

# Serum and sputum $\alpha_2$ macroglobulin in patients with chronic obstructive airways disease

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**ABSTRACT** Serum  $\alpha_2$ macroglobulin concentrations were measured in patients with chronic obstructive airways disease and an age-matched group of control subjects. The mean serum level of  $\alpha_2$ macroglobulin was significantly lower in bronchitic subjects with acute chest infections than the mean value of the controls. No significant differences were observed between serum  $\alpha_2$ macroglobulin values in controls, subjects with "emphysema", and bronchitic patients who did not have chest infections. Sputum  $\alpha_2$ macroglobulin concentrations were compared in sputum samples from bronchitic patients with and without acute chest infections. The protein was detected ( $> 0.2$  mg/l) in 94% of sputum samples from infected subjects but only 60% of non-infected sputum samples. Concentrations of  $\alpha_2$ macroglobulin in infected samples were significantly higher than the non-infected samples. Sputum/serum concentration ratios of  $\alpha_2$ macroglobulin were also significantly higher in infected samples but this difference was eliminated by "correcting" the values with the albumin sputum/serum ratios of the same samples. The results suggest that  $\alpha_2$ macroglobulin concentrations are higher in bronchial secretions during chest infection because of increased transudation from the blood rather than the presence of significant local secretion.

Pulmonary emphysema in man may be the result of an imbalance between elastolytic enzymes and their inhibitors within the lung such that enzyme activity predominates.<sup>1</sup> The subsequent damage to lung elastin leads to structural changes resulting in emphysema. The bronchial secretions contain several protease inhibitors and protect the lung from enzymatically-induced emphysema in experimental animals.<sup>2</sup> Alpha<sub>1</sub>-antitrypsin is the major serum inhibitor of elastase in man and subjects with severe plasma deficiency of this protein are particularly susceptible to the development of emphysema.<sup>3</sup> Alpha<sub>1</sub>-antitrypsin enters the lung secretions by diffusion from the blood plasma<sup>4</sup> and hence its contribution to the elastase inhibitory capacity in the lung is reduced as a result of serum deficiency.<sup>5</sup> However, most patients with emphysema have normal circulating levels of  $\alpha_1$ antitrypsin and in these subjects alternative explanations must be found to account for their disease.

The other major plasma inhibitor of elastolytic

enzymes is  $\alpha_2$ macroglobulin ( $\alpha_2$ M) but its large size restricts its movement by diffusion into the lung secretions from the blood plasma.<sup>4</sup> Nevertheless, alveolar macrophages have been shown to synthesise and secrete  $\alpha_2$ M in vitro<sup>6</sup> and therefore the concentrations of this protein in the lung secretions may not depend upon diffusion alone. The presence of  $\alpha_2$ M in the lung secretions may be important for two reasons. Firstly,  $\alpha_2$ M can work in conjunction with  $\alpha_1$ antitrypsin by accepting proteases from it, facilitating their removal by the cells of the reticulo-endothelial system, including macrophages.<sup>7,8</sup> Secondly,  $\alpha_2$ M inhibits endopeptidases such as elastase by sterically "trapping" the enzymes, leaving the active sites chemically intact.<sup>9</sup> Small substrates such as elastin precursors may gain access to the enzyme's active site and be degraded.<sup>10</sup> Thus a substantial amount of  $\alpha_2$ M in the lung secretions may potentiate the effect of elastolytic enzymes by shielding them from more effective inhibitors such as  $\alpha_1$ antitrypsin.

The present study was designed to assess the possible contribution of  $\alpha_2$ M to the elastase inhibitors of the bronchial secretions of a group of patients with bronchitis. In particular we wished to determine whether its concentrations in the secretions are determined by transudation from serum or whether a

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Table Details of the subjects studied, showing age, sex, serum  $\alpha_2$ M concentrations and ratio (expressed as per cent) of forced expired volume in one second/forced vital capacity ( $FEV_1/FVC$ )

Subject group	Number of patients	Age (yr) $\pm$ SD	$FEV_1/FVC$ (%)	Sex (% male)	Serum $\alpha_2$ M (mg/l)
1 Bronchitis not infected	53	58.4 $\pm$ 11.4	39.5 $\pm$ 11.7	85	1905 $\pm$ 586
2 "Emphysema"	15	58.7 $\pm$ 8.6	40.4 $\pm$ 10.3	73	2145 $\pm$ 565
3 Bronchitis chest infection	17	59.2 $\pm$ 12.0	43.2 $\pm$ 13.7	94	1535 $\pm$ 369
4 Controls	42	57.1 $\pm$ 10.4	75.1 $\pm$ 6.5	71	2214 $\pm$ 775

significant amount of local production occurs. A similar group of patients with active chest infections was also studied to assess the effect of inflammation upon the  $\alpha_2$ M concentrations in the secretion.

