

Releasability of human basophils: Cellular sensitivity and maximal histamine release are independent variables

Donald W. MacGlashan, Jr., MD, PhD Baltimore, Md.

Within the atopic and nonatopic population, basophils display a wide variation in the extent of histamine release when challenged with an optimal concentration of polyclonal anti-IgE antibody. We have tested the hypothesis that the maximum percent histamine release obtainable with this stimulus is an indicator of basophil sensitivity. We have defined basophil sensitivity, in the context of IgE-mediated release, as the number of cell-surface IgE molecules required to obtain a half-maximal IgE-mediated response, and we have examined this parameter in the basophils of 39 donors. Sensitizing basophils with various densities of antigen-specific IgE, and measuring histamine release at these measured densities, allowed a determination of basophil sensitivity in 38 of the donors. Basophil sensitivities ranged from 300 to 40,000 molecules per basophil (point of 50% maximal response), although most donors fell in the 800 to 8000 range, with 1900 molecules per basophil as the median response. The maximal response, after challenge with an optimal concentration of either polyclonal or monoclonal anti-IgE antibody, ranged from 8% to 94% (median, 50%). There was no correlation between basophil sensitivity and the maximum histamine release obtained by a challenge with anti-IgE antibody. Therefore two independent variables appeared to control the IgE-mediated response in basophils, similar to the parameters of efficacy and potency in drug studies. In the IgE-mediated response one parameter is the maximum histamine release obtainable through IgE-mediated mechanisms, and the second parameter is basophil sensitivity as defined above. As a byproduct of these studies, we also found that the density of unoccupied high-affinity receptors for IgE antibody was uncorrelated with the total receptor density (range, 4200 to 572,000 receptors per basophil). Indeed, the density of unoccupied receptors was remarkably constant across this large range of total receptor densities. (J ALLERGY CLIN IMMUNOL 1993;91:605-15.)

Key words: Human basophils, IgE, IgE receptors, histamine release, releasability

A number of years ago the concept of releasability was introduced into the literature to describe an intrinsic property of the basophil or mast cell that predetermined its ability to respond to IgE-mediated stimulation. Often this concept was associated with the maximum histamine release (HR) that could be obtained with any stimulus that caused the aggregation of cell-surface IgE antibody, although other measures

of basophil function (and occasionally mast cells) have been used. These include variables such as the response to an optimal concentration of anti-IgE antibody,¹⁻⁸ the response to simple buffers alone (spontaneous release plus or minus simple compounds such as deuterium oxide),⁹⁻¹¹ age-related responses to anti-IgE antibody,¹² measurements of cell-surface IgE antibody,¹³⁻¹⁵ and the response of basophils to various drugs.¹⁶ It is generally appreciated that differences in the basophil or mast cell response during an allergic reaction could well determine the differences observed in the severity of the reaction among atopic donors, although proof of this assertion has not been forthcoming. Part of the problem is that the relevant parameter of the cell response may not have been examined yet.

Several studies have suggested that antigen must induce the aggregation of at least 100 molecules of IgE antibody on the basophil or mast cell surface

From The Johns Hopkins University School of Medicine, Johns Hopkins Asthma and Allergy Center, Baltimore.

Received for publication Oct. 14, 1991.

Revised June 25, 1992.

Accepted for publication Sept. 9, 1992.

Reprint requests: Donald W. MacGlashan, Jr., MD, PhD, The Johns Hopkins University School of Medicine, Johns Hopkins Asthma and Allergy Center, Unit Office 3A.62, 301 Bayview Blvd., Baltimore, MD 21224.

Copyright © 1993 Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/1/42454

Abbreviations used

PIPES:	Piperazine- <i>N,N'</i> -bis-2-ethanesulfonic acid
BPO:	Benzylpenicilloyl
HSA:	Human serum albumin
BPO ₂₁ -HSA:	Human serum albumin conjugated with an average of 21 BPO groups/molecule
PAG:	PIPES buffer (25 mmol/L) containing human serum albumin and 0.1% glucose
PAGCM:	PAG buffer containing 1 mmol/L CaCl ₂ and MgCl ₂
anti-IgE:	Goat polyclonal anti-IgE (PS myeloma) antibody
PAG-EDTA:	PAG with 1.0 mmol/L ethylenediamine tetraacetic acid
HR:	Histamine release

membrane before a measurable secretory response follows.^{17, 18} However, these estimates suggest only the minimum cross-linking signal that is required under special circumstances to initiate this reaction; little is known about the number of cell-surface IgE molecules needed for a response in the general population. In a pilot study several years ago we noted that among a small group of donors considerable variation existed in the number of cell-surface IgE molecules required to cause measurable HR.¹⁵ In this study we introduced a somewhat different definition of basophil releasability, which we termed basophil sensitivity, in an attempt to further identify parameters responsible for differences in basophil releasability. The new parameter was defined as the density of cell-surface antigen-specific IgE antibody required to cause 25% HR from cells stimulated with an optimal concentration of antigen. However, this pilot study generally focused on donors whose basophils would release greater than 50% of their available histamine when presented with an optimal IgE-mediated stimulus like polyclonal anti-IgE antibody. In some of the studies mentioned herein, it was noted that maximal HR itself varies from 0% to 100% among basophils obtained from a broader screening of the population. Our first question after the pilot study was whether it was possible to measure our sensitivity parameter in any donor, regardless of maximum release obtainable. We also hypothesized that basophil sensitivity and maximal HR were manifestations of one basic property of the cells. It seemed reasonable to speculate that poor maximum release after stimulation with a strong stimulus like polyclonal anti-IgE antibody reflected very low sensitivity, that is, the basophil response would require high densities of cell-surface IgE. The current study is designed to address these questions.

MATERIALS AND METHODS**Buffers**

Buffer consisted of the following: PIPES (piperazine-*N,N*-bis-2-ethanesulfonic acid) (Sigma Chemical Co., St. Louis, Mo.), stock buffer, 25 mol/L of PIPES containing 110 mmol/L of NaCl, 5 mmol/L of KCl, and 40 mmol/L NaOH, adjusted to pH 7.3 and stored at 10 times the above concentration. PAG, PIPES (1 X), containing 0.003% human serum albumin (HSA) (Miles Laboratories Inc., Elkhart, Ind.) and 0.1% glucose, PAGCM, PAG with 1.0 mmol/L CaCl₂ and 1.0 mmol/L MgCl₂, PAG-EDTA (ethylenediamine tetraacetic acid), PAG with 1.0 mmol/L EDTA. Acetate elution buffer contained 0.05 mol/L sodium acetate, 0.085 mol/L NaCl, 10 mmol/L EDTA, and 0.03% HSA at pH 7.3. Lactic acid elution buffer contained 0.01 mol/L lactic acid, 0.14 mol/L NaCl, and 0.005 mol/L KCl, pH 3.9.

