

Allergens, IgE, mediators, inflammatory mechanisms

Enhanced expression of high-affinity IgE receptor (FcεRI) α chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells

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Background: IgE-dependent activation of mast cells and basophils through the high-affinity IgE receptor (FcεRI) is involved in the pathogenesis of allergen-induced immediate and late responses.

Objective: We investigated the expression and cellular distribution of FcεRI in the nasal mucosa after allergen challenge in patients with summer hay fever.

Methods: Fourteen grass pollen-sensitive patients and seven normal control subjects underwent nasal challenge with grass pollen and allergen diluent in random order separated by 2 weeks. Nasal airway caliber was monitored by acoustic rhinometry, and nasal biopsy was performed at 6 hours. Messenger RNA for FcεRI was determined by using reverse-transcription polymerase chain reaction, and FcεRI protein expression was determined by immunohistology with a mouse monoclonal antibody (22E7) and a rabbit polyclonal antibody (997) directed against the α subunit. Co-localization of FcεRI receptors was performed by using double-immunostaining methods.

Results: In atopic subjects, there was a significant early decrease in nasal airway caliber, which extended up to 6 hours after allergen challenge. FcεRI mRNA levels were elevated at 6 hours ($p = 0.03$). Cells expressing FcεRI protein were increased in patients with atopic rhinitis compared with normal control subjects ($p = 0.03$). Further increases in FcεRI⁺ cells were observed after allergen challenge only in the atopic group ($p = 0.02$). Double immunohistochemistry revealed that the majority of FcεRI⁺ cells were mast cells (64%), followed by macrophages (20%), eosinophils (4%), and dendritic cells (2%), with 10% FcεRI⁺ cells being unidentified.

Conclusions: Our results demonstrate increased FcεRI expression during allergen-induced rhinitis and highlight a potential target for treatment. (*J Allergy Clin Immunol* 1997; 100:78-86.)

Key words: Allergic rhinitis, FcεRI receptors, IgE

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).^{1,2} Although the serum half-life of free IgE is only a few days, mast cells may remain sensitized by IgE for many months because of high-affinity binding to the IgE receptor.^{1,2} FcεRI is intimately involved in the allergic mediator release process. Cross-linking of the FcεRI, even in the absence of IgE, results in mast-cell triggering.³ In sensitized individuals, 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 has also been shown to be IgE-dependent.^{4,5} Late cutaneous biopsy specimens obtained after allergen challenge have demonstrated IgE-bound antigen-presenting cells, such as epidermal Langerhans cells and dermal dendritic cells.^{6,7} More recently, it has been demonstrated that Langerhans cells,^{8,9} dermal dendritic cells,^{8,10} peripheral blood monocytes,¹¹ and eosinophils^{12,13} may all express the high-affinity receptor for IgE (FcεRI). On the other hand, there is no previous *in vivo* evidence for the presence of FcεRI receptors in human allergic rhinitis.

In this study we hypothesized that FcεRI-bearing cells are increased in the target organ (the nasal mucosa) in patients with allergic rhinitis and that local allergen challenge induces upregulation of FcεRI expression at both messenger RNA and protein levels. We also attempted to determine whether mast cells are the predominant FcεRI-bearing cells and assessed the phenotype of other cells, which might express FcεRI.

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Abbreviations used

APAAP:	Alkaline phosphatase-anti-alkaline phosphatase
mAb:	Monoclonal antibody
NAC:	Nasal airway caliber
PCR:	Polymerase chain reaction

METHODS

Patients

Fourteen patients (12 men and 2 women) with a history of grass pollen seasonal allergic rhinitis and seven nonatopic, normal subjects (6 men and 1 woman) took part in the study (Table I). Subjects were recruited from the Allergy Clinic and the Nose Clinic at the Royal Brompton Hospital. All subjects were nonsmokers. Patients with rhinitis were chosen on the basis of: (1) a history of nasal blockage, sneezing, and rhinorrhea during the grass pollen season for more than 3 years; (2) a strongly positive skin wheal response (>5 mm) to prick testing with timothy grass pollen extract (*Phleum pratense*; Soluprick, ALK, Horsholm, Denmark); and (3) positive RAST test result (score ≥ 2 on a scale of 0 to 6) for the same grass pollen extract. The study was performed outside the grass pollen season, and the subjects were free of symptoms during testing. Normal volunteers were free of symptoms and had negative skin test responses to a panel of 11 common aeroallergens including pollens, animal danders, molds, and house dust mite (Soluprick, ALK). All subjects refrained from taking any form of medication for at least 1 month before the study began and throughout the study period. None of the subjects had a history of upper respiratory tract infection in the preceding 4 weeks, nasal polyps, infective sinusitis, nasal surgery, or nasal deformities. None of the patients had received immunotherapy in the previous 5 years. 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 on two occasions separated by 2 weeks. Baseline nasal airway caliber (NAC) was measured by using an acoustic rhinometer (GM Instruments, Glasgow, U.K.). This was followed by bilateral nasal challenge with grass pollen extract or allergen diluent. Acoustic rhinometry was performed on both sides at 5, 10, 15, 30, 60 minutes and then hourly until 6 hours after nasal challenge. Subjects recorded symptoms of nasal blockage and itching on a 10 cm visual analog scale. The number of sneezes was counted. At 6 hours, nasal biopsy was performed.

