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Does IgE Bind to and Activate Eosinophils from Patients with Allergy?1

Hirohito Kita,2 Masayuki Kaneko, Kathleen R. Barretmes, Deborah A. Weiler, Andrew W. Schimming, Charles E. Reed, and Gerald J. Gleich

Human eosinophils have been reported to express both the mRNA and protein for the high affinity IgE receptor (FcεRI); it is speculated that this receptor plays a role in eosinophil mediator release in allergic diseases. However, questions still remain. How much of the FcεRI protein is actually expressed on the cell surface of the eosinophil? If they are present, are these IgE receptors associated with effector functions of eosinophils? To address these issues, we studied blood eosinophils from patients with ragweed hay fever. A high level of low affinity IgG receptor (FcγRII, CD32), but no expression of FcεRI, was detectable on the eosinophil surface by standard FACS analysis. However, after in vitro sensitization with biotinylated chimeric IgE (cIgE), cell-bound cIgE was detected by PE-conjugated streptavidin. This cIgE binding was partially inhibited by anti-FcεRI mAb, suggesting that eosinophils do express minimal amounts of FcεRI detectable only by a sensitive method. Indeed, FACS analysis of whole blood showed that eosinophils express ~0.5% of the FcεRI that basophils express. When stimulated with human IgE or anti-human IgE, these eosinophils did not exert effector functions; there was neither production of leukotriene C4 or superoxide anion nor any detectable degranulation response. In contrast, eosinophils possessed membrane-bound human IgG and showed functional responses when stimulated with human IgG or anti-human IgG. Thus, IgG and/or cytokines, such as IL-5, appear to be more important for eosinophil activation in allergic diseases than IgE. The Journal of Immunology, 1999, 162: 6901–6911.

EOSinophils play important roles in the pathophysiology of bronchial asthma and other allergic diseases (reviewed in Ref. 1). In such diseases, mediators released by T cells, epithelial cells, and other inflammatory cells induce migration of eosinophils from blood into the affected tissues (reviewed in Ref. 2). Subsequently, various stimuli trigger eosinophil activation, resulting in the local release of a series of inflammatory mediators, such as lipid metabolites (2, 3), superoxide anion (4), and toxic cationic granule proteins (5). Indeed, eosinophilic infiltration in the epithelium and submucosa of the airways has been a hallmark of the pathology of asthma and allergic rhinitis (6, 7). Correlations have been observed between the number of infiltrating eosinophils and asthma disease severity (6). In addition, pulmonary segmental allergen challenge in allergic individuals causes eosinophil recruitment into the airways; this is associated with the release of eosinophil granule proteins and an increase in vascular permeability (8, 9). In spite of this strong association between eosinophils and allergic diseases, the activation mechanism(s) of eosinophils in vivo are largely unknown.

A critical feature of the allergic response is the release of histamine and other inflammatory mediators from mast cells and basophils after allergen cross-links specific IgE Ab occupying high affinity IgE receptors (FcεRI) (10). Furthermore, epidemiological studies show a close correlation between serum IgE levels and the prevalence and severity of the bronchial asthma (11). Thus, there is converging evidence to support a role for IgE in the pathophysiology of allergic diseases in humans. Earlier, eosinophils were considered to express only low affinity IgE receptor (FcεRII) (12); however, recent studies by Gounni et al. (13) demonstrated that eosinophils express FcεRI and that they release inflammatory mediators and exert cytotoxicity to helminths through this receptor. Subsequent immunohistochemical and immunocytochemical studies on patients with allergic diseases showed that local allergen provocation induces expression of FcεRI by eosinophils infiltrating into the airways (14) and skin (15, 16). Furthermore, FcεRI was detected in blood eosinophils from patients with various allergic diseases (17). Therefore, it is reasonable to speculate that FcεRI plays a role in the eosinophil secretory process in allergic diseases.

There are caveats to this conception. Most of the patient studies were performed by histologic examination using in situ hybridization and immunocytochemical techniques. Therefore, little is known about the actual expression of FcεRI protein on the eosinophil surface. For example, in human T cells, the low affinity IgG receptor proteins (FcγRII, CD32) are stored within the cytoplasm but are not expressed on the cell surface (18), and their surface expression was detected only by a highly sensitive biotin-avidin flow cytometric technique (19). Furthermore, the functional significance of the FcεRI expression by eosinophils from patients with allergic diseases is also unknown. To address these questions, we studied blood eosinophils from patients with ragweed hay fever during the peak of the allergy season. We had three goals. First, we examined whether FcεRI proteins are expressed on the surface of the eosinophil, and we compared the expression levels to known FcεRI-bearing cells, such as basophils. Second, we examined whether FcεRI confers IgE binding to eosinophils. Finally, we examined whether IgE receptors stimulate mediator release by these eosinophils.

