

Functional consequences of Fc ϵ RI α up-regulation by IgE in human basophils

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Abstract: These studies examine the functional changes that occur after up-regulation of Fc ϵ RI α by immunoglobulin E (IgE) for human basophils. Basophils were cultured with and without IgE antibody (PS myeloma IgE or anti-gp120-specific IgE) for 1 week and challenged with anti-IgE, anti-Fc ϵ RI α , or antigen for histamine and IL-4 secretion. There were no statistically significant changes in their response to anti-IgE or anti-receptor antibodies, as compared with controls incubated for the same period, whereas receptor expression increased an average of 4-fold. There was increased responsiveness to antigenic challenge, most notably at suboptimal concentrations of antigen (gp120 peptide-ovalbumin conjugate). For a 6-fold difference in cell surface density of gp120-specific IgE, there was a 2.2-fold change in antigen potency or 3-fold increases in histamine release at lower antigen concentrations. Similar results were found for secretion of IL-4. Basophil sensitivity, which is a measure of the density of antigen-specific IgE required for 50% of maximal secretion, was used to determine whether up-regulation of Fc ϵ RI α was coordinated with up-regulation of other components of the IgE-signaling pathway. The results indicated up-regulation of Fc ϵ RI is not always accompanied by changes that allow sensitivity to be maintained. These results indicate that functional up-regulation does occur but that its magnitude may be modulated because not all components of the signaling pathway are up-regulated in a balanced manner. *J. Leukoc. Biol.* 68: 479–486; 2000.

Key Words: Fc ϵ RI α · immunoglobulin E · basophils

INTRODUCTION

It has been demonstrated that immunoglobulin E (IgE) antibody directly regulates the expression of the high-affinity IgE receptor on mast cells and basophils [1–8]. More recent studies using human basophils have also indicated that up-regulation by IgE results from the interaction of IgE with Fc ϵ RI rather than through some other IgE-binding protein [5]. The mechanisms of the up- or down-regulation mediated by IgE remain unknown and are under investigation. However, it is apparent from studies in mice that up-regulation of Fc ϵ RI is

accompanied by a better response to IgE-dependent stimuli [6]. For example, up-regulation of Fc ϵ RI on bone marrow-derived mast cells during 1 week of culture with IgE led to marked increases in IL-4 secretion and modest changes in serotonin release. Based on a variety of studies over the last several decades, it is not surprising that enhanced functionality accompanies the increased expression of Fc ϵ RI, especially when the starting cell surface density is quite low, as it is in relatively young bone marrow mast cells from mice. Similar experiments are not as straightforward for the human basophil. To culture basophils for an extended period, interleukin 3 (IL-3) must be included in the culture medium, and IL-3 itself is a powerful means to alter basophil function. In addition, the starting density of receptors on basophils is relatively high, so that the degree of receptor up-regulation is often quite modest [3]. Therefore, recently published studies have not focused on the functional changes that might accompany the up-regulation of Fc ϵ RI in human basophils. The current studies begin to explore the characteristics of any changes that might occur.

There is an additional reason for exploring the nature of any changes that are related to secretion. It is now recognized that there may be components of the IgE-mediated signaling pathway that are rate-limiting. Examples might be the src-family kinase, lyn [9, 10], or the β subunit of Fc ϵ RI itself [11], which has been demonstrated to be unnecessary for cell surface expression of Fc ϵ RI α [12–14]. Although there is no direct proof that these two particular components are required for signaling in human basophils, all available evidence indicates that they are. It is possible that there are other required components that may be rate-limiting. For the human histamine-containing Fc ϵ RI-expressing cells studied to date, freshly isolated or developed in culture, up-regulation has only been demonstrated for Fc ϵ RI α , not other components of the receptor or any other accessory molecules related to IgE-mediated signaling. Rather than attempt to evaluate the up-regulation of all potential components, we have asked whether the characteristics of the functional response can provide some insight into whether up-regulation of Fc ϵ RI α is balanced by concordant up-regulation of other components of IgE-mediated signaling.

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MATERIALS AND METHODS

Buffers

PIPES (Piperazine-N,N-bis-2-ethanesulfonic acid) (Sigma, St. Louis, MO) stock buffer; 25 mM PIPES containing 110 mM NaCl, 5 mM KCl, and 40 mM NaOH, adjusted to pH 7.3 and stored at 10× the above concentration. PAG; PIPES (1×) containing 0.003% human serum albumin (HSA) (Miles, Elkhart, IN) and 0.1% glucose; PAGCM, PAG with 1.0 mM CaCl₂ and 1.0 mM MgCl₂; PAG-EDTA, PAG with 1.0 mM ethylenediaminetetraacetate (EDTA). Acetate elution buffer contained 0.05 M sodium acetate, 0.085 M NaCl, 10 mM EDTA, and 0.03% HSA at pH 7.3 [15]. Heparin-EDTA buffer was stored as a 50× solution; 0.1 M EDTA and 500 µg/ml heparin (Sigma). Lactic acid buffer; 0.01 M lactic acid, 0.14 M NaCl, 0.005 M KCl, pH 3.9. Sensitization buffer (for rapid sensitization); RPMI-1640 (Life Technologies), 0.025% HSA, 1× heparin/EDTA buffer.