Reagents

Polyclonal goat antihuman IgE was prepared as described previously¹⁹; the antibody used for these studies represented the IgG fraction of goat serum prepared by DE-52 chromatography (Whatman Ltd., Maidstone, England). Mouse monoclonal IgG antihuman IgE (HP6061) was provided by Dr. Robert Hamilton. This antibody was prepared from an established clone and purified to 2 mg/ml from ascites with use of diethylaminoethyl anion exchange and hydroxyapatite chromatography.

Subjects

Donors originally consisted of 58 adult subjects. They were randomly selected among the personnel of the Department of Clinical Immunology. Donors were not excluded on the basis of atopy, but only four were atopic by history. Nonreleasing donors were excluded because the current method of sensitivity determination would have been difficult for these donors. Donors did not have any notable medical problems other than atopy and were not using medications at the time of blood drawing.

Cell preparation

Venous blood was drawn by venipuncture, and the cells were separated on a single-step Percoll (Pharmacia Diagnostics, Inc., Fairfield, N.J.) gradient.²⁰ In brief, blood was centrifuged over Percoll (specific gravity, 1.080 gm/ml). The mononuclear cell layer was then collected and washed with EDTA saline solution, PAG-EDTA, and twice with PAG and then was used as described in the following paragraphs.

Cell challenge

Two basic protocols were used for testing, and these are shown in Fig. 1. In the first evaluation cells were tested for endogenous IgE and unoccupied receptor densities (see following text), their response to challenge with specific antigen (after sensitization, see following text), or anti-IgE antibody. Dose response curves for both polyclonal and monoclonal anti-IgE antibody were examined to obtain the optimal or maximum release value. To determine the max-

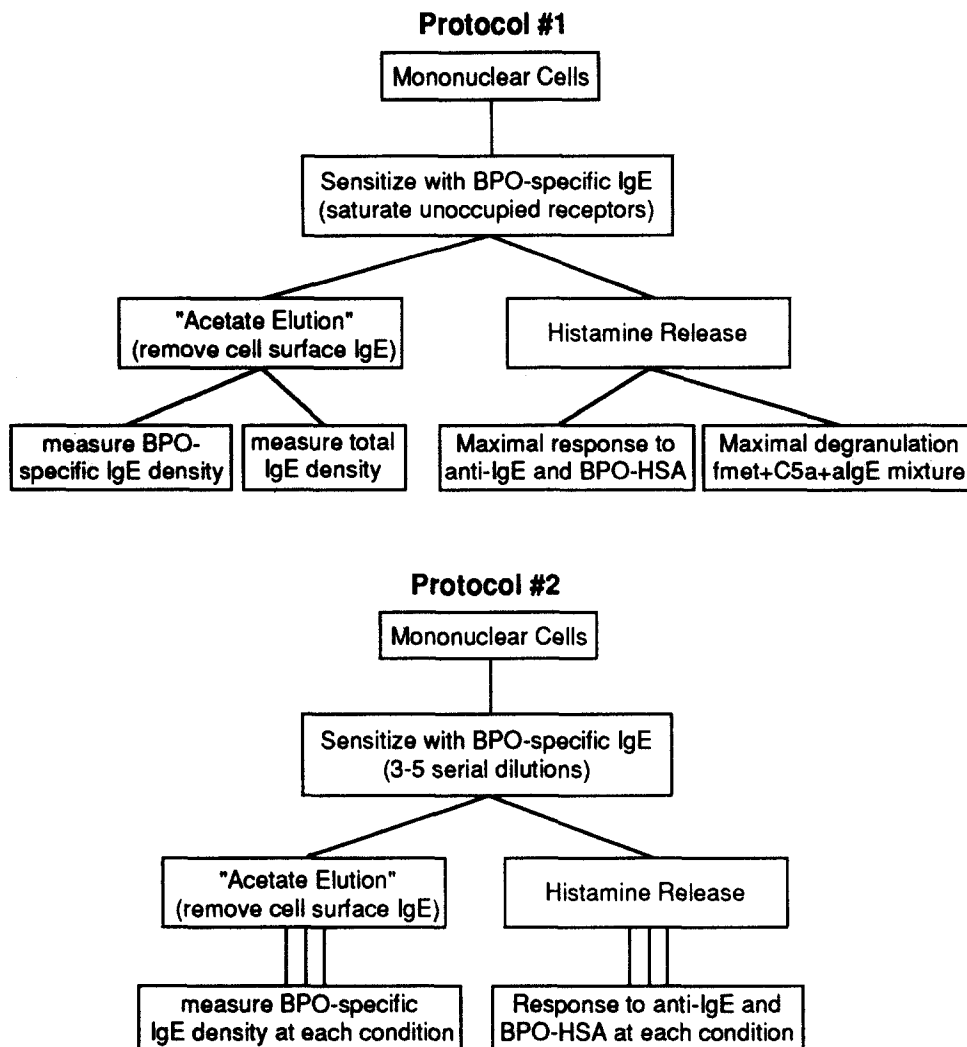


FIG. 1. Schematic of the two protocols used to examine basophil sensitivity.

imal degranulation response to physiologic stimuli we also challenged cells with a mixture of 0.1 $\mu\text{g/ml}$ polyclonal anti-IgE antibody, 1 $\mu\text{mol/L}$ fMET (formyl-methionine-leucine-phenylalanine) peptide (Sigma Chemical Co.), and 0.15 $\mu\text{g/ml}$ recombinant human C5a (gift of Henry Showell, Pfizer, Groton, Conn.). The simultaneous challenge with these three stimuli delivers a strong degranulation signal to the basophil and generally results in nearly 100% degranulation. The point of this particular challenge was to ensure that a less than 100% HR to anti-IgE antibody did not result from an inability of the cell to release completely. Cells were resuspended in PAGCM then challenged with stimulus for 45 minutes at 37° C. The final volume was usually 1.0 ml. After 45 minutes samples were centrifuged, and the supernatants were collected for the histamine assay.