Nasal allergen challenge

Nasal challenge was undertaken by using a hand-held pump spray (Fisons Ltd., Loughborough, U.K.), which delivered 0.13 ml per actuation to each nostril.¹⁴ The pump was placed in one nostril, while occluding the contralateral nostril, and activated once during quiet inspiration. The procedure was then repeated in the opposite nostril. A total dose of 1000 biological units (500 BU per actuation) of timothy grass pollen extract (ALK) was delivered to each nostril. This dose has previously been shown to provoke both immediate and late-phase nasal responses.¹⁵ On the control day, allergen diluent was used.

Nasal biopsy

Nasal biopsy was performed by using cup and ring (Geritsma) forceps as previously described.¹⁶ Local anesthesia was established by placing a cotton wool carrier holding 3% cocaine and epinephrine (1 in 1000) under the inferior turbinate adjacent to the site chosen for biopsy. A 2.5 mm diameter nasal biopsy specimen was taken from the inferior turbinate. The subjects were then observed for 30 minutes. Any slight bleeding was controlled by local pressure to the nose or occasional application of a silver nitrate stick.

Processing of nasal biopsy specimens

Nasal biopsy specimens were prefixed in 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline for 2 hours, followed by 15% sucrose/phosphate-buffered saline for 1 hour, and then subsequently in 15% sucrose in phosphate-buffered saline overnight. Biopsy specimens were then embedded in OCT (BDH, U.K.) on a small piece of card, snap-frozen in isopentane precooled in liquid nitrogen, and stored at -80°C . Nasal biopsy specimens from eight atopic subjects¹⁻⁸ were cut into two halves, and one half was snap-frozen in isopentane precooled in liquid nitrogen and stored at -80°C for polymerase chain reaction (PCR) analysis.

Detection and PCR analysis of mRNA for FcεRI-α chain

The procedure for detection and PCR analysis of mRNA for FcεRI-α chain has been described previously.¹⁷ Nasal biopsy specimens from eight atopic subjects¹⁻⁸ were used for PCR analysis. Total RNA was isolated by using Ultraspec RNA isolation system (Biotex Laboratories, Houston, Texas) according to the manufacturer's protocol. RNA was then reverse-transcribed, and PCR was conducted in an air thermocycler as described previously.^{17,18} The complementary DNA for β-actin and water were used in lieu of reverse-transcribed cDNA for positive and negative controls, respectively. After the reaction, the amplified product was electrophoresed on 3% Nusieve 3:1 agarose gel (Sigma Ltd., Poole, U.K.). The amplified product was then identified on the gel by the anticipated molecular size and by Southern hybridization.¹⁷ The gels were exposed to Biomax x-ray films (Kodak, Hemel Hempstead, England) for 2 hours before development; and the bands were excised from the gel, placed in 20 ml of scintillation fluid, and counted in a liquid scintillation counter.

The primer pairs were designed from the published cDNA sequence data.¹⁹ Both primers were custom-designed by using the software program "Primer" (Scientific and Education Software, State Line, Pa.). Nucleotide sequences of the primers used were as follows.

FcεRI-α sequences were 5':CCT TAC TGT TCT TCG CTC CA and 3':CTG AAG ACT TCC AGG TAC AC. β-Actin sequences were 5':GTG GGG CGC CCA AGG CAC CA and 3':CTC CTT AAT GTC ACG CAC GAT TTC.

Immunohistology

Lymphocytes, macrophages, mast cells, Langerhans cells, and eosinophils. Six microliter sections of nasal biopsy specimens obtained at 6 hours after challenge in all subjects were cut on a cryostat, mounted on slides, and air-dried overnight at 37°C . Sections were then fixed in paraformaldehyde. The alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique was used to process the biopsy specimens in order to phenotype cells.¹⁵ The following monoclonal antibodies (mAbs) were used: CD3 (Becton Dickinson, Cowley, Oxon, U.K.); EG2 (Pharma-

PCR Amplified Products of β -actin and Fc ϵ RI α -chain Found in Nasal Biopsies From Atopic Patients

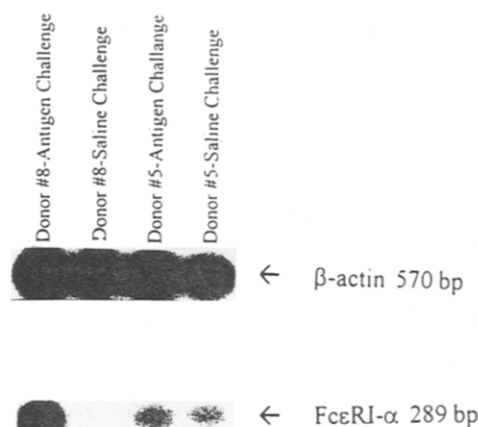


FIG. 1. Expression of mRNA for Fc ϵ RI α -chain and β -actin (control) by human nasal biopsy tissue. Tissue was processed for reverse transcription, and PCR was performed in the presence of [α - ^{32} P]-deoxycytidine triphosphate. The gel was exposed to an x-ray film for 2 hours. Expression of β -actin was approximately equal in all samples. When challenged with saline solution, subject 8 did not express Fc ϵ RI mRNA, whereas subject 5 expressed a low level of Fc ϵ RI. However, both patients expressed induced Fc ϵ RI mRNA at increased levels in nasal biopsy specimens when challenged with allergen.

