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

Hirohito Kita,[2] Masayuki Kaneko, Kathleen R. Bartemes, 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 C_4 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.

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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 pruritis 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 (mIgG1))[3] 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 1×10^6 eosinophils. Anti-CD11b mAb (Bear1, mIgG1), anti-FcεRII (CD23) mAb (9P25, mIgG1), anti-CD25 mAb (B1.49.9, mIgG2a), anti-CD32 mAb (2E1, mIgG2a), and anti-CD69 mAb (TP1.55.3, mIgG2b) were purchased from Immunotech (Westbrook, ME). Control mouse myeloma Ig, including mIgG1, mIgG2a, and mIgG2b, were obtained from ICN Pharmaceuticals (Costa Mesa, CA). PE-conjugated F(ab')₂ fragments of sheep IgG anti-mIgG 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 IgG 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 Cortex Biochem (San Leandro, CA). We performed all the experiments with both of these IgE preparations and found that the results were virtually identical. Therefore, only results from our myeloma IgE preparation(s) are shown. Purified human serum IgG was purchased from ICN Pharmaceuticals. F(ab')₂ fragments of goat IgG anti-human IgG and F(ab')₂ fragments of goat IgG anti-human IgE were purchased from ICN Pharmaceuticals and DiaMed (Windham, ME), respectively. F(ab')₂ fragment of goat IgG was obtained from Jackson ImmunoResearch Laboratories, West Chester, PA.

Cell preparation

Eosinophils were isolated from six patients and six normal subjects by a magnetic cell line separation system (MACS, Becton Dickinson, San Jose, CA) as described previously with minor modifications (20). Briefly, 60 ml of venous blood anticoagulated with 50 U/ml heparin were diluted with PBS at a 1:1 ratio. Diluted blood was overlaid on isotonic Percoll solution (density, 1.085 g/ml, Sigma) and centrifuged at $1000 \times g$ for 30 min at 4°C. The supernatant and mononuclear cells at the interface were carefully removed, and erythrocytes in sediment were lysed by two cycles of hypotonic water lysis. Isolated granulocytes were washed twice with PIPES buffer (25 mM PIPES, 50 mM NaCl, 5 mM KCl, 25 mM NaOH, 5.4 mM glucose, pH 7.4) containing 1% alpha calf serum (HyClone Laboratories; Logan, UT). An approximately equal volume of anti-CD16-conjugated magnetic beads (Miltenyi Biotec, Auburn, CA) was added to the cell pellet. After 60 min of incubation on ice, cells were loaded onto the separation column positioned in the strong magnetic field of the MACS. Cells were eluted three times with 5 ml of PIPES buffer with defined calf serum. The purity of eosinophils counted by Randolph's stain was regularly >98%.

Purified eosinophils were washed and suspended in reaction medium and then used immediately.

Basophils were obtained as the mononuclear cell layer after density gradient centrifugation from the same donor used for eosinophil isolation. Briefly, heparinized venous blood was centrifuged over Histopaque 1077 (Sigma), following the procedure recommended by the manufacturer. The mononuclear cell layer was collected, washed with PIPES buffer, suspended in reaction medium, and used immediately. The purity of basophils counted by Wright-Giemsa staining of cytospin preparations was between 0.5% and 2%.

Flow cytometric analysis

Eosinophil activation markers. Expression of CD25, CD69, and CD11b on eosinophils was measured by flow cytometry as an indicator of eosinophil activation (21–23). To avoid purification-induced activation or inadvertent subset selection, total leukocyte samples were stained with mAb and eosinophils were electronically gated during FACS analysis. Briefly, 5 parts of heparinized whole blood from patients and normal subjects were incubated 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 lysing solution (Becton Dickinson). Buffy coat leukocytes were washed with PBS containing 0.1% NaN₃ and 1% BSA (PAB buffer). The cells were resuspended in PAB buffer at 2×10^7 cells/ml and added to 96-well plates at 100 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 IgG diluted in PAB buffer at 1:400 and incubated in the dark for 30 min. The samples were washed and fixed with 1% paraformaldehyde in PAB. FACS analysis was performed within 2 h using a FACScan 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 cytospin 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 50,000 total 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 FcγRII (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 PAB buffer 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 PAB buffer and mixed with 100 μl of 1:400 dilution of PE-conjugated F(ab')₂ fragments of sheep anti-mouse IgG. After an additional incubation on ice for 30 min in the dark, the cell pellets were washed twice with PAB buffer and fixed with 1% paraformaldehyde. Fluorescence analysis was conducted in a FACScan 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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[3] 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 lactic acid buffer to remove in vivo bound IgE molecules as described above. Eosinophils and other leukocytes were then washed with sensitization buffer (RPMI 1640 medium supplemented with 25 mM HEPES, 1% BSA, 0.1% NaN$_3$, pH 7.3) and resuspended in the medium at 5×10^6 cells/ml and 2×10^7 cells/ml, respectively. Aliquots (100 μl) of each cell suspension were added to 15-ml polypropylene conical tubes and mixed with human IgE (50 μg/ml), human IgG (50 μg/ml), or biotinylated cIgE (25 μg/ml). In some experiments, to block the binding of IgE to FcϵRI, cells were pretreated with a saturating concentration of anti-FcϵRI mAb (15-1, 10 μg/ml) for 30 min on ice before in vitro sensitization with cIgE. Cells were incubated for 2 h at 4°C with gentle mixing and washed twice with PAB buffer. To detect IgE or IgG bound on the cell surface, cells were incubated with saturating amounts of FITC-conjugated goat anti-human IgE or FITC-conjugated 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 10,000 cells from each sample using logarithmic amplification and presented as the ΔMFC between the test Ab (e.g., anti-human IgE) and control Ab by Becton Dickinson Lysis II software. To analyze binding of cIgE to individual cell populations of buffy coat leukocytes, cells were electronically gated based on their unique green autofluorescence and side scatter characteristics. Two-dimensional dot-plot analysis of the green fluorescence intensity and side scatter of buffy coat leukocytes showed four distinct populations, including lymphocytes, monocytes, neutrophils, and eosinophils (see *Results* for detail). Basophils fell within the population of lymphocytes. However, when basophils were stained with biotinylated cIgE and PE-conjugated streptavidin, they formed one unique population in green fluorescence/side scatter dot-plot analysis due to their extremely strong PE fluorescence. Thus, individual cell populations were easily identified by their unique green autofluorescence and side scatter characteristics; appropriateness of the identification of each population had been confirmed by positive staining for appropriate Ab (CD9 for eosinophils and basophils, CD16 for neutrophils, and CD14 for monocytes) as well as morphological analysis of cytospin preparations of sorted cells. The cells were electronically gated, and the binding of cIgE was measured by examining the intensity of PE fluorescence. Fluorescence intensity was determined on 50,000 total cells from each sample using logarithmic amplification, which was converted to the linear equivalent by Becton Dickinson Lysis II software. Data are presented as the ΔMFI between test samples (e.g., biotinylated cIgE plus PE-conjugated streptavidin) and control (PE-conjugated streptavidin alone).

