

Regulatory role of prostaglandin E in allergic histamine release with observations on the responsiveness of basophil leukocytes and the effect of acetylsalicylic acid *

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This study examined the relationship between prostaglandin E (PGE) production and antigen-induced histamine release by leukocytes of asthmatic patients. The maximum amount of antigen-induced histamine release from leukocytes of different donors did not correlate with serum ragweed-specific IgE levels and correlated inversely with the basal PGE release from cultured leukocytes. The relationship between maximum histamine release and serum ragweed-specific IgE levels appeared to be influenced by the degree of PGE release. Patients with lower histamine release relative to ragweed-specific IgE levels had elevated PGE release. Based on these findings it can be hypothesized that spontaneously produced PGE might be a determinant of the responsiveness of basophil leukocytes to immunologic stimulation. This hypothesis was supported by demonstration of a positive correlation between the degree of inhibition of total basal PGE production in leukocyte suspensions during a short period of incubation and the degree of enhancement of antigen-induced histamine release by acetylsalicylic acid (ASA). The results indicate that, although the presence of IgE antibody in the blood may be the prerequisite for allergic histamine release from human leukocytes, endogenous PGE may be an important regulator of the release reaction. The results also suggest that idiosyncratic reactions to ASA and other nonsteroidal anti-inflammatory drugs observed in the allergic population may be at least partly due to impairment of the regulator function of endogenous PGE in the IgE-mediated histamine release reaction.

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When leukocytes of individuals with atopic disease are stimulated in vitro with the appropriate antigens, the cells frequently release significant amounts of histamine.¹ This antigen-induced release of histamine is caused by the interaction between the basophil leukocyte-bound immunoglobulin E (IgE) and the antigen molecules.²

The histamine release is strongly inhibited by exogenously added prostaglandin E compounds (PGEs)³ which are a group of naturally occurring acidic lipids possessing varied and potent biological activities.⁴ Since PGs are known to be produced by most mammalian tissues,⁴ PGE may be synthesized by the blood cells and influence the histamine release. The present study was conducted to study the possible role of endogenous PGE in the control of antigen-induced IgE-mediated release of histamine from human leukocytes.

TABLE I. Measurement of antigen-induced histamine release (HR), ragweed-specific serum IgE antibody levels, and baseline prostaglandin E (PGE) release by cultured leukocytes

Patient	HR (%) [*]	Serum IgE (% RAST) [†]	HR/RAST ratio [‡]	PGE release [§]
1	0	103	0	32
2	51	52	0.98	5.4
3	25	96	0.26	27
4	0	29	0	11
5	23	102	0.23	16
6	80	74	1.1	2.4
7	95	112	0.85	2.4
8	32	174	0.18	11
9	73	184	0.40	1.2
10	6	13	0.5	4.8
11	64	36	1.8	5.2
12	72	39	1.9	5.7
13	96	77	1.3	2.5
14	63	46	1.4	5.2
15	5	102	0.05	2.4
Mean	46	83	0.7	5.8¶

^{*}Maximal release by optimal antigen concentration, % of total cellular level.

[†]Ragweed antigen-specific IgE antibody levels as determined by the radioallergosorbent technique. Results expressed as % of a positive standard (100%).

[‡]Percent histamine release divided by % RAST levels.

[§]PGB₁ equivalent in culture supernatant, ng/5 × 10⁶ cells in RPMI 1641 medium with fetal calf serum/day.

||Arithmetic mean.

¶Mean from logarithmic values.

MATERIALS AND METHODS

Selection of patients

Leukocytes and sera were obtained from individuals who had asthma and demonstrable serum ragweed-specific IgE by the radioallergosorbent technique (RAST).⁵ These individuals were over 15 yr of age, of equal sex distribution, and did not have a history of aspirin idiosyncrasy. They were either asymptomatic or experiencing mild wheezing and/or rhinitis when venous blood was obtained for study. They had not taken any systemic medications for more than 48 hr or any anti-inflammatory drugs for at least 1 wk prior to blood sampling and were not receiving immunotherapy.

