

Expression of the High-affinity Receptor for IgE on Bronchial Epithelial Cells of Asthmatics

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Bronchial epithelial cells are the first cells to come into contact with inhaled pneumoallergens. It has been suggested that these cells may play an important role in the allergic response, and indeed bronchial epithelial cells of some atopic asthmatic subjects have been shown to express the low-affinity receptor for IgE on their surface. In this report we demonstrate, using bronchial biopsies, that bronchial epithelial cells of some asthmatic subjects express both the alpha and gamma chains of the high-affinity receptor for IgE (FcεRI) on their surface and that they are capable of fixing IgE. Second, using reverse transcription-polymerase chain reaction, we show that both control and asthmatic subjects have messenger RNA for FcεRI. Finally, we demonstrate that this receptor may be functional since stimulation of the cells with the antibody to the alpha chain of FcεRI results in the liberation of 15-hydroxyeicosatetraenoic acid from epithelial cells of asthmatic, but not control, subjects or subjects suffering from chronic bronchitis. These data suggest that bronchial epithelial cells from at least some asthmatic subjects express a functional high-affinity receptor for IgE and it is therefore possible that these cells may be able to interact directly with inhaled allergens. **Campbell, A. M., I. Vachier, P. Chanez, A. M. Vignola, B. Lebel, J. Kochan, P. Godard, and J. Bousquet. 1998. Expression of the high-affinity receptor for IgE on bronchial epithelial cells of asthmatics. *Am. J. Respir. Cell Mol. Biol.* 19:92–97.**

Bronchial epithelial cells are likely to play an important role in the pathogenesis of asthma. For many years epithelial cells were considered to have the relatively simple role of a barrier while being involved in the secretion of mucus and the removal of noxious agents by their cilia. More recently these cells have been shown to have a much broader range of activities, including the release of eicosanoids and endopeptidases and degradation of neuropeptides and fibronectin, which is involved in the regeneration of the normal epithelium (1–4). In asthma, epithelial cells are in an activated state, as shown by the increased release of inflammatory mediators (5) and expression of surface markers (6). These cells are likely to participate in the cellular network underlying the airways inflammation, either directly or by the recruitment of other cell types. However, the exact mechanisms by which epithelial cells are activated still remains to be elucidated.

Two types of receptors for the Fc part of IgE have been described. FcεRI has a high affinity for IgE (10^{-9} to 10^{-10} M)

and, although FcεRI was thought to be restricted to basophils and mast cells, it has recently been observed that this receptor is also present on eosinophils from patients with hypereosinophilic syndrome (7) on monocytes from allergic subjects (8) and on Langerhans cells (9). The high-affinity receptor for IgE consists of four chains: 1 α-chain, 1 β-chain, and 2 γ-chains. Of these, it has been demonstrated that only the α-chain is necessary for the binding of IgE, whereas β- and γ-chains are not required (10). A second receptor for IgE, FcεRII, has a lower affinity for IgE (10^{-6} to 10^{-7} M) and is expressed on many cells, including the ciliated epithelial cells of some asthmatic subjects (11), B and T lymphocytes, monocytes and macrophages, platelets, possibly eosinophils, and epidermal Langerhans cells (for review see ref. 12).

In this study we have used immunohistochemical techniques to investigate the possibility that bronchial epithelial cells from asthmatic subjects may be capable of playing a direct role in the allergic reaction by determining the presence of FcεRI and IgE in bronchial biopsies from asthmatic and control subjects.

Materials and Methods

Subjects

Thirty-two asthmatic patients ranging in age from 18 to 56 yr were studied. Asthma was defined according to the cri-

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Abbreviations: 15-hydroxyeicosatetraenoic acid, 15-HETE; reverse transcription-polymerase chain reaction, RT-PCR.