Reagents

Anti-antibody, 22E7, was a gift from Dr. Jarema Kochan at Hoffman LaRoche (Little Falls, NJ). Polyclonal goat anti-human IgE was prepared as described previously [16]; the antibody used for these studies represented the IgG fraction of goat serum prepared by DE-52 chromatography. Purified IgE-PS myeloma was a gift from Dr. T. Ishizaka [17]. Anti-gp120 chimeric IgE and gp120 peptide-ovalbumin conjugate (gp120-OVA) were gifts from Dr. Frances M. Davis of Tanox Biosystems (Houston, TX) and were prepared by methods previously described [18]. IL-3 was obtained from Biosource (Camarillo, CA).

Cell preparation

We used two types of basophil preparations. Many of the studies used cells obtained from leukapheresis and were prepared as previously described [19]. Basophil purities in these preparations ranged from 15 to 95% with a median of 33. In some experiments, cells were isolated by the double-Percoll method used for fresh blood [20]. The blood was diluted with EDTA-saline, centrifuged at 500g for 15 min to obtain a buffy coat. The buffy-coat cells were diluted in saline and layered onto a 2-step Percoll gradient, 1.072 g/ml/1.082 g/ml as described previously [21]. After centrifugation at 700g for 20 min, the interface between the 1.072 Percoll/plasma upper layer and the 1.082 lower Percoll layer was harvested and washed as above (basophil purities of 8–45%). We noted in the text which cell preparations we used.

Cell culture

To modulate FcεRIα expression, enriched basophil preparations were cultured in Iscove's modified Dulbecco's media (IMDM) (Life Technologies) containing 2% FCS, 40 µg/ml gentamicin, and 10 ng/ml of IL-3 with or without IgE. The total cell density was 2×10^6 /ml and culturing was done in 96-, 24-, or 6-well tissue culture-treated plates (Costar, Cornell, NY). For secretion of IL-4 and IL-13, basophils were cultured in C-IMDM (IMDM conditioned with 5% heat-inactivated [56°C for 20 min.] FCS, nonessential amino acids [Life Technologies], and 10 µg/ml gentamicin), as reported elsewhere [20]. Cytokine protein measurements and percent histamine released in the same culture supernatants were assessed by enzyme-linked immunosorbent assays and automated fluorimetry, respectively [20].

Sensitization methods

Previous studies established that 3–10 µg/ml of IgE would optimally up-regulate expression of FcεRIα in 1-week cultures [3]. For many of the experiments, anti-gp120 hIgE was used at 5–10 µg/ml in these cultures. For the experiments shown in Figures 3, 5, and 6, anti-gp120 IgE was mixed with PS myeloma IgE so that the total concentration of IgE was maintained at 10 µg/ml. The concentrations of gp120-IgE were 10 µg/ml (with no PS myeloma), 3.3, 1.1, 0.34, and 0.11 µg/ml (the last four with PS myeloma IgE at 6.7, 8.9, 9.66, and 9.89 µg/ml, respectively). Two groups of cells were cultured for 1 week in IMDM medium supplemented with nonessential amino acids, 2% FCS, 40 µg/ml gentamicin, and L-glutamine: one group of five conditions, where IgE was included at the concentrations noted above for the entire week, and a second group of five conditions that were sensitized with the above concentrations of gp120-IgE 1 h before harvesting the cells (at the end of the 1-week culture).

IgE sensitivity curves for cytokine secretion (Fig. 3) were assessed using basophils passively sensitized with varying levels of gp120-IgE and then challenged with specific antigen. For most, but not all, of the experiments, passive sensitization was performed by first removing receptor-bound IgE using a lactic acid protocol modified from that previously reported. Briefly, basophil-enriched cell suspensions prepared by double-Percoll density centrifugation were washed 1× with 1 ml of ice-cold 0.9% NaCl in 1.5-ml tubes and centrifuged at 150g for 5 min. Cell pellets were resuspended in 0.25 ml ice-cold lactic acid buffer for 30 sec before adding 1 ml sensitization buffer with IgE. After 10 min on ice, the cells were centrifuged, the supernatants aspirated, and the pellets resuspended once again in sensitization buffer. Cells were aliquoted in 100-µl volumes, to which varying amounts of gp120-IgE mixed with PS myeloma IgE were added as described above. After incubating 30 min at 37°C, the cells were washed 3× with PAG.

