High-affinity IgE Receptor (FcεRI)-Bearing Cells in Bronchial Biopsies from Atopic and Nonatopic Asthma

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Asthma is characterized by bronchial mucosal inflammation. Although allergen-induced activation of cells binding allergen-specific immunoglobulin E (IgE) through high-affinity receptors (FcεRI) is believed to play some role in asthma, inappropriate synthesis of total or allergen-specific IgE cannot be demonstrated in some ("intrinsic") patients despite the fact that the nature of the bronchial inflammation is similar to that in atopic ("extrinsic") asthmatics. We have studied the numbers and phenotype of FcεRI-bearing cells in bronchial biopsies from 12 atopic and 10 nonatopic asthmatic patients and compared our findings with 10 atopic and 12 nonatopic control subjects using single and double immunohistochemistry. Significantly increased numbers of FcεRI-bearing cells were identified in bronchial biopsies from atopic and nonatopic asthmatics and atopic control subjects when compared with normal controls ($p = 0.001$, 0.006, and 0.0006, respectively). In asthmatics and atopics the majority of FcεRI-bearing cells were identified as mast cells and macrophages; a much smaller percentage were eosinophils. We conclude that elevated numbers of high-affinity IgE receptor–bearing cells are a feature of bronchial biopsies of asthmatic subjects, irrespective of their atopic status. Humbert M, Grant JA, Taborda-Barata L, Durham SR, Pfister R, Menz G, Barkans J, Ying S, Kay AB. High-affinity IgE receptor (FcεRI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma.

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The biologic activities of immunoglobulin E (IgE) are contingent upon the binding of its Fc region to specific cellular receptors, which may be of high (FcεRI) or low (FcεRII) affinity (1). Classically described on mast cells and basophils (1, 2), high-affinity IgE receptors have been recently shown to be present on Langerhans cells (3, 4), eosinophils (5), and monocytes (6). FcεRI-mediated cellular activation is dependent on the cross-linking of bound surface IgE molecules. This typically occurs in subjects who inappropriately produce IgE against antigens ("allergens") encountered at mucosal surfaces. Once sensitized, exposure of atopic subjects to allergen causes IgE cross-linking and cellular activation with the release of mediators which include, in the case of mast cells and basophils, histamine, eicosanoids, and cytokines.

Asthma is associated with eosinophil-rich inflammation of the bronchial mucosa (7). IgE-mediated release of mediators from sensitized cells is believed to play a role in the pathogenesis of this inflammation, since exposure of sensitized atopic asthmatics to allergens causes immediate and late bronchoconstriction, as well as bronchial hyperresponsiveness (8), whereas removal of such subjects from allergen exposure ameliorates the disease (9). However, a small subgroup of patients with asthma are not demonstrably atopic, and it has been suggested that there may be atopic and nonatopic variations of the disease (10). Nonatopic (so-called "intrinsic") asthmatics are skin prick test negative to common aeroallergens and their total serum IgE concentrations are within the "normal range." It is uncertain whether these patients represent asthma with a different mechanism or similar immunopathology with allergy to as yet undetected allergen. It has been shown in epidemiologic studies that, irrespective of atopic status, total serum IgE concentrations relate closely to the prevalence of self-reported asthma (11), suggesting that IgE-mediated mechanisms may play a role in nonatopic asthma despite the fact that these patients have no detectable allergen-specific IgE. As a first step in investigating this possibility, we hypothesized that both atopic and nonatopic asthma are characterized by increased numbers of FcεRI-bearing cells in the bronchial mucosa. Our aims were to evaluate the numbers and define the phenotypes of these cells in atopic and nonatopic asthmatics and control subjects.

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METHODS

Patient Population

The clinical and demographic characteristics of the 22 asthmatics (12 atopic, 10 nonatopic) and the 22 control subjects (10 atopic, 12 nonatopic) are shown in Table 1. Subjects with asthma were included on the basis of history and demonstrated reversible airway obstruction (more than 20% variability of the forced expiratory volume in one second [FEV_1] either spontaneously or after inhaled β_2 agonists), increased airway responsiveness to histamine (concentration producing a 20% decrease from baseline in FEV_1 [PC_{20}] $\leq$ 6 mg/ml), or both. Bronchial responsiveness to histamine was measured using the method described by Hargreave and coworkers (12). Asthma clinical severity was assessed according to the scoring system of Aas (13). Atopy was defined by a positive skin prick test (wheal diameter of over 3 mm following subtraction of the negative control) to one or more aeroallergens (including house dust mite, mixed grass pollen, mixed tree pollen, mixed molds, cat fur, and dog dander). By use of the CAP system (Pharmacia Diagnostics, Uppsala, Sweden) all atopic patients demonstrated a positive radioallergosorbent test (RAST > 0.70 IU/ml) to one or more allergens. Nonatopic subjects were defined by negative skin test to a wide range of local common aeroallergens in the presence of a positive histamine control. The nonatopic subjects also exhibited a serum IgE concentration within the normal range (0–150 IU/ml) and negative RAST tests to 25 common aeroallergens. All IgE and RAST measurements were performed in the same laboratory. Five atopic control subjects had mild to moderate seasonal or perennial rhinitis (n = 2 and n = 3, respectively) and five were asymptomatic. All atopic control subjects had a lifetime absence of wheeze