1 Address correspondence and reprint requests to Dr. Hirohito Kita, Department of Immunology, Mayo Clinic, Rochester, MN 55905. E-mail address: kita.hirohito@mayo.edu

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3 Address correspondence and reprint requests to Dr. Hirohito Kita, Department of Immunology, Mayo Clinic, Rochester, MN 55905. E-mail address: kita.hirohito@mayo.edu
Materials and Methods

Patients

Thirty patients, 18–60 yr of age, with moderate to severe ragweed allergic rhinitis and 14 healthy nonatopic subjects were studied. The patients had a history of ragweed hay fever, a positive skin test to ragweed extract, and an elevated level of IgE Ab for short ragweed (305–2190% of normal, median 1114%). Patients were excluded if they were receiving any forms of glucocorticoid treatment within the preceding 6 mo or immunotherapy within the preceding 12 mo. Each patient kept a diary of daily symptom scores for nasal congestion, pruritus or discharge, ocular pruritus, eye watering, eye redness, and pruritus of the ear or palate to validate their clinical reaction to airborne ragweed allergen. Normal control subjects had no clinical or laboratory evidence of atopy. Blood samples were obtained from patients and control subjects during the peak of hay fever season in Rochester, MN (end of August) and used as described below. The study was approved by the Institutional Review Board of the Mayo Clinic and Mayo Foundation.

Antibodies

Anti-FcεRI α subunit mAb (15-1, mouse IgG1 (mlG1)) and biotinylated chimeric mouse/human IgE monoclonal protein (cIGE) were kind gifts from Dr. J.-P. Kinet, Beth Israel Deaconess Medical Center, Harvard Medical School (Boston, MA). Concentration-response experiments to establish saturating concentrations for cIGE to sensitize human eosinophils in vitro and for 15-1 to inhibit the sensitization of cIGE were performed before this study began (our unpublished results); the saturating concentrations were ~25 µg/ml for cIGE and 10 µg/ml for 15-1 per 1 × 10^7 eosinophils. Anti-CD11b mAb (Bear1, mlG1), anti-FcεRII (CD23) mAb (9P25, mlG1), anti-CD25 mAb (B1.49.9, mlG2a, anti-CD32 mAb (2E1, mlG2a), and anti-CD69 mAb (TP1.55.3, mlG2b) were purchased from Immunotech (Westbrook, ME). Control mouse myeloma Ig, including mlG1, mlG2a, and mlG2b, were obtained from ICN Pharmaceuticals (Costa Mesa, CA). PE-conjugated F(ab)2 fragments of sheep IgG anti-mlgG and PE-conjugated streptavidin were purchased from Sigma Chemical (St. Louis, MO) and Becton Dickinson (Mountain View, CA), respectively. FITC-conjugated goat IgG anti-human IgG, FITC-conjugated goat anti-human IgE, and FITC-conjugated goat anti-mouse IgM were from BioSource International (Camarillo, CA).

Reagents for cell functional analyses

We used myeloma IgE purified from serum from a patient with multiple myeloma as described elsewhere (5). Purified human IgE was also purchased from Saints with a different capability (20). Briefly, 60 ml of venous blood was centrifuged over Histopaque 1077 (Sigma) and centrifuged at 1000 g. The supernatant and mononuclear cells at the interface were carefully re-suspended with 1 part of 6% hetastarch in 0.9% NaCl (Hespan, DuPont Pharmaceuticals, Wilmington, DE) for 45 min at 37°C to sediment erythrocytes. The buffy coat was collected, and the remaining erythrocytes were lysed by erythrocyte lysis solution (Becton Dickinson). Buffy coat leukocytes were washed with PBS containing 0.1% NaN3 and 1% BSA (PBAbuffer). The cells were resuspended in PBAbuffer at 2 × 10^6 cells/ml and plated in 96-well plates at 1 µl/well. One microgram of anti-CD11b, anti-CD25, anti-CD69, or isotype-matched control mouse Ig was added separately to individual wells and incubated for 30 min at 4°C. After washing, cells were resuspended with 100 µl of PE-conjugated F(ab')2 fragments of sheep anti-mouse IgE diluted in PBAbuffer at 1:400 and incubated in the dark for 30 min. The samples were washed and fixed with 1% formaldehyde. PBAbuffer was used to wash the pellets prior to analysis was performed within 2 h using a FACSscan flow cytometer (Becton Dickinson). Established programs were used to ensure consistent instrument settings (e.g., laser power, compensation, gain, scaling, etc.) among experiments. Two-dimensional dot-plot analysis of the green fluorescence intensity and side scatter revealed four distinct populations of leukocytes, including lymphocytes, monocytes, neutrophils, and eosinophils. Eosinophils were identified as a cell population with the strongest green autofluorescence and side scatter as previously described (24). Appropriateness of the identification of the eosinophil population was confirmed by positive staining for anti-CD9 mAb (Immunotech) as well as morphological analysis of cytosin preparation of sorted cells. The cells were electronically gated on this eosinophil population, and the expression of activation markers was measured by examining the intensity of PE fluorescence. Fluorescence intensity was determined on the same cell population using PE-conjugated anti-FcεRII mAb (9P25) and PE-conjugated anti-FcεRI mAb (15-1), anti-FcεRII mAb (9P25), or anti-FcεRI mAb (2E1), or the same amount of control mouse Ig. After an incubation for 30 min on ice, the cell pellets were washed twice with cold PBAbuffer and mixed with 100 µl of 1:400 dilution of PE-conjugated F(ab')2 fragments of sheep anti-mouse IgE. After an additional incubation on ice for 30 min in the dark, the cell pellets were washed twice with PBAbuffer and fixed with 1% parformaldehyde. Fluorescence analysis was conducted in a FACSscan flow cytometer. Fluorescence intensity was determined on 10,000 cells from each sample using logarithmic amplification and presented as the differences in the mean fluorescence channel (ΔMFC) between the test mAb (e.g., anti-CD11b) and isotype-matched control mouse Ig by Becton Dickinson Lysis II software.

