Clin. exp. Immunol. (1981) **45**, 124–130.

The immunological measurement of 'free' secretory piece and its relationship to local IgA production

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(Accepted for publication 16 January 1981)

SUMMARY

Free secretory piece (FSP) was studied in the sputum of patients with chronic bronchitis. The concentrations were higher both in the presence of infection and when greater quantities of total secretory piece were present compared to the amount of 11S IgA. A significant inverse correlation was found between the amount of FSP and 11S IgA in the individual samples when standardized for their total secretory piece content ($2P < 0{\cdot}01$). This suggests that the amount of FSP in any sample is partly dependent upon the amount of 11S IgA. The techniques employed offer a method for the study of the secretory IgA system in biological fluids. The preliminary results suggest that the secretory IgA system is independent of the systemic IgA system.

INTRODUCTION

Secretory IgA consists of several components produced independently by plasma cells and epithelial cells (Bienenstock & Befus, 1980). The bronchial secretions contain IgA in both its monomeric and dimeric forms (Reynolds & Newball, 1974) as well as secretory piece bound to the dimeric IgA (as secretory IgA) and unbound as 'free' secretory piece (Merrill *et al.*, 1980). It is therefore necessary to measure each of these components when assessing the integrity of the secretory IgA system in individual patients and patient groups.

The separation of monomeric and dimeric IgA in secretions can be performed with relative ease (Stockley, Afford & Burnett, 1980a). Total secretory piece can also be easily measured immunologically (Stockley & Burnett, 1980). However, the identification of 'free' secretory piece alone is more complex. Several techniques have been employed to assess the presence and amount of 'free' secretory piece in biological fluids:

(1) Column chromatography or thin-layer chromatography with crossed immunoelectrophoresis (Stockley *et al.*, 1980a) to separate the two forms of secretory piece by virtue of their different molecular sizes.
(2) Prediction of the presence of 'free' secretory piece knowing the dimeric IgA and total secretory piece content of a secretion from the formula derived by Stockley *et al.* (1980a).
(3) Bidirectional rocket immunoelectrophoresis can separate the 'free' secretory piece by causing it to migrate cathodally whilst secretory IgA migrates towards the anode (Kosaka, Asahina & Kobayashi, 1980).
(4) The immunological detection of antigenic components of secretory piece that are exposed when the protein is 'free' but covered when it is associated with IgA (Merrill *et al.*, 1980).

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The last method is the most direct and the present study was designed to raise and evaluate an antibody specific for 'free' secretory piece. This paper describes the preparation of the antibody and its use for measuring 'free' secretory piece in the sputum. The results are compared with the measurements of the other components of the secretory IgA system.

METHODS

Preparation of antibody to 'free' secretory piece. The method was essentially that of Bradwell *et al.* (1976). In brief, sputum was obtained from a patient with IgA deficiency. Previous studies had shown this sample to contain a large quantity of immunoreactive secretory piece but very little IgA. Furthermore, the secretory piece was demonstrated to be dissociated from IgA by thin-layer chromatography and crossed immunoelectrophoresis (Stockley *et al.*, 1980a).

The sample underwent two-dimensional immunoelectrophoresis into agarose with an intermediate gel containing anti-IgA (Seward Laboratories BA 02) and a further gel containing anti-secretory piece (Seward Laboratories BA 08) in the second dimension (Fig. 1). The secretory piece precipitation arc was removed as shown (Fig. 1); a sheep was immunized with the preparation together with Freund's complete adjuvant. Two intramuscular injections were given 1 month apart with a further injection 2 months later. Two weeks after the final injection, the sheep was bled. The IgG fraction of the resulting immune serum was obtained by ion-exchange chromatography on DE 52 cellulose (Whatman). The whole sheep serum was applied to the column in 0·03 molar phosphate buffer, pH 7·2. Non-adherent protein was collected (this being the IgG-rich fraction).

Modification and assessment. Whole human whey was fractionated on a Sephadex G-150 column (4·4 × 90 cm) and the fractions containing secretory piece and IgA were identified using the appropriate antisera (Fig. 2).