TABLE 1

CLINICAL DETAILS OF PATIENTS AND CONTROL SUBJECTS

Patient No.	Age/Sex	Blood Eosinophils (%)	Serum Ige (*IU/ml*)	SPT and RAST	FEV_1 (*% predicted*)	FVC (*% predicted*)	Histamine PC_{20} (*mg/ml*)	Aas Score
Nonatopic controls								
1	32/F	4.0	16	−ve	110	111	> 16	
2	39/F	0.3	7	−ve	110	102	> 16	
3	25/F	1.7	3	−ve	119	121	> 16	
4	23/M	1.0	18	−ve	90	99	> 16	
5	21/M	1.6	29	−ve	92	88	> 16	
6	42/M	3.1	5	−ve	100	102	> 16	
7	21/F	1.4	5	−ve	118	114	> 16	
8	21/F	1.0	3	−ve	106	99	> 16	
9	20/M	3.0	6	−ve	99	93	> 16	
10	19/M	1.3	77	−ve	96	92	> 16	
11	27/F	2.1	43	−ve	99	92	> 16	
12	19/M	5.0	47	−ve	94	99	> 16	
Median	22/NA	1.7	12	NA	100	99	NA	
Atopic controls								
13	28/M	1.8	315	Grass, HDM	98	104	> 16	
14	22/M	2.5	107	HDM	125	120	> 16	
15	33/M	1.7	152	HDM	123	128	> 16	
16	21/M	4.5	241	Cat, HDM	121	112	> 16	
17	28/M	3.6	83	HDM	100	98	> 16	
18	36/M	2.9	38	Grass	100	101	> 16	
19	23/M	3.6	77	HDM	107	108	> 16	
20	18/M	1.2	343	Grass	98	91	> 16	
21	22/M	1.5	225	Grass, HDM	111	113	> 16	
22	22/M	4.4	55	HDM	109	120	> 16	
Median	23/NA	2.7	130	NA	108	110	NA	
Atopic asthmatics								
23	24/M	3.6	253	HDM	72	93	0.06	4
24	26/M	3.9	351	Cat, grass, HDM	79	95	0.02	4
25	28/F	1.1	57	Cat, grass, HDM	100	111	2.99	3
26	42/M	2.3	330	Cat, grass, HDM	85	99	0.14	4
27	39/M	5.2	3,749	Cat, grass, HDM	81	86	3.49	1
28	36/F	1.5	62	Grass, HDM	100	118	3.61	1
29	55/M	1.6	180	Cat, grass, HDM	56	68	0.16	4
30	26/F	6.1	78	Cat, grass, HDM	86	103	3.66	3
31	42/M	5.3	295	Grass, HDM	104	111	1.63	1
32	55/F	4.0	178	HDM	64	91	2.76	4
33	23/M	4.8	207	Grass, HDM	102	100	2.02	3
34	37/M	5.7	304	Cat, grass	55	89	0.02	4
Median	37/NA	3.8	230	NA	83	97	1.83	3.5
Nonatopic asthmatics								
35	62/M	10.6	75	−ve	61	71	0.50	4
36	61/M	10.0	17	−ve	58	64	0.25	4
37	53/F	14.2	79	−ve	92	94	0.12	3
38	58/M	9.2	40	−ve	112	102	1.00	2
39	52/F	7.3	108	−ve	64	86	2.04	4
40	54/F	10.5	46	−ve	78	89	6.00	2
41	49/F	5.7	29	−ve	96	106	1.34	4
42	54/F	11.4	108	−ve	73	132	NT	4
43	39/F	5.7	33	−ve	102	112	0.12	1
44	46/M	4.1	99	−ve	70	80	NT	3
Median	53/NA	9.6	61	NA	76	92	0.75	3.5

Definition of abbreviations: HDM = house dust mite; SPT = skin prick test; NA = not applicable; NT = not tested.

or chest tightness, normal baseline lung function with less than 20% FEV$_1$ reversibility either spontaneously or after inhaled β_2 agonists, and histamine $PC_{20} > 16$ mg/ml. All subjects were nonsmokers and had not taken oral corticosteroids for the previous 2 mo, or inhaled corticosteroids for the previous 2 wk. All asthmatics were treated with inhaled β_2 agonists only. The study was approved by the Ethics Committees of the Royal Brompton Hospital, London and the Hochgebirgsklinik Davos and all subjects gave written informed consent.

Fiberoptic Bronchoscopy

Bronchoscopy was performed in all subjects (asthmatics and controls) following premedication with 2.5 mg salbutamol by nebulizer and 0.6 mg of atropine and 5 to 10 mg midazolam administered intravenously. Local anesthesia of the vocal cords and trachea was induced with 2% and 4% lidocaine. After inspection of the airways, biopsies were taken from the right middle, lower, and upper lobe bronchi using Olympus alligator forceps (model FB15C). This procedure was well tolerated and the safety of our bronchoscopy protocol was prospectively monitored in all patients and controls. Bronchial biopsies were fixed immediately in freshly prepared 4% paraformaldehyde (BDH, Poole, UK) in phosphate-buffered saline (PBS, pH 7.4) for 2 h, washed twice (1 h) with 15% sucrose (Sigma, Poole, UK) in PBS, embedded in OCT compound (Bayer, Basingstoke, UK), snap-frozen and stored at −80° C to be used later in immunohistochemistry (IHC).

Immunohistochemistry

Enumeration of numbers of macrophages, mast cells, eosinophils, and FcεRI$^+$ cells. Immunohistochemistry was performed as previously described (14) by the alkaline phosphatase-antialkaline phosphatase method. After a wash with Tris-buffered saline (TBS), sections were incubated for 30 min with a variety of mouse monoclonal antibodies including anti-CD68 (macrophages; Becton Dickinson, Mountain View, CA), anti-tryptase (mast cells; Dako Ltd., High Wycombe, UK), anti-EG2 (cleaved form of eosinophil cationic protein, a marker of activated eosinophils; Sanbio BV, Amsterdam, the Netherlands), anti-CD3 (pan T lymphocytes, leu4; Becton Dickinson), and a noncompetitive monoclonal antibody (i.e., unable to block the binding of IgE to FcεRI) to the FcεRI α subunit (22E7; a kind gift of Drs. R. Chizzonite and J. P. Kochan, Hoffmann-LaRoche, Nutley, NJ; final concentration 10 μg/ml) (15). The sections were then washed and incubated with rabbit immunoglobulins to mouse immunoglobulins (Dako) for 30 min. After an additional wash in fresh buffer, sections were incubated with soluble complexes of alkaline phosphatase and monoclonal mouse antialkaline phosphatase (Dako) for 30 min, and subsequently developed with Fast Red TR/Naphthol AS-MX (Sigma). Isotype-matched monoclonal antibodies of irrelevant specificity were included as negative controls.

