

# 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

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*Basophils from approximately one fifth of the population were found to be unresponsive (nonreleasers), in terms of both histamine and leukotriene release, to an IgE cross-linking stimulus, such as anti-IgE antibody. Although unresponsive to any IgE-mediated stimulation, these basophils responded to non-IgE-mediated stimuli, such as the phorbol ester, 12-O-tetradecanoyl phorbol-13 acetate, the calcium ionophore, A23187, and to formyl-methionyl-leucyl-phenylalanine peptide. These stimuli produced equal dose-response curves in both releaser (basophils able to respond with >5% histamine release to anti-IgE antibody) and nonreleaser basophils. Nonreleaser basophils possessed statistically similar densities of cell-surface IgE antibody (287,000 versus 400,000 IgE molecules per basophil for releaser and nonreleaser basophils, respectively), and with 12-O-tetradecanoyl phorbol-13 acetate as a probe of anti-IgE-induced cross-linking, the IgE on nonreleaser basophils was found to be cross-linked by the polyclonal anti-IgE antibody used for these studies. Interleukin-3 (IL-3) has previously been demonstrated to enhance markedly both histamine and leukotriene release in human basophils. However, IL-3 was unable to convert nonreleasing basophils into releasing basophils, as measured by anti-IgE antibody. IL-3 equivalently enhanced formyl-methionine peptide-induced release in both releaser and nonreleaser basophils, suggesting that the lack of an effect on anti-IgE-induced release was not due to a lack of IL-3 receptors. Although there are several possible interpretations of these data, these results and results of our previous studies of protein kinase C activation and cytosolic  $Ca^{++}$  elevations in human basophils suggest that nonreleasing basophils have a defect in early signal transduction, possibly involving the influx of  $Ca^{++}$ . (J ALLERGY CLIN IMMUNOL 1990;85:1020-9.)*

Cross-linking of IgE antibody on the cell surface of mast cells and basophils initiates the release of a number of potent inflammatory mediators.<sup>1</sup> It is generally accepted that the density of cell-surface IgE controls the extent of the reaction, since the density determines the magnitude of the signal transmitted to the interior of the cells.<sup>2</sup> For example, it was demonstrated many years ago that there was a good correlation between the sensitivity of basophils of atopic

## Abbreviations used

|                    |                                                          |
|--------------------|----------------------------------------------------------|
| HR:                | Histamine release                                        |
| TPA:               | 12-O-tetradecanoyl phorbol-13 acetate                    |
| Con A:             | Concanavalin A                                           |
| PKC:               | Protein kinase C                                         |
| fMet peptide:      | Formyl-methionine-leucine-phenylalanine                  |
| PIPES:             | Piperazine-N-N'-bis-2-ethanesulfonic acid                |
| HSA:               | Human serum albumin                                      |
| PAG:               | PIPES buffer (25 mmol/L) containing HSA and 0.1% glucose |
| PAGCM:             | PAG buffer containing 1 mmol/L of $CaCl_2$ and $MgCl_2$  |
| EDTA:              | Ethylenediaminetetraacetic acid                          |
| LTC <sub>4</sub> : | Leukotriene C <sub>4</sub>                               |
| D <sub>2</sub> O:  | Deuterium oxide (heavy water)                            |
| Sr:                | Strontium                                                |
| IL-3:              | Interleukin-3                                            |
| DAG:               | Diacylglycerol                                           |

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patients to antigenic stimulation and the antigen-specific IgE titer.<sup>3</sup> More recent controlled experiments support this belief.<sup>4</sup> However, it is less well accepted that the sensitivity of a given patient's cells to an IgE-mediated signal may influence the severity of the allergic disease process. This concept has fallen into the catchall category of "releasability," the intrinsic ability of the cell to respond to stimulation.

Although the basophil and mast cell are not entirely similar in their response characteristics, the basophil remains useful as a surrogate of the IgE-mediated mast cell response.<sup>5</sup> The basophil is especially useful since the responses of basophils obtained from well-defined donor populations can be compared. Several methods have been used to determine basophil releasability or sensitivity<sup>6-12</sup>: (1) monitoring spontaneous HR in normal buffers or buffers in which water has been replaced with D<sub>2</sub>O, (2) stimulation of mixed leukocyte preparations with a concentration of polyclonal anti-IgE antibody that is optimal for HR, and (3) determining the density of antigen-specific IgE required to obtain 25% HR after challenge with an optimal concentration of specific antigen.<sup>12</sup> The first method had led to reports that atopic subjects and atopic subjects with asthma can be differentiated from nonatopic donors on the basis of spontaneous release.<sup>6, 7</sup> The mechanisms underlying this characteristic remain obscure. The second method has the advantage of simplicity as well as testing a more understandable parameter.<sup>9-11</sup> There is the problem that it may not determine cell sensitivity because the maximal amount of HR with a physiologic stimulus, or the efficacy of a stimulus, is not clearly related to the potency of a stimulus. However, we have previously found that the amount of leukotriene release after a challenge with an optimal concentration of anti-IgE antibody correlated with cellular sensitivity, as measured by the third method,<sup>12</sup> whereas the magnitude of HR correlated less well. The third method does extract the desired parameter, cellular sensitivity, but it requires much greater effort and has yet to be applied to a study of the general population.

