

IgE-mediated basophil releasability is influenced by intrinsic factors and by IgE on the cell surface

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Experiments were done to clarify the mechanisms associated with releasability of histamine. First, washed leukocytes from 23 asthmatic patients sensitive to mite allergen were challenged with Der p 1, a major allergen isolated from *Dermatophagoides pteronyssinus*, or anti-IgE. A significant correlation was observed between the ratio of Der p 1-specific IgE titer to total IgE level (S/T) in the patient's plasma and either the reactivity (maximal percentage of histamine release; $r_s = 0.514$, $P = 0.016$, $n = 23$) or the sensitivity (the minimum allergen concentration required to achieve 25% histamine release; $r_s = -0.790$, $P = 0.0002$) to Der p 1. Additionally, the reactivity to Der p 1 was significantly correlated with that to anti-IgE ($r_s = 0.690$, $P = 0.0012$), indicating that an intrinsic cellular property may be one of the contributing factors in immunologic histamine release. In a second series of experiments, sinus mast cells were passively sensitized with immunoglobulins prepared from the patient's plasma. A statistically significant correlation was found between either the reactivity or the sensitivity to Der p 1 and S/T, thus indicating that S/T is an indicator of the releasability of histamine. When basophils or mast cells were passively sensitized with mouse IgE and subsequently stimulated with antimouse IgE, the reactivity to antihuman IgE was significantly correlated with that to antimouse IgE ($r_s = 0.966$, $P = 0.0023$, $n = 11$). These observations suggest that an intrinsic cellular property regulates reactivity in immunologic histamine release. Taken together, our results suggest that an intrinsic cellular property, as well as specific IgE antibody levels on the cell surface, is an important factor in determining histamine release in response to IgE-dependent activation.

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Cross-linking of IgE molecules that are bound to high-affinity IgE receptors ($Fc_\epsilon RI$) on mast cells and basophils triggers secretion of various chemical mediators of immediate-type hypersensitivity. Other cell types including eosinophils and monocytes have been reported to possess $Fc_\epsilon RI$ and may directly participate in allergic reactions (1, 2). However, there has been no evidence of mediator release from these cells after cross-linking of IgE molecules. Thus, mast cells and basophils are considered to be the two principal cell types involved in the initiation of allergic reactions (3–6).

When washed leukocytes from atopic patients were incubated with the relevant allergen *in vitro*, IgE-dependent activation led to degranulation and

concomitant release of histamine. The histamine-release test is an established model of allergic reaction. Recent studies have demonstrated that both basophil histamine release and bronchial hypersensitivity to mite are significantly improved after 40 days of allergen avoidance and deteriorate shortly after exposure to allergen (7), thus indicating that basophil releasability is correlated with the pathogenesis of allergic disorders. Two parameters, i.e., sensitivity and reactivity, have been used to assess release in the histamine-release test (8). The minimum concentration of allergen that is required for a given percentage of histamine release is regarded as an indication of cell sensitivity, and reactivity is defined as the maximal percentage of histamine release

at optimal concentration of allergen (8). There is no definitive evidence as to what factors influence releasability of histamine.

In this study, Der p 1, which is a major allergen extracted from *Dermatophagoides pteronyssinus* (9), was used for the histamine-release test to avoid complications associated with the use of crude allergen, because patients may respond variably to some allergen components contained in a crude extract. We studied the factors involved in regulating histamine release in three experimental models. First, releasability was evaluated in basophils from individual patients with respect to total IgE and allergen-specific IgE levels in the plasma. In the second model, we performed the experiments using cells passively sensitized with globulin fractions from the patient's plasma, in which histamine release could be examined in relation to only IgE antibody levels on the cell surface, independent of the intrinsic cellular property. Finally, experiments were carried out to examine the intrinsic cellular property, in which cells were stimulated with either antihuman IgE or anti-mouse IgE after sensitization with mouse IgE.

Material and methods

Chemicals

Reagents were purchased from the following suppliers: affinity-purified goat antibody to human IgE (anti-IgE) from Kirkegaard & Perry Lab. Inc., USA; and antimouse IgE (clone LO-ME-2) from the Experimental Immunology Unit, Belgium. Der p 1 was isolated from *D. pteronyssinus* by Dr H. Yasueda in our laboratory, as previously reported (10). Mouse IgE was purified from ascitic fluid of BALB/c mice bearing the TIB 142 hybridoma (American Type Culture Collection, USA).