During the course of these studies it was noted that the serum concentrations of  $\alpha_2$ M in patients with bronchitis were low compared with our control sera collected from normal subjects. The study was therefore extended to include a larger group of bronchitic subjects in order to confirm this finding.

### Methods

The details of the subjects studied are summarised in the table. In brief they consisted of four groups of subjects: (1) those with chronic cough and sputum production who had chronic airflow obstruction ( $FEV_1/FVC < 70\%$ ) and no evidence of chest infection; (2) a similar group of patients with little or no sputum production who had radiological features consistent with pulmonary emphysema;<sup>11</sup> (3) a group of obstructive bronchitic patients who had an acute chest infection characterised by increased cough and sputum production, increasing dyspnoea, pyrexia, and a positive bacterial culture from their sputum; and (4) a control group of laboratory staff or outpatients seen at least two months after an uncomplicated myocardial infarction.

Venous blood samples were collected from all patients, allowed to clot and the serum was collected and stored at  $-70^\circ\text{C}$ .

Sputum samples were collected from patients with chronic bronchitis (group 3 and some of group 1) over a four-hour period, during which the venous blood sample was also taken. After an aliquot of sputum was sent for routine bacteriology, the remainder was ultra-centrifuged at 54 000 g for 90 m at  $3^\circ\text{C}$  to obtain the supernatant which was stored at  $-70^\circ\text{C}$ .

Concentrations of  $\alpha_2$ M and albumin were measured in the sputum and serum samples by rocket immunoelectrophoresis<sup>12</sup> with reference to a standard serum obtained from Seward Laboratory, London.

The lower limit of detection of  $\alpha_2$ M in sputum was 0.2 mg/l. When  $\alpha_2$ M was detectable in sputum, the sputum/serum ratio of the protein was calculated. When  $\alpha_2$ M was not detectable the sputum/serum

ratio was taken as zero. The difference between  $\alpha_2$ M sputum/serum ratios of infected and non-infected patients and  $\alpha_2$ M sputum/serum ratios "corrected" (divided) by corresponding albumin sputum/serum ratios in infected and non-infected sputum were tested using the Kolmogorov two-sample test.

The standardised normal deviate<sup>13</sup> was used to test differences in mean serum  $\alpha_2$ M concentrations between the four subject groups studied (table).

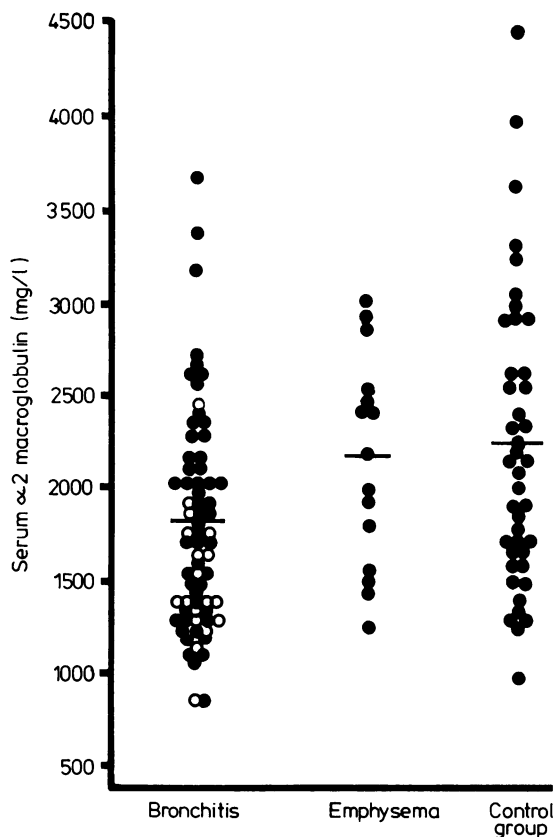


Fig 1 *Alpha-2-macroglobulin concentrations in serum. Open circles show the values in individual bronchitic subjects who had acute chest infections. Horizontal bars denote mean values.*

## Results

### SERUM STUDY

The mean serum  $\alpha_2$ M concentrations in the subject groups studied are shown in the table and individual concentrations are plotted in fig 1.

