

Dendritic cells in normal and asthmatic airways: expression of the α subunit of the high affinity immunoglobulin E receptor (Fc ϵ RI- α)

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Summary

Background Immunoglobulin E (IgE) plays an important role in asthma, with total serum IgE levels closely related to both clinical expression of the disease and airway hyperresponsiveness. IgE binds to a high affinity cell-surface receptor (Fc ϵ RI) which is present on mast cells and which has also recently been demonstrated on cutaneous dendritic cells. If pulmonary dendritic cells were also able to express this receptor, this would have important implications with regard to their potential role in asthma.

Objectives The aims of the study were to investigate the expression of the α subunit of the high affinity IgE receptor (Fc ϵ RI- α) in normal and asthmatic airways, and to analyse its cellular provenance with particular emphasis on the dendritic cell.

Methods Bronchial biopsy specimens were obtained using fiberoptic bronchoscopy from 10 atopic asthmatics and nine non-atopic non-asthmatic control subjects. Specimens were processed into glycolmethacrylate resin and analysed by immunohistochemistry using specific monoclonal antibodies against Fc ϵ RI- α , and against tryptase and CD1a, markers for mast cells and dendritic cells, respectively.

Results The numbers of dendritic cells were significantly higher in the airways of asthmatics compared with those of control subjects ($P < 0.02$). Analysis of sequential sections revealed that the α subunit of Fc ϵ RI was localized to both mast cells and dendritic cells. The proportion of dendritic cells expressing Fc ϵ RI- α was significantly increased in the asthmatic group ($P < 0.003$).

Conclusion These results support the hypothesis that dendritic cells play an important role in allergic asthma although the functional significance of Fc ϵ RI- α expression needs further investigation.

Keywords: asthma, biopsy, bronchoscopy, dendritic cells, Fc ϵ RI, immunoglobulin ϵ

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Introduction

Immunoglobulin E (IgE) plays an important role in the pathophysiology of asthma and other allergic conditions, with elevated total serum IgE levels closely related to both clinical expression of asthma and airway

hyperresponsiveness [1,2]. IgE binds to specific cell-surface receptors, providing an important trigger mechanism for the release of inflammatory mediators. The high affinity receptor for IgE, termed Fc ϵ RI, is a hetero-oligomer composed of an α chain, a β chain, and two disulphide-linked γ chains [3]. The α chain is necessary for IgE binding and receptor internalization whereas the γ chains are important in the process of signal transduction. The role of the β chain is currently uncertain as it appears not to be

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essential for receptor function [4]. FcεRI was originally described on mast cells and basophils, cells that can be activated by cross-linking of two or more receptor-bound IgE molecules by specific allergen [5]. Recently it has been reported that epidermal dendritic cells (DCs) (Langerhans cells) can also express FcεRI [6–8]. This may enhance their antigen-presenting function by facilitating the capture and internalization by Langerhans cells of antigens which can be subsequently presented to T-lymphocytes [9,10].

Mast cells and DCs are present in the bronchial mucosa of both normal and asthmatic subjects. As assessed by immunohistochemistry, numbers of mast cells are not increased in asthma, although ultrastructural features of degranulation are more frequent in asthmatic airways [11,12]. DCs are also present in human airways, where they form a tightly meshed network within the bronchial epithelium [13,14]. These pulmonary DCs function efficiently as antigen-presenting cells [13], and form a dynamic population which can rapidly increase in number and activation in response to local inflammatory stimuli [15]. The CD1a antigen is widely used as a marker for tissue DCs [16]. It has recently been demonstrated that numbers of CD1a+ DCs are increased in the airways of atopic asthmatic subjects compared with atopic non-asthmatics, and these cells have been reported to induce the selective proliferation of a TH₂-like subpopulation of T-lymphocytes [17]. If pulmonary DCs were also able to express FcεRI, this would have important implications with regard to their potential role in atopic asthma.

The aims of the present study were to investigate the expression of the IgE-binding α subunit of FcεRI (FcεRI-α) in normal and asthmatic airways and to analyse the cellular provenance of this receptor in the airways, with particular emphasis on the dendritic cell.

