

IL-4 upregulates FcεRI α-chain messenger RNA in eosinophils

Nobuhisa Terada, MD,^a Akiyoshi Konno, MD,^a Yoshie Terada, MD,^a Setsuya Fukuda, PhD,^b Tetsuji Yamashita, PhD,^b Tatsuya Abe, PhD,^c Hiroko Shimada, PhD,^c Kazuto Ishida, PhD,^c Kentaro Yoshimura, PhD,^c Youichi Tanaka, MD,^d Chisei Ra, MD, PhD,^e Kazuo Ishikawa, MD,^f and Kiyoshi Togawa, MD^f Chiba, Tokyo, Nagasaki, and Akita, Japan

By using the reverse transcription polymerase chain reaction and Southern blot hybridization, we demonstrated that FcεRI α-chain (FcεRIα) messenger RNA was expressed in eosinophils purified from the peripheral blood of patients with allergic rhinitis and that this expression was enhanced by IL-4. However, studies in which flow cytometry or immunostaining was used did not reveal the expression of FcεRIα protein on eosinophils from peripheral blood. Neither IL-4 alone nor the combination of IL-4 and other cytokines could induce detectable FcεRIα protein; nevertheless, they do express FcεRIα mRNA. Double-labeling immunostaining on cryostat sections of nasal mucosa clearly demonstrated that some FcεRIα-positive cells were eosinophil cationic protein-positive, which confirms their eosinophilic nature. Not all the eosinophil cationic protein-positive cells express an FcεRIα signal. Considering that FcεRIα mRNA was detectable in four of five samples of eosinophils from those patients with nasal allergy and that only one of five eosinophil samples from normal subjects expressed FcεRIα mRNA, the level of FcεRI expression may be correlated with the activation of eosinophils. It seems very likely that some other unidentified factors are required for the process from the expression of FcεRIα mRNA to that of FcεRI as a protein. (J ALLERGY CLIN IMMUNOL 1995; 96:1161-9.)

Key words: IL-4, high-affinity IgE receptor, eosinophil, nasal allergy

The high-affinity IgE receptor (FcεRI) was considered to be present only on basophils and mast cells, but its presence also on Langerhans' cells, dendritic cells,¹ monocytes,² and eosinophils³ has been recently reported. The expression of FcεRI on eosinophils is important in that it suggests the possibilities of direct contact of antigen and eosinophils, followed by subsequent degranulation, and the function of antigen presentation of eo-

Abbreviations used

BSA:	Bovine serum albumin
ECP:	Eosinophil cationic protein
FcεRIα:	High-affinity IgE receptor α-chain
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
INF-γ:	Interferon-γ
mAb:	Monoclonal antibody
PBS:	Phosphate-buffered saline
RT-PCR:	Reverse transcription polymerase chain reaction
TNF-α:	tumor necrosis factor-α

From ^aDepartment of Otorhinolaryngology, Chiba University, School of Medicine; ^bResearch and Development Division and Department of Gene Analysis, Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo; ^cDepartment of Parasitology, Akita University, School of Medicine; ^dDepartment of Dermatology, Nagasaki University School of Medicine; ^eDepartment of Immunology, Juntendo University School of Medicine, Tokyo; and ^fDepartment of Otorhinolaryngology, Akita University, School of Medicine.

Supported in part by grants from Sandoz Ltd., Tokyo, Japan. Reprint requests: Nobuhisa Terada, MD, Department of Otorhinolaryngology, Chiba University School of Medicine, 1-8-1 Inohana, Chiba 260, Japan.

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sinophils.⁴ Meanwhile, the expression of the low-affinity IgE receptor (FcεRII/CD 23) is known to be subject to regulation by various cytokines. For example, IL-4, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF) cause enhancement of the expression, whereas interferon-γ (INF-γ) causes suppression.⁵

By using the reverse transcription polymerase

TABLE I. Expression of FcεRIα mRNA in peripheral blood eosinophils of patients with nasal allergy and normal subjects

Patient No.	Group	Age (yr)	Sex	Antigen	Eosinophilia in nasal smear	FcεRI mRNA
1	Nasal allergy	16	M	HD, M, JC, R, Mu	3+	+
2	Nasal allergy	19	M	HD, R	2+	+
3	Nasal allergy	33	F	HD, M	+	+
4	Nasal allergy	27	F	HD, M, Mu	2+	—
5	Nasal allergy	11	M	HD, M, JC, Mu	+	+
1	Normal	29	M	—	—	—
2	Normal	28	F	—	—	—
3	Normal	40	M	—	—	+
4	Normal	15	M	—	—	—
5	Normal	17	M	—	—	—

HD, House dust; M, mites; JC, Japanese cedar; R, ragweed pollen; Mu, mugwort pollen.

chain reaction (RT-PCR) and Southern blot hybridization, we demonstrated in this study that the FcεRI α-chain (FcεRIα) messenger RNA was expressed in eosinophils, purified from the peripheral blood of patients with allergic rhinitis, and that this expression was enhanced by IL-4. However, the studies in which flow cytometry or immunostaining was used did not reveal the expression of FcεRIα on either freshly prepared or IL-stimulated eosinophil samples. Nor did the simultaneous stimulation with other cytokines reveal the occurrence of FcεRIα expression as protein. In nasal mucosa some FcεRIα-positive cells are eosinophil cationic protein (ECP)-positive, which confirms their eosinophilic nature.

