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Lung secretion sol-phase proteins: comparison of sputum with secretions obtained by direct sampling

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ABSTRACT The protein content of tracheal secretions and of bronchial lavage and bronchoalveolar lavage fluid was compared with that of sputum in 33 patients who underwent fibreoptic bronchoscopy. The secretion-to-serum concentration ratios for albumin, α_1-antitrypsin, α_1-antichymotrypsin, and immunoglobulin A (IgA) fell progressively as samples were obtained from further down the bronchial tree, probably reflecting greater sample dilution. The secretion-to-serum protein ratios when standardised for the corresponding albumin ratio were similar in all secretions studied. In particular, the IgA ratios were about eight times those of albumin and α_1-antichymotrypsin ratios were about twice those of albumin, suggesting similar degrees of "local production" of these proteins in all secretions studied. Some patients showed considerable differences in IgA ratios between sputum and bronchial lavage fluid. The significance of these differences is not clear.

Studies of the protein content of the lung secretions may provide information about the pathogenesis of lung diseases. Sputum is frequently studied because it is easy to collect and is therefore suitable for investigating large groups of patients. It is, however, a mixture of secretions from different areas of the bronchial tree and may not be representative of all parts of the respiratory tract. Furthermore, sputum is variably contaminated with saliva, although this effect may be largely dilutional.[1]

The problem of salivary contamination can be overcome by direct sampling of secretions during fibreoptic bronchoscopy, and this technique allows secretions to be selectively sampled from areas of the bronchial tree most relevant to the disease being studied. This means an invasive procedure, however, and the technique is less suitable for large population studies. Furthermore, the secretions obtained are also subject to variable dilution by anaesthetic solutions, used during the bronchoscopy, and by saline, which is used for lavaging the lung to obtain secretions from the lower respiratory tract.

The purpose of the present study was to compare the protein composition of sputum with that of secretions obtained from different areas of the bronchial tree during fibreoptic bronchoscopy.

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Methods

Thirty-three patients (eight female) were studied. The average age was 62 years (range 29 to 77 years) and most (28) had chronic cough and sputum production with irreversible airflow obstruction (mean forced expiratory ratio (FEV_1/FVC) 58·9%, SD 12%). The patients underwent fibreoptic bronchoscopy for a variety of reasons—15 patients had pulmonary neoplasia, 14 unexplained haemoptysis, and four interstitial lung disease.

Fibreoptic bronchoscopy was performed after premedication with intramuscular atropine 0·6 mg and intravenous diazepam 10 mg. The bronchoscope was passed transnasally after local anaesthesia with lignocaine and passed between the vocal cords after spraying them with two 2-ml aliquots of 4% lignocaine solution.

SECRETIONS STUDIED

Sputum This was collected as free from saliva as possible over a three-hour period from 19 patients on the morning of bronchoscopy, before premedica-

tion. Samples were centrifuged at 54 000 *g* for 90 minutes to obtain the sol phase, which was stored at −70°C with a corresponding serum sample until analysis.

Tracheal secretions These were obtained from 23 patients and consisted of all secretions aspirated into a sterile trap from the vocal cords to the carina as the bronchoscope was advanced.

Bronchial lavage fluid This consisted of secretions aspirated from either the right or the left main bronchus after the instillation of 5–10 ml of sterile normal saline. The procedure was performed on 10 patients.

Bronchoalveolar lavage fluids The bronchoscope was gently wedged in a segmental or subsegmental bronchus of either the right middle lobe or the lingula in 31 of the patients. Aliquots of 20 ml of sterile normal saline were instilled and then gently aspirated into a sterile trap. The procedure was repeated until 60–120 ml had been instilled. The volume of fluid aspirated for analysis varied widely, from 10 to 40 ml.

In patients with obvious neoplasms the lavaged secretions were obtained from the clinically unaffected lung. The tracheal secretions and bronchial lavage and bronchoalveolar lavage fluids obtained were centrifuged at 54 000 *g* for 90 minutes and stored with corresponding serum samples at −70°C until subsequent analysis.

PROTEINS STUDIED

Before estimation of protein concentrations in bronchoalveolar lavage fluids the samples were concentrated, by a known factor (5–10-fold), with an Amicon pressure-filtration system with a UM2 membrane (molecular weight cut-off 2000 daltons). The protein concentrations obtained were then divided by this known concentration factor to obtain the protein concentration of the secretion in the unconcentrated lavage fluid.