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teria of the American Thoracic Society (13) and all patients had a reversible airways obstruction characterized by an increase of 15% of predicted forced expiratory volume in 1 s (FEV₁) values after inhalation of 200 µg of salbutamol. The clinical severity of asthma was assessed according to the score of Aas (14), a scale which can be used to grade chronic asthma from very mild forms (score of 1) to incapacitating disease requiring permanent medication (score of 5). None of the subjects was a current smoker and none of them had smoked within the previous 2 yr. No subject had had any bronchial or respiratory tract infection during the month preceding the test. Patients were excluded from the study if they had had a severe exacerbation of asthma requiring hospitalization during the month preceding the study; had taken systemic corticosteroids of any form during the previous 2 mo; had inhaled corticosteroids the previous month; had inhaled nedocromil sodium, sodium cromoglycate, or ketotifene the week before; or had inhaled theophylline in the previous 48 h. The use of β 2-agonists was stopped for 6 h prior to bronchoscopy. Allergy was defined by positive skin-prick tests and clinical history.

Twenty-two normal volunteers ranging in age from 17 to 70 yr were enrolled in the study. None had any previous history of lung disease, while four subjects were allergic as assessed by positive skin-prick tests and clinical history. All subjects had normal lung-function test results.

Five patients with chronic bronchitis were also studied, ages 56 to 73 yr.

Immunostaining on Bronchial Biopsies

Biopsies were performed using "alligator" forceps in the sub-segmental bronchi of the left lobe; care was taken to avoid carinae, since metaplasia may be observed on these sites. The biopsies were fixed in Histochoice® (Amresco, Solon, OH) and embedded in paraffin blocks. Sections 2-µm thick were cut and mounted onto slides. The sections were rehydrated in double-distilled water and then permeabilized using methanol/acetone (1:1, vol:vol, -20°C).

The detection system used for the visualization of staining was based on the horseradish peroxidase-labeled streptavidin biotin method (Dako, Glostrup, Denmark). Endogenous peroxidase activity was quenched by incubating the section with 3% hydrogen peroxide for 5 min. The primary antibodies used were a polyclonal antibody against IgE (Dako) or monoclonal antibodies against either the α - (22E7) or γ - (4D8) chains of Fc ϵ RI. Data regarding the specificity of the two latter antibodies are given by Riske and colleagues (15) and Schoneich and associates (16). These two antibodies were used at a final concentration of 10 and 8 µg/ml, respectively. Control slides were prepared without exposure to the primary antiserum or following its substitution with similar dilution of an irrelevant antibody of the same isotype. At least 300 cells were counted using a phase contrast microscope (Zeiss Axioskop, Oberkochen, Germany) by two independent observers who were unaware of the clinical details of the subjects. Staining was considered to be positive when more than 50% of epithelial cells were stained.

To test the specificity of the staining of Fc ϵ RI, in six cases the antibody (22E7 or an anti-cytokeratin mono-

clonal antibody [mAb] [clone MNF116; Dako] [irrelevant staining]) was incubated with the soluble receptor (100 µg/ml) for 15 min prior to performing the staining procedure. This step had the effect of blocking staining with 22E7 but had no effect on staining with the anti-cytokeratin antibody.

Adjacent sections were stained with hematoxylin and eosin for routine pathologic examination.

Stimulation of Cells with 22E7

Cells were obtained by bronchial brushing, carried out as previously described, using fiberoptic bronchoscopy (17). After centrifugation, the cells were washed three times and resuspended in 1 ml of RPMI (GIBCO, Paisley, UK). Macrophages were removed by incubating the cells on plastic plates in a humidified incubator under an atmosphere of 95% air and 5% CO₂ for 1 h at 37°C. The viability of the nonadherent cells was assessed by exclusion of trypan blue stain. The epithelial origin of cells was characterized after cytocentrifugation using May Grunwald Giemsa (MGG) staining and an anti-cytokeratin mAb (MNF116; Dako). The purity of epithelial cells recovered was similar in normal, chronic bronchitis (none of whom suffered from allergies), and asthmatic subjects and was always over 97% of cells recovered. The viability of the epithelial cells was $43 \pm 13\%$ (mean $\pm$ SD) for normal and chronic bronchitis subjects, and $16 \pm 8\%$ in allergic asthmatic subjects. The other cells were mainly macrophages and lymphocytes; no mast cells (as assessed by MGG staining or toluidine blue staining) or eosinophils (as assessed by MGG staining) were detected.

