

Increased Expression of High Affinity IgE (FcεRI) Receptor-α Chain mRNA and Protein-bearing Eosinophils in Human Allergen-induced Atopic Asthma

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FcεRI receptors play an important role in allergen-induced mediator release and antigen presentation by mast cells, basophils, and monocyte/macrophages in atopic disorders. The expression of FcεRI by tissue eosinophils in atopic asthma after allergen challenge has not been established. For this reason we attempted to identify mRNA and protein product + FcεRIα eosinophils in cytopins made from bronchoalveolar lavage (BAL) from atopic asthmatics (n = 9) and nonatopic normal subjects (n = 4) 24 h after segmental challenge with allergen or diluent. Messenger RNA for FcεRIα was determined using *in situ* hybridization and FcεRIα protein expression by immunocytochemistry using a mouse monoclonal antibody 22E7. Colocalization of FcεRIα receptors to eosinophils was performed using chromotrope 2R. When compared with a control challenge, segmental challenge with *Dermatophagoides pteronyssinus* induced significant BAL eosinophilia (p = 0.007). The total number of BAL FcεRIα mRNA and protein-positive cells also increased in asthmatics, median values 2 (0.7–7.2) and 11.5 (0.6–65.0) × 10⁶ cells (p = 0.02) and 0 (0–0.3 × 10⁶) and 3.1 × 10⁶ (0.45 – 162.5 × 10⁶) cells (p = 0.007), respectively, for mRNA and protein. Net increases in FcεRIα+ cells correlated with the net increases in BAL eosinophils (r = 0.98, p = 0.0001 for mRNA and r = 0.72, p = 0.02 for protein). Colocalization studies with chromotrope 2R revealed that only 4% of FcεRIα+ cells were eosinophils after control challenge and, in contrast, 85 to 95% of FcεRIα+ cells were eosinophils after allergen. There were no differences in the numbers of FcεRIα+ cells or eosinophils in normal control subjects. Our results demonstrated that local endobronchial allergen provocation in atopic asthmatics results in increased synthesis and expression of FcεRIα predominantly on BAL eosinophils. Rajakulasingam K, Till S, Ying S, Humbert M, Barkans J, Sullivan M, Meng Q, Corrigan CJ, Bungre J, Grant JA, Kay AB, Durham SR. Increased expression of high affinity IgE (FcεRI) receptor-α chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma.

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Asthma is characterized by mucosal inflammation of the airways and is closely related to atopy, which is characterized by enhanced IgE responses to common environmental allergens. Epidemiologic studies link total serum IgE concentrations with the incidence of asthma symptoms and bronchial hyper-responsiveness (1, 2). An important characteristic of IgE is its ability to bind to mast cells and basophils with high affinity through its Fc portion to the IgE receptor (FcεRI) (3, 4). FcεRI comprises an α chain, a β chain, and two disulphide-linked γ chains. Alpha chain is involved in IgE binding and re-

ceptor internalization, resulting in the allergic mediator release process, whereas β chain plays a role in signal transduction (3, 4). Cross-linking of the FcεRI, even in the absence of IgE, results in mast-cell triggering (5). In sensitized persons, the interaction of FcεRI-bound IgE with the relevant antigen elicits an immediate reaction characterized by mast-cell degranulation and release of preformed mediators and cytokines.

Apart from mediating the immediate response, the allergen-induced late phase skin reaction (LPR) has also been shown to be IgE-dependent (6, 7). Biopsies of allergen-induced cutaneous late phase reactions have demonstrated IgE-bound antigen-presenting cells such as epidermal Langerhans' cells and dermal dendritic cells (8, 9). More recently, it has been demonstrated that Langerhans' cells (10, 11), dermal dendritic cells (10, 12), peripheral blood monocytes (13), and eosinophils (14–16) may all express the high affinity receptor for IgE (FcεRI). FcεRI, therefore, is being considered as a critical component of the effector arm of the allergic response in me-

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diating both early and late phase responses. We have recently demonstrated elevated numbers of FcεRIα+ cells in the bronchial submucosa of patients with stable, mild asthma compared with control subjects (17). Colocalization studies revealed that the majority of FcεRIα+ cells were identified as mast cells and macrophages and a much smaller percentage were eosinophils. However, a variable proportion (0 to 40%) of these eosinophils were FcεRIα+. Because of the relatively small numbers of eosinophils in this baseline study, we felt it important to reevaluate expression of FcεRIα by bronchial eosinophils after allergen challenge.