Sensitization

The two protocols noted previously sensitized the leukocytes with antigen-specific IgE, although in the first protocol only a complete sensitization of unoccupied IgE

receptors was examined by sensitizing cells with the highest concentration of IgE (5 $\mu\text{g/ml}$). In the second protocol the cells were sensitized with various concentrations (5 $\mu\text{g/ml}$ as the highest concentration and a series of 1:3 dilutions) of either penicillin (BPO) or GP120-specific IgE antibody (provided by Drs. Frances Davis and Tse-Wen Chang of Tanox Biosystems, Inc., Houston, Texas) to generate a series of cell-surface IgE densities. Purified BPO-specific IgE, BPO₇-HSA, and chimeric human IgE specific for the human immunodeficiency virus (HIV) protein GP-120 peptide were obtained by procedures described previously.^{21,22} Cells were incubated with IgE antibody for 20 minutes in PAG containing 1 mmol/L EDTA and 10 $\mu\text{g/ml}$ heparin at 37° C. After washing once, the cells were layered over 1 ml of chelated newborn calf serum and centrifuged for 5 minutes to rapidly separate the cells from their diluted sensitization buffer. The cells were further washed twice in PAG, and an aliquot was taken to determine HR. The remainder were incubated for 60 minutes at 37° C to allow IgE to elute from low-affinity receptors. After centrifugation, the pellets were treated with acetate buffer (see following text) to elute ba-

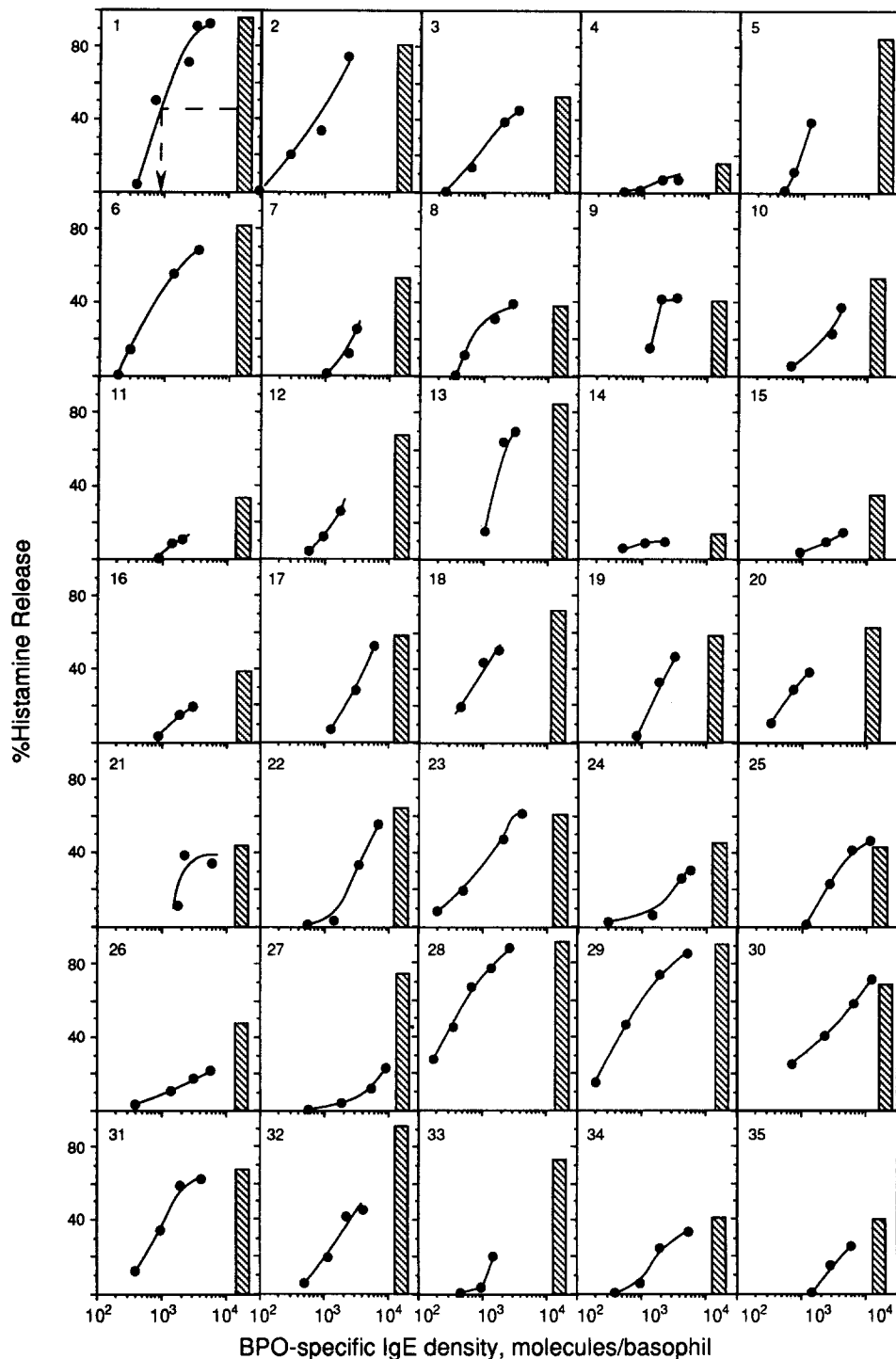


FIG. 2. Sensitivity profiles for 35 of the 38 selected donors. Basophils were sensitized with various concentrations of BPO-specific IgE (see text) and then tested for the BPO-specific IgE density and HR response to an optimal concentration of BPO-HSA. Filled circles plot these data. Bar columns show response to an optimal concentration of anti-IgE antibody in the same experiments. **Panel 1.** Graphic example of how basophil sensitivity was determined (see methods for a further description). Horizontal broken line is located at the point of 50% of the anti-IgE-induced response. Vertical broken line indicates number of IgE molecules required for this half-maximal response.

sophil IgE antibody, and the supernatant was neutralized and tested for IgE concentration by total IgE RIST or BPO-RAST (see following text). Cell counts (alcian blue positive cells) obtained before elution allowed a calculation of the receptor density to be made (the amount of IgE measured by RIST or RAST divided by cell count with the result expressed as IgE molecules per basophil). Previous studies demonstrated that sensitization with the highest concentration of BPO-specific IgE effectively measured the unoccupied receptor density.¹⁸ The cells being tested for HR were challenged with either anti-IgE antibody (0.2 µg/ml) or BPO₂₁-HSA at 0.5 µg/ml (previously determined to be optimum for HR at all levels of basophil sensitization). Note that by this method all donors were tested with the same antigen-specific IgE antibody and the same antigen. This general procedure and the acetate elution technique have been described in detail in previous publications.^{13, 15, 18, 21, 23}

Acetate elution

After the aliquoted cells were centrifuged the pellets were resuspended in ice-cold acetate buffer, pH 3.7, and incubated in an ice bath for 10 minutes. After a brief centrifugation (15,000 g), the supernatant was removed and neutralized with 1N NaOH. These samples were frozen immediately for later measurement of total and specific IgE by RIST or RAST. Acid elution was done in duplicate. Basophil counts (alcian blue staining)²⁴ made before elution were also done in duplicate.