Receptor expression

Expression of receptors for IgE and IgG, including FcεRI, FcεRII (CD23), and FcγRI (CD32), by eosinophils was examined by a standard FACS technique using isolated eosinophils. To release putative in vivo bound IgE molecules from the surface of the eosinophil, eosinophils were subjected to acid stripping as described previously (25). Briefly, isolated eosinophils were washed with cold 0.15 M NaCl. The pellets were treated with lactic acid buffer (130 mM NaCl, 5 mM KCl, 10 mM lactic acid, pH 3.9) for 3.5 min. Cells were washed with PBAbuffer and suspended in the same buffer at 5 × 10^6 cells/ml. Aliquots (100 µl) of cell suspension were added to 15-ml polypropylene conical tubes, mixed with saturating amounts of anti-FcεRI mAb (15-1), anti-FcεRII mAb (9P25), or anti-FcγRII mAb (2E1), or the same amount of control mouse Ig. After an incubation for 30 min on ice, the cell pellets were washed twice with cold PBAbuffer and mixed with 100 µl of 1:400 dilution of PE-conjugated F(ab')2 fragments of sheep anti-mouse IgE. After an additional incubation on ice for 30 min in the dark, the cell pellets were washed twice with PBAbuffer and fixed with 1% paraformaldehyde. Fluorescence analysis was conducted in a FACSscan flow cytometer. Fluorescence intensity was determined on 10,000 cells from each sample using logarithmic amplification and presented as the differences in the mean fluorescence channel (ΔMFC) between the test mAb (e.g., 15-1) and isotype-matched control mouse Ig by Becton Dickinson Lysis II software.

Ig binding

Binding of IgE or IgG to eosinophils and other leukocyte populations was examined before and after in vitro passive sensitization with these Ig. Isolated eosinophils or buffy coat leukocytes were obtained as described

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2 Abbreviations used in this paper: mIgG, mouse IgG1; cIgE, chimeric mouse/human IgE; EDN, eosinophil-derived neurotoxin; HSA, human serum albumin; LT, leukotriene; PAB, PBS containing 0.1% NaN3, and 1% BSA; ΔMFC, differences in the mean fluorescence channel; EIA, enzyme immunoassay.
above. One portion of cells was stained immediately for the analysis of cell-bound IgE or IgG in vivo (see below). The other portion of cells was passively sensitized in vitro with IgE or IgG. First, cells were treated with a saturating concentration of anti-FcεRI or 50 μg/ml of F(ab’2) fragments of goat anti-human IgG, or the same amounts of control (FITC-conjugated goat anti-mouse IgM). Alternatively, cell-bound biotinylated cIgE was visualized by PE-conjugated streptavidin. After incubation on ice for 30 min in the dark, cell pellets were washed twice with PAB buffer and fixed with 1% paraformaldehyde. Fluorescence analysis was conducted in a FACScan flow cytometer. To analyze IgE, cIgE, or IgG bound to isolated eosinophils, fluorescence intensity was determined on 50,000 total cells from each sample using logarithmic amplification and presented as the MFI between test samples or paired Student’s t test. Statistical significance was assessed using the Mann-Whitney U test or paired Student’s t test.

**Results**

**Expression of activation markers by eosinophils from patients with hay fever**

Generally, in Rochester, MN, ragweed hay fever season starts in early August, peaks at the end of August, and continues until the end of September. In the current study, patients’ symptoms increased in parallel to the pollen counts and peaked during the peak pollination period (late August through early September). Similarly, patients’ peripheral blood eosinophil counts increased significantly during the peak hay fever season from the values before the season (284 ± 41/μl at peak season vs 141 ± 19/μl before the season (early July); n = 30, p < 0.01). Initial FACs analyses of peripheral blood eosinophils validated in vivo “activation” of the patients’ eosinophils. These cells expressed significantly higher levels of several eosinophil cell surface activation markers, including CD25, CD69, and CD11b, at the peak hay fever season compared to normal subjects (Fig. 1). The increased expression of CD11b was especially pronounced. In addition, when stimulated in vitro with 1 μM FMLP, patients’ eosinophils released significantly larger amounts of the eosinophil granule protein, EDN, than normal subjects’ eosinophils (27.3 ± 5.6 and 7.8 ± 1.1% of total EDN for patients and normal subjects, respectively; n = 6, p < 0.05). These findings suggest that blood eosinophils from patients with hay fever are “activated” compared with those from normal
Expression of IgE and IgG receptors by eosinophils from patients with hay fever

We next examined whether patients’ eosinophils can bind IgE or IgG on their surfaces and whether they express receptors for these Ig. FACS analysis of cell-bound IgE with FITC-conjugated anti-IgE Ab showed that none or minimal amounts of IgE were present on the surface of freshly isolated eosinophils (Figs. 2 and 3A). When these eosinophils were incubated with IgE in vitro for 2 h, the amounts of bound IgE significantly increased ($p < 0.01$, Fig. 3A), suggesting that these eosinophils have the capacity to bind IgE. Freshly isolated eosinophils bound detectable amounts of IgG on their surface (Figs. 2 and 3A), and the levels increased further by in vitro incubation with IgG (Fig. 3A, $p < 0.01$). Thus, patients’ eosinophils were able to bind both IgE and IgG in vitro although the amounts of IgE bound in vivo were negligible.