METHODS

Patients

Nine patients (four male and five female) with a history of house-dust-mite-sensitive asthma and four nonatopic, normal subjects (three male and one female) took part in the study (Table 1). Subjects were recruited from the Allergy Clinic and from the general public after an advertisement in the local newspaper. All subjects were nonsmokers. Asthmatic subjects were chosen on the basis of (1) a clear history of asthma symptoms, (2) histamine PC₂₀ value of < 16.0 mg/ml in the previous 2 wk, (3) a strongly positive skin wheal response (> 5 mm) to prick testing with *Dermatophagoides pteronyssinus* extract (Soluprick; ALK, Horsholm, Denmark), and (4) positive RAST test for the same house dust mite extract (Table 1). Normal volunteers were asymptomatic, had negative skin responses to a panel of 11 common aeroallergens, including house dust mite, animal danders, moulds, and grass pollen (Soluprick; ALK), and negative RAST tests for the same panel of allergen extracts. None of the subjects was receiving oral steroids or theophylline preparations during the previous 6 mo, and inhaled steroids were withheld for 72 h prior to the study. None of the subjects had a history of lower respiratory tract infection in the preceding 4 wk or of any current medical illness other than atopic asthma. Subjects with FEV₁ of less than 80% predicted and histamine PC₂₀ < 1.0 mg/ml were excluded. None of the patients had received immunotherapy in the previous 5 yr. The study was approved by the Ethics Committee of the Royal Brompton National Heart and Lung Hospital, London, and informed written consent was obtained from each subject.

Study Design

Subjects were asked to attend the laboratory to undergo two fiberoptic bronchoscopies separated by 24 h. Fiberoptic bronchoscopy was

undertaken as previously described (17). In brief, all subjects received nebulized albuterol (2.5 mg) and intravenous atropine (0.6 mg) and midazolam (0 to 10 mg) 10 min prior to the procedure. Oxygen was delivered via the nasal canulae throughout the procedure, and oxygen saturation was monitored by pulse oximetry. Local anaesthesia of the upper airways was induced with 2 to 4% lidocaine. The bronchoscope (BFP20; Olympus Corp., Lake Success, NY) was then passed through the nares or mouth, and as much as 10 ml of lidocaine were introduced through the bronchoscope to anaesthetize the vocal cords and lower airways. After inspection of the bronchial tree, the bronchoscope was wedged in the lateral subsegment of the right middle lobe to undertake a control challenge with 5 ml of sterile saline solution prewarmed to 37° C. The bronchoscope was then passed into the lateral subsegment of lingula of the left lung, and 5 ml of prewarmed saline containing 100 BU of allergen (*Der p*) were instilled. Allergen and control challenge sites were randomly chosen for individual subjects. The appearance of the airways was then observed for 5 min. If there was no visible reaction to 100 BU of allergen, a further 5 ml of saline containing 400 BU of allergen were then instilled into the allergen challenge site and was observed for 5 min to confirm an airway response (18). The subjects were then observed closely, and a second bronchoscopy was performed with the same premedications and oxygenation at 24 h after segmental allergen challenge. After inspection of the bronchial tree, BAL was performed using 120 ml of prewarmed 0.9% saline solution at each challenge site. All subjects were then observed closely for 2 h.

Processing of Bronchoalveolar Lavage Fluid

BAL fluid was passed through two layers of sterile gauze to remove debris and then centrifuged at 500 × *g* for 10 min at 4° C. The pellet was then resuspended in RPMI 1640 (Chester Beatty Laboratories, London, UK) supplemented with 2 U/ml sodium heparin. The fluid was then centrifuged again at 500 × *g* for 10 min. The cells were then resuspended at 0.25 to 0.5 × 10⁶ cells/ml in RPMI-1640 medium (GIBCO, Paisley, UK) supplemented with 100 IU/ml penicillin/streptomycin (GIBCO), 2 mM L-glutamine (GIBCO), and 5% human AB serum (Sigma, Poole, Dorset, UK); 100 μl of cells were used per poly-L-lysine-coated slide and mounted on Shandon 190005 filter cards and then centrifuged in a Shandon 2 machine for 5 min at 500 rpm (Shandon Ltd, Runcorn, Cheshire, UK). Slides were then air-dried and fixed in 4% paraformaldehyde (PFA) for 30 min at room temperature, and this procedure was repeated again. Cytospins were then immersed in 15% sucrose for 30 min and then rinsed in PBS to remove sucrose. The cytopins were then dried overnight at 37° C incubator. The slides were stored at -80° C pending analysis.