Flow cytometry

A flow cytometric technique incorporating light scatter characteristics was used to quantify cell surface IgE and FcεRIα chain expression on basophils as described [22]. Cell surface IgE that was specific for gp120 peptide was detected using a monoclonal antibody (AB19-4, Tanox). Using enriched basophil preparations at purities >25%, this antibody can detect <1,000 molecules of gp120-specific IgE. This antibody does not detect other cell-bound IgE. Cells not sensitized with gp120-specific IgE showed flow cytometric distributions equal to nonspecific IgG1 labeling of cells sensitized with gp120-specific IgE. Cell surface expression of FcεRIα chain was detected using a mouse IgG1 anti-human FcεRIα chain monoclonal antibody (22E7, provided J. Kochan, Roche, Mont Clair, NJ [23]) and was compared with labeling with an identical concentration of irrelevant mouse IgG1 (Coulter, Hialeah, FL). The 22E7 antibody has been shown to recognize an epitope that is unaffected by FcεRIα occupancy [23]. Aliquots of cells were labeled in phosphate-buffered saline containing 0.2% HSA with 1 mg/ml human IgG to minimize nonspecific binding to FcγR [22]. Each of the monoclonals were used at concentrations predetermined to be optimal for labeling. Binding of monoclonals was detected using saturating concentrations of R-phycoerythrin-conjugated polyclonal goat anti-mouse IgG (Tago, Burlingame, CA). An EPICS Profile flow cytometer (Coulter) was used to analyze fluorescent signals after excitation at 488 nm. "Bitmap" gates, intermediate between the forward and side scatter characteristics of lymphocytes and monocytes, were used to select for a population of cells that were predominantly basophils. Because the cells were already enriched in basophils, these bitmaps can select a population of cells that is generally >80% basophils, with the primary contaminants being lymphocytes. Data are expressed as the mean fluorescence in labeled cells minus the mean fluorescence of IgG1 controls. Day-to-day variability in the sensitivity of the flow cytometer was corrected by noting or adjusting the photomultiplier tube voltage to generate the same signal for a set of standard calibration beads (Immunochek, Coulter).

In previous studies [3], the flow cytometric measurements were calibrated examining the fluorescence staining of six donors' basophils that spanned a moderate range of staining intensities (7–120 fluorescent units or 8,000–140,000 FcεRI per basophil) and simultaneously assessing receptor or IgE density by the acetate elution method described above. 22E7 staining (ordinate) compared with total FcεRI density by acetate elution (after sensitizing with PS myeloma IgE as described above) was linear with a slope of 0.00084 (i.e., a fluorescence measurement of 100 represents ~120,000 receptors) with $r = 0.963$. gp120-specific IgE density as detected with AB19-4 anti-id antibody was previously calibrated by sensitizing RBL-SX38 cells (expressing a transfected human FcεRI) with gp120-IgE, stripping and measuring total IgE in a RIST [24]. When combined with cell counts, these measurements established the cell surface gp120-IgE density with which the fluorescent measurements were compared.

Statistics

Some statistical comparisons were made with a Student's t-test, whereas others were made with a nonparametric Wilcoxon's signed rank statistic. If error bars are shown, they represent the standard error of the mean unless otherwise indicated.

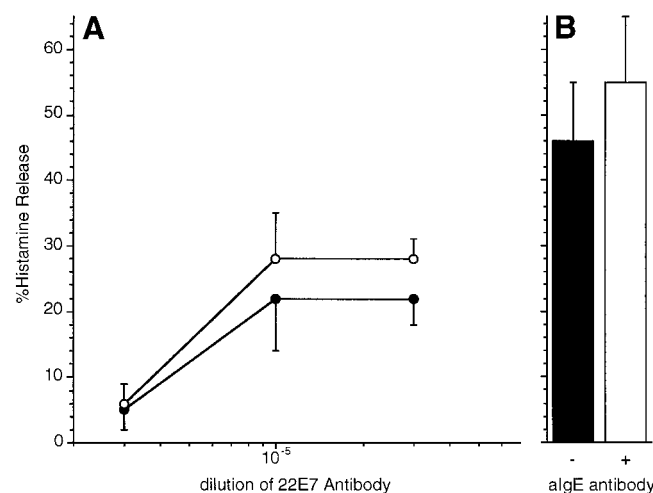


Fig. 1. Differences in IgE-mediated histamine release in cells cultured with and without IgE for 1 week ($n = 3$). Enriched basophils were cultured with (○) or without (●) 5 μ g/ml PS myeloma in media containing 10 ng/ml of IL-3. After 7 days, the cells were harvested and challenged with three concentrations of anti-Fc ϵ RI α antibody, 22E7 (panel A), or one concentration of anti-IgE antibody (panel B, - and + indicate the absence or presence of IgE in the culture, respectively). Supernatants were analyzed for histamine content. Not indicated, receptor expression increased by 4 ± 1 -fold in the cells cultured with IgE.

RESULTS

Our first experimental protocols were straightforward; we used both anti-IgE and anti-Fc ϵ RI α antibodies to determine if the dose response curves to these stimuli differed in cells that been exposed to IgE for 1 week versus those that had not. For these experiments, partially enriched basophils were cultured for 1 week in the presence or absence of PS myeloma IgE at 5 μ g/ml in IMDM media containing 10 ng/ml of IL-3. Expression of Fc ϵ RI α was determined on day 0 and day 7 by flow cytometry using 22E7 as the detection antibody. On day 7, cells were harvested and challenged with several concentrations of 22E7 or polyclonal anti-IgE antibody. As can be seen in **Figure 1**, there was no statistically different response between the two groups of cells and any concentration of antibody. Receptor expression increased an average of 4 ± 1 -fold. Based on studies where flow cytometric measurements of 22E7 binding were calibrated against receptor density measurements by the acetate strip method, these cells started with $\sim 15,000$ receptors on day 0 and ended with 53,000 receptors on day 7. The cells incubated without IgE expressed $\sim 15,000$ receptors on day 7 (no statistical change from day 0).

