

Blood eosinophils from atopic donors express messenger RNA for the α , β , and γ subunits of the high-affinity IgE receptor (Fc ϵ RI) and intracellular, but not cell surface, α subunit protein

Susan J. Smith, PhD,^a Sun Ying, MD,^a Qui Meng, MD,^a Mark H. F. Sullivan, PhD,^a Julia Barkans, BSc,^a Onn Min Kon, MD,^a Bhupinder Sihra, MRCP,^a Mark Larché, PhD,^a Francesca Levi-Schaffer, PhD,^b and A. Barry Kay, MD^a London, United Kingdom, and Jerusalem, Israel

Background: Blood eosinophils from hypereosinophilic donors were previously reported to possess the functional high-affinity IgE receptor (Fc ϵ RI), so providing a potential mechanism to account for eosinophil degranulation in atopic allergic disease. Furthermore, tissue eosinophils from allergic tissue reactions were shown to be mRNA⁺ for the α , β , and γ subunits of Fc ϵ RI and gave positive immunostaining with an anti-Fc ϵ RI- α antibody. Recent studies, however, revealed negative surface staining on peripheral blood eosinophils, but intracellular Fc ϵ RI- α protein was identified by Western blot analysis.

Objective: Our purpose was to examine on peripheral blood eosinophils from atopic subjects (1) surface expression and mRNA for Fc ϵ RI- α , (2) up-regulation of Fc ϵ RI- α by allergy-associated tissue factors, and (3) Fc ϵ RI- α -dependent release of eosinophil peroxidase (EPO).

Methods: We measured (1) Fc ϵ RI mRNA expression by *in situ* hybridization, (2) Fc ϵ RI- α by flow cytometry and immunocytochemistry (with use of nonpermeabilized and permeabilized cells), and (3) Fc ϵ RI- α -dependent release of EPO.

Results: Eosinophils from atopic donors had negligible surface expression of Fc ϵ RI- α , which was not enhanced by culture with IgE, IL-3, IL-4, IL-5, GM-CSF, or fibronectin or coculture with fibroblasts. Permeabilization, however, revealed appreciable intracellular staining for Fc ϵ RI- α . The majority of eosinophils were mRNA⁺ for the α , β , and γ subunits of Fc ϵ RI. Small but significant ($P = .03$) increases in α chain mRNA expression were observed after coculture of eosinophils with fibroblasts but not with IgE, IL-4, or fibronectin. Cross-linking of Fc ϵ RI on the surface of eosinophils from atopic donors did not lead to detectable EPO release.

Conclusion: Human blood eosinophils express negligible, non-

functional membrane Fc ϵ RI- α but have intracellular Fc ϵ RI- α protein and mRNA expression for the α , β , and γ subunits. (*J Allergy Clin Immunol* 2000;105:309-17.)

Key words: Eosinophils, high-affinity IgE receptor (Fc ϵ RI), allergy, atopy

Over the years there has been considerable debate regarding the presence and function of the eosinophil membrane-bound high-affinity IgE receptor (Fc ϵ RI). A functional surface Fc ϵ RI would provide eosinophils with a mechanism of degranulation because deposition of granule-derived basic proteins is a characteristic feature of allergic tissue reactions. Previous studies reported that human eosinophils from hypereosinophilic disorders possessed functional Fc ϵ RI.¹ Fc ϵ RI- α immunoreactive eosinophils were also observed in bronchial biopsy specimens from asthmatic patients at baseline,² in bronchoalveolar lavage fluid (BAL) after segmental allergen challenge,³ and in allergen-induced rhinitis.⁴ Furthermore, human tissue eosinophils from allergen-induced late-phase skin reactions expressed messenger RNA (mRNA) for the α , β , and γ subunits of Fc ϵ RI and gave positive immunostaining with anti-Fc ϵ RI- α antibodies.^{5,6} However, eosinophils from the peripheral blood of atopic patients had negligible specific mean fluorescence (smf) for Fc ϵ RI- α , compared with blood basophils and monocytes,⁷ although soluble intracellular Fc ϵ RI- α has been detected by immunoprecipitation and Western blot analysis.⁸

In the current study we have attempted to show that blood eosinophils express mRNA for the α , β , and γ subunits of Fc ϵ RI, confirming our previous findings on tissue eosinophils from human atopic skin.^{5,6} We have also determined whether expression of mRNA for the subunits of Fc ϵ RI or surface expression of Fc ϵ RI- α could be modulated by incubation with IL-3, IL-5, and GM-CSF as well as IgE, fibronectin, and coculture with fibroblasts. We have also confirmed and extended the observations of intracellular Fc ϵ RI- α with use of flow cytometry of permeabilized cells as well as direct visualization of cytopins with use of immunocytochemistry. Finally, because Kita et al⁹ recently reported that eosinophils did not release either leukotriene C₄ or superoxide anion when incubated with either human IgE or antihuman IgE

From the ^aDepartment of Allergy and Clinical Immunology, Imperial College School of Medicine, National Heart and Lung Institute, London, United Kingdom, and the ^bDepartment of Pharmacology, School of Pharmacy, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Supported by the Aimwell Charitable Trust. S. J. S. is a recipient of the Isaac and Myrna Kaye Travelling Fellowship (Imperial College School of Medicine, London, United Kingdom, and the Hebrew University, Jerusalem, Israel).

Received for publication Aug 20, 1999; revised Oct 18, 1999; accepted for publication Oct 20, 1999.

Reprint requests: A. B. Kay, MD, Allergy and Clinical Immunology, Imperial College School of Medicine, National Heart and Lung Institute, Dovehouse Street, London, SW3 6LY, United Kingdom.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/1/103826

Abbreviations used

APAAP:	Alkaline phosphatase antialkaline phosphatase
BAL:	Bronchoalveolar lavage
cDNA:	Complementary DNA
EPO:	Eosinophil peroxidase
FcεRI:	High-affinity IgE receptor
ISH:	In situ hybridization
mfi:	Mean fluorescence intensity
mRNA:	Messenger RNA
PI:	Propidium iodide
smf:	Specific mean fluorescence

antibodies, we investigated whether eosinophils could be triggered to release an eosinophil granule protein, eosinophil peroxidase (EPO), by cross-linking FcεRI-α.

