

Regulation of Surface FcεRI Expression on Human Eosinophils by IL-4 and IgE

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Key Words

Eosinophils · FcεRI · IgE · IL-4 · Flow cytometry

Abstract

Background: Recent studies have demonstrated that eosinophils from allergic patients express low levels of FcεRI on their surface, but the regulatory mechanisms of eosinophil surface FcεRI expression are not fully understood. We investigated whether IL-4 and IgE, which are reported to regulate surface FcεRI expression on human mast cells, are able to affect surface FcεRI expression in normal human eosinophils. **Methods:** Eosinophils purified from peripheral blood were cultured with IL-5 and with or without IL-4 and/or IgE, and surface FcεRI expression was analyzed by flow cytometry using an anti-FcεRI mAb, CRA-1. **Results:** Apparent FcεRI expression (~1% of mast cell FcεRI levels) was observed in eosinophils cultured with both IL-4 and IgE. A combination of IL-4 (≥ 1 ng/ml) and IgE (≥ 0.5 μg/ml) was necessary for the maximal induction of surface FcεRI expression. In the presence of IL-4 and IgE, eosinophils cultured for 2 days demonstrated low but statistically significant levels of

surface FcεRI, which reached a plateau after 7 days of culture. However, cross-linkage of surface FcεRI molecules by CRA-1 or anti-IgE did not induce any eosinophil activation. **Conclusions:** IL-4 and IgE can affect the levels of surface FcεRI on normal human eosinophils. FcεRI expression on eosinophils may be regulated by a mechanism similar to that in mast cells.

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Introduction

Numerous studies have shown that eosinophils are active participants in allergic diseases as well as helminth infections [1–3]. Locally activated eosinophils are an important source of chemical mediators, including cytotoxic granule proteins and lipid mediators; through their release, this cell type is believed to play a proinflammatory role [1–4]. In allergic diseases such as asthma, tissue eosinophilia and locally secreted eosinophil granule proteins are characteristic features, which are related to the severity of the allergy [5]. In addition, patients with helminth infections demonstrate deposition of an eosinophil-de-

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rived cytotoxic protein, major basic protein, around the parasites [4]. Although mediator release is a critical event in the expression of the biological function of eosinophils, the mechanisms accounting for eosinophil activation *in vivo* have not been fully determined.

While it is clear that mast cells and basophils express a high-affinity receptor for IgE (FcεRI) on their surface and exert antigen- and IgE-dependent functions, other cell types such as Langerhans cells, monocytes, dendritic cells and megakaryocytes/platelets have also been reported to express functional FcεRI [6–12]. Several studies have demonstrated that human eosinophils are also FcεRI-bearing cells [13–19]. It has been shown that FcεRI can be detected at low levels on the surface of blood eosinophils from patients with allergy [15] or marked eosinophilia [13]. In 1994, Gounni et al. [13] reported that eosinophils from subjects with eosinophilia showed surface FcεRI expression, and that cross-linked FcεRI on the surface can transduce activation signals in eosinophils leading to IgE-dependent cell-mediated cytotoxicity and mediator release. Although these findings indicate that human eosinophils can express surface FcεRI, the regulatory mechanism of their surface FcεRI expression has not yet been identified. Furthermore, it is not known whether upregulation of surface FcεRI expression can be induced in eosinophils from normal donors. Based on our previous reports that IL-4 and IgE strongly upregulate surface FcεRI expression in mast cells [20], we investigated whether these same factors are also capable of regulating surface FcεRI expression in normal human eosinophils.

Materials and Methods

Reagents

The reagents used in the experiments were mostly the same as described previously [20]. Myeloma human IgE and human rIL-4 were purchased from Biodesign (Kennebunk, Maine, USA) and Peprotech (London, UK), respectively. Human rIL-5 was kindly donated by the Suntory Institute of Biomedical Research (Mishima, Osaka, Japan). For the detection of FcεRI expression, we used a mouse mAb, CRA-1, which can bind to the human FcεRI α chain whether or not it is occupied by IgE [20, 21]. A murine isotype-matched mAb, IgG2bκ (MOPC 141), which usually demonstrates almost no nonspecific binding to eosinophils, was purchased from ICN Pharmaceutical (Aurora, Ohio, USA). Recombinant soluble FcεRI α chain (rsFcεRIα) was prepared as described previously [22, 23].