We then examined whether the up-regulation would effect the dose-response curve for an antigenic stimulus. For these experiments, the cells were incubated for 7 days with or without 5 μ g/ml of anti-gp120 IgE antibody. As above, receptor densities were measured by flow cytometry and, on day 7, the cells harvested and challenged with gp120 peptide-OVA conjugate at several concentrations. **Figure 2** shows the dose response for the two groups of cells, which in this instance was statistically different for most concentrations of antigen. Receptor expression, as measured by 22E7, increased 4.2 ± 1.5 -fold. As measured by an anti-idiotypic antibody, AB19-4,

for which the flow cytometric measurements have been previously calibrated against acid elution measurements, cells incubated without gp120-IgE were loaded with $\sim 33,000$ gp120-IgE molecules per cell, whereas those incubated for 1 week with gp120-IgE were found to be loaded with 188,000 gp120-IgE molecules per cell.

We speculated that histamine release might not be a good detector for the changes occurring in cells that already possessed a moderate density of IgE receptors (20,000–35,000 based on the flow cytometric studies and comparing results with previously calibrated values of 22E7 binding). In previous murine studies, IL-4 was a sensitive detector of up-regulation, at least at the low starting point found in the control mice examined [6]. At the low densities of cell surface IgE on mouse mast cells that had not yet been cultured with IgE, there was no IL-4 secretion after stimulation, whereas serotonin release was significant. After IgE-mediated up-regulation of Fc ϵ RI and stimulation with antigen, IL-4 secretion was marked [6]. Therefore, we examined two facets of the basophil response in context with histamine release: IL-4 and IL-13 secretion. In previous studies, we described a technique to assess the sensitivity of human basophils, with sensitivity defined as the number of antigen-specific IgE molecules required to attain 50% of the cell's maximum response [15] (see discussion for the background leading to this definition). If IL-4 or IL-13 secretion were differentially sensitive to the strength of the stimulus, the sensitivity curves for histamine, IL-4, and IL-13 release would be different, and, if different (in the correct direction), then up-regulation of receptor expression by IgE could lead to results similar to those observed in murine mast cells for IL-4 secretion. Therefore, we first examined the sensitivity curves for the secretion of histamine, IL-4, and IL-13. For these experiments, it was not necessary to know the abso-

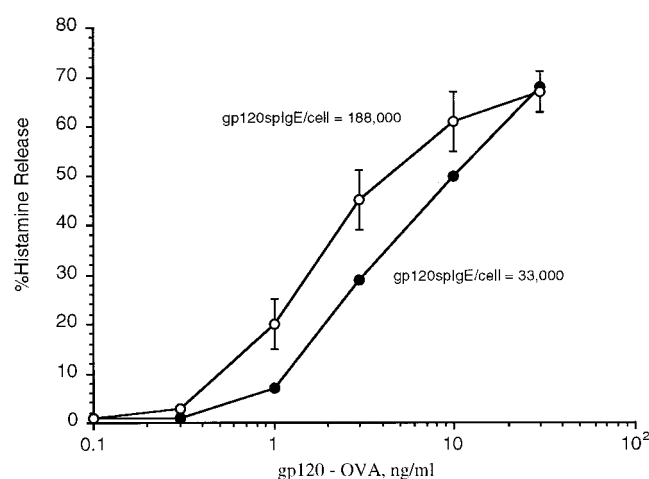


Fig. 2. Differences in IgE-mediated histamine release in cells cultured with and without IgE for 1 week ($n = 5$). Enriched basophils were cultured with (○) or without (●) 5 μ g/ml gp120-specific IgE in media containing 10 ng/ml of IL-3. Before harvesting on day 7, 5 μ g/ml of gp120-specific IgE was added to the cell cultures not previously treated with IgE. After 1 h, all cultures were harvested and the washed cells challenged with six concentrations of gp120-OVA. Supernatants were analyzed for histamine content. Not indicated, receptor expression increased by 4.2 ± 1.5 -fold in the cells cultured with IgE for 1 week.

