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Expression of the High Affinity IgE Receptor by Neutrophils of Individuals with Allergic Asthma is Both Minimal and Insensitive to Regulation by Serum IgE

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Abstract

We evaluated the hypothesis that serum IgE regulates neutrophil FcεRI expression in the same manner as described for other FcεRI⁺ cells. FcεRI expression by neutrophils of 40 asthma subjects and 20 control subjects did not correlate with serum IgE levels, whereas FcεRI expression by basophils of the same subjects showed a highly significant correlation. The level of FcεRI expression by neutrophils of both asthma and control subjects was approximately 1% of that for basophil FcεRI expression. IgE⁺ neutrophils were minimally detectable, and FcεRI α subunit was not detected in Western blots of neutrophil membranes and cytosol. The neutrophil FcεRI did not support anti-IgE stimulated superoxide release or IgE-induced increase in neutrophil survival. We conclude that FcεRI expression by neutrophils of both asthma patients and control individuals is minimal at best and that, if present, neutrophil FcεRI expression, unlike that of other human FcεRI⁺ cells, is not regulated by serum IgE.

Keywords

Allergic asthma; Neutrophils; Basophils; High affinity IgE receptor; IgE antibody; Fc receptors

Introduction

Neutrophils are key effector cells in both the innate and adaptive immune responses against bacterial and fungal pathogens and also contribute to the pathogenesis of many inflammatory disorders, including several in the lung [1–3]. In this context, neutrophils have become strongly implicated in the pathogenesis of severe asthma, both in acute exacerbations of chronic asthma

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and in the steroid-resistant form of asthma [4–6]. Indeed, a distinct neutrophilic phenotype of asthma has been described [7].

The expression by neutrophils of multiple immunoreceptor tyrosine-based activation motif (ITAM)-linked Fc receptors for IgG and IgA provides an essential link between neutrophil activation and the adaptive immune response [8,9]. It has been reported that neutrophils of individuals with mild asthma also express the high affinity receptor for IgE (FcεRI) [10,11], which is the predominant ITAM-linked immunoglobulin receptor expressed on human mast cells and basophils [12]. Neutrophils of the asthma subjects responded to IgE-mediated activation in a variety of manners, including production of IL 8 [10], induction of cyclooxygenase-2 expression and resultant production of prostaglandin E₂ and thromboxane A₂ [13,14], and release of eosinophil cationic protein [15]. In contrast, neutrophils of non-asthmatic individuals did not express FcεRI or respond to IgE-mediated activation in the same studies [10,11,13,15]. The IgE antibody has also been reported to prolong survival of neutrophils isolated from asthma patients in culture [11].

Expression of the FcεRI is a steady state process that reflects both the rate of synthesis and the rate of unoccupied receptor loss from the cell surface [12,16]. Whereas exposure to cytokines or viral infection can induce or increase synthesis of the receptor [16–19], and therefore operate independently of IgE, IgE itself regulates receptor loss. Specifically, occupancy of the FcεRI by IgE blocks endocytosis of FcεRI [20], thus shifting the steady state balance such that the level of FcεRI expression increases as the level of serum IgE increases [21–23]. The ability of serum IgE to regulate FcεRI expression on basophils, mast cells, monocytes, and plasmacytoid dendritic cells in this manner, and in turn their IgE-mediated reactivity, is now well established [12,23–25]. Accordingly, the regulation of FcεRI expression by serum IgE is believed to be an important component in the therapeutic benefit of anti-IgE monoclonal antibody (omalizumab) therapy for allergic asthma [26–28]. Thus, the finding that neutrophils of individuals with mild allergic asthma, but not neutrophils of normal individuals, expressed FcεRI [10,11] suggested that serum IgE levels would also regulate FcεRI expression by neutrophils in the same manner as reported for the other FcεRI⁺ cells [12,23–25]. The present study was performed to examine the relationship between serum IgE level and FcεRI expression by neutrophils of asthma patients with differing degrees of disease severity.