METHODS**Eosinophil purification and culture**

Eosinophils were purified from the peripheral blood of atopic subjects, as previously described, with use of a combination of density gradient centrifugation and immunoselection.¹⁰ The eosinophils were >98% pure, as determined by staining with Kimura, and >97% viable, as determined by trypan blue and propidium iodide (PI) dye exclusion. Cells were cultured (1×10^6 mL⁻¹) for 20 hours in RPMI containing 2 mmol/L L-glutamine, 100 U mL⁻¹ penicillin, and 100 μg mL⁻¹ streptomycin and supplemented with 10% heat-inactivated FCS (Sigma-Aldrich, Dorset, United Kingdom). Culture media were supplemented with IgE (10 μg mL⁻¹), IL-4 (2.5 ng mL⁻¹) (R & D Systems, Oxfordshire, United Kingdom), or plasma fibronectin (100 μg mL⁻¹) (Sigma-Aldrich), or the eosinophils were cocultured with either human lung (MRC-5) or human foreskin (HS27) fibroblasts (0.5×10^6 mL⁻¹), which were obtained from ECACC, Porton Down, United Kingdom.

Preparation of cytopins for immunocytochemistry and in situ hybridization

Cytopins for immunocytochemistry and in situ hybridization were prepared with use of either freshly purified eosinophils or eosinophils that had been cultured for 20 hours. Cytopins were made on Polysine microscope slides (BDH, Essex, United Kingdom) with a Shandon 2 Cytopin (Shandon Southern Instruments, Cheshire, United Kingdom). The eosinophils were resuspended in PBS at a concentration of 1×10^6 cells mL⁻¹ and 100 μL of cell suspension was used per slide and centrifuged on the slide for 3 minutes at 400 revolutions/min.

For immunocytochemistry, the cytopins were air dried at room temperature overnight and then frozen at -20°C before staining. For in situ hybridization (ISH), the cytopins were air dried for 30 minutes, fixed in 4% paraformaldehyde (BDH) in PBS pH 7.4 for 10 minutes, washed with 15% sucrose in PBS, pH 7.4 (Sigma-Aldrich), for 10 minutes, dried at 37°C overnight, and then stored -70°C.

In situ hybridization

The complementary DNA (cDNA) fragments encoding FcεRI-α (bp 25-936), β (bp 43-802), and γ (bp 55-475) subunits were kindly provided by Dr J-P Kinet (Molecular Allergy and Immunology Section, National Institute of Allergy and Infectious Diseases, Rockville, Md). These cDNA fragments of FcεR subunits were

inserted into the appropriate vectors (pGEM) (Promega, Southampton, United Kingdom) and linearized to produce antisense and sense riboprobes. Sulfur 35-labeled riboprobes were prepared with SP6 or T7 RNA polymerases (Promega) to generate antisense or sense probes, respectively. The permeabilization, prehybridization, and ISH protocols were as described previously.^{3,5,11} The slides were counted blind in a coded random order by 2 observers, with a variation of less than 5% between the counts obtained. The binding of riboprobes to the cells was estimated by a modification of the method of Borghi et al.¹² The numbers of silver grains associated with each cell were counted and the data classified into 5 groups dependent on the numbers of associated silver grains: group 1, 5 to 10; group 2, 11 to 20; group 3, 21 to 30; group 4, 31 to 40; and group 5, >41. A minimum of 100 cells in each sample was counted if possible; in the small number of samples with fewer cells present, all cells were counted. A weighted mean¹³ was calculated as follows:

$$\frac{\sum([Group\ number] \times n[cells\ in\ group])}{\sum n}$$

This method has the advantage that cells expressing very high levels of mRNA, above which the silver grains cannot be counted accurately, may still be included within the analysis. This was particularly important for the γ subunit, which was expressed by all cells at high intensity. These weighted means were then compared by the Mann-Whitney *U* test to assess whether different culture conditions affected the expression of any of the FcεRI subunits.

Monoclonal antibodies

Murine mAb 22E7 was a kind gift of Drs R. Chizzonite and J. P. Kochan, Hoffman La Roche, Nutley, NJ,¹⁴ and 3B4 was a kind gift from Professor H. Gould, King's College, London, United Kingdom; like 22E7, this antibody also recognizes a region on human FcεRI-α that is not competitive with IgE.

Flow cytometry

FcεRI-α surface expression on eosinophils was determined with FITC-labeled 22E7 as described.⁷ The eosinophils were washed with PBS containing 0.5% BSA and 0.1% azide and resuspended at a concentration of 1×10^6 mL⁻¹. One hundred microliters of cell suspension was incubated with 10 μL of 100 μg mL⁻¹ of FITC-labeled 22E7 or FITC-labeled mouse IgG1 for 20 minutes at room temperature. The cells were washed with PBS containing 0.5% BSA and 0.1% azide and resuspended in 50 μL of Isoton-II and 150 μL of PI solution at 50 μg mL⁻¹ (Beckman Coulter) and then stored for 15 minutes at 4°C before acquisition on the flow cytometer.

The eosinophils were analyzed on a Coulter Epics Elite flow cytometer. Nonviable eosinophils (as denoted by positive PI staining) were omitted from analyses to exclude any staining that may be the result of intracellular FcεRI-α given increased membrane permeability of nonviable cells.

Determination of intracellular FcεRI-α, by flow cytometry, was performed on purified eosinophils, as previously described.¹⁵

Immunocytochemistry

Cytopins were stained with use of the alkaline phosphatase-antialkaline phosphatase (APAAP) method. The slides were brought to room temperature and fixed in 4% paraformaldehyde in PBS, pH 7.4, for 10 minutes and washed in 15% sucrose in PBS, pH 7.4, for 10 minutes. The cytopins were then stained with either 22E7 or 3B4 at 10 μg mL⁻¹. Rabbit antimouse Ig and APAAP were purchased from Dako.