Leukotriene (LT) C$_4$ production

Eosinophil production of LTC$_4$ was performed in 96-well tissue culture plates as described previously with minor modifications (26). Isolated eosinophils were stimulated by cross-linking cell surface IgE or IgG receptors by human IgE, human IgG, anti-human IgE or anti-human IgG, immobilized onto the wells of tissue culture plates. Wells of 96-well flat-bottom Costar 3596 tissue culture plates (Costar, Cambridge, MA) were coated overnight at 4°C with 50 μl of serial dilutions of human IgE or human IgG, or 50 μg/ml of F(ab')$_2$ fragments of goat anti-human IgG, goat anti-human IgE or goat IgG (control). After aspiration of the solution, 50 μl of 2.5% human serum albumin (HSA, Sigma) was added to each well to block the nonspecific protein binding sites. After incubation for 2 h at 37°C and 5% CO$_2$, wells were washed twice with 0.9% NaCl before use. Freshly isolated eosinophils were washed with HBSS supplemented with 10 mM HEPES, 20 mM L-serine and 5 mM glutathione and resuspended in the same medium at 5×10^5 cells/ml. Aliquots of cell suspensions (200 μl) were added to the wells and incubated for 1 h at 37°C and 5% CO$_2$. The supernatants from each well were collected and frozen at -70°C or assayed immediately. Concentrations of LTC$_4$ in the sample supernatants were measured by ELISA using an LTC$_4$ kit (Cayman Chemical, Ann Arbor, MI) following the procedure recommended by the manufacturer. The sensitivity of the assay was 4.8 pg/ml. All experiments were conducted in duplicate.

Superoxide anion production

Eosinophil superoxide production was induced by Ig or anti-Ig immobilized onto tissue culture plates and measured by reduction of cytochrome c as previously described (27, 28). Briefly, IgE, IgG, anti-IgE, or anti-IgG were immobilized onto polystyrene 96-well flat-bottom tissue culture

plates as described above. Freshly isolated eosinophils were washed and resuspended in HBSS with 10 mM HEPES and 100 μM cytochrome c at 5×10^5 cells/ml. Cell suspension (100 μl) was dispensed onto the wells, followed by 100 μl of medium alone. Immediately after addition of stimuli, the reaction wells were measured for absorbance at 550 nm in a microplate autoreader (Thermomax, Molecular Devices, Menlo Park, CA), followed by repeated readings. Between absorbance measurements, the plate was incubated at 37°C. Superoxide anion generation was calculated with an extinction coefficient of 21.1×10^3cm^{-1} M^{-1} for reduced cytochrome c at 550 nm and was expressed as nanomols of superoxide produced per 10^5 cells.

Eosinophil and basophil degranulation

Eosinophils and basophils were stimulated with anti-human IgE or anti-human IgG, either immobilized onto the wells of tissue culture plates or in solution, or FMLP (Calbiochem-Novabiochem, San Diego, CA) in solution. Polystyrene 96-well flat-bottom tissue culture plates were coated with 50 μg/ml F(ab')$_2$ fragments of goat anti-human IgE, goat anti-human IgG, or goat IgG (control); blocked with 2.5% HSA; and washed with saline as described above. Plates used for anti-IgE, anti-IgG, or FMLP in solution were blocked with HSA. Freshly isolated eosinophils or basophil preparations were suspended in RPMI 1640 supplemented with 10 mM HEPES and 0.1% HSA at 5×10^5 cells/ml and 1×10^6 cells/ml, respectively. Aliquots of cell suspensions (100 μl) were added to the wells and stimulated with serial dilutions of F(ab')$_2$ fragments of goat anti-human IgE, goat anti-human IgG or goat IgG (control), or 1 μM FMLP (100 μl). Cells incubated in the wells coated with anti-Ig received 100 μl of medium alone. Cells were incubated for 60 min (basophil preparations) or 180 min (eosinophils) at 37°C and 5% CO$_2$. After incubation, supernatants were collected and stored at -20°C until assayed. To quantitate basophil degranulation, the concentration of histamine in the sample supernatants was measured by histamine enzyme immunoassay (EIA) kit (Immunotech) following the procedure recommended by the manufacturer. To quantitate eosinophil degranulation, the concentration of eosinophil-derived neurotoxin (EDN) in the sample supernatants was measured by RIA. The RIA is a double-Ab competition assay in which radioiodinated EDN, rabbit anti-EDN, and burro anti-rabbit IgG are used, as reported elsewhere (5). Total cellular histamine and EDN contents were measured simultaneously using supernatants from cells lysed with 0.5% Nonidet P-40 detergent. The sensitivities of histamine EIA and EDN RIA were 0.2 nM and 2.0 ng/ml, respectively. All assays were done in duplicate.