Another question remains. Do eosinophils express Fc receptors accountable for binding these Ig? To detect IgE receptors, eosinophils were pretreated with lactic acid to remove any bound IgE and incubated with anti-receptor mAb and PE-conjugated secondary Ab. Surprisingly, no receptors for FcεRI or FcεRII were detected by 15-1 or 9P25 mAb, respectively, in any of the six patients (Figs. 2 and 3B). In contrast, FcyRII was easily detectable on the same eosinophils (Figs. 2 and 3B), consistent with the IgG binding capacity of eosinophils. The experiments were repeated without removing IgE from eosinophils; neither FcεRI nor FcεRII could be detected on these nonstripped eosinophils (data not shown).

Analysis of FcεRI expression by biotinylated clgE

Perhaps we were unable to detect FcεRI on eosinophils because of the sensitivity limitations of the standard FACS technique. Therefore, to enhance the signals for FACS analysis, we removed any IgE bound to the cells by lactic acid treatment, then incubated them in vitro with biotinylated clgE, and visualized them with PE-conjugated streptavidin. Fig. 4A shows that the binding of clgE to eosinophils was clearly detectable. Furthermore, the clgE binding was inhibited by pretreatment of cells with anti-FcεRI mAb (15-1) (Fig. 4B); the isotype-matched control Ig for anti-FcεRI did not affect clgE binding to eosinophils. Similar analyses of eosinophils from six patients showed that this observation is reproducible and that the binding of clgE was significantly blocked by anti-FcεRI mAb (Fig. 5A, $p < 0.01$). The inhibitory effects of anti-FcεRI mAb were variable among the patients, suggesting a wide variance in the expression levels of FcεRI. Interestingly, eosinophils from normal subjects also bound clgE, and the binding was also inhibited partially but significantly by anti-FcεRI mAb (Fig. 5A, $p < 0.05$). The amounts of bound clgE levels as well as the amounts of clgE-binding inhibited by anti-FcεRI mAb tended to be higher in eosinophils from patients compared with eosinophils from normal subjects. Recent studies on mast cells and basophils show that the density of cell surface FcεRI is regulated by blood IgE levels (29, 30). In eosinophils, we also found that the expression of FcεRI, as estimated by anti-FcεRI-inhibitable binding of clgE,
showed a strong linear correlation with serum levels of total IgE (Fig. 5B, $r^2 = 0.941$).

**Whole blood analysis of FcεRI expression**

Although we could detect FcεRI expression by the cIgE-biotin-streptavidin technique (Figs. 4 and 5), the failure to detect by the standard FACS technique (Figs. 2 and 3) suggested that the density of FcεRI expressed on the surface of the eosinophil is extremely low. Basophils are known to express a high density of FcεRI (31) and blood monocytes from patients with allergy also express FcεRI (32). Therefore, we compared the expression levels of FcεRI by eosinophils to other leukocytes. To this end, total leukocytes were sensitized with biotinylated cIgE and visualized by PE-conjugated streptavidin and flow cytometry. The individual cell populations were electronically gated on their unique green autofluorescence and side scatter characteristics. This strategy avoided unanticipated cell activation during the isolation procedures, avoided inadvertent subset selection, and enabled simultaneous analyses of

![FIGURE 3. Binding of Ig to eosinophils (A) and expression of Fc receptors by eosinophils (B). In A, freshly isolated eosinophils were divided into two portions. One portion of cells (presensitization) were stained immediately with FITC-conjugated goat anti-human IgE or FITC-conjugated goat anti-human IgG, or FITC-conjugated control Ab. The other portion of cells (postsensitization) were stripped with lactic acid and passively sensitized in vitro with human IgE (50 μg/ml) or human IgG (50 μg/ml) for 2 h at 4°C. Cells were then stained with FITC-conjugated goat anti-human IgE or FITC-conjugated goat anti-human IgG, or FITC-conjugated control Ab. Fluorescence analysis was conducted in a FACSscan flow cytometer. Data are presented as the differences in the mean fluorescence channel (ΔMFC) between the test mAb (e.g., anti-IgE) and control Ab. Each dot represents the result from an individual subject, and the results from the same subject are connected by a line. Significant differences between the groups are shown. In B, isolated eosinophils were treated with lactic acid to remove cell surface IgE. Cells were then stained with saturating amounts of anti-FcεRI mAb (15-1), anti-FcεRII mAb (9P25), anti-FcγRII (2E1), or isotype-matched control mouse Ig followed by PE-conjugated F(ab')2 fragments sheep anti-mouse IgG. Fluorescence analysis used a FACSscan flow cytometer. Data show the differences in the mean fluorescence channel (ΔMFC) between the test mAb (e.g., 15-1) and isotype-matched control Ab. Each dot represents the result from an individual subject.](http://www.jimmunol.org/)