Materials and methods

Study subjects

Asthma subjects were recruited from outpatients visiting the Section of Allergy/Immunology at Rush University Medical Center. The asthma subjects were non-smokers, had a positive history of physician-diagnosed mild intermittent to severe persistent asthma for at least one year, and had a confirmed IgE-mediated sensitivity to one or more aeroallergens as measured by a positive RAST value obtained within the preceding two years or by skin testing with a prick/aeroallergen panel (Hollister-Stier Laboratories, Spokane, WA) at the time of enrollment. Exclusion factors included having been treated with omalizumab within the preceding 12 months, having a respiratory infection, receiving allergen immunotherapy, having a history of alcohol or drug abuse, or being pregnant. All patients underwent a baseline pulmonary function test (pre-test only) and completed the Asthma Control Test [29] (QualityMetric Inc., Lincoln, RI). Asthma medications used in the preceding two weeks prior to enrollment were recorded. The asthma subjects were classified as mild intermittent, mild persistent, moderate persistent, or severe persistent asthmatics according to National Heart Blood Lung Institute guidelines [30]. Healthy non-smoking, non-asthmatic subjects were recruited from personnel at Rush University Medical Center and from the general population to serve as controls. The control subjects had a negative history for atopic disease, had a negative skin test to the prick/

aeroallergen skin test panel at the time of enrollment, and had normal pulmonary function (> 80 % FEV1) at the time of enrollment.

Antibodies and reagents

Mouse monoclonal antibody (mAb) 22E7 (IgG1) specific for human FcεRI α-subunit (FcεRIα) was kindly provided by Dr. J. Kochan (Roche Pharmaceuticals, Nutley, NJ); mouse IgG1 was purchased from R & D Systems (Minneapolis, MN). Mouse anti-FcεRI α-chain mAb (clone CRA-1; IgG2b) and mouse IgG2b were purchased from eBioscience (San Diego, CA). Phycoerythrin (PE)-conjugated mouse anti-human CD123, mouse anti-human IgE (clone G7-18; IgG2a), and FITC-conjugated annexin V were purchased from BD Biosciences (San Jose, CA). AlexaFluor (AF) 647-conjugated goat anti-mouse IgG1, AF647-conjugated goat anti-mouse IgG (H + L chains), and mouse IgG2a were purchased from Invitrogen (Carlsbad, CA). Horseradish peroxidase-conjugated goat anti-mouse Ig (H + L chains) was purchased from Southern Biotechnology (Birmingham, AL). The IgE myeloma (HU-IgE Myeloma JK) was obtained from Hybridoma Reagent Laboratories (Baltimore, MD). Human IgG (Gammagard) was obtained from Baxter, Inc. (Deerfield, IL), and human IgA was obtained from Sigma-Aldrich (St. Louis, MO). Human recombinant GM-CSF was purchased from Peprotech (Rocky Hill, NJ). Goat anti-human IgE was purchased from Immunology Consultants Laboratory (Newberg, OR).

Cell isolation

Neutrophils and peripheral blood mononuclear cells (PBMC) were isolated under sterile conditions from freshly drawn venous blood. Briefly, the blood was diluted 1:1 with sterile saline (Baxter) containing 4 mM EDTA, was overlaid onto a 3-ml cushion of lymphocyte separation medium (Lonza, Walkersville, MD), and was centrifuged at 300 g for 30 min at 4° C. For experiments that required larger starting volumes of blood, the diluted blood was overlaid onto 10-ml cushions of lymphocyte separation medium. Neutrophils were isolated from the granulocyte/erythrocyte pellet by two or three cycles of brief hypotonic lysis in ice-cold H₂O; isotonicity was restored by addition of 2X concentrated Hanks Balanced Salt Solution (HBSS) (without Ca²⁺ and Mg²⁺) containing 5 mM HEPES, pH 7.4 [31]. The PBMC were recovered at the plasma:separation medium interphase, were diluted 1:3 with sterile saline, and were collected by centrifugation at 250 g for 10 min at 4° C. The PBMC were washed once in HBSS (without Ca²⁺ and Mg²⁺), and the neutrophils and PBMC were each suspended in RPMI 1640 for cell counting. Neutrophils isolated in this manner are routinely > 95 % viable as measured by reactivity with FITC-annexin V and by forward light-scatter and side light-scatter profiles (results not shown).