The concentrations of albumin, α_1-antitrypsin, α_1-antichymotrypsin, and IgA were measured in all the secretions, monospecific antisera produced in the Birmingham University immunodiagnostic research laboratory being used. The results were expressed as percentages of a standard reference serum (100% standard ≡ albumin 44·4 g/l; α_1-antitrypsin 2·04 g/l; α_1-antichymotrypsin 0·43 g/l; IgA1·57 g/l). The secretion-to-serum concentration ratios were calculated to overcome variations between individual patients in the serum concentrations of the acute-phase proteins α_1-antitrypsin and α_1-antichymotrypsin, as described previously.[2]

The secretion-to-serum concentration ratios of α_1-antitrypsin, α_1-antichymotrypsin, and IgA were "standardised" for albumin by dividing each one by the corresponding albumin ratio to allow comparison of the relative protein concentrations of each secretion and to assess the presence and degree of local protein production.[3]

Results

The individual secretion-to-serum albumin concentration ratios for each sample are shown in figure 1. The range was wide in all secretions studied (median values, with ranges, are given in table 1).

There was a progressive decrease in the values the more peripherally the secretions were obtained from the bronchial tree. This is emphasised in figure 2, which shows the albumin results for three secretions obtained from the same nine patients. Similar results were found for secretion-to-serum α_1-antitrypsin, α_1-antichymotrypsin, and IgA concentration ratios (table 1).

"Standardisation" of the protein secretion-to-serum ratios for albumin (that is, division by the

Table 1 *Secretion-to-serum protein concentration ratios* (median values with ranges in parentheses)*

	Sputum (n = 19)	*Tracheal secretions (n = 23)*	*Bronchial lavage fluid (n = 10)*	*Bronchoalveolar lavage fluid (n = 31)*
Albumin	0·71 (0·23–18·42)	0·53 (0·038–1·62)	0·044 (0·019–0·84)	0·024 (0·001–0·43)
α_1-antitrypsin	0·86 (0·31–8·64)	0·29 (0·028–1·08)	0·038 (0·014–0·59)	0·025 (0·001–0·27)
α_1-antitrypsin "corrected" for albumin	1·04 (0·47–2·80)	0·75 (0·095–1·75)	0·84 (0·50–1·88)	0·79 (0·13–1·42)
α_1-antichymotrypsin	2·73 (0·79–4·49)	0·83 (0·096–2·10)	0·20 (0·033–1·32)	0·048 (0·01–0·99)
α_1-antichymotrypsin "corrected" for albumin	1·96 (0·15–15·13)	2·34 (0·51–14·47)	2·32 (1·3–10·53)	2·00 (0·36–35·5)
IgA	9·20 (1·80–49·3)	5·22 (0·59–6·55)	0·56 (0·20–1·20)	0·39 (0·05–13·7)
IgA "corrected" for albumin	7·40 (0·60–44·7)	8·50 (2·02–40·3)	9·30 (1·43–21·6)	7·75 (1·27–661·7)

*The α_1-antitrypsin, α_1-antichymotrypsin, and IgA values are "corrected" for (that is, divided by) the corresponding albumin result. All values are multiplied by 100 for convenience.

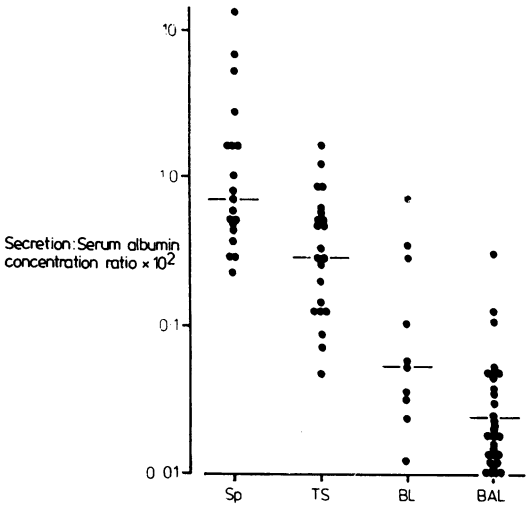

Fig 1 *Secretion-to-serum albumin concentration ratios (values × 10² for convenience). Each point represents the result from a single patient; horizontal lines are the median value for each secretion. Sp—sputum; TS—tracheal secretions; BL—bronchial lavage fluid; BAL—bronchoalveolar lavage fluid.*

