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Bronchial mucosal manifestations of atopy: a comparison of markers of inflammation between atopic asthmatics, atopic nonasthmatics and healthy controls

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ABSTRACT: We studied the role of atopy, as defined by positive skin tests to common inhalant allergens, in allergic bronchial inflammation.

Endobronchial biopsies were taken *via* the fibreoptic bronchoscope in 13 symptomatic atopic asthmatics, 10 atopic nonasthmatics, and 7 normals.

The numbers of mast cells, identified in the submucosa by immunohistochemistry using the AA1 monoclonal antibody against tryptase, were no different between the three groups, but electron microscopy showed that mast cell degranulation, although less marked in atopic nonasthmatics, was a feature of atopy in general. The numbers of eosinophils, identified by immunohistochemical staining using the monoclonal anti-eosinophil cationic protein antibody, EG2, were greatest in the asthmatics, low or absent in the normals and intermediate in the atopic nonasthmatics. In both atopic groups eosinophils showed ultrastructural features of degranulation. Measurements of subepithelial basement membrane thickness on electron micrographs showed that the collagen layer was thickest in the asthmatics, intermediate in the atopic nonasthmatics and thinnest in the normals.

The results suggest that airways eosinophilia and degranulation of eosinophils and mast cells, as well as increased subepithelial collagen deposition, are a feature of atopy in general and suggest that the degree of change may determine the clinical expression of this immune disorder.

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Accumulating evidence from postmortem and bronchoscopy studies [1–6] has established mucosal inflammation as a major feature of asthma. Characteristically, asthmatic airways are infiltrated with inflammatory cells, predominantly eosinophils, although increases in other cell types, including lymphocytes and mast cells, have also been reported [4, 7]. There is now evidence that inflammatory cells in the airways are also in a state of heightened activity, as suggested by studies of lymphocyte activation markers [6, 8], findings of increased mediator levels in bronchoalveolar lavage (BAL) [9, 10], and electron microscopic observations of mast cell and eosinophil degranulation [11].

A link between atopy, defined by the presence of skin test positivity to common inhalant allergens, and the clinical expression of asthma has been established, in that allergen challenge in the laboratory results in bronchoconstriction, enhanced airway responsiveness [12], and airway inflammation [5, 13]. Although in a proportion of patients developing asthma later in life it is not possible to elicit positive skin responses, the concentration of serum immunoglobulin E (IgE) has recently been shown by BURROWS *et al.* [14] to relate closely to the expression of asthma irrespective of the presence of atopy, suggesting that in most patients suffering from this disease IgE-mediated mechanisms may play an important role. However, it remains unclear why only a proportion of atopic individuals develop lower airways disease, whilst the vast majority remains either asymptomatic or suffers from allergic manifestations restricted to other sites such as the nose, eye and skin.

To improve understanding of the relevance of atopy to the airway histopathological changes and clinical expression of asthma, we have extended our previously reported study of atopic asthma [11] to those atopic subjects with a history of allergic rhinitis but no lower airway symptoms. In addition to numbers and ultrastructural appearance of airway mucosal mast cells and eosinophils, we have compared the three subject groups with respect to thickness of the subepithelial collagen layer of the lamina reticularis. We have related these pathological changes in the airways to

skin wheal responses to common allergens and total serum IgE as indices of atopy as well as to airway function and methacholine responsiveness.

Subjects and methods

All subjects underwent skin prick testing with a series of common inhalant allergens *(Dermatophagoides pteronyssinus, Dermatophagoides farinae,* mixed grass pollens, cat fur, dog hair, feathers and a mixture of moulds; Bencard, Brentford, UK) according to the method of PEPYS [15], and the greatest wheal diameters were recorded after 15 min. Subjects were classified as being atopic if skin prick tests resulted in a wheal reaction which was at least 3 mm greater than that produced by physiological saline control. The sums of the greatest diameters of skin wheal responses to three of the most common inhalant allergens in the UK *(Dermatophagoides pteronyssinus,* mixed grass pollens and the greater of the responses to cat fur and dog hair) were used as an index of an individual's atopy.