The mean  $\alpha_2$ M concentrations were similar in the control group and patients with "emphysema" ( $2p < 0.1$ ). However, the mean  $\alpha_2$ M concentration of all bronchitic patients (groups 1 and 3) was significantly lower ( $2p < 0.02$ ) than that of the control group (Fig. 1). Similarly the mean  $\alpha_2$ M level of the infected bronchitic subjects (group 3) was significantly lower than that of the control group ( $2p < 0.02$ ) and the mean value of bronchitics without infection ( $2p < 0.02$ ). However, no significant difference was observed between the  $\alpha_2$ macroglobulin concentrations of non-infected bronchitics, the "emphysematous" patients and control subjects ( $2p > 0.1$ ).

No significant differences were observed between the mean  $\alpha_2$ M concentrations of smokers and non-smokers in any of the subject groups.

### SPUTUM STUDY

Alpha-2-macroglobulin was not detectable ( $< 0.2$  mg/l) in eight (40%) of the 20 non-infected sputum samples. In the remaining 60% the  $\alpha_2$ macroglobulin levels ranged from 0.4-26.4 mg/l (mean value 7.6 mg/l  $\pm$  S.D. 6.6 mg/l). The  $\alpha_2$ macroglobulin concentrations in sputum samples from patients with chest infections were on average higher than those of non-infected subjects ( $2p < 0.02$ ) being detectable in 16 of 17 (94%) (range 2.7-1009 mg/l mean 93.4 mg/l  $\pm$  125 mg/l).

The sputum/serum concentration ratios of  $\alpha_2$ macroglobulin (Fig. 2) were also increased during acute chest infection ( $2p < 0.02$ ). However, there was no longer a significant difference ( $2p > 0.05$ ) when these ratios were "corrected" by the corresponding sputum/serum albumin values (fig 3).

## Discussion

The finding of the present study that serum  $\alpha_2$ M levels were on average lower in bronchitic patients than in controls was not expected in view of the results of Barnett *et al.*<sup>14</sup> They demonstrated no significant difference between serum  $\alpha_2$ M in patients with obstructive lung disease and age-matched controls, although none of their patients was reported to have a chest infection. However, we could only demonstrate evidence of reduced plasma  $\alpha_2$ M levels in bronchitic subjects who had sputum production and chest infection. The remaining bronchitic patients had  $\alpha_2$ M levels similar to the

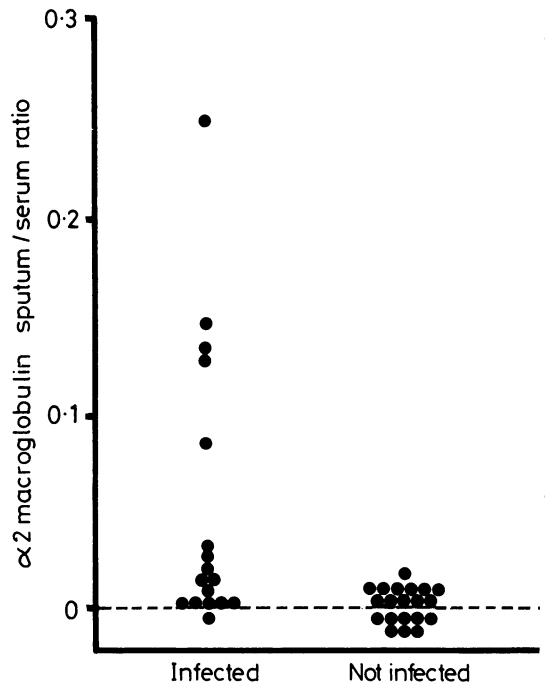


Fig 2 Sputum/serum concentration ratios of  $\alpha_2$ macroglobulin in bronchitic subjects with and without chest infection. Individual values below the dotted line are those in which sputum  $\alpha_2$ M concentrations were below the limit of detection.

control subjects. This suggests that reduced serum  $\alpha_2$ M concentrations are a feature of the infection rather than the bronchitis alone. The mechanism responsible for low plasma concentrations of  $\alpha_2$ M is not known but could be caused by reduced synthesis of the protein or increased catabolism resulting from  $\alpha_2$ M complexing with endopeptidases of lysosomal or bacterial origin.<sup>7</sup>