Acetylsalicylic acid (aspirin, ASA) solution

ASA was obtained from Sigma Chemical Company, St. Louis, Mo. (aspirin, 2-acetoxy benzoic acid, crystalline, Lot 60C-3610), and dissolved in Tyrode solution⁶ containing 0.25% gelatin (Atlantic Gelatin, United States Pharmacopeia 250 Bloom A, Lot 7303003 General Foods Corp., Woburn, Mass.) immediately prior to use (Tyrode-gelatin, pH 7.40 ± 0.05). The solutions used in this study, with 2.5, 25, and 250 µg of ASA/ml, had the same pH as those without ASA. ASA in concentrations of 3 mg/ml or

TABLE II. Relationship between histamine release (HR), specific serum IgE level (RAST), and baseline prostaglandin E (PGE) release by cultured leukocytes*

	HR (% of total cellular level)	% RAST	HR/RAST ratio
A. Spearman's rank order correlation test† (Rs, N = 15)			
RAST	+0.15	—	—
	NS‡		
HR/RAST ratio	+0.75	—0.61	—
	p < 0.01	p < 0.05	
PGE release	—0.58	—0.11	—0.17
	p < 0.05	NS	NS
B. Analysis of the data comparing patients with high and low PGE release (Student's t test)			
PGE release			
>5.8§ (n = 5)	16	101	0.13
<5.8 (n = 10)	61	74	1.0
P	<0.02	NS	<0.01

*The data in Table I were analyzed.

†Colton, T.: Statistics in medicine, Boston, 1974, Little, Brown & Co.

‡Statistically insignificant (p > 0.05).

§PGB₁ equivalent, ng/5 × 10⁶ cells/day, mean from log-converted values.

greater lowered the pH. Sodium chloride solution with the same osmolarity as 250 µg/ml of ASA was used as a control.

Leukocyte histamine release

Antigen-induced release of histamine was performed with the cells suspended either in tris buffer without human serum⁷ or in Tyrode-gelatin.⁶ Briefly, leukocytes were separated from heparinized blood by dextran sedimentation followed by differential centrifugation. The cells were washed, resuspended in the buffer, and, after preincubation for 25 min with and without ASA, stimulated with varying concentrations of ragweed pollen extract at 37° C for 45 min (4.0 × 10⁻¹ to 4.0 × 10⁻³ protein nitrogen units [PNU]/ml of dialyzed crude short ragweed pollen extract, 40,000 PNU/ml, Center Laboratory, Port Washington, N. Y.). The amount of histamine release was determined by spectrophotofluorometry and expressed as percentage of the total cellular level. The value of maximal release from individual leukocyte preparations was used for analysis. The variation of differences between duplicate histamine release experiments was less than 5% (90% confidence limit) of the greater value of a pair of samples.

Leukocyte preparation and PGE assay

For the study of PGE synthesis by leukocytes, aliquots were obtained from the leukocyte suspensions also used for the histamine release studies. The pooled supernatants of the leukocyte culture with 5 × 10⁶ cells/ml in RPMI 1640 medium with 10% fetal calf serum (Grand Island Biological

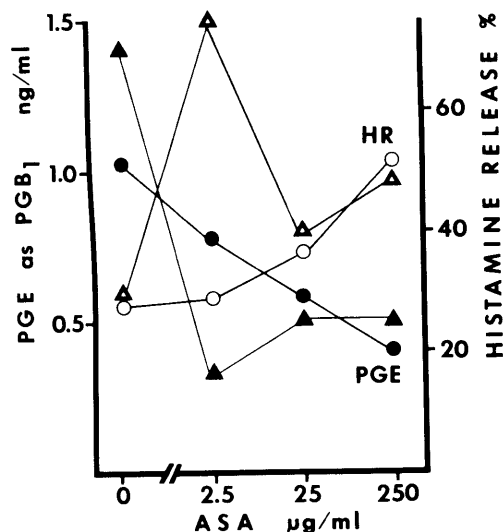


FIG. 1. The effects of acetylsalicylic acid (ASA) on PGE production and histamine release (HR) by peripheral leukocytes. Representative results: Patient 1 (circles) had a ragweed-specific serum IgE level (RAST) of 106%. Patient 2 (triangles) had a RAST of 117%. Total net baseline PGE synthesis (closed circles and triangles) was measured by the use of the whole leukocyte suspension in Tyrode-gelatin solution after 25 min of incubation at 37° C with and without ASA. Histamine release (open signs) was performed also after the same period of preincubation of identical aliquots of leukocytes in the same medium under the same conditions.