Measurement of neutrophil and basophil FcεRI expression

Neutrophils or PBMC (500,000 cells of each) were transferred to 1.5-ml centrifuge tubes pre-rinsed with 1 % bovine serum albumin and were incubated in 90 µl of phosphate-buffered saline/0.25 % bovine serum albumin/1 mM EDTA (PBE) containing 0.05 % human IgG for 5 min on ice to block non-specific antibody binding. The PBMC then were incubated with 2 µg/ml of PE-conjugated anti-CD123 along with 2 µg/ml of anti-FcεRIα mAb 22E7 or mouse IgG1 isotype control for 30 min at 4° C. For neutrophils, the PE-conjugated anti-CD123 was omitted from the staining mixture, and the concentrations of the mAb 22E7 and isotype control were increased to 5 µg/ml to compensate for the potentially increased number of FcεRI⁺ cells. The cells were washed twice in PBE and then were incubated with AF647-conjugated goat IgG anti-mouse IgG1 (1:1000 dilution in PBE) for 30 min on ice. The cells were washed once in PBE and were suspended in 1 % formaldehyde in PBS for analysis. Fluorescence intensities of 30,000 PBMC and 10,000 neutrophils were measured using a FACSCalibur instrument (BD Biosciences), which was calibrated daily with fluorescent beads to maintain uniformity of the

instrument settings throughout the study. The neutrophil preparations routinely contained less than 2 % PBMC as determined by the forward light-scatter and side light-scatter profiles. Basophil FcεRI expression was quantified as the difference in the median fluorescence intensity (MFI) of CD123⁺ cells stained with mAb 22E7 and with the isotype control. Neutrophil FcεRI was quantified as the difference in the MFI of cells stained with mAb 22E7 and with the isotype control. For some analyses, the neutrophils and PBMC were also incubated with anti-FcεRIα mAb CRA-1 (10 µg/ml), with anti-IgE mAb G7-18 (10 µg/ml), or with the same concentration of isotype controls. Bound mAb CRA-1 and bound mAb G7-18 were detected by subsequent incubation with AF647-conjugated goat IgG anti-mouse IgG (H + L chain-specific) as described above. Expression was measured and quantified as described above. The percentages of FcεRI⁺(mAb 22E7⁺) neutrophils were also determined in some experiments. Non-specific background reactivity was set at 1 % positive cells after staining with the mouse IgG1 isotype control. In all cases, non-viable neutrophils were eliminated from the analyses by gating electronically on the viable neutrophil population (> 95 % of the cells) defined by the forward light-scatter and side light-scatter profiles.

Western blot analysis

Membrane and cytosolic subcellular fractions of neutrophils were prepared as described previously [31]. Briefly, neutrophils isolated as above were suspended at a concentration of 10⁸ cells/ml in ice-cold extraction buffer (50 mM Tris-HCl (pH 7.5), 2 mM EGTA, 50 mM 2-mercaptoethanol, 1 mM phenylmethanesulfonyl fluoride, 2 mM 4-(2-aminoethyl) benzenesulfonyl fluoride, 10 µg/ml leupeptin, 10 µg/ml pepstatin, and 4 mM difluorophosphate). The cells were sonicated twice for 12 sec each time on ice, the sonicate was centrifuged at 800 × g to remove unbroken cells and cell debris, and the resultant supernatant was centrifuged at 150,000 × g for 90 min at 4° C. The cell pellet (membrane fraction) was suspended in extraction buffer by brief sonication on ice, and the membrane and cytosol fractions were stored in aliquots at -80° C until analysis. A whole cell lysate of purified basophils was prepared as described previously [20] and was stored at -80° C until use. Polyacrylamide (10 %) gel electrophoresis and Western blot analysis were performed as described previously [31] but using 2.5% bovine serum albumin as the blocking reagent. The blots were probed with 0.56 µg/ml of mAb 22E7 or with the same concentration of mouse IgG1 in Tris-buffered saline containing 0.1% Tween (TBST). The blots were incubated with horseradish peroxidase-conjugated goat anti-mouse Ig (1:15,000 dilution in TBST), and the protein bands were developed using Amersham ECL Western blotting analysis system (GE Healthcare, Piscataway, NJ). Densitometry was performed using an AlphaImager and AlphaEase FC analysis program (Alpha Innotech; San Leandro, CA).

Measurement of neutrophil superoxide production

Superoxide production was measured as described previously [32]. Briefly, neutrophils (2 × 10⁵) were incubated with or without 10 µg/ml goat anti-human IgE in HEPES-buffered HBSS containing 300 µg/ml human serum albumin and 50 µM cytochrome c for 60 min at 37° C in a Synergy HT microplate reader (Bio-Tek; Winooski, VT). Incubations were performed in wells in a 96-well flat bottom tissue culture plate that had been incubated with 100 µl of 2 mg/ml human serum albumin for 2 hr at 37° C to block non-specific protein binding sites. As positive controls, cells were also incubated in wells that had been pre-coated with human IgG or human IgA by incubating 50 µl of the indicated concentrations of IgG or IgA overnight at 4° C before subsequent incubation with the human serum albumin as above. In all cases, the wells were rinsed twice with PBS before use, and total incubation volumes were 200 µl. Absorbance at 550 nm was recorded at 15 min intervals for 120 min, and superoxide production was calculated as described previously [32]. Results are presented for the 60 min time point.