On the basis of positive history of wheeze and attacks of breathlessness, associated with increased diurnal peak expiratory flow variability, 13 subjects (5 female; mean age 28 yrs, range 19–66 yrs) were classified as asthmatic. All were symptomatic at the time of study and treated only with salbutamol. Ten subjects (3 female; mean age 26 yrs, range 20–44 yrs), without past or present asthmatic symptoms, but with a history of other atopic diseases, including allergic rhinitis, allergic conjunctivitis and eczema (n=3), were classified as atopic nonasthmatics. Seven nonatopic, symptom-free subjects (3 female; mean age 17 yrs, range 21–45 yrs) were designated as normal controls.

The study was performed outside the pollen season. For at least two months prior to bronchoscopy none of the asthmatics had received any disease-modifying drugs. None of the atopic nonasthmatics or normal control subjects was taking any medication for at least 2 weeks preceding the study. The study was approved by the Southampton Hospitals and University Ethics Subcommittee and patients gave their written informed consent.

Measurement of total serum IgE

Total serum IgE was measured using the enzyme-linked immunosorbent assay (ELISA) in a sample of blood drawn on the day of bronchoscopy.

Measurement of FEV_1 and PC_{20} methacholine

Forced expiratory volume in one second (FEV_1) was measured on the day of methacholine challenge using a dry wedge spirometer (Vitalograph, Buckinghamshire, UK). Airway responsiveness was measured by methacholine challenge between 2 and 5 days before fibreoptic bronchoscopy according to a method modified from CHAI *et al.* [16], as reported previously [11], with concentrations of methacholine (Sigma, Poole, UK), ranging from 0.03–16 $mg \cdot ml^{-1}$. The cumulative provocative concentration of methacholine ($PC_{20}M$) was calculated by linear interpolation between the last two points of the log-dose-response curve. The top concentration of methacholine used in the atopic non-asthmatics and normal controls was 16 $mg \cdot ml^{-1}$, and if this failed to cause a >20% fall, the cumulative $PC_{20}M$ was recorded as being >32 $mg \cdot ml^{-1}$.

Bronchoscopy

Fibreoptic bronchoscopy was performed according to the National Institute of Health guidelines [17] and our previously reported protocol [11]. After premedication with nebulized salbutamol and ipratropium bromide, *i.m.* atropine and *i.v.* midazolam to achieve mild sedation, the bronchoscope (Olympus 1T20, Tokyo, Japan) was introduced through the nose or mouth under local anaesthesia and with supplemental oxygen. Using alligator forceps (Olympus FB 15C) two biopsies were taken of subcarinae in the lower and middle lobes of the right lung.

Processing of endobronchial biopsies

One coded biopsy sample was processed into Araldite resin for immunohistochemistry as reported previously [11]. For each cell type, five sections 1 μm thick were cut at 10 μm intervals to avoid overlapping of cells, and the positively stained nucleated cells were counted by a blinded observer. Mast cells were demonstrated using a mouse monoclonal antibody (AA1) directed to tryptase of human mast cell granules [18]. Eosinophils were stained using a mouse monoclonal antibody, EG2 (Cambridge, Bioscience, Cambridge, UK), which is specific for the cationic protein (ECP) present in eosinophil granules [19]. Antibody binding was displayed by peroxidase-labelled avidin-biotin complexes as described previously [11]. The submucosa was delineated on the Video Interactive Display System (VIDS II) and its area calculated using software for measurement of area (AMS Analytical Measuring Systems, Cambridge, UK). The cell counts of the entire submucosal area, excluding glands and blood vessels, were expressed as the number of $cells \cdot mm^{-2}$.

The second coded biopsy was processed into Spurr resin for electron microscopy as reported previously [11]. Electron micrographs of mast cells and eosinophils were taken for observation of the ultrastructure of their granules. As described previously [20], the thickness of the lamina reticularis layer of collagen was measured using Vernier callipers on electron micrographs of well-orientated sections at a magnification of ×5,000. Five points were measured for each specimen by a blinded observer and the mean used for analysis.