The fractions containing IgA also contained immunoreactive secretory piece and it was assumed that this represented the complete secretory IgA molecule (fractions 102–124) with no 'free' secretory piece. Fractions 140–170, where no IgA was detected, were assumed to contain secretory piece predominantly in its 'free' form.

The antibody raised to SP was firstly adsorbed with normal human serum and then a pooled sample of fractions 102–124 to remove any cross-reactivity with serum proteins and secretory piece on IgA. This adsorbed antibody retained some activity against the sputum preparation and the

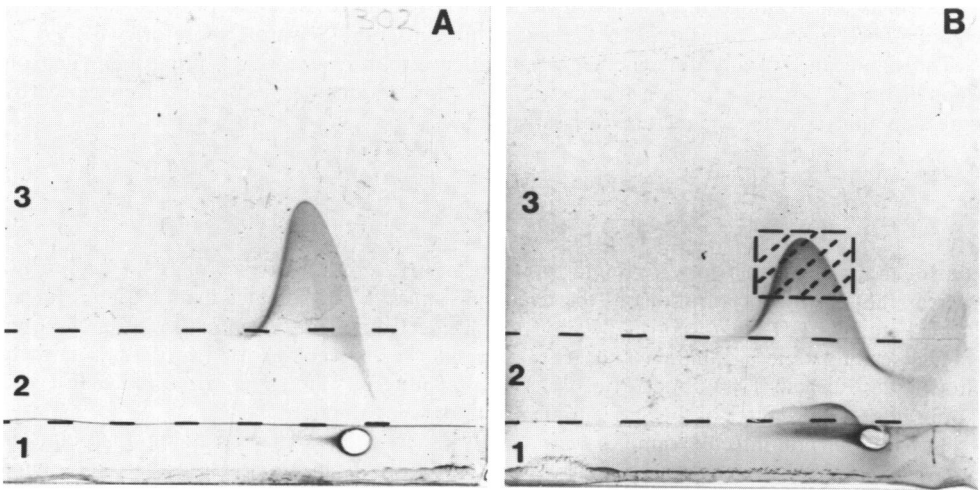

Fig. 1. Two-dimensional immunoelectrophoresis of the sputum rich in secretory piece. The sample was electrophoresed from right to left in plain agarose (1) and subsequently from bottom to top into agarose containing antibody to secretory piece (3). The intermediate gel (2) in plate A was blank and that in plate B contained anti-IgA. The hatched area (plate B) was removed and used to immunize a sheep.

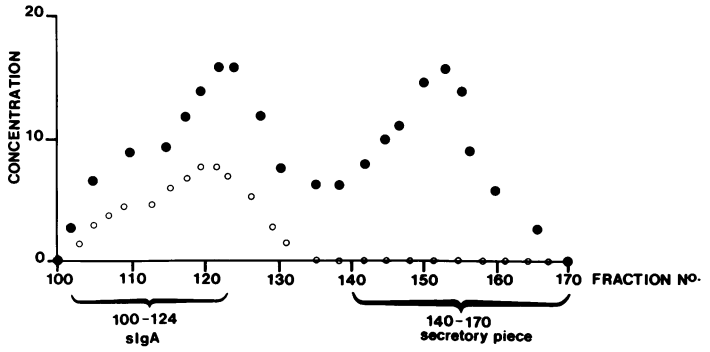

Fig. 2. The elution profile of human whey after fractionation on Sephadex G150. The vertical axis is the concentration in arbitrary units and the horizontal axis the fraction number. Immunoreactive IgA (o) and secretory piece (•) are shown for each of the fractions.

pooled whey fractions 140–170 containing 'free' secretory piece but no activity to fractions 102–124. This modified antibody was assessed by immunoelectrophoresis against a variety of body fluids to ensure that only one precipitation line persisted. Absence of cross-reactivity with lactoferrin (Seward Laboratories BX 65), free light chain (Seward Laboratories BA 28, BA 29) and free J chain (Nordic Immunological Laboratories 14–1173 and 14B–1173) was confirmed by Ouchterlony immunodiffusion using the appropriate antibodies, the initial sputum preparation and whey fractions 140–170.