Lactic acid elution

The procedure for removing basophil IgE has been described elsewhere.²⁵⁻²⁷ The current modification reduces the incubation with lactic acid buffer to 30 seconds to minimize basophil damage (see Appendix). In brief, after the cells were washed in saline solution the pellet was gently resuspended at room temperature in the residual saline solution, and 5 ml of lactic acid buffer was added for 30 seconds. The reaction was stopped by the addition of 40 ml of ice-cold PAG buffer and the cells allowed to sit undisturbed for 10 minutes before they were centrifuged and handled further.

Histamine assay

All reactions, performed in duplicate, were stopped by centrifugation, and the supernatant was removed for histamine analysis. In each experiment perchloric acid at a 1.6% final concentration was added to duplicate tubes to determine total histamine content. Histamine was assayed by an automated fluorometric technique.²⁸ The percentage of HR was calculated from the ratio of sample to total histamine after spontaneous release was subtracted from both.

Whole blood HR

To determine whole blood HR, blood was anticoagulated with heparin (20 units/ml of blood), brought to 37° C, and immediately added to tubes containing PAGCM buffer plus

or minus stimulus (0.5 ml blood plus 0.5 buffer plus or minus polyclonal anti-IgE antibody). After a 30-minute incubation, the supernatant was harvested after being centrifuged and stored frozen until assay by the radioenzymatic assay.^{29, 30} Total histamine content of the blood was obtained by boiling 0.1 ml of blood for 10 minutes and harvesting the supernatant. Controls in these experiments included both serial dilutions of the total histamine samples to determine linearity and the "spiking" of total histamine and other samples with known amounts of histamine. In general, the assay response to total histamine dilutions was similar to the standard curve, and the ratio of expected histamine content to measured content for the spiked samples was 1.01 ± 0.04 ($n = 32$, from a previous study³¹).

Calculation of basophil sensitivity

The plot of BPO-HSA-induced HR versus BPO-specific IgE density typically generated a point near 50% of the maximum anti-IgE-induced HR. Simple linear extrapolation of the data near the 50% point was used to determine the number of IgE molecules required for 50% of the maximal anti-IgE-induced HR. Maximal HR was taken to be the response to an optimal concentration of polyclonal or monoclonal anti-IgE antibody.

RESULTS

To first determine whether sensitization of any donor with purified BPO-specific IgE could result in a measurable response to BPO-HSA, protocol No. 1 (see Methods and Fig. 1) was used with 58 donors. The average maximal IgE-mediated release (after challenge with an optimal concentration of either monoclonal or polyclonal anti-IgE (see Appendix), was $50\% \pm 3\%$, and the average ratio of the HR response to BPO-HSA in these sensitized cells to maximal HR was 0.67 ± 0.08 . Only one donor showed no response to BPO-HSA (with 47% HR to anti-IgE antibody), whereas most responded quite well. In these experiments the density of both endogenous IgE on basophils and BPO-specific IgE (which equals unoccupied Fc_εRI receptor density¹⁸) were also determined. As expected from previous studies,^{13, 15, 31} there was no correlation between the endogenous IgE density and maximal HR after polyclonal anti-IgE antibody ($R_s = 0.009$). In addition, as found in a previous study,³¹ maximal anti-IgE-induced HR followed a distribution centered around 50% (as noted previously, nonreleasing donors were excluded because further information concerning sensitivity would have been difficult to obtain from these donors' cells³¹). The challenge of donors with the mixture of receptor-mediated stimuli (anti-IgE/fMET peptide/C5a, see Methods) on average resulted in $93\% \pm 1\%$ HR. In other words, this mixture of physiologic stimuli could induce the basophils of any donor

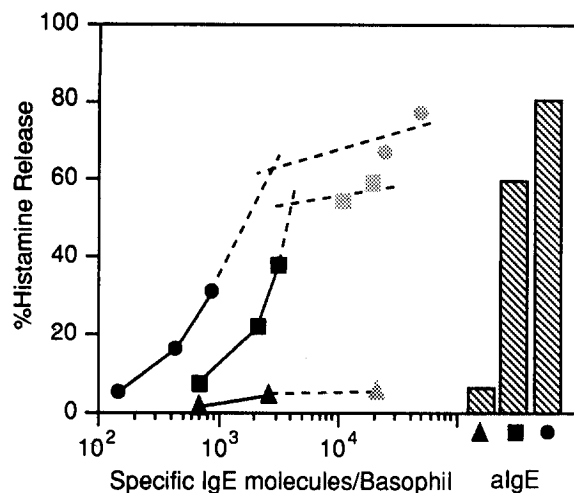


FIG. 3. Sensitivity profiles for cell preparations treated with lactic acid. Plot shows sensitivity profiles for three donors plotted in the manner of Fig. 2. *Filled symbols* represent response to BPO-HSA after sensitization of basophils not treated with lactic acid. *Bar columns* again represent anti-IgE response (symbols for the line plots and bar plots are matched), and *partially filled grey symbols* represent the response to BPO-HSA from basophils first treated with lactic acid, to remove a fraction of the endogenous IgE, and then resensitized. *Broken lines* represent extrapolations from the two extremes of the profiles.

to fully degranulate. These results indicated that the sensitivity parameter could be determined in most donors. Of the original 58 donors, 19 chose not to continue with the study and one donor was unresponsive to BPO-HSA and could not be further studied, leaving 38 to be characterized in greater detail.

