Cells containing IgA subclasses in bronchi of subjects with and without chronic obstructive lung disease

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SUMMARY Necropsy specimens were obtained from the lungs of 10 subjects who had no history of lung disease, 10 who had died with chronic bronchitis, and 10 with bronchiectasis. Tissue sections were stained for IgA1 or IgA2 using the immunoperoxidase technique, and the number of cells in the bronchi stained for these proteins was counted. The total number of IgA positive cells was increased in bronchitic and bronchiectatic lungs compared with those from control subjects. The number of IgA2 positive cells was similar in those with bronchitis and bronchiectasis and significantly higher than in controls. Similarly, cells containing IgA1 were increased in the lungs of subjects with chest disease but were higher in those with bronchitis than in those with bronchiectasis. The proportion of IgA2:total IgA containing cells was similar in sections from controls (mean (SD) 25 (5.0)%) and those with bronchiectasis (mean (SD) 24 (4)%), but lower in those with bronchitis (mean (SD) 19 (5.0)%). The results show that cells containing IgA1 predominate in the major bronchi but that the proportion of cells containing IgA2 is higher than in non-mucosal lymphoid tissues. Bronchitis and bronchiectasis are associated with greater numbers of cells producing IgA in the bronchi, and this is consistent with increased local production of IgA in the lung secretions of bronchitic subjects.

Immunoglobulin A (IgA) is thought to protect the respiratory tract from infection by pathogens.¹ It is the most abundant immunoglobulin class in lung secretions, unlike the blood plasma, where IgG predominates.² Most (about 90%) of the blood IgA is monomeric³; in lung secretions about 50–70% is present as polymeric IgA and most of this is secretory IgA,^{4,5} indicating active secretion of polymeric IgA by secretory component mediated transport.⁶ Furthermore, although 10–20% of blood IgA is of the IgA2 subclass, the proportion of IgA2 is higher in lung secretions, representing about 30% of the total IgA.⁷ These observations, showing differences in the IgA composition of blood and lung secretions suggest that most lung IgA is produced locally within pulmonary tissues, although some is also derived from the blood by transudation.⁸

IgA plasma cells are most abundant in the glands and lamina propria of the major bronchi, suggesting

that most of the IgA in lung secretions is produced in the upper airways, although some IgA plasma cells have also been seen in the small bronchi, bronchioles, and alveolar septa.^{9,10}

The factors which influence local synthesis of IgA in the lung are poorly understood. It has been proposed, on the basis of indirect methods, that deficient local IgA production may be responsible for recurrent infection and morbidity in chronic bronchitis.^{9,11} This concept of “local deficiency” was supported by the report of reduced numbers of IgA plasma cells in the bronchi of bronchitics.⁹ The assessment of local IgA synthesis, however, is difficult in the presence of lung inflammation, as transudation of proteins including IgA from the plasma is increased.⁸ Recent studies measuring secretory IgA or polymeric IgA in the lung secretions from patients with chronic bronchitis have shown a significant increase in the amounts of these locally produced components of IgA during infective exacerbations.^{4,12,13} These results showed that most subjects with chronic bron-

chitis were capable of producing more IgA locally in response to local bacterial infection.

In view of these findings the present study was designed to reinvestigate, by immunohistochemistry, the numbers of plasma cells producing IgA in the bronchi of subjects with chronic obstructive lung disease. We wished to determine whether a study of the number of cells containing IgA in the bronchi of subjects with chronic bronchitis supported the hypothesis of local deficiency of IgA plasma cells or, alternatively, whether it suggests a competent bronchial IgA system. We also compared the results with those from subjects with bronchiectasis, a chronic lung condition characterised by continual lung infection. Specifically, plasma cells containing the IgA1 or IgA2 subclasses were counted to establish the proportion of cells containing these proteins and the influence of chronic lung disease on their distribution.

Material and methods

LUNG TISSUE

Necropsy tissue specimens were obtained within eight hours of death from 10 subjects (age range 27–81 years, mean 57), who had no recent history or pathological evidence of acute or chronic lung disease; from 10 subjects (64–83 years, mean 71) who had died with clinical and postmortem evidence of bronchiectasis, originating from a viral infection in childhood; and from 10 subjects (59–83 years, mean 65) who had died with chronic bronchitis. Four of these last group of subjects had died of their bronchitis and six had "incidental" bronchitis which did not cause their deaths. Histological diagnoses were made using the criteria of Dunnill.¹⁴ Necropsy tissue was considered to be appropriate for this study as we have shown previously that IgA staining of bone marrow cells seen in necropsy specimens reflects the numbers seen in biopsy material.¹⁵

IMMUNOPEROXIDASE STAINING OF TISSUES

The lung tissue samples were fixed in 10% formal saline, processed to paraffin wax, and sectioned to 3 μ m thickness. The sections were dewaxed, taken to Tris hydrochloric acid buffer, (pH 7.8) and endogenous peroxidase blocked with 0.1% hydrochloric acid in methanol for 30 minutes. After washing in buffer the sections were incubated for 45 minutes in mouse monoclonal antiserum to IgA1 (antiserum M4D8) or IgA2 (SCAB1/2E2) which were prepared in the department of immunology, University of Birmingham. The characteristics of these antisera have been described elsewhere.¹⁶ After further washing the sections were incubated for 45 minutes in horseradish peroxidase conjugated rabbit antiserum to mouse IgG (department of immunology, Univer-

sity of Birmingham). The peroxidase reaction was visualised by incubation with 1 mg/10 ml 3,3'-diaminobenzidine in Tris hydrochloric acid buffer containing one drop of 30% hydrogen peroxide. The sections were counterstained with haematoxylin, dehydrated, and mounted in synthetic medium.