METHODS

Monoclonal antibodies and cytokines

Monoclonal antibody (mAb) AER37 (anti-FcεRIα) was prepared as previously described.⁶ An mAb to anti-IL-4 was purchased from Genzyme (Boston, Mass.). Human recombinant IL-5 was kindly provided by Dr. N. Tsuruoka (Suntory Institute for Biomedical Research, Osaka, Japan). Other recombinant cytokines were purchased from Genzyme.

Eosinophil purification

Eosinophils were isolated from the peripheral blood of five patients with nasal allergy (3 male and 2 female subjects; mean age, 21.2 ± 3.9 years) and five normal control subjects (4 male subjects and 1 female subject; mean age, 25.8 ± 4.5 years) (see Table I for clinical details). A diagnosis of nasal allergy was made on the basis of a typical history of perennial nasal allergy symptoms, eosinophilia in nasal smears, and a positive RAST response to house dust and/or mites. The normal

control subjects were selected from among those who met the following requirements: absence of eosinophilia in nasal smears; negative RAST response to house dust, mites, Japanese cedar pollen, ragweed pollen, and mugwort pollen; IgE less than 300 U/ml; and no symptoms of nasal hyperreactivity, such as sneezing and watery nasal discharge.

Sedimentation leukocyte fractions were obtained by using dextran. Eosinophils were then isolated by means of negative immunomagnetic selection with CD16 microbeads and the MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany).⁷ Eosinophils were occasionally purified by using combined density-gradient centrifugation and negative selection with CD16 microbeads. Isolates, consisting of more than 99% eosinophils with a viability of greater than 95%, as judged by using trypan blue exclusion, were used for the gene analysis study. Isolates consisting of more than 97% eosinophils with a viability of greater than 95% were used in the other study.

Cell culture

Eosinophils were cultured in flat-bottomed 24-well plates in RPMI 1640 medium containing 10% fetal calf serum (Hyclone Laboratories, Logan, Utah), 100 U/ml penicillin G, and 100 mg/ml streptomycin at 37° C in the presence of 5% CO₂. Cytokine stimulation of eosinophils was conducted by adding cytokine solution to RPMI 1640 medium. Eosinophils were collected 6 and 24 hours after the addition of cytokines. The expression of FcεRI mRNA in eosinophils was determined 6 hours after the addition of cytokines by using a reverse transcription polymerase chain reaction (RT-PCR) and Southern blot analysis. The surface expression of FcεRIα on eosinophils was determined by flow cytometric analysis and immunohistochemical study, 24 hours after the addition of cytokines.

TABLE II. Sequences used in this study

Gene	Sequence	Expected fragment (bp)
FcεRI α-chain		536
5' primer	5'-CTGTTCTTCGCTCCAGATGGCGT-3'	
3' primer	5'-TACAGTAATGTTGAGGGGCTCAG-3'	
Internal	5'-CCTTGTACACATCCCAGTTCCTCCAACCAT-3'	
Histamine H ₂ R		331
5' primer	5'-CACAGCCTCTTCCTTTTGCC-3'	
3' primer	5'-GTCGAGGCTGATCATGAAGAGG-3'	
Internal	5'-GAAGACCTTGCCAAAGCTCCACTTGCAGGA-3'	
β-actin		305
5' primer	5'-CTTCTACAATGAGCTGCGTG-3'	
3' primer	3'-TCATGAGGTAGTCAGTCAGG-3'	

Collection of samples from inferior nasal mucosa

Samples from turbinal mucosa were collected from a patient with perennial allergy sensitized to house dust and/or mites. Samples from inferior nasal mucosa were obtained during surgery for submucosal resection.