A number of important control experiments exist that preceded the more detailed sensitivity curve studies, and these are covered in detail in the Appendix. The sensitivity curves of 35 of the 38 donors are shown in Fig. 2 (the remaining three are shown in Fig. 3, see following text). In addition, the response to an optimal concentration of polyclonal anti-IgE is also shown in the column bar in each panel. In our previous study we defined basophil sensitivity as the density of BPO-specific IgE required to obtain 25% HR after challenge with an optimal concentration of BPO-HSA. Because this study examines a large range of maximum histamine values, this definition was modified to define sensitivity as the density of BPO-specific IgE required for half-maximal release (by anti-IgE antibody). For most of these curves, it was relatively straightforward to determine the density of cell surface BPO-specific IgE required to initiate a response that was 50% of the maximum (Fig. 2).

For several donors the 50% value was more difficult to determine because the best response to BPO-HSA

was well below the maximum (e.g., donors shown in panels 27 and 33; Fig. 2). We also considered the possibility that for some donors, BPO-HSA-induced release might exceed anti-IgE-induced release if the cells could be sensitized with higher densities of BPO-specific IgE. Therefore we used the technique of lactic acid elution to remove a small fraction of the endogenous cell-surface IgE to resensitize the cells with a higher density of BPO-specific IgE than could be obtained by "natural" sensitization. The original protocol was designed to remove all the cell-surface IgE and yet retain cell function.²⁵ However, we have found that donors' basophils are altered to varying extents by the procedure. Indeed, for some donors lysis of their basophils can occur. For our purposes, only a small amount of the endogenous IgE needed to be removed, since median IgE densities were greater than 100,000 (see following text), so the procedure was modified to treat the cells for only 30 seconds (versus the 3- to 5-minute procedure used in previous studies²⁵). Preliminary experiments (see Appendix) indicated that there was little effect on the natural sensitivity of basophils. We then used an abbreviated sensitization protocol on 13 of the donors in the study to test for release at higher densities of BPO-specific IgE. Of the 13 experiments performed with lactic acid "stripping," only three donors' cells were unaffected by the treatment, that is, the maximal HR to anti-IgE antibody was the same with and without lactic acid treatment. Sensitivity curves for these three donors' cells are shown in Fig. 3 (Figs. 2 and 3 therefore show all 38 of the sensitivity curves in the study). These results indicated that high densities of BPO-specific IgE result in the same release as the response induced by anti-IgE antibody. The figure shows dotted lines extrapolated from the two regions of the curve, suggesting that a plateau occurs in this type of donor, which is similar to the plateaus observed for many of donors shown in Fig. 2 (e.g., panels 1, 8, 9, 14, 21, 23, 25, 28, 29, and 31). For the remaining 10 donors tested with lactic acid treatment, the treatment had variable effects on anti-IgE-induced HR. On average, the ratio of anti-IgE-induced HR after lactic acid treatment to anti-IgE-induced release without treatment was 0.83 ± 0.06 . However, in all cases, in the range of 20,000 to 90,000 BPO-specific IgE molecules/basophil, which is 20- to 50-fold higher than obtainable under conditions of "natural" sensitization, HR after BPO-HSA and anti-IgE was equal (mean antigen HR/anti-IgE HR, 0.95 ± 0.06 vs 0.35 ± 0.05 without lactic acid treatment in these selected donors).

These results indicate that the HR response of a donor's basophils to anti-IgE antibody does not give

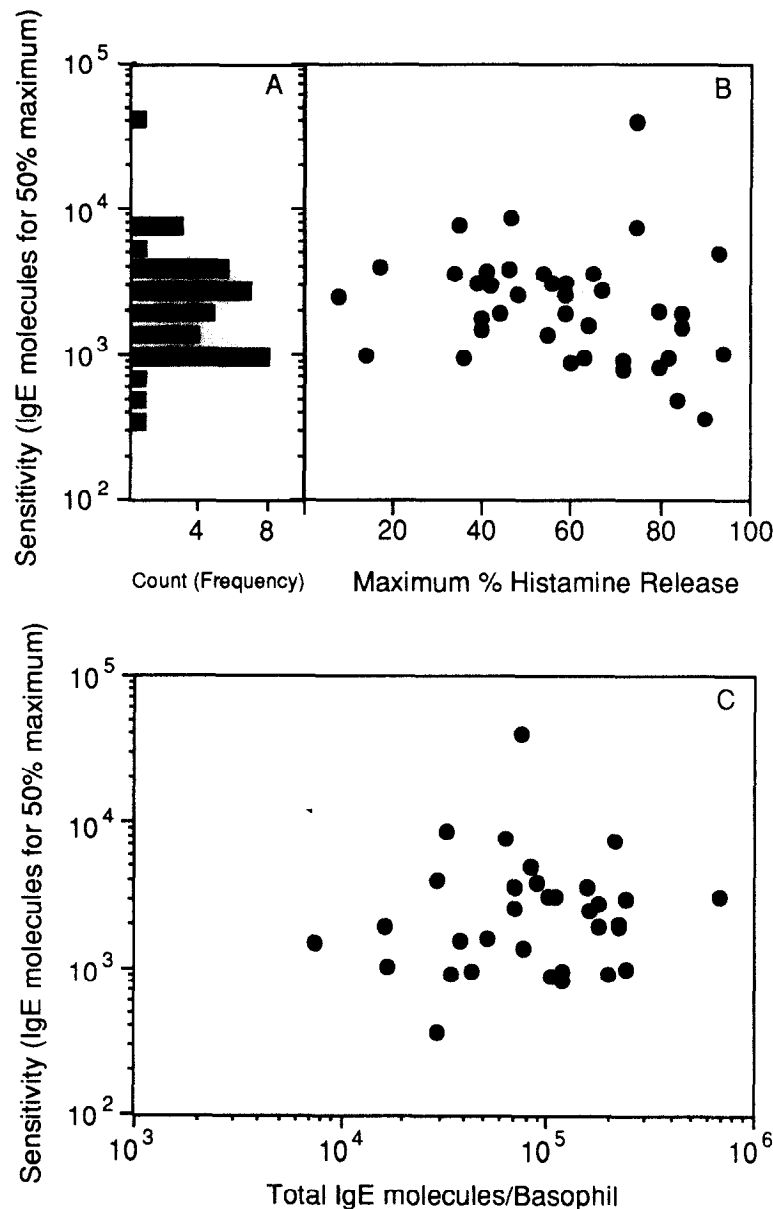


FIG. 4. Correlation plots for the relationship between basophil sensitivity and maximum IgE-mediated HR (**panel B**) and endogenous IgE density (**panel C**). Sensitivity measurement is expressed as number of IgE molecules per basophil required to generate a half maximal response (the maximum determined by the polyclonal anti-IgE response, see text). **Panel A**, Frequency distribution of the sensitivities for the 38 donors (count refers to number of donors at a particular range of sensitivity values).