Cell Preparation and Culture

Human eosinophils were isolated from venous blood drawn from consenting volunteers with no history of allergic diseases, such as bronchial asthma, allergic rhinitis, atopic dermatitis, and chronic

urticaria. The eosinophils were purified by Percoll density gradient centrifugation followed by negative selection using anti-CD16 magnetic beads, as described previously [24]. The purity and viability of the eosinophil preparations were >99 and >95%, respectively. After washing three times, purified eosinophils were resuspended in RPMI 1640 medium (GIBCO, Grand Island, N.Y., USA) supplemented with 10% FCS (Dainippon Pharmaceutical, Osaka, Japan), rIL-5 at 10 ng/ml, and, from GIBCO BRL, 2 mM *L*-glutamine, antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) and antimycotics (0.4 µg/ml amphotericin B). The cells were then cultured in 12- or 24-well flat-bottom culture plates (IWAKI Glass, Chiba, Japan) with or without human IL-4 and/or IgE at 37 °C for 7 days unless otherwise specified.

Flow-Cytometric Analysis for FcεRI

Cells were harvested and washed twice with PBS supplemented with 3% FCS and 0.1% NaN₃, and then incubated with CRA-1 or control mAb (IgG2bκ) at 5 µg/ml for 60 min on ice. The cells were washed and stained with FITC-conjugated F(ab')₂ fragment of goat anti-mouse IgG at 7 µg/ml (fluorophore/protein ratio on a molar basis: 2.7, Jackson ImmunoResearch Labs, West Grove, Pa., USA) for 30 min on ice. The stained cells were then washed, and the fluorescence intensity of each cell was measured by flow cytometry (EPICS XL System II, Coulter, Tokyo, Japan). The median values of fluorescence intensity of the eosinophils were converted to the number of the molecules of equivalent soluble fluorochromes (MESF) using Quantum 25 microbeads (Flow Cytometry Standards, San Juan, P.R., USA) on each day an experiment was performed, according to the specifications of the manufacturer [20].

In some experiments, surface IgE binding on eosinophils was analyzed using FITC-conjugated goat anti-human IgE (Biosource, Camarillo, Calif., USA). FITC-conjugated goat IgG (Jackson ImmunoResearch) was used as negative control.

Assay of Mediator Release and Calcium Influx

RPMI 1640 supplemented with 0.1% human serum albumin (HSA) was used for mediator release reactions. Following culture for 7 days, eosinophils were sensitized with myeloma IgE at 1 µg/ml for 2 h at 37 °C, washed and added to plastic plates containing CRA-1, control mouse IgG2b, goat anti-human IgE (Medical & Biological Laboratories, Nagoya, Japan) or ionophore A23187 (Calbiochem, La Jolla, Calif., USA). The plates were then incubated at 37 °C for 4 h, and the supernatants were harvested following centrifugation. The concentrations of eosinophil-derived neurotoxin (EDN) were measured using a RIA kit (Pharmacia, Uppsala, Sweden). EDN release was expressed as the percentage of the total cellular EDN content. The concentrations of leukotriene (LT) C₄ in supernatants were measured using an EIA kit (Cayman, Ann Arbor, Mich., USA). All measurements were performed at least in duplicate.

Ca²⁺ influx in eosinophils was assessed using Fura-2 AM (Dojindo, Tokyo, Japan) and measured with a fluorescence spectrometer (F-2500, Hitachi, Tokyo, Japan).

Statistical Analysis

Data are presented as the mean ± SEM. The statistical significance of differences between various treatment groups was assessed by the two-tailed Student's *t* test.