Extraction of total RNA and RT-PCR

RNA extraction, cDNA synthesis, and the RT-PCR were performed essentially as described elsewhere.^{8,9} Briefly, total RNA was extracted by using the guanidium isothiocyanate cesium chloride method, and synthesis of complementary DNA was performed with a cDNA Synthesis Kit (Boehringer, Mannheim, Germany). A cDNA equivalent to 500 ng of total RNA was amplified in a 50 μl reaction volume containing 200 mmol/L (each) of deoxyribonucleoside triphosphates, 3 units of Ampli-taq DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn.), 25 pmol of 5' primer and 3' primer, and a 10-fold PCR buffer (1.5 mmol/L MgCl₂, 50 mmol/L KCl, 10 mmol/L Tris-HCl, and 100 mg of gelatin, pH 8.3). PCR was performed in a DNA thermal cycler (Quick Thermo Systems; Nippon Genetics, Tokyo, Japan). PCR was performed for 30 cycles under the following conditions: (1) pre-denaturing at 94° C for 5 minutes, (2) denaturing at 94° C for 2 minutes, (3) annealing at 55° C for 2 minutes, (4) extension at 72° C for 2 minutes, and (5) denaturing at 94° C for 2 minutes. Steps 3 to 5 were repeated for 32 cycles, then re-extension was done at 72° C for 5 minutes.

The sequences of the sense primers and the antisense primers used in this study are listed in Table II.^{10,11}

Southern blot analysis

Specificity of the PCR product for FcεRI was determined by Southern blot analysis.¹² A portion (10 μl) of the PCR product was electrophoresed with 3% agarose gel, and after denaturing with alkaline solution, was

transfected to a nylon membrane filter for Southern blotting. After transfer, DNA was fixed by UV irradiation, and hybridization was performed by using a human FcεRI probe labeled with ³²P by random primed DNA labeling. The conditions for hybridization were: prehybridization at 42° C for 2 hours and hybridization at 42° C for 1 day. The wash conditions were: 2X (1X standard saline citrate = 150 mmol/L NaCl and 15 mmol/L trisodium citrate, pH = 7.0) and 0.1% sodium dodecylsulfate at room temperature for 20 minutes performed twice, and 0.2X standard saline citrate and 0.1% sodium dodecylsulfate and at 52° C for 15 minutes, performed three times. X-ray film was then exposed to the product for 1 hour.

The sequences of the internal probes used in this study are listed in Table II.

Flow cytometric analysis

For the detection of surface FcεRIα on eosinophils, 5 × 10⁵ eosinophils were incubated with 2% goat serum in 1% bovine serum albumin (BSA)/phosphate-buffered saline (PBS) for 30 minutes at 4° C. Then, fluorescein isothiocyanate-conjugated anti-FcεRIα (AER37, 0.5 mg/10⁶ cells), or fluorescein isothiocyanate-conjugated anti-mouse IgG2b control antibody (Dako, Glostrup, Denmark) in PBS for 30 minutes at 4° C. After washing, cells were analyzed with flow cytometry (EPICS Elite; Coulter Corp., Miami, Fla.). The percent of positive cells at the peak of fluorescence for each sample was determined.

Single-labeling immunostaining procedures

The FcεRIα⁺ human basophil cell line KU812 served as a positive control. KU812 was purchased from Riken Cell Bank (Tsukuba, Japan). Cytopreparations, fixed in 4% paraformaldehyde, were incubated with mAb AER 37 (1:50 dilution) and developed with the avidin-biotin-peroxidase complex method.¹³

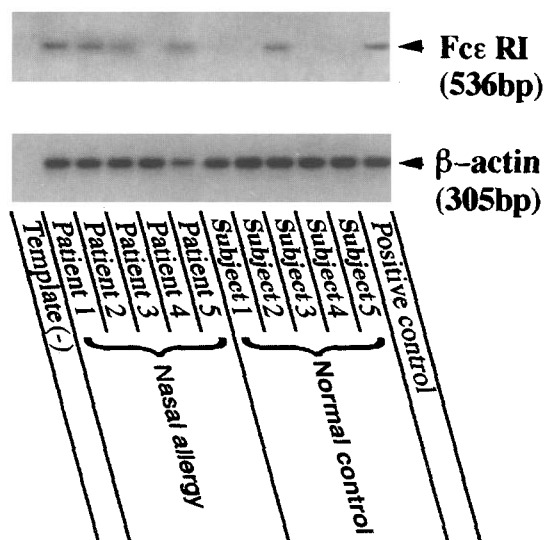


FIG. 1. Expression of FcεRIα mRNA in peripheral blood eosinophils of patients with nasal allergy and normal control subjects. See Table I for clinical details. PCR-assisted Southern blot analysis was performed as described in the text. Total RNA was isolated from fresh peripheral blood eosinophils. Sterilized water was used as a negative control. The human basophil cell line KU-812 was used as a positive control. Amounts of β-actin were adjusted to be almost equal between samples.