TABLE 1
DEMOGRAPHIC DETAILS

Subject No.	Sex	Age (yr)	Medications	Skin Prick Tests	RAST to HDM (IU/ml)	PC ₂₀ Histamine (mg/ml)
Asthmatics						
1	M	26	S, Bec	HDM, G, C, D	12.5	3.66
2	M	29	S	HDM, G, C	13.1	4.24
3	F	27	S	HDM, G, C	49.8	1.69
4	F	36	S, Bec	HDM, G, T	9.4	3.61
5	F	34	S, Bec	HDM, G, C, D	> 100	1.3
6	M	25	S, Bec	HDM, G	47.3	2.02
7	F	21	S	HDM, C	83.3	14.0
8	F	25	S, Bec	HDM, G, C	2.3	1.9
9	F	23	S, Bec	HDM, G, C	29	1.43
Mean		27.3			38.5	2.02
Normal subjects						
1	M	22		Neg	Neg	> 32
2	F	21		Neg	Neg	> 32
3	M	21		Neg	Neg	> 32
4	M	19		Neg	Neg	> 32
Mean		20.8				> 32

Definition of abbreviations: S = salbutamol; Bec = beclomethasone; HDM = house dust mite; G = grass; C = cat; D = dog; T = tree.

Differential Cell Count

A differential cell count was performed on unfixed cytopspins of BAL cells using May-Grunwald Giemsa stain.

In Situ Hybridization

The cDNA fragment encoding FcεRI α (bp 25–936) was kindly provided by Dr. J.-P. Kinet (Molecular Allergy and Immunology Section, National Institute of Allergy and Infectious Diseases, Rockville, MD) (19). This cDNA segment was inserted into the RNA expression vector (pGEM7) (Promega, Southampton, UK) and linearized to produce antisense and sense riboprobes. ³⁵S-labeled riboprobes were prepared with Sp6 or T7RNA polymerase (Promega) to generate antisense or sense probes, respectively. *In situ* hybridization (ISH) of cytopspins prepared from BAL cells were performed according to a protocol that has been validated previously (16), with some modifications. In brief, cytopspins were made permeable by immersion in 0.3% Triton × 100 in PBS for 10 min. After a brief washing in PBS, slides were further made permeable by exposure to proteinase K (Promega) solution (1 mg/ml in 20 mM TRIS-HCl and 1 mM EDTA at pH 7.2) for 20 min at 37° C, the activity of which was then terminated by immersion in 4% paraformaldehyde/PBS for 5 min. To inhibit nonspecific binding of ³⁵S-labeled probes, slides were treated with 10 mM iodoacetamide and

N-ethyl-maleimide (Sigma) for 30 min at 37° C and then treated with 0.5% acetic anhydride and 0.1 M triethanolamine for 10 min before air-drying. Slides were hybridized with ³⁵S-labeled riboprobes (either antisense or sense at 10⁶ cpm per slide) at 55° C overnight. After hybridization, the slides were washed under high-stringency conditions (65° C, 0.1 SSC). After dehydration, slides were immersed in K-5 emulsion (Ilford Ltd, Ilford, Essex, UK) and exposed for 2 weeks at 4° C. The autoradiographs were developed in developing solution (D-19; Eastman, Kodak Co., Rochester, NY), fixed with Hypam (Ilford), counterstained with hematoxylin and chromotrope 2R (BDH, Poole, UK) for 30 min. For negative controls, slides were hybridized with a sense probe or pretreated with RNase (Sigma) prior to hybridization with the antisense probe.

Immunocytochemistry

A noncompetitive murine monoclonal antibody 22E7 (a kind gift from Drs. R. Chizzonite and J. P. Kochan, Hoffman La Roche Inc., Nutley, NJ; concentration, 10 μg/ml) directed against the α chain of FcεRI was used for immunocytochemistry using the APAAP method (17). As a negative control, the primary antibody was replaced with nonspecific mouse IgG.

Cells on cytopsin slides were first treated with 1% hydrogen peroxide and 0.02% sodium azide in PBS to block endogenous peroxi-