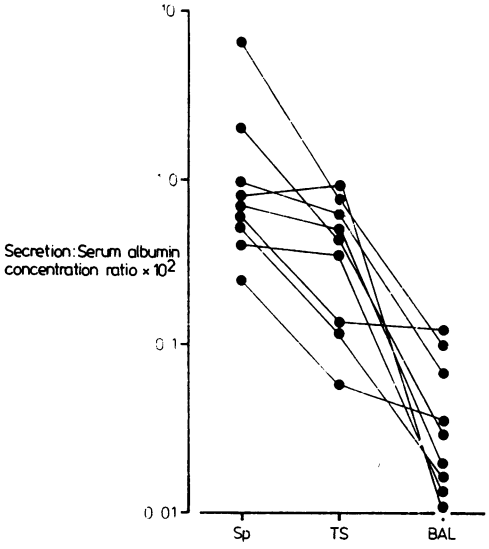

Fig 2 *Secretion-to-serum albumin concentration ratios (× 10²) for nine patients from whom matched sputum (Sp), tracheal sucking (TS), and bronchoalveolar lavage (BAL) samples were available. Each point represents the result from one patient; the lines join matched samples.*

corresponding albumin value) gave similar results in all secretions studied. The results for α_1-antitrypsin were close to unity, whereas IgA ($\simeq$ 8) and α_1-antichymotrypsin ($\simeq$ 2) usually had a greater ratio than did albumin (table 1).

Median results (with ranges) for paired samples (that is, secretions obtained from the same patients) are shown in table 2. The median results were similar to those obtained from the whole group of patients. Secretion-to-serum albumin ratios in sputum were significantly higher than those in tracheal secretions ($2p < 0{\cdot}01$), which in turn were higher than those in bronchoalveolar lavage fluid ($2p < 0{\cdot}001$). There were no significant differences between the "corrected" ratios of α_1-antitrypsin, α_1-antichymotrypsin, and IgA for any two secretions studied.

Data from individual patients are shown in more detail in figures 3 and 4. Four secretions were obtained from nine patients and data for those individuals are shown in table 3. For those patients there was also a progressive decrease in the secretion-to-serum protein ratios as sampling progressed down the bronchial tree (for clarity data for albumin only are shown). The corrected secretion-to-serum ratios for α_1-antitrypsin, α_1-antichymotrypsin, and IgA were similar in each secretion.

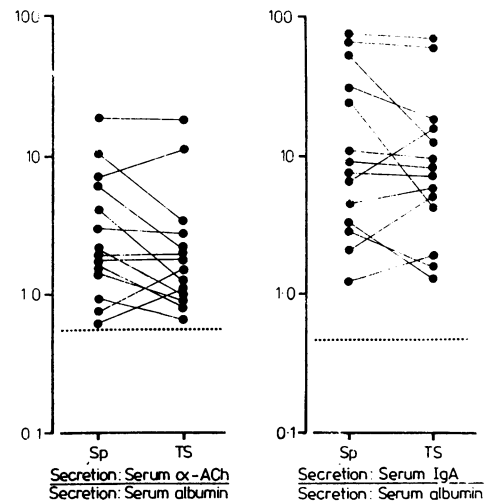

Fig 3 *Secretion-to-serum α_1-antichymotrypsin and IgA concentration ratios "corrected" for albumin: results in 14 patients from whom matched sputum (Sp) and tracheal sucking (TS) samples were available. Each point represents the result from one patient and the lines join paired samples. The broken lines represent the average value that would be expected for each protein if it behaved like albumin (that is, entering lung secretions by simple transudation). Values above this line are consistent with "local" production of the protein. α_1-ACh—4a_1-antichymotrypsin.*

Table 2 *Secretion-to-serum protein concentration ratios* for paired data (median values with ranges in parentheses)*