Double-labeling immunostaining procedure

Double-labeling immunostaining was performed as follows. Cryostat sections were fixed in 4% paraformaldehyde and rinsed in PBS three times. Then they were incubated overnight at 4°C with anti-FcεRIα mAb (AER37, 1:50) in 5% BSA/PBS. After being washed three times, they were incubated consecutively for 1-hour periods at room temperature with: (1) fluorescein isothiocyanate-conjugated anti-mouse IgG (1:50; Organon Teknika Corp., Durham, N.C.) in 5% BSA/PBS, (2) anti-ECP (Pharmacia, Uppsala, Sweden; 1:10) in 5% BSA/PBS, and (3) rhodamine-conjugated anti-mouse IgG (1:50; Organon Teknika Corp.) in 5% BSA/PBS. After being washed three times, the sections were mounted and viewed with fluorescence microscopy.

Statistical assessment

The results were expressed as means $\pm$ SEM. Statistical significance was determined by using the Student's paired *t* test or the paired Wilcoxon test, and a *p* value of less than 0.05 was considered statistically significant.

RESULTS

FcεRIα mRNA was detectable in four of five samples of eosinophils from those patients with nasal allergy. In contrast, only one of five eosinophil samples from normal subjects expressed FcεRIα mRNA (Fig. 1) (Table I). As expected,

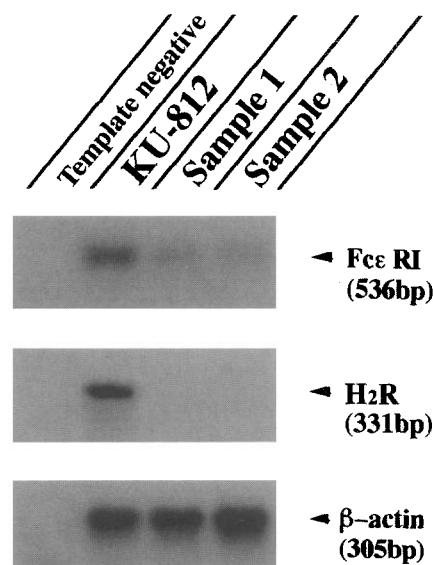


FIG. 2. Expression of FcεRIα mRNA and histamine H₂ receptor mRNA in eosinophil-rich samples. PCR-assisted Southern blot analysis was performed as described in the text. Note that both FcεRIα mRNA and histamine H₂ receptor mRNA are observed in the human basophil cell line KU-812. Peripheral blood eosinophils, however, expressed FcεRIα mRNA but not histamine H₂ receptor mRNA.

KU-812 cells expressed a strong signal for FcεRIα mRNA. To test the possibility that a few basophils were contaminating eosinophil-rich samples and presenting FcεRIα mRNA levels, we carried out PCR for histamine H₂ receptor mRNA, which is considered to be expressed on mast cells and basophils.¹⁴ As shown in Fig. 2, histamine H₂ receptor mRNA expression is detected in KU-812 cells but not in eosinophil-rich samples, thus excluding contaminating basophils as the source of the observed FcεRIα mRNA levels in our samples.

The effect of IL-4 on FcεRI mRNA was investigated by using eosinophils from patients with nasal allergy.

After stimulation for 6 hours with IL-4, eosinophils showed enhancement of FcεRI mRNA levels in five of five samples (representative data shown in Fig. 3, A). FcεRIα mRNA levels reached peak levels within the first 6 hours of stimulation with IL-4 and rapidly returned to baseline levels within the next 18 hours (data not shown). Both IL-5 and GM-CSF weakly upregulated FcεRI mRNA levels in only one of five samples (Fig. 3, B, case 2). Other cytokines such as IL-2, INF-γ, and tumor necrosis factor-α (TNF-α) did not affect FcεRIα mRNA levels (data not shown).