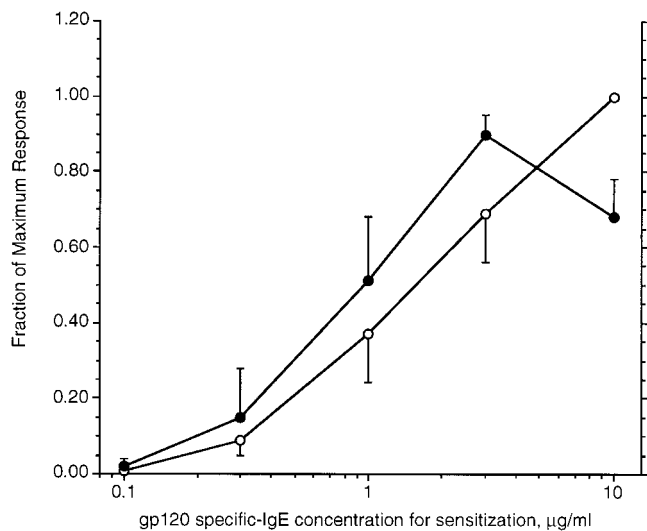


Fig. 3. Sensitivity of the basophil response with respect to histamine or IL-4 release. Enriched basophils, treated with lactic acid, were sensitized with five concentrations of gp120-specific IgE (total IgE concentration was held constant at 10 μ g/ml by diluting a 10 μ g/ml gp120-specific IgE stock with media containing PS myeloma at 10 μ g/ml. After sensitization for 30 min, the cells were washed and challenged with an optimal concentration of gp120-OVA (50 ng/ml) in C-IMDM media and the cells cultured for 4 h. After harvesting the supernatants, histamine and IL-4 contents were determined (see Materials and Methods). The resulting data is plotted as the fraction of release obtained for the condition giving the best response. For histamine release (●), this was always the release from cells sensitized with 10 μ g/ml of gp120-specific IgE, whereas for IL-4 release (○), the maximum often occurred (4 of 6 experiments) in cells sensitized with 3 μ g/ml of gp120-specific IgE, means $\pm$ SEM. Histamine release at the maximum averaged $54 \pm 8\%$ and maximum IL-4 release was not strictly computed because the total cell number used for each challenge was not measured.

lute number of IgE molecules loaded into unoccupied receptors, only the relative response of each secreted mediator from basophils sensitized with different amounts of IgE. **Figure 3** shows these results. Serial dilutions of anti-gp120 IgE were used to sensitize basophils, which were then washed and challenged in IMDM medium containing 5% FCS and gentamicin (see Materials and Methods). Supernatants were harvested at 4 and 24 h. The figure shows that the sensitivity curves were similar for the release of the IL-4 and histamine (data for IL-13 was similar but not shown). Similar results were

found for cells not treated with lactic acid (data not shown). The similarity in sensitivity for the three mediators implies that up-regulation of Fc ϵ RI by IgE should not lead to results seen in murine mast cells (see Discussion). To verify this conclusion, we examined a single preparation of basophils for IL-4 secretion after 7 days of culture with and without IgE antibody. As observed for histamine release, there was a 2-fold difference in antigen potency for cells up-regulated with or without gp120-specific IgE for 1 week (data not shown).

As noted in the introduction, we have thus far only demonstrated IgE-mediated up-regulation of Fc ϵ RI α in human basophils, and a variety of studies indicate that there may be elements of the signaling pathway that may be rate-limiting and, therefore, also require up-regulation for secretion to be enhanced. We have developed an alternative method for examining cell responsiveness that should also address the issue concerning rate-limiting accessory molecules. **Figure 4** illustrates the logic behind the procedure. Basophil sensitivity is defined as the density of antigen-specific IgE molecules required for 50% of the cells maximum IgE-mediated response. In the complete method (unlike the experiments shown in Figure 3), the amount of antigen-specific IgE that is present on a sensitized cell is measured. Thus, for each sensitization condition, two parameters are assessed: the density of antigen-specific IgE and the amount of histamine release after challenge with an optimal concentration of antigen. The sensitivity curve labeled '1' in Figure 4 is based on both the number of antigen-specific IgE molecules per cell, which are plotted along the abscissa and the overall strength of the signal reflected in the histamine release, is plotted on the ordinate. In this depiction, the signal strength is essentially dependent on all those factors that are not Fc ϵ RI α /IgE itself, represented by the filled circles. After up-regulation of Fc ϵ RI α , Figure 4 depicts no change in the non-Fc ϵ RI α accessory factors, therefore, the signal generated is the same as in the original cells. However, because cell-surface antigen-specific IgE is measured and is increased due to up-regulation of Fc ϵ RI α , the point that is plotted is shifted to the right. Therefore, the entire curve is shifted to the right. On a per Fc ϵ RI α /IgE basis, the basophil appears less sensitive. The figure shows this for a case where Fc ϵ RI α density increases by ~ 4 -fold without changes in the accessory molecules. On the other hand, if accessory molecules also increase in proportion to Fc ϵ RI α , then the

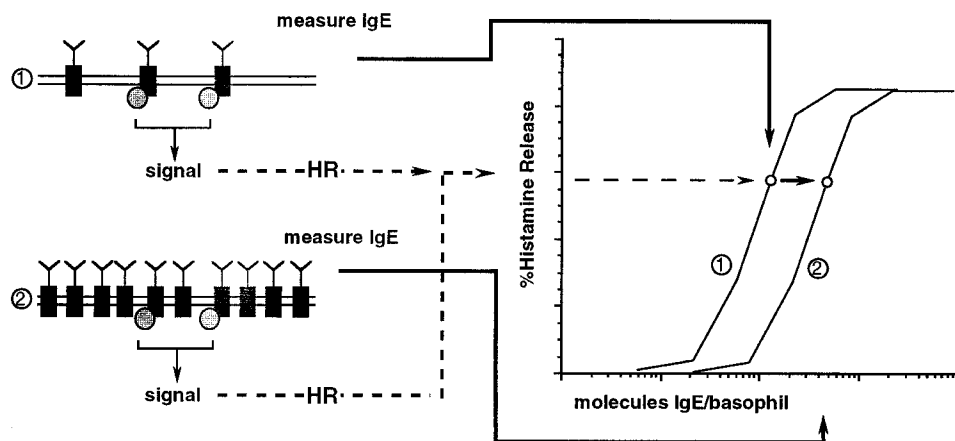


Fig. 4. Idealized representation of up-regulation of Fc ϵ RI α during conditions where accessory signaling molecules are present in rate-limiting quantities. See text for a detailed description.

relative sensitivities should appear identical. If accessory molecules increased more than Fc ϵ RI α , then the cells would appear more sensitive. Finally, if Fc ϵ RI α is the rate-limiting species, and all other participating species are in excess, then increasing Fc ϵ RI α without increasing the accessory molecules also results in the two curves being similar or identical.