Results

Surface Expression of FcεRI on Normal Human Eosinophils Cultured with IL-4 and IgE

In the first series of experiments, we examined whether surface expression of FcεRI was observed on eosinophils from non-allergic donors by flow-cytometric analysis. However, surface FcεRI was below detectable levels in fresh eosinophils from each of the 4 tested donors (data not shown). In the case of human cultured mast cells, recent reports demonstrated that IgE upregulates the expression of FcεRI, and that IL-4 acts synergistically with IgE to enhance surface FcεRI expression [20]. IL-4 is also reported to enhance FcεRIα mRNA levels in human mast cells [25] and eosinophils [26]. Based on these findings, we next cultured eosinophils with or without IgE (at 1 μg/ml) and/or IL-4 (at 10 ng/ml), and the surface expression of FcεRI was analyzed by flow cytometry. As shown in figure 1, no significant expression of FcεRI was observed in eosinophils cultured for 7 days without IL-4 or IgE, or eosinophils cultured with IL-4 alone, or IgE alone (fig. 1a), although minimal levels of FcεRI expression cannot be ruled out through the interference by the high autofluorescence of eosinophils. In some donors (2 out of 6 tested donors), eosinophils demonstrated a somewhat enhanced level of surface FcεRI expression after culture with IL-4 alone (note the slightly greater difference in the height of IgG2b-marked bars and CRA-1-marked bars in fig. 1b). On the other hand, apparent FcεRI expression was consistently detected on eosinophils cultured for 7 days with a combination of IL-4 and IgE (fig. 1a, 1b, 2a), and the levels of FcεRI expression indicated in MESF values over negative control cells varied from 630 to 1,580 among the 6 subjects. To confirm that CRA-1 mAb specifically recognizes the surface FcεRI expressed on eosinophils, we examined whether the binding of CRA-1 mAb to eosinophils was inhibited by rsFcεRIα. Following culture for 7 days with IL-4 plus IgE, eosinophils were harvested, washed and preincubated with or without various concentrations of rsFcεRIα for 10 min at 4°C, before adding CRA-1. As shown in figure 2b, rsFcεRIα at 5 μg/ml and higher concentrations almost completely inhibited CRA-1 binding to eosinophils cultured with both IL-4 and IgE. Pretreatment with rsFcεRIα at 5 and 25 μg/ml also strongly inhibited CRA-1 binding to human basophils (data not shown). These results imply that induction of CRA-1-binding ability in eosinophils by culture with IL-4 and IgE for 7 days reflects surface expression of FcεRI.