Co.) under 95% O₂-5% CO₂ were used for the assay of released PGE. The culture supernatant was removed each day and fresh medium added to the cells. The daily supernatants from each separate cell culture were individually pooled for 3 days for subsequent PGE assay. The culture medium contained 0.25 ng/ml of PGE present in the calf serum. This amount of PGE was subtracted from each of the observed PGE levels.

The effect of ASA on histamine release and on the total net basal PGE production was studied with the identical leukocyte suspensions prepared simultaneously from the leukocytes of the same patients. The total net production was measured with the use of the mixed leukocyte suspension in Tyrode-gelatin solution with and without ASA after a 25-min incubation at 37° C. Histamine release was also performed after the same period of preincubation in the identical medium.

Extraction and chromatographic separation of PGE was performed according to the procedure described by Zusman and associates.⁸ In brief, the samples and external standards (PGE₁*) were brought to pH 3.5 with 1N HCl. PGs were extracted with ethylacetate, which was then removed by evaporation. The dried extract was dissolved in benzene-ethylacetate-methanol (60:40:2) mixture and applied to a silicic acid column. The PGA fraction was first removed with benzene-ethylacetate (60:40), then PGE was eluted with benzene-ethylacetate-methanol. The recovery of tracer [³H] PGE₂ (prostaglandin E₂, 5,6,8,11,12,14,15-³H [n], 160,000 mCi/mmol, Amersham-Searle Co., Arlington

Heights, Ill.) was 50% to 80% throughout extraction and chromatography. PGE thus obtained was converted to PGB by alkaline dehydration,⁹ and the amount was measured by competitive radioimmunoassay with the use of anti-PGB₁ rabbit antibody (Clinical Assays, Inc., Cambridge, Mass.) and [³H] PGB₁ (Clinical Assays) as a competing ligand. The competitive binding curve of PGB₁* against [³H] PGB₁ was parallel to that obtained with PGB₂ (made by alkaline dehydration of PGE₂*), and the 50% binding inhibition dose of PGB₂ was approximately 6 times greater than that of PGB₁. The cross-reactivity of PGB₁ compared with the 50% binding inhibition dose of PGB₂ was less than 5%. The PGE levels were expressed in terms of PGB₁ equivalents. The variation of assay values in duplicate samples of PG synthesized by the whole cell suspension was 8 ± 6% (mean ± standard deviation).

Ragweed-specific IgE

Sera obtained simultaneously with the leukocytes were stored at -20° C. Ragweed-specific IgE antibodies were measured by the RAST method described by Ceska and co-workers.⁵ In brief, ragweed pollen antigen was chemically coupled to filter paper discs. The disc was then added to the sera containing IgE antibodies. The disc was washed, labeled anti-IgE antibodies were added, and the radioactivity on the disc was measured. The amount of IgE in the sample was compared with a standard curve obtained with various concentrations of pooled reaginic sera obtained from highly ragweed-sensitive patients. The specific IgE antibody level thus obtained was expressed as percentage of this standard. The average variation in duplicate assays was ±10%.

Histamine release/specific IgE (HR/RAST) ratio

Some results of the present study are expressed in terms of the HR/RAST ratio. This ratio describes the relationship between the maximal amount of immunologic histamine release (HR) and the amount of specific IgE antibody in patient's serum (RAST).

Increasing amounts of IgE in fluid phase (serum) results in the binding of greater numbers of the IgE molecules to basophil receptors according to the mass action law¹⁰ and is accompanied by generally greater amounts of maximal histamine release upon antigenic challenge of the basophil leukocytes.¹¹ Thus, higher specific serum IgE levels should be associated with proportionately higher histamine release, although there has been no experimental basis to assume that HR should be in exact linear proportion to RAST under a given condition. However, the results of previous^{12, 13} and present studies with a population of different patients do not always show this association, i.e., higher RAST values generally have not always been accompanied by proportionately higher HR values among different patients.

The HR/RAST ratio was obtained by dividing the amount of maximal histamine release as expressed in per-

*Gift of Dr. John E. Pike, The Upjohn Company, Kalamazoo, Mich.

centage of total leukocyte histamine by the serum specific IgE concentration as expressed in percentage of the concentration in the pooled reference sera.