The second method of determining cellular sensitivity does reveal an interesting aspect to the releasability issue. Although there is wide variability in the response of basophils to anti-IgE antibody, it is particularly intriguing that 10% to 20% of the donors have basophils that consistently fail to release histamine in response to this stimulus. There are also anecdotal observations that such donors can cycle in and out of responsiveness during very long time periods. These cells may represent a special case of "releasability" or they may be part of a continuum, but in either case, elucidation of the cause for their lack of response may provide insight into an important aspect

of basophil control. Furthermore, to understand the underlying cause of releasability differences among the donors, we need to study strongly contrasting responses, and these donors provide such contrasting responses. This study was initially designed to answer the one question that could trivialize this problem (at least from the standpoint of releasability): Do the basophils of these donors lack or possess very low densities of cell-surface IgE antibody? Once this question was settled, other aspects of the basophil response could be examined.

## MATERIAL AND METHODS

### Buffer

Buffer consisted of the following: PIPES (Sigma Chemical Co., St. Louis, Mo.), stock buffer, 25 mmol/L of PIPES containing 110 mmol/L of NaCl, 5 mmol/L of KCl, and 40 mmol/L of NaOH, adjusted to pH 7.3, and stored at 10 times (10 ×) the above concentration, PAG, PIPES (1 ×), containing 0.003% HSA (Miles Laboratories, Inc., Elkhart, Ind.) and 0.1% glucose, PAGCM, and PAG with 1.0 mmol/L of EDTA. Acetate elution buffer was 0.05 mol/L of sodium acetate, 0.085 mol/L of NaCl, 10 mmol/L of EDTA, and 0.03% of HSA at pH 3.7.

### Reagents

Polyclonal goat antihuman IgE was prepared as described previously<sup>13</sup>; the antibody used for these studies represented the IgG fraction of goat serum prepared by DE-52 chromatography (Whatman Ltd., Maidstone, England). Three times crystallized, lyophilized Con A, calcium ionophore A23187, fMet peptide, and TPA were purchased from Sigma Chemical Co. Mouse monoclonal IgG antihuman IgE was a gift from Dr. Robert Hamilton (Tanox Corp., Houston, Texas). Recombinant human IL-3 was expressed in yeast and purified to homogeneity by a combination of ion exchange and reverse-phase high-performance liquid chromatography techniques.<sup>43</sup>

### Subjects

Donors consisted of adult subjects who were randomly selected from the personnel of the Department of Clinical Immunology. The donors were mostly nonatopic. For the purpose of this study, a nonreleaser was defined as a donor whose basophils released <5% histamine in response to any concentration of anti-IgE antibody on three occasions. Donors were asked if they had any medical problems, especially any known history of atopy (asthma, allergic rhinitis, chronic urticaria, atopic dermatitis, etc.), and if they were receiving any medications.

### Cell preparation

Venous blood was obtained by venipuncture, and the cells were separated on a single-step Percoll gradient.<sup>15</sup> Briefly, blood was centrifuged over Percoll (specific gravity, 1.080 g/ml). The mononuclear cell layer was then collected and washed in large volume with EDTA saline, PAG-EDTA, and