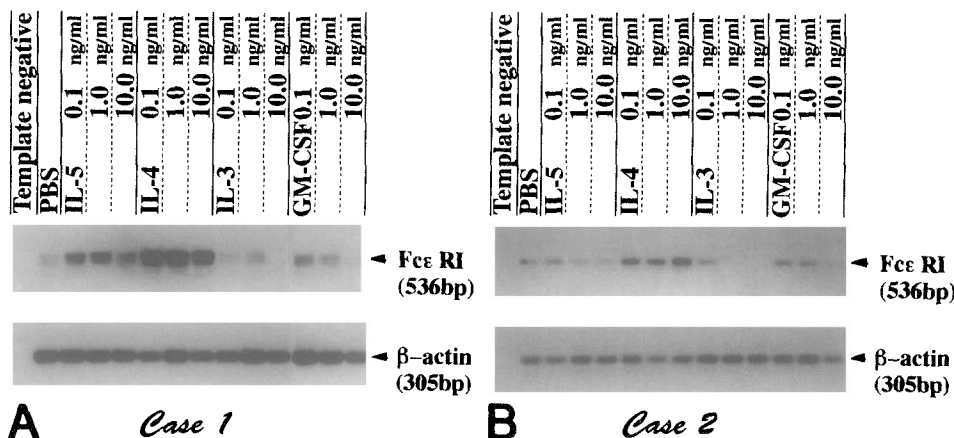


FIG. 3. Effect of cytokines on the expression of FcεRIα mRNA in peripheral blood eosinophils. Representative experiments. (A) Case 1 (nasal allergy). (B) Case 2 (nasal allergy). Total RNA was isolated from peripheral blood eosinophils after incubation for 6 hours with various concentrations of recombinant human IL-5, IL-4, IL-3, and GM-CSF. PCR-assisted Southern blot analysis was performed as described in the text. Sterilized water was used as a negative control. The human basophil cell line KU-812 was used as a positive control. Amounts of β-actin were adjusted to be almost equal between samples.

An mAb to IL-4 suppressed the IL-4-induced enhancement of FcεRIα mRNA levels (Fig. 4).

Flow cytometric analysis revealed that eosinophils from peripheral blood do not express FcεRIα protein. Neither IL-4 alone nor the combinations of IL-4 and other cytokines induce detectable FcεRIα protein; nevertheless, they do express FcεRIα mRNA (Table III). In concordance with these data, an immunohistochemical study showed that the percentage of FcεRIα-positive eosinophils, freshly purified from peripheral blood, was 4.2%. The stimulation with IL-4 alone or with combinations of IL-4 and other cytokines did not increase the percentage of FcεRIα-positive eosinophils (Table IV).

Fig. 5 shows representative samples of FcεRIα-positive eosinophils and negative eosinophils.

In nasal mucosa many FcεRIα-positive cells can be seen from the epithelial layer to the deep layer (Fig. 6, A). No FcεRIα-positive cells were observed when cryostat sections were incubated with isotype-matched control antibody (Fig. 6, B). Some FcεRIα-positive cells exhibited typical eosinophil structure with polylobed nuclei (Fig. 6, C).

Double-labeling experiments on cryostat sections clearly demonstrated that some FcεRIα-positive cells are ECP-positive, confirming their eosinophilic nature (Fig. 7, arrows). Note that not all of the ECP-positive cells express an FcεRIα signal.

DISCUSSION

Recent information has suggested that FcεRI is present not only on mast cells and basophils but

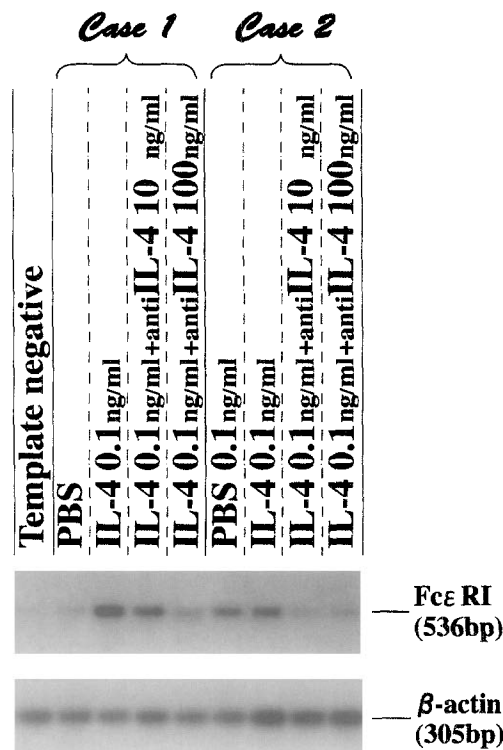


FIG. 4. Effect of anti-monoclonal IL-4 antibody on the IL-4-induced enhancement in FcεRIα mRNA levels. Eosinophils were incubated for 6 hours with 0.1 ng/ml recombinant human IL-4 in the presence or absence of various concentrations of anti-monoclonal IL-4 antibody.

also on other cell types.¹⁻³ Eosinophils were previously considered to have only FcεRII. However, recently, Gounni et al.³ have reported that IgE binding to the eosinophils, purified from the ve-

TABLE III. Effect of cytokines on expression of FcεRI protein in peripheral blood eosinophils