After washing, the cell pellet was resuspended to a concentration of approximately 100,000 viable cells per milliliter in phosphate-buffered saline containing calcium chloride (2.5 mM) and magnesium chloride (0.5 mM), and prewarmed at 37°C for 5 min prior to the addition of 22E7 (1 µg/ml) or calcium ionophore A23187 (2.5 µM) for 45 min. The release reactions were stopped by centrifugation at 4°C. Supernatants were stored at -20°C prior to the assay of mediators. 15-hydroxyeicosatetraenoic acid (HETE) was measured by radioimmunoassay (Amersham, Rainham, UK) and histamine was measured by enzyme immunoassay (Immunotech, Luminy, France).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed on cells obtained by bronchial brushing, as described above, from seven control and seven asthmatic subjects. Positive control experiments were also performed on peripheral blood basophils. Total cellular RNA was obtained by lysing cells in RNAzol. Briefly, the extraction was performed in three steps: homogenization of pellets in RNAzol and chloroform (centrifugation at $12,000 \times g$ for 15 min at 4°C), precipitation with 1 vol isopropanol at 4°C for 15 min (centrifugation at $12,000 \times g$ for 15 min at 4°C), and washing with 70% ethanol solution, followed by drying. The RNA pellet was then solubilized in 20 µl H₂O and stored at -80°C. The quantity of RNA was calculated by OD₂₆₀ spectrophotometry. The integrity of the purified RNA was determined by visualization of the 28S and 18S ribosomal RNA bands after electrophoresis through a 1% agarose-formaldehyde gel.

Total RNA samples were subjected to RT with oligo dT used as a template-primer. First-strand synthesis was performed over 1 h at 37°C in 50 μ l of a reaction mixture using 200 U Moloney murine leukemia virus reverse transcriptase in 1 $\times$ RT buffer, 0.5 mM deoxyadenosine triphosphate, 0.5 mM deoxyguanosine triphosphate, 0.5 mM deoxycytidine triphosphate, 0.5 mM deoxythymidine triphosphate, 10 mM dithiothreitol, 20 U of RNasin ribonuclease inhibitor, and 0.25 μ g of oligo dT. The reaction mixture was heated to 98°C for 5 min in order to inactivate reverse transcriptase.

The sequence of the PCR primer used is as follows: Fc ϵ RI α (536 bp) (sense 5'GGC GTG TTA GCA GTC CCT CAG AAA3', antisense 5'CTC ACG CGG AGC TTT TAT TAC AGT3'); Fc ϵ RI γ (314 bp) (sense 5'GCA GTG GTC TTG CTC TTA CTC C3', antisense 5'GCC AAC CAT GAG GGC TGG AAG AAC C3'); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (171 bp) (sense 5'TCG CCA GCC GAG CCA CAT3', antisense 5'GGA ACA TGT AAA CCA TGT AGT TG3').

PCR assays contained 0.5 U TAQ DNA polymerase,

0.5 μ M of each oligonucleotide primer, 0.2 M, 0.5 or 5 μ l of the RT reaction mixture, 50 mM KCl, 10 mM Tris HCl (pH 9), 0.1% Triton X-100, 5 to 15% of glycerol, and 1.5 mM magnesium chloride. An amplification sequence of 40 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min was used. PCR products were analyzed by electrophoresis in Tris borate EDPA buffer with ethidium bromide-stained 3.5% low-melting agarose gel.

Statistical Analysis

Statistical analyses were performed using the Friedman test with Dunn's post test (a variation of the Bonferroni test); the statistical package used was GraphPad (San Diego, CA).

Results

Fc ϵ -receptor Expression

Positive staining of ciliated epithelial cells (> 50% of cells) with 22E7 was seen in 11 of 21 asthmatic patients and in

