

DISSOCIATION OF IgE FROM RECEPTORS ON HUMAN BASOPHILS

I. Enhanced Passive Sensitization for Histamine Release¹

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Leukocytes of only one of 11 nonatopic donors could be passively sensitized for histamine release elicited by ragweed extract. A short incubation in an unbuffered isotonic saline at pH 3.9 or in an 0.01 M lactic acid/lactate-buffered isotonic saline at pH 3.9 dissociated from 4×10^5 to less than 3×10^4 IgE molecules per basophil from washed leukocytes of several in a series of six atopic and 11 nonatopic donors. After such treatment, leukocytes of only one of the 11 nonatopic donors could not be sensitized for histamine release. Basophils of the four ragweed-sensitive donors lost their sensitivity to ragweed after the treatment, but all could be passively resensitized; for three of these donors the level of release approximated their original reactivity. Leukocytes of the two mold-sensitive donors could be passively sensitized to ragweed allergens after but not before treatment.

Four plasma samples from histamine release-positive volunteers were used for sensitization of treated leukocytes of each cell donor; three were consistently effective and one was consistently ineffective. The positive plasmas had concentrations of antigen E-specific IgE of over 100 ng/ml, which accounted for 17 to 23% of the total IgE; the inactive one had less than 5 ng/ml of specific IgE. For each cell donor, all three samples of active plasma mediated quite similar histamine release, but there was a spectrum of donor cell reactivity ranging from 23 to 70% release. These results suggest that basophils from each donor, atopic or nonatopic, had a maximal potential for *in vitro* sensitization, which was only attained if the plasma contained appropriate, but yet to be fully defined, concentrations of specific and total IgE.

Several unexpected results were obtained. Treated leukocytes from some individuals were sensitized for mediator release to a greater extent by sixfold diluted than undiluted plasma. In addition, a 4-hr incubation with plasma at 37°C, but not at 25°C or 0°C, was less effective than were shorter incubation periods.

Treated leukocytes should be useful in studying kinetic and equilibrium parameters of IgE binding to specific receptors on human basophils. Analogous treatments should also be useful in sensitization and measurement of IgE-receptor interactions of mast cell populations.

Anaphylaxis and allergy are generally elicited by the bridging of IgE molecules that are bound to specific receptors on mast cells and basophils. The bonding between receptor and IgE on the basophil and mast cell is of high affinity and is not readily broken by washing under physiologic conditions, in contrast to the bonding of IgE to lymphocytes and monocytes. Washed human leukocytes therefore contain basophils with receptors that are most often occupied by endogenous IgE. Kinetic studies of IgE binding done with such cells, especially initial binding measurements, will be mostly representative of the unoccupied receptors. Some of these receptors may be unoccupied because they have lower average affinity for IgE than the occupied receptors. With cultured cells, such as those of the rat basophil leukemia series, all the receptors are unoccupied and reliable data have been obtained (1). The occupancy of IgE receptors on human basophils is illustrated by the low success rate in passive sensitization for IgE-mediated histamine release (2). The removal of IgE from its receptors would provide a better starting point for the determination of various parameters of passive sensitization. Acid treatment of basophils reversibly dissociated essentially all of the IgE detectable by autoradiography, but the resultant cells were reported to be incontinent of their histamine (3). A similar procedure was developed to enumerate basophil receptors by measurement of the IgE that could be eluted, apparently without investigation of the functional capacity of the treated basophils (4). A more recent study of IgE uptake by acid-treated human basophils has been made (5). Equilibrium binding of IgE was measured, but no determination was made of the biologic activity of the basophils.

It was assumed that in previous attempts to dissociate IgE reversibly from human basophils, conditions incompatible with cellular integrity were used. This possibility has now been studied and was found to be correct. Various aspects of disruption of the IgE-receptor bond and of passive sensitization of the treated cells have been investigated.

MATERIALS AND METHODS

Buffers. A solution consisting of 0.12 M NaCl, 0.005 M KCl, 0.025 M tris hydroxymethylaminomethane (tris)³ was adjusted to pH 7.6 and was used as a suspension medium for cells. For histamine release, Ca⁺⁺ and Mg⁺⁺ were added at final concentrations of 0.6 and 1.0 mM, respectively. To dissociate basophil-bound IgE, a solution of 0.14 M NaCl and 0.005 M KCl was adjusted to pH 3.5 (3.5S). Alternatively, a solution of 0.13 M NaCl, 0.005 M KCl, and 0.01 M lactic acid was adjusted with 1 N NaOH to pH 3.9 (LS). For comparison a solution of 0.05 M sodium acetate, 0.085 M NaCl, 0.01 M EDTA, and 0.03% human serum albumin was adjusted to pH 3.7 at 0°C with HCl (4). In addition, a solution of the same composition was adjusted to pH 4.0 (5).