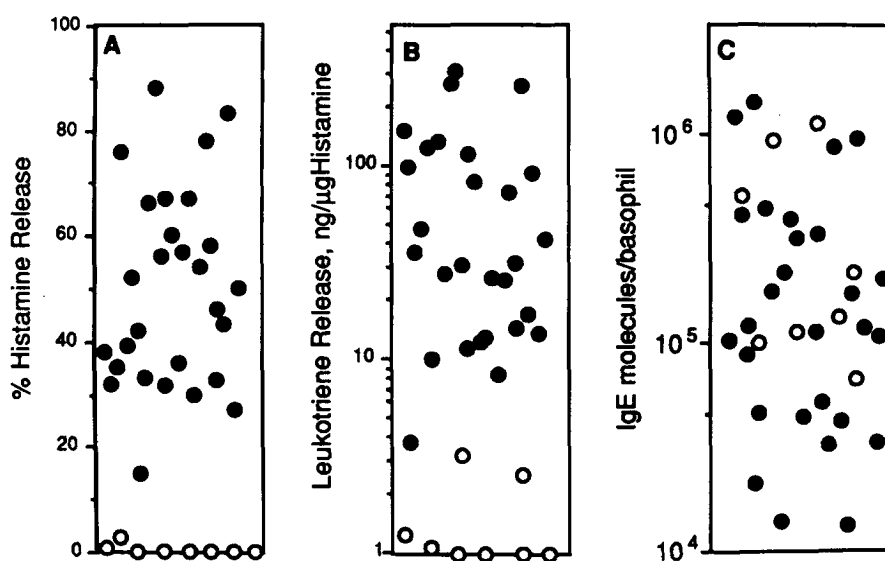


**FIG. 1.** Anti-IgE-induced mediator release in 36 randomly selected donors. **A-B,** Scattergrams that represent percent of HR and LTC<sub>4</sub> release at 0.1  $\mu$ g/ml of anti-IgE antibody for releasers (●) and nonreleasers (○). Average percent of HR was  $48\% \pm 21\%$  versus  $0.6\% \pm 1\%$  for releasers and nonreleasers, respectively. The average LTC<sub>4</sub> release was  $74.2 \pm 79.6$  ng/ $\mu$ g of histamine for releasers and  $0.9 \pm 1.39$  ng/ $\mu$ g of histamine for nonreleasers. **C,** Scattergram distribution of IgE antibody density levels on the same donors. The average IgE density was 287,000 IgE molecules per basophil versus 400,000 IgE molecules per basophil for releasers (●) and nonreleasers (○), respectively.

twice with PAG and then was used directly for the challenge experiments.

### Cell challenge

Cells were resuspended in PAGCM and then challenged with goat anti-IgE, Con A, TPA, and calcium ionophore A23187 or fMet peptide at various concentrations for 45 minutes at 37° C. The final volume was usually 0.5 ml, and usually, if LTC<sub>4</sub> was measured, 2 ml of blood was obtained for each experimental tube. For HR, only 1 ml of blood was needed for each tube. After 45 minutes, samples were centrifuged, and the supernatant was collected for histamine assay.

### Binding study

Cells were incubated in the absence of calcium with PAG and indicated concentrations of goat anti-IgE antibody for 90 minutes at 37° C. Control cells were treated in a similar manner without anti-IgE. After 90 minutes, PAG with two times CM were added to yield a final concentration of 1 mmol/L of CaCl<sub>2</sub> and MgCl<sub>2</sub>, and the cells were challenged with different concentrations of TPA. The reaction was allowed to proceed for an additional 45 minutes. Samples were then centrifuged, and the supernatant was collected for the histamine assay.

### Overnight culture

Percoll preparations of basophils were cultured overnight (37° C) at a total cell concentration of  $5 \times 10^6$  ml in RPMI 1640 medium containing 25 mmol/L of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer,

100  $\mu$ g/ml of gentamycin sulfate, and 0.3% HSA (2 by 24 ml well culture plates). Recombinant IL-3 was used at a concentration (200 U/ml or 11 ng/ml) previously demonstrated to be optimal for enhancement of mediator release while the direct induction of HR was minimized from donor basophils.<sup>16\*</sup>

### Histamine assay

All reactions, performed in duplicate, were stopped by centrifugation, and the supernatant was removed for histamine and LTC<sub>4</sub> analysis. In each experiment, perchloric acid at a 1.6% final concentration was added to some tubes to determine total histamine content. Histamine was assayed by an automated fluorometric technique.<sup>17</sup> 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 U/ml of blood), brought to 37° C, and immediately added to tubes containing PAGCM buffer plus/minus stimulus (0.5 ml of blood plus 0.5 ml of buffer). After a 30-minute incubation, the supernatant was harvested after centrifugation and stored frozen until assay by the radioenzymatic assay.<sup>18, 19</sup> Total histamine content of the

\*In collaboration with Dr. Bruce Bochner, we have recently demonstrated, with flow cytometry, that our polyclonal anti-IgE antibody binds in expected quantities to nonreleaser basophils (unpublished observations).

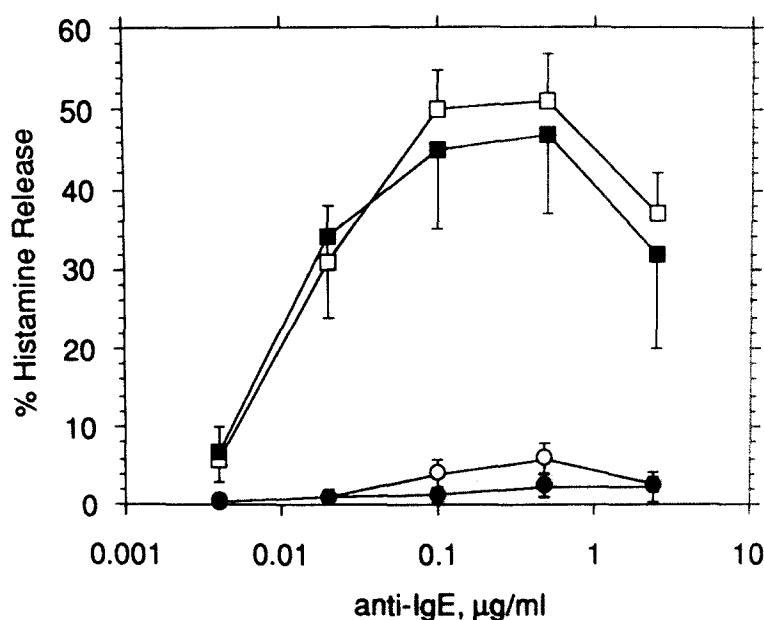


FIG. 2. Comparison of HR after challenge with anti-IgE antibody in whole blood versus Percoll-separated leukocytes; nonreleasers/Percoll (●), nonreleasers/whole blood ( $n = 3$ ) (○), releasers/Percoll (■), releasers/whole blood ( $n = 5$ ) (□).

blood was obtained by boiling 0.1 ml of blood for 10 minutes and harvesting the supernatant. Controls in these experiments included 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/measured content for the spiked samples was  $1.01 \pm 0.04$  ( $n = 32$ ).