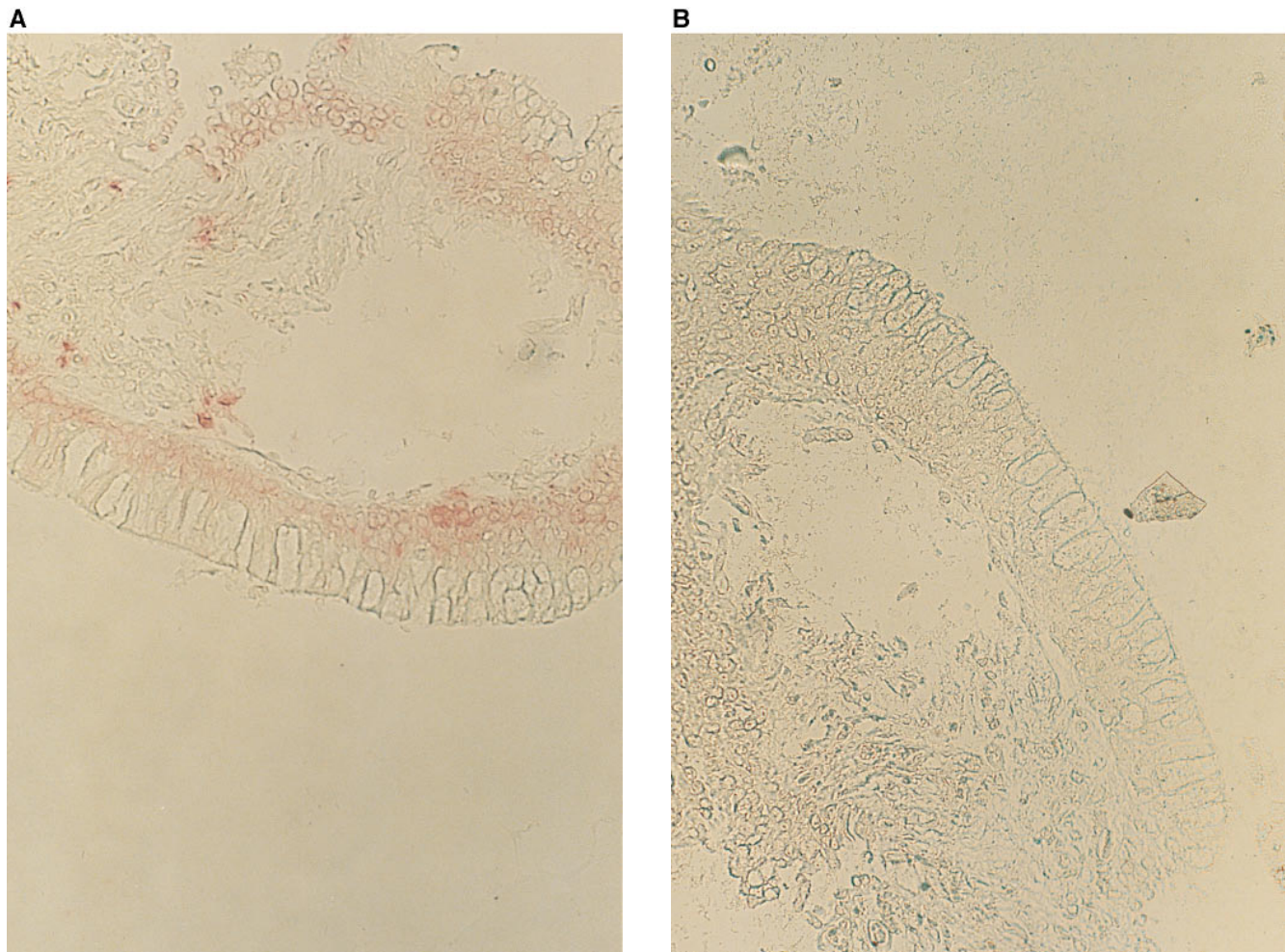


Figure 1. (A) Bronchial biopsy from an atopic asthmatic subject stained using an antibody to the α -chain of Fc ϵ RI. Positive cells (probably mast cells) can be seen in the submucosa. (B) The same biopsy stained with an antibody to the α -chain of Fc ϵ RI following pre-incubation of the antibody with the soluble α -chain of Fc ϵ RI. In both cases, the detection system used for the visualization of staining was based on the horseradish peroxidase-labeled streptavidin biotin method (Dako).