Briefly, the slides were incubated with the primary mAbs (both used at 10 μg mL⁻¹) in PBS containing 0.1% saponin and 2% normal human serum overnight at room temperature. After the slides

were washed in PBS, they were treated with rabbit antimouse IgG (1:30) for 30 minutes and then APAAP (1:30) for 30 minutes. An isotype-matched (mouse IgG1) mAb of irrelevant specificity and omission of primary layer antibodies were included as negative controls. Positive cells stained red after development with Fast Red (Sigma-Aldrich).

Basophil histamine release assays

The basophil histamine release assay was performed by spectrofluorimetry as described previously.¹⁶ The percentage of histamine release for each control and test substance was calculated from the mean histamine release (in nanograms per milliliter) with the following equation:

$$\text{Percent release} = \frac{(\text{Sample} - \text{Spontaneous})/(\text{Total} - \text{Spontaneous})}{1} \times 100$$

Eosinophil peroxidase release

For the EPO assay Maxisorp Microtitre plates (Gibco Life Technologies, Renfrewshire, United Kingdom) were coated with 100 μL of either human IgA (Sigma-Aldrich) or F(ab')₂ fragments of 22E7 or F(ab')₂ fragments of an IgG1 control antibody (ICN Biologicals, Buckinghamshire, United Kingdom). The antibody fragments were resuspended in PBS (0.01 mol/L, pH 7.4). The antibodies were incubated on the plates overnight at 4°C. The plates were then washed 3 times with PBS and then blocked for 1 hour at room temperature with PBS containing 5% FCS. The plates were subsequently washed with RPMI containing 10% FCS. Eosinophils, resuspended at a concentration of $2 \times 10^6 \text{ mL}^{-1}$ in RPMI containing 10% FCS, were added to the wells, 250 μL per well, and incubated for 3 hours at 37°C. The supernatants were assayed for EPO spectrofluorimetrically as described by White et al.¹⁷

Statistical analysis

Statistical analyses were performed with a statistical package (Minitab for Windows, Minitab Release 9.2, Minitab, State College, Pa). Nonparametric statistics were used throughout the study. The Mann-Whitney *U* test was used for intergroup comparisons.

RESULTS

Highly purified human peripheral blood eosinophils from atopic donors (>98% purity) expressed mRNA for the α , β , and γ subunits of Fc ϵ RI (Table I and Fig 1). The percentages (mean $\pm$ SEM) of eosinophils expressing mRNA were α chain (68.8 ± 2.2), β chain (77.8 ± 3.2), and γ chain (81.1 ± 4.7). After culture of eosinophils for 20 hours with IgE, IL-4, and fibronectin, there were no significant differences in mRNA expression. However, coculture with HS27 fibroblasts, but not combinations of IgE, IL-4, fibronectin, and fibroblasts, resulted in a small but significant up-regulation of α -chain expression ($P = .03$) (Fig 2). Expression of the β and γ chains was not significantly affected by any of these conditions.

There was negligible surface expression of Fc ϵ RI- α on human blood eosinophils, confirming previous work.⁷ However, after permeabilization with saponin there was an increase in fluorescence with all preparations of eosinophils studied stained by 22E7 ($n = 6$), smf (median and range) 0.81 (0.41-1.00) compared with surface Fc ϵ RI- α expression 0.11 (0.10-0.12) (Figs 3 and 4). Clear immunostaining with anti-Fc ϵ RI- α antibodies

(22E7 and 3B4) was also observed with permeabilized, but not with unpermeabilized, peripheral blood eosinophil cytopins (Fig 5).

Surface expression of Fc ϵ RI- α was not enhanced after incubation for 20 hours with IgE, IL-4, fibronectin, or HS27 (Fig 6) or MRC-5 fibroblasts (data not shown). The smf of the eosinophils stained with FITC-labeled 22E7, whether freshly isolated (data not shown) or after 20 hours in culture with various supplements (Fig 6), was very low (smf always <0.5, typically ~0.2) compared with basophils (smf ~50-110). Similarly, IL-3, IL-5, and GM-CSF did not cause up-regulation of surface expression of Fc ϵ RI- α , whereas a marked increase in smf of the eosinophil activation marker CD69 was observed (Fig 7).

When eosinophils were incubated on wells coated with F(ab')₂ antibody fragments of 22E7, there was no detectable release of EPO (ie, <0.3 $\mu\text{g mL}^{-1}$ of EPO/10⁶ cells). In contrast, IgA-stimulated eosinophils released an average of 6.31 $\mu\text{g mL}^{-1}$ of EPO/10⁶ cells ($n = 6$) when incubated on wells coated with 20 $\mu\text{g mL}^{-1}$ human IgA (Fig 8). Basophils released up to 56% of their total histamine (Fig 8) when incubated in suspension with F(ab')₂ fragments of 22E7. Under these culture conditions (ie, cells in suspension) there was no detectable release of EPO from purified blood eosinophils.

DISCUSSION

In this report we have demonstrated that eosinophils have intracellular stores of Fc ϵ RI- α by both flow cytometry and immunocytochemistry. With use of these techniques we have shown direct localization of this receptor to the eosinophil, which is not possible with the technique of Western blotting, as previously reported.⁸ Furthermore, comparison of the fluorescence intensity of basophils and permeabilized eosinophils that have been incubated with a fluoro-chrome-labeled anti-Fc ϵ RI- α antibody and analyzed by flow cytometry, indicates that the intracellular stores of Fc ϵ RI- α in eosinophils are considerably less (1%-2%) than those expressed on the surface of basophils. We also demonstrate that the majority of blood eosinophils express mRNA for Fc ϵ RI- α , β , and γ subunits. Coculture of the eosinophils with fibroblasts significantly up-regulated mRNA Fc ϵ RI- α expression. However, there was no significant up-regulation of Fc ϵ RI- α on the surface of the eosinophils under the same culture conditions, so therefore we conclude that the allergy-associated factors, which cause up-regulation of Fc ϵ RI- α on mast cells and basophils, do not enhance receptor expression on eosinophils. Although we could not enhance the expression of Fc ϵ RI- α , we still attempted to determine whether the membrane-bound receptor was functional. By the use of an antibody against Fc ϵ RI- α to directly cross-link the receptor, we were unable to detect EPO release. Thus we show by direct methods that Fc ϵ RI- α is present in negligible levels on the surface of peripheral blood eosinophils from atopic subjects, although there are intracellular stores, and that this receptor is nonfunctional in terms of EPO release.