Histamine release from patient basophils

After informed consent was obtained, venous blood was drawn from 23 asthmatic patients (mean age 45.6 ± 17.5 , eight females, 15 males) who were selected on the basis of positive skin test and enhanced specific IgE for mite allergen. Heparinized venous blood (10 ml) supplemented with 0.5 ml of 0.1 M EDTA solution (pH 7.4) was layered on top of three discontinuous gradients of Percoll solution (1.070, 1.079, and 1.090 g/ml) and centrifuged at 700 g for 15 min at 20°C, as described previously (11). After plasma had been collected from the top layer of the tube, the cells were carefully collected with a Pasteur pipette from both the Percoll layer with a density of 1.079 g/ml and the interface pellicle between the Percoll layers with densities of 1.070 and 1.079 g/ml. As over 80% of basophils were distributed in the band

and the Percoll layer (11), this method of collecting leukocytes is superior to the conventional technique using sedimentation with dextran solution, which recovered 50–60% of basophils. This method enabled us to make about 20 samples from 10 ml of blood.

After washing twice in saline, the cells were adjusted to about 2×10^4 basophils per 0.45 ml Tyrode's buffer containing 0.1% human serum albumin (HSA), and the cells in 0.45 ml were transferred to a plastic tube. After preincubation for a few minutes at 37°C, 0.05 ml of solution of Der p 1 or anti-IgE was added to the tube, and the tube was incubated at 37°C for 30–45 min. We used eight concentrations of Der p 1 (33.3, 11.1, 3.3, 1.1, 0.33, 0.11, 0.033, and 0.01 ng/ml) and six concentrations of anti-IgE (1, 0.33, 0.1, 0.033, 0.01, and 0.0033 µg/ml). The tubes were centrifuged at 400 g for 5 min at 4°C, and aliquots of the supernatant were assayed for histamine content by an automated fluorometric assay. The results were expressed as percentage of histamine release after subtraction of the spontaneous release which occurred during incubation in Tyrode's buffer containing 0.1% HSA.

Preparation of globulin fraction of plasma from atopic donors

After one volume of saturated ammonium sulfate solution had been added to an equal volume of plasma, the solution was allowed to stand for 1 h at room temperature. Then the tube was centrifuged at 1200 g for 20 min. After removal of the supernatant, the precipitate was washed with a 1/2 dilution of saturated ammonium sulfate solution. The precipitate was dissolved in and dialyzed against phosphate-buffered saline (PBS) (12). The dialysate was concentrated to the original volume of plasma with Molcut (mol. wt. cutoff 30 000, Nihon Millipore Ltd, Japan), and stored at -20°C.

Passive sensitization of dispersed mast cells or basophils

Sinus mucosa dissected from patients with chronic sinusitis was treated by enzymatic digestion, as described previously (13). Dispersed sinus mast cells or basophils were treated with a solution of 0.13 M NaCl, 0.005 M KCl, and 0.01 M lactic acid (pH 3.9) for 2 min at 4°C (14). After the cells had been washed with PBS containing 0.2% bovine serum albumin, they were incubated for 1 h at 37°C with gentle mixing with 0.2 ml of globulin fraction prepared from the plasma. In some experiments, the cells were incubated with mouse IgE (10 µg/ml) under the same conditions.

Assay of specific IgE titer to Der p 1 and total IgE level

Der p 1 was covalently coupled to paper disks activated with CNBr according to the method of Ceska et al. (15), and Der p 1-specific IgE titer was measured by the radioallergosorbent test (RAST), as previously described (10). Results were quantitated by reference to a control curve with disks coupled to Japanese cedar pollen extracts and pooled sera from patients allergic to Japanese cedar that had been substandardized against the Phadebas RAST reference system (Pharmacia, Sweden) in our laboratory. The results were extrapolated from the control curve and were expressed as PRU/ml (16). Total plasma IgE level was quantitated with a commercially available radioimmunoassay kit (Dainabot, Japan).

Statistical analysis

All data were calculated as the mean $\pm$ SD. Correlations between parameters were determined by means of Spearman's rank correlation test.