RESULTS

The maximal amounts of histamine released from leukocytes on antigenic stimulation, the ragweed-specific serum IgE levels, HR/RAST ratio, and PGE levels in the supernatants of cultured leukocytes in 15 asthmatic patients are shown in Table I. Since the amounts of PGs spontaneously released from the blood cells of different individuals distribute more normally on logarithmic scale,¹⁴ the mean PGE release in different groups (5.8 ng PGB₁ equivalent/ 5×10^6 cells/day) was calculated from log-converted values. The relationship among the observed values was statistically analyzed and the results are summarized in Table II.

The maximal histamine release did not correlate with serum ragweed-specific IgE levels (RAST), but it did have a statistically significant inverse correlation with PGE release (Table II, A). The HR/RAST ratio correlated with the histamine release and had an inverse correlation with specific serum IgE antibody levels. For the total group, the PGE release did not show a significant relationship to HR/RAST on Spearman's rank order analysis. However, when the data were analyzed on the basis of high (above mean) and low (below mean) PGE release, there was an important observation (Table II, B). High PGE releasers were found to have significantly lower HR/RAST ratios and maximal histamine release than the low PGE releasers.

Generally, the addition of ASA significantly enhanced the histamine release and simultaneously inhibited total net basal PGE synthesis. As shown in Fig. 1, there was a dose-related inhibition by ASA of PGE production accompanied by a proportionate enhancement of the maximal amount of antigen-induced histamine release. As exemplified by the two representative cases shown in the figure, the dose-related patterns of the response in PGE synthesis and histamine release to the *in vitro* administration of ASA showed some variation among the cells from different patients. However, there always was an inverse relationship between the changes in PG synthesis and histamine release.

Table III shows the effects of 250 μ g/ml of ASA on basal net total PGE production and histamine release in the blood cells from 15 asthmatic patients. The addition of ASA caused a statistically significant decrease in PGE production from a mean of 0.8 ng PGB₁ equivalent without ASA to 0.5 ng with ASA. The same dose (250 μ g/ml) of ASA also caused a statistically significant increase in the maximal his-

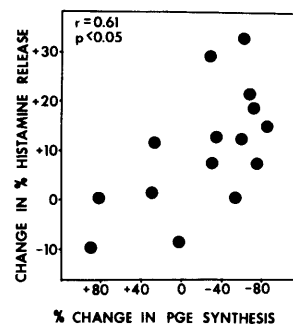


FIG. 2. The relationship between the change in PGE synthesis and the change in histamine release caused by the addition of 250 μ g/ml of ASA. This set of patients was different from that studied in Table I. The change in PGE synthesis as detected in the total cell suspension is shown in percent of baseline level in the absence of ASA in the medium. The change in histamine release is expressed in absolute difference in percent histamine release value because certain patients failed to release (0%) in the absence of ASA.

tamine release from a mean of 24% to 34%. In certain cases, 250 μ g/ml of ASA inhibited histamine release and also caused enhancement of PGE production (Table III). Although in these experiments the control PGE production and histamine release showed no correlation with each other on either nonparametric correlation analysis or on comparison of subgroups (Table IV), the changes in the basal total PGE production by the cells was inversely proportional to the changes in maximal histamine release upon the *in vitro* administration of ASA (Fig. 2).

DISCUSSION

The PGE assay measures both PGE₁ and PGE₂. In addition to PGB₁ converted from PGE₁ in the sample, the anti-PGB₁ antibodies used can bind PGB₂ which is formed from PGE₂ on dehydration. Thus, based on the cross-reactivity data described above, approximately 6 times as much PGE₂ as the PGE₁ levels measured could have been present in the specimen. Both PGE₁ and PGE₂ strongly inhibit the antigen-induced histamine release from human leukocytes *in vitro* and are equally effective in this function.³ Thus, the present PGE assay seems to be valid in studying the role of PGE in the histamine release reaction.

Although antigen-induced histamine release is believed to represent the release of intracellularly stored preformed histamine, the PG release is the result of synthesis by the cells.⁴ Although a PGE-metabolizing enzyme was not present in the medium, the released PG might re-enter the cells and be inactivated by any specific cellular enzymes. Thus, the PGE levels observed in the culture supernatants or total cell suspension probably reflect the net PGE production in the whole cell suspension.