### Surface IgE density determination

After the initial washing steps, the cells were incubated at room temperature for 30 to 45 minutes to allow further dissociation of IgE from low-affinity receptors on monocytes or lymphocytes. After centrifugation, the pellets were resuspended in ice-cold acetate buffer, pH 3.7, and incubated in an ice bath for 10 minutes.<sup>14</sup> After a brief centrifugation (15,000  $g$ ), the supernatant was removed and neutralized with 1 N NaOH. These samples were frozen immediately for later measurement of IgE by radioimmunosorbent test. Acid elution was done in triplicate. Basophil counts (alcian blue staining),<sup>20</sup> made before elution, were also done in triplicate.

### Radioimmunoassay

The radioimmunoassay for  $\text{LTC}_4$  has been described previously.<sup>21</sup>  $\text{LTC}_4$  was assayed in duplicate for each sample tube.

### Statistics

In comparisons of data from different donor groups, releasers versus nonreleasers, the Mann-Whitney U test was used. Bars (see Fig. 3) represent the standard deviation.

## RESULTS

In our study of 36 randomly selected adult donors, eight (22%) donors fit the criteria of nonreleasers. Although human basophils were found to be significantly less responsive to anti-IgE antibody in patients with chronic idiopathic urticaria,<sup>22</sup> prolonged incubation with steroids<sup>23</sup> or theophylline,<sup>24</sup> none of the above conditions was present among the nonreleasers to explain the poor response of basophils to anti-IgE antibody. The dose-response curves to anti-IgE antibody were examined in the range 0.02 to 0.5  $\mu\text{g/ml}$  for all donors, and the optimal concentration of anti-IgE antibody was found to be between 0.1 and 0.5  $\mu\text{g/ml}$  for releasers. The dose-response curve was then expanded, with concentrations ranging from 0.02 to 100  $\mu\text{g/ml}$  for nonreleasers, and basophils remained unresponsive throughout the tested range. A wide range of HR is illustrated in Fig. 1, A, and  $\text{LTC}_4$  release in Fig. 1, B, from basophils on challenge with 0.1  $\mu\text{g/ml}$  of anti-IgE antibody. Based on our definition, the releasers and nonreleasers were clearly separated. Nonreleasers also generated significantly less  $\text{LTC}_4$  than releasers (Mann-Whitney,  $p < 0.01$ ) in response to anti-IgE antibody.

We were concerned that the act of isolating mononuclear cells by Percoll separation led a selective loss in IgE-mediated responsiveness in nonresponder donors, that is, that the nonreleaser status was a technical artifact of cell preparation. Therefore, we challenged basophils in freshly isolated whole blood with anti-IgE antibody and measured HR with the radioenzy-

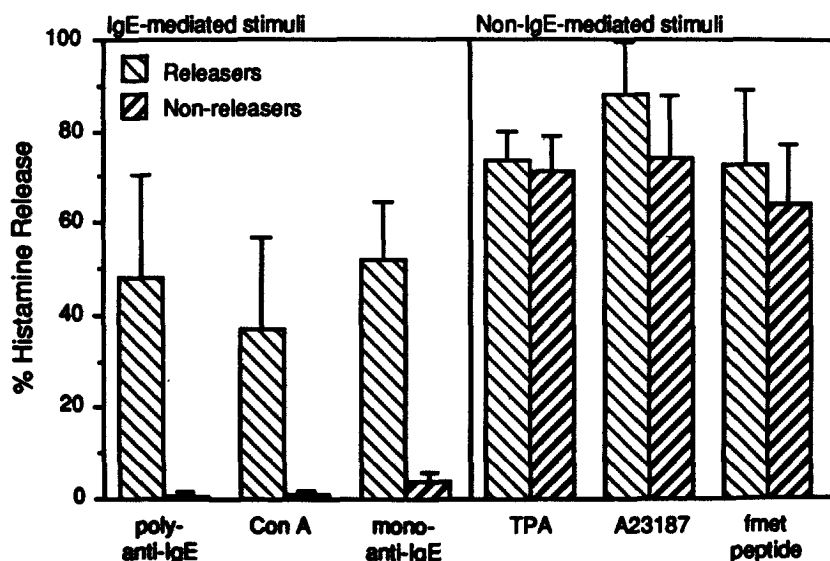


**FIG. 3.** Comparison of HR induced by IgE-mediated and non-IgE-mediated stimuli between releasers and nonreleasers. The average percent of HR when (left panel) releaser and nonreleaser cells were challenged at optimal concentration of IgE-mediated stimuli: poly-anti-IgE (0.1  $\mu$ g/ml), Con A (5  $\mu$ g/ml), and mono-anti-IgE (1:100 dilution). In contrast the average percent of HR (right panel) induced by non-IgE-mediated stimuli.

matic assay. In five releasers and three nonreleasers, the dose-response curves to anti-IgE antibody in whole blood and Percoll preparations were essentially superimposable (Fig. 2).