	Control mAb	anti-FcεRI		Control mAb	anti-FcεRI
Freshly purified eosinophil			IL-4 + TNF	2.9 ± 0.4	4.8 ± 1.5
PBS	3.8 ± 0.5	4.0 ± 0.5	IL-4 + IL-5	3.1 ± 1.4	4.5 ± 1.4
IL-5	3.8 ± 0.4	4.2 ± 1.0	IL-4 + IL-3	3.6 ± 1.0	4.5 ± 1.0
IL-4	3.2 ± 0.5	3.5 ± 0.8	IL-4 + GM-CSF	3.1 ± 1.2	4.1 ± 2.0
IL-3	3.0 ± 1.3	5.6 ± 0.6	IL-4 + IL-2	2.7 ± 0.5	4.9 ± 0.5
GM-CSF	3.6 ± 1.0	4.4 ± 0.4	IL-4 + IL-5 + IL-3	3.4 ± 0.3	5.4 ± 1.8
TNF-α	3.5 ± 1.3	3.6 ± 0.6	IL-4 + TNF + IL-2	4.2 ± 0.5	4.2 ± 2.1
IL-2	2.6 ± 0.7	3.4 ± 0.6	IL-4 + IL-5 + GM-CSF	3.0 ± 1.3	5.6 ± 1.9
INF-γ	3.4 ± 0.7	4.3 ± 1.4	IL-4 + IL-3 + IL-5 + GM-CSF	3.6 ± 1.0	4.9 ± 1.0
	2.9 ± 1.2	4.3 ± 1.1	IL-4 + IL-5 + IL-3 + GM-CSF + INF-γ + IL-2	3.5 ± 1.3	5.7 ± 0.9
			KU-812	3.7	49.7

Results of flow cytometry studies are expressed as percent positive cells.

TNF, Tumor necrosis factor.

nous blood of patients with hypereosinophilia, was inhibited by an anti-FcεRIα chain mAb. They also reported that on those eosinophils FcεRI mRNA was expressed and that the surface expression of FcεRIα protein on eosinophils was confirmed by using immunostaining and flow cytometry. They further demonstrated that FcεRI on eosinophils was involved in the release of eosinophil peroxidase and the induction of cytotoxicity against *Schistosoma mansoni*, suggesting that FcεRI had functional activities. These findings are important when we try to understand the possibilities of eosinophils having a link with antigen through FcεRI, which in turn results in the release of eosinophil-derived chemical mediators or granule proteins, and eosinophils having a function as antigen-presenting cells.⁴ Among the tetrameric structures of FcεRI, the α-chain subunit binds IgE with high affinity.¹⁵

In this study we attempted to determine whether the expression of FcεRIα occurred on eosinophils taken from the peripheral blood of patients with allergic rhinitis and normal control subjects. As shown in Fig. 1, FcεRIα mRNA was detectable in four of five samples of eosinophils from those patients with nasal allergy. In contrast, only one of the five eosinophil samples from normal subjects expressed FcεRIα mRNA. Because the RT-PCR assay has extreme sensitivity, we cannot completely exclude the possibility of detection of FcεRIα mRNA derived from basophilic cells, which might have been contaminated in our eosinophil samples. However, the fact that no basophilic cell-derived histamine H₂ receptor¹⁴ mRNA was detected on the eosinophil fraction may allow us to conclude that the majority of FcεRIα mRNA is eosinophil-derived (Fig. 2). These results suggest that there are some physiologic substances that prime eo-

sinophils and upregulate FcεRIα mRNA in the nasal mucosa and/or in the serum of patients with nasal allergy. The expression of the low-affinity IgE receptor (FcεRII/CD23) is known to be subject to regulation by various cytokines; for instance, IL-4, IL-3, and GM-CSF cause enhancement of the expression, whereas INF-γ causes suppression.¹⁵

We then attempted to clarify what regulation was induced on FcεRIα mRNA by cytokine stimulation.

Only IL-4, from among various cytokines, caused the enhancement of FcεRIα mRNA expression (Fig. 3). This enhancement seems to be specific for IL-4, because it was almost completely inhibited in the presence of anti-IL-4 mAb (Fig. 4). IL-4 was initially shown to be strongly involved in IgE production and has been recently shown to have a variety of other actions; IL-4 induces the expression of vascular cell adhesion molecule-1 (VCAM-1) on vascular endothelial cells,¹⁶ possibly promoting the selective infiltration of eosinophils in allergic diseases by enhancing the expression of VCAM-1, because very late antigen-4, a ligand of VCAM-1, is expressed on eosinophils but not on neutrophils.¹⁶ Our recent study has raised the possibility that IL-4 increased the expression of IL-5 receptor α-chain mRNA on eosinophils and accelerated the migration of eosinophils by IL-5¹⁷ (unpublished data). It therefore seems likely that IL-4 acts positively as an enhancer of the adhesion, migration, and degranulation of eosinophils.