Materials and methods

Subjects

Ten atopic asthmatic subjects (eight male) with mildly symptomatic disease, receiving treatment only with inhaled β₂-sympathomimetics as required, and nine non-atopic healthy volunteers (six male) on no regular medication took part in the study. Between 1 and 2 weeks prior to bronchoscopy, subjects attended for determination of their atopic status and measurement of their FEV₁ and airway reactivity. Atopy was defined as a skin weal response of diameter 3 mm or greater, in the presence of negative (saline) and positive (histamine acid phosphate) controls, in response to skin-prick testing with one or more of the following common aeroallergens: *Dermatophagoides*

pteronyssinus, mixed grasses, mixed feathers, cat fur and dog hair (Bencard, Brentford, UK). Methacholine inhalation challenge was undertaken using a method modified from that of Chai *et al.* [18]. After 15 min rest, subjects inhaled five breaths of 0.9% saline (pH 7.4), each breath taken from functional residual capacity to total lung capacity, followed at 3 min intervals by doubling incremental concentrations of methacholine (Sigma, Poole, UK) from 0.03 to 16 mg/mL. All solutions were administered via an Inspiron Mini-neb nebulizer with mouthpiece (CR Bard International Limited, Sunderland, UK), driven by compressed air at a flow of 8 L/min. FEV₁ was measured one and 3 min after inhalation of five breaths of each solution using a dry wedge spirometer (Vitalograph, Vitalograph Ltd, Buckingham, UK) with the highest of three values recorded at each time point. The percentage fall in FEV₁ from the post-saline baseline value was plotted against the cumulative concentrations of inhaled methacholine on a logarithmic scale, and the inhalation challenge discontinued either when a 20% fall in FEV₁ had been achieved or when the highest concentration of agonist had been reached. The PC₂₀ was obtained by linear interpolation between the last two points of the log₁₀ concentration-response plot. When a 20% fall in FEV₁ was not achieved with the highest concentration of methacholine, the cumulative value was recorded as >32 mg/mL.

The clinical and physiological details of the subjects studied are summarized in Table 1. All subjects gave written informed consent and the study was approved by the Southampton Combined University and Hospitals Ethics Committee.

Fibreoptic bronchoscopy and bronchial biopsy

Fibreoptic bronchoscopy was performed as described in our previously published protocol [19], conforming to current NHLBI guidelines [20]. All subjects received premedication with 0.6 mg atropine im, and nebulized salbutamol 5 mg and ipratropium bromide 0.5 mg. Midazolam was administered to achieve mild sedation and lignocaine 4% and 1% spray was used for local anaesthesia of the upper airways. An Olympus BFIT20 fibreoptic bronchoscope (Olympus Co., Tokyo, Japan) was inserted via the nasal or the oral route, and further aliquots of lignocaine 1% were applied to the lower airways. Mucosal biopsies were obtained from the middle lobe carina using FB15E alligator biopsy forceps (Olympus). Continuous supplemental oxygen was administered throughout the procedure via nasal cannulae and oxygen saturation was monitored by digital oximetry (Pulsox-7, Minolta, Middlesex, UK). Close clinical supervision was maintained during the bronchoscopy procedure and for a period of at least 4 h afterwards.

Table 1. Clinical and physiological characteristics of the subjects studied

Subject	Sex	Age (years)	FEV ₁ * (% predicted)	PC ₂₀ "" (mg/mL)
Asthmatics				
1	M	52	80	0.46
2	M	21	76	0.62
3	F	27	106	2.99
4	M	31	101	0.50
5	M	20	104	4.98
6	M	37	88	0.43
7	M	40	47	0.29
8	M	27	101	0.09
9	F	36	82	0.11
10	M	27	80	0.14
Normal subjects				
1	F	20	110	>32
2	M	20	105	>32
3	M	19	108	>32
4	M	18	118	14
5	M	18	117	>32
6	F	22	116	>32
7	M	60	93	>32
8	M	26	100	>32
9	F	19	110	>32

*Forced expiratory volume in 1 second.

""Cumulative provocative concentration of methacholine resulting in a 20% fall in FEV₁ from baseline.

Tissue processing and immunohistochemistry

Biopsies were placed immediately into ice-cooled acetone, containing 20 mM iodoacetamide and 2 mM phenylmethylsulphonylfluoride (PMSF) to inhibit protease activity, and fixed overnight at -20°C . The following day, specimens were processed into glycolmethacrylate (GMA) resin (Polysciences, Northampton, UK) as previously described [21].