Measurement of neutrophil survival

Neutrophils (10^6) were cultured without or with the indicated concentrations of myeloma IgE or 1 ng/ml GM-CSF in RPMI 1640 containing 100 U/ml of penicillin, 100 µg/ml of streptomycin, 2 mM L-glutamine, and 10 % heat-inactivated autologous serum in 48-well tissue culture plates for 18 hr at 37° C in a 5% CO₂ atmosphere. Total culture volume was 0.5 ml. Cultures were stopped by centrifugation at 300 × g for 10 min at 4° C, and neutrophil viability was measured by flow cytometry after incubation of the neutrophils with FITC-conjugated annexin V as described by the manufacturer. The fluorescence intensity of 10,000 cells was measured, and neutrophil viability was expressed as the percentage of cells negative for FITC-conjugated annexin V.

Measurement of serum total IgE

Venous blood was drawn into a BD vacutainer SST tube (BD, Franklin Lakes, NJ), as allowed to stand for 30 min at room temperature, and was centrifuged at 900 g for 20 min at 4° C. The serum was carefully aspirated and was stored in aliquots at -70° C until measurement of total IgE content by ELISA (Alpha Diagnostic International, San Antonio, TX). The detection limit of the assay was 1 IU/ml.

Statistical analysis

Correlations between FcεRI expression and serum IgE levels were calculated using the Pearson correlation coefficient after conversion of the absolute serum IgE levels to the log₁₀ values. Results of grouped data are presented as the mean ± SE or as the median, upper and lower quartiles, and range. Statistical significance was analyzed using ANOVA, and post-hoc analysis of significant differences was performed using Tukey's multiple comparisons test. Differences were considered significant for p values of less than 0.05.

Results

Profile of study subjects

Forty subjects with allergic asthma and 20 non-allergic, non-asthmatic control subjects participated in the study. The asthma subjects represented all four stages of asthma severity according to the National Heart Lung Blood Institute guidelines and had total serum IgE levels of 1 to 1041 IU/ml, with a range in the serum IgE levels noted among subjects within each category of asthma severity (Table 1). Control subjects had total serum IgE levels of 1 to 71 IU/ml, and 14 of the 20 control subjects had a total serum IgE level of less than 10 IU/ml. All asthma subjects except two were being treated at the time of the study with a short-acting β-agonist alone or some combination of a short acting β-agonist, an inhaled corticosteroid, a long-acting β-agonist, or a leukotriene modifier/receptor antagonist (Table 1). One asthma subject had used an inhaled corticosteroid within 12 hr of the study visit. No other asthma subject had used any medications for 12 hr prior to the visit. None of the asthma subjects had received anti-IgE (omalizumab) therapy in the preceding 12 months. Two asthma subjects had been treated with omalizumab prior to that time. Their serum IgE levels at the time of entry into the present study were 219 IU/ml and 535 IU/ml.

Neutrophil FcεRI expression is independent of serum IgE level

Expression of FcεRI by neutrophils and, as a positive control, by basophils of the control and asthma subjects was determined by flow cytometry using mAb 22E7, which reacts with either free or IgE-liganded FcεRIα [33]. Representative results obtained with neutrophils and basophils of a control subject and of an asthma subject are shown in Fig. 1. To assess the relationship between FcεRI expression and the level of serum IgE, FcεRI expression was quantified as the difference between the median fluorescence intensity (MFI) of cells incubated