Cells were incubated with serial dilutions of gp120-IgE for 1 week. This established the sensitivity curve for cells up-regulated by IgE (i.e., serial dilutions could not be done after up-regulation because all up-regulated Fc ϵ RI α would be occupied by the IgE used for up-regulation). However, for each dilution of gp120-IgE, the same degree of total Fc ϵ RI α up-regulation needed to be maintained. In this way, the hypothetical increase in accessory molecules would be the same for each condition with only the density of gp120-specific IgE varying among conditions. Therefore, the IgE concentration was held constant at 10 μ g/ml by diluting the gp120-IgE with medium containing 10 μ g/ml of PS myeloma. The assumption was that gp120-IgE antibody and PS myeloma IgE antibody induced the same degree of receptor up-regulation. This assumption was tested in pilot experiments by comparing the potency of these two IgE antibodies for up-regulating in 1-week cultures. In data not shown from five experiments, the relative potency of the two IgE antibodies was found to be statistically identical. This assumption was also born out by the equivalent amount of Fc ϵ RI α up-regulation observed for each of the different conditions (as detected by 22E7 binding). Partially enriched basophil preparations (these experiments required $\sim$ 10 million basophils to compensate for losses during the 1-week culture) were divided on day 0 into a group incubated without IgE and those incubated with IgE. Those cells cultured without IgE for 1 week were sensitized 1 h before harvesting the cells (see Materials and Methods). The cells were analyzed

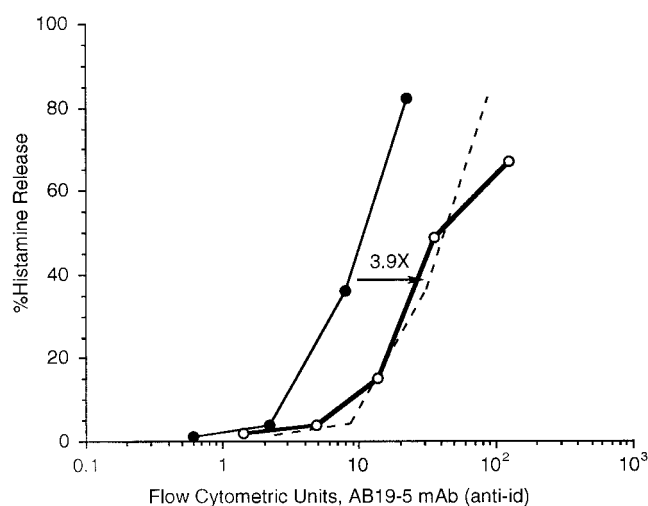


Fig. 5. Sensitivity curves for cells cultured with (○) or without (●) IgE for 7 days from one experiment. See text for complete description of the protocol. After culture, the harvested cells were either (1) challenged with an optimal concentration of gp120-OVA (50 ng/ml) and histamine release measured or (2) labeled for flow cytometry using anti-idiotypic antibody (AB19-4). Mean net fluorescence for the flow cytometric distributions is shown on the abscissa. The dotted line represents the idealized shift of the no IgE curve (●) 4-fold to the right (the magnitude of receptor up-regulation).

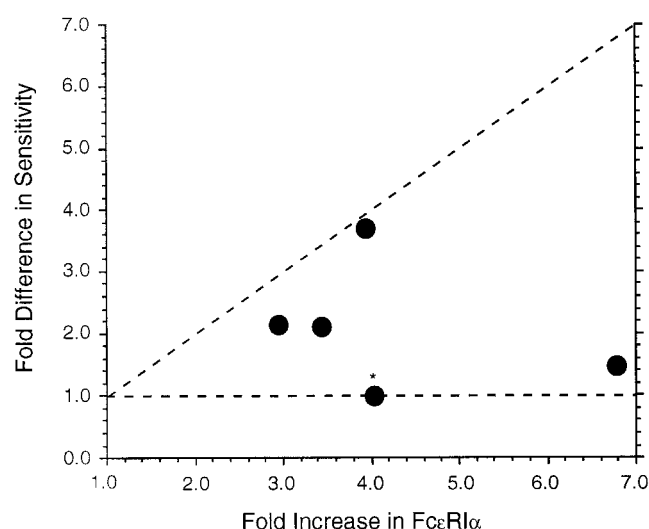


Fig. 6. Relationship between the difference in sensitivity for cells $\pm$ up-regulation and the increase in receptor expression ($n = 5$). Each point represents the data from one experiment where both the fold increase in Fc ϵ RI α expression and the fold difference in sensitivity between cells cultured with or without IgE was determined.

for histamine release in response to an optimal concentration of gp120-OVA (50 ng/ml) or subjected to flow cytometry using either 22E7 or anti-idiotypic antibody AB19-4 (anti-idiotypic antibody for the gp120-specific IgE).