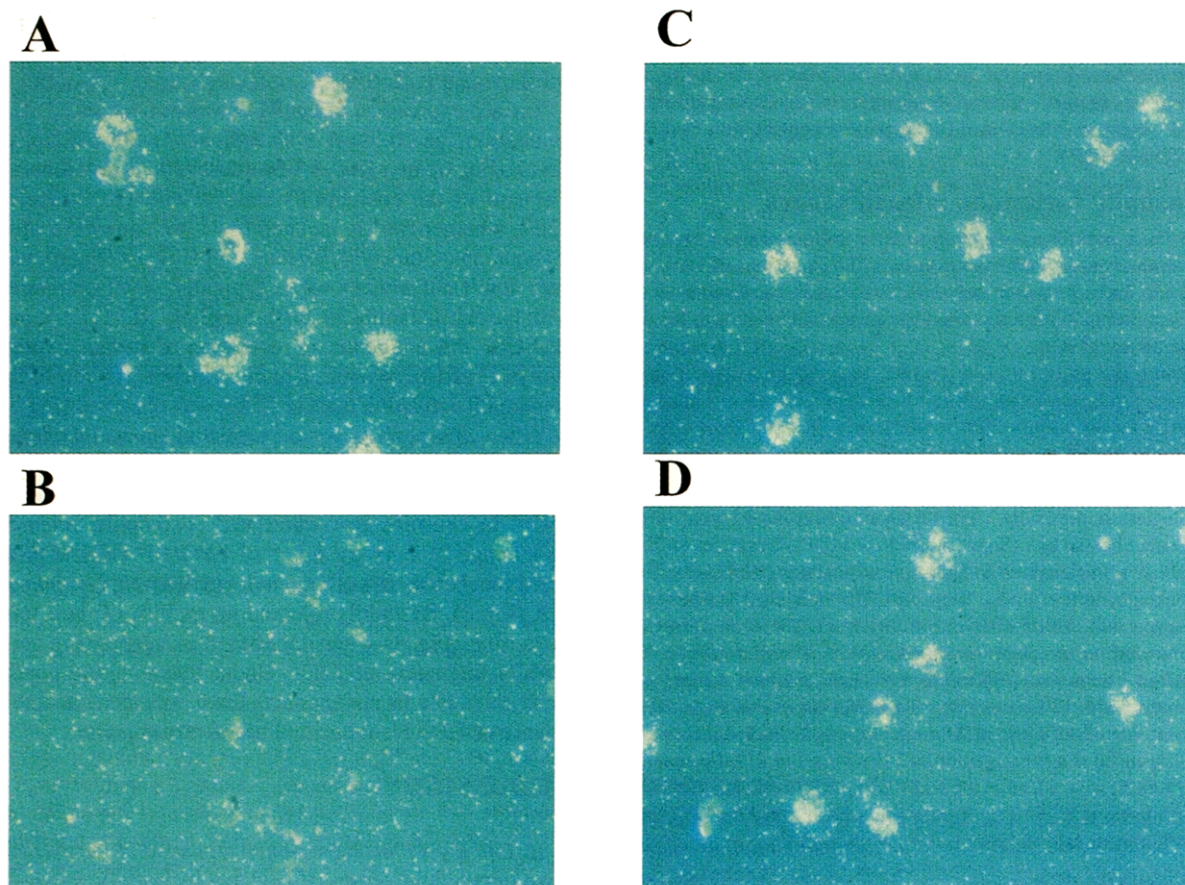


FIG 1. ISH of purified eosinophils. mRNA for the 3 subunits of FcεRI were detected with antisense cDNA riboprobes for the α, β, and γ chains (**A**, **C**, and **D**, respectively). **B**, Corresponding sense control for α chain.

TABLE I. Percentage of eosinophils expressing mRNA transcripts for 3 subunits (α, β, and γ) of human FcεRI

Subject No.	α	β	γ
1	69.2	85.3	87.7
2	78.3	87.4	93.2
3	61.7	81.7	70.5
4	66.7	71.8	71.3
5	69.0	70.1	70.8
6	67.6	70.3	93.2
Mean ± SEM	68.8 ± 2.2	77.8 ± 3.2	81.1 ± 4.7

Eosinophils were either freshly purified (subjects 1 and 2) or cultured in RPMI containing 10% FCS for 20 hours (subjects 3 to 6). Results are expressed as mean ± SEM.

Previous studies indicated that there was negligible surface expression of FcεRI on eosinophils compared with monocytes and basophils in flow cytometry with use of either a competitive (15-1) or noncompetitive (22E7) mAb to FcεRI-α.⁷ However, eosinophils infiltrating allergen-induced late-phase cutaneous reactions in human atopic subjects were mRNA⁺ for the α, β, and γ chains of FcεRI.⁵ Furthermore, the FcεRI-α protein colocalized to approximately 50% to 80% of eosinophils at all the time points studied (6, 24, and 48 hours). It was

unclear, however, whether immunoreactivity with the anti-α chain antibodies on fixed tissue sections reflected surface or intracellular α chain protein. To address this question, we have used peripheral blood eosinophils from atopic donors (1) to determine whether mRNA for the α, β, and γ subunits are present in circulating cells and (2) to compare surface with intracellular immunoreactivity with use of anti-FcεRI-α antibodies. Thus we confirmed that blood eosinophils, like tissue eosinophils, express mRNA for the 3 subunits (Table I and Fig 1). Attempts to modify mRNA expression by culturing in IgE, IL-4, fibronectin, or fibroblasts revealed a small but significant increase in α chain expression by coculture with fibroblasts. The biologic significance of this observation is uncertain but might suggest limited capacity to up-regulate α chain mRNA expression in a tissue environment. We were unable however, to confirm the finding of Terada et al,¹⁸ who observed that blood eosinophils from subjects with atopic rhinitis expressed mRNA for FcεRI-α, but not the protein, and that this was up-regulated by incubation with IL-4.