Results

Der p 1 or anti-IgE induced histamine release, which increased in a concentration-dependent manner up to maximal release of histamine and diminished at higher allergen concentrations, in all 23 patients. The Der p 1 concentration necessary for maximal histamine release was 7.9 ± 10.3 ng/ml (range 0.33–33.3), while maximal histamine release always occurred at 100 or 333 ng/ml for anti-IgE. The mean maximal

histamine release (reactivity) was $62.7 \pm 24.9\%$ for Der p 1 and $54.0 \pm 26.5\%$ for anti-IgE. The basophil sensitivities defined as the minimum concentrations required for 25% histamine release to Der p 1 and anti-IgE ranged from 0.01 to 10 ng/ml (geometric mean; 0.16 ng/ml, 1 SD; 0.02–1.23) and from 3 to 300 ng/ml (geometric mean; 12.6 ng/ml, 1 SD; 3.7–42.5), respectively.

Relationship between IgE antibody levels in plasma and basophil histamine release

The correlation between Der p 1- or anti-IgE-induced histamine release and IgE levels in 23 different patients is summarized in Table 1. The total IgE concentration and Der p 1-specific IgE titer were 494.2 ± 426.7 IU/ml (89.5–1846) and 43.6 ± 37.3 PRU/ml (2.0–126), respectively, and the ratio of Der p 1-specific IgE titer to total IgE level (S/T) ranged from 0.017 to 0.31 (0.106 ± 0.075).

In Der p 1-induced histamine release, both the sensitivity and the reactivity were significantly correlated with S/T (Table 1, Fig. 1). There was a significant, but weaker, correlation between the sensitivity and the Der p 1-specific IgE antibody titer (Table 1). These observations seem to reflect indirectly the existence of a significant correlation between Der p 1-specific IgE titer and S/T in the plasma ($r_s = 0.687$, $P = 0.0013$). However, we were unable to find any significant correlation between anti-IgE-induced histamine release and plasma IgE levels (Table 1). On the other hand, when the cellular reactivity to anti-IgE was compared with that to Der p 1, a significant correlation was found (Fig. 2,