Figure 5 shows the results from one of these experiments. The total Fc ϵ RI α expression differed by 4-fold between the two groups of cells (those treated for 1 week with IgE vs. those without IgE and sensitized just before harvesting). Likewise, it can be seen that the relative sensitivity was shifted by $\sim$ 4-fold. This result parallels that shown schematically in Figure 4. However, we found that results varied among preparations of basophils. Because the amount of up-regulation differed for each preparation, it was more instructive to plot individual experiments as shown in **Figure 6**. On the abscissa is the fold up-regulation of Fc ϵ RI α , on the ordinate in the fold difference in the two sensitivity curves. A priori, there is no reason to preclude sensitivity being greater after up-regulation, which would plot a point on the negative ordinate, but this was not observed. The dotted line at 45° represents the location for points where, under the model of some accessory elements being rate-limiting, one would find up-regulation of Fc ϵ RI α in the absence of up-regulation of any other accessory element. Data points that fall on the horizontal line would be less interpretable. As noted above, this latter situation could result from coordinated up-regulation of all components where accessory species are rate-limiting or from uncoordinated upregulation when Fc ϵ RI α is the rate-limiting species. From this perspective, in three experiments, functional up-regulation of all relevant Fc ϵ RI signaling molecules appeared uncoordinated, either lying on the 45° line or between the two dotted lines. In two experiments, the sensitivity curves were identical and, thus, the points lay on the horizontal line. For one of these experiments (marked with an asterisk in Figure 6), a day 0 sensitivity curve was also generated; for this one experiment the sensitivity at day 7 (in cells not treated with IgE) was

~2-fold lower than the sensitivity of day 0, indicating a modest loss of responsiveness in cells cultured for 7 days despite the presence of IL-3.

DISCUSSION

These studies demonstrated three main points. First, within the range of cell-surface IgE densities it is possible to control in studies of peripheral blood basophils, up-regulation of receptor expression had some effect on mediator release only if antigen was used to stimulate the cells. Changes were least apparent when stimulating cells with anti-IgE or anti-receptor antibodies. As noted by Conroy et al. [25], there is no relationship between the cell-surface density of IgE and the response to anti-IgE antibody. We later showed that this result occurs because a maximal response in human basophils requires densities of cell-surface IgE <10,000 [15, 26]. Because the original Conroy studies operated in a range >10,000 IgE/basophil, there should have been little correlation. Likewise, in the current studies, starting densities of IgE were >10,000 molecules, so little or no increase in release after up-regulation by IgE, using 22E7 or anti-IgE as a probe, would have been expected as well. Although it might be expected that there would be little change in maximal release, it was surprising that there was little increase at suboptimal concentrations of 22E7. However, for reasons not yet clear, we have observed that for polyclonal goat anti-IgE antibody, the half-widths of the dose response curves are very constant throughout a large range of cell-surface IgE densities [27]. Whether this kind of phenomenon applies to 22E7 is not yet known, but the data suggests that it would apply.

Changes in function are generally better appreciated by using antigens to aggregate specific IgE. The experiments shown in Figure 2 continue to support this view. The range of antigen-specific IgE that was examined was more appropriate to search for up-regulation and, because the stimulus was an antigen, broadening of the dose response curve was expected. However, there was a 6-fold difference in the density of gp120-specific IgE between the two conditions and only a 2.2-fold increase in potency. For some of the lower concentrations of antigen, there was only a 3-fold increase in absolute histamine release between the two levels of receptor expression. One context for evaluating the significance of these changes can be found in the murine studies where up-regulation of Fc ϵ RI α caused marked changes in IL-4 secretion. In these experiments, the fold change in secretion was certainly much greater than the fold change in receptor expression. We could find no evidence that release of cytokines was any more or less sensitive to stimulation than degranulation and that up-regulation of this response was, therefore, similar to histamine release. The studies in mice had the advantage that starting cell-surface densities of Fc ϵ RI were very low. However, the implication of the murine studies is that if a sensitivity curve were generated for mouse mast cells or basophils like the one shown in Figure 3, the curve for IL-4 secretion would be different than the one for serotonin release, possibly 3–10-fold shifted to the right of the curve for serotonin release [6]. Our results for human basophils indicate little difference in the curves, or possibly an

IL-4 sensitivity curve that is shifted leftward of the histamine release curve, a result opposite of that expected for murine cells. Based on the sensitivity curves shown in Figure 3, we would have predicted that up-regulation of IL-4 release after incubation for 7 days with IgE would be no different than that of histamine release. This was verified experimentally. One caveat to the studies shown in Figure 3 is that the IL-4 sensitivity curve shows an optimum in cells that were sensitized with 3 μ g/ml of gp120-IgE. This characteristic, where too much signaling causes a decreased IL-4 response, has been observed for stimulation with anti-IgE antibody [19, 28]. Our working hypothesis is that the observation reflects the increasing rates of desensitization at higher levels of signaling so that a short-lived secretory event like degranulation is less effected by an increased rate of desensitization than is a long-lived secretory event like IL-4 release.