Statistical analysis

Data are presented as mean $\pm$ SEM from the numbers of experiments indicated. 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 ($284 \pm 41/\mu$l at peak season vs $141 \pm 19/\mu$l 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 (21–23), compared with eosinophils from 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 $7.8 \pm 1.1\%$ 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
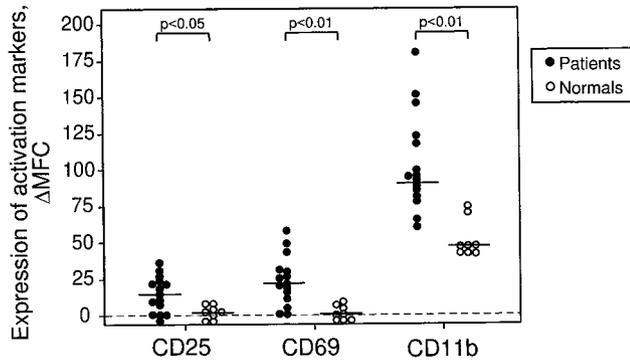
FIGURE 1. Expression of cell surface activation markers by eosinophils. Leukocytes from patients with hay fever or normal subjects were stained with anti-CD25, anti-CD69, or anti-CD11b mAb or isotype-matched control mouse Ig followed by PE-conjugated F(ab')$_2$ fragments of sheep anti-mouse IgG. Cells were analyzed by FACScan flow cytometry, and eosinophils were electronically gated as described in *Materials and Methods*. Data are presented as the differences in the mean fluorescence channel (ΔMFC) between the test mAb (e.g., anti-CD11b) and isotype-matched control mouse Ig. Each dot represents the result from an individual subject. Significant differences between patient and normal groups are shown.

subjects, presumably due to exposure to cytokines and other inflammatory mediators in vivo.

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, FcγRII 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 cIgE

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 cIgE, and visualized them with PE-conjugated streptavidin. Fig. 4A shows that the binding of cIgE to eosinophils was clearly detectable. Furthermore, the cIgE 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 cIgE binding to eosinophils. Similar analyses of eosinophils from six patients showed that this observation is reproducible and that the binding of cIgE 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 cIgE, and the binding was also inhibited partially but significantly by anti-FcεRI mAb (Fig. 5A, $p < 0.05$). The amounts of bound cIgE levels as well as the amounts of cIgE-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 cIgE,

FIGURE 2. FACS analyses of the Ig bound on the eosinophil cell surface and the expression of Fc receptors. To analyze the Ig bound on the surface of the eosinophil (sIgE and sIgG), freshly isolated eosinophils were incubated with saturating amounts of FITC-conjugated goat anti-human IgE (*B*) or FITC-conjugated goat anti-human IgG (*C*), or the same amounts of control (FITC-conjugated goat anti-mouse IgM). To analyze the expression of Fc receptors (FcεRI, FcεRII, and FcγRII), 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 (*D*)), anti-FcεRII mAb (9P25 (*E*)), anti-FcγRII (2E1 (*F*)), or isotype-matched control mouse Ig followed by PE-conjugated F(ab')$_2$ fragments of sheep anti-mouse IgG. Fluorescence analysis used a FACScan flow cytometer. The forward scatter/side scatter dot-plot (*A*) and histograms of FITC or PE fluorescence (*B–F*) are shown.
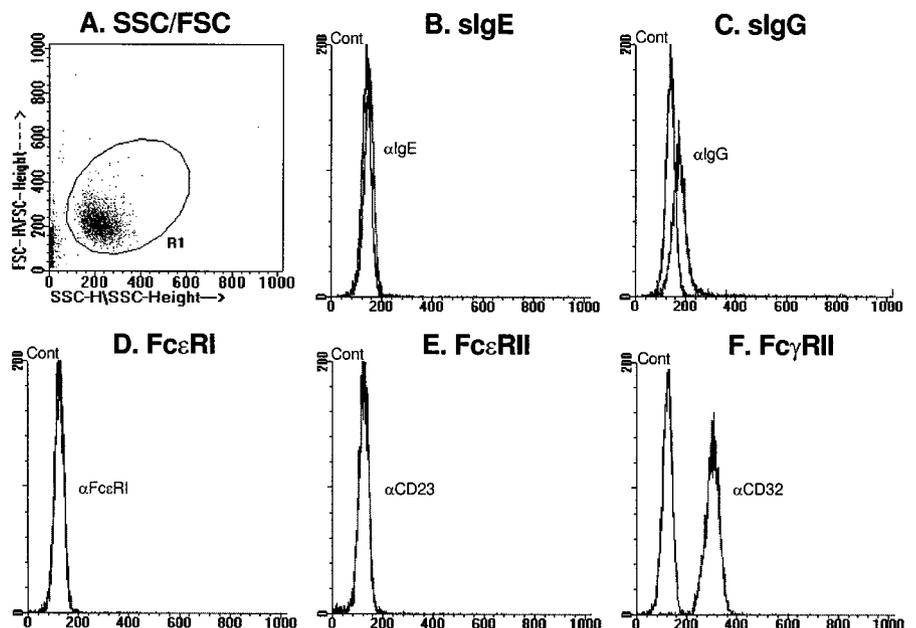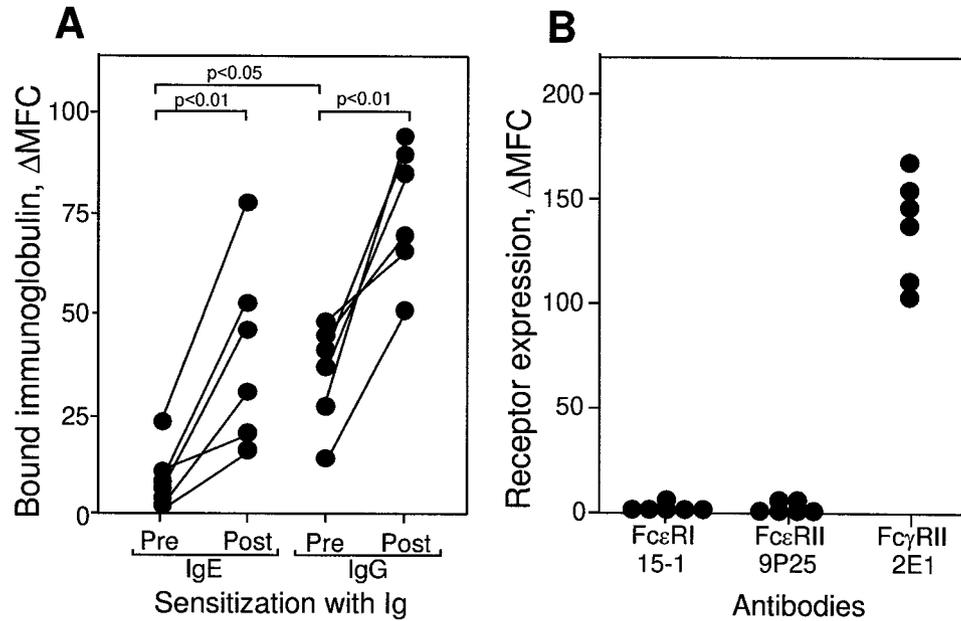
FIGURE 3. Binding of Ig to eosinophils (*A*) and expression of Fc receptors by eosinophils (*B*). In *A*, freshly isolated eosinophils were divided into two portions. One portion of cells (presensitization) were stained immediately with FITC-conjugated goat anti-human IgE or FITC-conjugated goat anti-human 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 FACScan 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 FACScan 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.

showed a strong linear correlation with serum levels of total IgE (Fig. 5*B*, $r^2 = 0.941$).