TABLE III. Effect of acetylsalicylic acid (ASA) on baseline total prostaglandin E (PGE) production and antigen-induced histamine release by leukocytes in Tyrode-gelatin medium

Patient*	PGE production†			Histamine release (%)‡		
	Control§	ASA, 250 µg/ml	Difference	Control	ASA, 250 µg/ml	Difference
1	1.4	0.28	-1.1	67	82	+15
2	1.1	0.39	-0.7	0	22	+22
3	0.77	0.53	-0.24	29	42	+13
4	1.1	0.51	-0.6	0	13	+13
5	1.1	0.49	-0.6	67	100	+33
6	0.53	0.69	+0.16	1	3	+2
7	0.55	0.73	+0.18	66	78	+12
8	1.5	0.50	-1.0	32	51	+19
9	1.0	0.51	-0.5	2	3	+1
10	0.60	0.64	+0.04	8	0	-8
11	0.83	0.25	-0.58	3	11	+8
12	0.96	0.69	-0.27	34	42	+8
13	0.60	1.2	+0.6	9	0	-9
14	0.48	0.90	+0.42	13	14	+1
15	0.46	0.35	-0.11	22	52	+30
Mean	0.8¶	0.5¶	-0.3**	24	34	+11††

*The patient group is not the same as the group shown in Table I.

†PGB₁ equivalent ng/ml of whole leukocyte suspension in Tyrode-gelatin/0.4 hr.

‡Maximal release by optimal concentration of antigen, % of total cellular level.

§NaCl isoosmolar as 250 µg/ml of ASA.

||[ASA] minus [control].

¶Mean from log-converted values.

**p < 0.05, paired t test.

††p < 0.01.

The amounts of PGE detected in the present experiments, with a mean value of 0.81 ng PGB₁ equivalent/ml (2.4×10^{-9} M) would be expected to inhibit the histamine release even when administered exogenously. Significant inhibition (20% to 55%) of leukocyte histamine release has been reported at 3×10^{-9} M of PGE, the 50% inhibition dose ranging from less than 3×10^{-9} M.³

The specific cell source of the PGE measured in this study is not known. The eosinophil-derived inhibitor (EDI) has been identified as PGE₁ and shown to be released from human eosinophil leukocytes.¹⁵ Thrombocytes which are present in the white blood cell preparation may contribute to the observed PGE production since they normally release PGs during the clotting process.⁴ The polymorphonuclear granulocytes¹⁶ and lymphocytes¹⁷ may also produce significant amounts of PGs. Since no nucleated human or other mammalian cells have been reported to have failed to produce PGs, it seems very likely that the human basophils may also produce PGs. In support of this speculation is the finding that the peritoneal mast cells of rat produced a PG-like substance along with the slow-reacting substance of anaphylaxis and histamine on exposure to compound 48/80.¹⁸

Only a minute amount of PGE intracellularly appears necessary for inhibiting the histamine release reaction. An excess of PGE would overflow into and is measured in the cell medium. It is also important to note that interaction of PGE with basophils after antigenic stimulation is ineffective for inhibiting histamine release.¹⁹ Thus, any PGE released by the basophils during antigenic stimulation would have no role in regulating the ongoing histamine release reaction. Simultaneous measurements of PGE and histamine following antigenic stimulation therefore would not provide an accurate means of assessing the relationship of these two agents.

The present study revealed an inverse correlation between the PGE production by cultured leukocytes and maximal histamine release (Table II). We wanted to further explore this relationship. Although elevated serum-specific IgE is associated with leukocyte histamine release,²⁰ the quantitative correlation between the two parameters has not always been significant.^{12, 13, 21} It has been suggested that the responsiveness of sensitized leukocytes to antigens for histamine release may be dependent on other factors in addition to specific-serum IgE levels.²¹⁻²⁴ The following factors might affect the relationship between histamine release and specific IgE levels (HR/RAST ratio): (1)

TABLE IV. Relationship between histamine release and baseline prostaglandin E (PGE) production by leukocytes in Tyrode-gelatin medium*

Histamine release and PGE production (Spearman's correlation test, †Rs = +0.12, NS‡)	
PGE production (high vs low PGE producers)	Mean maximal histamine release (%)
>0.81§ (n = 8)	27
<0.81 (n = 7)	25
Difference	2% (NS, t test)

*The data in Table III were analyzed.