![FIGURE 4. FACS analysis of cIgE binding to eosinophils. Isolated eosinophils were treated with lactic acid to remove in vivo bound IgE molecules. Fluorescence analysis used a FACSscan flow cytometer, and a histogram of PE fluorescence is shown. A shows the binding of cIgE to eosinophils. Cells were passively sensitized with medium alone (filled gray area) or 25 μg/ml biotinylated cIgE (solid black line) for 2 h at 4°C and stained with PE-conjugated streptavidin. B shows the inhibitory effect of anti-FcεRI on eosinophil cIgE binding. Cells were preincubated without Ab (gray area), with 10 μg/ml anti-FcεRI mAb (15-1, bold black line), or with 10 μg/ml isotype-matched control mouse Ig (thin black line) for 30 min at 4°C. Cells were then sensitized with 25 μg/ml biotinylated cIgE for 2 h at 4°C and stained with PE-conjugated streptavidin.](http://www.jimmunol.org/)
different cell populations from the same sample. As shown in Fig. 6, A and B, green fluorescence/side scatter dot-plot of leukocytes incubated with PE-streptavidin alone revealed four distinct cell populations, including lymphocytes plus basophils, monocytes, neutrophils, and eosinophils. When cells were sensitized with biotinylated IgE and stained with PE-streptavidin, we found that IgE binds to all the cell populations to various degrees (Fig. 6C). Notably, basophils were intensely stained, forming a unique population distinct from the lymphocyte cluster in PE fluorescence reading (Fig. 6C) and even in the green fluorescence reading (Fig. 6).

**FIGURE 5.** Binding of IgE to eosinophils. In A, isolated eosinophils were treated with lactic acid to remove in vivo bound IgE molecules. Cells were preincubated with or without 10 μg/ml anti-FcεRI mAb (15-1), sensitized with 25 μg/ml biotinylated IgE, and stained with PE-conjugated streptavidin. Fluorescence analysis used a FACScan flow cytometer. Data are presented as the differences in the mean fluorescence channel (ΔMFC) between test sample (e.g., biotinylated IgE plus PE-conjugated streptavidin) and control (PE-conjugated streptavidin alone). Each dot represents the result from one subject, and data from the same subject are connected by a line. Significant differences with vs without anti-FcεRI mAb pretreatment are shown. B shows the correlation between serum IgE concentrations and anti-FcεRI (15-1)-inhibitable binding of IgE. Anti-FcεRI-inhibitable binding of IgE was calculated as follows: (ΔMFC of cells preincubated without anti-FcεRI mAb and then incubated with biotinylated IgE and PE-conjugated streptavidin) — (ΔMFC of cells preincubated with anti-FcεRI mAb and then incubated with biotinylated IgE and PE-conjugated streptavidin). Each dot represents the result from one subject; ○, normal subjects; ●, data from patients with hay fever.

**FIGURE 6.** FACS analyses of whole blood for IgE binding to leukocytes. Buffy coat leukocytes were treated with lactic acid buffer to remove in vivo bound IgE molecules. Cells were preincubated without (A–D) or with (E and F) anti-FcεRI mAb (15-1). Cells were then passively sensitized with (C–F) or without (A and B) 25 μg/ml biotinylated IgE, and stained with PE-conjugated streptavidin. Fluorescence analysis used a FACScan flow cytometer. Two-dimensional dot-plot analysis of the green fluorescence intensity and side scatter of leukocytes showed four distinct populations (B), including lymphocytes (Ly), monocytes (Mo), neutrophils (Ne), and eosinophils (Eo). Basophils (Ba) fell within the population of lymphocytes. However, when stained with biotinylated IgE and PE-conjugated streptavidin (D), basophils (Ba) formed one unique population in green fluorescence/side scatter dot-plot analysis (C) due to their extremely strong PE fluorescence. The identity of the individual cell populations was confirmed as described in Materials and Methods.
6D). The distribution of cell clusters in green fluorescence/side scatter dot-plot did not change otherwise (Fig. 6D). Interestingly, when leukocytes were pretreated with anti-FceRI mAb, the binding of cIgE to the basophil cluster was completely inhibited (Fig. 6E). In contrast, the inhibitory effects of anti-FceRI mAb on cIgE binding to eosinophil and neutrophil clusters were not apparent in the dot-plot.

The quantitative analyses of cIgE binding and the inhibitory effects of anti-FceRI mAb (15-1) are shown in Fig. 7. Binding of cIgE to leukocytes was observed in the following order: basophils (5062 ± 221) > neutrophils (346 ± 23) > eosinophils (124 ± 15) = monocytes (90 ± 9) (means ± SEM of ΔMFI, n = 27). Pretreatment of cells with anti-FceRI mAb completely inhibited the cIgE binding to basophils (99.0 ± 0.1% of inhibition, n = 27), suggesting that FceRI plays a major role in cIgE binding to basophils. The binding of cIgE to monocytes was inhibited moderately but significantly by anti-FceRI mAb in 24 of 27 patients (45.0 ± 5.5% of inhibition, p < 0.01), suggesting roles for FceRI and for other binding site(s) in cIgE binding to monocytes. In contrast, neutrophils anti-FceRI mAb minimally affected the binding of cIgE (5.1 ± 5.3% of inhibition), and the inhibitory effects of anti-FceRI mAb varied considerably among individuals as evidenced by a wide range of Δ(W/O-With) values. The effects of anti-FceRI mAb on eosinophils were between those on monocytes and neutrophils, namely significant (p < 0.01) but small and variable inhibitory effects (18.2 ± 3.8% of inhibition). The binding of cIgE to leukocytes due to FceRI was observed in the following order: basophils (5015 ± 223) > monocytes (40 ± 12) > eosinophils (25 ± 7) > neutrophils (13 ± 13) (means ± SEM of the ΔMFI with or without anti-FceRI mAb). Thus, although the expression of FceRI by eosinophils is detectable, the level is strikingly less than that by basophils and less than that by monocytes.