Kings et al.<sup>25</sup> found that different anti-IgE preparations induced variable responses from the same donor's basophils. Therefore, to examine whether the lack of response to anti-IgE antibody was restricted to this particular goat polyclonal anti-IgE antibody preparation, cells were challenged with two additional IgE cross-linking reagents, Con A and mouse ascites monoclonal IgG antihuman IgE antibody. Con A is believed to cross-link cell-surface IgE via the carbohydrate portion of the IgE molecule. We have also tested a mouse monoclonal antibody with high affinity for human IgE Fc. Con A dose-response curves were tested from 0.2 to 25  $\mu$ g/ml. Mouse monoclonal IgG anti-IgE antibody was examined from 1:100 to 1:10,000 dilution. Nonreleaser basophils remained unresponsive to these two additional IgE cross-linking stimuli. The nonrelease status could be generalized to all the nonspecific IgE-mediated stimuli tested, as illustrated in Fig. 3, left panel.

Since the calcium ionophore A23187, TPA, and FMLP peptide are able to induce HR from human basophils by non-IgE-mediated mechanisms,<sup>26-28</sup> we used these stimuli to examine the degranulating ability of releaser and nonreleaser basophils. The TPA dose-response curves were tested in the range of 3 to 100 ng/ml (30 to 50 ng/ml being maximal), and the re-

lease curves were essentially identical for both releasers and nonreleasers (see below). The calcium ionophore A23187 dose-response curves were examined from 30 to 1000 ng/ml. The releaser and nonreleaser basophils response to calcium ionophore A23187 was maximal between 500 to 1000 ng/ml, and in both cases HR was nearly 100%. The fMet peptide dose-response curves, in the concentration range of  $10^{-9}$  to  $10^{-6}$  mmol/L, were examined with optimal responses obtained in the range from  $10^{-7}$  to  $10^{-6}$  mmol/L. The mean HR in response to these three different stimuli at the optimal concentrations, illustrated in Fig. 3, were not statistically different between releasers and nonreleasers, nor were the dose-response curves to TPA or fMet peptide different in a limited examination of eight randomly chosen releasers and the eight known nonreleasers. This demonstrates that degranulation mechanisms were intact in nonreleaser basophils, and an elevation of either PKC activity or intracellular calcium were sufficient for secretion. In addition, these cells were able to generate a non-IgE-mediated signal in response to fMet peptide stimulation with subsequent HR.

The cell-surface density of IgE antibody was also measured in these two groups of donors. As illustrated in Fig. 1, C, the nonrelease state was not correlated to the surface IgE density. There was no statistically significant difference in the surface IgE density of basophils from releasers and nonreleasers (Mann-Whitney,  $p > 0.05$ ). Therefore, nonreleaser baso-

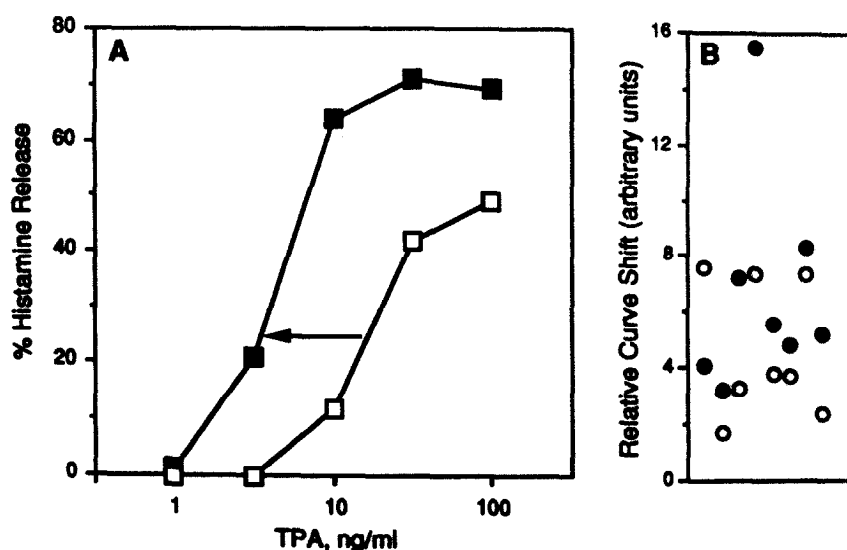


FIG. 4. Anti-IgE-antibody binding induced a TPA dose-response curve shift in releaser and non-releaser cells. Cells were incubated with 0.1  $\mu$ g/ml of anti-IgE in the absence of calcium for 90 minutes. Control ( $\square$ ) and pretreated cells ( $\blacksquare$ ) were then challenged with TPA. A, Length of a horizontal line that extends from the point of 50% maximal HR on the control curve to anti-IgE pretreated curve was used to measure the changes induced by pretreatment of cells with anti-IgE in both releasers ( $n = 8$ ) and nonreleasers ( $n = 8$ ). B, Change in cell sensitivity to TPA after pretreatment with anti-IgE in releasers ( $\bullet$ ) and nonreleasers ( $\circ$ ).