with the 22E7 mAb and that of cells incubated with the isotype control mAb. As expected, FcεRI expression by basophils displayed a highly significant correlation with the level of serum IgE when evaluated for all 60 subjects (Fig. 2A). The median values for FcεRI receptor expression increased from 343 and 532 net MFI for basophils of control and asthma subjects, respectively, within the lowest range (1–10 IU/ml) of serum IgE levels to 1808 net MFI for basophils of asthma subjects within the highest range (>100 IU/ml) of serum IgE levels (Fig. 2C). In contrast, FcεRI expression by neutrophils did not correlate with the level of serum IgE (Fig. 2B) and, indeed, the median level of receptor expression was < 23 net MFI at all levels of serum IgE (Fig. 2D). No differences were noted in the levels of FcεRI expression by neutrophils of control and asthma subjects (Fig. 2D). In results not shown, the levels of basophil FcεRI expression also correlated significantly with serum IgE levels for control subjects alone ($r^2=0.76$; $n=20$) and within each subcategory of asthma subjects (r^2 values of 0.55–0.81; n values = 7–12). The level of FcεRI expression by neutrophils of asthma subjects with mild persistent disease was inversely correlated ($r^2=0.78$; $n=7$) with the serum IgE levels, but no correlation was observed between the levels of neutrophil FcεRI expression and IgE levels for any of the other subject groups (r^2 values of 0.01–0.37; n values = 8–12) or for the control subjects ($r^2=0.01$; $n=20$). In addition, no differences were noted in the relationships between basophil or neutrophil FcεRI expression and serum IgE levels for asthma patients who had used (r^2 values = 0.53 and 0.03 for basophils and neutrophils, respectively; $n=23$) or not used (r^2 values = 0.68 and 0.13 for basophils and neutrophils, respectively; $n=17$) inhaled corticosteroids, and neither basophil nor neutrophil FcεRI expression correlated with FEV1 values (results not shown). The percentages of FcεRI⁺ (mAb 22E7⁺) neutrophils also did not differ significantly between control (5 ± 1 %; mean $\pm$ SE) and asthma (8 ± 2 %) subjects when evaluated for the first 16 control subjects and first 21 asthma subjects enrolled in the study.

Low neutrophil FcεRI expression is paralleled by minimally detectable bound IgE

A second anti FcεRIα mAb (CRA-1) [34] used by others to evaluate FcεRI expression by neutrophils [11,35] was added to the experimental protocol while the study was in progress. The mAb CRA-1 yielded a positive correlation ($r^2 = 0.897$; $p < 0.001$) between basophil FcεRI expression and serum IgE levels for the six control subjects and 18 asthma subjects studied (results not shown). In contrast, FcεRI expression by neutrophils of the same subjects did not exceed 3 MFI at any level of serum IgE (results not shown). Doubling the concentration of the CRA-1 mAb or of the AF67-conjugated secondary antibody did not increase the level of mAb CRA-1 reactivity (results not shown). The presence of neutrophil-bound IgE was also assessed by addition of FITC-conjugated anti-IgE to the experimental protocol for six of the same asthmatic subjects and three of the same control subjects. Basophils of all nine subjects were positive for bound IgE (Fig. 3A), and the level of FITC-anti-IgE reactivity paralleled the level of FcεRI expression determined using either mAb 22E7 or mAb CRA-1. In contrast, the neutrophils of the same nine subjects had minimally detectable levels (< 10 MFI) of bound IgE (Fig. 3B). The serum IgE levels for the nine subjects ranged from 5–215 IU/ml (results not shown).

The FcεRIα subunit is not detectable in neutrophil membranes or cytosol

Given the low levels of neutrophil FcεRI expression, neutrophil membrane and cytosolic subcellular fractions were probed for the presence of the immature (46–48 kD) and the mature (55–65 kD) forms of FcεRIα [12,36] by Western blot. The mAb 22E7 detected both the immature and diffuse mature FcεRIα proteins in a lysate of 5×10^5 purified basophils (Fig. 4A), but did not detect either the immature or mature FcεRIα proteins in membrane or cytosolic fractions (each equivalent to 10^6 neutrophils) isolated from neutrophils of two asthma subjects (Fig. 4A and B, respectively). The mAb 22E7 reacted non-specifically with an approximately 60 kD protein in the membrane fraction and in the basophil lysate in Fig. 4A as well as with an approximately 62–63 kD protein in the membrane fraction in Fig. 4B. Densitometry of

results obtained with neutrophil membrane fractions of three different asthma subjects indicated, however, that the intensity of the 22E7 reactivity was increased by only 1.54 ± 0.16 fold (mean $\pm$ SE; $n=3$) as compared to isotype control (results not shown). The intensity of an approximately 29 kD protein that reacts non-specifically with the horseradish peroxidase-conjugated goat anti-mouse Ig (results not shown) was used to normalize the values for differences in sample loading. The same pattern was observed in a Western blot performed with membranes corresponding to 3×10^6 neutrophils of one asthma subject (results not shown). The mAb 22E7, in contrast did not react specifically with any protein band that corresponded to the immature Fc ϵ RI α protein in either the membrane or cytosol fractions (Fig. 4A and 4B), even after prolonged exposure (40 min) exposure of the film (results not shown). The 22E7 mAb also did not detect the immature Fc ϵ RI α protein in the membrane or cytosol fractions of neutrophils of a third asthma subject (results not shown).