Data analyses

Comparisons of logarithmically transformed $PC_{20}M$ and IgE values and the FEV_1 (% predicted) values were made by one way analysis of variance and the two-tailed paired Student's t-test. The mean submucosal cell counts as well as collagen layer thickness and sums of skin wheal responses were compared between groups using the Kruskal-Wallis test and the Wilcoxon's signed rank test for paired samples. Correlations between the logarithmically transformed values of IgE and $PC_{20}M$ were sought by linear regression. Relationships between skin wheal responses and $PC_{20}M$, as well as between both mast cells and eosinophils and collagen thickness were tested by Spearman's rank correlation.

Results

The sum of the maximal diameters of wheal responses to three most common allergens in the asthmatics (median 20 mm, range 8–54 mm) was greater than in the atopic nonasthmatics (median 12 mm, range 7–26 mm), but this failed to reach significance (p=0.06). Total serum IgE levels were highest in the asthmatic group (geometric mean 180 IU, range 10–6,048 IU), and although significantly greater (p<0.001) than those measured in the normal controls (geometric mean 17 IU, range 10–39 IU) the difference failed to reach significance (p=0.07) when compared with the atopic nonasthmatic group (geometric mean 47 IU, range 10–546 IU) (fig. 1). Serum IgE levels were significantly higher (p<0.05) in the atopic nonasthmatics than in the normal controls.

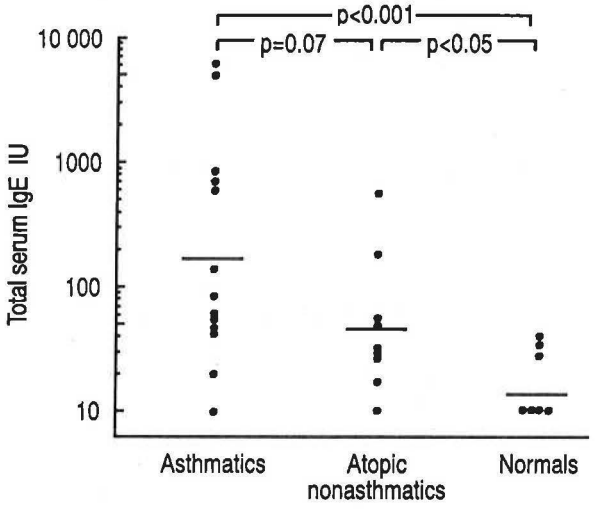

Fig. 1. – Total immunoglobulin E (IgE) concentrations in the asthmatics, atopic nonasthmatics and healthy controls (normals). Horizontal bars indicate geometric mean values.

The mean±SD percentage of predicted FEV_1 values measured on the day of methacholine challenge were lower in the asthmatics (89±11%) than in both the atopic nonasthmatics (106±9.6%, p<0.001) and the normal controls (108±12%, p<0.005), which were not significantly different. The asthmatics had a range of $PC_{20}M$ from 0.06–5.68 mg·ml^{-1} (geometric mean $PC_{20}M$ 0.69 mg·ml^{-1}), whereas in the normal control group $PC_{20}M$ could not be obtained with the highest tested concentration. Three atopic nonasthmatics had a $PC_{20}M$ <16 mg·ml^{-1}, with one subject achieving a value within the asthmatic range (4.8 mg·ml^{-1}).

The concentration of total serum IgE in the asthmatics was weakly but significantly inversely related to the $PC_{20}M$ (r=-0.55, p<0.05) but was not related to the atopic index as defined by skin testing. There was no relationship between the skin allergen responses and $PC_{20}M$.