We confirmed our previous flow cytometry data, which indicated that human eosinophils have extremely low surface expression of FcεRI.⁷ Similar findings have recently been reported by Kita et al,⁹ who also found

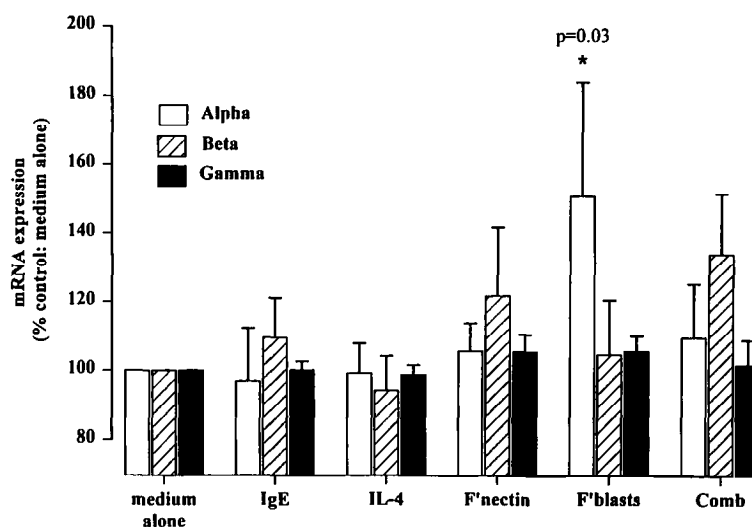


FIG 2. mRNA expression for 3 FcεRI subunits, α, β, and γ, on eosinophils cultured for 20 hours under different conditions (see Methods for details). Combination of reagents included coculture of eosinophils with human foreskin fibroblasts (HS27) in medium supplemented with both IgE (10 μg mL⁻¹) and IL-4 (2.5 ng mL⁻¹).

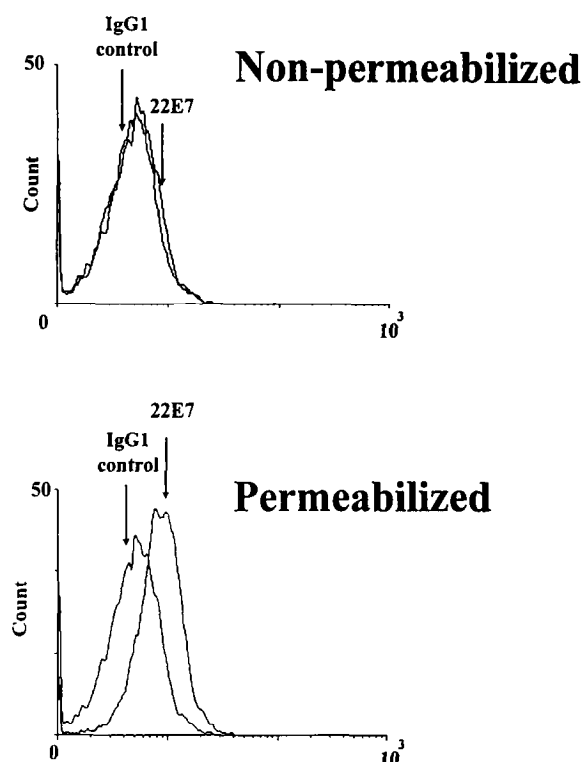


FIG 3. Example of flow cytometry of permeabilized and nonpermeabilized peripheral blood eosinophils stained with anti-FcεRI-α. Purified, freshly isolated eosinophils were stained with 22E7 or IgG1 isotype control and detected with phycoerythrin-labeled goat antimouse IgG.

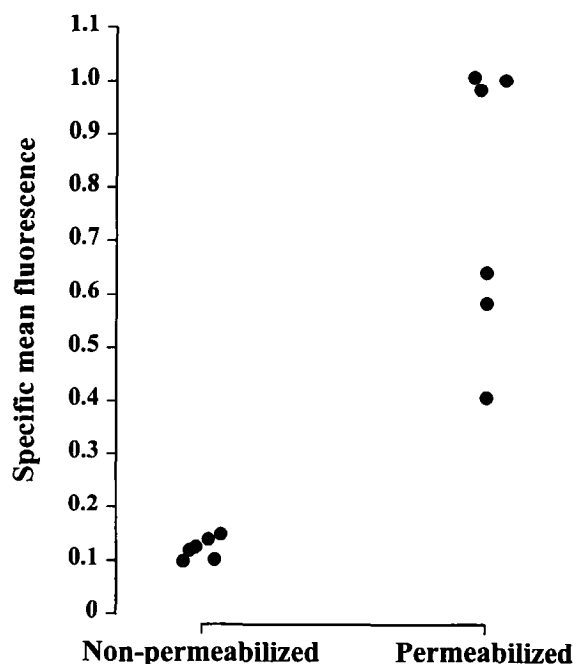


FIG 4. Flow cytometry of permeabilized and nonpermeabilized peripheral blood eosinophils. Results are expressed as smf of 22E7, which is calculated as the difference between mean fluorescence intensity (mfi) of IgG1 control and mfi of 22E7. Mean smf of nonpermeabilized eosinophils was 0.123 compared with 0.771 for permeabilized cells ($P = .005$).

minimal amounts of surface expression of FcεRI. Furthermore, Seminario et al⁸ have found little or no cell surface IgE or high-affinity IgE receptors, as analyzed by immunofluorescence and flow cytometry. These investi-

gators also observed that culture of eosinophils for up to 11 days in the presence, or absence, of IgE or IL-4 (conditions that enhanced FcεR on other cells)^{19,20} failed to induce any detectable surface FcεR on eosinophils. Again, we have confirmed these findings by incubating cells for shorter periods of time (ie, 20 hours) with IgE