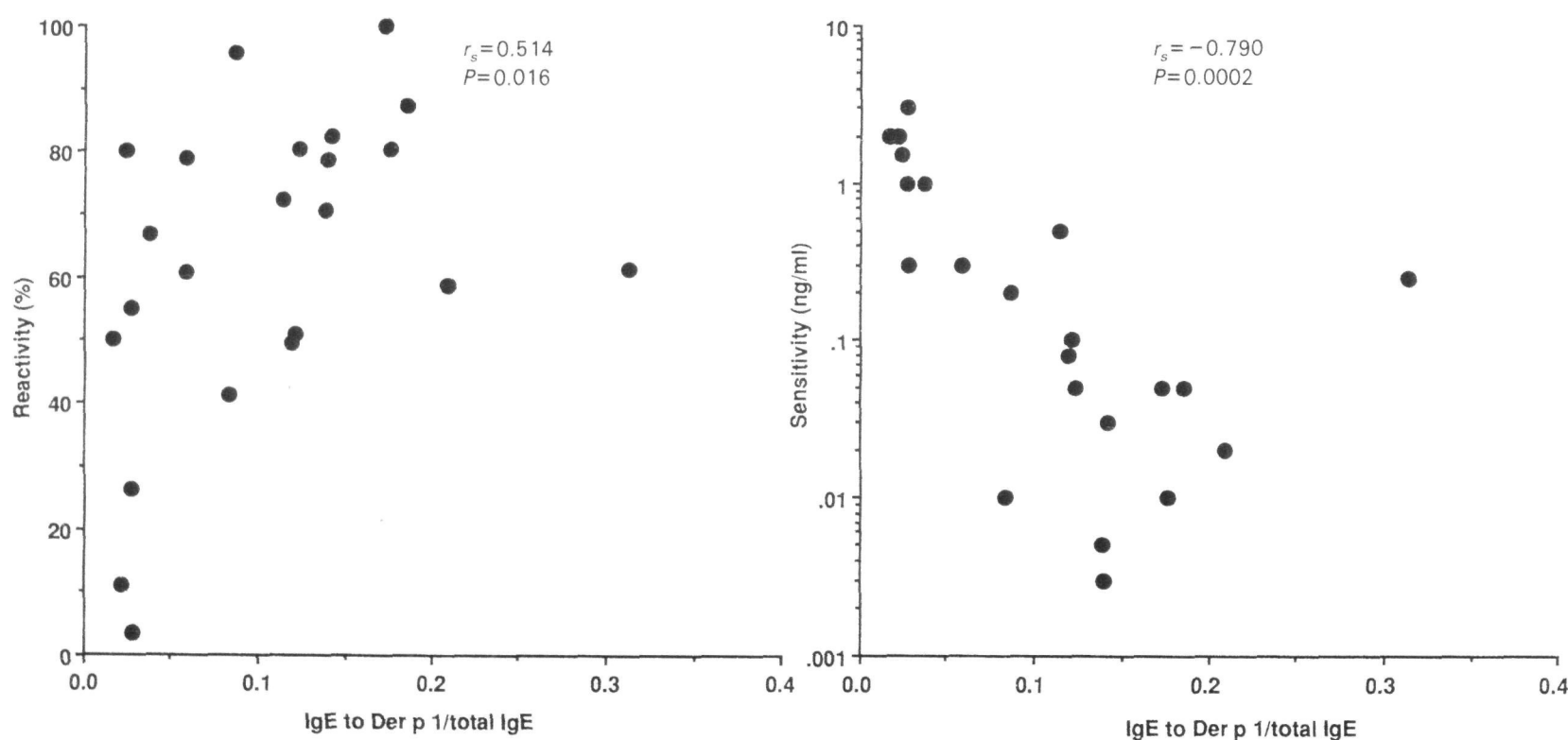


Fig. 1. Correlation between Der p 1-induced histamine release from basophils and ratio of Der p 1-specific IgE titer to total IgE level in plasma.

Table 1. Correlation between basophil histamine release in response to Der p 1 or anti-IgE stimulation and IgE antibody levels in plasma

	Der p 1		Anti-IgE	
	Reactivity	Sensitivity	Reactivity	Sensitivity
Total IgE (IU/ml)	$r_s=0.03$	$r_s=0.187$	$r_s=0.085$	$r_s=-0.135$
494.2±426.7 IU/ml (89.5–1846)	$P=0.888$	$P=0.382$	$P=0.690$	$P=0.526$
Specific IgE to Der p 1	$r_s=0.391$	$r_s=-0.450$	$r_s=-0.076$	$r_s=-0.285$
43.6±37.3 PRU/ml (2.0–126)	$P=0.067$	$P=0.035$	$P=0.721$	$P=0.181$
Specific IgE to Der p 1/total IgE	$r_s=0.514$	$r_s=-0.790$	$r_s=-0.008$	$r_s=-0.352$
0.106±0.075 (0.017–0.31)	$P=0.016$	$P=0.0002$	$P=0.970$	$P=0.099$