Flow cytometric and immunostaining studies, however, failed to reveal the expression of FcεRIα on eosinophils, irrespective of whether samples were freshly prepared or IL-4-stimulated. Nor did simultaneous stimulation with other cytokines reveal an occurrence of FcεRIα expression as protein (Fig. 5, Tables III and IV). The reason that

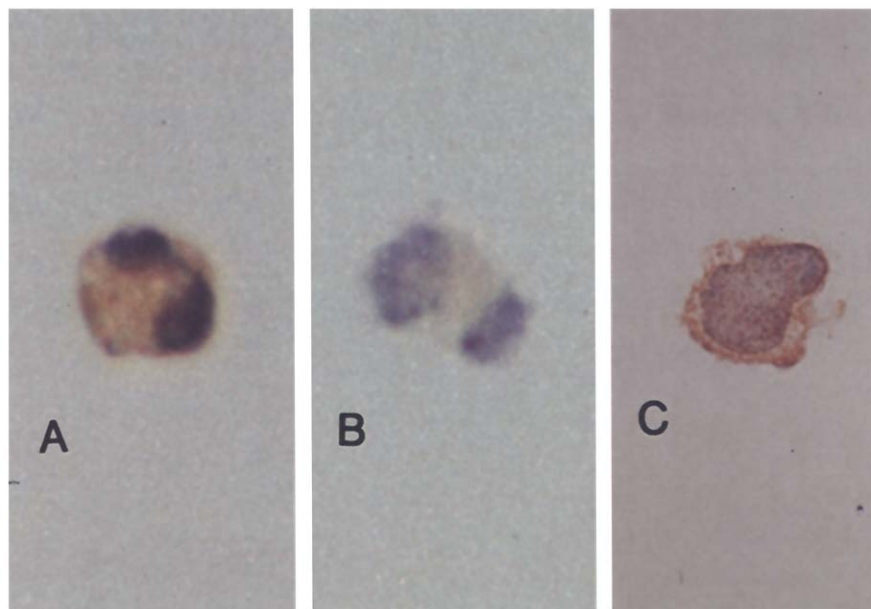


FIG. 5. Immunostaining pattern of FcεRIα-positive eosinophils purified from peripheral blood. Some eosinophils were FcεRIα-positive, as shown in **A**. Almost all eosinophils were FcεRIα-negative (**B**). The FcεRI⁺ human basophil cell line KU812 (**C**) served as a positive control.

TABLE IV. Effect of cytokines on expression of FcεRI protein in peripheral blood eosinophils: Results of immunostaining

	Control mAb	anti-FcεRI		Control mAb	anti-FcεRI
Freshly purified eosinophil	3.6 ± 0.4	4.2 ± 0.3	IL-4 + TNF	4.1 ± 0.2	5.1 ± 1.0
PBS	3.6 ± 0.3	3.9 ± 0.4	IL-4 + IL-5	4.5 ± 1.4	4.9 ± 1.9
IL-5	3.4 ± 0.7	4.6 ± 1.2	IL-4 + IL-3	3.2 ± 1.0	4.9 ± 1.1
IL-4	3.3 ± 0.8	5.8 ± 1.7	IL-4 + GM-CSF	4.5 ± 1.4	5.3 ± 2.0
IL-3	3.7 ± 0.7	4.9 ± 0.9	IL-4 + IL-2	3.9 ± 0.5	4.9 ± 0.5
GM-CSF	4.5 ± 1.2	4.6 ± 0.4	IL-4 + IL-5 + IL-3	4.2 ± 1.2	3.9 ± 1.8
TNF-α	4.6 ± 0.7	5.1 ± 1.4	IL-4 + TNF + IL-2	4.2 ± 0.5	4.2 ± 2.1
IL-2	2.8 ± 0.9	4.5 ± 1.4	IL-4 + IL-5 + GM-CSF	3.8 ± 1.3	5.6 ± 1.9
INF-γ	3.5 ± 0.8	3.8 ± 1.1	IL-4 + IL-5 + IL-3 + GM-CSF + INF-γ + IL-2	4.1 ± 1.4	6.1 ± 1.2
			KU-812	4.5	61.2

Results of assays are expressed as percent positive cells.

peripheral blood eosinophils do not express FcεRIα as protein, even after IL-4 stimulation, is not clear at present. However, the following can be considered as some of the possible causes: (1) low level of mRNA expression, (2) rapid shedding of FcεRI and transformation into a soluble form, (3) occurrence of intracellular but not surface FcεRI expression in peripheral blood eosinophils, and (4) lack of protein synthesis after transcription for some reason. In nasal mucosa many FcεRIα-positive cells could be found from the epithelial layer to the deep layer (Fig. 6, *A*). Some FcεRIα-positive cells exhibited typical eosinophil structure with polylobed nuclei (Fig.