Roles of IgE and IgG receptors for degranulation of basophils and eosinophils

In the next series of experiments, we examined the functional significance of IgE receptors for mediator release by eosinophils. The engagement of IgE receptors on basophils and mast cells by ligation of bound IgE with anti-IgE Ab is commonly used to trigger various effector functions of these cell types, including degranulation and production of lipid mediators and cytokines (reviewed in Refs. 33 and 34). Therefore, we investigated whether anti-IgE Ab induces degranulation of eosinophils and compared the responses of basophils and eosinophils using cells isolated from the same donors. When basophils were stimulated with 1 μg/ml anti-IgE Ab, eosinophils released a large amount of histamine (Fig. 8A, 85.7 ± 4% of total histamine, n = 6). The effect of 10 μg/ml anti-IgE Ab in solution was weaker, but substantial amounts of histamine were released (51.3 ± 10.5% of total, n = 6). In contrast, 1 μg/ml anti-IgE Ab in solution failed to induce EDN release from eosinophils (Fig. 8B), and higher concentrations of anti-IgE Ab (5–50 μg/ml) did not induce EDN release from eosinophils (data not shown). Furthermore, immobilized anti-IgE Ab induced histamine release from basophils but not EDN release from eosinophils. In contrast, immobilized anti-IgG Ab induced both basophil histamine release and eosinophil EDN release.

Production of LTC₄ and superoxide anion by eosinophils

The experiments described above suggest that a degranulation response is provoked by anti-IgE Ab in basophils, but not in eosinophils. Perhaps the amounts of IgE mounted on eosinophils are too small to trigger sufficient signals for cellular function when cross-linked by anti-IgE Ab. Furthermore, if the affinity of the IgE binding sites is low, the sites may be partially occupied and partially unoccupied in freshly isolated eosinophils (see Figs. 2 and 3). Therefore, to characterize completely the eosinophil responses to Ig, we stimulated eosinophils with Ig itself or anti-Ig Ab immobilized onto tissue culture plates and examined the production of an eicosanoid, LTC₄. As shown in Fig. 9, ligation of unoccupied IgG receptors by immobilized IgG provoked LTC₄ production in a concentration-dependent manner. In contrast, ligation of unoccupied IgE receptors (or binding sites) with immobilized IgE failed to induce LTC₄ production. Similarly, ligation of cell-bound IgG by anti-IgG Ab stimulated eosinophil LTC₄ production; however, anti-IgE Ab did not induce significant production of LTC₄. Furthermore, as shown in Fig. 10A, eosinophils produced superoxide anion.
anion in a time-dependent manner when stimulated with immobilized IgG; immobilized IgE stimulated only minimal superoxide production. In addition, anti-IgG Ab, but not anti-IgE Ab, induced eosinophil superoxide production (Fig. 10B). Altogether, these findings suggest that eosinophil degranulation and production of LTC4 and superoxide anion are only minimally induced by IgE-dependent stimuli, such as IgE or anti-IgE Ab, whereas these functions are induced vigorously by IgG-dependent stimuli.

Discussion

Eosinophils and their inflammatory products play important roles in the pathophysiology of bronchial asthma and other allergic disorders (reviewed in Ref. 1). Allergen-specific IgE is one of the major components in allergic diseases in humans, and the ligation of specific IgE bound to FcεRI on mast cells and basophils triggers production and release of inflammatory mediators from these cells (reviewed in Refs. 33 and 34). Because eosinophils from hypereosinophilic patients may express FcεRI (13), it has been suggested that FcεRI plays an important role in mediator release by eosinophils in allergic disease (35). However, we have made four major observations suggesting that this is unlikely. First, by standard FACS analysis, eosinophils from patients with hay fever during the peak of the season express no or minimal levels of FcεRI or FcεRII on their surfaces (Figs. 2 and 3); however, these eosinophils do express eosinophil activation markers and FcγRII. Second, eosinophils are able to bind IgE (Fig. 3); however, in contrast to basophils, the contribution of FcεRI toward eosinophil IgE binding is minor (Fig. 7). Third, IgE-dependent stimuli fail to provoke the release of inflammatory mediators by eosinophils (Figs. 8, 9, and 10). Fourth, in contrast, eosinophils from the same donors express FcγRII (Fig. 2) and bind IgG (Fig. 3), and their effector functions are triggered by IgG-dependent stimuli (Figs. 8, 9, and 10). Therefore, eosinophils from patients with ragweed hay fever express negligible FcεRI or FcεRII. Unlike mast cells and basophils, factors other than IgE, such as IgG and cytokines, likely play roles in activation of eosinophils in allergic diseases.