TABLE 1
Immunostaining on bronchial biopsies obtained from allergic and nonallergic asthmatic subjects

Subject	Allergy	22E7 (Anti- α -chain)	4D8 (Anti- γ -chain)	22E7 + Soluble α -chain	Anti-cytokeratin + Soluble α -chain	Anti-IgE
1	Yes	+	+	—	+	+
2	Yes	—	—	ND	ND	
3	Yes	—	—	ND	ND	
4	No	—	—	ND	ND	
5	Yes	—	—	ND	ND	—
6	No	—	—	ND	ND	
7	Yes	—	—	ND	ND	
8	Yes	+	+	—	+	—
9	Yes	+	+	—	+	+
10	No	—	—	ND	ND	—
11	Yes	+	+	—	+	
12	Yes	+	+	ND	ND	+
13	No	—	—	ND	ND	
14	Yes	+	+	—	+	
15	No	—	—	ND	ND	
16	Yes	+	+	ND	ND	
17	Yes	+	+	—	+	
18	Yes	—	—	ND	ND	
19	Yes	+	+	ND	ND	
20	Yes	+	+	ND	ND	+
21	Yes	+	+	ND	ND	

Staining was considered positive when more than 50% of epithelial cells were stained. Allergy was defined by positive skin-prick tests and clinical history. ND = not done.

none of the normal subjects, nonasthmatic allergic subjects, or patients with chronic bronchitis (Figure 1A; Table 1), suggesting that the increased expression is specific to asthma and not simply due to an underlying inflammation in the airways or to allergy. The staining appeared to be

predominantly limited to basal cells with very low staining levels on ciliated cells. All subjects with a positive α -chain immunoreactivity also expressed the γ -chain. In order to test the specificity of the staining, the sections were pre-incubated with the soluble α -chain of Fc ϵ RI; this completely

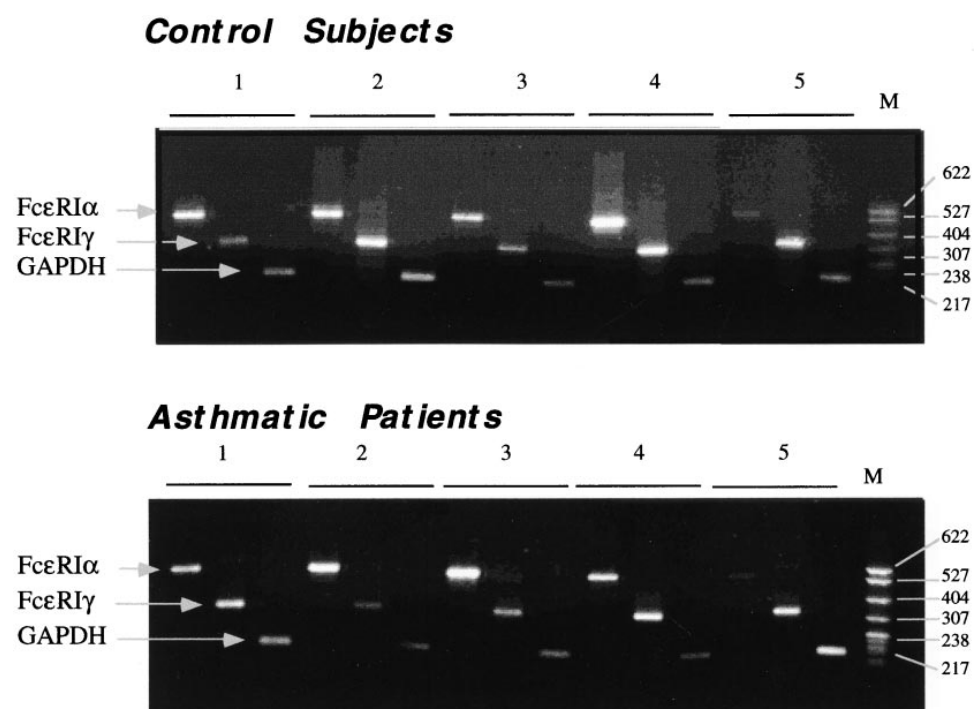


Figure 2. RT-PCR showing that epithelial cells express mRNA for both the α - and γ -chain of Fc ϵ RI and the housekeeping gene GAPDH.

blocked the staining due to the α -chain of Fc ϵ RI (Figure 1B) but did not interfere with the staining due to an anti-cytokeratin antibody (Table 1).