†Colton, T.: Statistics in medicine, Boston, 1974, Little, Brown & Co.

‡Statistically insignificant ($p > 0.05$).§PGB₁ equivalent ng/ml cell suspension/0.4 hr, mean from log-converted values.

There is nonlinearity of the relationship between HR and RAST, e.g., if HR which cannot be higher than the value of 100% should be compared with RAST which can be without a theoretic upper limit, patients with extremely high RAST would have a relatively low HR/RAST even if they had maximal (100%) HR. (2) Any physicochemical anomaly of the specific IgE molecule might cause a loss of biological activity in IgE-mediated reactions. (3) The affinity between specific IgE and antigen might be important in influencing the basophil-stimulating capacity of the antigen-antibody complex. (4) The affinity between specific IgE and basophil receptor might also be important.¹⁰ (5) IgE antibodies with other specificities might block basophil receptors.²⁵ (6) The biochemical conditions (reactivity or responsiveness) of basophil leukocytes may determine the amount of histamine released upon antigen-antibody interaction on their cell surface.^{21, 24}

In this study, histamine release and serum IgE were compared in relationship to PGE production which would influence the biochemical condition of the leukocyte. Patients with high PGE release were found to have significantly lower maximal histamine release than patients with low PGE release. Furthermore, these high PGE releasers have relatively low or no leukocyte histamine release in face of significant amounts of specific serum IgE (expressed as low HR/RAST ratio). Patients with relatively greater histamine release in relationship to serum IgE antibody levels (a higher HR/RAST ratio) had lower PGE release.

Based on the generally inverse relationship observed between PGE release and histamine release, we hypothesized that PGE synthesized by certain or all subgroups of the white blood cells may be an active regulator of the *in vitro* histamine release reaction in the human basophil leukocyte. This hypothesis was strongly supported by the result of further experiments with the use of ASA, a well-known PG syn-

thesis inhibitor²⁶ which significantly inhibited PGE synthesis also in the present experimental system in which the leukocytes of different asthmatic patients were studied. The inhibition of PGE synthesis was accompanied by a significant enhancement of histamine release under identical conditions in the same experiments. Furthermore, the degree of the PGE synthesis inhibition and the degree of the histamine release enhancement in different patients showed statistically significant positive correlation.

Asthmatic patients sensitive to ASA are also known to be sensitive to other nonsteroidal anti-inflammatory agents.²⁷ ASA idiosyncrasy of this type has been speculated to be due to the inhibition of PG synthesis by ingested ASA.²⁸ Reduced PGE production may adversely affect lung function if PGE, a bronchodilator,⁴ normally acts on bronchial smooth muscles to control the muscle tone.²⁸ However, it also seems reasonable to assume that the ASA-induced reduction of PGE synthesis and consequent loss of PGE inhibition of mediator release reaction may be at least partly responsible for idiosyncratic reactions to ASA.

Although the basal PGE release by the cultured leukocytes had significant inverse correlation with histamine release, the basal total PGE production during 25-min incubation did not show significant relation to control histamine release in the above-mentioned experiments designed to demonstrate the effect of ASA. The leukocyte culture, with fresh medium replacing the old every 24 hr, may have minimized any effects of medications which some of the patients had been taking orally until 48 hr prior to the start of the experiments. The incubation for 25 min in Tyrode-gelatin may not have allowed the leukocytes either to recover from any drug effects or to metabolically equilibrate themselves in the new medium. Moreover, it should be noted that even though this particular correlation between the two parameters (histamine release and basal PGE production

during 25-min incubation in Tyrode-gelatin medium) failed to be significant, studies with ASA in this experiment supported findings of the other experiments.

The degree of correlation between PGE synthesis and histamine release generally may be influenced not only by technical variation of measurements but also other factors such as beta adrenergic and membrane adenosine-5'-triphosphatase activities,^{29, 30} which might also regulate the mediator release reaction. These possibilities are currently under investigation in our laboratories.

Authors' note added in proof

A recent paper (Conroy et al.: *J. IMMUNOL.* **118**:1317, 1977) showed that leukocytes from donors having approximately the same cell-bound IgE concentration varied widely in their sensitivity to anti-IgE for histamine release. The importance of the responsiveness of the cells to antigen stimulation thus seems further supported.

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