The expression of IgE receptors by eosinophils has been a controversial issue. Murine eosinophils do not express FcεRI or FcεRII.

![FIGURE 8](http://www.jimmunol.org/)

**FIGURE 8.** Degranulation of basophils and eosinophils induced by anti-IgE or anti-IgG Ab. Isolated eosinophils or basophil preparations were stimulated with 1 μg/ml F(ab')2 fragments of goat anti-human IgE (soluble stimulus), or F(ab')2 fragments of goat anti-human IgE, goat anti-human IgG, or goat IgG (control) immobilized onto 96-well tissue culture plates at 50 μg/ml (immobilized stimuli). Cells were incubated for 60 min (basophil preparations) or 180 min (eosinophils) at 37°C and 5% CO2. To quantitate basophil degranulation, the concentration of histamine in the sample supernatants was measured by histamine EIA kit. To quantitate eosinophil degranulation, the concentration of EDN in the sample supernatants was measured by RIA. Total cellular histamine and EDN contents were measured using supernatants from cells lysed with 0.5% Nonidet P-40 detergent. Each dot represents the result from an individual subject.

![FIGURE 9](http://www.jimmunol.org/)

**FIGURE 9.** LTC4 production by eosinophils stimulated with IgE- or IgG-dependent stimuli. Isolated eosinophils were stimulated with serial dilutions of human IgE or human IgG immobilized onto 96-well tissue culture plates, or with F(ab')2 fragments of goat anti-human IgE, goat anti-human IgG, or goat IgG (control) immobilized onto tissue culture plates at 50 μg/ml. Cells were incubated for 1 h at 37°C and 5% CO2, and concentrations of LTC4 in the sample supernatants were measured by LTC4 ELISA kit. Data show means ± SEM from three experiments using cells from three patients with hay fever.

![FIGURE 10](http://www.jimmunol.org/)

**FIGURE 10.** Superoxide anion production by eosinophils stimulated with IgE- or IgG-dependent stimuli. Isolated eosinophils were stimulated with human IgE or human IgG immobilized onto 96-well tissue culture plates at 10, 30, or 100 μg/ml, or with F(ab')2 fragments of goat anti-human IgE, goat anti-human IgG or goat IgG (control) immobilized onto tissue culture plates at 50 μg/ml. The kinetics of superoxide production were examined by reduction of cytochrome c using a microplate autoreader. Between absorbance measurements, the plate was incubated at 37°C. Data are presented as means ± SEM from six experiments using cells from six different patients with hay fever.
Although human eosinophils were considered to express only the low affinity IgE receptor (FcεRII) (12, 37–39), a more recent study by the same investigators demonstrated that eosinophils from hyper eosinophilic patients express FcεRI (13). This latter report triggered clinical studies to examine eosinophil expression of FcεRI. For example, the transcription of mRNA for α-, β-, and/or γ-chains of FcεRI in eosinophils was detected during allergen-induced late phase cutaneous reactions in patients with allergy (16). In addition, local allergen provocation induced expression of FcεRI protein by eosinophils infiltrating into the airways (14) and skin (15, 16). However, other investigators showed that the expression of FcεRI protein was not detected on the surface of the eosinophil (40, 41). In addition, we showed that eosinophils from patients with hay fever express very low numbers of FcεRI on their surface, which can be detected by the biotin-streptavidin technique but not by the standard FACS technique. Although the reasons for the discrepancies in our observations and some of the previous studies are unknown, we speculate that synthesis of receptor proteins and surface expression of the receptor proteins are regulated under different mechanisms. In other words, the detection of receptor proteins by immunohistochemistry may not necessarily represent the expression of the proteins on cell surface. For example, human T cells contain proteins for low affinity IgG receptor (FcγRII, CD32) within the cytoplasm, but these receptors are not expressed on the cell surface. A similar scenario is known for FcγRII (CD16) in eosinophils (42). In addition, the earlier studies often used eosinophils from patients with hematologic or immunologic disorders, including idiopathic hypereosinophilic syndrome and lymphomas. Perhaps disease heterogeneity accounts for the receptor expression heterogeneity.

The strikingly different responses to IgE between basophils and eosinophils are apparent in this study. From the MFI of FACS analysis, ~50 times more clgE was bound to basophils than to eosinophils (Fig. 7). Furthermore, >99% of clgE binding to basophils was inhibited by anti-FcεRI mAb pretreatment, suggesting that FcεRI is the predominant IgE receptor in basophils. In contrast, anti-FcεRI mAb only partially (18%) inhibited clgE binding to eosinophils. Thus, we estimate that eosinophils from these ragweed hay fever patients express ~0.5% of the FcεRI compared to basophils. In addition, the basophil degranulation response was triggered by ligation of FcεRI, either by anti-IgE Ab or by immobilized IgE; these same stimuli failed to induce degranulation or release of mediators from eosinophils. Interestingly, treatment of patients with atopy with humanized anti-IgE Ab resulted in ~96% decrease in the expression of FcεRI and ~40% decrease in anti-IgE-induced histamine release response by basophils (30). Because eosinophils possess only ~0.5% of the FcεRI possessed by basophils, this paucity of FcεRI expression by eosinophils may explain the heterogeneous responses of eosinophils and basophils to FcεRI ligation. Perhaps eosinophils lose or basophils acquire their capacity to express high levels of FcεRI during their differentiation from common progenitors (43).