Sputum studies. Sputum samples were collected as free from saliva as possible, over a 4-hr period, from 31 patients (16 with active chest infection) with chronic cough and sputum production. The samples were ultracentrifuged at 54,000 ***g*** for 90 min to obtain the sol phase which was stored at −70°C. The samples were subsequently analysed for the presence of 'free' secretory piece by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965). Pooled fractions 140–170 were used as the standard and each value expressed as a percentage.

Each sample had been previously assessed for its albumin, IgA and total secretory piece content (Stockley & Burnett, 1980) as well as the immunological proportions of IgA present as secretory (11S) and monomeric (7S) IgA (Stockley, Afford & Burnett, 1980b). The secretory piece results were expressed as a percentage of a standard human whey (Seward Laboratories BR 08) and the remaining proteins as a percentage of a standard human serum (Seward Laboratories BR 99).

The significance of any difference between patient groups was assessed using the Wilcoxon Mann–Whitney rank test for non-parametric data. Correlations between results were tested by regression analysis using the method of least squares.

RESULTS

Four of the patients studied were found to have a partial serum deficiency of IgA (< 700 mg/l) whereas the serum concentrations in the remaining subjects were greater than 850 mg/l (mean infected patients 3,517 mg/l, s.d. $\pm$2,079; mean non-infected patients 2,914 mg/l, s.d. $\pm$1,409).

The secretory piece, albumin and 11S IgA results have been reported in detail elsewhere (Stockley & Burnett, 1980; Stockley *et al.*, 1980b). The results are summarized in Table 1 and expressed as a percentage of the appropriate serum or whey standard.

Two of the non-infected samples were clearly different from the remaining 15 (points in parentheses, Fig. 3). For this reason, they were omitted from the main analysis and are considered separately with the four patients with partial serum IgA deficiency. This omission did not alter the significance of any observed differences or correlation and hence did not affect the overall conclusions.

The average values for FSP in the infected and non-infected samples are summarized in Table 1.

Table 1. Average sputum results for secretory piece (SP), free secretory piece (FSP), 11S IgA and albumin for infected and non-infected patients.

	Infected ($n=16$)	Non-infected ($n=15$)	Significance ($2P$)
Sputum FSP (% standard)	115·26 (51·48)	79·46 (77·34)	<0·01
Sputum SP (% standard)	54·96 (13·89)	41·24 (20·57)	n.s.
11S IgA (% standard)	14·58 (5·66)	6·77 (4·76)	<0·01
Albumin (% standard)	4·26 (5·05)	0·65 (0·63)	<0·0025
FSP/SP	2·20 (1·09)	2·06 (1·10)	n.s.
FSP/11S IgA	9·56 (6·09)	21·19 (20·02)	n.s.
FSP/albumin	82·52 (86·58)	399·70 (920·40)	<0·05

Data expressed as a percentage of the relevant standard (see Methods section). The FSP/11S IgA, FSP/SP and FSP/albumin ratios are shown together with the significance ($2P$) of any difference between infected and non-infected groups. Figures in parenthesis are 1 s.d.

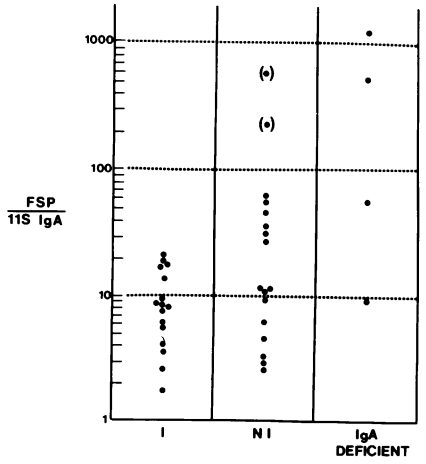

Fig. 3. FSP/11S IgA ratio. The vertical axis is the ratio on a logarithmic scale. Each point indicates the results for one patient. The results for the infected (I), non-infected (NI) and partial IgA-deficient patients are shown. The points in parentheses are discussed separately (see Results). P = n.s. for infected and non-infected patients.