Anti-IgE does not stimulate neutrophil superoxide production

Based on a report that anti-IgE stimulated a superoxide-dependent induction of cyclooxygenase synthesis and activity in neutrophils [13], we evaluated the ability of anti-IgE to stimulate release of superoxide production. In three experiments, 10 μ g/ml of anti-IgE did not stimulate any superoxide production over a 60 min incubation period (Fig. 5A). In contrast, IgG and IgA each stimulated concentration-dependent release of superoxide in the same experiments when immobilized at the indicated concentrations. The anti-IgE stimulated histamine release by human basophils in previous experiments [37].

IgE does not prolong neutrophil survival

To confirm a previous observation that IgE prolongs neutrophil survival in culture [11], neutrophils were cultured with IgE myeloma, with GM-CSF as a positive control, or in RPMI culture medium alone for 20 hr. Approximately 38 % of the neutrophils remained viable, as determined by the absence of FITC-annexin-V staining, after culture in the RPMI medium alone (Fig. 5B). Culture in the presence of 100 ng/ml of IgE myeloma ($n=5$) or 500 ng/ml of IgE myeloma ($n=3$) did not produce an increase in the percentage of viable neutrophils. In contrast, the percentage of viable neutrophils increased to approximately 60 % ($n=5$) after culture in the presence of the GM-CSF positive control in the same experiments. The median level of neutrophil Fc ϵ RI expression in the experiments was 26 ± 10 MFI as determined using mAb 22E7 (results not shown).

Discussion

It is now well established that serum IgE regulates expression of Fc ϵ RI on basophils, mast cells, monocytes, and dendritic cells [12,23–25] through stabilization of the Fc ϵ RI [20]. Based on findings that neutrophils of patients with allergic asthma, but not those of non-allergic individuals, express Fc ϵ RI [10,11], we initiated the present study to determine if serum IgE levels also regulate Fc ϵ RI expression by neutrophils. The levels of Fc ϵ RI expression by neutrophils and, as a positive control, basophils of control subjects and subjects with allergic asthma were quantified by reactivity with mAb 22E7, which recognizes the Fc ϵ RI α -subunit of both free and IgE-liganded receptor [33]. The results demonstrate that neutrophil Fc ϵ RI expression remained constant over a range (1–1,041 IU/ml) of serum IgE levels for all study subjects. The Fc ϵ RI expression by basophils of the same subjects correlated as expected [21–23] with the serum IgE levels. In contrast to the reports by Gounni and colleagues [10,11,35], the levels of Fc ϵ RI expression by neutrophils of the control and asthma subjects overlapped in the present study, as determined by either the net MFI or the percentage of 22E7⁺ neutrophils. Whereas they reported that approximately 50 % of the neutrophils of allergic asthma subjects are Fc ϵ RI⁺ as opposed to 5 % of the neutrophils of healthy controls [10,11,35], only approximately 8 % of the neutrophils of allergic asthma subjects were Fc ϵ RI⁺ versus 5 % of

the neutrophils of healthy controls in the present study as determined using mAb 22E7. A statistically significant inverse correlation between FcεRI expression and serum IgE levels was observed with neutrophils of asthma subjects with mild intermittent disease. We think, however, that this finding is probably an anomaly due to the small sample (n=7), as a significant correlation was not obtained for any other asthma subject group or for the control group. Indeed, no differences in the levels of FcεRI expression by neutrophils of asthma subjects with differing degree of asthma severity could be discerned. We also detected minimal reactivity of a second anti-FcεRIα mAb, (CRA-1) [34], with neutrophils of both the asthma subjects and control subjects. The mAb CRA-1 recognizes an epitope that is either the same as or overlaps with the epitope recognized by mAb 22E7 [23] and has been reported to also react with approximately 50 % of the neutrophils of allergic asthma subjects [11,35]. A basis for the difference between the results described here and those of Gounni and colleagues [10,11,35] is not immediately evident, although the use of different methods for neutrophil isolation cannot be excluded. The present study used neutrophils obtained by standard density gradient centrifugation, whereas the neutrophils were further enriched by positive selection using anti-CD16-coupled magnetic beads in the other studies [10,11,35].