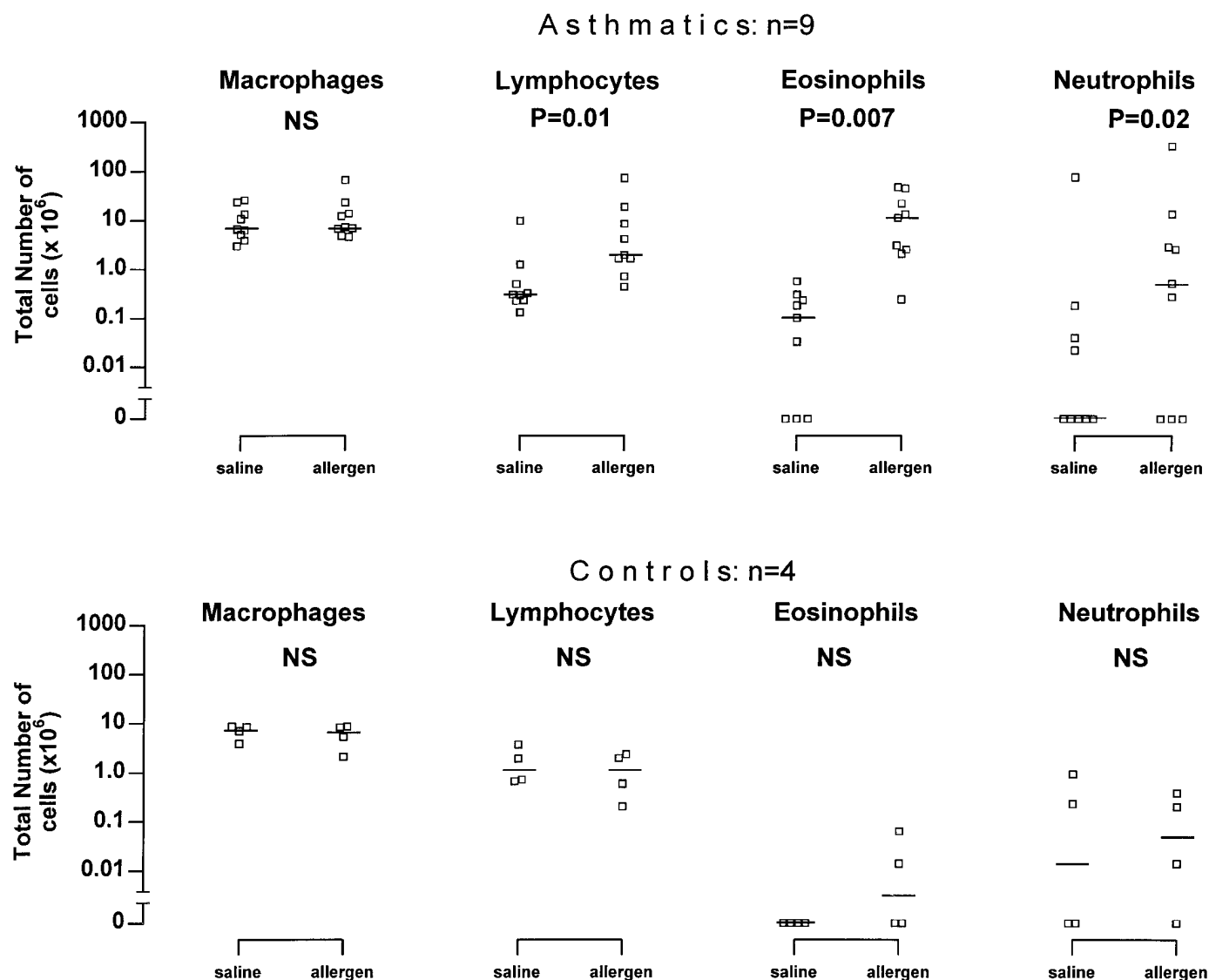


Figure 1. Composition of cells in bronchoalveolar lavage in atopic asthmatic (n = 9) and normal (n = 4) subjects after saline and allergen challenge. Between groups, the net increases in cell counts (allergen minus saline) are compared. Horizontal bars represent median values.