Immunohistochemistry was also performed with a rabbit polyclonal antibody with reactivity against the human FcεRI α subunit described in reference 16 (997; a kind gift of Dr. J.-P. Kinet, Molecular Allergy and Immunology Section, National Institute of Allergy and Infectious Diseases, National Institute of Health, Rockville, MD; final concentration 1/500) by the peroxidase antiperoxidase technique. Sections were briefly washed in Tris-buffered saline (TBS, pH 7.2) between each experimental step. After pretreatment with 3% hydrogen peroxide (Sigma) in TBS to inhibit endogenous peroxidase and 1% bovine serum albumin (Sigma) in TBS to block nonspecific labeling (30 min each), sections were incubated overnight at room temperature with the primary antibody (997). Sections were then incubated with a swine anti-rabbit antibody (Dako), followed by soluble complexes of rabbit antibody to horseradish peroxidase and horseradish peroxidase (Dako) (30 min each). Positive cells stained brown after development with 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma). Controls included irrelevant rabbit immunoglobulins and rabbit serum (both Dako). Slides were counted blind to the patients' clinical status using a squared eyepiece graticule by two independent investigators, as previously described (17). Results were expressed as the total numbers of positive cells per mm^2 of submucosa. The coefficient of variability of the differences between the counts obtained from all slides by both observers was less than 5%.

Identification of phenotype of FcεRI$^+$ cells by double immunohistochemistry. To examine the phenotype of FcεRI α subunit-bearing cells, cryostat sections were studied by double IHC. Sections were briefly washed in TBS between each experimental step. After pretreatment with 3% hydrogen peroxide and by 1% bovine serum albumin both in TBS (30 min each), mouse anti-human phenotype monoclonal antibodies (CD68, EG2, tryptase, or CD3) together with rabbit anti-human FcεRI α subunit (997) were applied overnight at room temperature to the section for simultaneous localization. The second layer consisted of a biotinylated goat anti-mouse antibody (Dako) together with a swine anti-rabbit antibody (30 min). The third layer included streptavidin alkaline phosphatase (Amersham, Bucks, UK) together with soluble complexes of rabbit antibody to horseradish peroxidase and horseradish peroxidase (30 min). Fast Red and DAB were sequentially used for the development of the cell phenotype and the FcεRI-specific staining, respectively. Controls included isotype-matched mouse monoclonal antibodies of irrelevant specificities and irrelevant rabbit immunoglobulins and serum. After development, cells expressing CD68, EG2, tryptase, or CD3 were stained red and FcεRI-bearing cells stained brown. Double positive cells stained for both colors. Slides were counted in duplicate at high-power magnification (×1,000). The colocalization of 997 staining with the tryptase, CD68, and EG2 phenotypic markers and the percentages of cells of each phenotype coexpressing FcεRI α subunit were then calculated.

Statistical Analysis

Data were analyzed using a statistical package (Minitab Release 7, Minitab Inc., State College, PA). Nonparametric statistical tests were used in the analysis, and median values are presented in the accompanying figures and tables. When multiple comparisons were made between groups, significant between-group variability was first established using the Kruskal-Wallis test. The Mann-Whitney U test was then used for intergroup comparison (with Bonferroni's correction). Correlation coefficients were obtained by Spearman's rank-order method. p Values < 0.05 were considered significant.

RESULTS

Despite the fact that their total serum IgE concentrations were within the normal range of the reference laboratory, nonatopic asthmatics had increased total serum IgE concentrations compared with nonatopic control subjects ($p = 0.015$), although levels were lower than in atopic asthmatics ($p = 0.010$) (Table 1). The numbers of EG2$^+$ eosinophils, tryptase$^+$ mast cells, CD68$^+$ macrophages, and CD3$^+$ T lymphocytes in bronchial biopsies from asthmatic patients and nonasthmatic control subjects are shown in Table 2. Irrespective of their atopic status, asthmatic patients were characterized by elevated numbers of bronchial mucosal eosinophils, compared with nonasthmatic control subjects ($p = 0.0001$). As indicated in Table 2, this eosinophilic inflammation was more pronounced in nonatopic asthmatics.

Bronchial biopsies from atopic and nonatopic asthmatics as well as atopic control subjects had statistically equivalent numbers of FcεRI-bearing cells which were significantly elevated when compared with nonatopic control subjects (Figure 1). FcεRI$^+$ cells

TABLE 2

NUMBERS OF EG2$^+$ EOSINOPHILS, TRYPTASE$^+$ MAST CELLS, CD68$^+$ MACROPHAGES, AND CD3$^+$ T LYMPHOCYTES IN BRONCHIAL BIOPSIES FROM ATOPIC AND NONATOPIC ASTHMATICS AND ATOPIC AND NONATOPIC CONTROL SUBJECTS*

Patient Group	EG2$^+$	Tryptase$^+$	CD68$^+$	CD3$^+$
Group I	0	38	48	58
Nonatopic controls, n = 12	(0–30)	(8–82)	(5–173)	(5–150)
Group II	5	46	38	63
Atopic controls, n = 10	(0–33)	(11–103)	(20–115)	(20–155)
Group III	13†	21	35	68
Atopic asthmatics, n = 12	(0–40)	(10–78)	(0–75)	(25–150)
Group IV	32‡	49	94	42
Nonatopic asthmatics, n = 10	(0–200)	(15–70)	(5–156)	(10–200)

* Values are median per mm^2, with range given in parentheses.
† $p < 0.03$ versus Group I.
‡ $p < 0.01$ versus Groups I and II.