	(n = 13)		*(n = 19)*	
	Sputum	*Tracheal secretions*	*Sputum*	*Bronchoalveolar lavage fluid*
Albumin × 100	0·65 (0·23–3·14)	0·49 (0·04–1·62)	0·71 (0·23–18·42)	0·03 (0·01–0·12)
α_1-antitrypsin "corrected"	1·05 (0·84–2·30)	0·87 (0·22–1·75)	1·04 (0·47–2·80)	0·75 (0·13–1·29)
α_1-antichymotrypsin "corrected"	2·14 (0·50–15·13)	2·00 (0·51–14·47)	1·96 (0·15–15·13)	2·05 (0·36–35·5)
IgA "corrected"	7·40 (1·26–44·7)	8·69 (2·02–40·31)	7·40 (0·6–44·7)	13·84 (1·27–661·7)

*The α_1-antitrypsin, α_1-antichymotrypsin, and IgA results are shown "corrected" for (that is, divided by) the corresponding albumin results (which are multiplied by 100 for convenience).

Table 3 *Secretion-to-serum protein concentration ratios* for nine patients from whom all four secretions were available (median values with ranges in parentheses)*

	Sputum	*Tracheal secretions*	*Bronchial lavage fluid*	*Bronchoalveolar lavage fluid*
Albumin × 100	0·84 (0·37–3·14)	0·49 (0·12–1·62)	0·044 (0·026–0·84)	0·029 (0·01–0·064)
α_1-antitrypsin "corrected"	1·05 (1·04–1·63)	0·75 (0·22–0·84)	0·76 (0·5–1·88)	0·79 (0·56–1·29)
α_1-antichymotrypsin "corrected"	2·14 (0·6–6·23)	1·16 (0·51–2·71)	2·32 (1·30–5·00)	1·63 (0·77–3·17)
IgA "corrected"	11·79 (1·26–34·3)	9·27 (2·02–15·42)	9·30 (1·43–21·62)	15·88 (13·84–34·4)

*The α_1-antitrypsin, α_1-antichymotrypsin, and IgA results are shown "corrected" for the corresponding albumin result (which is multiplied by 100 for convenience).

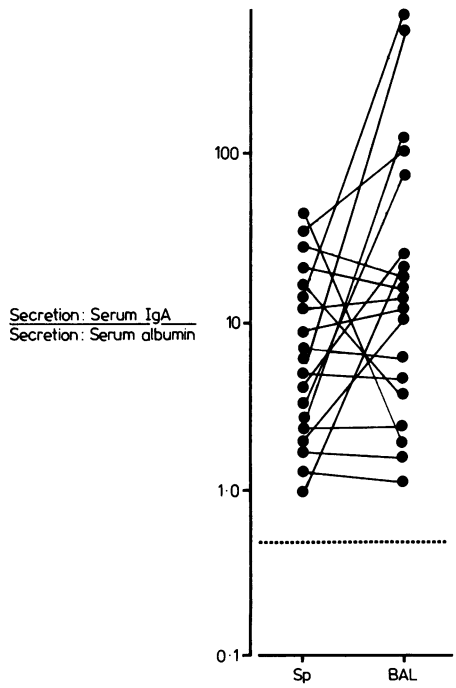

Fig 4 *Secretion-to-serum IgA concentration ratios "corrected" for albumin: results from 19 patients from whom matched sputum (Sp) and bronchoalveolar lavage (BAL) samples were obtained. Each point represents the result from one patient and the lines join paired samples.*

Discussion

The study of the protein content of lung secretions may provide clues to the pathogenesis of emphysema[4] and the adult respiratory distress syndrome[5] and help in the diagnosis of lung cancer.[6–8] Sputum is readily obtained from patients but is a mixture of secretions from all regions of the lung and nasopharynx. Furthermore, it is frequently contaminated with saliva to a variable extent, although the effect may be largely dilutional[1]; and it is not certain how representative sputum is of events in the more distal parts of the lung.

The sol-phase proteins albumin and α_1-antitrypsin are both thought to enter the lung secretions by simple diffusion from the serum and their secretion-to-serum ratios are similar in sputum[1] and bronchoalveolar lavage fluid.[3] IgA is produced locally within the lung and this is reflected in higher concentrations in sputum[9] and bronchoalveolar lavage fluid[3 8 10] than could be predicted on the basis of diffusion alone. But although local production of α_1-antichymotrypsin is found in the sol phase of sputum of patients with chronic obstructive bronchitis[1 2] Tegner failed to confirm it in bronchial lavage fluid from normal subjects.[11] The present study was designed to assess the relative amounts of locally produced IgA and α_1-antichymotrypsin in secretions from various parts of the bronchial tree to determine whether sputum is representative of other secretions in this respect.