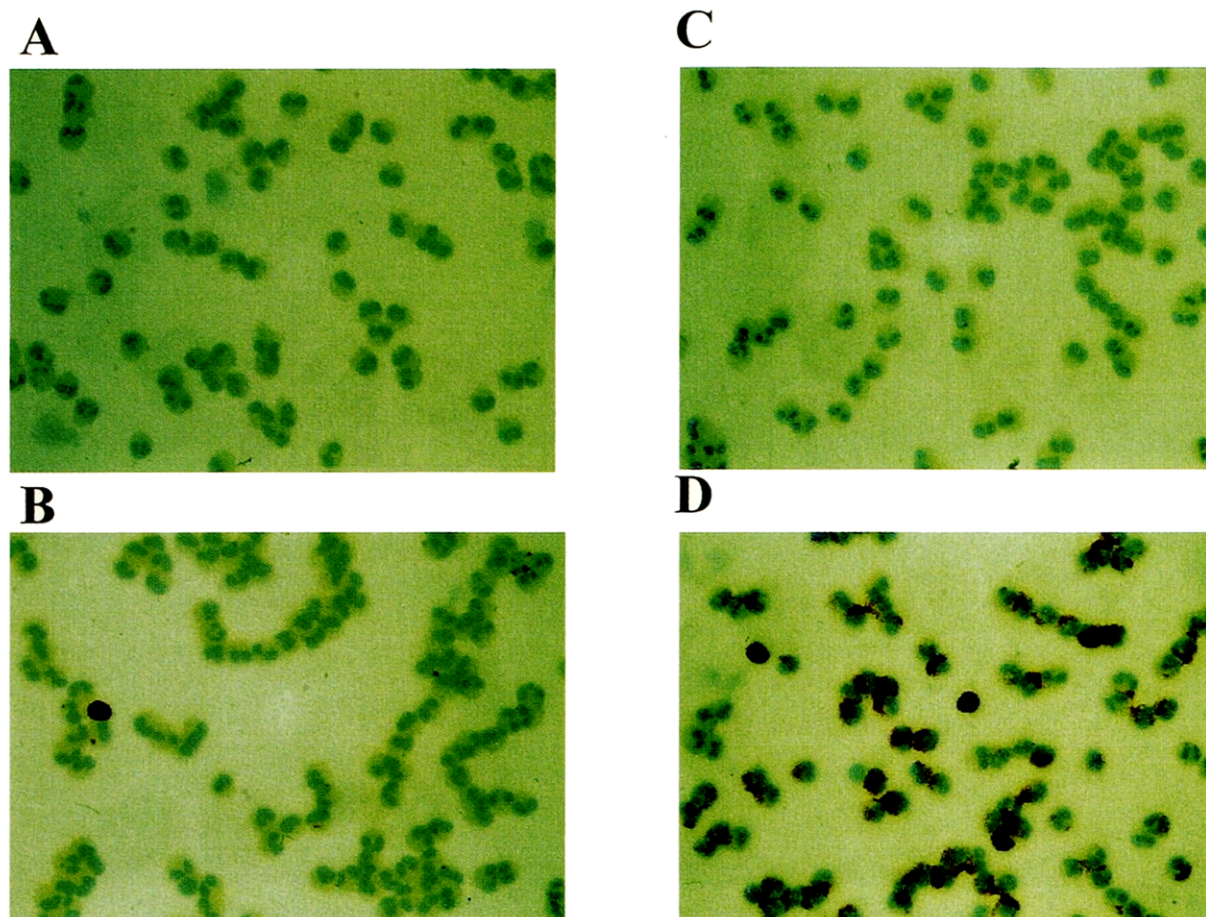


FIG 5. Immunocytochemistry of permeabilized and nonpermeabilized eosinophils. Peripheral blood eosinophil cytopins stained with IgG1 control or 22E7 without (**A** and **B**) and with (**C** and **D**) saponin permeabilization. Staining of a single cell having morphologic appearances of a basophil is observed in nonpermeabilized staining with 22E7 preparation (**B**). In contrast, after permeabilization with saponin, eosinophils are clearly staining with 22E7 (**D**) but not with IgG1 control (**C**).

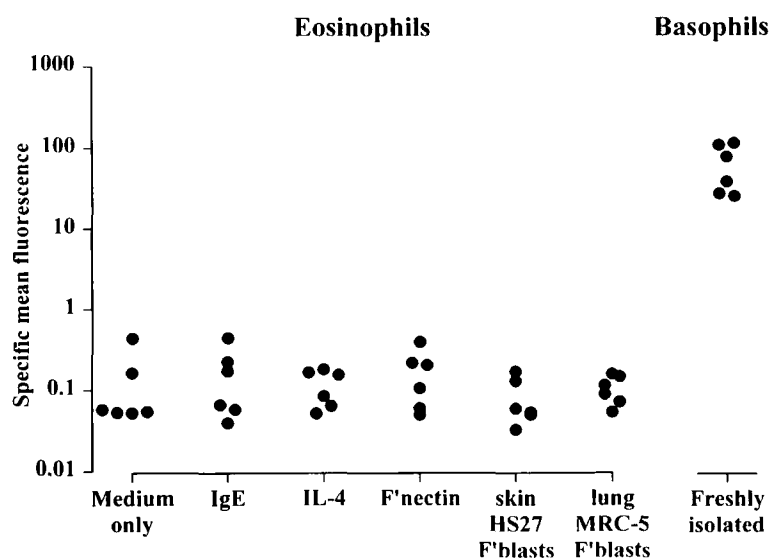


FIG 6. Expression of α subunit of Fc ϵ RI on eosinophils under differing culture conditions (see Methods for details) compared with freshly isolated basophils.