IgE Expression

The expression of IgE using immunocytochemistry on bronchial biopsies was detected in four of seven allergic asthmatic patients (Table 1). IgE was observed only on bronchial epithelial cells, which also expressed the high-affinity receptor. None of the six normal nonallergic subjects, the four nonasthmatic allergic subjects, or the five patients with chronic bronchitis expressed IgE on the surface of the bronchial epithelial cells.

Expression of Messenger RNA (mRNA)

Using PCR we demonstrated that both normal subjects and asthmatic patients express the gene for both the α - and γ -chains of Fc ϵ RI (Figure 2). Although undetectable by staining, it is possible that a very small percentage of metachromatic cells was present. However, in experiments using basophils we found that if the expression was due to contamination by basophils alone the level of contamination would have to be over 25% in order to achieve the level of expression observed in epithelial cells from asthmatic subjects. The presence of mRNA for α - and γ -chains of Fc ϵ RI in cells from normal subjects but the absence of the receptors on the membrane suggests a possible post-transcriptional activation in cells from asthmatics.

Release of 15-HETE and Histamine

Following challenge with 1 μ g/ml 22E7, only asthmatic subjects were able to release 15-HETE (Figure 3) ($P < 0.01$). It also appeared that allergy plays an important role, as nonallergic asthmatic subjects either did not respond to

challenge with 22E7 or responded to a lesser extent than most allergic asthmatics. No normal subjects or patients with chronic bronchitis responded to challenge with 22E7 by the release of 15-HETE, although release was seen following stimulation with calcium ionophore. No detectable release of histamine (detection limit of the assay: 20 pg ml⁻¹) was observed either from unchallenged cells or following challenge with either 22E7 or calcium ionophore. No detectable histamine was found in the lysed cell pellets (average histamine content of basophils or mast cells from the mucosa: 1–2 pg/cell), which again suggests that there were no contaminating metachromatic cells.

Discussion

This study shows that bronchial epithelial cells of asthmatic but not normal subjects express the high-affinity receptor for IgE. Although it has been demonstrated that epithelial cells can express Fc ϵ RII, to our knowledge this is the first time that these cells have been shown to express Fc ϵ RI. However, due to the possible presence of the low-affinity receptor, we cannot confirm the type of receptor to which the IgE is bound.

We used bronchial biopsies to investigate whether epithelial cells were capable of expressing Fc ϵ RI or IgE because this allows us to examine the cells while guarding the structure of the bronchi. It also allows the identification of other cell types within the epithelium or submucosa that may react with the antibodies. In addition, several cuts can be performed from the same section, allowing a direct comparison of techniques. The specificity of any immunohistochemical reaction has to be ascertained. The antibodies against IgE and Fc ϵ RI are highly specific, and pre-incubation of the antibody to Fc ϵ RI with the soluble receptor blocked staining. Moreover, only epithelial cells and mast