The secretion-to-serum concentration ratios for all proteins became progressively lower from sputum to bronchoalveolar lavage fluid. This effect is likely to be caused by the progressive dilution of the secretions. The tracheal secretions were diluted by variable amounts of the lignocaine used to anaesthetise the vocal cords. Bronchial lavage samples were further diluted by lignocaine and by the normal saline used during the lavage procedure. Finally, bronchoalveolar lavage fluids were greatly diluted by 60–120 ml saline. To overcome this uncertain dilutional element, it is conventional to "standardise" the secretion by comparison with the albumin concentration. Albumin is thought to enter lung secretions by simple diffusion from serum. A protein which is either "locally produced" or preferentially concentrated within a lung secretion will be present in a greater concentration relative to albumin than in the corresponding serum.[1] This simple technique for recognising local production can produce anomalous results when proteins of larger molecular size than albumin are studied, particularly in the presence of lung inflammation,[9] which affects the permeability of the surface epithelium of the bronchial mucosa. It remains, however, a useful method for comparing different secretions in the same patient, since the relative protein concentrations should be similar unless the proportions of proteins produced locally or the degree of inflammation in various parts of the bronchial tree are also different.

The albumin and α_1-antitrypsin secretion-to-serum concentration ratios were similar in each secretion studied, confirming that the two proteins are behaving in the same way throughout the lung, entering all secretions by simple diffusion from serum. The IgA ratios were greater than albumin in all secretions studied, reflecting "local production" throughout the bronchial tree and providing confirmation that sputum is representative of this phenomenon. This confirms the findings of Masson *et al,* who showed similar IgA results in sputum and bronchial aspirates,[12] and Mogi *et al,* who found a similarity between nasal, laryngeal, and tracheobronchial secretions,[13] although the latter study included patients with laryngeal inflammation.

Evidence of local IgA production was also found in the bronchoalveolar lavage fluids studied here (with about eight times the albumin concentration), confirming the work of Reynolds and Newball[3] and Warr *et al.*[10] In the present study the median bronchoalveolar lavage fluid-to-serum IgA concentration ratio "standardised" for albumin was about eight times that of albumin, whereas the values of Warr *et al,* derived from normal subjects, were 12–16 times greater than albumin.[10] Similarly, Reynolds and Newball found IgA ratios about 12–14 times greater than albumin in normal subjects, although less (8–10 times the albumin ratio) in patients with a variety of intrathoracic lesions (either minimal lung parenchymal lesions located in an upper lobe or asymmetrical mediastinal enlargement). This difference might be interpreted as reflecting reduced "local" IgA production in the patients of the present study and that of Reynolds and Newball. Patients from both these studies are likely, however, to have had some degree of bronchial inflammation compared with normal subjects and this may give the impression of less "local" IgA production where it is normal.[9] The more complex techniques recently described by Stockley *et al*[14] may overcome this problem.

Our study confirms comparable "local production" of α_1-antichymotrypsin in both sputum and other lung secretions. This protein is an inhibitor of proteolytic enzymes and may protect tissues from damage by leucocyte cationic proteases.[15] The results are at variance with those of Tegner,[11] who found no evidence for local production of α_1-antichymotrypsin in bronchial lavage fluid of normal subjects. For reasons discussed previously,[2] however, the results of Tegner are difficult to interpret in the absence of paired serum samples and they were from normal subjects, who may differ in this respect from patients with bronchitis. Only five of our subjects did not have chronic cough with sputum production and their results were not clearly different from the remaining 28 patients. The problem therefore remains unresolved and will require further studies.

In the present study centrifugation at 54 000 *g* for 90 minutes was used to obtain the sol phase, which contains the freely diffusable proteins, from the sputum samples and also to remove debris from the other secretions studied. This speed was chosen to enable comparison with our previous studies on lung secretions.[1 2 4 9 14] There is no standard method of preparation of lung secretions for immunological analysis, although other workers, using a variety of centrifugation techniques,[3 8 10 12] have obtained results in general agreement with those presented in the present paper. We consider that variations in centrifugation are unlikely to influence the results.