cia, Milton Keynes, U.K.) mAb to recognize eosinophils; tryptase, mAb that recognizes mast cells (Chemicon, International Inc., Temecula, Calif.); CD68 (macrophages) (Dako, High Wycombe, U.K.); and CD1a (OKT-6), mAbs to recognize dendritic cells (a kind gift from Dr. Wyke Fokkens, Ziekenhuis dijkzigt, Rotterdam, The Netherlands). Sections of human tonsils were included as positive controls. For negative control preparations, the primary antibody was replaced with either nonspecific mouse IgG or Tris-buffered saline.

Fc ϵ RI-bearing cells. A noncompetitive murine mAb (22E7; a kind gift from Drs. Roger Chizzonite and John P. Kochan, Hoffman La Roche Inc., Nutley, N.J.; concentration 10 μ g/ml) directed against the α chain of Fc ϵ RI was used for single immunohistochemistry with the APAAP technique as described above.¹⁵

Double immunocytochemistry

This method was used to identify the phenotype of Fc ϵ RI α subunit-bearing cells in nasal biopsy specimens. For this method, a rabbit polyclonal antibody to the human Fc ϵ RI α subunit (997, a kind gift from Dr. Jean-Pierre Kinet) was used. Murine mAb 22E7 was not used because of the possibility of cross-reactions with other murine antibodies used for detecting cell phenotypes. First, endogenous peroxidase was blocked by pretreatment with 3% hydrogen peroxide and 1% bovine serum albumin. Mouse anti-human mAbs against CD68, CD3, CD1a, tryptase, and EG2 were applied together with rabbit anti-human Fc ϵ RI α subunit (997) antibody overnight at room temperature. A second layer, consisting of a biotinylated goat anti-mouse antibody (Dako) and a swine anti-rabbit antibody (Dako), was applied for 30 minutes. A third layer made of streptavidin alkaline phosphatase (Amersham, Bucks, U.K.),

TABLE I. Demographic details of patients studied

Subject No.	Age (yr)	Sex	Atopy	Total IgE (IU/ml)
Normal group				
1	24	F	Neg	16
2	27	M	Neg	17
3	20	M	Neg	21
4	37	M	Neg	67
5	22	M	Neg	46
6	22	M	Neg	89
7	26	M	Neg	205
Mean	25.4			66
SEM	2.1			25
Rhinitis group				
1	23	M	G, T	650
2	27	F	G, T	257
3	37	M	G, D	278
4	22	M	G, C, T, D	714
5	31	M	G	171
6	27	M	G, C	130
7	28	M	G	594
8	20	M	G, T	217
9	26	M	G	245
10	27	M	G, M	186
11	24	M	G, T, C	468
12	20	M	G, M	343
13	24	M	G, C, D	1137
14	24	F	G	51
Mean	25.7			389
SEM	1.2			79

Positive skin prick test responses to: G, grass pollen; T, tree pollen; C, cat; D, dog; M, molds.

together with soluble complexes of rabbit antibody to horseradish peroxidase and rabbit anti-horseradish peroxidase (Dako), was applied for 30 minutes. After this, Fast Red TR and 3,3'-diaminobenzidine tetrahydrochloride were sequentially applied for detection of cell phenotype and Fc ϵ RI α subunit, respectively. Cells bearing CD68, EG2, tryptase, CD1a, or CD3 phenotypes were detected as red-stained cells; and those bearing an Fc ϵ RI α subunit phenotype appeared brown. Those cells that had both phenotypes (double-positive) stained both colors. For controls, isotype-matched nonspecific mouse mAbs and rabbit immunoglobulins were used.

Quantification

For single and double immunocytochemistry, slides were counted in blinded fashion and in random coded order by using a BH2 microscope (Olympus Corp., Lake Success, N.Y.) fitted with an eyepiece graticule. Sections were counted at a magnification of $\times 200$, and double-immunostained cells were confirmed at a magnification of $\times 1000$ under oil immersion. For both techniques, at least two sections were stained for each antibody, and four to five grids per section were counted. Results were expressed as the number of cells per square millimeter. For double immunocytochemistry, the co-localization of 997 staining with the CD68, CD3, CD1a, tryptase, and EG2 phenotypic markers and the percentage of cells of each phenotype co-expressing Fc ϵ RI α -subunit were