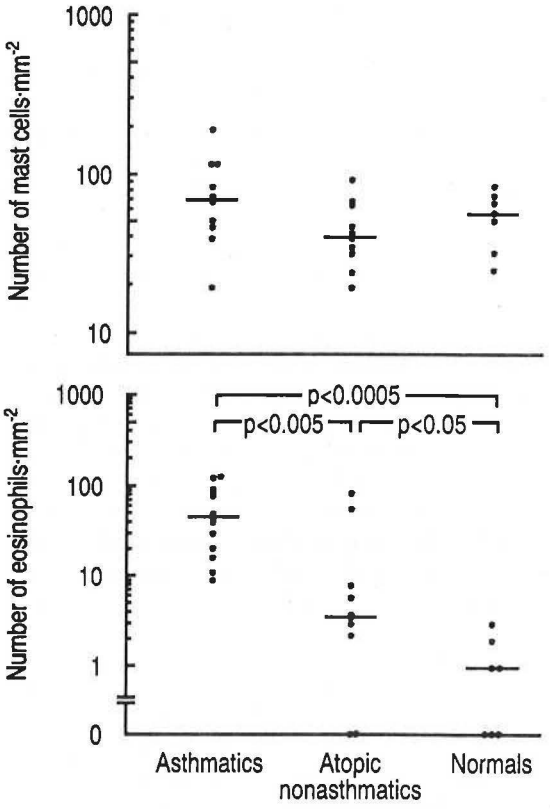

Fig. 2. – Numbers of mast cells (upper panel) and eosinophils (lower panel) in the submucosa of atopic asthmatics, atopic nonasthmatics and healthy controls (normals). There were no significant differences in mast cell numbers between the groups.

Inflammatory cell populations

There was no significant difference between the asthmatic (median 53 cells·mm^{-2}, range 19–192 cells·mm^{-2}) and either the atopic nonasthmatic (median 22 cells·mm^{-2}, range 20–44 cells·mm^{-2}) or normal control groups (median 56 cells·mm^{-2}, range 24–83 cells·mm^{-2}) (fig. 2). Eosinophil numbers were highest in the asthmatics (median 47 cells·mm^{-2}, range 9–126 cells·mm^{-2}) and lowest in the normals (median 1 cell·mm^{-2}, range 0–3 cells·mm^{-2}), with those of atopic nonasthmatic subjects (median 3.6 cells·mm^{-2}, range 0–85 cells·mm^{-2}) being intermediate and significantly different from both the asthmatics (p<0.005) and

the normal controls ($p<0.05$) (fig. 2). There was no relationship between total serum IgE and either mast cell or eosinophil numbers in both atopic groups.