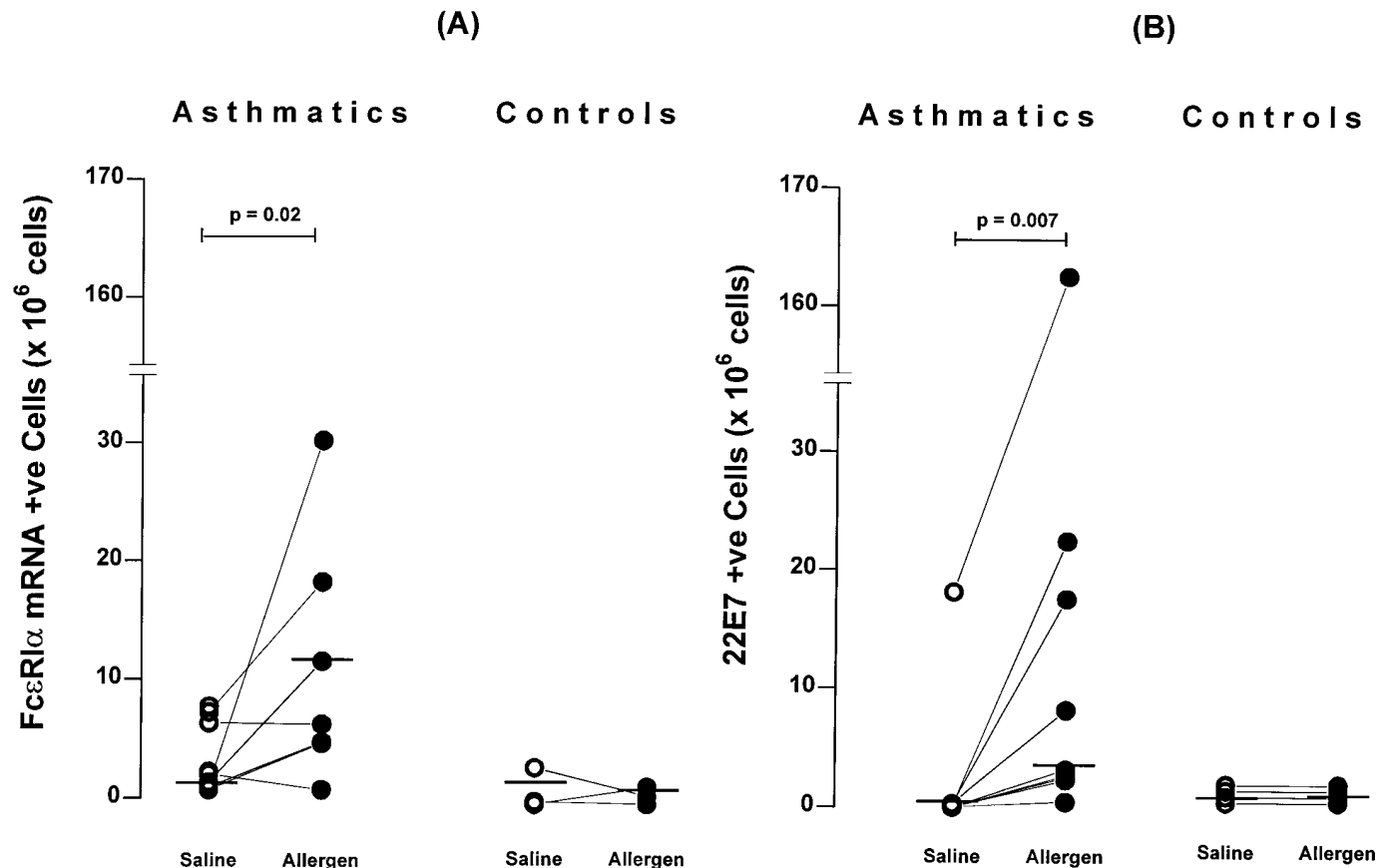


Figure 2. Expression of FcεRIα (A) mRNA and (B) protein in BAL cells in atopic asthmatic (n = 9) and normal (n = 4) subjects after saline (open circles) and allergen (closed circles) challenge. Horizontal bars represent median values.

dase. Immunocytochemistry was then performed using a vector kit (Vector, Peterborough, UK) consisting of rabbit serum, noncompetitive murine monoclonal antibody 22E7 (10 µg/ml), biotinylated rabbit antimouse antibody, and avidin biotin complex. The reaction was developed in SG substrate (Vector). 22E7+ cells stained grey/black. Slides were then washed in water after immunocytochemistry and counterstained with chromotrope 2R (BDH) for 45 min. The slides were then dehydrated and mounted in DPX.

TABLE 2
PERCENTAGE OF FcεRIα mRNA+ CELLS COLOCALIZED TO CHROMOTROPE 2R+ CELLS AFTER SALINE (CONTROL) AND SEGMENTAL ALLERGEN CHALLENGE

Subject No.	Control	Allergen
Asthmatics		
1	0	58
2	4	96
3	5	83
4	4	89
5	29	85
6	22	89
7	13	86
8	2	80
9	0	68
Median	4	85
Normal subjects		
1	11	0
2	0	0
3	0	0
Median	0	0

Quantification

Slides were counted blind in a random coded fashion using a BH2 microscope (Olympus Corp.) fitted with an eyepiece graticule. For *in situ* hybridization studies, hybrids between FcεRIα chain mRNA and cRNA probes were localized as dense collections of silver grains in photographic emulsion overlying individual cells. Cells expressing FcεRIα chain-specific mRNA were quantified in terms of the number of cells with overlying silver grains. For each subject (control and allergen challenged), at least 200 total BAL cells were counted. Chromotrope 2R+ cells (red color) were considered as eosinophils. For immunocytochemistry, counts were performed at magnification ×200 and cells double-stained with FcεRIα+/chromotrope 2R+ were confirmed at magnification ×1,000 under oil immersion. For both techniques, random fields on slides were counted. Within-observer mean coefficient of variation for cell counts was always less than 5%.

Statistical Analysis

Data were analyzed using a Microsoft Excel statistical package. Wilcoxon's matched-pairs signed-rank test was used for within-group comparisons. Between groups, the net increases in cell counts (allergen minus control) were compared using the Mann-Whitney U test. Correlation coefficients were obtained using Spearman's rank analysis; p values < 0.05 were considered significant.