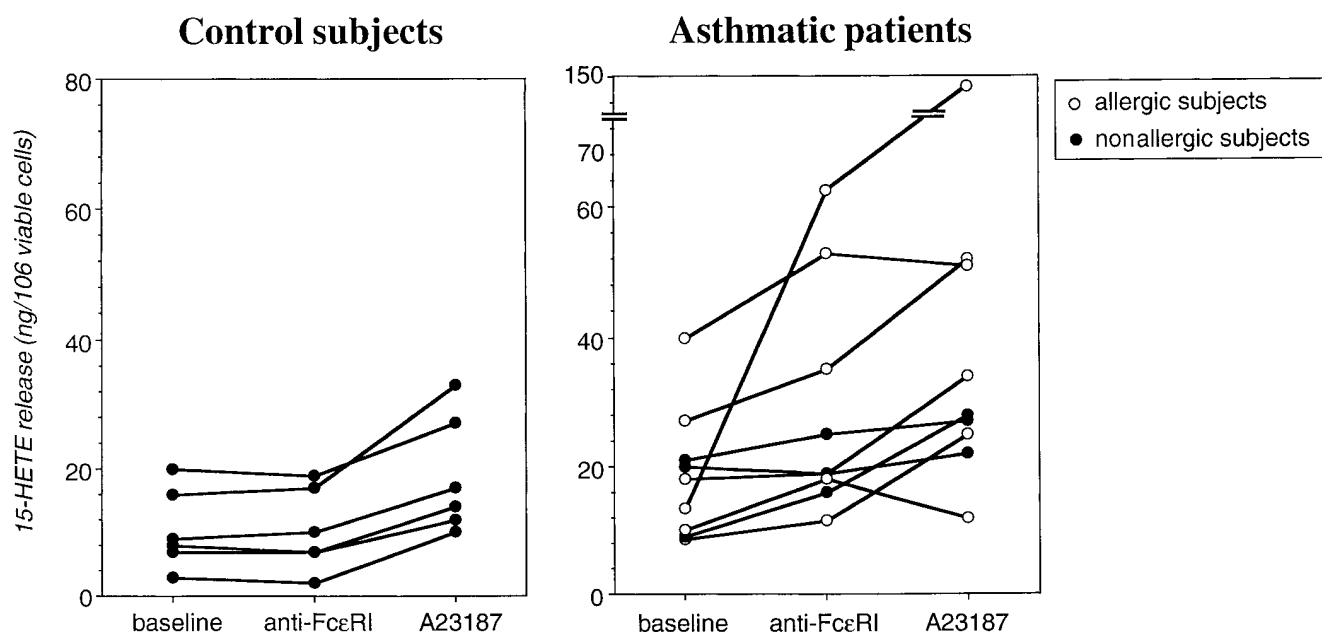


Figure 3. Release of 15-HETE from bronchial epithelial cells obtained by brushing following challenge with either calcium ionophore A23187 (2.5 μ M) or anti- α -chain of Fc ϵ RI (1 μ g/ml) for 45 min in allergic and nonallergic (controls and chronic bronchitis) subjects.

cells were positive for FcεRI, whereas cells comprising the submucosa were negative. This reaction was further confirmed by the presence of immunostaining for IgE on the same cells. Interestingly, IgE was not detected on macrophages in the biopsies; this may be due to the facts that macrophages express only the low-affinity receptor for IgE and that the bound IgE is easily shed from the receptor and is therefore not found.

We have previously demonstrated that 15-HETE is the major eicosanoid released from bronchial epithelial cells following challenge with calcium ionophore (5). If bronchial epithelial cells express the high-affinity receptor for IgE, then it should be possible to activate them using an IgE-dependent stimulus. However, we have previously demonstrated the presence of the low-affinity receptor for IgE on the bronchial epithelial cells of some asthmatics and therefore stimulation using anti-IgE or allergen would not be sufficient to distinguish whether the cells were being activated via their low- or high-affinity receptors. For this reason we used the anti-receptor antibody that has been shown to be suitable in the activation of human basophils (18). In addition, the use of anti-IgE could activate other cell types that express the low-affinity receptor for IgE. Only bronchial epithelial cells from allergic asthmatic patients responded well to stimulation with the anti-receptor antibody. Although other cell types present in the lung are capable of the generation of 15-HETE, it appears unlikely that the 15-HETE measured in these experiments is derived from macrophages because in our studies there is no expression of the high-affinity receptor for IgE on these cells. FcεRI has recently been shown to exist on cell types other than mast cells or basophils, including eosinophils and monocytes. However, it is unlikely that the 15-HETE measured in the supernatants is derived from these cell types since (1) no mast cells or eosinophils were detected in the cell preparations, (2) there was no release of histamine, and (3) the purity of the epithelial cells was > 97%.

This study indicates that FcεRI and IgE can be detected on epithelial cells but only in asthmatic subjects. Thus, it appears that cells from asthmatics but not from control subjects can be induced to express FcεRI. The mediators that can induce FcεRI are still unclear; however, cytokines such as tumor growth factor-beta can potentiate the expression of this receptor at least on monocytic cell lines (19).

This study suggests that bronchial epithelial cells may be triggered by an IgE-anti-IgE mechanism that may be of importance in allergic asthma. Thus, in addition to indirect activation by cytokines and inflammatory mediators such as histamine, it seems that bronchial epithelial cells represent a key cell type in bronchial inflammation.

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