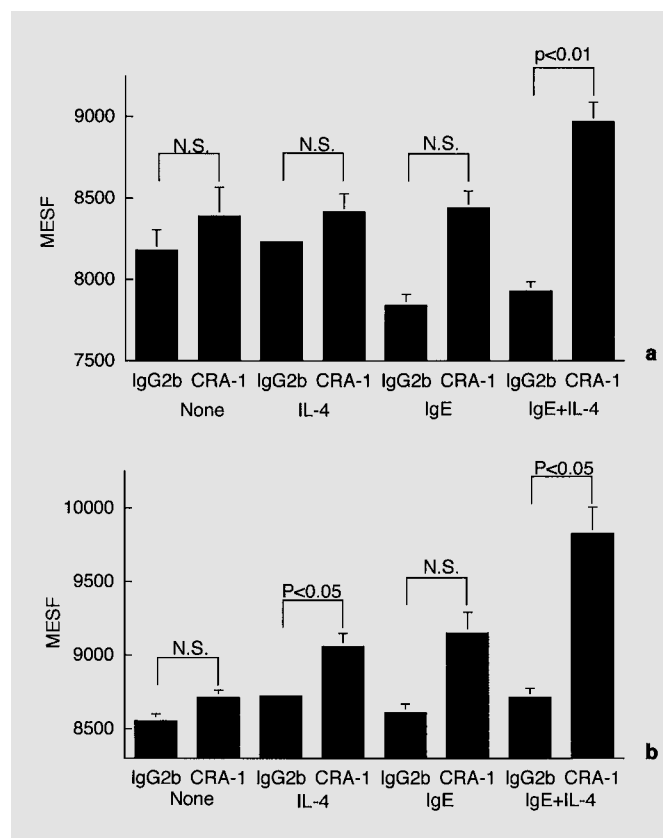


Fig. 1. The surface CRA-1-binding capacity of eosinophils is regulated by IL-4 and IgE. Purified eosinophils from non-allergic donors were cultured with or without IL-4 (10 ng/ml) and/or IgE (1 μg/ml) for 7 days at 37°C. Cells were incubated with CRA-1 or control mAb (IgG2bκ) for 60 min on ice, stained with FITC-conjugated F(ab')₂ fragment of goat anti-mouse IgG for 30 min, and analyzed by flow cytometry. The median values of fluorescence intensity of eosinophils were converted to MESF units. **a** and **b** are two representative results of six experiments using eosinophils from 6 separate donors. Bars represent the SEM of triplicate determinations.

Requirement of Both IL-4 and IgE for Maximal Induction of Surface FcεRI Expression in Eosinophils

We previously reported that even 0.05 μg/ml of IgE was able to slightly enhance the surface expression of FcεRI in cultured human mast cells [20]. A similar situation was observed with eosinophils. When added together with IL-4 at 10 ng/ml, IgE dose-dependently enhanced the surface FcεRI level of eosinophils, and significant FcεRI expression was observed in eosinophils cultured for 7 days with IgE at 0.05 μg/ml compared with that in eosinophils cultured without IgE, but with IL-4 (fig. 3). The greatest enhancement of FcεRI expression on eosinophils was observed when IgE at 0.5 μg/ml or higher concentra-