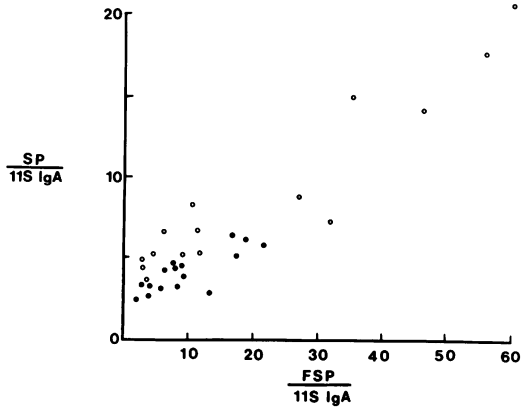

Fig. 4. Relationship of SP to FSP. The SP and FSP values of each sample standardized for 11S IgA are shown. (•) Infected samples and (o) non-infected samples. Correlation coefficients: for infected samples $r = 0{\cdot}811$, for non-infected samples $r = 0{\cdot}948$; for both groups together $r = 0{\cdot}933$.

The range was wide, from 33·6% standard to 339·6% standard. The concentrations of FSP were higher in the infected compared to the non-infected samples (Table 1). When the individual values were standardized for their albumin content (divided by the albumin concentration), the difference was reversed (Table 1). However, no difference was found when the values were standardized for their 11S IgA content (Table 1). The FSP/11S IgA values ranged from 6·25 to 21·36 in the infected samples and 2·57 to 60·00 in the non-infected samples (Fig. 3).

Relationship of FSP to SP. There was no difference between the FSP/SP ratios for the infected and non-infected samples (Table 1). The values ranged from 0·52 to 4·67. The absolute values of FSP and SP failed to correlate in both infected and non-infected samples. However, the FSP and SP values were significantly correlated ($2P < 0{\cdot}001$) when standardized for the 11S IgA content of the samples (Fig. 4).

Correlation of FSP with 11S IgA. The absolute values for 11S IgA and FSP failed to correlate in the infected and non-infected samples. However, when each non-infected sample was standardized for its total SP content, there was a significant inverse correlation ($2P < 0{\cdot}01$; $r = -0{\cdot}632$; Fig. 5).

Standardization of the samples for albumin failed to produce a significant correlation between the FSP and 11S IgA values in either the infected or non-infected samples ($r = 0{\cdot}301$; $2P > 0{\cdot}1$).

IgA deficiency. Of the four patients with partial serum IgA deficiency, two had very high FSP levels in sputum compared to the remaining patients (2,158 and 1,426% standard pool). The other two had similar FSP levels to the patients with normal serum IgA (223·7 and 63·8%).

The two subjects with high FSP values also had the highest total SP values of all patients studied

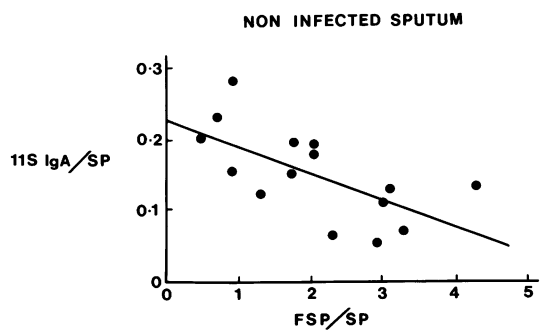

Fig. 5. Relationship of FSP to 11S IgA. The 11S IgA and FSP concentrations of each non-infected sample standardized for total secretory piece. Each point represents one sample and the regression line is drawn. Significance of the relationship: $2P < 0{\cdot}01$.

and similarly their FSP/SP ratios were greater (7·32 and 10·83). The FSP/11S IgA ratios in these patients were also higher than the infected and non-infected patients as shown in Fig. 3 (526·3 and 1,218·8).

The two serum IgA-deficient patients with low FSP concentrations in their sputum were similar to the infected and non-infected patients for their secretion 11S IgA concentration (23·06 and 1·12% standard), FSP/SP ratio and FSP/11S IgA ratio (Fig. 3).