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
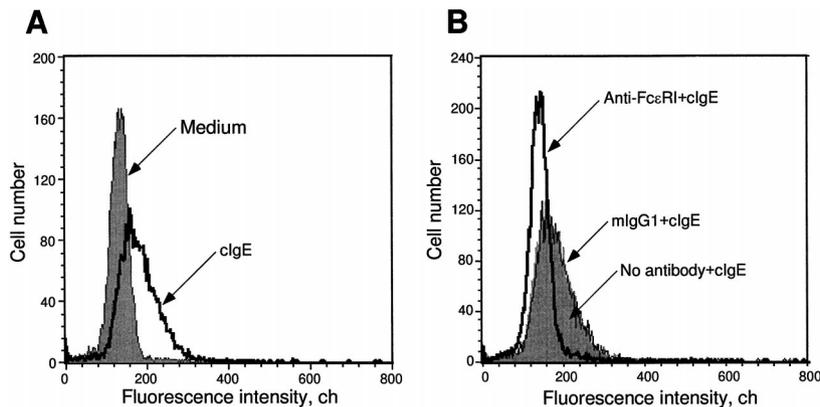

FIGURE 4. FACS analysis of cIgE binding to eosinophils. Isolated eosinophils were treated with lactic acid to remove in vivo bound IgE molecules. Fluorescence analysis used a FACScan flow cytometer, and a histogram of PE fluorescence is shown. *A* shows the binding of cIgE to eosinophils. Cells were 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.
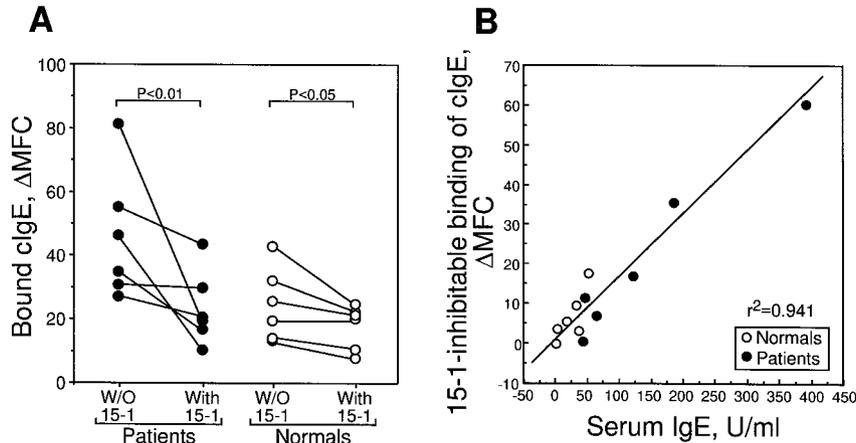
FIGURE 5. Binding of cIgE to eosinophils. In *A*, isolated eosinophils were treated with lactic acid to remove in vivo bound IgE molecules. Cells were preincubated with or without 10 μg/ml anti-FcϵRI mAb (15-1), sensitized with 25 μg/ml biotinylated cIgE, and stained with PE-conjugated streptavidin. 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 cIgE 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 cIgE. Anti-FcϵRI-inhibitable binding of cIgE was calculated as follows: (ΔMFC of cells preincubated without anti-FcϵRI mAb and then incubated with biotinylated cIgE and PE-conjugated streptavidin) $-$ (ΔMFC of cells preincubated with anti-FcϵRI mAb and then incubated with biotinylated cIgE and PE-conjugated streptavidin). Each dot represents the result from one subject; $\bigcirc$, normal subjects; $\bullet$, data from patients with hay fever.

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 without sensitization with cIgE revealed four distinct cell populations, including lymphocytes plus basophils, monocytes, neutrophils, and eosinophils. When cells were

sensitized with biotinylated cIgE and stained with PE-streptavidin, we found that cIgE binds to all the cell populations to various degrees (Fig. 6*C*). Notably, basophils were intensely stained, forming a unique population distinct from the lymphocyte cluster in PE fluorescence reading (Fig. 6*C*) and even in the green fluorescence reading (Fig.
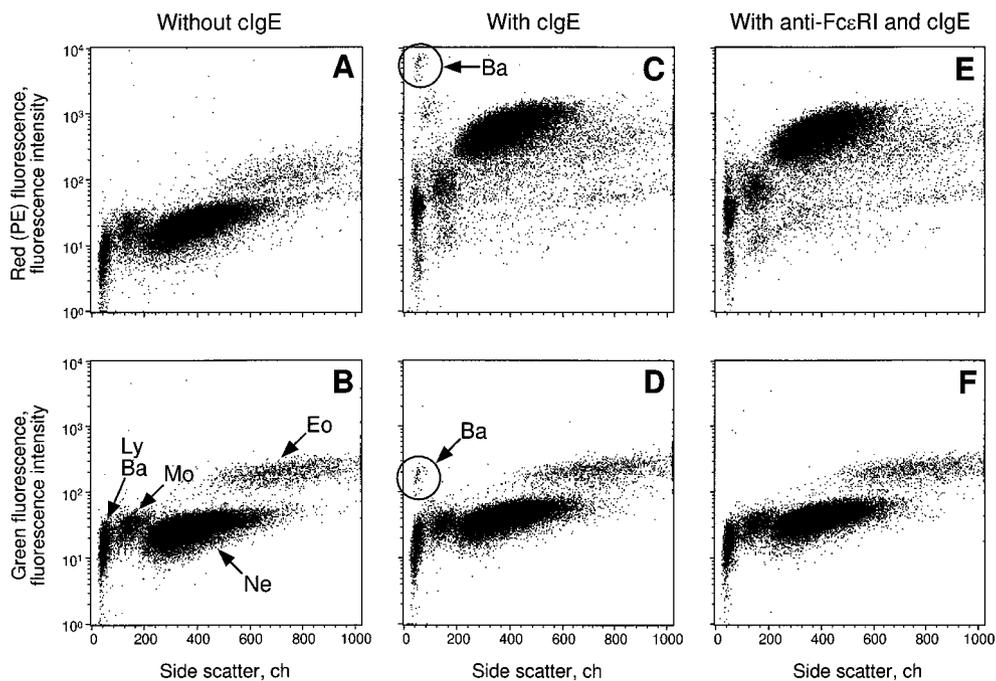