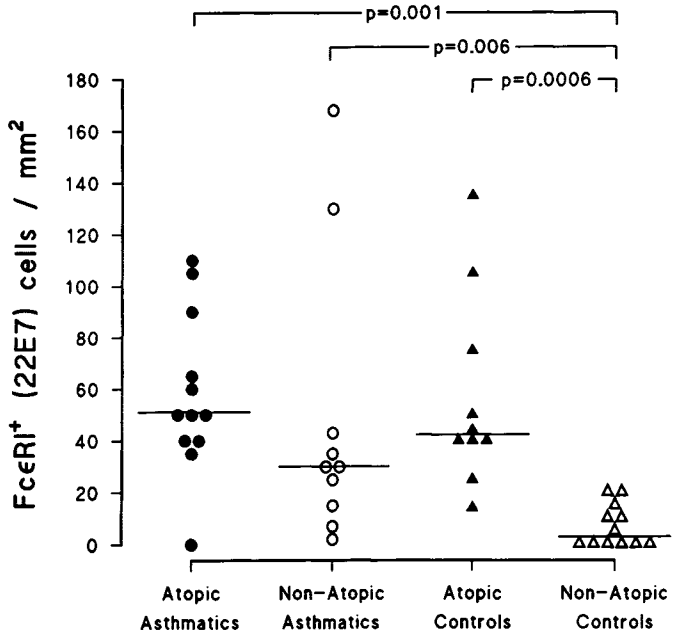

Figure 1. The distribution of FcεRI-bearing cells (identified with antibody 22E7) in bronchial biopsies from atopic asthmatics (n = 12), nonatopic asthmatics (n = 10), atopic control subjects (n = 10), and nonatopic control subjects (n = 12).

were mainly confined to the bronchial submucosa (Figure 2A) with a similar distribution using both FcεRI-specific antibodies (22E7 and 997) (not shown). In asthmatics, the numbers of 22E7$^+$ cells/mm^2 submucosa did not correlate with the patients' disease severity evaluated by the Aas score or histamine PC_{20} ($r = 0.21$ and -0.35, respectively, $p > 0.20$), whereas a positive correlation was demonstrated with the log total IgE serum concentrations ($r = 0.62$, $p = 0.002$).

To determine whether FcεRI immunoreactivity was localized to mast cells, macrophages, and eosinophils, double IHC was performed in 14 individuals (four atopic controls, six atopic asthmatics, and four nonatopic asthmatics). When the data were examined in terms of the percentages of bronchial cells of a given phenotype coexpressing 997, it was found that a majority of tryptase$^+$ mast cells were 997$^+$ (Figures 2B and 3). A large proportion of the CD68$^+$ macrophage population were 997$^+$ (Figures 2C and 3). A much lower proportion of activated EG2$^+$ eosinophils expressed 997 although this was not observed in all subjects (Figures 2D and 3). No CD3/997 double positive cells were observed (not shown). When the data were examined in terms of the percentages of 997$^+$ cells coexpressing tryptase, CD68, or EG2, it was found that a majority of the 997$^+$ cells were tryptase$^+$ mast cells and CD68$^+$ macrophages; 3% of the 997$^+$ cells were EG2$^+$ eosinophils and 2% were unidentified (Figure 4).

DISCUSSION

In this study we have identified increased numbers of FcεRI$^+$ cells in bronchial biopsies of subjects with asthma, irrespective of their atopic status, as well as atopic individuals without asthma as compared with nonatopic control subjects. Although this study was not designed to test FcεRI function in asthma, evidence of elevated numbers of FcεRI-bearing bronchial cells in most cases of skin test negative (so-called intrinsic) asthmatics is of considerable interest because it raises the possibility that asthma may be associated with IgE-mediated processes despite the absence of detectable allergen-specific IgE, as evidenced by negative skin prick and RAST tests in these patients. The fact that total serum IgE concentrations were significantly higher in nonatopic asthmatics than nonatopic normal subjects (although still within the laboratory "normal range") supports this view. Furthermore the data are consistent with two previous epidemiologic studies showing that the prevalence of asthma was closely related to serum IgE concentrations when adjusted for age and sex (11) and that airway hyperresponsiveness appears to be closely linked to total IgE concentrations in children, even in subjects with no history of atopic disease (18). The nonatopic asthmatic patients in this study were older than atopic asthmatics, reflecting the described clinical difference between these groups (10). Despite the observation that serum IgE concentrations decline with age (11), elevated serum IgE concentrations and FcεRI expression were observed in these patients. This suggests that the age of the patients was not a confounding factor in our analysis. Thus, in nonatopic asthma there may be as yet unidentified allergen(s) or antigen(s) capable of binding IgE. Our results are also compatible with a role for virus-specific IgE (19) or autoantibodies to FcεRI in this condition, as previously suggested in chronic idiopathic urticaria, a disease characterized by basophil and mast cell release of histamine and other vasoactive mediators in the absence of any clear allergic, infectious, drug-induced, or physical cause (20). Hide and colleagues demonstrated histamine-releasing IgG autoantibodies against the FcεRI α subunit in the circulation of certain patients with chronic idiopathic urticaria (20). The presence of FcεRI α subunit-bearing cells in the bronchial mucosa of intrinsic asthmatics also suggests that autoantibody-induced cross-linking of IgE receptors may possibly be a mechanism in the pathogenesis of this disease.