The results obtained here indicate that neutrophil expression of FcεRI, as determined by reactivity with mAb 22E7, is quite low compared to that of basophils. Indeed, the median value for FcεRI expression by neutrophils of asthma subjects who had a serum IgE level within the range of 10–100 IU/ml was approximately 15 net MFI units, whereas the median value for FcεRI expression by basophils of the same subjects was approximately 1500 net MFI units. Previous studies have determined that the density of basophil FcεRI surface expression is approximately 150,000 receptors per cell in the presence of 100 ng/ml IgE [22]. Using these numbers, we estimate that neutrophil expression of FcεRI is only about 1 % of that for basophils or approximately 1,500 FcεRI per neutrophil. It has been shown that a CD2⁺ subset of human monocytes of both allergic asthmatic subjects and non-atopic controls exhibit increased FcεRI expression that correlates with serum IgE levels [25]. As such, the possibility that FcεRI expression may be increased in a small subset of neutrophils cannot be excluded, although the 22E7 mAb produced a uniform histogram for neutrophil reactivity (cf Fig. 1). Consistent with the low level of FcεRI expression, surface-bound IgE was minimally detectable, at best, with neutrophils (cf Fig. 3B).

It has been reported previously that anti-IgE antibody immunoprecipitates a 45–48 kD protein identified as the FcεRI α-subunit in lysates of neutrophils after preincubation of the neutrophils with IgE or the IgE Fc fragment [10]. This finding suggested that the mature FcεRIα-subunit expressed on neutrophil plasma membranes is distinct from the 55–65-kD (average 60-kD) fully processed and glycosylated mature FcεRI α-subunit expressed on the surface of basophils and mast cells [12,36]. Given the minimally detectable level of surface IgE on neutrophils in our study (cf Fig. 3B), we sought to detect the FcεRI α-subunit protein in neutrophil membrane and cytosolic subcellular fractions by Western blot analysis. The mAb 22E7 did not detect either the 45–48 kD FcεRI α-subunit reported previously [10] or the more conventional 55–65 kD FcεRI α-subunit [12,36] in the neutrophil membrane fraction. The mAb 22E7 did react with an approximately 62–63 kD band in the membrane fraction. The reactivity, however, was only approximately 50 % greater than that obtained with the isotype control antibody, thus arguing against a specific reaction. It is reasonable to argue that the Western blot analysis is not sufficiently sensitive to detect the FcεRI α protein when expressed at such a low level. The mAb 22E7, however, also failed to detect the immature form of the FcεRI α-subunit in either the neutrophil membrane fraction or in the cytosol fraction. The immature form of the FcεRI α subunit is present intracellularly within the endoplasmic reticulum of FcεRI⁺ cells as a more abundant 48–50-kD protein [12,36]. Together, these findings indicate that expression of the FcεRIα in neutrophils is, at best, so low as to be undetectable by conventional Western blot analysis.

Previous reports indicate that the neutrophil FcεRI is functionally active, even though expressed at very low levels. Anti-IgE or specific antigen have been reported to stimulate production of IL-8 [10] and eosinophil cationic protein [15] as well as induce cyclooxygenase-2 expression and resultant synthesis of prostaglandin E₂ and thromboxane A₂ [13,14]. The latter was correlated with production of intracellular superoxide [13] and, thus, raised the possibility of IgE-mediated superoxide release. Anti-IgE at the same concentration as used in the previous study [13] did not, however, stimulate any superoxide release by neutrophils. We also were unable to confirm a pro-survival effect of IgE antibody on neutrophils [11], either at the same 100 ng/ml concentration of IgE used in the previous study [11] or at a 5-fold higher concentration of IgE. Consistent with our negative findings, it has been reported that IgE does not prolong survival of human cord blood-derived mast cells [38] in contrast to its ability to prolong survival of mouse mast cells [39]. We have not evaluated the other IgE-mediated responses reported for neutrophils [10,13–15] or the possibility of additional IgE-mediated actions. Indeed, the level of FcεRI expression by plasmacytoid dendritic cells [40] appears comparable to that described here for neutrophils, and stimulation by anti-IgE reciprocally down-regulates TLR9 expression by the dendritic cells [40].