6, *C*). Double-labeling experiments on cryostat sections clearly demonstrated that some FcεRIα-positive cells are ECP-positive and hence to be of eosinophilic nature (Fig. 7). It is of interest to note that ECP-positive cells do not always express the FcεRIα signal. In this regard, we recently demonstrated that EG2-positive cells more frequently express FcεRIα (unpublished data). The rate of EG2-positive cells, constituting one of the indices of eosinophil activation,¹⁷ is 16.5% ± 10% in the peripheral blood of normal subjects, whereas it is as high as 61.7% ± 18% in those with Japanese cedar pollinosis.¹⁸ It is almost 100% in samples of nasal mucosa from

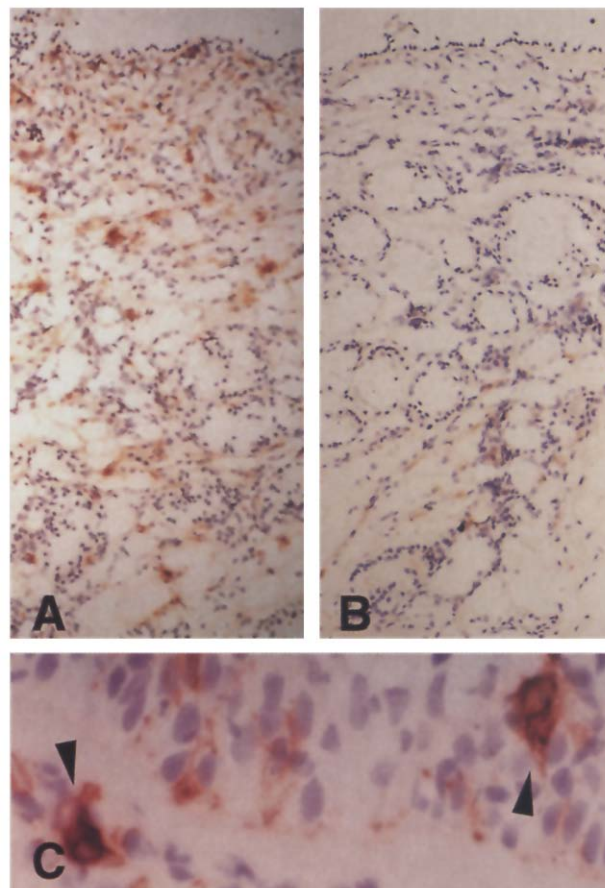


FIG. 6. Demonstration of Fc ϵ RI α -positive cells in cryostat sections of nasal mucosa. Many Fc ϵ RI α -positive cells are visible from the epithelial layer to the deep layer (**A**). Some Fc ϵ RI α -positive cells exhibited typical eosinophil structure with polylobed nuclei (**C**, arrows). No Fc ϵ RI α -positive cells were observed when sections were incubated with isotype-matched control antibody (**B**).

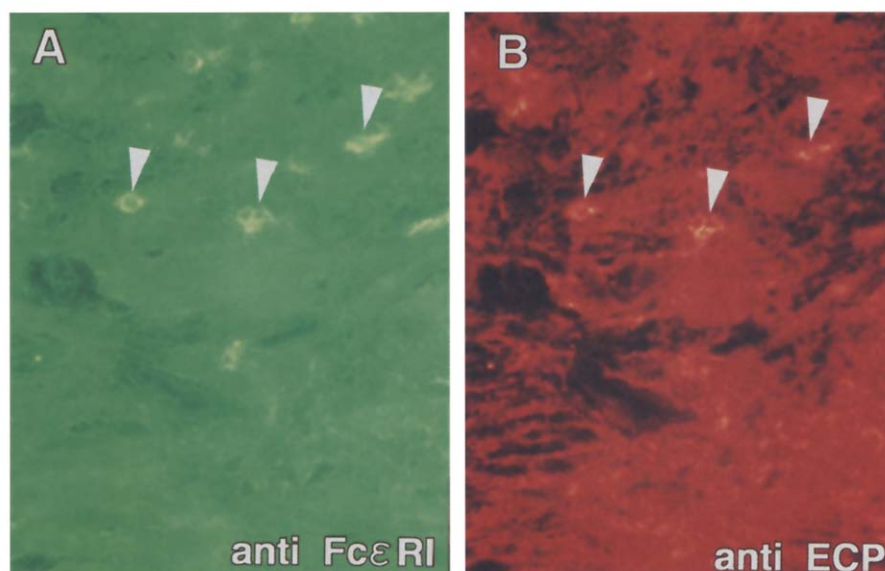


FIG. 7. Demonstration of Fc ϵ RI α and ECP double-positive cells in cryostat sections of nasal mucosa. Double-labeling immunostaining for Fc ϵ RI α and ECP-positive cells was performed as described in the text. Note that some ECP-positive cells are Fc ϵ RI α -positive (arrows).

patients with nasal allergy.¹⁹ The level of FcεRI expression may be correlated with the activation of eosinophils. It can be surmised that some other still unidentified factors may be involved in the expression of FcεRI protein.

The characteristics of FcεRIα-positive eosinophils and their functional role in nasal mucosa remain to be elucidated. Moreover, potentiated FcεRIα expression induced by simultaneous stimulation with cytokines, especially IL-4, and chemical mediators, such as leukotriene and platelet activating factor, must be determined.

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