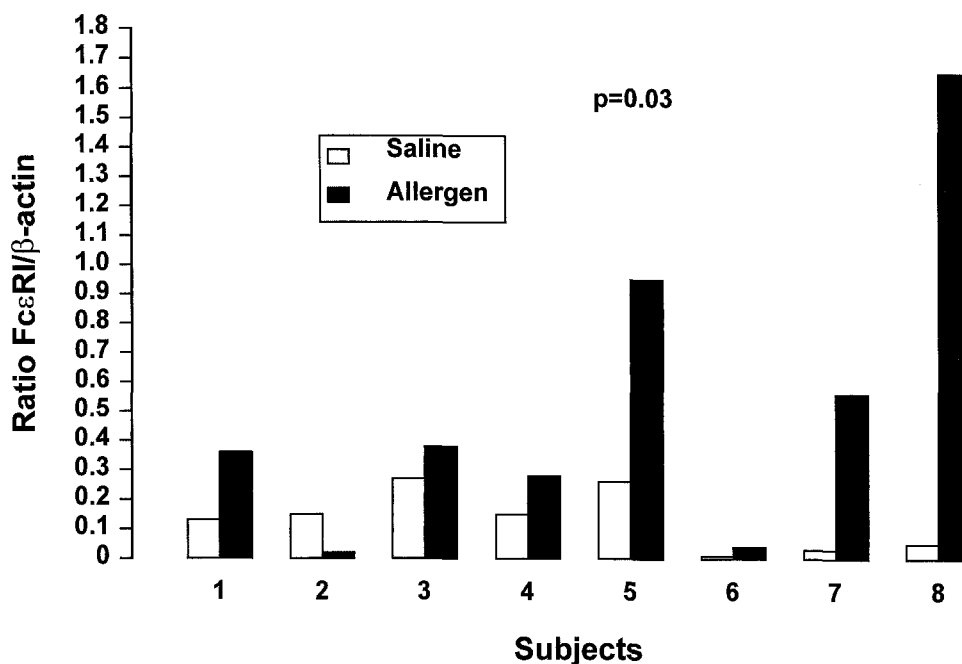


FIG. 2. Ratios of FcεRI mRNA counts to β-actin mRNA from allergen- and saline-challenged patients. Reverse-transcription PCR samples were labeled with [α - ^{32} P]-deoxycytidine triphosphate; and resultant bands were cut from the gel, placed in scintillant, and counted in a liquid scintillation counter. Counts were analyzed as a ratio of counts per minute of FcεRI to counts per minute of β-actin. Allergen caused an increase in FcεRI in most atopic subjects at 6 hours, with subjects 5 and 8 expressing the highest level of the receptor.

TABLE II. Immunohistology of nasal biopsy specimens after saline and allergen challenges in atopic patients with rhinitis and normal subjects

mAb	Patients with atopic rhinitis (n = 14)			Normal subjects (n = 7)		
	Control day (median)	Allergen day (median)	Median difference	Control day (median)	Allergen day (median)	Median difference
CD3	127 (76, 288)	238 (118, 295)	37 (-80, 188)	171 (11, 282)	202 (116, 553)	111 (-60, 383)
CD1a	4 (1, 20)	10 (3, 13)	0 (-2, 2)	3 (0, 8)	4 (1, 13)	3 (-2, 4)
EG2 (eosinophils)	8 (2, 13)	31 (6, 109)	22* (6, 109)	6 (0, 23)	7 (4, 11)	0 (-3, 4)
Tryptase (mast cells)	22 (15, 30)	33 (17, 45)	8 (-11, 17)	29 (6, 40)	29 (6, 52)	4 (-14, 19)
CD68 (macrophages)	138 (109, 189)	170 (136, 214)	30 (-45, 70)	168 (95, 212)	124 (99, 191)	4 (-38, 44)

Cell counts are expressed as median number of cells/mm² (25th to 75th percentile range).

**p* = 0.005.

calculated. Within-observer mean coefficient of variation for cell counts was always less than 5%.

For PCR, quantification was performed by labeling the samples with α-phosphorous 32 deoxycytidine triphosphate; and the resultant bands were cut from the gel, placed in scintillant, and counted in a liquid scintillation counter. The counts were analyzed as a ratio of counts per minute of FcεRI to counts per minute of β-actin.

Statistical analysis

Data were analyzed by using a Microsoft statistical package (Microsoft Excel, Microsoft Corporation). Baseline acoustic rhinometric values (NAC) were compared by using Wilcoxon's signed-rank test. Changes in NAC in response to allergen and saline solution were expressed as a percentage change from the baseline value. NAC was plotted against the time in minutes after allergen challenge. The response was quantified both as the maximal increase in NAC and the area under each time