With regard to asthma pathogenesis, we have demonstrated that both atopic and nonatopic asthmatics show elevated bronchial mucosal expression of FcεRI, as compared with nonatopic control subjects. Expression of bronchial FcεRI did not correlate with symptomatology or airway hyperresponsiveness in asthma. Furthermore, bronchial mucosal FcεRI expression was not specific for asthma since it was also observed in the atopic nonasthmatics. Taken together these data are compatible with the hypothesis that elevated bronchial mucosal FcεRI expression is necessary, but not sufficient, for the development of both atopic and nonatopic asthma. Thus, additional factors must be essential for the development of asthma. It remains possible, however, that elevated FcεRI expression in the bronchial mucosa of nonatopic asthmatics is an epiphenomenon reflecting local ongoing inflammation promoting nonspecific FcεRI upregulation through the release of various mediators. For instance, T cell–derived interleukin-9 is believed to play a role in the modulation of FcεRI on inflammatory cells (21). In order to address the specificity of our findings to asthma, it will be of interest to study FcεRI mucosal expression in bronchial tissue from other chronic inflammatory respiratory diseases.

We confirm that the tissue mast cell is a prominent FcεRI α subunit–bearing cell *in vivo*. The lack of significant mast cell staining with both anti-FcεRI antibodies in nonatopic control subjects presumably reflects a much lower IgE receptor density in this population, emphasizing the fact that FcεRI may be expressed at variable density *in vivo* (22, 23). The positive correlation demonstrated between the numbers of FcεRI$^+$ bronchial cells and serum IgE concentrations has to be analyzed with caution. Although serum IgE may not interfere directly with bronchial mucosal cells, our results are in keeping with former human studies indicating a correlation between serum IgE concentrations and numbers of IgE molecules per basophils (22, 23). Similarly, mucosal FcεRI expression could be modulated by local IgE

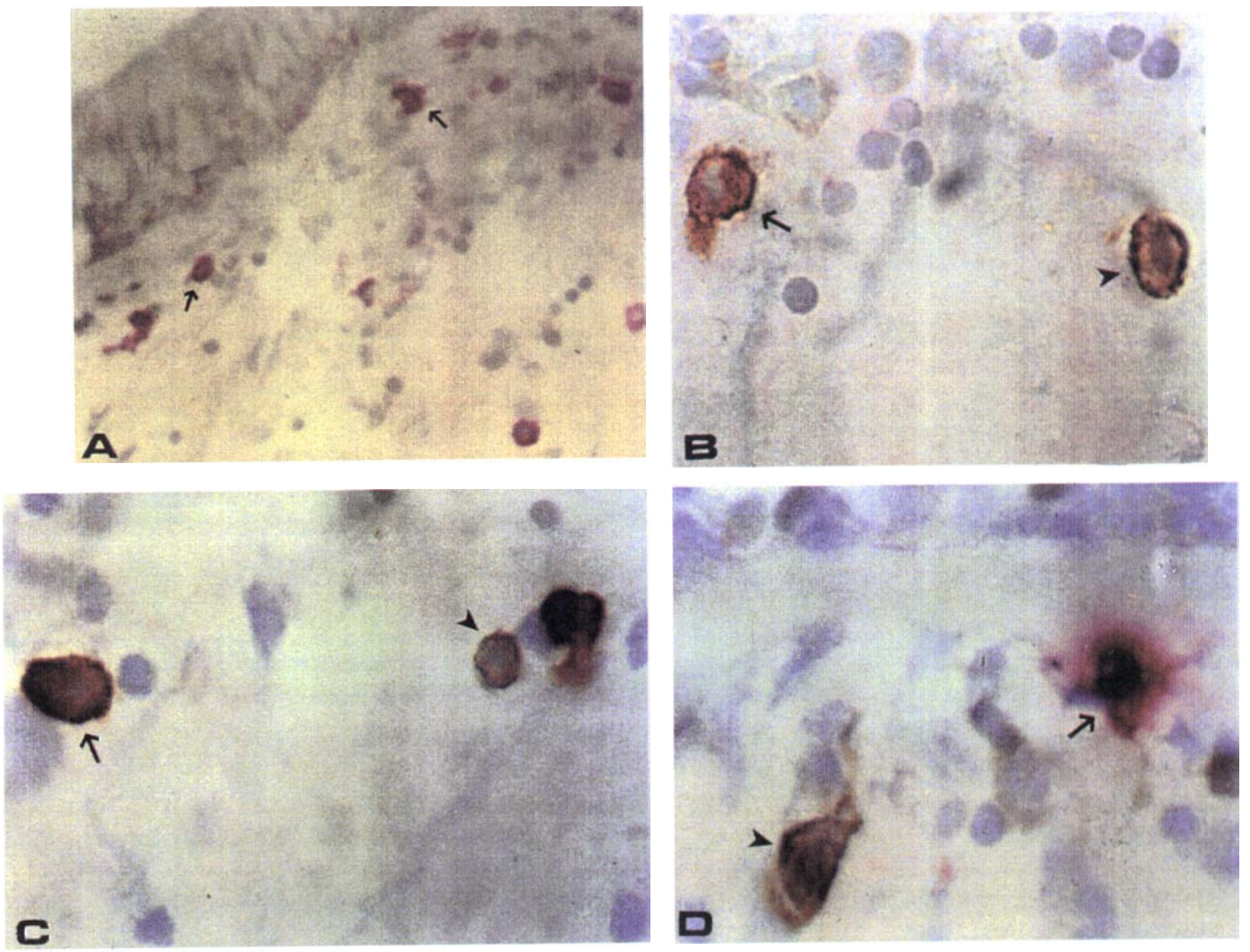

Figure 2. (*A–D*) Cryostat sections of bronchial biopsies in asthma. (*A*) FcεRI$^+$ cells (*arrows*) in nonatopic asthma (antibody 22E7, magnification ×400). (*B*) Tryptase$^+$/FcεRI$^+$ double-stained mast cell (*arrow*). (*C*) CD68$^+$/FcεRI$^+$ double-stained macrophage (*arrow*) and (*D*) ED2$^+$/FcεRI$^+$ double-stained eosinophil (*arrow*) in atopic asthma. Arrowheads indicate FcεRI$^+$ single-stained cells (*B–D*: antibody 997, magnification ×1,000).