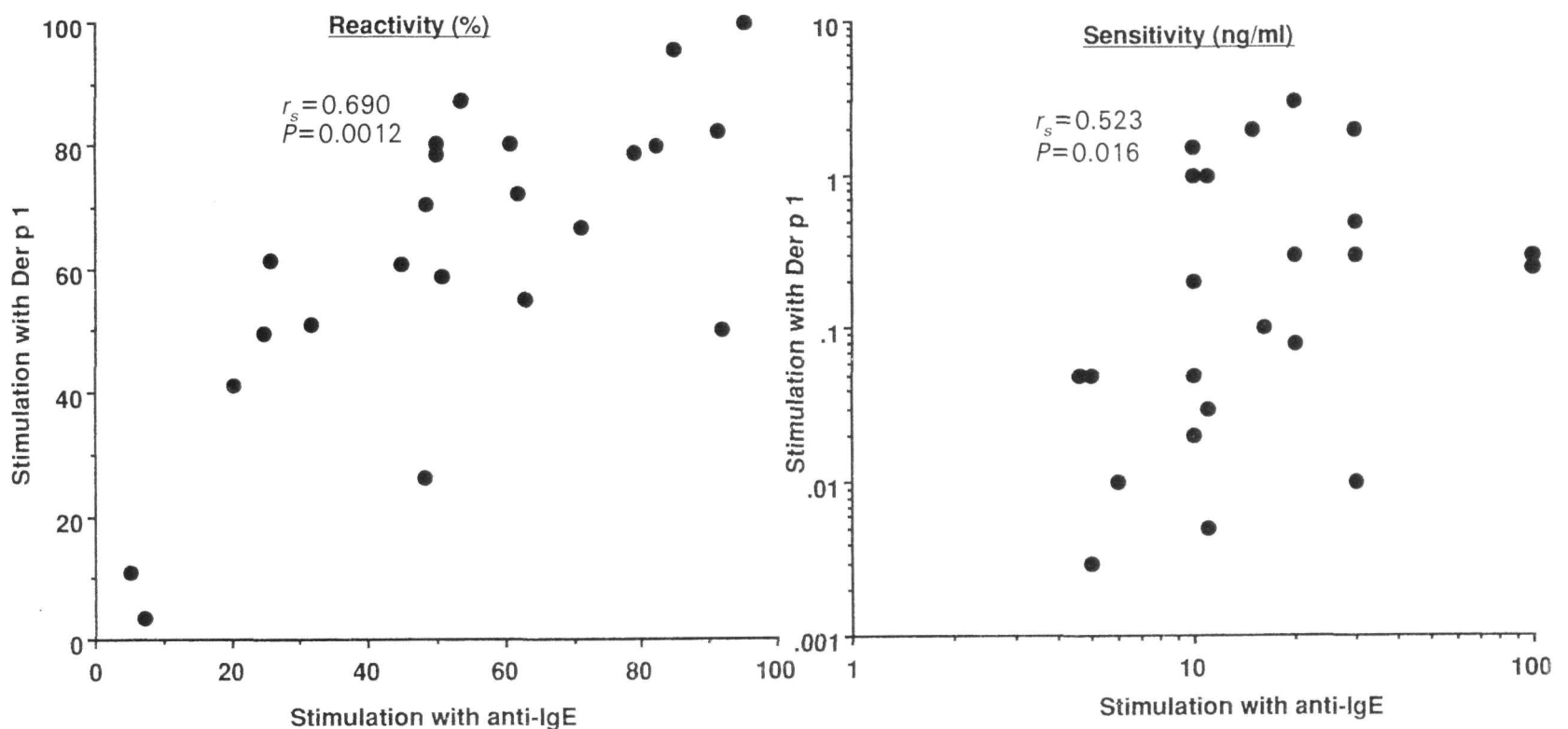


Fig. 2. Correlation between anti-IgE-induced and Der p 1-induced basophil histamine release in 23 asthmatic patients sensitive to Der p 1.

$r_s = 0.690$, $P = 0.0012$), indicating that an intrinsic cellular property may be one of the contributing factors to immunologic histamine release. In addition, the cellular sensitivity to anti-IgE was correlated with that to Der p 1 ($r_s = 0.523$, $P = 0.016$). A significant negative correlation was observed in anti-IgE-induced histamine release between the reactivity and the sensitivity ($r_s = -0.557$, $P = 0.0089$); however, the correlation did not reach a statistically significant level in Der p 1-induced histamine release ($r_s = -0.373$, $P = 0.079$).

Histamine release from passively sensitized cells

After treatment with the acidic buffer in the same manner as basophils, mast cells that dispersed from sinus mucosa obtained from patients with chronic sinusitis were passively sensitized with the IgE-containing globulin fraction from patient plasma (17).

In two different experiments, nine or seven globulin fractions were randomly selected from 23 patients' plasma samples, which were pooled in the

experiments shown in Table 1, for passive sensitization. These fractions contained 68.9–464 IU/ml of total IgE and 2.5–84.5 PRU/ml of Der p 1-specific IgE antibody, and S/T ranged from 2.8 to 18.6 (12.4 ± 7.6). The results depicted in Fig. 3 show that Der p 1 induced mast cells from donor M. N., which were sensitized with globulin fractions from nine different plasma samples, to release histamine in a dose-dependent manner at Der p 1 concentrations from 0.03 to 33 ng/ml. Two measures of histamine release were determined from each dose-response curve, in which the sensitivity was expressed as the minimum concentration of Der p 1 required for 10% histamine release because of the relatively low level of histamine release.