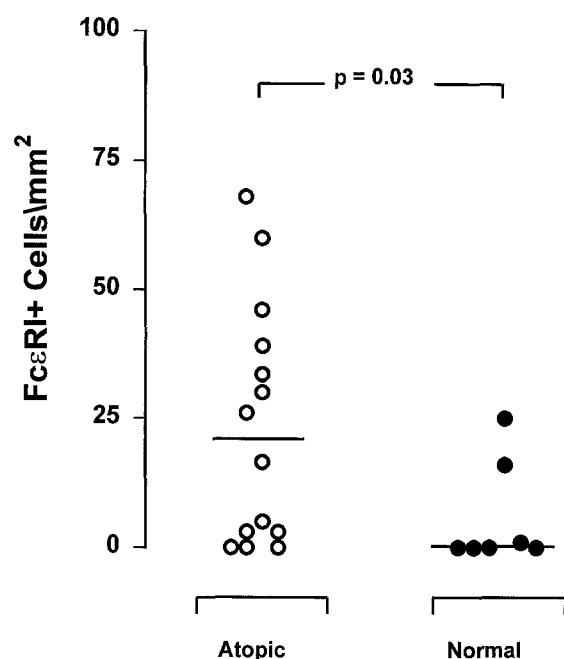


FIG. 3. Number of cells expressing FcεRI in nasal biopsy specimens from atopic patients with rhinitis and normal subjects. Horizontal bars represent median values.

response curve, which was calculated by trapezoid integration. 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 by using the Mann-Whitney U test. Correlation coefficients were obtained by using Spearman's rank analysis. Probability (*p*) values of less than 0.05 were considered significant.

RESULTS

Clinical data

Clinical data for the patients and normal volunteers studied are shown in Table I. The two groups were well matched for age, gender, and baseline NAC. No change in NAC for either group was observed on the control day after saline challenge. Atopic subjects reported symptoms of rhinorrhea, nasal itching, sneezing, and nasal blockage immediately after allergen challenge. There were, however, no significant late nasal symptoms at 2 to 6 hours after challenge, although six atopic subjects reported late nasal blockage during this period. Acoustic rhinometry detected a marked, immediate (5 minutes, $p < 0.005$) decrease in NAC, followed by a continuous reaction up to 6 hours after allergen challenge ($p < 0.05$). No significant change in NAC after allergen challenge was observed for the normal subjects.

FcεRI α-chain mRNA

PCR was used to investigate FcεRI α-chain mRNA expression in nasal biopsy specimens from eight atopic subjects. Expression of mRNA was quantified as a ratio

to β -actin. On the control day, a low level of Fc ϵ RI mRNA was detectable in seven of eight nasal biopsy specimens from those patients with atopic rhinitis (Fig. 1); the median number of copies of mRNA encoding Fc ϵ RI (relative to β -actin) in nasal biopsy specimens was 0.14. After nasal allergen challenge at 6 hours, all eight nasal biopsy specimens expressed Fc ϵ RI, and this was increased ($p = 0.03$) in seven of eight biopsy specimens; the median value was 0.37 (Fig. 2).

Cell infiltration

On the control day, there was no significant difference in the median number of EG2⁺, CD3⁺, CD68⁺, CD1a⁺, and tryptase-positive cells in the nasal submucosa between patients and normal volunteers (Table II). When compared with the control day, there were significant increases in eosinophils (EG2⁺) at 6 hours after allergen challenge in atopic patients; median values were 31 cells/mm² ($p = 0.005$) (Table II). In contrast, no increases were observed after allergen in nonatopic, normal subjects (Table II). When the magnitudes of the net increase in cells (allergen minus saline) were compared between atopic and nonatopic subjects, significant increases were still seen for EG2⁺ cells ($p = 0.009$). Median cell counts for CD3⁺, CD68⁺, CD1a, and tryptase-positive cells were increased at 6 hours after allergen challenge, although not significantly compared with the control day, and no changes were observed for these cell types in nonatopic subjects (Table II).

FcεRI α-chain protein (immunohistochemistry data)

FcεRI⁺ cells were mainly confined to the nasal submucosa with a few cells detectable within the nasal epithelium. The median numbers of FcεRI⁺ cells in the nasal submucosa on the control day were 21 cells/mm² for patients with atopic rhinitis and 0 cells/mm² for normal nonatopic subjects ($p = 0.03$) (Fig. 3). After nasal allergen challenge, increase in FcεRI⁺ cells in the nasal mucosa at 6 hours was seen in atopic subjects from 21 to 47 cells/mm² ($p = 0.02$) (Fig. 4). When the magnitudes of net increases in cell counts (allergen minus saline) were compared, significant increases in the FcεRI⁺ cells were still seen in atopic subjects with median increases of 16 and 0 cells/mm² ($p = 0.02$) for atopic and normal subjects, respectively (Fig. 4). FcεRI⁺ cells were also found in the nasal epithelium of five patients with rhinitis and three normal subjects. However, the cell counts were low in number and were not significantly different between control and allergen challenge days.

Double immunohistochemistry

To determine the phenotype of FcεRI⁺ cells, double immunohistochemistry was performed on allergen-challenged nasal biopsy specimens from seven atopic subjects. When the data were expressed as percentage of FcεRI⁺ cells co-expressing tryptase, CD68, EG2, or CD1a, the majority of FcεRI⁺ cells were found to be