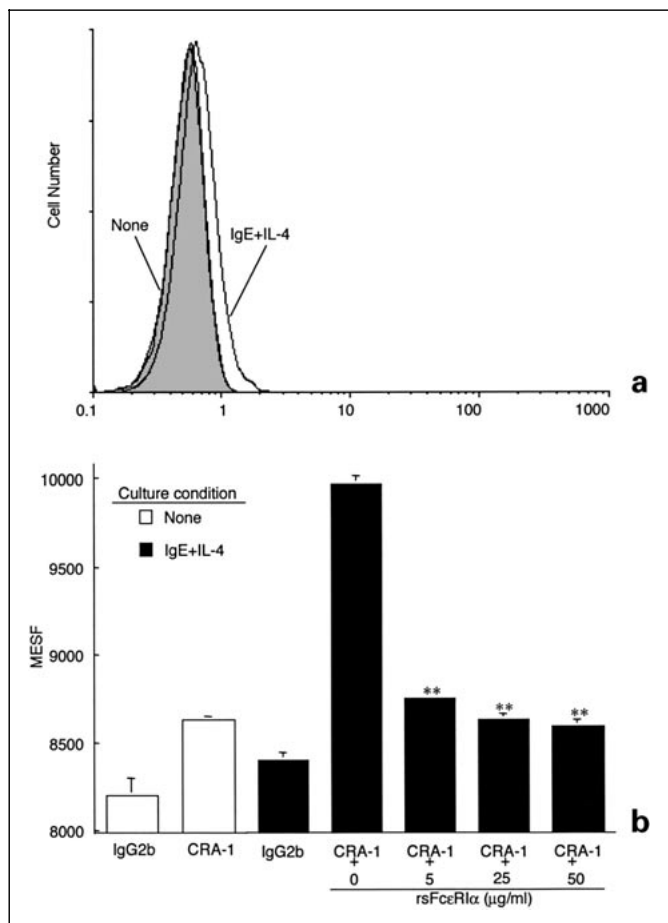


Fig. 2. **a** Analysis of surface expression of FcεRI on cultured eosinophils. Purified eosinophils were cultured for 7 days without ('none') or with as (IgE + IL-4) IgE (1 μg/ml) plus IL-4 (10 ng/ml). Solid lines indicate the fluorescence of eosinophils stained using CRA-1 mAb. Negative control stain of cells using control IgG2b mAb instead of CRA-1 is shown as shaded area. Data shown are representative of six separate experiments, and fluorescence intensities of the cells converted to MESF units are included in **b**. **b** Recombinant soluble FcεRIα blocks the binding of CRA-1 to eosinophils. Eosinophils cultured with IL-4 and IgE for 7 days (closed columns) were incubated with or without rsFceRIα at the indicated concentrations for 10 min at 4°C, prior to addition of CRA-1. Cells were then washed twice, stained and analyzed by flow cytometry. The MESF values of cells cultured with neither IL-4 nor IgE are also indicated (open columns). IgG2b in the figure denotes MESF values of cells stained with control IgG2b mAb instead of CRA-1 mAb before flow cytometry. Bars represent the SEM of triplicate experiments. ** $p < 0.01$, vs. MESF of eosinophils cultured with IL-4 and IgE, but not treated with rsFceRIα before flow cytometry.

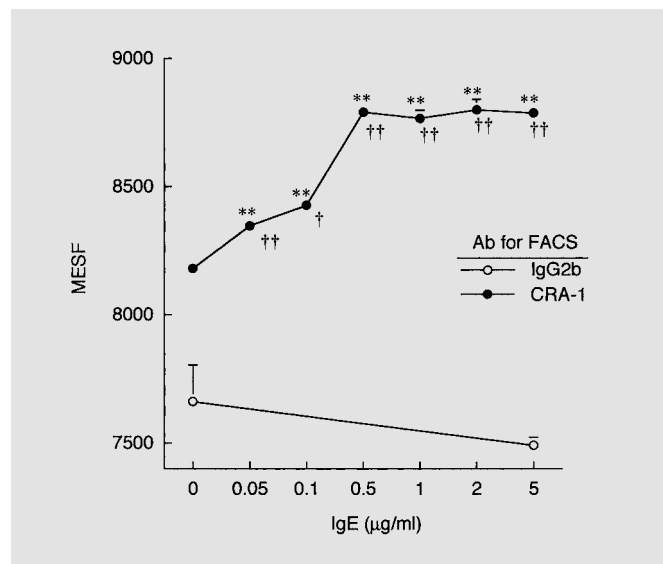


Fig. 3. Dose-dependent effects of IgE on surface FcεRI expression in eosinophils. Eosinophils were cultured for 7 days at 37°C with IL-4 (10 ng/ml) and IL-5 (10 ng/ml) in the presence or absence of IgE at the indicated concentrations. Data are representative of two separate experiments using eosinophils from separate donors, showing similar results. All data are mean $\pm$ SEM of triplicate experiments. ** $p < 0.01$, vs. MESF of cells stained with control IgG2b following 7-day culture without IgE. † $p < 0.05$, †† $p < 0.01$, vs. MESF of cells stained with CRA-1 following culture without IgE.

tions was included in culture medium supplemented with IL-4 (10 ng/ml).

In the next series of experiments, we tested the effects of various concentrations of IL-4 on surface FcεRI expression, using eosinophils cultured for 7 days with or without IgE at 1 μg/ml (fig. 4). As assessed by flow cytometry, MESF values for cells cultured with IL-4 alone (10–100 ng/ml) were only slightly higher than those for cells cultured without IL-4. However, when IL-4 at 0.1–100 ng/ml was added to the culture medium together with IgE at 1 μg/ml, eosinophils exhibited significantly elevated levels of surface FcεRI expression, with the maximal enhancement of surface FcεRI induced by IL-4 at 1–100 ng/ml.