The results of the correlation tests from the experiments using two different mast cell samples (M. N. and H. T.) are presented in Table 2. In both samples, the reactivity and the sensitivity were significantly correlated with S/T in the globulin fractions. Two parameters of histamine release were also correlated with the Der p 1-specific IgE titer in the experiment using donor sample M. N., but not in

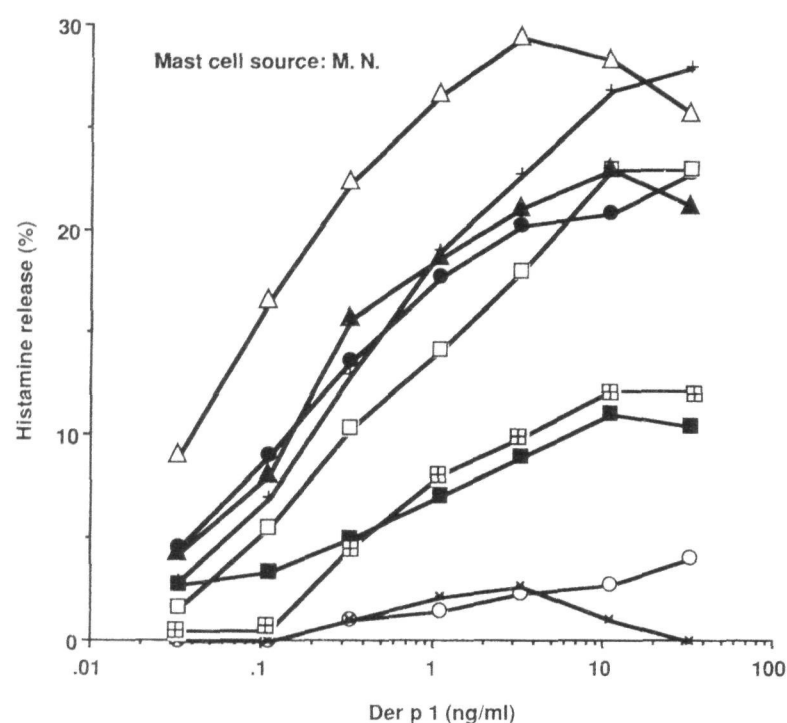


Fig. 3. Der p 1-induced histamine release from passively sensitized sinus mast cells. After dispersed sinus mast cells were treated with lactic acid buffer, cells were passively sensitized with nine different globulin fractions from patients' plasma.

Table 2. Correlation between antibody levels in globulin fractions and histamine release from sensitized mast cells with Der p 1. Dispersed sinus mast cells from donor M.N. or H.T. were passively sensitized with nine or seven different globulin fractions from atopic plasma, respectively

Mast cell source	Reactivity		Sensitivity	
	M.N.	H.T.	M.N.	H.T.
Total IgE	$r_s=0.611$ $P=0.084$	$r_s=0.50$ $P=0.221$	$r_s=-0.234$ $P=0.566$	$r_s=-0.290$ $P=0.517$
Specific IgE to Der p 1	$r_s=0.904$ $P=0.011$	$r_s=0.750$ $P=0.066$	$r_s=-0.883$ $P=0.031$	$r_s=-0.638$ $P=0.154$
Specific IgE to Der p 1/total IgE	$r_s=0.770$ $P=0.029$	$r_s=0.893$ $P=0.029$	$r_s=-0.811$ $P=0.047$	$r_s=-0.886$ $P=0.048$

that using donor sample H. T. The lack of significant correlation in the experiment using mast cells from donor H. T. may be attributed to the fact that the single globulin (one of seven) used in the experiment contained a total IgE concentration and a Der p 1-specific IgE titer which were markedly high (total IgE 1940 IU/ml and Der p 1-specific IgE titer 93.5 PRU/ml), compared with 102–546 IU/ml and 3.4–62 PRU/ml for the other six fractions, respectively.