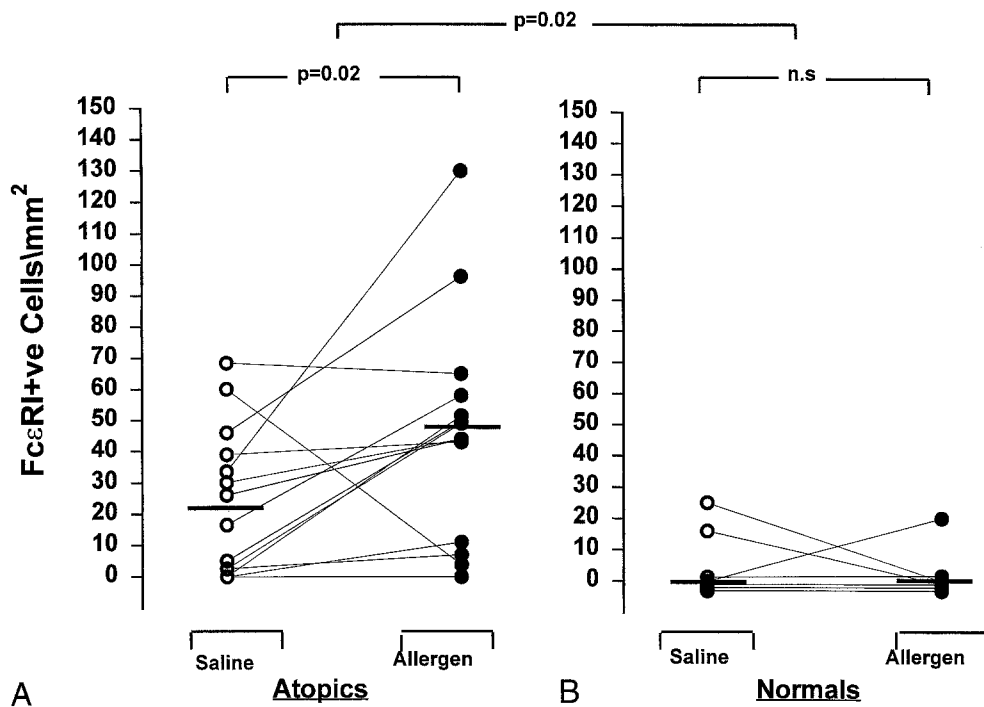


FIG. 4. FcεRI⁺ cells in nasal biopsy specimens from atopic patients with rhinitis (A) and normal subjects (B) after saline challenge (open circles) and allergen challenge (filled circles). Between groups, net increases in cell counts (allergen minus saline) are compared. Horizontal bars represent median values.

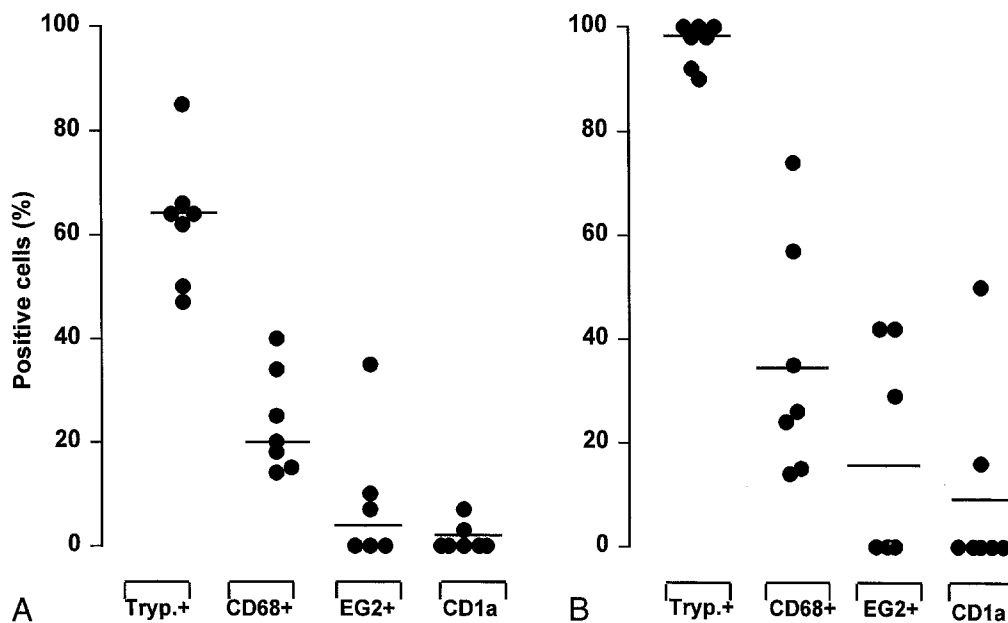


FIG. 5. A, Distribution of FcεRI⁺ cells that were mast cells (Tryp⁺), macrophages (CD68⁺), eosinophils (EG2⁺), and dendritic cells (CD1a⁺) in nasal biopsy specimens of seven atopic subjects at 6 hours after allergen challenge. B, Distribution of mast cells, macrophages, eosinophils, and dendritic cells that were FcεRI⁺ in nasal biopsy specimens of seven atopic subjects at 6 hours after allergen challenge.

mast cells (64%), followed by macrophages (20%), eosinophils (4%), and dendritic cells (2%) (Fig. 5, A, and Fig. 6) and 0% T lymphocytes. Ten percent of FcεRI⁺ cells were not double-immunostained with the

above markers and remain unidentified. We also examined the percentage of each cell phenotype within the nasal mucosa that co-expressed the FcεRI α subunit. Almost all (98%) mast cells co-expressed the FcεRI α