QUANTITATION OF IGA POSITIVE CELLS

Tissue sections containing major first order bronchi were investigated. The numbers of cells stained positively for IgA1 or IgA2 were counted in 10 separate random fields on each section using an objective at a magnification of 40. To prevent recounting of cells a simple eyepiece graticule was used. The random fields counted included subepithelial seromucoid glands and stroma.

Differences between cell counts in the subject groups were tested using Student's *t* test.

Results

The cells staining for IgA1 exceeded those staining for IgA2 in all groups of subjects (figure).

The numbers of cells positive for IgA2 were similar in the lung sections from chronic bronchitics (mean (12) 51 cells) and bronchiectatics (mean (18) 56, but both groups had significantly higher numbers than the controls (mean (4.9) 24 two tailed $p < 0.001$). Similarly, sections from subjects with chronic bronchitis and those with bronchiectasis had larger num-

Figure Cells which stained for IgA1 or IgA2 in necropsy lung tissue sections from 10 subjects who had died with chronic bronchitis, 10 who had bronchiectasis, and 10 who had no evidence of lung disease (controls). Each point represents number of positive cells counted in 10 microscope fields from one subject. Horizontal bars denote mean values.

bers of IgA1 positive cells than the controls (mean (14.8) 73 cells, two tailed $p < 0.001$), although the numbers of IgA1 positive cells were higher in sections from chronic bronchitis (mean (32) 218) than those from the group of bronchiectasis (mean (24) 175 two tailed $p < 0.005$).

The proportion of IgA2 cells (per cent of total IgA positive cells) was similar in sections from the control group (mean (4.8) 24.8%) and bronchiectasis (mean (4.2) 23.7%) but significantly lower in the chronic bronchitis (mean (4.6) 19% two tailed $p < 0.001$).

The total numbers of IgA positive cells in sections from chronic bronchitis (mean (32) 269 cells) and bronchiectasis (mean (39) 230) were significantly higher than those of the control subjects (mean (17) 98 two tailed $p < 0.001$).

Discussion

Although IgA is thought to be important in protecting mucosal surfaces such as those of the lungs, its full role is uncertain. In particular, the biological importance of the higher proportions of the IgA2 subclass in mucosal secretions⁷ is not known, and functional differences between the subclasses are obscure. The IgA2 subclass is, however, resistant to proteolysis by the IgA proteases that are produced by many pathogenic bacteria.¹⁷ The stability of IgA2 in the presence of infection, when bacterial proteinases are likely to be released, would maintain its functional characteristics better than IgA1 which would be more easily damaged by proteolytic cleavage.

In a small series (four samples) Andre *et al*¹⁸ reported that 27–33% of the IgA positive cells in human bronchus produced IgA2. Thus the lung, like other secretory tissue, contains a higher proportion of cells producing IgA2 than “non-secretory” lymphoid tissue.^{19 20}

In the present study we observed a proportion of IgA2:IgA1 cells in the normal human bronchus similar to that reported by Andre *et al*,¹⁸ and this seems to reflect the proportions of these IgA subclasses found in the lung secretions.⁷

Deficiency of serum and lung secretion IgA has been reported in subjects with chronic bronchitis and recurrent infections, including those associated with bronchiectasis.^{13 21 22} Soutar⁹ suggested that a deficiency of plasma cells producing IgA in the respiratory tract was a feature of subjects with fatal chronic bronchitis. We have been unable to confirm this observation, either in the group of bronchitis as a whole or in the four who had died of their disease. On the contrary, we observed significantly increased numbers of cells containing IgA in the bronchi of subjects with “fatal” or “incidental” chronic bronchitis.

The results are consistent with our previous obser-

vations that most affected subjects seem to be capable of responding to infective exacerbations with an increase in IgA concentrations in their lung secretions.^{4 12 13} Similarly, subjects who had died with another chronic lung disease, bronchiectasis, had increased numbers of cells containing IgA in the bronchi compared with subjects who had died without evidence of lung disease. The results of this study suggest, therefore, that most subjects with chronic obstructive lung disease do not have an associated general deficiency of cells producing IgA in the lung, although these conditions can be a consequence of systemic or local IgA deficiency in some subjects.^{13 22}

The higher number of cells containing IgA in lungs of subjects who had died with chronic obstructive lung disease was represented by an increase in both IgA subclasses, and the proportion of IgA2:IgA1 cells was similar in those with bronchiectasis and controls. The subjects with chronic bronchitis, however, showed a preferential increase in the proportion of IgA1 cells compared with IgA2, resulting in a decreased proportion of the IgA2 cells.

The reasons for this observation are unknown. It would be valuable to determine whether the lung secretion concentrations of the IgA subclasses reflect the cell populations and if the increases lead to local secretion of more polymeric or secretory IgA. Further studies into the factors regulating the differential stimulus of plasma cells producing the IgA subclasses in the lungs are clearly indicated.

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