phils possess as much surface IgE as releaser basophils, a quantity that should be capable of providing a signal for degranulation if the surface IgE was cross-linked by anti-IgE antibody.<sup>12</sup>

Schleimer et al.<sup>27</sup> demonstrated that pretreatment of human basophils with anti-IgE in the absence of calcium produced cells that were twofold to tenfold more sensitive to TPA. This curve shift was generally found to be a result of an enhanced rate of release. Warner and MacGlashan<sup>29</sup> found that to maintain the curve-shift phenomenon, IgE cross-links must also be sustained at all times. It was suggested that the increased sensitivity of anti-IgE-pretreated cells to TPA was probably due to preactivation of PKC by anti-IgE antibody-induced cross-links. Such activation has now also been demonstrated.<sup>16, 29</sup> Schleimer et al.<sup>27</sup> also observed in a restricted study that a nonreleaser basophil also displayed an increased sensitivity preincubated with anti-IgE antibody. We then used these established facts to demonstrate that anti-IgE was able to bind\* and cross-link the cell-surface IgE in non-releaser basophils. The relative TPA curve shift resulting from prior incubation of the cells with anti-IgE antibody for 90 minutes in the absence of calcium

(which fully desensitized the basophils for any subsequent IgE-mediated release) was compared for the two groups of donors. For these experiments, eight randomly chosen releasers (eight random drawings from the pool of 28 releasers described above) and eight known nonreleasers were studied. A typical TPA curve shift is illustrated in Fig. 4, A, in both releasers and nonreleasers. The curve shift was calculated by taking the ratio of TPA concentration required for 50% of maximal HR on the control curve, divided by TPA concentration required for the same HR on the anti-IgE antibody pretreated curve. The relative curve shifts are illustrated in Fig. 4, B, for releasers and nonreleasers. The TPA dose-response curves shifted twofold to sevenfold in nonreleasers, and threefold to 15-fold in releasers. There was no statistical difference between these two groups of donors. Therefore, these data suggested that anti-IgE antibody effectively bound and cross-linked cell-surface IgE on nonreleaser basophils.

There are several agents ( $D_2O$ ,<sup>30</sup> cytochalasin B,<sup>30</sup> Sr,<sup>31</sup> and IL-3<sup>32</sup>) that enhance HR from basophils challenged with anti-IgE antibody. Many of these agents are nonphysiologic and cause modest enhancement. We and other investigators have recently found that IL-3 is a powerful up regulator of basophil responsiveness.<sup>33-34</sup> Although a brief preincubation with IL-3 will up regulate or "prime" the basophil response, we have found that overnight culture of mononuclear

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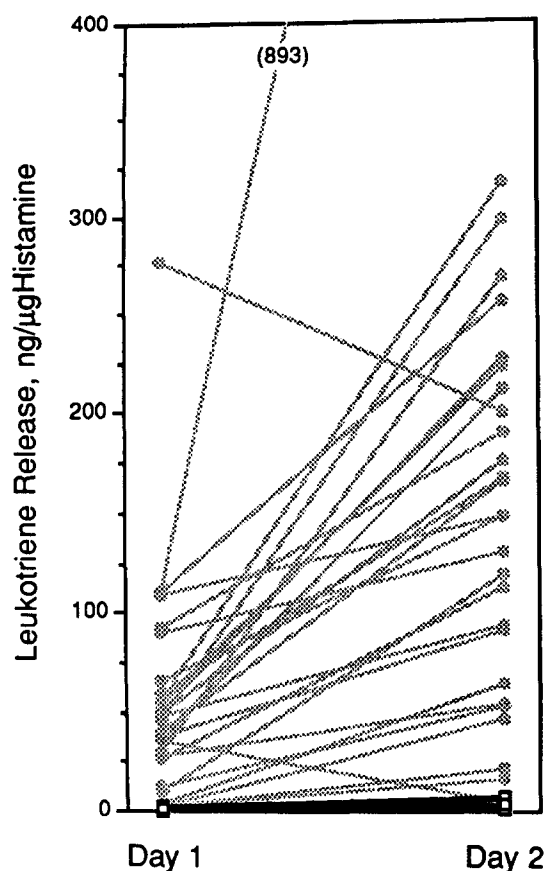


**FIG. 5.** Leukotriene release distribution before and after overnight culture with 11 ng/ml of recombinant IL-3. Change in leukotriene release, measured as nanograms of LTC<sub>4</sub> per micrograms of histamine for cells challenged with 0.1 μg/ml of anti-IgE antibody before culture and after culture with 11 ng/ml of IL-3. Open squares indicate the nonreleasing donors; average leukotriene release: day 1, 43 ± 9 ng/μg of histamine; day 2 control, 25 ± 7 ng/μg of histamine; day 2 plus IL-3, 135 ± 27 ng/μg of histamine.