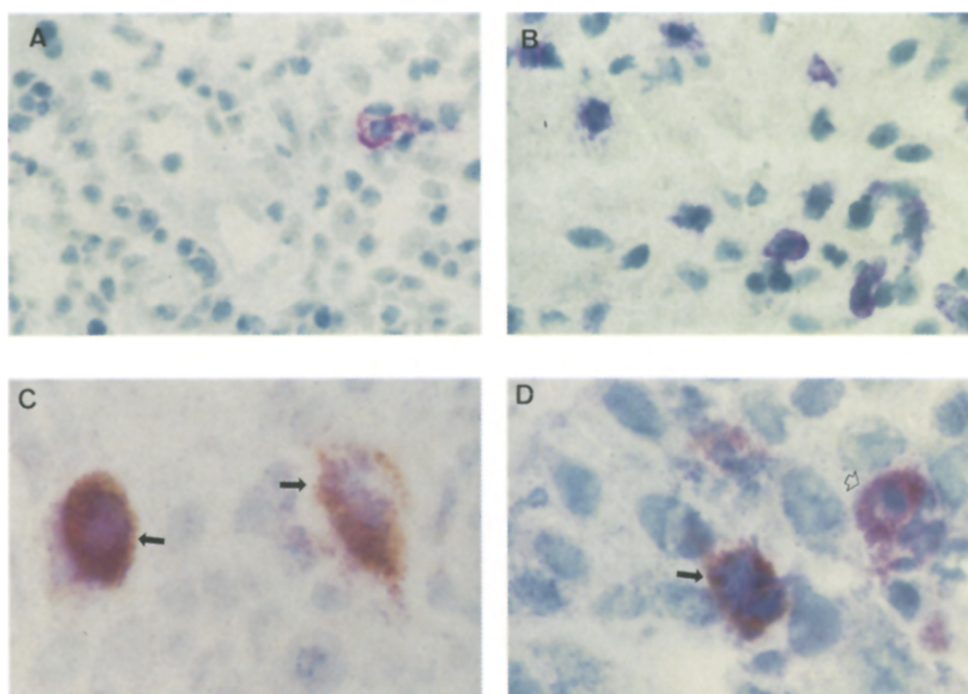


FIG. 6. Serial sections of a nasal biopsy specimen from an atopic subject 6 hours after saline challenge (**A**) and allergen challenge (**B**). Sections of nasal biopsy specimens were single-immunostained with antibody 22E7 against FcεRI α subunit by using the APAAP method and developed with fast red (magnification $\times 400$). Sections of nasal biopsy specimens double-immunostained with antibody 997 and mAbs against tryptase (mast cells) (**C**) and CD68 (macrophages) (**D**). Double-immunostained cells are indicated by *solid arrowhead*; single-immunostained cells against FcεRI are indicated by *open arrowhead*.

subunit. This was followed by CD68⁺ macrophages (35%). A smaller percentage of EG2⁺ eosinophils (15%) and CD1a⁺ cells (12%), but no CD3⁺ T lymphocytes (0%), co-expressed the FcεRI α subunit (Fig. 5, *B*).

DISCUSSION

We have demonstrated increased numbers of FcεRI α subunit-bearing cells in the nasal mucosa of subjects with atopic rhinitis. Upregulation of both FcεRI mRNA and FcεRI⁺ cells was observed after allergen provocation. We confirmed that the predominant FcεRI α -immunoreactive cells were tissue mast cells (64%), followed by macrophages (20%); some patients had FcεRI⁺ expression by eosinophils and dendritic cells. Our data support the role of the high-affinity IgE receptors during late responses and highlight this receptor as a potential target for therapy.

We chose a 6-hour antigen challenge model on the basis of a previous study, which demonstrated that nasal challenge with 320 BU of grass pollen allergen resulted in late symptoms of nasal blockage in 50% of patients between 3 and 10 hours.¹⁵ Our study included patients with rhinitis acting as their own control subjects, and the median changes (allergen-saline) were compared with those changes in a control group of nonatopic normal subjects. All atopic subjects in our study clearly demonstrated immediate nasal symptoms such as increased

sneezing, rhinorrhea, nasal blockage, and nasal pruritus. Consistent with this, we objectively observed a significant drop in NAC at 5 to 10 minutes after allergen challenge. There were, however, no significant reports of nasal symptoms between 2 and 6 hours after challenge, despite the sustained reduction in NAC, for up to 6 hours. In this study six of the 13 atopic subjects tested did experience late nasal blockage at 2 to 6 hours. The timing was variable such that the mean data at each time point failed to achieve significance. On the other hand, the prolonged decrease in NAC indicates a continuous response to allergen, and consistent with this, nasal biopsy specimens obtained at 6 hours after allergen challenge demonstrated marked tissue eosinophilia. The observation that no physiologic or pathologic changes were observed in nonatopic control subjects indicates that the changes observed in atopic subjects require specific sensitization and are not due to effects of the allergen preparation per se.

