissue inhibitor of metalloproteinase balance

Conflicting reports exist as to whether a relative preponderance of MMP-9 or TIMP-1 favours collagen deposition and remodelling (recently reviewed by Atkinson and Senior [23]). It might be assumed that MMP-9 would be associated with collagen breakdown, and an excess of TIMP-1 might inhibit this process and permit the accumulation of excess collagen.

Two interesting studies have recently demonstrated that the MMP-9/TIMP-1 ratio is inversely correlated to airway wall thickness in adult asthmatics [40], and to RBM thickening in severely asthmatic children [41]. Sputum MMP-9/TIMP-1 ratio has also been shown to be positively correlated to FEV1 (i.e. increased TIMP-1 is associated with airflow obstruction) [30].

Other airway inflammatory conditions, particularly those associated with neutrophilic inflammation such as bronchiectasis, have demonstrated elevated levels of MMP-9 [42, 43]. Cystic fibrosis also demonstrates a thickened reticular basement membrane with deposition of collagen types I and III [44]. Levels of MMP-7, MMP-8 and MMP-9 are also elevated in children ventilated for acute respiratory failure [39].

The relationship between airways remodelling, airway inflammation and symptoms of wheeze and cough has been difficult to elucidate. Remodelling occurs early in childhood asthma [6]. Arguably, loss of lung function or potential lung function in asthma also occurs in early childhood [45, 46]. However, it is not known whether remodelling contributes to loss of lung function or is even a marker for it. The relevance of airways remodelling to fixed airways obstruction in childhood has been questioned by one small case series in severely asthmatic children. This study demonstrated normal measures of airway function after intensive steroid therapy in spite of bronchoscopic evidence of remodelling [47]. Conversely, these children demonstrated severe symptoms prior to therapy, in spite of little evidence of airways inflammation.

In the NA and AO groups, the levels of MMP-8 and MMP-9, and consequently the ratios of MMP-8 and MMP-9 to TIMP-1, lie in between those values obtained for normal children and asthmatic children. There is evidence of ongoing

inflammation in children in clinical remission from asthma [32, 48, 49]. Atopy is clearly associated with asthma, but even NAs are more likely to have airway hyper-responsiveness [50]. There is the unproven suggestion from our data that these groups may also have deranged ECM metabolism. This may influence lung function or the development or recurrence of asthmatic symptoms in these groups.

### *Neutrophils and matrix metalloproteinases*

Elevated levels of MMP-8 and MMP-9 are found in conjunction with airway neutrophilia. Both are neutrophil products. MMP-9 levels have previously been shown to be linked to neutrophil counts in serum [25], and sputum [28] in asthma; BALF following allergen challenge [29]; and in cerebrospinal fluid in neurological diseases [51, 52]. MMP-9 may also influence recruitment of neutrophils by processing of IL-8 [53].

The association between TIMP-1 and neutrophils is less strong. The co-production of TIMP-1 and MMP-9 is not as closely linked in neutrophils as in other cells [54]. TIMP-1 is secreted by many cells as a heterodimer complexed with pro-MMP-9. The production of TIMP-1 by neutrophils may be more finely regulated, with much of it stored in separate vesicles from MMP-9 [54]. TIMP-1 can also be produced by epithelial cells and eosinophils, with which it is weakly correlated in our study. TIMP-1 can inhibit many of the metalloproteinases including MMP-8 and MMP-9. It is involved in other processes, including regulation of the cell cycle and mitogenesis [23].

Neutrophilic inflammation is seen in acute exacerbations of asthma, viral infections and with viral-associated wheeze [55]. Previous studies have shown that asthma exacerbations with elevated neutrophils also have an accompanying increase in MMP-9, which may even be detected in the serum [24, 25]. Intermittent exacerbations may lead to temporary increases in MMP-9. In our study, the positive correlation of the MMP-9/TIMP-1 ratio with BAL neutrophils suggests that under conditions of neutrophilic inflammation, increases in MMP-9 may be greater than increases in TIMP-1. However, one might speculate that if the rise in TIMP-1 is more sustained than the rise in MMP-9, there may subsequently develop a relative excess of TIMP-1.

We have also shown that MMP-9 is reduced in asthmatic children compared with normal children. This contributes at least in part to the overall reduction in the ratio of MMP-9 to TIMP-1 seen in asthmatic children in this study. Asthmatic children have elevated levels of exhaled nitric oxide even during asymptomatic periods [32, 49], and it has been shown in other disease processes that nitric oxide may down-regulate the production of MMP-9 without affecting TIMP-1 [56].

### *Study limitations*

The study of airways disease in childhood is problematic, and our study shares several of its limitations with previous work. MMPs are easily measured in BALF, albeit in relatively low concentrations. However, the reproducibility of BAL work can be difficult [57]. The ERS argue for standardized reporting of BAL measurements per millilitre of BALF recovered as we have done [58]. The recording of MMPs and

TIMPs per milligram of protein alters the strength of the correlations presented here although not to a degree that greatly affects our conclusions. The study is cross-sectional, whereas a longitudinal study would help to answer some of the questions about the progression of airways remodelling and asthma symptoms.

The strong negative correlation of macrophages with all the proteins measured is probably a statistical artefact. As the percentage of other cells such as neutrophils (with which these proteins are positively correlated) increases, the percentage of macrophages decreases. This explanation is corroborated by the fact that correlations of proteins with neutrophils persisted when both percentage and absolute cell counts were used. The correlation with macrophages disappeared when the percentage count was replaced with the absolute cell count.

Alternatively, it has been argued that the presence of alveolar macrophages suggests lower airways sampling and the levels of MMP-8, MMP-9 and TIMP-1 may differ depending on which part of the bronchial tree is sampled.

## **Conclusions**

In conclusion, MMP-9 levels, MMP-9 activity and MMP-9/TIMP-1 ratio all appear to be reduced in currently well children with asthma. This study presents further evidence of a derangement in the metabolism of the ECM in the airways of young children with mild asthma. This may be associated with airway wall thickening, and may have implications for the future treatment of asthma.

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