As mentioned previously, two of the non-infected sputum samples seemed different from the remainder. Their concentrations of FSP were similar but the FSP/SP ratios (5·53 and 10·01) and the FSP/11S IgA ratios (569·4 and 223·0) were higher than the remaining non-infected subjects. These two patients, despite similar sputum albumin content, had the lowest 11S IgA concentrations in their sputum of any patient so far studied (0·32 and 0·54% standard).

DISCUSSION

Secretory piece is produced by mucosal epithelial cells and binds dimeric IgA to form the complete secretory IgA molecule. This binding is thought to be the mechanism responsible for the preferential transport of dimeric IgA into the external secretions (Bienenstock & Befus, 1980). Much of the secretory piece in the external secretions is bound to secretory IgA, though varying proportions of unbound or 'free' secretory piece have been identified in saliva and jejunal fluid (Strober *et al.*, 1976) and bronchial secretions (Merrill *et al.*, 1980).

In the present studies, FSP was identified in every sample although the concentrations were higher in the presence of infection. The reason for this increase is not clear. It may be the result of increased secretory piece production or release from the epithelial cells during the inflammatory reaction. Alternatively, dissociation of secretory IgA molecules, because of the greater proteolytic activity of the infected samples (Stockley & Burnett, 1979), could have resulted in a greater quantity of FSP. However, the latter explanation is less likely since no degradation of secretory IgA has been observed in purulent sputum even when kept for 4 weeks at 4°C (Schiotz, Clemmensen & Hoiby, 1980). Furthermore, the FSP/SP ratio was similar in the infected and non-infected samples despite the greater intrinsic proteolytic activity of the infected samples.

The concentrations of FSP varied over a wide range for both the infected and non-infected samples. However, the interpretation of individual protein concentration in sputum samples is complicated by varying degrees of fluid content and sample contamination by saliva. The major effect of contamination by saliva is dilution of the sputum protein concentrations (Stockley *et al.*, 1979). This is likely to apply to FSP measurement since Strober *et al.* (1976) found no FSP in unconcentrated saliva of subjects with normal serum IgA using conventional immunological techniques similar to those employed here. The effect of variable sample dilution is overcome by comparing protein ratios in the same sample and usually albumin is used as the standard. Results are conventionally expressed as protein concentration per unit of albumin in order that different samples might be compared. However, the albumin content of sputum is also dependent upon the degree of bronchial inflammation and increases in the presence of infection (see Results) because of increased protein transudation. Thus standardizing locally produced protein concentrations for the diffused albumin concentration could be misleading (Stockley & Burnett, 1980). For this reason, we compared the *locally* produced components (FSP, SP and 11S IgA) with each other in order that individual samples might be compared.

The FSP/11S IgA ratio seemed lower in the infected samples though this failed to reach statistical significance. This result suggests that if FSP rises in infection, it is less than the increase in 11S IgA production. However, further studies will be required to clarify this point.

There was a significant correlation between the FSP/11S IgA ratios and the SP/11S IgA ratios for both the infected and non-infected samples. The results suggest that if there is a greater immunological excess of SP over 11S IgA the relative concentration of FSP is higher. This finding would suggest that the amount of FSP in any sample depends partly upon the amount of 11S IgA which is bound to the total secretory piece present.

Further support for this concept is the inverse relationship between FSP and 11S IgA

standardized for total SP in the non-infected samples (Fig. 5). This relationship did not exist in the infected samples which may reflect disproportionate changes in secretory piece and IgA production in those samples.

Secretory piece and IgA are produced by different cells within the mucous membrane and secretory piece production can continue in IgA deficiency (McClelland, Shearman & Van Furth, 1976; Strober *et al.*, 1976). Thus if local IgA production were decreased and secretory piece production maintained, this would be reflected in a high FSP/11S IgA ratio.