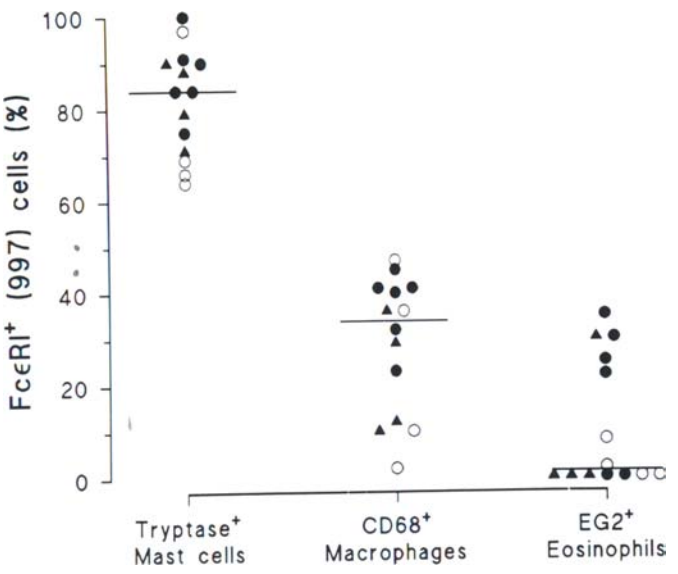

Figure 3. Percentage of tryptase$^+$ mast cells, CD68$^+$ macrophages, and EG2$^+$ eosinophils expressing FcεRI (identified with antibody 997 in bronchial biopsies from six atopic asthmatics (*solid circles*), four nonatopic asthmatics (*open circles*), and four atopic control subjects (*solid triangles*).

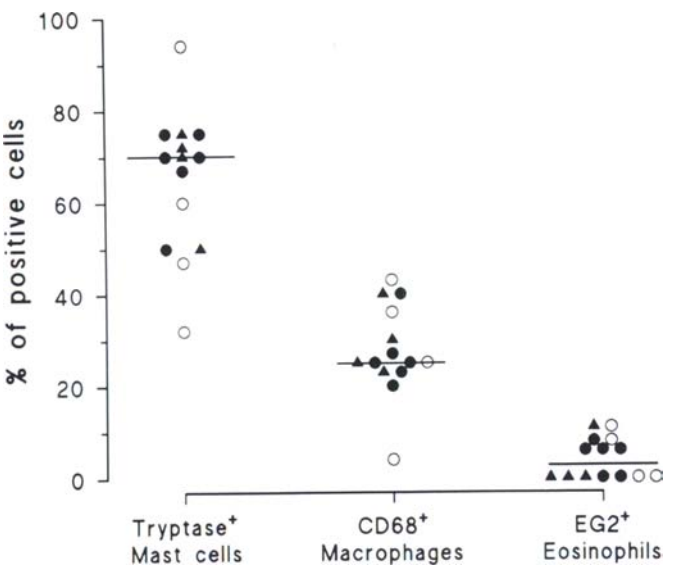

Figure 4. Percentage of FcεRI-bearing cells (identified with antibody 997) expressing tryptase, CD68, or EG2 in bronchial biopsies from six atopic asthmatics (*solid circles*), four nonatopic asthmatics (*open circles*) and four atopic control subjects (*solid triangles*).

production within the bronchial mucosa of asthmatic individuals. Further studies are needed to analyze FcεRI regulation in the bronchial mucosa of atopic and nonatopic asthma.

The presence of FcεRI-bearing macrophages in the bronchial mucosa, the true site of the asthmatic inflammatory process, suggest that IgE-dependent activation of mononuclear phagocytes may play a pathogenic role in tissue inflammation. This may result in the synthesis of proinflammatory mediators by these cells and/or enhanced allergen presentation (24–27). Maurer and coworkers have reported that IgE-dependent antigen presentation allows presentation to T cells in a way that may be more effective than non-IgE-dependent antigen presentation, as shown in peripheral blood mononuclear cells enriched for monocytes (27). Although low-affinity IgE receptors may also mediate IgE-dependent antigen trapping and presentation, it has been also shown in cultures of monocytes from atopic donors that competitive antibodies to FcεRI but not competitive antibodies to FcεRII could largely abrogate detectable binding of IgE to monocyte (27).

In this study, the numbers of mast cells and macrophages were not significantly elevated in bronchial biopsies from asthmatics, as compared with control subjects, although there was a trend for increased bronchial mucosal CD68$^+$ macrophage counts in nonatopic asthma. Nevertheless, mast cells and macrophages accounted for the large majority of FcεRI-bearing cells in asthma, and were hardly stained in normal controls. We therefore speculate that FcεRI expression could be a marker of activated bronchial mucosal mast cells and macrophages in atopic and/or asthmatic subjects.

We here confirm that bronchial mucosal activated eosinophils are a prominent feature of asthmatic inflammation (7). In addition, a low and variable proportion of bronchial mucosal eosinophils expressed FcεRI in our patient population, indicating the possible relevance of FcεRI$^+$ eosinophils in eosinophil-induced tissue damage. Soussi Gounni and coworkers have shown that peripheral blood eosinophils from hypereosinophilic donors were FcεRI$^+$ and that FcεRI-bearing eosinophils could be detected in the skin of a patient with atopic dermatitis (5). Recent observations from our laboratory indicate that a small proportion of tissue eosinophils from biopsies of allergen-induced cutaneous late-phase reaction express FcεRI (28). The physiologic relevance of FcεRI-bearing eosinophils in allergic disease in still unclear, although FcεRI-mediated IgE-dependent mediator release could contribute to the marked eosinophil degranulation observed in allergic inflammation (29). Similarly, FcεRI may play a role in antigen presentation by eosinophils (30).