RESULTS

Clinical

Relevant details of the patients and normal volunteers studied are shown in Table 1. The two groups were well matched for age and sex. All asthmatics had circulating *Der p* specific IgE, as demonstrated by positive RAST tests (mean, 38.5 IU/ml), whereas this was not detectable in the nonatopic control sub-

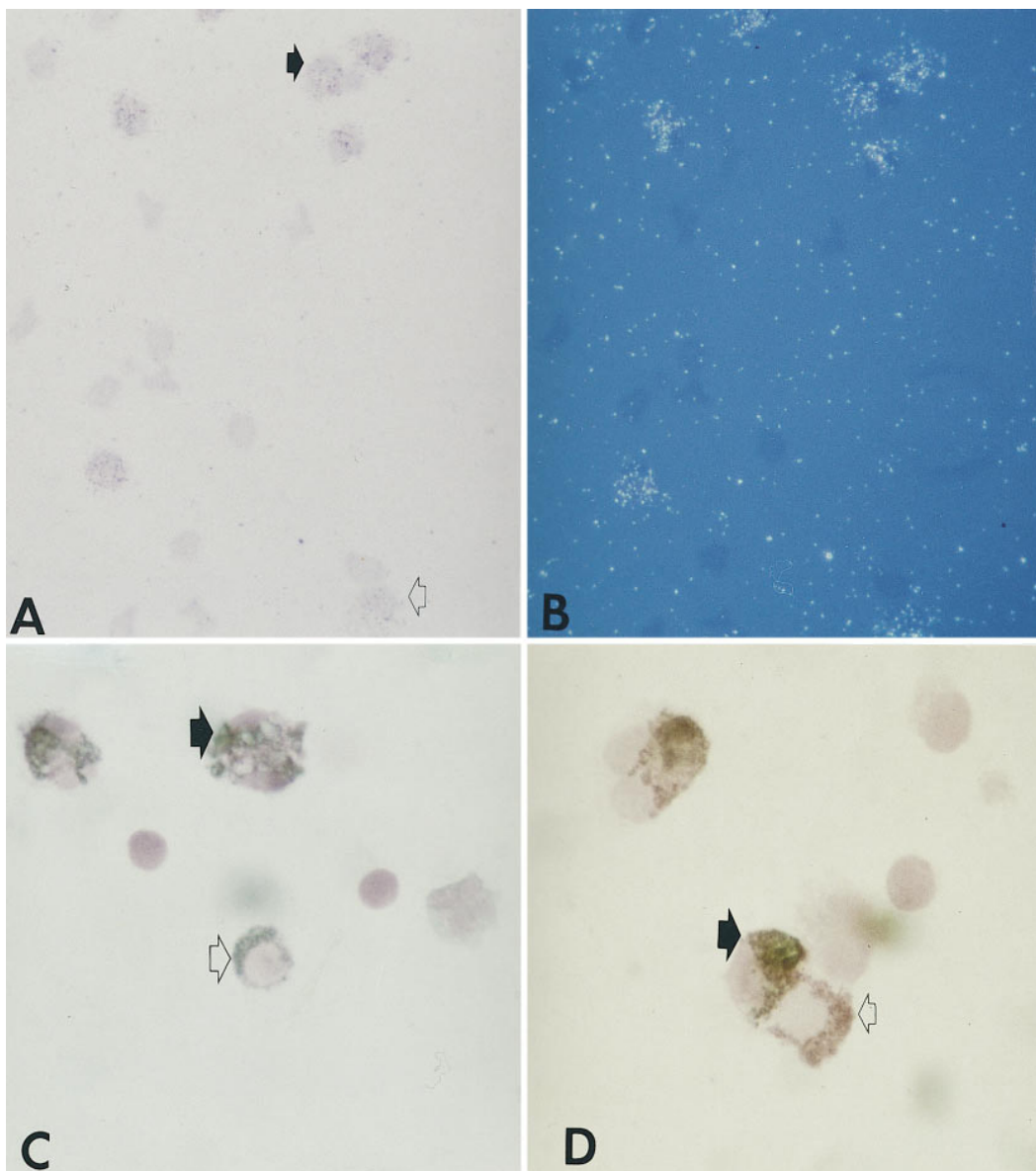


Figure 3. BAL cytospin from an atopic subject 24 h after segmental allergen challenge. A and B: Hybridization *in situ* with FcεRIα chain antisense probe and counterstained with chromotrope 2R (solid arrow) and single mRNA+ cells (open arrowhead) (A: bright field; B: dark field). C and D: Cytospins immunostained with 22E7 and counterstained with chromotrope 2R. Double-stained cells are indicated by solid arrowhead; single-stained cells against FcεRI (C) and eosinophils (D) are indicated by open arrowhead.

jects (Table 1). The asthmatics also demonstrated airway hyperresponsiveness to inhaled histamine; mean value being 2.02 mg/ml ($p < 0.05$). Seven of the nine asthmatic subjects reacted to the instillation of *Der p* allergen by visible constriction of the challenged bronchus. This was accompanied by local wheezing determined by auscultation. At 24 h, slight mucosal swelling was visible in some of these patients, but the airway caliber appeared normal. No change in airway caliber was visible after saline control challenge. On the other hand, nonatopic normal subjects demonstrated no visible changes in airway caliber or hyperemia after both allergen and saline challenges.