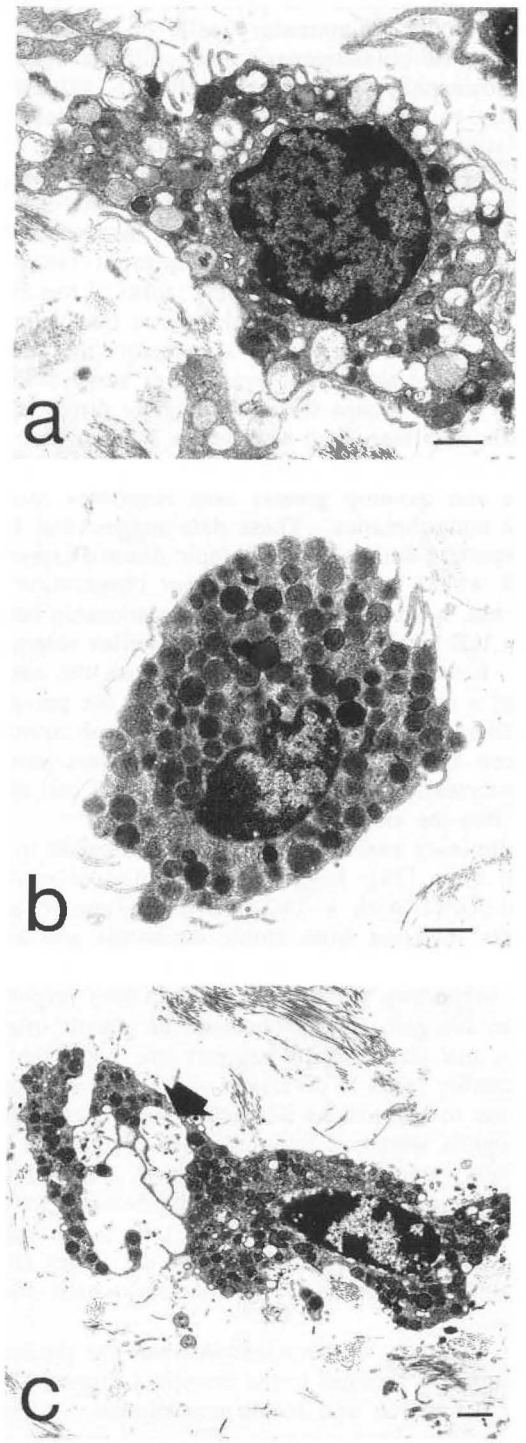

Fig. 3. – Mast cells in the bronchial submucosa: a) of an asthmatic; b) of a healthy control subject. In biopsies of asthmatic subjects mast cells were seen with ultrastructural appearance suggestive of piecemeal degranulation (a) and with degranulation channels compatible with the anaphylactic type of degranulation (c), (uranyl acetate-lead citrate stain; transmission electron microscopy; bar=1 μm).

Ultrastructural appearance of mast cells and eosinophils

Electron microscopy showed that whilst all of the mast cells of normals contained intact granules displaying a lattice and scroll pattern (fig. 3b) those in asthmatics showed varying degrees of degranulation (fig. 3a and c). Some mast cells in the asthmatics displayed features suggestive of piecemeal degranulation, consisting of partial loss of granular substance with remnant granular content being present within intact granular membranes (fig. 3a), whilst others contained degranulation channels characteristic of the anaphylactic type of degranulation (fig. 3c). Although most mast cells in the atopic nonasthmatic subjects were also fully granulated, occasional cells showed evidence of a lesser degree of degranulation of both piecemeal and anaphylactic types.

In both atopic groups eosinophils were seen by electron microscopy to display a similar degree of granule heterogeneity, with apparent dissolution of granular matrix and core, frequently yielding an inverse staining pattern whereby the normally darker core stained more lightly than the matrix. As no eosinophils were seen by electron microscopy in the sections from normal controls, no comparison of their ultrastructure was possible with the other two subject groups.

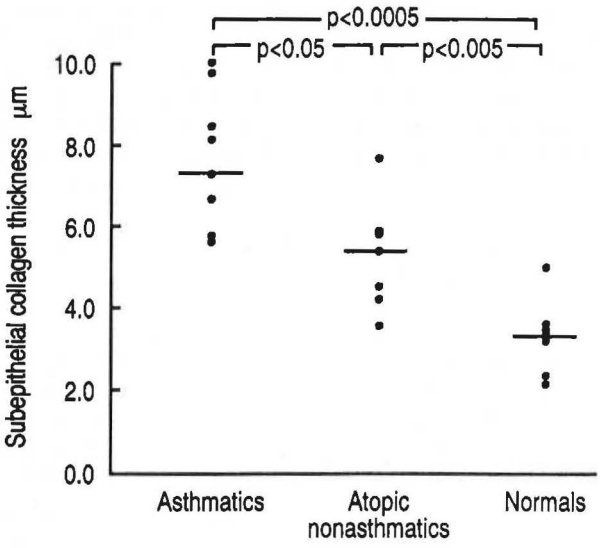

Fig. 4. – Subepithelial collagen thickness in the asthmatics, atopic nonasthmatics and healthy controls (normals). Horizontal bars indicate median values.

Subepithelial collagen

The thickness of the subepithelial collagen layer was greatest in the asthmatics (median 7.3 μm, range 5.6–10 μm), intermediate in the nonasthmatics (median 5.4 μm, range 3.6–7.7 μm, $p<0.05$) and least in the normals (median 3.4 μm, range 2.2–5, $p<0.0005$) (fig. 4), and the differences were significant between

all three groups. There was no relationship between either mast cell or eosinophil numbers and the collagen thickness in either of the studied groups.