Then, the question remains as to which receptors or binding sites contribute to the binding of myeloma IgE (Figs. 2 and 3) and clgE (Figs. 4 through 7) by eosinophils. The results of our experiments and a review of the literature provide some insights. As shown in Figs. 2 and 3, none to minimal expression of FcεRI or FcεRII (CD23) was detectable using 15-1 or 9P25 mAb, respectively, and the standard FACS technique. These findings are also consistent with previous reports, demonstrating negligible binding of anti-FcεRI mAb (clones 22E7 and 15-1) (17) and a panel of mAb against CD23 (44, 45) to eosinophils. Therefore, the expression levels of high affinity IgE receptor (FcεRI) and low affinity IgE receptor (FcεRII) on eosinophils are likely too low to play major roles in IgE binding. Furthermore, the minimal or lack of binding of IgE on eosinophils in vivo (Figs. 2 and 3) suggests that IgE binding to eosinophils is of low affinity. Besides these IgE Fc receptors, an IgE-binding molecule belonging to an S-type lectin family, called Mac-2/eBp, can also bind IgE through the carbohydrate recognition domain (46). Mac-2/eBp is expressed by various cell types, including neutrophils and eosinophils (47, 48). Indeed, Ab against Mac-2/eBp strongly inhibited IgE binding and IgE-dependent activation of human neutrophils (47). Therefore, by analogy to neutrophils, Mac-2/eBp is a good candidate molecule for IgE-binding sites on eosinophils. Our findings, showing large amounts of IgE binding to both eosinophils and neutrophils independent of FcεRI (Fig. 7), are consistent with this speculation. Alternatively, IgE may bind nonspecifically through its carbohydrate moiety to lectin-like binding sites on the surface of the eosinophil.

Two other questions remain. Because previous histological studies (14–16) could not differentiate between the proteins within the cells and those on the cell surface, the first question involves the surface expression of FcεRI by tissue eosinophils. As shown in Fig. 5B, the levels of FcεRI expression by eosinophils show a positive correlation with serum IgE levels, suggesting that the expression of FcεRI by eosinophils is regulated by IgE-dependent mechanisms similar to those in mast cells (29) and basophils (30). In addition, IL-4 enhances the expression of mRNA and protein for the α-chain of FcεRI (40). It is well known that IL-4 is expressed in the local tissues of patients with allergy (49, 50); a more recent report suggests that IgE may be produced by B cells in the airway tissues in patients with allergy (51). Therefore, the tissue microenvironment may be optimal for FcεRI expression, suggesting that the surface expression of FcεRI by tissue eosinophils may be higher than the surface expression in peripheral blood eosinophils. On the other hand, even in the optimal tissue environment, the ability of eosinophils to express FcεRI on their surfaces may be limited. For example, eosinophils obtained from bronchoalveolar lavage fluids from patients with allergy do not express detectable levels of FcεRI on their surfaces (41). Eosinophils cultured for up to 11 days with myeloma IgE or IL-4, conditions known to up-regulate FcεRI on basophils, failed to induce any detectable surface FcεRI (41). Furthermore, Terada et al. (40) found that after culture with IL-4, eosinophil production of FcεRI α-subunit protein was increased, but expression of FcεRI was not detectable. In preliminary studies, we were not able to detect FcεRI by standard FACS analysis on eosinophils cultured for 3 days with human myeloma IgE in the presence of various cytokines, such as IL-5, IL-3, IL-4, TNF-α and fibroblast supernatants (data not shown).

The second question concerns the interaction of IgE with other eosinophil agonists. We found that anti-IgE Ab or immobilized IgE by themselves failed to provoke eosinophil mediator release (Figs. 8 through 10). However, a previous report also suggests that IgE mediates eosinophil killing of schistosomula of Schistosoma mansoni when cells are stimulated with lipid mediators, such as platelet-activating factor or LTB4, without the apparent expression of FcεRI (52). Therefore, IgE may collaborate with other eosinophil agonists to provoke some functions of the cells, such as cellular cytotoxicity. In preliminary studies, IgE did not enhance EDN release from eosinophils stimulated with platelet-activating factor or IL-5 (H. Kita and M. Muraki, unpublished observation). Furthermore, it is also possible that FcεRI or IgE may be involved in other functions than secretory process of eosinophils, such as Ag presentation, as proposed in other cell types (53).

Remaining questions aside, our study clearly shows that the surface expression of FcεRI by blood eosinophils from patients with hay fever is minimal and that, in contrast to IgG, IgE does not
comparisons of responses to IgE by eosinophils and basophils

medium release of inflammatory mediators from these eosinophils. Therefore, previous observations regarding the expression of FcεRI and FcεRII by blood eosinophils from patients with eosinophilia may not be directly applicable to patients with allergy. Although our study may be limited because only patients with hay fever were studied, our observations raise questions about the current conception that eosinophil IgE receptors play a role in the pathophysiology of allergic diseases. The observations in IgE knockout mice, which demonstrated eosinophilic inflammation and increased bronchial hyperreactivity in the absence of IgE (54), may in part be applicable to humans; i.e., factors other than IgE may act as trigger(s) for mediator release by eosinophils in allergic inflammation.

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