We then examined the time course of the effects of IL-4 and/or IgE. As shown in figure 5, obvious enhancement of surface FcεRI expression was observed after 2-day culture of eosinophils with IL-4 (10 ng/ml) and IgE (1 μg/ml), and the surface FcεRI level continued to increase gradually with time. In eosinophils cultured for 14 days with IL-4 and IgE, however, FcεRI expression was not higher than in cells cultured for 7 days (data not shown). In eosino-

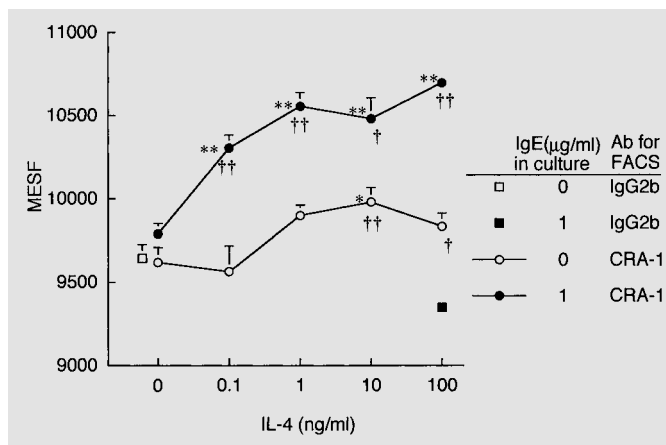


Fig. 4. Dose-dependent effects of IL-4 on surface FcεRI expression in eosinophils. Eosinophils were cultured for 7 days at 37°C with or without IgE (1 μg/ml), in the presence or absence of IL-4 at the indicated concentrations. Bars represent the SEM of triplicate experiments. * $p < 0.05$, ** $p < 0.01$, vs. MESF of eosinophils stained with control IgG2b following 7-day culture. † $p < 0.05$, †† $p < 0.01$, vs. MESF of cells stained with CRA-1 following culture with the same concentration of IgE (i.e. 0 or 1 μg/ml), but without IL-4. Another experiment showed similar results.

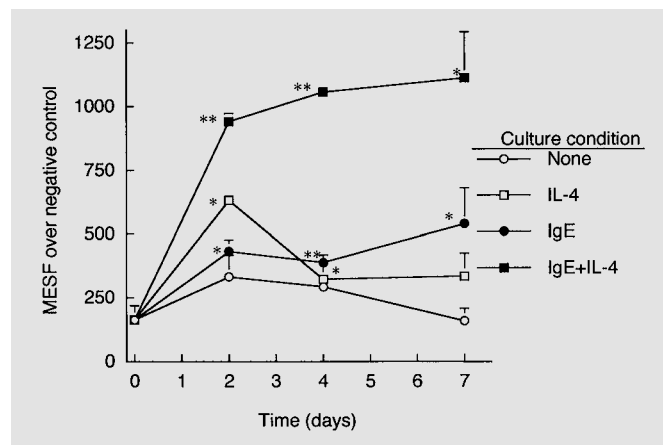


Fig. 5. Time course of surface FcεRI expression on eosinophils cultured with or without IL-4 and/or IgE. Eosinophils were cultured with or without IL-4 (10 ng/ml) and/or IgE (1 μg/ml) for up to 7 days, and surface FcεRI expression was analyzed by flow cytometry. Data are expressed as specific MESF units of eosinophils calculated by subtracting the MESF value of corresponding cells stained with control IgG2b analyzed at each time point of the experiment. Data are mean $\pm$ SEM of triplicate experiments. * $p < 0.05$, ** $p < 0.01$, vs. specific MESF of eosinophils on day 0. Another experiment showed similar results.