Elevated numbers of basophils have been measured in the sputum of patients with bronchial asthma (31) and basophil recruitment into the lumen of the lower airways has been demonstrated in late-phase reactions following local allergen provocation (32). The role of these cells in asthma, however, remains controversial. Despite the absence of a basophil-specific marker, our study shows that only 2% of the total FcεRI$^+$ cells do not correspond to mast cells, macrophages, or eosinophils. No significant immunostaining was associated to the bronchial epithelial cells or any structural cells of the bronchi. Therefore tissue basophils, epithelial cells, and structural cells of the bronchi do not seem to represent a significant proportion of FcεRI$^+$ bronchial cells in our study population.

FcεRI is a tetrameric complex of one α-, one β-, and two γ-chains (1, 2). The FcεRI α subunit is a transmembrane molecule with an extracellular segment containing two immunoglobulin-related domains, the second of which is necessary for IgE binding (15). The FcεRI α subunit is associated with the β- and γ-chains in the cell membrane. These chains are required for insertion of the α-chain into the cell membrane and for signal transduction following allergen interaction with IgE. Genetic studies have already suggested a role for regulation of FcεRI expression in the pathogenesis of atopy. Targeted disruption of the FcεRI α-chain gene in mice has produced animals resistant to cutaneous and systemic anaphylaxis, demonstrating that FcεRI is necessary for the initiation of IgE-dependent anaphylactic reactions (33). Furthermore a genetic linkage has recently been demonstrated between inappropriate IgE synthesis and chromosome 11q13 (34). The FcεRI-β subunit is a candidate atopy-causing locus on chromosome 11q: indeed the β subunit of the FcεRI also lies on chromosome 11q13, and a common variant of FcεRI-β subunit has been demonstrated among unrelated nuclear families with allergic asthmatic probands (Ile181Leu substitution within the fourth transmembrane domain of the molecule) (34, 35).

In conclusion our data extend our knowledge of the relationship between atopy, asthma, IgE, and FcεRI. We have shown that atopy is characterized by increased numbers of FcεRI-bearing bronchial cells irrespective of the patients' asthmatic status and that so-called intrinsic asthmatics have increased number of FcεRI-bearing bronchial cells in the absence of obvious allergic triggers. Moreover we provide evidence that FcεRI-bearing cells in atopics include mast cells, macrophages, and eosinophils, suggesting that all these cell types may be implicated in IgE-dependent inflammation in atopy and asthma. The demonstration of a positive correlation between the numbers of FcεRI-bearing bronchial cells and the serum IgE concentrations provides further evidence that patients with atopy and asthma may be genetically predisposed to produce inappropriate IgE responses to various stimuli and to have increased numbers of FcεRI-bearing cells and/or higher FcεRI cellular density in various body compartments including the bronchial mucosa. Our data are consistent with the hypothesis that bronchial mucosal FcεRI expression is necessary but not sufficient for the asthma phenotype in adults. Additional factors including environmental influences are required for the development of asthma in genetically predisposed individuals.

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References

1. Sutton, B. J., and H. J. Gould. 1993. The human IgE network. *Nature* 366:421–428.
2. Metzger, H. The receptor with high affinity for IgE. 1992. *Immunol. Rev.* 125:37–48.
3. Bieber, T., H. de la Salle, A. Wollenberg, J. Hakimi, R. Chizzonite, J. Ring, D. Hanau, and C. de la Salle. 1992. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (FcεRI). *J. Exp. Med.* 175:1353–1365.
4. Wang, B., A. Rieger, O. Kilgus, K. Ochiai, D. Maurer, D. Fodinger, J.-P. Kinet, and G. Stingl. 1992. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. *J. Exp. Med.* 175:1285–1290.
5. Soussi Gounni, A., B. Lamkhioued, K. Ochiai, Y. Tanaka, E. Delaporte, A. Capron, J.-P. Kinet, and M. Capron. 1994. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 367:183–186 (erratum 368:473).
6. Maurer, D., E. Fiebiger, B. Reininger, B. Wolff-Wininski, M.-H. Jouvin, O. Kilgus, J.-P. Kinet, and G. Stingl. 1994. Expression of functional high affinity immunoglobulin E receptor (FcεRI) on monocytes of atopic individuals. *J. Exp. Med.* 179:745–750.
7. Bousquet, J., P. Chanez, J.-Y. Lacoste, G. Barneon, N. Ghanavian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, and F.-B. Michel. 1990. Eosinophilic inflammation in asthma. *N. Engl. J. Med.* 323:1033–1039.
8. Cartier, A., N. C. Thompson, P. A. Frith, R. Roberts, and F. E. Hargreave. 1982. Allergen-induced increase in bronchial responsiveness