For immunohistochemistry, $2\text{ }\mu\text{m}$ sections were cut by ultramicrotomy, floated on 0.2% (v/v) ammonia solution for 1 min, picked up onto poly L-lysine-coated slides, and dried at room temperature for 1–4 h. Sections were pre-treated with a solution of 0.1% (w/v) sodium azide and 0.3% (v/v) hydrogen peroxide for 30 min to inhibit endogenous peroxidase activity. After 3×5 min washes in Tris-buffered saline (TBS) pH 7.6, blocking medium, consisting of Dulbecco's modified Eagle's medium, 10% fetal calf serum and 1% bovine serum albumin, was applied for 30 min. The following mouse IgG1 monoclonal antibodies (MoAbs) were then applied to sequential sections: MoAb 15.1 specific for Fc ϵ RI- α (a gift from Dr J. P. Kinet, National Institute of Allergy and Infectious Diseases,

Rockville, MD, USA), anti-human tryptase (MoAb AA1) [22], anti-CD1a (OKT6; Ortho Diagnostics, UK), and anti-IgE (Immunotech, Marseilles, France). After overnight incubation, sections were washed three times in TBS and a biotinylated rabbit anti-mouse secondary antibody (Dako Ltd, High Wycombe, UK) was applied for 2 h. After further washes in TBS, bound antibody was demonstrated using a streptavidin-biotin-horseradish peroxidase complex detection system (Dako Ltd). Aminoethylcarbazole was used as a chromogen, resulting in a red reaction product, and sections were counterstained with Mayer's haematoxylin. Control slides were similarly treated but with the primary antibody either omitted or replaced by an unrelated mouse IgG1 MoAb (MOPC21; Sigma Chemical Co., Poole, UK) used at the same concentration as that of MoAb 15.1.

Quantification and image analysis

Light microscopy was performed using a Leitz Ortholux II microscope (Ernst Leitz, Wetzlar, Germany) at a magnification of $\times 63$ and with the aid of an eyepiece graticule. For each biopsy, all positively stained cells were counted in the

entire area of subepithelial tissue, excluding glands and blood vessels, and within the bronchial epithelium. The subepithelial area and the length of epithelial basement membrane were calculated using the Video Interactive Display System with appropriate software (Analytical Measuring Systems, Cambridge, UK). Cell counts were expressed per mm² subepithelial tissue and per mm basement membrane length.

Colocalization of cells in adjacent sections was performed using a computerized system of cell recognition (Biocom 200 Image Analyser, Biocom, Les Ulis, France). For each section, all of the positively stained cells were localized and compared with those in adjacent sections.

Statistical analyses

Data for age and FEV₁ were expressed as mean ± SEM, and for methacholine PC₂₀ as geometric mean [range]. As airway reactivity data and cell numbers were not normally distributed, these measurements were log-transformed and square root-transformed, respectively, prior to statistical analysis. Comparisons between the asthmatic group and the normal subjects were then made using unpaired *t*-tests, and correlations between cell counts and physiological measurements were sought using Pearson's test. For the colocalization studies, percentages of immunoreactive cells were expressed as median [range] and comparison of these data between groups was undertaken using the Mann-Whitney *U*-test. Statistical analysis was performed using StatView 4.02 for Macintosh (Abacus Concepts, Berkeley, CA, USA). A *P*-value was considered significant when <0.05.

Results

There was no significant difference between the asthmatic and control subjects in age (31.8 ± 3.1 vs 24.7 ± 4.5 years, *P* = 0.20) or sex. The asthmatic subjects had significantly lower FEV₁% predicted measurements than the control subjects (86.5 ± 5.7 vs 108.6 ± 2.8 *P* = 0.004). The asthmatic subjects had varying degrees of airway hyperreactivity (0.45 [0.09–4.98] mg/mL) whereas all the control subjects had normal airway reactivity (>32 [14–>32] mg/mL). The bronchoscopy procedure was well-tolerated in all cases.

The numbers of subepithelial cells expressing the four markers in normal and asthmatic airways are shown in Fig. 1. No immunostaining was identified either in the absence of the primary antibody or in the presence of the mouse IgG₁ isotype control. CD1a⁺ DCs were evident both within the bronchial epithelium, where they were mainly visible as a meshwork of dendritic processes, and in the subepithelial tissue. Numbers of DC nuclei at both sites

were significantly higher in the asthmatics compared with the normal subjects (median 7 vs 1 cells/mm², *P* = 0.017, and 3 vs 0 cells/mm, *P* = 0.0087, for subepithelial and intraepithelial DCs, respectively). No significant correlation could be demonstrated between numbers of subepithelial DCs and either FEV₁% predicted or airway reactivity, either when considering the asthmatic and control groups separately or together.