Two of the non-infected sputum samples from patients with normal serum IgA had high FSP/11S IgA ratios (points in parentheses, Fig. 3). The results suggest that some patients with normal *systemic* IgA production have deficient *local* IgA production. Similarly, two of the serum IgA-deficient subjects had high FSP/11S IgA ratios consistent with a generalized deficiency of local IgA production. However, the remaining two patients with serum IgA deficiency had similar FSP, 11S IgA concentrations and FSP/11S IgA ratios to the majority of non-infected samples suggesting normal local IgA production.

In conclusion, the preparation of an antibody specific for 'free' secretory piece is described. Comparisons with the amount of 11S IgA in sputum samples suggest that four groups of subjects exist:

(1) Those with normal systemic and local IgA production.
(2) Those with deficient systemic and local IgA production.
(3) Those with deficient systemic and normal local IgA production.
(4) Those with normal systemic but deficient local IgA production.

The techniques described offer a method for assessing the integrity of the local IgA system in biological samples.

The authors wish to acknowledge the encouragement of Professor I. C. M. MacLennan and the typing of Mrs C. Seymour. Dr R. A. Stockley was in receipt of grants from the Medical Research Society, West Midlands Regional Health Authority, The Endowment Fund and Boehringer Ingelheim.

REFERENCES

BIENENSTOCK, J. & BEFUS, A.D. (1980) Mucosal immunology. *Immunology*, **41,** 249.

BRADWELL, A.R., BURNETT, D., RAMSDEN, D.B., BURR, W.A., PRINCÉ, H.P. & HOFFENBERG, R. (1976) Preparation of a monospecific antiserum to thyroxine binding globulin for its quantitation by rocket immunoelectrophoresis. *Clin. Chim. Acta*, **71,** 501.

KOSAKA, T., ASAHINA, T. & KOBAYASHI, N. (1980) Different quantification of SIgA and SC by two-directional rocket method. *Immunology*, **40,** 597.

MCCLELLAND, D.B.L., SHEARMAN, D.J.C. & VAN FURTH, R. (1976) Synthesis of immunoglobulin and secretory component by gastro-intestinal mucosa in patients with hypogammaglobulinaemia or IgA deficiency. *Clin. exp. Immunol.* **25,** 103.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2,** 235.

MERRILL, N.W., GOODENBERGER, D., STROBER, W., MATTHAY, R.A., NAEGEL, G.P. & REYNOLDS, H.Y. (1980) Free secretory component and other proteins in human lung lavage. *Am. Rev. respir. Dis.* **122,** 156.

REYNOLDS, H.Y. & NEWBALL, H.H. (1974) Analysis of proteins and respiratory cells from human lungs by bronchial lavage. *J. Lab. clin. Med.* **84,** 559.

SCHIOTZ, P.O., CLEMMENSEN, I. & HOIBY, N. (1980) Immunoglobulins and albumin in sputum from patients with cystic fibrosis. A study of protein stability and presence of proteases. *Acta Pathol. Microbiol. Scand.* **88,** 275.

STOCKLEY, R.A., AFFORD, S.C. & BURNETT, D. (1980a) A method for the study of local IgA production using radial immunodiffusion and thin layer chromatography. *J. Immunol. Methods*, **38,** 151.

STOCKLEY, R.A., AFFORD, S.C. & BURNETT, D. (1980b) Assessment of 11S and 7S IgA in sputum. *Am. Rev. respir. Dis.* **122,** 959.

STOCKLEY, R.A. & BURNETT, D. (1979) Alpha$_1$ antitrypsin and leukocyte elastase in infected and non-infected sputum. *Am. Rev. respir. Dis.* **120,** 1081.

STOCKLEY, R.A. & BURNETT, D. (1980) Local IgA production in patients with chronic bronchitis: effect of acute respiratory infection. *Thorax*, **35,** 202.

STOCKLEY, R.A., MISTRY, M., BRADWELL, A.R. & BURNETT, D. (1979) A study of plasma proteins in the sol phase of sputum from patients with chronic bronchitis. *Thorax*, **34,** 777.

STROBER, W., KRAKAUER, R., KLAEVEMAN, H.L., REYNOLDS, H.Y. & NELSON, D.L. (1976) Secretory component deficiency. A disorder of the IgA immune system. *N. Engl. J. Med.* **294,** 351.