Table 1. Analysis of EDN release from eosinophils stimulated with CRA-1 Ab or ionophore A23187

Stimulus	Culture condition			
	none	IL-4	IgE	IgE+IL-4
Control mouse IgG2b	12.1 $\pm$ 2.0	9.5 $\pm$ 1.8	15.2 $\pm$ 2.4	9.7 $\pm$ 1.4
CRA-1	7.8 $\pm$ 1.0	6.7 $\pm$ 1.0	10.9 $\pm$ 1.6	7.0 $\pm$ 0.7
Ionophore A23187	22.8 $\pm$ 0.9	22.6 $\pm$ 2.4	24.1 $\pm$ 2.2	19.6 $\pm$ 0.8

Purified eosinophils were cultured with or without IL-4 (10 ng/ml) and/or IgE (1 μg/ml), in the presence of IL-5 (10 ng/ml) at 37°C for 7 days. The cultured eosinophils were sensitized with myeloma IgE at 1 μg/ml for 2 h at 37°C, washed and stimulated with CRA-1 Ab (10 μg/ml), control mouse IgG2b Ab (10 μg/ml) or ionophore A23187 (1 μg/ml) for 4 h at 37°C. Data show the percentage release of the total EDN content of the eosinophils (mean $\pm$ SEM of three experiments using cells from separate donors).

phils cultured with IL-4 alone, slight enhancement of FcεRI was detected on day 2, but thereafter surface FcεRI decreased with time. Culture with IgE alone demonstrated only marginal enhancement of surface FcεRI expression in eosinophils. Essentially identical results were observed with eosinophils from another donor.

Analysis of Function of FcεRI on Eosinophils

To investigate the function of FcεRI expressed on eosinophils, we examined Ca²⁺ influx in cultured eosinophils. However, no Ca²⁺ influx was observed in eosinophils, cultured for 7 days with IL-4 (10 ng/ml) and IgE (1 μg/ml), in response to cross-linkage of surface FcεRI by CRA-1 mAb [27], or to further treatment with goat anti-mouse IgG Ab (data not shown). In accordance with the absence of Ca²⁺ influx, a granule-associated mediator,

EDN, was not released from eosinophils cultured for 7 days under any of the four conditions, i.e., culture medium with or without IL-4 and/or IgE, followed by stimulation with CRA-1 mAb (table 1). Although IgE binding to the surface of eosinophils cultured with IgE plus IL-4 was confirmed by flow cytometry, again no EDN release was detected following stimulation with goat IgG anti-human IgE (anti-IgE-induced maximal release: 9.4%; spontaneous release: 9.0%, $n = 2$). On the other hand, Fc ϵ RI-independent stimulation with Ca ionophore A23187 induced both Ca²⁺ influx and the release of EDN from cultured eosinophils (table 1). Cross-linkage of Fc ϵ RI by CRA-1 also failed to induce the release of a lipid mediator, LTC₄, from eosinophils cultured for 7 days with or without IL-4 and/or IgE, although ionophore A23187 elicited apparent LTC₄ release from cultured eosinophils (data not shown).

Discussion

Although surface Fc ϵ RI expression was hardly detectable on normal human blood eosinophils, we have observed that several days of incubation of the cells with IL-4 and IgE resulted in low but significant levels of surface Fc ϵ RI expression. This enhanced expression of Fc ϵ RI in eosinophils cultured in the presence of IL-4 plus IgE, as demonstrated by increased binding of anti-Fc ϵ RI α mAb CRA-1, was confirmed by the finding that rsFc ϵ RI α efficiently inhibited CRA-1 binding to eosinophils (fig. 2b). Interestingly, eosinophils cultured with both IL-4 and IgE exhibited a potent, sustained increase in the surface Fc ϵ RI level, whereas cells cultured with IL-4 alone or IgE alone did not. These findings indicate that IL-4 and IgE acted synergistically on eosinophils to induce surface Fc ϵ RI expression. Recently, Seminario et al. [28] reported that not only eosinophils cultured with either IL-4 or IgE, but also cells cultured with both factors failed to induce any detectable surface Fc ϵ RI. Although our findings on the action of IL-4 alone or IgE alone on eosinophil Fc ϵ RI expression are in line with their results, their findings show a clear contrast to the results presented herein in terms of the effects of IL-4 plus IgE; this may be explained by the differences in the experimental protocols for detecting surface Fc ϵ RI [19]. Our flow-cytometric procedure, which includes semiquantification of cellular fluorescence, may have enabled us to detect low but significant expression of Fc ϵ RI on the surface of eosinophils. Thus, in future studies analyzing variations or in vivo regulatory mechanisms of the surface Fc ϵ RI level on normal

human peripheral eosinophils [19], a more sensitive assay method will be appropriate.