The activity of IgE depends on its interaction with specific receptors on effector cells. Two types of IgE receptors have been cloned^{1,2}: high-affinity (FcεRI) and low-affinity (FcεRII) (CD23) receptors. The FcεRI is a multi-subunit protein, consisting of an α chain, which binds to IgE, a β chain, and two γ chains. Antibodies directed against the α chain of the receptor have been shown to block IgE binding. Cross-linking of FcεRI in the absence of IgE results in mast cell histamine re-

lease.³ Genetic studies have demonstrated that targeted disruption of the FcεRI gene in mice made these animals resistant to development of anaphylaxis.²⁰ FcεRI has also been shown to mediate IgE-dependent schistosomal killing.¹² In a proportion of patients with chronic idiopathic urticaria, autoantibodies against the FcεRI receptor have been shown to provoke histamine release in vivo and in vitro, indicating the importance of FcεRI receptors in mediating type 1 responses in the skin.²¹ Furthermore, passive sensitization of human and rhesus monkey lung tissue has been shown to be blocked by human FcεRI-IgG and humanized anti-IgE mAb MaE11.²² In peripheral blood monocytes, targeting of FcεRI receptors through allergen-specific IgE has been shown to greatly enhance allergen presentation to T cells.²³ Eosinophils may be involved in antigen presentation in allergic diseases through IgE linked to FcεRI.²⁴ Late responses have been shown to be IgE-dependent. Thus the human cutaneous late-phase reaction could be reproduced by cross-linking IgE receptors by use of an F(Ab)₂ antibody against human IgE.⁴ Moreover, the allergen-induced cutaneous late-phase reaction could be transferred from a sensitive donor to a naive subject passively by intradermal donor serum. Pretreatment of the serum in ways that removed IgE resulted in loss of ability to transfer the late response.⁵ The FcεRI α subunit has recently been shown to be expressed on eosinophils and monocytes, as well as on mast cells and basophils.^{1, 2, 11-13, 24} Taken together, these studies provide strong evidence for the role of the high-affinity IgE receptors in human allergic responses.

After the immediate allergic reaction, in some patients, nasal symptoms recur and a second round of mediator release occurs at 6 to 24 hours after allergen challenge.²⁵ Our previous studies^{15, 26-28} have shown that tissue eosinophilia and epithelial migration of tryptase-positive mast cells occur during natural grass pollen exposure²⁷ and that increases in CD4⁺ and CD25⁺ T cells and in eosinophils occur 24 hours after nasal allergen challenge.^{15, 26, 28} Furthermore, increased cytokine expression for IL-3, IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor was also seen in nasal biopsy specimens obtained at 24 hours after allergen challenge.²⁸ The increases in nasal lavage mediators and expression of cytokines were shown to be suppressed by pretreatment with a topical corticosteroid.²⁹ Thus local generation of IL-4 would promote IgE production, and on the other hand, IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor after T-cell activation would promote tissue eosinophilia, features in keeping with IgE-dependent allergic tissue inflammation. In support of this, Terada et al.³⁰ have shown that only IL-4, among other cytokines, caused upregulation of FcεRI mRNA expression in blood eosinophils obtained from patients with atopic rhinitis.

The presence of higher numbers of FcεRI-bearing cells in the nasal mucosa of patients with asymptomatic rhinitis out of season indicates that increased FcεRI expression occurs even in the absence of allergen expo-

sure in sensitive (atopic) subjects. Using double immunohistochemistry, we have confirmed that the tissue mast cell is the predominant FcεRI α subunit-bearing cell in the human nasal mucosa. We also provide evidence that tissue macrophages and a variable percentage of eosinophils and dendritic cells also express FcεRI. Basophils are also known to be rich in FcεRI receptors,³¹ although in the absence of a specific marker for tissue basophils, it was not possible to assess the contribution of basophils. However, any such contribution from tissue basophils would be less than 10% of cells in this study. In this study we did not assess whether FcεRI expression by eosinophils is constitutively expressed or only occurs after allergen exposure. Because mast cell and macrophage numbers remain unchanged after allergen challenge, the observed increase in FcεRI⁺ cells in the nasal mucosa after allergen challenge was probably due to a combination of an increase in receptor expression and an influx of FcεRI-bearing eosinophils. However, confirmation of this would require additional double immunohistochemistry studies with 997 antibody and phenotype markers on nasal biopsy specimens obtained at baseline. Our results suggest that IgE-dependent activation of mononuclear phagocytes and eosinophils may also play a role in nasal allergic inflammation either by enhanced mediator release or enhanced allergen presentation.

In conclusion, we demonstrate that expression of the high-affinity receptor for IgE (FcεRI) is increased in the nasal mucosa of atopic subjects and that allergen challenge results in significant late increases in FcεRI at both mRNA and protein levels. The FcεRI α subunit is present mainly on mast cells but also on macrophages and eosinophils. These observations highlight the role of the FcεRI α subunit in early and chronic nasal responses in allergic rhinitis. Whether strategies directed against the FcεRI α subunit, such as use of the soluble receptor or antibodies directed against the receptor, might modify the response to allergen remains to be determined.

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