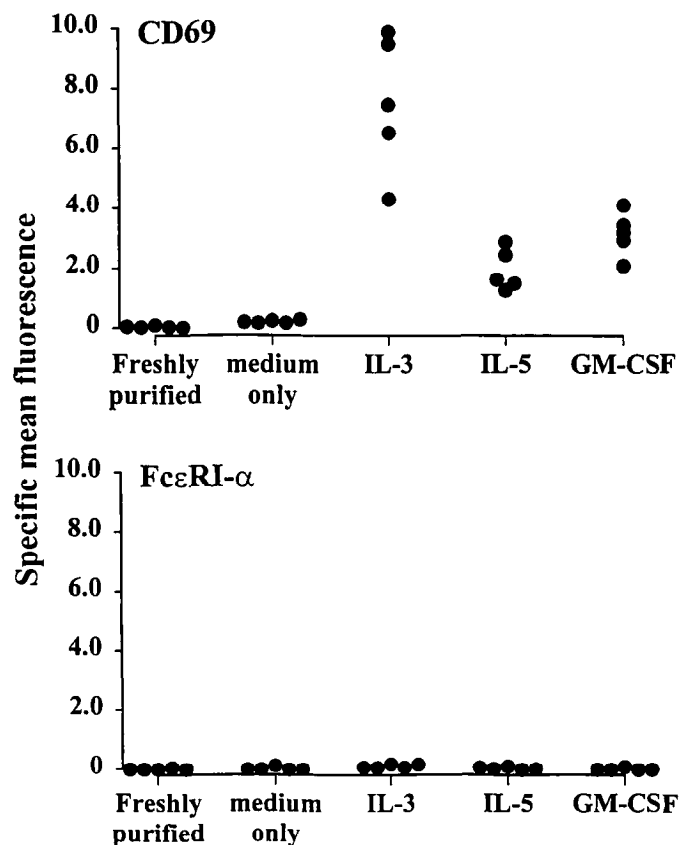


FIG 7. Effect of IL-3, IL-5, and GM-CSF on surface expression of eosinophils that were cultured for 20 hours in presence of 10 ng mL⁻¹ of either IL-3, IL-5, or GM-CSF compared with culture in medium alone (RPMI containing 10% FCS) and freshly isolated. Cells were stained with FITC-labeled 22E7 or FITC-labeled IgG1 isotype control. Results are expressed as smf of 22E7, which is calculated as difference between mfi of IgG1 control and mfi of 22E7.

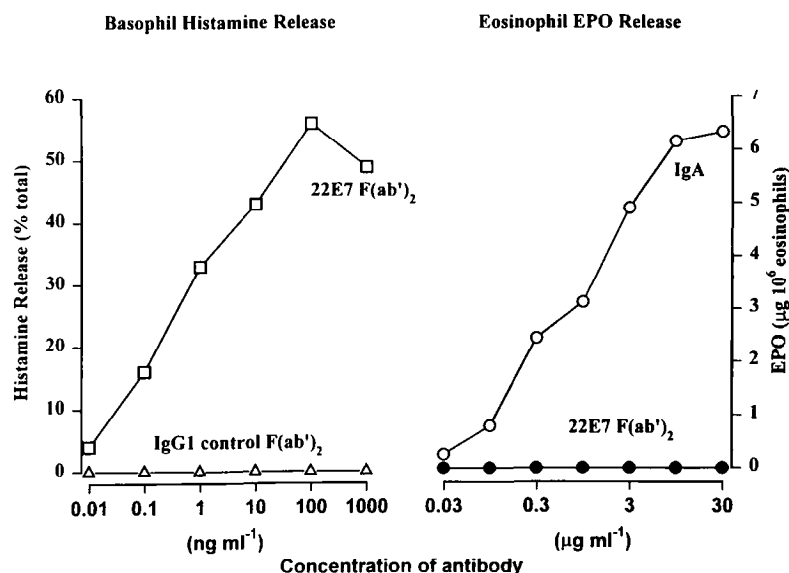


FIG 8. Histamine release from basophils and EPO release from eosinophils. Eosinophils were incubated in microtiter plates coated with either human IgA (open circles) or F(ab')₂ fragments of 22E7 (closed circles), n = 6. Histamine release from PBMCs containing basophils incubated with F(ab')₂ fragments of 22E7 (open squares) or IgG1 (open triangles), n = 5.

and IL-4 and with tissue elements including fibronectin or fibroblasts. Thus attempts to mimic tissue conditions do not appear to increase cell surface expression of FcεRI. However, expression of FcεRI-α protein could readily be detected by the use of permeabilized cells with either flow cytometry (Figs 3 and 4) or immunohistochemistry (Fig 5). This confirms our previous work in which FcεRI-α was detectable on cytopins from eosinophils from BAL cells obtained after allergen challenge.³ In the current study we show that permeabilization of human blood eosinophil cytopins was necessary for positive eosinophil staining with 22E7, whereas previously BAL cells did not require permeabilization. This may simply reflect greater fragility of the BAL cells in respect of penetration of the antibody. Thus we have confirmed the findings of Seminario et al⁸ on intracellular expression of FcεRI-α by human eosinophils. These workers used immunoprecipitation and Western blot analysis of eosinophil lysates with an mAb specific for FcεRI-α, whereas we used both flow cytometry of permeabilized cells as well as the direct approach of immunocytochemistry.

A previous report indicated that FcεRI on eosinophils was functional in terms of killing parasitic larvae.¹ However, Kita et al⁹ found that IgE neither bound to nor activated eosinophils from patients with allergic diseases. Our current work confirms the findings of Kita et al in respect to nonfunctional surface FcεRI-α. Thus F(ab')₂ anti-FcεRI-α antibody fragments did not release detectable amounts of EPO from eosinophils, although it did induce dose-dependent histamine release from basophils. Nevertheless, appreciable EPO release was observed from peripheral blood eosinophils with use of IgA-coated wells as the stimulus.

In summary, eosinophils express mRNA for the α, β, and γ subunits of FcεRI. Although there was negligible surface expression of FcεRI-α, there was clear intracellular staining of permeabilized cells, as shown both by flow cytometry and immunocytochemistry. The significance of intracellular FcεRI-α is uncertain. One possibility is that it may regulate the biologic effect of IgE by binding and neutralizing its action, although this is speculative and requires confirmation. One way to reconcile the observation of lack of surface FcεRI with that of the previous study by Soussi-Gouni et al¹ is to conclude that eosinophils from atopic individuals are substantially different from eosinophils in the hypereosinophilic disorders. Because blood and tissue eosinophils, even from atopic subjects, express mRNA for the 3 subunits of FcεRI, there may be posttranslational mechanisms present in rare eosinophilic disorders that are not observed with eosinophils in allergic disease. Nevertheless, our data suggest that FcεRI is unlikely to be important in eosinophil degranulation in atopic allergic disease, although we cannot exclude the possibility that tissue eosinophils, as opposed to blood eosinophils, have functional FcεRI.