Induction of surface Fc ϵ RI expression by IL-4 and IgE was consistently observed in all of the eosinophil preparations obtained from 6 healthy, non-allergic donors, although the maximal level of Fc ϵ RI varied among the donors. These results suggest that the demonstration of surface Fc ϵ RI on blood eosinophils in previous reports [13, 15] may be related to soluble factors, including IL-4 and IgE. Indeed, the presence of surface Fc ϵ RI on blood eosinophils was demonstrated in patients with atopy [15] and marked eosinophilia [13]. Although the serum concentrations of IL-4 and IgE were not reported in those studies, it is reasonable to speculate that the predominance of IL-4 and IgE in allergic diseases or parasitic infections may favor the expression of Fc ϵ RI on eosinophils, leading to a more complex interplay of effector cells in the pathogenesis of allergic inflammation, or to an enhanced host response to parasites.

However, we could not detect any function of eosinophil Fc ϵ RI because neither a granule-associated mediator EDN release, lipid mediator LTC₄ release nor Ca²⁺ influx was observed in eosinophils cultured with IL-4 and IgE followed by stimulation with CRA-1 mAb or anti-IgE Ab. Our results are in clear contrast to the findings of Gounni et al. [13] who showed that eosinophils from hypereosinophilic patients express surface Fc ϵ RI and elicit Fc ϵ RI-mediated mediator release and antibody-dependent cell-mediated cytotoxicity [13]. The difference between our results and their findings may be due to several reasons. First, the level of surface Fc ϵ RI detected in this study may be too low to cause Fc ϵ RI-dependent eosinophil activation. Compared with cultured human mast cells demonstrating a surface Fc ϵ RI level of $\sim 10^5$ to 10^6 MESF units as assessed by flow cytometry using CRA-1 mAb [20], cultured eosinophils possessed very low levels (up to 1.7×10^3 MESF units as demonstrated by the specific binding of CRA-1) of surface Fc ϵ RI (presumably $<1,000$ receptors/cell), i.e. as low as 1% or less of those of mast cells. Such a low level of surface Fc ϵ RI may be insufficient to transduce a signal leading to stimulation of eosinophils derived from normal donors. Secondly, we cannot formally exclude the possibility that some factors other than IL-4 or IgE are present in allergic subjects and induce enhancement of the surface Fc ϵ RI level, and/or augmentation of the mediator release of eosinophils. Analyzing the effect of IL-5, which was included in the culture medium to enhance the survival of eosinophils, on the action of IL-4 and IgE on Fc ϵ RI will be of importance. Further study is necessary to clarify this point.

While the regulatory mechanisms of FcεRI expression in a variety of FcεRI⁺ cells have not been fully elucidated, IL-4 and/or IgE are reported to potently upregulate the surface FcεRI level in human mast cells [20] and basophils [29]. The effect of IL-4 and IgE on surface FcεRI expression is potentially important because enhancement of surface FcεRI expression by IL-4 and/or IgE is associated with increased function of mast cells in response to IgE-mediated stimulation [20, 30]. Our present study suggests that surface FcεRI expression on human eosinophils, which can also be enhanced by IL-4 and IgE, may be regulated by a mechanism similar to that in mast cells and basophils. In this context, it will be interesting to analyze to what extent IL-4 and IgE affect surface FcεRI expression in other FcεRI⁺ cells such as dendritic cells, macrophages and platelets. Elucidation of the precise mecha-

nisms of FcεRI expression in various cell types will provide more insight into the pathogenesis of allergic diseases.

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