an indication as to the sensitivity of the cell. Fig. 4 demonstrates this point with two correlation plots. In Fig. 4, *panel B* there was no correlation ($R_s = 0.32$, $p = 0.0594$) between the maximum HR and the sensitivity (number of IgE molecules for 50% of the maximal IgE-mediated HR) of the cell. The sensitivity parameter was obtained from the plots in Figs. 2 and 3 and ranged from the highest sensitivity of ≈ 300 molecules of IgE to the lowest at 40,000 (Fig. 4,

panel A). Despite this wide range, this parameter generally shows the same range of variation as maximal HR, with 93% of the cells lying between 800 and 8000 molecules of IgE (median, 1900), for a 10-fold variation. Fig. 4, *panel C* shows the lack of correlation between the total IgE density and the same sensitivity parameter ($R_s = 0.083$, $p = 0.63$).

It is interesting to note that the density of unoccupied $Fc\epsilon RI$ receptors (as measured by the conditions

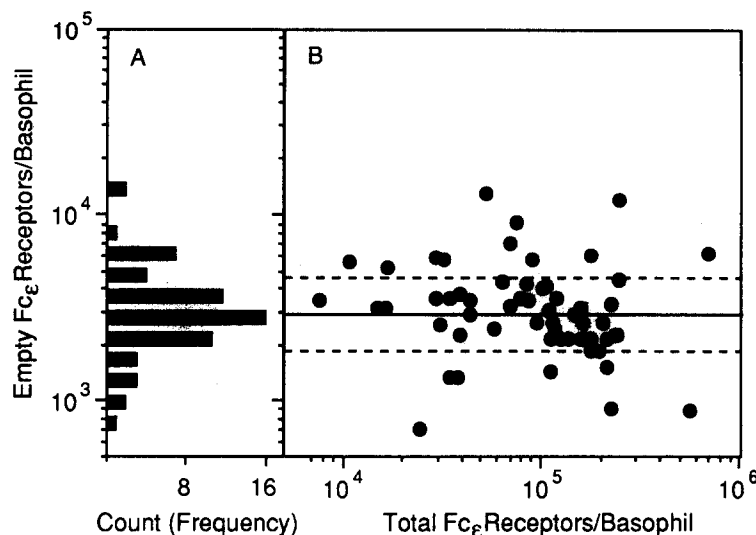


FIG. 5. Relationship between unoccupied Fc ϵ RI receptor densities and total receptor density. **Panel A**, Frequency distribution (count refers to number of donors at a particular range of values) for unoccupied receptors ($n = 58$ from the protocol no. 1 screening); **Panel B**, Correlation between these two variables.

of optimal BPO-specific IgE sensitization) was relatively constant. With a 200-fold range in the total receptor density, empty receptors showed only an eightfold range and no correlation to total receptor density. Fig. 5 shows this distribution, where the median unoccupied receptor density was 3000 and the median total receptor density was 105,000.

DISCUSSION

Previous studies have demonstrated that selected donors, whose basophils responded well to anti-IgE antibody, could respond to antigen with as few as 100 to 200 molecules of cell surface IgE.^{15, 18} These same studies indicated that there was a 30-fold range of sensitivity (number of IgE molecules for 50% of the maximum IgE-mediated response) among selected donor basophils, and these studies also suggested that the relationship between the maximal response and sensitivity might be poor. The current studies have sought to clarify the issue. Studying a wide range of HR, we found no correlation between maximal HR and basophil sensitivity (defined as the density of antigen-specific IgE required for a half-maximal response). In addition, the data indicated that a single measurement of IgE density and anti-IgE antibody-dependent HR would yield an inadequate description of basophil sensitivity but did show that this parameter could be measured for most donors with use of the sensitization protocol described. It is surprising that basophil sensitivity for this broader selection of donors also ranged only 30-fold among donors (with one ex-

ception), the variation observed in earlier studies. Indeed, for most donors, the sensitivity ranged only 10-fold. We expected that poorly releasing basophils would be better separated from basophils that released 80% to 90%, since our simplest assumption was that the maximal HR of basophils had at least a weak relationship to cellular sensitivity. Although we did not expect to see sensitivities much lower than 300 (i.e., 50% maximal response with fewer than 300 molecules of antigen-specific IgE), because this probably means that fewer than 150 cross-links would be signaling the cell (at least two IgE molecules per cross-link), we were surprised that more donor's basophils did not appear to have sensitivities in the 10,000 to 100,000 range.

This study also indicated that nearly all donors can be studied in this manner. Because we are interested in whether cellular sensitivity, in conjunction with maximal HR, plays a role in the atopic state or determines the severity of a patient's disease, future studies are possible to examine this parameter in these contexts. Given the relatively narrow range of sensitivities, it may be possible to simply ask whether sensitivities less than 1000 (i.e., 50% maximal response with fewer than 1000 molecules of antigen-specific IgE) can be found in a distinctive population of patients. This is the value noted in our earlier studies where these donors' basophils released leukotrienes in 10- to 100-fold higher amounts than the average release for the population. One might speculate that during the allergic reaction antigen must

TABLE I. Dose response curves for polyclonal anti-IgE antibody with basophils isolated by standard Percoll methods and basophils challenged in heparinized blood ($n = 4$)

anti-IgE*	Donors								Averages	
	1		2		3		4		Percoll	Blood
	P	B	P	B	P	B	P	B		
0.04	11	9	14	13	1	2	11	18	9 ± 3	11 ± 3
0.2	21	16	25	23	3	5	19	21	17 ± 5	16 ± 4
1.0	41	30	34	27	8	11	37	31	30 ± 8	25 ± 5
2.5	40	24	22	24	6	7	28	36	24 ± 7	23 ± 6

An analysis of variance (ANOVA) calculation indicates no statistical difference between the Percoll and blood preparations.

P, Percoll; B, blood.