FIGURE 6. FACS analyses of whole blood for cIgE binding to leukocytes. Buffy coat leukocytes were treated with lactic acid buffer to remove in vivo bound IgE molecules. Cells were preincubated without (*A–D*) or with (*E* and *F*) anti-FcϵRI mAb (15-1). Cells were then passively sensitized with (*C–F*) or without (*A* and *B*) 25 μg/ml biotinylated cIgE, 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 cIgE 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*.
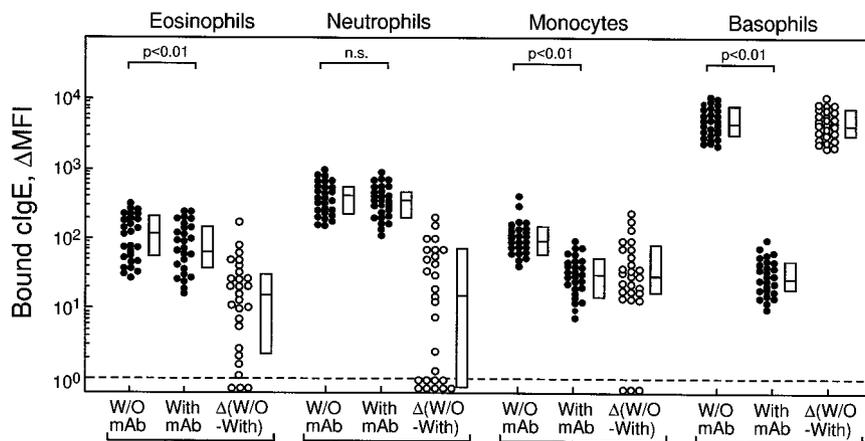
FIGURE 7. Binding of cIgE to leukocyte populations and the inhibitory effects of anti-FcεRI mAb (15-1). Buffy coat leukocytes were treated with lactic acid buffer, preincubated with or without FcεRI mAb, and sensitized with cIgE. The bound cIgE was visualized by PE-conjugated streptavidin and analyzed by a FACScan flow cytometer. Individual cell populations were electronically gated, based on their unique green fluorescence and side scatter characteristics, as shown in the dot plots of Fig. 6, *B* and *D*. PE fluorescence intensity was determined on 50,000 total cells from each sample using logarithmic amplification, which was converted to the linear equivalent by Becton Dickinson Lysis II software. Data are presented as the differences in the mean fluorescence intensity (ΔMFI) between test sample (e.g., biotinylated cIgE plus PE-conjugated streptavidin) and control (PE-conjugated streptavidin alone). Each dot represents an individual subject, and the statistical differences with or without preincubation with anti-FcεRI mAb are shown. Δ(W/O-With) was calculated by subtracting (ΔMFI value without anti-FcεRI mAb pretreatment) from (ΔMFI value with anti-FcεRI mAb pretreatment) for each individual subject. Open bars and horizontal bars show the 25th to 75th percentile range and median of the data points, respectively.

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-FcεRI mAb, the binding of cIgE to the basophil cluster was completely inhibited (Fig. 6E). In contrast, the inhibitory effects of anti-FcεRI 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-FcεRI mAb (15-1) are shown in Fig. 7. Binding of cIgE to leukocytes was observed in the following order: basophils (5062 ± 221) $\gg$ neutrophils (346 ± 23) > eosinophils (124 ± 15) $\cong$ monocytes (90 ± 9) (means $\pm$ SEM of ΔMFI, $n = 27$). Pretreatment of cells with anti-FcεRI mAb completely inhibited the cIgE binding to basophils ($99.0 \pm 0.1\%$ of inhibition, $n = 27$), suggesting that FcεRI plays a major role in cIgE binding to basophils. The binding of cIgE to monocytes was inhibited moderately but significantly by anti-FcεRI mAb in 24 of 27 patients ($45.0 \pm 5.5\%$ of inhibition, $p < 0.01$), suggesting roles for FcεRI and for other binding site(s) in cIgE binding to monocytes. In contrast, in neutrophils anti-FcεRI mAb minimally affected the binding of cIgE ($5.1 \pm 5.3\%$ of inhibition), and the inhibitory effects of anti-FcεRI mAb varied considerably among individuals as evidenced by a wide range of Δ(W/O-With) values. The effects of anti-FcεRI mAb on eosinophils were between those on monocytes and neutrophils, namely significant ($p < 0.01$) but small and variable inhibitory effects ($18.2 \pm 3.8\%$ of inhibition). The binding of cIgE to leukocytes due to FcεRI was observed in the following order: basophils (5015 ± 223) $\gg$ monocytes (40 ± 12) > eosinophils (25 ± 7) > neutrophils (13 ± 13) (means $\pm$ SEM of the ΔMFI with or without anti-FcεRI mAb). Thus, although the expression of FcεRI 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 in solution, they released a large amount of histamine (Fig. 8A, $85.7 \pm 4\%$ 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 ($51.3 \pm 10.5\%$ 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 ($5\sim50$ 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
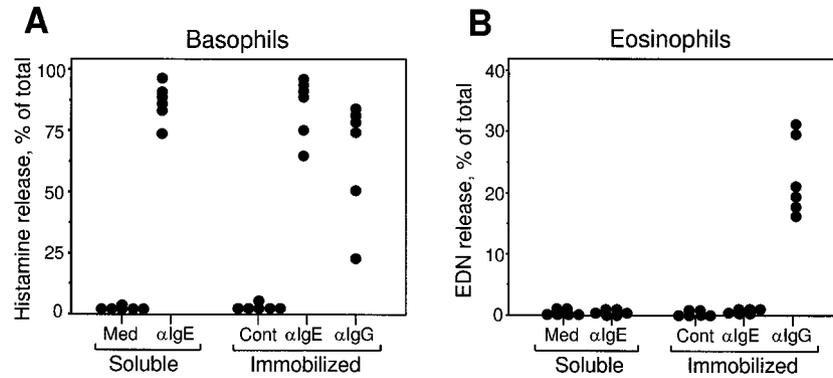
FIGURE 8. Degranulation of basophils and eosinophils induced by anti-IgE or anti-IgG Ab. Isolated eosinophils or basophil preparations were stimulated with 1 μg/ml F(ab')$_2$ fragments of goat anti-human IgE (soluble stimulus), or F(ab')$_2$ fragments of goat anti-human IgE, goat anti-human IgG, or goat 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% CO_2. 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.