We thank Drs Brigitte Haselden, Nasreen Khan, Alison Macfarlane, and William Oldfield and Ms Karen Shirley for the clinical

classification of the subjects used and Dr Douglas Robinson for critical review of the manuscript. We also thank Professor Hannah Gould, King's College, London, United Kingdom, for the chimeric mouse-human IgE antibody and for the mAb recognizing FcεRI-α, called 3B4. Drs Richard Chizzonite and Jarema Kochan, Hoffman La Roche Inc., Nutley, NJ, generously provided the anti-FcεRI-α mAb, called 22E7. The cDNA fragments encoding FcεRI-α (bp 25-936), β (bp 43-802), and γ (bp 55-475) subunits were kindly provided by Dr Jean-Pierre Kinet, Molecular Allergy and Immunology Section, National Institute of Allergy and Infectious Diseases, Rockville, Md. We thank Dr Amanda Bennett and Professor Martin Church (University of Southampton, United Kingdom) for the histamine measurements.

REFERENCES

1. Soussi-Gouni A, Lamkhioued B, Ochiai K, Tanaka Y, Depaport E, Capron A, et al. High affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 1994;367:183-6.
2. Humbert M, Grant JA, Barata LT, Durham SR, Pfister R, Menz G, et al. High affinity IgE receptor (FcεRI)-bearing cells in bronchial biopsies from atopic and non-atopic asthma. *Am J Respir Crit Care Med* 1996;153:1931-7.
3. Rajakulasingam K, Till S, Ying S, Humbert M, Barkans J, Sullivan M, et al. Increased expression of high affinity IgE receptor (FcεRI) α chain mRNA and protein bearing eosinophils in human allergen-induced atopic asthma. *Am J Crit Care Med* 1998;158:233-40.
4. Rajakulasingam K, Durham SR, O'Brien F, Humbert M, Barata LM, Reece L, et al. 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. *J Allergy Clin Immunol* 1997;100:78-86.
5. Barata LT, Ying S, Grant JA, Humbert M, Barkans J, Meng Q, et al. Allergen-induced recruitment of FcεRI⁺ eosinophils in human atopic skin. *Eur J Immunol* 1997;27:1236-41.
6. Ying S, Barata LT, Meng Q, Grant JA, Barkans J, Durham SR, et al. High-affinity immunoglobulin E receptor (FcεRI)-bearing eosinophils, mast cells, macrophages and Langerhans' cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *Immunology* 1998;93:281-8.
7. Sihra BS, Kon OM, Grant JA, Kay AB. Expression of high affinity IgE receptors (FcεRI) on peripheral blood basophils, monocytes and eosinophils in atopic and non-atopic subjects: relationship to total serum IgE concentrations. *J Allergy Clin Immunol* 1997;99:699-706.
8. Seminario M-C, Saini SS, MacGlashan DW, Bochner BS. Intracellular expression and release of FcεRI-α by human eosinophils. *J Immunol* 1999;162:6893-900.
9. Kita H, Kaneko M, Bartemes KR, Weiler DA, Schimming AW, Reed CE, et al. Does IgE bind to and activate eosinophils from patients with allergy? *J Immunol* 1999;162:6901-11.
10. Hansel TT, Pound JD, Pilling D, Kitas GD, Salmon M, Gentle TA, et al. Purification of human blood eosinophils by negative selection using immunomagnetic beads. *J Immunol Methods* 1989;122:97-103.
11. Ying S, Meng Q, Barata LT, Corrigan CJ, Barkans J, Assoufi B, et al. Human eosinophils express messenger RNA encoding RANTES and store and release biologically active RANTES protein. *Eur J Immunol* 1996;26:70-76.
12. Borghi H, Calle A, Sesboue R, Bourgiugnon J, Diarra-Mehrpour M, Martin JP. Light microscopical detection of inter-alpha-trypsin inhibitor and its different mRNAs in cultured hepatoma Hep G2 cells using immunocytochemical and in situ hybridisation techniques. *Histochem J* 1994;26:252-61.
13. Spiegel MR. Schaum's outline series: theory and problems of statistics. 2nd ed. London: McGraw-Hill; 1988.
14. Kochan J, Pettine L, Hakimi K, Kinet JP. Isolation of the gene coding for the alpha subunit of the human high affinity receptor. *Nucleic Acid Res* 1988;16:3584.
15. Kon OM, Sihra BS, Till SJ, Corrigan CJ, Kay AB, Grant JA. Unstimulated basophils in atopic and nonatopic subjects express intracellular interleukin-4: detection by flow cytometry. *Allergy* 1998;53:891-6.

16. Hasleden BM, Kay AB, Larché M. Immunoglobulin E-independent major histocompatibility complex restricted T cell peptide-induced late asthmatic reactions. *J Exp Med* 1999;189:1885-94.
17. White SR, Kulp GVP, Spaethe SM, Alstyne EV, Leff AR. A kinetic assay for eosinophil peroxidase activity in eosinophils and eosinophil conditioned media. *J Immunol Methods* 1991;44:257-63.
18. Terada N, Konno A, Terada Y, Fukada S, Yamashita T, Abe T, et al. IL-4 upregulates FcεRI α-chain messenger RNA in eosinophils. *J Allergy Clin Immunol* 1995;96:1161-9.
19. Saini SS, MacGlashan DW, Sterbinsky SA, Togias A, Adelman DC, Lichtenstein LM, et al. Down-regulation of human basophil IgE and FcεRI-α surface densities and mediator release by anti-IgE infusions is reversible in vitro and in vivo. *J Immunol* 1999;162:5624-30.
20. Yamaguchi M, Lantz CS, Oettgen HC, Katone IM, Fleming T, Miyajima I, et al. IgE enhances mouse mast cell FcεRI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J Exp Med* 1997;185:663-72.