BAL Differential Cell Counts

The absolute numbers of macrophages, lymphocytes, neutrophils, and eosinophils in lavaged bronchial segments after con-

trol saline and allergen challenge of atopic asthmatics and normal control subjects are shown in Figure 1. After segmental challenge with *Der p*, there was a significant increase in the absolute numbers of BAL eosinophils, median values of 0.15×10^6 (0 to 0.55×10^6) and 11.1×10^6 (0.5 to 46.5×10^6) cells ($p = 0.007$) for control and allergen, respectively (Figure 1). BAL lymphocytes and neutrophils also showed significant increases after allergen (Figure 1). On the other hand, no significant changes in BAL macrophages was observed after allergen provocation (Figure 1).

BAL FcεRIα+ Cell Count

When compared with a control (diluent) challenge, *in situ* hybridization studies demonstrated a 6-fold increase in FcεRIα mRNA+ cells after allergen; median values were 2 (0.7 to 7.2) and $11.5 (0.6 \text{ to } 65.0) \times 10^6$ cells for control and allergen, re-

TABLE 3
PERCENTAGE OF CELLS COLOCALIZING FcεRIα PROTEIN
(22E7) AND CHROMOTROPE 2R AFTER SEGMENTAL
ALLERGEN CHALLENGE

Asthmatics (No.)	FcεRIα+ Cells Coexpressing Chromotrope 2R (%)
1	96
2	95
3	97
4	93
5	96
6	79
Median	95

spectively (Figure 2A). Net increases in FcεRIα mRNA+ cells correlated with the net increases in BAL eosinophils ($r = 0.98$, $p = 0.0001$). Similarly, the total numbers of FcεRIα chain protein-bearing cells also increased after allergen; median values were 0 (0 to 0.30×10^6) and 3.1×10^6 (0.45 to 162.5×10^6) cells ($p = 0.007$) (Figure 2B). Net increases in FcεRIα+ cells correlated with the net increases in BAL eosinophils ($r = 0.72$, $p = 0.02$). There was no significant change in FcεRIα+ cells in BAL after allergen in normal control subjects (Figure 2).

Colocalization of FcεRIα mRNA and Protein to BAL Eosinophils

In situ hybridization studies in nine asthmatics demonstrated that only 4% of FcεRIα mRNA+ cells were colocalized to chromotrope 2R+ eosinophils after saline (control) challenge (Table 2). On the other hand, 85% of FcεRIα mRNA+ cells colocalized to eosinophils after segmental allergen challenge (Table 2 and Figure 3A and B). The eosinophil numbers were very low in BAL after control challenge (1 to 2 per whole fields counted), and 75% of them expressed FcεRIα chain mRNA. Segmental allergen challenge resulted in significant BAL eosinophilia (Figure 1), and 80% of these eosinophils showed positive hybridization signals for FcεRIα mRNA.

In order to confirm colocalization of FcεRIα chain protein to eosinophils, colocalization studies with 22E7 and chromotrope 2R were performed in six asthmatic subjects who had appreciable numbers of 22E7+ cells by single staining. These studies revealed that 95% (93 to 96%) of FcεRIα+ cells stained with chromotrope 2R (Table 3 and Figure 3C and D). We also examined the percentage of chromotrope 2R+ cells in BAL that were immunostained with 22E7; 91% (26 to 100%) of 2R+ cells coexpressed the FcεRIα subunit. There were no changes in FcεRIα+ cells, eosinophils, or other cell populations in normal control subjects after allergen challenge (Figures 1 and 2).

DISCUSSION

We have demonstrated increased numbers of FcεRIα mRNA and protein-bearing cells in BAL of subjects with atopic asthma after segmental allergen challenge. We confirmed that the predominant FcεRIα+ cells (85 and 93%) in BAL after allergen exposure were eosinophils and 80 to 91% of the eosinophils expressed both mRNA and protein for FcεRIα+. The fact that similar changes were not observed in the nonatopic control subjects suggests that these changes are dependent upon the presence of allergen-specific IgE and not upon some nonspecific effect of locally instilled allergen.

Allergen-induced increases in BAL FcεRIα+ cells were observed in the asthmatics within the time frame of the late

phase bronchoconstrictor response to global allergen bronchial challenge, which is well known to be associated with a dose-dependent influx of inflammatory cells, particularly eosinophils, lymphocytes, and neutrophils, into the bronchial lumen 18 to 24 h after allergen exposure (18, 20, 21). The advantage of the segmental challenge model is that it allows simultaneous challenge with allergen and diluent in remote bronchial segments, thus ensuring that each subject acts as his or her own control. The disadvantage is that global bronchoconstrictor responses cannot be measured. Nevertheless, seven of the nine atopic subjects in our study clearly demonstrated an immediate segmental bronchial response 5 to 10 min after allergen challenge, and this observation coupled with the characteristic inflammatory cell influx 24 h later provide compelling evidence that the observed allergen-induced increases in FcεRIα+ cells in the asthmatics occurred within the context of a late phase inflammatory response to allergen challenge.