*anti-IgE antibody concentration in micrograms per milliliter.

penetrate the mucosal barriers and that the depth of penetration is limited. The more sensitive the cells in the reaction, be they basophils or mast cells, the more cells deep in the tissue are likely to respond, which therefore might result in a more severe reaction. A future study might also examine whether cytokines, which are thought to be present in tissues, shift all donor responses into the highly sensitive range. Finally, we are interested in whether there is a common mechanism that accounts for the range of sensitivities observed. This study indicates that the basophil has two independent means of regulating its response to IgE-mediated signals, through regulation of cellular sensitivity and its maximal response. We will be interested in whether this indicates that there are two major control points in the release reaction, which once understood, could be exploited to down-regulate the cells' response. Given the large number of possible biochemical steps in this release reaction, comparing low- and high-sensitivity donors might shed some light on the important biochemical steps.

These studies brought to light a second unexpected observation, that the unoccupied Fc ϵ RI density is relatively constant despite a large variation in the total receptor density. Previous studies have noted that the Fc ϵ RI density is well correlated with the serum IgE antibody titer.¹⁴ It is not known whether the IgE titer induces changes in the receptor density or whether the two parameters are under the control of another mechanism. Studies in rat basophilic leukemia cells indicate that there is no effect of IgE on receptor expression,³² and the information for human basophils is not available, although unpublished studies by our group have not shown an effect of IgE on receptor expression. However, the constant expression of unoccupied receptors noted in the present study would suggest that occupied receptors would signal the cell to express more receptor or that the balance of receptor cycling

or synthesis degradation or both is altered by receptor occupancy. In these situations one might find the observations shown in Fig. 5. However, further study is needed to clarify this issue.

In summary, these studies demonstrated that basophil sensitivity is not reflected in the response to anti-IgE antibody. We had also hoped that if this correlation did not exist, then sensitivity could be determined easily by passive sensitization with purified antigen-specific IgE. In all but one instance, an approximate measure of the basophil sensitivity could be determined for almost any type of donor. Purified IgE is probably required to generalize the method for any donor, since some donor's unoccupied receptor densities were low enough that only maximal sensitization with antigen-specific IgE led to a reasonable response. Although nearly all donors' basophils could be sensitized, a sensitivity determination required two or three sensitization conditions. It would have been simpler if only one sensitization condition were required; however, different slopes in the sensitivity curves as well as the occurrence of donors with very sensitive basophils required additional points to make the determination.

APPENDIX

Whole blood histamine release

Using nonreleasing donors and donors where HR after polyclonal anti-IgE antibody was greater than 70%, we have previously shown that the HR obtained after Percoll isolation of the leukocytes accurately reflected the release after a challenge of basophils in heparinized blood.³¹ We have extended these observations to include donors whose basophils release 20% to 40% of their histamine in response to anti-IgE antibody to ensure that results from this subset of the population were also not an artefact of Percoll isolation. The HR dose response curves to anti-IgE an-

tibody for four donors were compared; heparinized blood versus Percoll separated cells. As we found previously, the dose response curves were virtually superimposable (Table I).

Independence of the results on the antigen-specific IgE used

It was necessary to determine whether the use of penicillin-specific IgE was providing an accurate representation of the antigen-induced HR response after a sensitization protocol that filled all unoccupied receptors. We were also particularly interested in donors whose anti-IgE-induced release was poor and whether antigen-induced release after sensitization could ever be much better than challenge with anti-IgE antibody. Some donors' cells were further tested by sensitizing the cells with a purified monoclonal chimeric IgE with specificity for the HIV protein, GP-120. Cells sensitized with this antibody were challenged with a GP-120 peptide-ovalbumin conjugate, and the HR in response to this antigen was compared with the polyclonal anti-IgE antibody-induced response (ratio of antigen response/aIgE response). This ratio averaged 0.61 ± 0.11 for cells sensitized with the GP-120-specific IgE and averaged 0.67 ± 0.08 for cells sensitized with BPO-specific IgE (and challenged with BPO-HSA). These data indicated that the ability of IgE to sensitize basophils was similar for the two different purified IgE preparations. Moreover, the sensitivity measurements should therefore be a reflection of the cells' intrinsic sensitivity regardless of the antigen-specific IgE used to make the determination. One other technical point, in this context, is the assumption that the density of BPO-specific IgE measured is an accurate measure of basophil densities only. This point has been discussed in detail in previous studies where we conclude, through an extensive series of experiments that confirm other earlier studies, that the procedure we use is not confused by IgE binding to lymphocytes or monocytes (the contaminants in these preparations).^{15, 18, 21}

Intradonor variability

We have studied the variability of the basophil response from the same individual over various intervals of time. Over a 1-year period, the HR response of a given donor to anti-IgE antibody was essentially the same. Release varied $\pm 20\%$ of its mean value, that is, HR of 30% ranged 20% to 40% (± 2 SD). The occasional donor can show larger variation over a period of 1 to 10 years, but this study was not designed to examine this variability. Although we have not examined other parameters with the same regularity, IgE receptor densities (occupied and empty) and ba-

sophil sensitivity (as defined in the Methods section) also have similar variability over time. For example, an examination of nine donors in which total IgE and unoccupied receptors densities were measured three to seven times showed an average standard deviation of $\pm 16\%$ of the log mean. Similarly, 5-point sensitivity curves were generated on two occasions for each of four donors, and the resulting 50% maximal release determinations (see following text) displayed a $\pm 19\%$ mean variability.

Effect of lactic acid treatment on basophil sensitivity

Using the brief 30-second lactic acid protocol we tested for an effect of the procedure on the sensitivity of the basophils using two donors' basophil preparations. The cells were divided into a group sensitized as usual (control curve) and a group first treated with lactic acid (30 seconds) followed by a similar series of sensitization conditions, although greater dilutions of BPO-specific IgE were used to generate levels of cellular sensitization similar to nonlactic acid treated cells. The two sensitivity curves for each donor showed similar midpoints (1600 vs 1800 molecules IgE for 50% maximal response, control vs lactic acid treatment, respectively), which was within the variability of this assay.

Monoclonal vs. polyclonal anti-IgE antibody

We wished to know whether the polyclonal anti-IgE antibody we used always generated maximal IgE-mediated release. We therefore examined broad dose response curves for a number of monoclonal antibodies and chose one that consistently gave strong release. Amount of release with this monoclonal antibody was no better than the polyclonal antibody. In fact, regression analysis of the release obtained with optimal concentration of either polyclonal or monoclonal antibody had a slope statistically indistinguishable from 1.00, a y-intercept indistinguishable from 0, and a correlation value of 0.93. Therefore we could not find an instance of polyclonal anti-IgE not reflecting the best IgE-mediated release obtainable. These results were also supported by the lactic acid "stripping" experiments discussed in the text where BPO-HSA-induced HR at high BPO-specific IgE densities was no better than anti-IgE-induced release.