Discussion

In this study we have shown that mucosal eosinophilia and activation of mast cells and eosinophils, as judged by structural features of degranulation, are a characteristic of atopy in general, with the extent of changes being related to the expression of the asthmatic state. In both atopic groups there was increased collagen deposition beneath the epithelium which was greatest in asthma and intermediate in atopic nonasthmatics, suggesting that bronchial fibrosis may be occurring in atopy in the absence of overt clinical asthma.

A role for the mast cell in the experimentally provoked early asthmatic response (EAR) to inhaled allergen has been firmly established [21]. Although mast cell numbers in BAL have been shown in some but not all studies to be elevated in asthma [3, 4], we have confirmed our previous observations of similar numbers of these cells in mucosal biopsies in asthmatics and normals [5] and have extended this observation to atopic nonasthmatics. The electron microscopic appearances in both atopic groups were compatible with the anaphylactic and piecemeal types of degranulation described by DVORAK and co-workers [22, 23] but were more pronounced in the asthmatics than in the atopic nonasthmatics. These structural observations are in keeping with reports of increased releasability of BAL mast cells in asthma [24], resulting in elevated histamine [25] and tryptase levels [26], and with the capacity of H_1-antihistamines to cause bronchodilatation in atopic asthmatics [27]. Our present finding of a mild loss of mast cell granular content in atopic nonasthmatics offers an explanation for the elevated histamine levels in BAL of allergic rhinitis patients, found by CASALE *et al.* [25] to be intermediate to those measured in atopic asthmatics and normals.

The potential of the eosinophil to produce inflammatory mediators such as leukotrienes and the arginine-rich basic proteins, major basic protein (MBP), localized to the granule core, and ECP and eosinophil peroxidase (EPO), localized to the matrix, has placed it as a major effector cell of allergic disease [21]. In our study mucosal eosinophilia, although most pronounced in atopic asthmatics, was shown to be another general feature of atopy. In both atopic groups these cells contained granules with features which have been attributed to eosinophil activation such as dissolution of granule cores. There is ample evidence that activated or "primed" eosinophils have the capacity to generate more mediators such as leukotriene C_4 (LTC_4) [28] and that they are hypodense when separated on discontinuous density centrifugation [29]. The role of activated eosinophils in the airways of atopic nonasthmatics is unclear, but they could contribute to a mild form of mucosal inflammation in the absence of clinical disease.

The presence of atopy and IgE-mediated allergic mechanisms appears also to play a role in the development of long-term consequences of allergic inflammation in the form of bronchial fibrosis. The mechanisms leading to excessive collagen deposition in atopy are unclear but probably involve enhanced production by inflammatory cells of factors which stimulate fibroblastogenesis and collagen synthesis. Both eosinophils [30] and mast cells [31, 32] have the capacity to activate fibroblasts, but the absence of correlation between cell numbers and collagen thickness suggests that the relationship is complex and probably related more to the functional status of these cells. By analogy with other diseases such as diabetes mellitus, in which basement membrane changes occur in the "subclinical" form of the disease [33], the present study would suggest that pathological changes in atopy can be seen before the development or in the absence of overt lower airways disease.

Both atopic groups were significantly different from normals with respect to total serum IgE concentration, with a tendency for asthmatics to have higher IgE levels and develop greater skin responses than the atopic nonasthmatics. These data suggest that IgE is an important determinant of atopic disease expression, which would be supported by our observation of a weak but, nevertheless, significant relationship between serum IgE levels and airway methacholine responsiveness. It would also be in keeping with the observation of a relationship between IgE and the prevalence of asthma [14]. However, the absence of correlation between total serum IgE and cell numbers points to the complexity of mechanisms involved in cell recruitment into the airways.