Asthmatic airways also contained more FcεRI-α⁺ cells than normal airways (median 20 vs 3 cells/mm², *P* = 0.053). No significant difference between asthmatic and normal subjects was found in the numbers of AA1⁺ cells (median 42 vs 33 cells/mm², *P* = 0.51) or IgE⁺ cells (median 21.5 vs 14 cells/mm², *P* = 0.49). There was a significant correlation between the numbers of FcεRI-α⁺ cells and IgE⁺ cells (*r* = 0.85, *P* = 0.0015). However, there were no significant correlations between the subjects' physiological parameters and numbers of cells immunoreactive for FcεRI-α, IgE or AA1.

Sections adjacent to those stained with 15.1 were stained with MoAbs against CD1a and tryptase in order to show the relationship between FcεRI-α expression and markers for DCs and mast cells, respectively (Fig. 2). This revealed that 15.1 immunoreactivity was localized to both cell types. The percentage of DCs that were 15.1⁺ was significantly higher in asthmatics than in normal subjects (80 [60–100]% vs 13 [0–50]%, *P* < 0.003). When comparing 15.1⁺ and tryptase⁺ cells in adjacent sections, there was no significant difference between asthmatics and normal subjects. Using a similar analysis to compare sequential sections, we found that in asthmatics 45 [18–62]% of FcεRI-α⁺ cells were also IgE⁺, whereas 50 [29–81]% of IgE⁺ cells expressed the α-chain of FcεRI.

Discussion

The recognition that asthma is characterized by a chronic inflammatory process in the airways [23] has drawn attention to the mechanisms responsible for the initiation and maintenance of this immunological reaction. Dendritic cells are of potential importance in this respect, both in view of their anatomical location [13,14] and their ability to present antigen efficiently to T-lymphocytes [13]. The aims of the present study were to quantify numbers of DCs in the bronchial mucosa in asthmatics and control subjects, and to investigate whether these cells are able to express the IgE-binding α chain of the high-affinity receptor for IgE, FcεRI-α.

In the present study and others [24], the MoAb OKT6, directed against CD1a, has been used as a marker for airway DCs. Other cells known to express this marker are either present in the airways at very low frequency (B-lymphocytes) [11,25] or absent altogether (cortical