In this study we took mRNA and protein expression of the α chain of the high affinity receptor for IgE (FcεRI) as an indicator of their potential to be activated by IgE-mediated mechanisms. Because cytopins were used in this study some immunostaining may reflect intracellular rather than membrane staining. The biologic importance of FcεRIα in mediating IgE-dependent immune reactions has been demonstrated in a number of different studies (5, 14, 22–27). It has been speculated that eosinophils may also be involved in antigen presentation in allergic diseases via IgE linked to FcεRI (28). FcεRIα has also been implicated in IgE-dependent schistosomal killing by eosinophils (14).

Our data show that a median of 80 to 91% of eosinophils recruited to the bronchial lumen of atopic asthmatics 24 h after local allergen challenge expressed the α chain of FcεRI, although there was some variability in this figure. Furthermore, eosinophils comprised a median of 85 to 95% of the total FcεRIα+ cells so recruited (Tables 2 and 3). Our results are consistent with those observed by Barata and colleagues (16) in the skin after allergen challenge. These findings contrast strikingly with the distribution of FcεRIα chain expression on cells within the bronchial mucosa and peripheral blood of stable, atopic asthmatics at “baseline” (i.e., in the absence of allergen challenge). We found some FcεRIα mRNA expression after control challenge in both asthmatics and normal subjects, but no significant protein expression was seen (Figure 2). This dichotomy might be due to the difference in sensitivity of the two methods employed. However, only a few eosinophils were present after control challenge expressing FcεRIα mRNA, and only 4% of FcεRIα mRNA+ cells were eosinophils at “baseline” (control challenge). Consistent with this, three recent studies on such patients have suggested that, in both the bronchial mucosa and the peripheral blood, mast cells/basophils and monocytes/macrophages constituted the majority of FcεRIα+ cells, whereas eosinophils made up only a small percentage (17, 29, 30). The remainder of the FcεRIα+ cells recovered from the bronchial lumen of the atopic asthmatics after allergen challenge in the present study were presumably other cells (monocyte/macrophages, mast cells/basophils and dendritic cells) known to express FcεRIα constitutively (12, 13, 17, 29, 30). Our data do not permit accurate quantification of the distribution of FcεRIα+ expression on these cells. Because the numbers of FcεRIα+ cells and eosinophils in the bronchial lumen of the atopic asthmatics after control challenge were in general very low, our data considered with the data referred to the above concerning FcεRIα expression in the blood and bronchial mucosa of these patients suggest a process whereby eosinophils expressing both mRNA and protein for FcεRIα are preferentially recruited to the bronchial

lumen of these patients. The mechanism of this process remains to be defined, but it might include synthesis and upregulation of FcεRIα expression on eosinophils in transit from the peripheral blood to the bronchial lumen after interaction with cytokines such as IL-5, IL-3, or GM-CSF (31), selective recruitment of FcεRIα-expressing eosinophils from the blood or bronchial mucosa, or both. Again, this process would appear to be IgE-dependent since it was not observed in the non-atopic control subjects in the present study after allergen challenge under identical conditions.

There are early indications that FcεRI expression on eosinophils may play an important role in mediating eosinophil function in inflammatory processes. In peripheral blood monocytes, allergen-specific IgE bound to surface FcεRI receptors has been shown to enhance allergen presentation to T cells, and it has been speculated that eosinophils may also present antigen in this way (26). Gounni and colleagues (14) provided the first evidence that cross-linking of FcεRI receptors on eosinophils obtained from patients with hypereosinophilic syndrome resulted in eosinophilic degranulation and participated in eosinophil-mediated cytotoxicity against *Schistosoma mansoni*. Release of cationic proteins such as major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EPO) are believed to mediate, at least in part, the bronchial epithelial damage characteristic of asthma (31).

We therefore propose that, *in vivo*, allergen exposure in sensitized asthmatics not only upregulates eosinophilopoiesis and eosinophil recruitment to the site of allergen exposure but also upregulates expression of FcεRIα mRNA and protein by these cells. Our study indicates that, in contrast to mast cells, macrophages, and dendritic cells, which constitutively express FcεRI, eosinophils may be the predominant cells in BAL expressing FcεRI after endobronchial allergen exposure. This raises the possibility that FcεRI-expressing eosinophils may therefore participate in IgE-mediated allergic inflammatory responses. Further studies should focus on the regulation and functional significance of FcεRI expression by eosinophils and their IgE-dependent activation.

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