Despite the decreased plasma levels of  $\alpha_2$ M in patients with acute chest infections, sputum concentrations of the protein in these subjects were significantly elevated and the sputum/serum concentration ratios were also increased. These results suggest that  $\alpha_2$ M levels in the bronchial secretions were elevated either because of a general increase in transudation of proteins from the blood plasma or because of local secretion, perhaps by alveolar macrophages.<sup>6</sup> Albumin enters the bronchial secretions from the blood by diffusion and this is dependent on the molecular size and serum concentration.<sup>4</sup> Inflammation in the lung results in increased transudation of albumin and other proteins from the blood.<sup>4 15</sup>

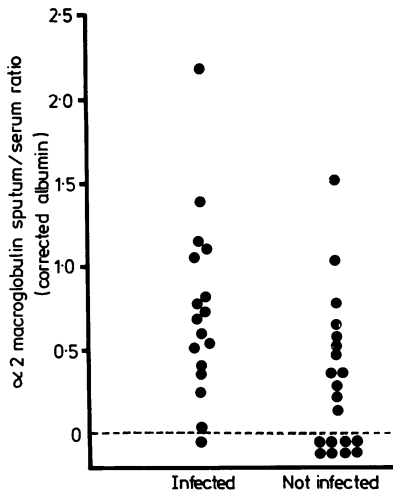


Fig 3 Sputum/serum concentration ratios of  $\alpha_2$ M "corrected" (divided by the albumin sputum/serum ratio. Values below the horizontal dotted line were those individuals whose sputum  $\alpha_2$ M values were below the limit of detection.

Correction of the sputum/serum concentration ratios of  $\alpha_2$ M with the albumin sputum/serum ratios eliminated the difference between the  $\alpha_2$ M values of infected and non-infected sputum samples. This suggests that the increased  $\alpha_2$ M concentrations in sputum during infections were mainly the result of inflammation-induced transudation from the blood plasma with little significant contribution from local secretion.

If  $\alpha_2$ M is locally secreted the present results suggest that this provides a minor contribution to the sputum concentrations in these subjects since the concentrations were generally found to be low and consistent with transudation from the blood.<sup>4</sup> However, some of the samples had sputum to serum  $\alpha_2$ M ratios greater than one when "corrected" for albumin (fig 3). This could indicate that local production does occur in some subjects and raises the possibility that the disease in these subjects may be associated with "local"  $\alpha_2$ M production deficiency.

In molar quantities, the  $\alpha_2$ M concentrations in sputum are (with the exception of some  $\alpha_1$ antitrypsin-deficient subjects) less than 5% of the  $\alpha_1$ antitrypsin molar concentrations.<sup>18</sup> Alpha<sub>1</sub>-antitrypsin inhibits protease in a one-to-one molar ratio;<sup>17</sup>  $\alpha_2$ M inhibits the enzymes in a 2:1 ratio.<sup>9</sup> The contribution of  $\alpha_2$ M in the protease inhibitory capacity of the bronchial secretions would therefore appear to be less than 10% of that contribution by  $\alpha_1$ AT. Furthermore, Tegner<sup>18</sup> has shown that all the plasma-derived inhibitors in non-purulent lavage fluids account for

only 10% of the elastase inhibitory capacity. The remaining 90% is caused by a locally secreted low-molecular weight bronchial inhibitor. However, elastolytic activity has only been demonstrated in lung secretions during chest infections,<sup>15,19</sup> and the levels of the low molecular weight inhibitor in purulent secretions are not known, although levels of  $\alpha_1$ AT<sup>15</sup> and  $\alpha_2$ M are increased. Nevertheless, most of the  $\alpha_1$ AT in infected sputum samples appears to be inactive<sup>20</sup> and the relatively low levels of  $\alpha_2$ M in these secretions may therefore complex with a greater proportion of proteases released from lysosomes or bacteria than the  $\alpha_1$ AT/ $\alpha_2$ M molar ratios suggest.

Endopeptidases that are complexed by  $\alpha_2$ M retain their enzymatic activity towards small substrates that can gain access to the active site of the enzyme within the  $\alpha_2$ M-enzyme complex.<sup>9</sup> Galdston *et al*<sup>10</sup> have shown that tropoelastin and solubilised elastin were degraded by elastase bound to  $\alpha_2$ M. They suggested that  $\alpha_2$ M-elastase complexes may contribute to the digestion of elastin precursors and therefore have a role to play in the development of pulmonary emphysema. This hypothesis has yet to be tested but it remains possible that the  $\alpha_2$ M present, despite its low concentrations in the bronchial secretions, could be a significant factor in the protease/inhibitor balance of the lung. This may be particularly important during infection when  $\alpha_2$ M is present in higher concentrations and enzyme levels within the lung are also increased.<sup>15</sup>

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