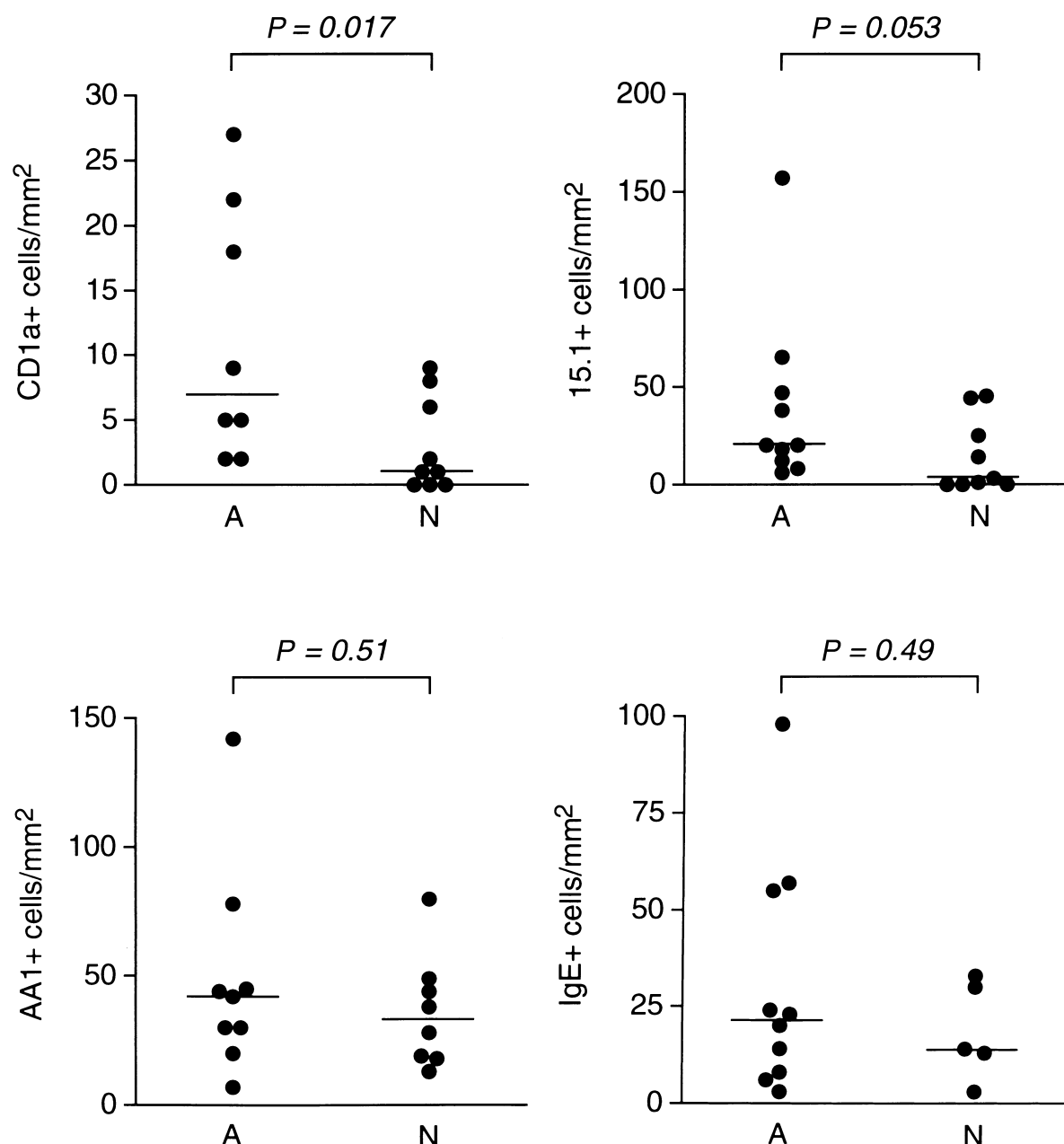


Fig. 1. Numbers of subepithelial cells staining with each of the four MoAbs in the airways of asthmatic (A) and of normal (N) subjects. Horizontal lines represent median values. Data were unavailable for anti-IgE in the case of four of the control subjects, and for anti-CD1a and AA1 in the case of two and one of the asthmatics, respectively.

thymocytes). Using this MoAb, we have found that numbers of both subepithelial and intraepithelial CD1a⁺ DCs are significantly increased in the airways of atopic asthmatics in comparison with non-atopic control subjects. This finding is consistent with other recent reports indicating that DCs are more numerous in the lower airways of atopic asthmatics in comparison with atopic non-asthmatic subjects [17], and in the nasal mucosa of grass pollen sensitive patients with

allergic rhinitis during the grass pollen season compared to non-allergic control subjects [26]. As there is little evidence that DCs are able to proliferate within the airway mucosa, these changes are likely to reflect alterations in their recruitment and/or egress, although the steady-state turnover of airway DCs is rapid with a half-life of 2 days [27]. Little is known about factors governing these processes. In allergic rhinitis, DC numbers in the nasal epithelium