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 LTC$_4$ 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
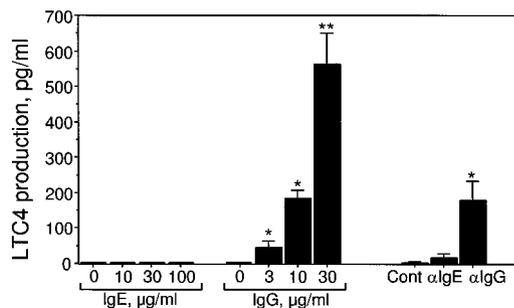

FIGURE 9. LTC$_4$ production by eosinophils stimulated with IgE- or IgG-dependent stimuli. Isolated eosinophils were stimulated with serial dilutions of human IgE or human IgG immobilized onto 96-well tissue culture plates, or with F(ab')$_2$ fragments of goat anti-human IgE, goat anti-human IgG, or goat IgG (control) immobilized onto tissue culture plates at 50 μg/ml. Cells were incubated for 1 h at 37°C and 5% CO_2, and concentrations of LTC$_4$ in the sample supernatants were measured by LTC$_4$ ELISA kit. Data show means $\pm$ SEM from three experiments using cells from three patients with hay fever.
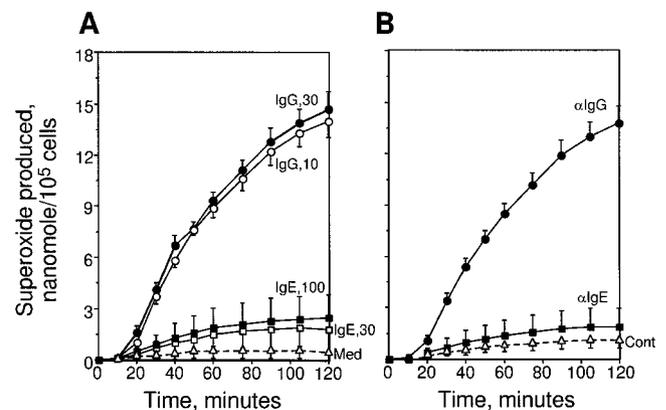

FIGURE 10. Superoxide anion production by eosinophils stimulated with IgE- or IgG-dependent stimuli. Isolated eosinophils were stimulated with human IgE or human IgG immobilized onto 96-well tissue culture plates at 10, 30, or 100 μg/ml, or with F(ab')$_2$ fragments of goat anti-human IgE, goat anti-human 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 $\pm$ SEM from six experiments using cells from six different patients with hay fever.

FcεRII (36). 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 hypereosinophilic 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 (18). A similar scenario is known for FcγRIII (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 cIgE was bound to basophils than to eosinophils (Fig. 7). Furthermore, >99% of cIgE 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 cIgE binding to eosinophils. Thus, we estimate that eosinophils from these ragweed hay fever patients express ~0.5% of the FcεRI compared with 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 cIgE (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/εBP, can also bind IgE through the carbohydrate recognition domain (46). Mac-2/εBP is expressed by various cell types, including neutrophils and eosinophils (47, 48). Indeed, Ab against Mac-2/εBP strongly inhibited IgE binding and IgE-dependent activation of human neutrophils (47). Therefore, by analogy to neutrophils, Mac-2/εBP 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 FcεR 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 LTB$_4$, without the apparent expression of FcεR (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

mediate 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.

Acknowledgments

References

1. Butterfield, J. H., K. M. Leiferman, and G. J. Gleich. 1995. Eosinophil-associated diseases. In *Samter's Immunologic Diseases*, Vol. 1, M. M. Frank, K. F. Austen, H. N. Claman, and E. R. Unanue, eds. Little, Brown, Boston, p. 501.

2. Kita, H., C. R. Adolphson, and G. J. Gleich. 1998. Biology of eosinophils. In *Allergy: Principles and Practice*. 5th Ed. E. Middleton, Jr., C. E. Reed, E. F. Ellis, N. F. Adkinson, Jr., J. W. Yunginger, and W. W. Busse, eds. Mosby-Year Book, St. Louis, p. 242.

3. Lee, T.-C., D. J. Lenihan, B. Malone, L. L. Roddy, and S. I. Wasserman. 1984. Increased biosynthesis of platelet-activating factor in activated human eosinophils. *J. Biol. Chem. 259:5526.*

4. Sedgwick, J. B., R. F. Vrtis, M. F. Gourley, and W. W. Busse. 1988. Stimulus-dependent differences in superoxide anion generation by normal human eosinophils and neutrophils. *J. Allergy Clin. Immunol. 81:876.*

5. Abu-Ghazaleh, R. I., T. Fujisawa, J. Mestecky, R. A. Kyle, and G. J. Gleich. 1989. IgA-induced eosinophil degranulation. *J. Immunol. 142:2393.*

6. Bousquet, J., P. Chanez, J. Y. Lacoste, G. Barneon, N. Ghavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, and F.-B. Michel. 1990. Eosinophilic inflammation in asthma. *N. Engl. J. Med. 323:1033.*

7. Naclerio, R. M., F. M. Baroody, A. Kagey-Sobotka, and L. M. Lichtenstein. 1994. Basophils and eosinophils in allergic rhinitis [Review]. *J. Allergy Clin. Immunol. 94:1303.*