Thus, the observations may be explained in terms of the relationship between the amount of IgE in the globulin fraction and the number of unoccupied $Fc_\epsilon RI$ on mast cell surfaces. It may be assumed that a limited number of vacant $Fc_\epsilon RI$ is insufficient for binding a relatively higher amount of IgE in the fraction. When the values were normalized to S/T, which should represent the concentration of Der p 1-specific IgE on the cell surface, a significant corre-

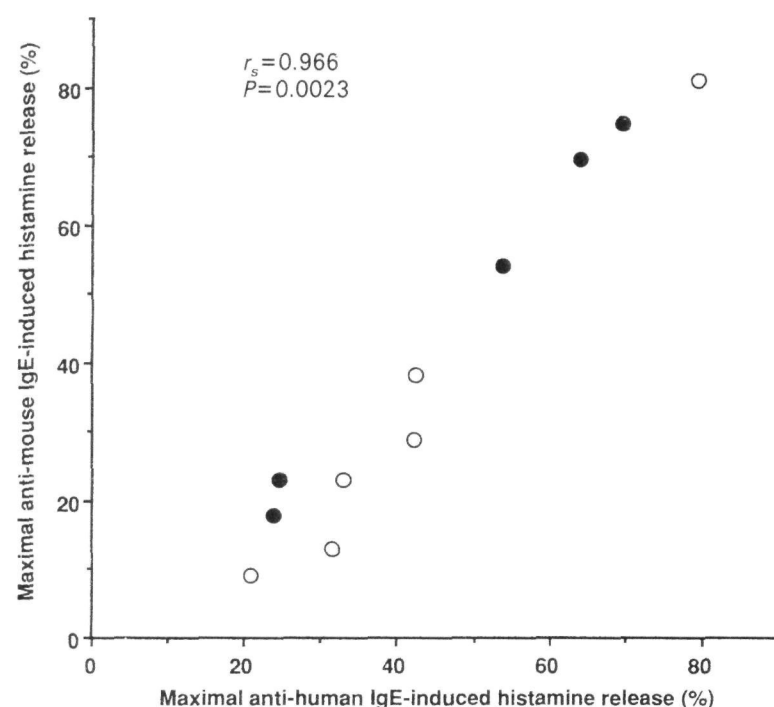


Fig. 4. Relationship between maximal antihuman IgE-induced histamine release and maximal antimouse IgE-induced histamine release in passively sensitized cells. Basophils (●); sinus mast cells (○).

lation was found between S/T and reactivity or sensitivity. The results strongly support the idea that the reactivity or the sensitivity to allergen was regulated by the concentration of allergen-specific IgE antibodies on the cell surface.

An aliquot of the leukocytes or dispersed mast cells was incubated with 10 μ g/ml of monoclonal mouse IgE after treatment with acidic buffer, and then the cells were stimulated with antimouse IgE. Cells in another aliquot were challenged with anti-human IgE without passive sensitization. The passively sensitized cells responded to antimouse IgE, and dose-dependent histamine release was always observed. Antimouse IgE did not release histamine from cells without passive sensitization, suggesting that antimouse IgE could not react with human IgE on the cells. When the maximal histamine release obtained upon stimulation with antihuman IgE was compared with that obtained upon stimulation with antimouse IgE, a statistically significant correlation was observed (Fig. 4, $r_s = 0.966$, $P = 0.0023$), thus indicating that an intrinsic cellular property is involved in regulation of histamine release from basophils and mast cells after cross-linking of $Fc_\epsilon RI$.

Discussion

Two parameters, i.e., sensitivity and reactivity, which correspond to efficacy and potency in drug studies, respectively, have been used as indicators of releasability of histamine. Earlier studies have suggested that there is no correlation between the reactivity (maximal percentage of histamine release) and the allergen-specific IgE titer in serum (18, 19). How-

ever, more recent studies have demonstrated the existence of a significant relationship between them (7, 20, 21). Thus, our work was designed to examine in greater detail factors which influence histamine release, and the contributions of IgE antibody levels to immunologic release of histamine were investigated in two experimental models. In the first, we examined basophil histamine release from individual patients sensitive to mite allergen. In the second model, we used dispersed mast cells which were passively sensitized with globulin fractions from the patient's plasma to address this question. In preliminary studies, purified basophils were treated with acidic buffer containing lactic acid, followed by staining with FITC-labeled anti-IgE. The concentration of membrane-bound IgE was below the detectable limit on the cell surfaces by analysis with a flow cytometer (data not shown). Stripping of the membrane-bound IgE resulted in only a slight decrease in histamine release elicited with anti-IgE but rendered the cells capable of passive sensitization. This model allowed us to examine the correlation between IgE levels on the cell surface and histamine release, independent of intracellular factors. We used Der p 1, a major allergen extracted from *D. pteronyssinus* (9, 10), in this study, because use of a crude allergen extract containing various kinds of allergen molecules leads to difficulties in interpreting the observations, and an isolated allergen is absolutely necessary to study the correlation between IgE antibody level and histamine release.