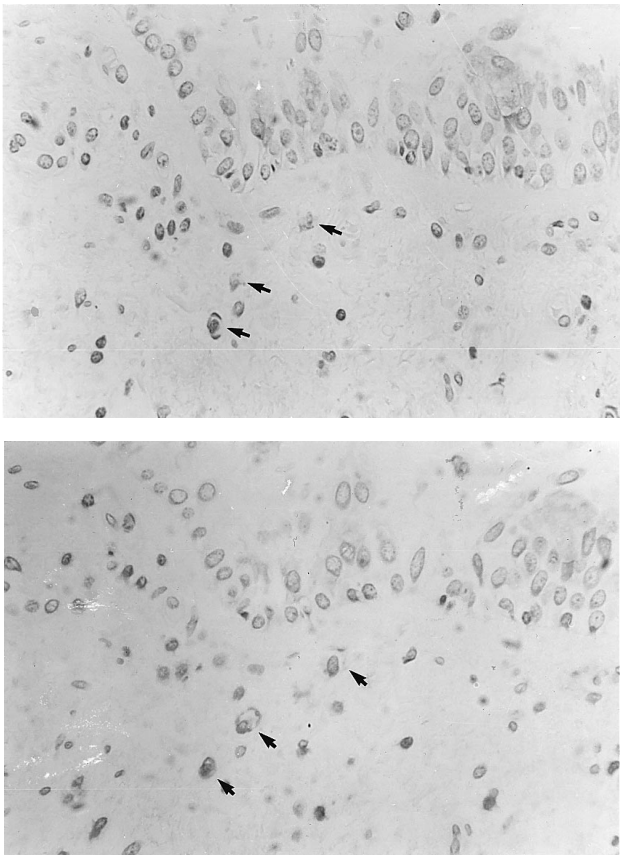


Fig. 2. Immunohistochemical staining with MoAbs OKT6 against CD1a (upper panel) and 15.1 against FcεRI-α (lower panel) in sequential 2 μm sections of a bronchial mucosal biopsy from a patient with allergic asthma. Individual cells are identified (arrows) staining with both antibodies, indicating that dendritic cells express FcεRI-α.

fluctuate with natural seasonal exposure to grass pollen [28], suggesting that the variability in DC numbers in asthmatic airways which we have observed in the present study may result from differences in levels of allergen exposure.

Recent reports that Langerhans cells in human skin express FcεRI [6,7] prompted us to investigate this possibility in the airways by immunohistochemistry using MoAb 15.1 which recognizes the α chain of FcεRI in the absence of bound IgE. By comparison of cells immunoreactive for 15.1 and CD1a in adjacent sections, we have been able to demonstrate for the first time that airway DCs, like their epidermal counterparts, express the FcεRI-α. Moreover the proportion of DCs that were FcεRI-α⁺ was increased in asthma. For technical reasons it has not been possible to demonstrate that the FcεRI detected on airway DCs in this study is functional, i.e. able to bind IgE-allergen complexes and transduce a signal. Providing this is the case, FcεRI expression by DCs may facilitate the capture and

internalization of allergens [10], thus highlighting a potential role for DCs in the induction and maintenance of chronic inflammation in asthma. Indeed it has recently been shown *in vitro* that monocytes present allergen to T-lymphocytes 100–1000-fold more efficiently if allergen uptake has been mediated by FcεRI on these cells [29].

Using MoAb 15.1, we found that the number of sub-epithelial cells bearing the high affinity receptor for IgE was also increased in asthma, although this was borderline with regard to statistical significance ($P = 0.053$). The lack of a more marked increase in the number of FcεRI-α⁺ cells is likely to result from the fact that, in agreement with previous studies [11,12], we were unable to demonstrate an increase in the number of mast cells in asthmatic airways, since these are a major cell type expressing the high affinity receptor for IgE [5]. No relationship could be established between expression of FcεRI and clinical parameters, and this may reflect the fact that MoAb 15.1 recognizes the α chain of FcεRI only in the absence of bound IgE.

Other cell populations may also contribute to the total expression of FcεRI in the airways. Basophils are well-known to express FcεR [5], but previous investigators have found few basophils in either BAL fluid [30] or biopsies [11] in asthma. It has recently been reported that eosinophils from hypereosinophilic subjects express this receptor [31], although this finding has yet to be confirmed in atopy. To establish whether 15.1-immunoreactive cells detected in the present study might include eosinophils, we have analysed adjacent sections immunostained with MoAb EG2 (directed against eosinophil cationic protein) as an eosinophil marker and MoAb 15.1. We were unable to demonstrate expression of FcεRI-α by eosinophils in bronchial tissue. FcεRI expression has also been reported on monocytes from atopic individuals [32]. In the present study it has not been possible to identify unambiguously cells of the monocyte/macrophage lineage since markers such as CD14 [24,33] and CD68 [34] can also be expressed by DCs. Finally, a preliminary report has suggested that bronchial epithelial cells in asthma can express both FcεRI and IgE [35]. In the present study, however, we did not detect immunostaining for either of these molecules localized to bronchial epithelial cells.

In summary, we have shown that numbers of DCs are increased in asthma, that these cells express the α chain of the high affinity IgE receptor, and that the proportion of DCs expressing this receptor is also increased in asthma. Taken together these results support the hypothesis that DCs play an important role in asthma and other forms of allergic inflammation. Expression of FcεRI is likely to increase the ability of DCs to capture allergens, which can then be processed and presented in the context of MHC class II to CD4⁺ T-lymphocytes. Further studies of the role of DCs in asthma will need to focus on the regulation and functional

significance of FcεRI expression, and on the repertoire of cytokines and other costimulatory signals expressed. An understanding of these processes may offer the potential to target DCs using novel therapeutic strategies in asthma.

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