Preliminary evidence in humans, analogous to studies in mice [34], suggests preferential selection of lymphocytes with a Th2-like phenotype in atopic patients suffering from atopic dermatitis and asthma [35, 36], with the resulting profile of secreted cytokines supporting the allergic inflammatory response to inhaled allergen. Thus, whatever the genetic origin of atopy, our data would support the view that this abnormality leads to diversion of the mucosal immune response to IgE and its interaction with mast cells and eosinophils which, if minimal, fails to produce lower respiratory tract symptoms. Although a mild form of airway hyperresponsiveness may be detected in atopic nonasthmatics, it would appear that these immune responses are greatly enhanced in the lower airways in asthmatic individuals, for reasons which are still unclear.

In conclusion, we have demonstrated the presence of inflammatory changes in the bronchial mucosa of both atopic asthmatics and atopic nonasthmatics. Because these inflammatory changes were most pronounced in asthmatics, and intermediate in atopic nonasthmatics, we suggest that they are related to the expression of clinical asthma and that serum IgE and atopy, as demonstrated by skin tests, are important determinants of the degree of inflammation in the airway mucosa and consequent expression of disease.

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References

1. Dunnill MS. – The pathology of asthma with special reference to changes in the bronchial mucosa. *J Clin Pathol*, 1960; 13: 27–33.
2. Dunnill MS, Massarella GR, Anderson JA. – A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmaticus, in chronic bronchitis and in emphysema. *Thorax*, 1969; 24: 176–179.
3. Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. – Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am Rev Respir Dis*, 1988; 137: 62–69.
4. Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM. – Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis*, 1987; 136: 379–383.
5. Beasley R, Roche WR, Roberts JA, Holgate ST. – Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis*, 1989; 139: 806–817.
6. Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles G, Assoufi B, Collins JV, Durham S, Kay AB. – Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable asthma. *Am Rev Respir Dis*, 1990; 142: 1407–1413.
7. Kelly CA, Stenton SC, Ward C, Bird G, Hendrick DJ, Walters EH. – Lymphocyte subsets in bronchoalveolar lavage fluid obtained from stable asthmatics and their correlations with bronchial responsiveness. *Clin Exp Allergy*, 1989; 19: 169–175.
8. Corrigan CJ, Hartnell A, Kay AB. – T lymphocyte activation in acute severe asthma. *Lancet*, 1988; i: 1129–1132.
9. Casale TB, Wood D, Richerson HB, Trapp S, Metzger WJ, Zavala D, Hunninghake GW. – Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with methacholine bronchial hyperresponsiveness. *J Clin Invest*, 1987; 79: 1197–1203.
10. Wardlaw AJ, Hay H, Cromwell O, Collins JV, Kay AB. – Leukotrienes, LTC_4 and LTB_4, in bronchoalveolar lavage in bronchial asthma and other respiratory diseases. *J Allergy Clin Immunol*, 1989; 84: 19–26.
11. Djukanović R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, Howarth PH, Holgate ST. – Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. *Am Rev Respir Dis*, 1990; 142: 863–871.
12. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. – Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy*, 1977; 7: 503–513.
13. Metzger WJ, Zavala D, Richerson HB, Moseley P, Iwamota P, Monick M, Sjoerdsma K, Hunninghake GW. – Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs. Description of the model and local airway inflammation. *Am Rev Respir Dis*, 1987; 135: 433–440.
14. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. – Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med*, 1989; 320: 271–277.
15. Pepys J. – Skin testing. *Br J Hosp Med*, 1975; 14: 412–421.
16. Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, Sheffer AL, Spector SL, Townley RG. – Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol*, 1975; 56: 323–327.
17. Summary and recommendations of a workshop on the investigative use of fiberoptic bronchoscopy and bronchoalveolar lavage in asthmatics. *Am Rev Respir Dis*, 1985; 132: 180–182.
18. Walls AF, Bennett AR, McBride HM, Glennie MJ, Holgate ST, Church MK. – Human mast cells tryptase: a biochemical marker for mast cell degranulation. *Biochem Soc Trans*, 1989; 17: 728–729.
19. Tai P-C, Spry CJF, Peterson C, Venge P, Olsson I. – Monoclonal antibodies distinguish between storage and secreted forms of eosinophil cationic protein. *Nature*, 1984; 309: 182–184.
20. Roche WR, Beasley R, Williams JH, Holgate ST. – Subepithelial fibrosis in the bronchi of asthmatics. *Lancet*, 1989; i: 520–524.
21. Djukanović R, Roche WR, Wilson JW, Beasley CRW, Twentyman OP, Howarth PH, Holgate ST. – Mucosal inflammation in asthma. State of the art. *Am Rev Respir Dis*, 1990; 142: 434–457.
22. Dvorak AM, Schulman ES, Peters SP, MacGlashan DW, Newball HH, Schleimer RP, Lichtenstein LM. – Immunoglobulin E-mediated degranulation of isolated human lung mast cells. *Lab Invest*, 1985; 53: 45–56.
23. Dvorak AM, Monahan RA, Osage JE, Dickersin GR. – Crohn's disease: transmission electron microscopic studies. II. Immunologic inflammatory response. Alterations of mast cells, basophils, eosinophils, and the microvasculature. *Hum Pathol*, 1980; 11: 606–619.
24. Flint KC, Leung KBP, Hudspith BN, Brostoff J, Pearce FL, Johnson NM. – Bronchoalveolar mast cells in extrinsic asthma: a mechanism for the initiation of antigen specific bronchoconstriction. *Br Med J*, 1985; 291: 923–926.
25. Casale TB, Wood D, Richerson HB, Trapp S, Metzger WJ, Zavala D, Hunninghake GW. – Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with methacholine bronchial responsiveness. *J Clin Invest*, 1987; 79: 1197–1203.
26. Wenzel SE, Fowler AA, Schwartz LB. – Activation of pulmonary mast cells by bronchoalveolar allergen challenge. *In vivo* release of histamine and tryptase in atopic subjects with and without asthma. *Am Rev Respir Dis*, 1988; 137: 1002–1008.
27. Rafferty P, Holgate ST. – Terfenadine (Seldane) is a potent and selective histamine H_1 receptor antagonist in asthmatic airways. *Am Rev Respir Dis*, 1987; 135: 181–184.
28. Shaw RJ, Walsh GM, Cromwell O, Moqbel R, Spry CJF, Kay AB. – Activated eosinophils generate SRS-A leukotrienes following IgG-dependent stimulation. *Nature*, 1985; 316: 150–152.
29. Wardlaw AJ, Kurihara K, Moqbel R, Walsh GM, Kay AB. – *In*: Clinical immunology and allergy: the allergic basis of asthma. A.B. Kay ed., London, 1988; pp. 15–36.
30. Pincus SH, Ramesh KS, Wyler DJ. – Eosinophils stimulate fibroblast DNA synthesis. *Blood*, 1987; 70: 572–574.