In the experiments of histamine release from basophils of individual patients, the correlation between Der p 1-specific IgE titer and the reactivity to Der p 1 was moderately significant, and a more significant correlation was observed between the reactivity and S/T (Table 1); this reaction should reflect the relative amount of cell-fixed IgE to Der p 1 (19). The correlation was quite similar to that observed in passively sensitized mast cells where the reactivity to Der p 1 was also correlated with S/T rather than the Der p 1-specific antibody titer (Table 2). These data support the idea that the concentration of specific IgE antibody on the cell surface has an operative effect in determining the reactivity of the cells to the allergen. This hypothesis is strengthened by the observation that the reactivity was significantly correlated with S/T, but not with specific IgE antibody titer in an experiment using mast cells from donor H. T.; this finding may be attributed to the fact that a single globulin fraction containing both a high total IgE concentration and a high specific IgE titer was used in the experiment. Thus, the concentration of Der p 1-specific IgE antibody on the cell surface plays a critical role in determining the reactivity of mast cells and basophils to Der p 1.

In accordance with data obtained by others, there

was no significant correlation between anti-IgE-induced histamine release and IgE levels in the plasma (18, 22). Moreover, when the cell reactivity to anti-IgE was compared with that to Der p 1, a highly significant correlation was observed between them ($r_s = 0.690$, $P = 0.0012$). The observations are compatible with previous studies (18, 23). The results seem to indicate that other modulatory factors may be involved in the reactivity to immunologic stimulation.

We tested this hypothesis in a separate study by observing whether histamine release in response to stimulation with antihuman IgE correlates with that induced by antimouse IgE stimulation, which triggers histamine release in a human IgE-independent manner. The results shown in Fig. 4 demonstrate a highly significant linear correlation between the reactivity to antihuman IgE and that to antimouse IgE, a correlation which clearly demonstrates the existence of an intrinsic factor or factors in the cells which regulate immunologic response to stimulation. Thus, in addition to the number of molecules of allergen-specific IgE antibody on the cell surface, an intrinsic property of the cells also plays an important role in determining immunologic release of histamine.

It is difficult to identify the mechanisms responsible for the intrinsic property. One possible explanation is that the intrinsic cellular property operating in the control of immunologic histamine release is dependent on genetic factors, although the precise mechanism of the factors has not yet been clarified (24). An alternative possibility is that this intrinsic cellular property may have been due to *in vivo* priming of basophils. It is well known that both histamine release and IgE antibody production are controlled by various cytokines (25, 26) and that T-cell-derived cytokines, including interleukin 3, interleukin 5, and granulocyte-macrophage colony-stimulating factor, as well as additional cytokines such as nerve growth factor and insulin-like growth factor, render basophils capable of potentiating histamine release and leukotriene C₄ generation in response to all established stimulants after short-term culture (27–34). However, there is no proof of a causal relationship between the intrinsic property and the specific IgE antibody levels.

In response to anti-IgE, the reactivity was inversely correlated with the sensitivity ($r_s = -0.557$, $P = 0.0089$). On the other hand, there was a poor correlation in Der p 1-induced histamine release between the two parameters ($r_s = -0.373$, $P = 0.079$). The difference appears to be associated with the observation that different donors showed varying dose-response curves and that the concentration necessary for maximal histamine release upon Der p 1 challenge varied over a wide range, from

0.33 to 33.3 ng/ml, as compared with that in response to anti-IgE challenge, in which maximal histamine release was always achieved at 100 or 333 ng/ml anti-IgE. However, the precise mechanisms underlying these observations remain obscure.

In summary, immunologic release of histamine may be divided into two aspects: that regulated by an intrinsic property of the cells and that regulated by the concentration of allergen-specific IgE antibody on the cell surface.

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