Another context in which to view the significance of the difference in antigen dose response curves in Figure 2 is found in the characteristics of basophil sensitivity. The control sensitivity curve shown in Figure 5 is representative of many other sensitivity curves demonstrated in previous studies. It can be seen that at low levels of sensitization, there are disproportionate changes in basophil function. For example, histamine release was 4% when gp120-specific IgE density was 2 flow units and 36% for 8 flow units. A similar disproportionality was observed in the recent studies of recovery from MAb E25 treatment in atopic patients. A critical point of these studies was the observation that a 3-fold change in receptor expression, at the low end of the receptor expression, was accompanied by a 5-fold increase in function [4]. The critical point of these studies is that disproportional changes in function versus cell-surface IgE occur most obviously at low densities of IgE, whereas the changes in function for the current studies occur at higher densities of cell-surface IgE.

A third context for evaluating the differences in antigen-induced response comes from the sensitivity studies shown in Figures 4–6. Ideally, it might seem that the best definition of sensitivity would be the number of cell-surface aggregates required to elicit a fixed amount of mediator release. However, it was shown both in human basophils and rat basophilic leukemia cells that not all aggregates can be considered equal with respect to the signals they initiate [29–31]. Therefore, such a definition of sensitivity would also have to specify the precise nature of the aggregate: its size, complexity (extent of branched aggregation), and duration. This is, of course, not practical. However, for comparisons among basophil donors, a reasonable approach is to define sensitivity on the basis of the number of antigen-specific IgE molecules per cell, provided that the same IgE were used on each basophil preparation examined and the same antigen used to stimulate the cells. Under these conditions, one could reasonably assume that the characteristics of the aggregates would be similar. This was done for previous studies of basophil sensitivity. A further consideration for defining sensitivity is to decide whether the response is expressed as a function of the absolute level of secretion (e.g., percent histamine release, fg of IL-4 secreted per basophil, etc.) or relative to the maximum release obtainable with any IgE-dependent stimulus. We previously showed that maximum release and number of molecules of IgE re-

quired for 50% of maximum release were independent parameters of cell function [15]. One parameter could change without the other changing. For the current studies, the preconditions for measuring sensitivity could have been relaxed somewhat because no comparisons were made between basophil preparations; only differences in sensitivity within preparations was determined. Furthermore, because the cells within each basophil preparation were cultured under the same conditions, with the exception of the presence or absence of IgE, we found no differences in the maximum release between the two conditions. In data generated for previous studies, we also noted that the qualitative characteristics of the sensitivity curves do not change if suboptimal, rather than optimal, concentrations of antigen are used to stimulate the cells after sensitization. The experiments shown in Figure 5, therefore, represent a well-controlled assessment of basophil sensitivity. The uncoordinated up-regulation of all the signaling components suggested by the results shown in Figures 5 and 6 should provide an additional context for evaluating the data in Figure 2. In other words, functional up-regulation within a given preparation of basophils may depend on more than up-regulation of Fc ϵ RI α alone. Variability among experiments might be explained by the varied degree of uncoordination (Fig. 6).

Growing evidence shows that not all components of the IgE-mediated signaling cascade are present in constant stoichiometric quantities. Beginning with the receptor itself, Fc ϵ RI β is not necessary for expression of Fc ϵ RI α . We will be reporting studies that indicate that α/β stoichiometry is not constant across a range of Fc ϵ RI α densities [32]. Because Fc ϵ RI β is an amplifier of the earliest stage of the signaling cascade [11], uncoordinated regulation of these two components alone could account for a large proportion of the variability in IgE-mediated function we observe. Some indication that such uncoordination of Fc ϵ RI β expression can be found in the studies of human liver-derived mast cells [33]. In these studies, culture with IL-4 up-regulated the mRNA for Fc ϵ RI α but not Fc ϵ RI β or Fc ϵ RI γ . Although levels of mRNA don't always readily translate to protein expression, these studies are suggestive.

Studies in RBL cells indicate that lyn kinase is a rate-limiting component of the reaction [9, 10]. Although these results are somewhat controversial and not necessarily applicable to human basophils, they raise the issue that increased function might only follow coordinated changes of any component in the signaling cascade that is otherwise rate-limiting. The studies shown in Figures 5 and 6 indicate that coordination of up-regulation is an issue, and, at a minimum, indicate by another means the possible variability in the results for different preparations. Taken at face value, these results indicate a new point of regulation in the responsiveness of these cells to stimulation. The factors regulating the coordinated up-regulation would be a future target of study. An important caveat for these in vitro studies is that the basophils are incubated for 1 week. Although IL-3 appears to maintain functionality, cells are lost at rates that vary among preparations. Either the average rate of loss reflects the natural life span of basophils in vivo, or we have yet to provide the correct mix of cytokines to sustain normal functionality in culture. Differential sensitivity to an inadequate cytokine environment

could explain the variable results shown in Figure 6. However, even if this were true, the results continue to indicate that up-regulation requires co-factors that determine the degree of balanced expression of all necessary components of the reaction.

In summary, IgE induces up-regulation of Fc ϵ RI α with a resulting increase in basophil responsiveness to antigenic challenge. However, the resulting up-regulation of function may be regulated by other factors, differing among preparations, because up-regulation of Fc ϵ RI α is not always coordinated with other yet-to-be-identified components of the IgE-mediated signaling cascade.

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