The IgA-to-albumin and α_1-antichymotrypsin-to-albumin ratios in tracheal suckings were similar to the values obtained in sputum from the same individuals. In this respect the secretions are biochemically comparable despite the presence of saliva and nasopharyngeal secretions in sputum. Secretion-to-serum ratios ("corrected" for albumin) in sputum were comparable to the values obtained in bronchoalveolar lavage fluid when the grouped data are considered. When matched samples from

the same individuals are examined, however, striking individual variation is revealed, particularly in the case of IgA (fig 4). The reason for these differences is not clear since the patients do not fall into distinct diagnostic groups. Further studies are required to determine the significance of this variation.

In conclusion, secretion-to-serum protein concentration ratios fall as sampling advances peripherally and this probably reflects greater sample dilution by anaesthetic and lavage fluid. The protein profile of sputum is similar to that of other secretions obtained during fibreoptic bronchoscopy, suggesting that a similar proportion of IgA and α_1-antichymotrypsin is locally produced in all secretions studied. The study of sputum proteins may well reflect changes in secretions from more distal areas of the lung. Major individual differences are seen in some patients, however, particularly with respect to IgA.

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References

[1] Stockley RA, Mistry M, Bradwell AR, Burnett D. A study of plasma proteins in the sol phase of sputum from patients with chronic bronchitis. *Thorax* 1979;**34**:777–82.

[2] Stockley RA, Burnett D. Alpha$_1$ antichymotrypsin in infected and non infected sputum. *Am Rev Respir Dis* 1980;**122**:81–8.

[3] Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med* 1974;**84**:559–73.

[4] Stockley RA, Burnett D. Alpha$_1$ antitrypsin and leucocyte elastase in infected and noninfected sputum. *Am Rev Respir Dis* 1979;**120**:1081–6.

[5] Lee CT, Fein AM, Lippman M, Holtzman MD, Kimbel P, Weinbaum G. Elastolytic activity in pulmonary lavage fluids from patients with Adult Respiratory Distress Syndrome. *N Engl J Med* 1981;**304**:192–6.

[6] Merrill WW, Goodenburger D, Strober W, Matthay RA, Naegel GP, Reynolds HY. Free secretory component and other proteins in human lung lavage. *Am Rev Respir Dis* 1980;**122**:156–61.

[7] Merrill WW, Goodman M, Matthay RA, Naegel GP, Vandevoorde JP, Myl AD, Reynolds HY. Carcinoembryonic antigen in the lung lining fluids of smokers and non smokers. *Am Rev Respir Dis* 1981;**123**:29–31.

[8] Mandel MA, Dvorak KJ, Worman LW, De Cosse JJ. Immunoglobulin content in the bronchial washings of patients with benign and malignant pulmonary diseases. *N Engl J Med* 1976;**295**:694–8.

[9] Stockley RA, Burnett D. Local IgA production in patients with chronic bronchitis: effect of acute respiratory infection. *Thorax* 1980;**35**:202–6.

[10] Warr GA, Martin RR, Sharp PM, Rossen RD. Normal human bronchial immunoglobulins and protein. Effects of cigarette smoking. *Am Rev Respir Dis* 1977;**116**:25–30.

[11] Tegner H. Quantitation of human granulocyte protease inhibitors in non purulent bronchial lavage fluids. *Acta Otolaryngol (Stockh)* 1978;**85**:282–9.

[12] Masson PL, Heremans JF, Prignot J. Studies on the proteins of human bronchial secretions. *Biochim Biophys Acta* 1965;**111**:466–78.

[13] Mogi G, Watanabe N, Maeda S, Umehara T. Laryngeal secretions. An immunochemical and immunohistological study. *Acta Otolaryngol* 1979;**87**:129–41.

[14] Stockley RA, Burnett D, Afford SC. The immunological measurement of "free" secretory piece and its relationship to local IgA production. *Clin Exp Immunol* 1981;**45**:124–30.

[15] Ohlsson K, Åkesson U. α_1Antichymotrypsin interaction with cationic proteases from granulocytes. *Clin Chim Acta* 1976;**73**:285–9.