8. Sedgwick, J. B., W. J. Calhoun, G. J. Gleich, H. Kita, J. S. Abrams, L. B. Schwartz, B. Volovitz, M. Ben-Yaakov, and W. W. Busse. 1991. Immediate and late airway response of allergic rhinitis patients to segmental antigen challenge: characterization of eosinophil and mast cell mediators. *Am. Rev. Respir. Dis. 144:1274.*

9. Collins, D. S., R. Dupuis, G. J. Gleich, K. R. Bartemes, Y. Y. Koh, M. Pollice, K. H. Albertine, J. E. Fish, and S. P. Peters. 1993. Immunoglobulin E-mediated increase in vascular permeability correlates with eosinophilic inflammation. *Am. Rev. Respir. Dis. 147:677.*

10. Sutton, B. J., and H. J. Gould. 1993. The human IgE network [Review]. *Nature 366:421.*

11. Sears, M. R., B. Burrows, E. M. Flannery, G. P. Herbison, C. J. Hewitt, and M. D. Holdaway. 1991. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N. Engl. J. Med. 325:1067.*

12. Capron, M., M. J. Truong, D. Aldebert, V. Gruart, M. Suemura, G. Delespesse, B. Tourvieille, and A. Capron. 1991. Heterogeneous expression of CD23 epitopes by eosinophils from patients: relationships with IgE-mediated functions. *Eur. J. Immunol. 21:2423.*

13. Gounni, A. S., B. Lamkhioued, K. Ochiai, Y. Tanaka, E. Delaporte, A. Capron, J. P. Kinet, and M. Capron. 1994. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature 367:183.*

14. Rajakulasingam, K., S. Till, S. Ying, M. Humbert, J. Barkans, M. Sullivan, Q. Meng, C. J. Corrigan, J. Bungre, J. A. Grant, A. B. Kay, and S. R. Durham. 1998. Increased expression of high affinity IgE (FcεRI) receptor-α chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. *Am. J. Respir. Crit. Care Med. 158:233.*

15. Barata, L. T., S. Ying, J. A. Grant, M. Humbert, J. Barkans, Q. Meng, S. R. Durham, and A. B. Kay. 1997. Allergen-induced recruitment of FcεRI+ eosinophils in human atopic skin. *Eur. J. Immunol. 27:1236.*

16. Ying, S., L. T. Barata, Q. Meng, J. A. Grant, J. Barkans, S. R. Durham, and A. B. Kay. 1998. 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 93:281.*

17. Sihra, B. S., O. M. Kon, J. A. Grant, and A. B. Kay. 1997. Expression of high-affinity IgE receptors (FcεRI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. *J. Allergy Clin. Immunol. 99:699.*

18. Sandilands, G. P., A. P. McLaren, D. Howie, and R. N. MacSween. 1995. Occult expression of CD32 (FcγRII) in normal human peripheral blood mononuclear cells. *Immunology 86:525.*

19. Mantzioris, B. X., M. F. Berger, W. Sewell, and H. Zola. 1993. Expression of the Fc receptor for IgG (FcγRII/CDw32) by human circulating T and B lymphocytes. *J. Immunol. 150:5175.*

20. Ide, M., D. Weiler, H. Kita, and G. J. Gleich. 1994. Ammonium chloride exposure inhibits cytokine-mediated eosinophil survival. *J. Immunol. Methods 168: 187.*

21. Walker, C., S. Rihs, R. K. Braun, S. Betz, and P. L. Bruijnzeel. 1993. Increased expression of CD11b and functional changes in eosinophils after migration across endothelial cell monolayers. *J. Immunol. 150:4061.*

22. Mawhorter, S. D., D. A. Stephany, E. A. Ottesen, and T. B. Nutman. 1996. Identification of surface molecules associated with physiologic activation of eosinophils: application of whole-blood flow cytometry to eosinophils. *J. Immunol. 156:4851.*

23. Matsumoto, K., J. Appiah-Pippim, R. P. Schleimer, C. A. Bickel, L. A. Beck, and B. S. Bochner. 1998. CD44 and CD69 represent different types of cell-surface activation markers for human eosinophils. *Am. J. Respir. Cell Mol. Biol. 18:860.*

24. Thurau, A. M., U. Schylz, V. Wolf, N. Krug, and U. Schauer. 1996. Identification of eosinophils by flow cytometry. *Cytometry 23:150.*

25. Pruzansky, J. J., L. C. Grammer, R. Patterson, and M. Roberts. 1983. Dissociation of IgE from receptors on human basophils. I. Enhanced passive sensitization for histamine release. *J. Immunol. 131:1949.*

26. Kita, H., R. I. Abu-Ghazaleh, S. Sur, and G. J. Gleich. 1995. Eosinophil major basic protein induces degranulation and IL-8 production by human eosinophils. *J. Immunol. 154:4749.*

27. Kaneko, M., S. Horie, M. Kato, G. J. Gleich, and H. Kita. 1995. A crucial role for β_2 integrin in the activation of eosinophils stimulated by IgG. *J. Immunol. 155:2631.*

28. Horie, S., and H. Kita. 1994. CD11b/CD18 (Mac-1) is required for degranulation of human eosinophils induced by human recombinant granulocyte-macrophage colony-stimulating factor and platelet-activating factor. *J. Immunol. 152:5457.*

29. Yamaguchi, M., C. S. Lantz, H. C. Oettgen, I. M. Katona, T. Fleming, I. Miyajima, J. P. Kinet, and S. J. Galli. 1997. 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. 185:663.*

30. MacGlashan, D. W., Jr., B. S. Bochner, D. C. Adelman, P. M. Jardieu, A. Togias, J. McKenzie-White, S. A. Sterbinsky, R. G. Hamilton, and L. M. Lichtenstein. 1997. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J. Immunol. 158:1438.*

31. Malveaux, F. J., M. C. Conroy, N. F. Adkinson, Jr., and L. M. Lichtenstein. 1978. IgE receptors on human basophils: relationship to serum IgE concentration. *J. Clin. Invest. 62:176.*

32. Maurer, D., E. Fiebiger, B. Reininger, B. Wolff-Winiski, M. H. Jouvin, O. Kilgus, J. P. Kinet, and G. Stingl. 1994. Expression of functional high affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. *J. Exp. Med. 179:745.*

33. Costa, J. J., P. F. Weller, and S. J. Galli. 1997. The cells of the allergic response: mast cells, basophils, and eosinophils [Review]. *J. Am. Med. Assoc. 278:1815.*