cell preparations with IL-3 produces a greater enhancement of HR and leukotriene release from anti-IgE- and fMet peptide-stimulated basophils. Remarkably, IL-3 can induce a hundredfold (average is approximately equal to ninefold) enhancement of leukotriene release, far greater than other enhancing agents. IL-3 could also maintain purified basophil function and increase basophil sensitivity, as measured by the density of antigen-specific IgE required for 25% HR<sup>32</sup> (unpublished observations). We hypothesized that the nonreleaser status reflected an *in vivo* condition in which there was little IL-3 up regulation and that *in vitro* culture with IL-3 might bring nonreleasing basophils into the release category. To test this hypothesis, we measured the basophil response to 0.1 μg/ml of anti-IgE antibody on the day blood was obtained (day 1) and after overnight

culture (≈18 hours) ± 200 U/ml (11 ng/ml) of IL-3 on 35 donors. Samples were obtained for both HR and leukotriene release. As we have previously found, leukotriene release is a very sensitive indicator of the effects of IL-3 in releasers. Although there was a slight increase in both HR and leukotriene release, IL-3 did not raise the response of the nonreleasers above the 5% HR point (Fig. 5). For nonreleasing basophils, HR and leukotriene release averaged 1.2% ± 0.4% and 0.3 ± 0.2 ng/μg of histamine before culture 0.6% ± 0.6% and 0.5 ± 0.3 ng/μg of histamine in day 2 control cells, and 2.0% ± 0.8% and 4 ± 1 ng/μg of histamine in day 2 plus IL-3 cells\* with the enhancement (day 2 IL-3) treated cells per day 2 control release) averaging 0.87 ± 0.42 and 3.3 ± 1.5 for HR and leukotriene release, respectively. Based on these data from weak releasers, we would have expected ratios >3 and >10 for HR and leukotriene release, respectively. As a positive control for the effects of IL-3 on nonreleasing basophils, we found that fMet peptide-induced release was equally enhanced in a subset of five releaser and three non-releaser donor basophils. We conclude that pretreatment of nonreleaser basophils with IL-3 did not correct the IgE-dependent "defect."

We also examined whether 44% D<sub>2</sub>O, 5 μmol/L of cytochalasin B, or 10 mmol/L of Sr<sup>++</sup> would enhance anti-IgE antibody-induced HR in four nonreleasing donors. However, as we found for IL-3, the nonreleasers responded slightly but were not converted to releasers. The net anti-IgE response in normal buffer was 0.8% ± 0.6% in the presence of 10 mmol/L of Sr<sup>++</sup>, 3% ± 1%; 44% D<sub>2</sub>O, 2% ± 1%; and 5 μmol/L of cytochalasin B, 2% ± 1%. Interestingly, poor releasers were enhanced markedly by Sr<sup>++</sup>; two such releasers (average release, 7%) were enhanced to 47% with 10 mmol/L of Sr<sup>++</sup> (see DISCUSSION).

## DISCUSSION

Previous studies have suggested that intrinsic "releasability" parameters are largely responsible for the poor response of some donors' basophils to anti-IgE antibody. For example, Ishizaka et al.<sup>35</sup> found that sensitized basophils from some donors failed to release histamine when basophils were challenged with appropriate antigen or anti-IgE antibody. However, the cells were able to release a significant amount of histamine in the presence of D<sub>2</sub>O. Conroy et al.<sup>36</sup> also

\*In three of seven experiments, the average of duplicate leukotriene determinations for anti-IgE-stimulated cells was greater than the spontaneous release, but not statistically different from, spontaneous release within the given assay. Therefore, these data were skewed to a more positive result.

found an enhanced response when basophils of these donors were challenged in the presence of cytochalasin B. However, these results could have occurred because of insufficient sensitization with antigen-specific IgE rather than true sensitivity differences between basophil preparations.

In our study, we found 22% of the donors were nonreleasers according to our definition, a value only slightly higher than the 15% found by Conroy et al.<sup>36</sup> Our studies demonstrated that the nonreleaser basophils possessed a sufficient number of surface IgE molecules, which were cross-linked by anti-IgE (as defined by the TPA studies), to mount a response. The nonrelease state appeared to be confined to IgE-mediated stimulation; fMet peptide, by binding to its specific receptors on basophils<sup>28</sup> and initiating a stimulatory event through a non-IgE pathway,<sup>37</sup> induced the release of an equivalent amount of HR in nonreleaser and releaser basophils. These data were also supported by the ionophore and TPA challenges, both of which bypass receptor-mediated events by either elevating cytosolic calcium directly or directly stimulating the activity of PKC. In contrast, Con A and monoclonal IgG anti-IgE antibody, which are IgE cross-link secretagogues, also failed to induce HR from the nonreleaser basophils. This suggests that a defect(s) is probably specific for the IgE-mediated transduction pathway.