REFERENCES

1. Marone G, Giugliano R, Lembo G, Ayala F. Human basophil releasability. II. Changes in basophil releasability in patients with atopic dermatitis. *J Invest Dermatol* 1986;87:19-23.
2. Marone G, Poto S, Celestino D, Bonini S. Human basophil releasability. III. Genetic control of human basophil releasability. *J Immunol* 1986;137:3588-92.

3. Casolaro V, Galeone D, Giacommo A, Sanduzzi A, Melillo G, Marone G. Human basophil/mast cell releasability. V. Functional comparisons of cells obtained from peripheral blood, lung parenchyma, and bronchoalveolar lavage in asthmatics. *Am Rev Respir Dis* 1989;139:1375-82.
4. Casolaro V, Spadaro G, Marone G. Human basophil releasability. VI. Changes in basophil releasability in patients with allergic rhinitis or bronchial asthma. *Am Rev Respir Dis* 1990;142:1108-11.
5. Busse WW, Swenson CA, Sharpe G, Koschat M. Enhanced basophil histamine release to concanavalin A in allergic rhinitis. *J ALLERGY CLIN IMMUNOL* 1986;78:90-7.
6. Flint KC, Leung KB, Hudspeth BN, Brostoff J, Pearce FL, Johnson NM. Bronchoalveolar mast cells in extrinsic asthma: a mechanism for the initiation of antigen specific bronchoconstriction. *BMJ [Clin Res]* 1985;291:923-6.
7. Flint KC, Leung KB, Hudspeth BN, Pearce FL, Brostoff J, Johnson NM. The function and properties of human lung mast cells. *Respiration* 1986;2:31-41.
8. May CD, Williams CS. Further studies of concordant fluctuation in sensitivity of peripheral leucocytes to unrelated allergens and the meaning of nonspecific "desensitization". *Clin Allergy* 1973;3:319-37.
9. May CD, Remigio L. Observations on high spontaneous release of histamine from leucocytes in vitro. *Clin Allergy* 1982;12:229-41.
10. May CD. High spontaneous release of histamine in vitro from leukocytes of persons hypersensitive to food. *J ALLERGY CLIN IMMUNOL* 1976;58:432-7.
11. Findlay SR, Lichtenstein LM. Basophil "releasability" in patients with asthma. *Am Rev Respir Dis* 1980;122:53-9.
12. Marone G, Poto S, di ML, Condorelli M. Human basophil releasability. I. Age-related changes in basophil releasability. *J ALLERGY CLIN IMMUNOL* 1986;77:377-83.
13. Conroy MC, Adkinson NFJ, Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977;118:1317-21.
14. Malveaux FJ, Conroy MC, Adkinson NFJ, Lichtenstein LM. IgE receptors on human basophils. Relationship to serum IgE concentration. *J Clin Invest* 1978;62:176-81.
15. MacGlashan DW, Peters SP, Warner J, Lichtenstein LM. Characteristics of human basophil sulfidopeptide leukotriene release: releasability defined as the ability of the basophil to respond to dimeric cross-links. *J Immunol* 1986;136:2231-9.
16. Marone G, Cirillo R, Genovese A, Marino O, Quattrin S. Human basophil/mast cell releasability. VII. Heterogeneity of the effect of adenosine on mediator secretion. *Life Sci* 1989;45:1745-54.
17. Fewtrell C, Metzger H. Larger oligomers of IgE are more effective than dimers in stimulating rat basophilic leukemia cells. *J Immunol* 1980;125:701-10.
18. MacGlashan DW, Lichtenstein LM. Studies of antigen binding on human basophils. I. Antigen binding and functional consequences. *J Immunol* 1983;130:2330-6.
19. Adkinson NF Jr. Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody. 2nd ed. Washington DC: American Society of Microbiology, 1980.
20. MacGlashan DW, Lichtenstein LM. The purification of human basophils. *J Immunol* 1980;124:2519-21.
21. MacGlashan DW, Mogowski M, Lichtenstein LM. Studies of antigen binding on human basophils. II. Continued expression of antigen-specific IgE during antigen-induced desensitization. *J Immunol* 1983;130:2337-42.
22. Sun LK, Liou NC, Sun NC, et al. Transfectomas expressing both secreted and membrane-bound forms of chimeric IgE with anti-viral specificity. *J Immunol* 1991;146:199-205.
23. MacGlashan DW, Lichtenstein LM. The transition from specific to nonspecific desensitization in human basophils. *J Immunol* 1981;127:2410-4.
24. Gilbert HS, Ornstein L. Basophil counting with a new staining method using alcian blue. *Blood* 1975;46:279-86.
25. Pruzansky JJ, Grammer LC, Patterson R, Roberts M. Dissociation of IgE from receptors on human basophils. I. Enhanced passive sensitization for histamine release. *J Immunol* 1983;131:1949.
26. MacDonald SM, Lichtenstein LM, Proud D, et al. Studies of IgE-dependent histamine releasing factors: heterogeneity of IgE. *J Immunol* 1987;139:506-12.
27. MacGlashan DW, Lichtenstein LM. Basic characteristics of human lung mast cell desensitization. *J Immunol* 1987;139:501-5.
28. Siraganian RP. An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Anal Biochem* 1974;57:383-94.
29. Brown MJ, Ind PW, Causon R, Lee TH. A novel double-isotope technique for the enzymatic assay of plasma histamine: application to estimation of mast cell activation assessed by antigen challenge in asthmatics. *J ALLERGY CLIN IMMUNOL* 1982;69:20-4.
30. Charlesworth EN, Hood AF, Soter NA, Kagey SA, Norman PS, Lichtenstein LM. Cutaneous late-phase response to allergen. Mediator release and inflammatory cell infiltration. *J Clin Invest* 1989;83:1519-26.
31. Nguyen KL, Gillis S, MacGlashan DW. A comparative study of releasing and nonreleasing human basophils: nonreleasing basophils lack an early component of the signal transduction pathway that follows IgE cross-linking. *J ALLERGY CLIN IMMUNOL* 1990;85:1020-9.
32. Isersky C, Rivera J, Mims S, Triche TJ. The fate of IgE bound to rat basophilic leukemia cells. *J Immunol* 1979;122:1926-36.