34. Schroeder, J. T., and D. W. MacGlashan, Jr. 1997. New concepts: the basophil [Review]. *J. Allergy Clin. Immunol. 99:429.*

35. Gounni, A. S., B. Lamkhioued, E. Delaporte, A. Dubost, J. P. Kinet, A. Capron, and M. Capron. 1994. The high-affinity IgE receptor on eosinophils: from allergy to parasites or from parasites to allergy? [Review]. *J. Allergy Clin. Immunol. 94:1214.*

36. de Andres, B., E. Rakasz, M. Hagen, M. L. McCormik, A. L. Mueller, D. Elliot, A. Metwali, M. Sandor, B. E. Britigan, J. V. Weinstock, and R. G. Lynch. 1997. Lack of Fcε receptors on murine eosinophils: implications for the functional significance of elevated IgE and eosinophils in parasitic infections. *Blood 89: 3826.*

37. Capron, M., T. Jouault, L. Prin, M. Joseph, J. C. Ameisen, A. E. Butterworth, J. P. Papin, J. P. Kusnierz, and A. Capron. 1986. Functional study of a monoclonal antibody to IgE Fc receptor (FcεRII) of eosinophils, platelets, and macrophages. *J. Exp. Med. 164:72.*

38. Capron, A., J. P. Dessaint, M. Capron, M. Joseph, J.-C. Ameisen, and A. B. Tonnel. 1986. From parasites to allergy: a second receptor for IgE. *Immunol. Today 7:15.*

39. Jouault, T., M. Capron, J. M. Balloul, J. C. Ameisen, and A. Capron. 1988. Quantitative and qualitative analysis of the Fc receptor for IgE (FcεRII) on human eosinophils. *Eur. J. Immunol. 18:237.*

40. Terada, N., A. Konno, Y. Terada, S. Fukuda, T. Yamashita, T. Abe, H. Shimada, K. Ishida, K. Yoshimura, Y. Tanaka, C. Ra, K. Ishikawa, and K. Togawa. 1995. IL-4 upregulates FcεRI α-chain messenger RNA in eosinophils. *J. Allergy Clin. Immunol. 96:1161.*

41. Seminario, M.-C., and B. S. Bochner. 1998. Eosinophil (EOS) possess intracellular α-chain of the high affinity IgE receptor but little or no expression on their surface. *J. Allergy Clin. Immunol. 101:S175 [Abstr.].*

42. Zhu, X., K. J. Hamann, N. M. Muñoz, N. Rubio, D. Mayer, A. Hernreiter, and A. R. Leff. 1998. Intracellular expression of FcγRIII (CD16) and its mobilization by chemoattractants in human eosinophils. *J. Immunol. 161:2574.*

43. Denburg, J. A., S. Telizyn, H. Messner, B. L. N. Jamal, S. J. Ackerman, G. J. Gleich, and J. Bienenstock. 1985. Heterogeneity of human peripheral blood

eosinophil-type colonies: evidence for a common basophil-eosinophil progenitor. *Blood 66:312.*

44. Hartnell, A., G. M. Walsh, R. Moqbel, and A. B. Kay. 1989. Phenotyping markers of human eosinophils. *J. Allergy Clin. Immunol. 83:192 [Abstr.].*

45. Ebisawa, M., R. P. Schleimer, C. Bickel, and B. S. Bochner. 1995. Phenotyping of purified human peripheral blood eosinophils using the blind panel mAb. In *Leukocyte Typing V: White Cell Differentiation Antigens*, Vol. 1. S. Schlossman, L. Boumsell, W. Gilks, J. Harlan, T. Kishimoto, C. Morimoto, J. Ritz, S. Shaw, R. Silverstein, T. Springer, T. Tedder, and R. Todd, eds. Oxford University Press, New York, p. 1036.

46. Drickamer, K. 1988. Two distinct classes of carbohydrate-recognition domains in animal lectins [Review]. *J. Biol. Chem. 263:9557.*

47. Truong, M. J., V. Gruart, J. P. Kusnierz, J. P. Papin, S. Loiseau, A. Capron, and M. Capron. 1993. Human neutrophils express immunoglobulin E (IgE)-binding proteins (Mac-2/εBP) of the S-type lectin family: role in IgE-dependent activation. *J. Exp. Med. 177:243.*

48. Truong, M. J., V. Gruart, F. T. Liu, L. Prin, A. Capron, and M. Capron. 1993. IgE-binding molecules (Mac-2/εBP) expressed by human eosinophils: implication in IgE-dependent eosinophil cytotoxicity. *Eur. J. Immunol. 23:3230.*

49. Robinson, D. S., Q. Hamid, S. Ying, A. Tsicopoulos, J. Barkans, A. M. Bentley, C. Corrigan, S. R. Durham, and A. B. Kay. 1992. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med. 326:298.*

50. Kay, A. B., S. Ying, V. Varney, M. Gaga, S. R. Durham, R. Moqbel, A. J. Wardlaw, and Q. Hamid. 1991. Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J. Exp. Med. 173:775.*

51. Ghaffar, O., S. R. Durham, K. Al-Ghamdi, E. Wright, P. Small, S. Frenkiel, H. J. Gould, and Q. Hamid. 1998. Expression of IgE heavy chain transcripts in the sinus mucosa of atopic and nonatopic patients with chronic sinusitis. *Am. J. Respir. Cell Mol. Biol. 18:706.*

52. Moqbel, R., G. M. Walsh, T. Nagakura, A. J. MacDonald, A. J. Wardlaw, Y. Iikura, and A. B. Kay. 1990. The effect of platelet-activating factor on IgE binding to, and IgE-dependent biological properties of, human eosinophils. *Immunology 70:251.*

53. Tkaczyk, C., M. Viguier, Y. Boutin, P. Frandji, B. David, J. Hebert, and S. Mecheri. 1998. Specific antigen targeting to surface IgE and IgG on mouse bone marrow-derived mast cells enhances efficiency of antigen presentation. *Immunology 94:318.*

54. Mehlhop, P. D., M. van de Rijn, A. B. Goldberg, J. P. Brewer, V. P. Kurup, T. R. Martin, and H. C. Oettgen. 1997. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc. Natl. Acad. Sci. USA 94:1344.*