to histamine: relationship to the late asthmatic response and change in airway calibre. *J. Allergy Clin. Immunol.* 70:170–177.
9. Dorward, A. J., M. J. Collof, N. S. MacKay, C. McSharry, and N. C. Thompson. 1988. Effect of house dust mite avoidance measures on adult atopic asthma. *Thorax* 43:98–105.
10. Rackemann, F. M. 1947. A working classification of asthma. *Am. J. Med.* 3:601–606.
11. Burrows, B., F. D. Martinez, M. Halonen, R. A. Barbee, and M. G. Cline. 1989. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N. Engl. J. Med.* 320:271–277.
12. Hargreave, F. E., G. Ryan, N. C. Thompson, P. M. O'Byrne, K. Latimer, E. F. Juniper and J. Dolovich. 1981. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J. Allergy Clin. Immunol.* 68:347–355.
13. Aas, K. 1981. Heterogeneity of bronchial asthma: sub-populations or different stages of the disease. *Allergy* 36:3–14.
14. Bradley, B. L., M. Azzawi, M. Jacobson, B. Assoufi, J. V. Collins, A.-M. A. Irani, L. B. Schwartz, S. R. Durham, P. K. Jeffery, and A. B. Kay. 1991. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J. Allergy Clin. Immunol.* 88:661–674.
15. Riske, F., J. Hakimi, M. Mallamaci, M. Griffin, B. Pilson, N. Tobkes, P. Lin, W. Danho, J. Kochan, and R. Chizzonite. 1991. High affinity human IgE receptor (FcεRI): analysis of functional domains of the α-subunit with monoclonal antibodies. *J. Biol. Chem.* 266:11245–11251.
16. Letourneur, O., S. Sechi, J. Willette-Brown, M. W. Robertson, and J.-P. Kinet. 1995. Glycosylation of human truncated FcεRI α chain is necessary for efficient folding in the endoplasmic reticulum. *J. Biol. Chem.* 270:8249–8256.
17. Bentley, A. M., G. Menz, C. Storz, D. S. Robinson, B. Bradley, P. K. Jeffery, S. R. Durham, and A. B. Kay. 1992. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma: relationship to symptoms and bronchial responsiveness. *Am. Rev. Respir. Dis.* 146:500–506.
18. Sears, M. R., B. Burrows, E. M. Flannery, G. P. Herbison, C. J. Hewitt, and M. D. Holdaway. 1991. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N. Engl. J. Med.* 325:1067–1071.
19. Welliver, R. C., D. T. Wong, M. Sun, E. Middleton, Jr., R. S. Vaughan, and P. L. Ogra. 1981. The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N. Engl. J. Med.* 305:841–846.
20. Hide, M., D. M. Francis, C. E. H. Grattan, J. Hakimi, J. P. Kochan, and M. W. Greaves. 1993. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N. Engl. J. Med.* 328:1599–1604.
21. Louahed, J., A. Kermouni, J. Van Snick, and J.-C. Renauld. 1995. IL-9 induces expression of granzymes and high-affinity IgE receptor in murine T helper clones. *J. Immunol.* 154:5061–5070.
22. Malveaux, F. J., M. C. Conroy, N. F. Adkinson, L. M. Lichtenstein. 1978. IgE receptors on basophils: relationship to serum IgE concentration. *J. Clin. Invest.* 62:176–181.
23. Mac Glashan, D. W., R. P. Schleimer, S. P. Peters. E. S. Schulman, G. K. Adams, A. K. Sobotka, H. H. Newball, and L. M. Lichtenstein. 1983. Comparative studies of human basophils and mast cells. *Fed. Proc.* 42:2504–2509.
24. Rankin, J. A., M. Hitchcock, W. Merill, M. K. Bach, J. R. Brashler, and P. W. Askenase. 1982. IgE-dependent release of leukotriene C_4 from alveolar macrophages. *Nature* 297:329–331.
25. Joseph, M., A.-B. Tonnel, G. Torpier, A. Capron, B. Arnoux, and J. Benveniste. 1983. Involvement of immunoglobulin E in the secretory processes of alveolar macrophages from asthmatic patients. *J. Clin. Invest.* 71:221–230.
26. Bieber, T. 1994. FcεRI on human Langerhans cells: a receptor in search of new functions. *Immunol. Today* 15:52–53.
27. Maurer, D., C. Ebner, B. Reininger, E. Fiebiger, D. Kraft, J.-P. Kinet, and G. Stingl. 1995. The high affinity IgE receptor (FcεRI) mediates IgE-dependent allergen presentation. *J. Immunol.* 154:6285–6290.
28. Taborda-Barata, L., M. Humbert, J. A. Grant, and A. B. Kay. 1995. High affinity IgE receptor (FcεRI) positive cells in allergic tissue inflammation include macrophages and eosinophils as well as mast cells (abstract). 9th International Congress of Immunology. San Francisco, 23–29 July 1995.
29. Broide, D. H., G. J. Gleich, A. J. Cuomo, D. A. Coburn, E. C. Federman, L. B. Schwartz, and S. I. Wasserman. 1991. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J. Allergy Clin. Immunol.* 88:637–648.
30. Weller, P. F., T. H. Rand, T. Barrett, A. Elovic, D. T. W. Wong, and R. W. Finberg. 1993. Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1α expression. *J. Immunol.* 150:2554–2562.
31. Kimura, I., Y. Tanizaki, K. Saito, K. Takahashi, N. Ueda, and S. Sato. 1975. Appearance of basophils in sputum of patients with bronchial asthma. *Clin. Allergy* 5:95–98.
32. Guo, C.-B., M. C. Liu, S. J. Galli, B. S. Bochner, A. Kagey-Sobotka, and L. M. Lichtenstein. 1994. Identification of IgE-bearing cells in the late-phase response to antigen in the lung as basophils. *Am. J. Respir. Cell Mol. Biol.* 10:384–390.
33. Dombrowicz, D., V. Flamand, K. K. Brigman, B. H. Koller, and J.-P. Kinet. 1993. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor α chain gene. *Cell* 75:969–976.
34. Sandford, A. J., T. Shirakawa, M. F. Moffatt, S. E. Daniels, C. Ra, J. A. Faux, R. P. Young, Y. Nakamura, G. M. Lathrop, W. O. C. M. Cookson, and J. M. Hopkin. 1993. Localisation of atopy and β subunit of high-affinity IgE receptor (FcεRI) on chromosome 11q. *Lancet* 341: 332–334.
35. Shirakawa, T., A. Li, M. Dubowitz, J. W. Dekker, A. E. Shaw, J. E. Faux, C. Ra, W. O. C. M. Cookson, and J. M. Hopkin. 1994. Association between atopy and variants of the β subunit of the high-affinity immunoglobulin E receptor. *Nature Genet.* 7:125–130.