31. Franzen L, Norrby K. – Local mitogenic effect of tissue mast cell secretion. *Cell Tissue Kinet*, 1980; 13: 635–642.
32. Roche WR. – Mast cells and tumors. The specific enhancement of tumor proliferation *in vitro*. *Am J Pathol*, 1985; 119: 57–64.
33. Siperstein MD, Unger RH, Madison LL. – Studies of muscle capillary basement membranes in normal subjects, diabetic, and prediabetic patients. *J Clin Invest*, 1968; 47: 1973–1999.
34. Street NF, Mossman TR. – Regulation of immune responses by T cells with different cytokine secretion. Proceedings of the XVIII Congress of the Collegium Internationale Allergologicum, Basel, (In Press).
35. Wierenga EA, Snoek M, De Groot C, Chrétien I, Bos JD, Jansen HM, Kapsenburg ML. – Evidence for compartmentalization of functional subsets of CD4+ T lymphocytes in atopic patients. *J Immunol*, 1990; 144: 4651–4656.
36. Romagnani S. – Increased numbers of Th2-like CD4+ T cells in target organs and in the allergen-specific repertoire of allergic patients. Possible role of IL-4 produced by non-T cells. Proceedings of the XVIII Congress of the Collegium Internationale Allergologicum, Basel, (In Press).