The locus for this defect is not yet clear, in part, since the sequence of events leading to degranulation remains unclear. Recent models of IgE-mediated activation have focused on two nearly ubiquitous second messengers, DAG<sup>17, 38, 42</sup> and inositol triphosphate.<sup>39, 40</sup> In univalent receptor-mediated responses, the generation of these two second messengers from membrane phospholipids is believed to occur very early in the sequence of events after ligand binding to receptor. Both of these messengers are probably generated after an IgE-mediated stimulus, since many studies in murine cells and cell lines, as well as recent studies in the human mast cell and basophil, demonstrate or implicate their formation. The use of TPA and ionophore should bypass the two or three steps preceding the elevation of cytosolic calcium by inositol triphosphate or activation of PKC by DAG and suggests that a defect occurs in the coupling of receptor cross-linking to second messenger generation. However, both these stimuli may not operate directly on the same pathways normally used by the IgE-mediated pathway and only serve to indicate that the cells can secrete. In a similar context, fMet peptide is now believed to operate by a very distinct biochemical cascade, and although this once again demonstrates that nonreleaser cells can secrete, it does not serve to localize the defect very precisely. It is interesting that anti-IgE antibody

induces a dose-response curve shift to TPA in the nonreleaser basophils. We have previously suggested that because anti-IgE antibody induces an increase in PKC activity, the response to TPA is improved. On this basis, we would speculate that anti-IgE induces increased PKC activity in nonreleaser basophils without the coincident rise in cytosolic calcium. However, if the reaction was this simple, we might have expected the kinetics of HR initiated with anti-IgE in nonreleasers to resemble the slow kinetics induced by TPA, which selectively activates PKC. This did not happen. In contrast, it may not be reasonable to assume that the PKC activity induced by anti-IgE would in itself mimic the activation by TPA. In other words, TPA may be activating additional PKC enzymes unrelated to enzymes used by IgE-mediated activation and thus evoke slow degranulation.

We have recently found that IgE-mediated HR correlated very well with elevations in cytosolic  $\text{Ca}^{++}$  ( $r_s = 0.79$ )\* and elevations in PKC activity ( $r_s = 0.90$ ).<sup>16</sup> In purified basophils that were unresponsive, there was no elevation in cytosolic  $\text{Ca}^{++}$  in response to anti-IgE antibody (the y intercept for this correlation was at the origin), whereas fMet peptide did produce its characteristic calcium response.<sup>†</sup> Although PKC activity is also correlated, nonreleasing basophils did exhibit a small, but statistically significant, increase in PKC activity ( $\approx 170\%$  of control). This information suggests that nonreleasing basophils lack the means to elevate cytosolic  $\text{Ca}^{++}$  levels, although they possess a partially disabled pathway leading to PKC activation. (Since DAG-stimulated PKC activity remains partially dependent on  $\text{Ca}^{++}$  levels, the lack of a  $\text{Ca}^{++}$  response might also influence an otherwise normal PKC activation.) Studies by Foreman et al.<sup>31</sup> indicated that  $\text{Sr}^{++}$  could enhance release in a nonreleaser. Their studies suggest that  $\text{Sr}^{++}$  uses the calcium-entry pathway with greater efficiency. We also observe remarkable enhancement by  $\text{Sr}^{++}$  in donors that exhibit release between 5% and 10%. However, the donors that we have classified as nonreleasing were only marginally affected by  $\text{Sr}^{++}$ . Nevertheless, as Foreman et al.<sup>31</sup> have speculated, our results might also indicate that nonreleasing donors completely lacked the apparatus that moves calcium into the cell.

It is also interesting that nonreleasers were nonatopic except for one donor who had an 8-year previously recorded positive skin test to ragweed but,

\*Warner JA, MacGlashan DW Jr. Signal transduction events in human basophils: a comparative study of the role of PKC in basophils activated by anti-IgE and fMet peptide [submitted].

†Warner JA, MacGlashan DW Jr. Stimulus-dependent leukotriene release from human basophils: a comparative study of C5a and fMet-leu-phe [submitted].

currently, had no correlated clinical symptoms in the fall. Whether the intrinsic sensitivity of the cells to stimulation modulates the allergic reaction in this particular donor remains speculative at this point. However, we have also found that nonreleaser basophils do not adhere to endothelium after stimulation with anti-IgE antibody,<sup>41</sup> a possible first step for basophils to infiltrate the tissue from the blood and to participate in the inflammatory reaction.

One alternative explanation of the lack of an IgE-mediated response in nonreleasers could be the presence of a strong inhibitory signal in vivo. The most efficacious-known natural inhibitors of basophil mediator release are the glucocorticosteroids. However, we have recently demonstrated that IL-3 reverses the effects of these steroids,<sup>32</sup> and the lack of an effect by IL-3 in the studies in this article suggests that endogenous steroids are not the operative inhibitor if there is an inhibitor responsible for this phenomenon. These results further emphasize the special case these donors represent in the releasability issue.

In summary, these studies establish that nonreleasing basophils possess sufficient cell-surface IgE antibody to mount a response. With the knowledge of our previous biochemical studies of basophil activation, these data suggest that nonreleasing basophils lack the means to elevate cytosolic  $Ca^{++}$  levels. Further studies to elucidate the precise step(s) in the complex biochemical signal that explain the nonreleaser basophils lack of response may be useful in understanding an IgE-specific control mechanism of mediator release.

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