The potential for IgE-mediated activation of neutrophils has direct implications for the potential roles for neutrophils in allergic asthma. Although we cannot conclude with certainty at the present time that neutrophils do indeed express a functional FcεRI receptor, we can conclude with certainty that, if neutrophils do express the receptor, the basal level of expression by neutrophils of allergic asthma subjects encompassing a spectrum of disease severity as well as by neutrophils of non-allergic, non-asthmatic individuals is quite low as compared to the level of basophil FcεRI expression. Moreover, the results unexpectedly, but clearly, demonstrate that neutrophil FcεRI expression, unlike that of other FcεRI⁺ human cells [12,23–25], is not regulated by serum IgE antibody. It has been reported that interleukin (IL)-4 and IL-9 can increase neutrophil FcεRI synthesis in vitro [35] and, thus, the possibility that neutrophils may transiently express higher levels of FcεRI in other circumstances that are then subject to regulation by IgE antibody cannot yet be excluded. In any event, the finding that serum IgE does not alter neutrophil FcεRI expression, at least for the low level of neutrophil FcεRI expression observed here, indicates that omalizumab therapy will not alter the level of baseline FcεRI expression by neutrophils of asthma patients.

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Fig. 1.

Representative histograms for basophil and neutrophil FcεRI expression. Basophils (CD123⁺ cells in PBMC) and neutrophils of an allergic asthma subject and of a control subject were incubated with anti-FcεRIα mAb 22E7 (filled peak) or mouse IgG1 (line). Bound antibody was detected by incubation with AF647-conjugated goat anti-mouse IgG1.

Fig. 2.

Basophil and neutrophil FcεRI expression as a function of serum IgE levels. FcεRI expression by basophils (CD123⁺ PBMC) and neutrophils of 20 control and 40 asthmatic subjects (MiI, mild intermittent (n=8); MiP, mild persistent (n=7); MoP, moderate persistent (n=12); SP, severe persistent n=13)) was measured as described for Fig. 1 and is quantified as the net MFI (difference between MFI for cells incubated with mAb 22E7 and for cells incubated with isotype control antibody). FcεRI expression is shown as a function of the serum IgE levels for each of the 60 subjects (A, B) and after grouping the serum IgE levels of the 60 subjects into tertiles (C, D). *p<0.05

Fig. 3.

Comparison of FcεRI expression and surface-bound IgE. FcεRI expression (mAbs 22E7 and CRA-1) and bound IgE are expressed as the net MFI for basophils (CD123⁺ PBMC) (A) and neutrophils (B) of 3 control subjects and 6 asthma subjects.

Fig. 4.

Western blot detection of FcεRI α-subunit. (A) A lysate of 5×10^5 purified basophils (Baso) and of membrane (M) and cytosol (C) fractions (equivalent to 10^6 neutrophils) isolated from neutrophils of an asthma subject were immunoblotted with mAb 22E7 or mouse IgG1 isotype control. (B) Western blot analysis of membrane and cytosol fractions (equivalent to 10^6 neutrophils) isolated from neutrophils of a second asthma subject.

Fig. 5.

Neutrophil Fc ϵ RI does not support IgE-mediated superoxide release or IgE-induced increase in neutrophil survival. (A) Neutrophils were incubated in buffer alone (Spont), with 10 μ g/ml of anti-IgE, or with IgG or IgA immobilized in tissue culture wells at the indicated concentrations. Results are expressed as the mean $\pm$ SE (n=3) for superoxide release after 60 min of incubation. (B) Neutrophils were cultured in medium alone (Spont), with GM-CSF, or with the indicated concentrations of IgE myeloma for 20 hr. Viability is quantified as the percentage of FITC-annexin V-negative cells. The results for 500 ng/ml of IgE are the mean $\pm$ SE for 3 experiments. All other values are the mean $\pm$ SE for 5 experiments. *P<0.05 compared to the values in medium alone.

Table 1

Characteristics of Asthma and Control Subjects

Controls	N	Sex M/F	Age	FEV1 (%)	ACT Score	Serum IgE (IU/ml)	Asthma Medications
Asthma Subjects	20	7/14	23–58	80–114	N/A	1–71	None
Mild Intermittent	8	2/6	14–51	81–100	20–25	3–286	SABA
Mild Persistent	7	3/4	13–44	79–103	18–25	2–215	SABA, ICS, LTM, LABA
Moderate Persistent	12	7/5	14–63	69–110	11–22	1–1041	SABA, ICS, LTM, LABA
Severe Persistent	13	7/6	13–57	24–107	10–23	1–535	SABA, ICS, LABA, LTM

SABA, short-acting β -agonist. LABA, long-acting β -agonist. ICS, inhaled corticosteroid, LTM, leukotriene modifier.