Leukocytes. Thirty milliliters of blood were obtained with informed consent from volunteers who were nonatopic or who were clinically sensitive to ragweed or mold allergens. The blood was collected in EDTA such that the

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³ Abbreviations used: tris, tris hydroxymethylaminomethane; 3.5S, unbuffered isotonic NaCl, KCl, pH 3.5; LS, 0.01 M lactate-buffered NaCl, KCl, pH 3.9.

resultant plasma was approximately 5 mM. Dextran (m.w. = 170,000) was added to a final concentration of 1% and the mixture was allowed to sediment for 45 to 60 min. The supernatant was centrifuged and the leukocytes were washed three times. Five to 12 aliquots were prepared as required for individual experiments.

Plasma. The plasma was retained from blood processed as described above. Some of the plasma used had been obtained before these experiments and had been stored at -20°C . Plasma A, B, C, and D contained antigen E-specific and total IgE, respectively, as follows: A: 175 and 930 ng/ml; B: 109 and 638 ng/ml; C: 221 and 941 ng/ml; D: less than 5 and 236 ng/ml. Donors A, B, C, and D each demonstrated specific histamine release from their washed leukocytes of 50% or more.

IgE determination. Plasma IgE and IgE eluted from basophils were assayed by the double antibody procedure of Gleich *et al.* (6). Specific IgE antibody to ragweed antigen E was determined by the polystyrene tube method of Zeiss *et al.* (7).

Basophil counts. Basophils were enumerated by the procedure of Gilbert and Ornstein (8).

Number of IgE molecules per basophil. Leukocytes that had been obtained from 30 ml blood were washed three times and resuspended in 5.2 ml buffered saline. One-milliliter aliquots were incubated under varying conditions and the supernatants were neutralized and assayed for IgE. An aliquot of the original suspension was counted for basophils and the number present in each eluted sample was determined. From the IgE eluted (expressed as molecules of IgE) and the number of basophils present, the mean number of IgE molecules dissociated per basophil was obtained.

Histamine release. Aliquots of leukocytes were incubated for 30 to 45 min at 37°C with concentrations of allergens that had been previously determined to produce maximal histamine release. After centrifugation, histamine in the supernatants and the cellular sediments was determined by the fluorometric procedure of Shore *et al.* (9) with 2 M citric acid used to stop fluorophore formation (10). The percentage of cellular histamine released was calculated from the relative fluorescent units of the supernatant in relation to the total of supernatant and cellular units.

Passive sensitization. Washed untreated or treated cells were incubated for 2 hr at 37°C with 10% plasma, except when otherwise indicated. The leukocytes were washed three times, resuspended, and incubated with the antigen relevant to the plasma used. Controls of irrelevant antigens or plasma were used. Histamine release was determined as described.

RESULTS

Preliminary experiments. The premise was that conditions used earlier to remove IgE from its receptors on the basophil surface impaired the functional integrity of the cell. The first possibility considered was the 0.05 M acetate buffer. Accordingly, an unbuffered solution of 0.14 M NaCl and 0.005 M KCl was adjusted to pH 3.5 with HCl (3.5S). A sample of washed human leukocytes suspended in this solution was found to have a pH of 3.9, which was maintained over a 10-min period. Leukocytes from a mold-sensitive and from a ragweed-sensitive donor, both exhibiting specific release of histamine of 24 and 51%, respectively, were incubated in 3.5S for periods of from 1.5 to 10 min at 24°C . Aliquots were rapidly neutralized with aqueous tris, centrifuged, washed, and resuspended in Ca^{++} , Mg^{++} buffer with or without specific antigen for 35 min at 37°C . Only 7 to 11% of the histamine was released in any of the aliquots, indicating that specific histamine release had been virtually abolished and that spontaneous release was acceptably low. Treated cells from the ragweed-sensitive donor, which were apparently desensitized, were resensitized by incubation for 1 hr at 24°C with 10% plasma from sensitive donors. After being washed three times, the cells were challenged with ragweed extract. These cells released 36% of their histamine; spontaneous release was 9%. These results indicated that not only did 3.5S remove significant quantities of receptor-bound IgE, but that a significant number of receptors were intact and could be resensitized for histamine release.

Passive sensitization of basophils from nonatopic donors. Washed leukocytes, without 3.5S treatment, obtained from 11 nonallergic donors were examined to determine whether they were in fact nonsensitive to ragweed antigen and whether they could be sensitized by incubation for 2 hr at 37°C with plasma

from one to three highly sensitive donors whose own leukocytes released greater than 50% of their histamine when challenged with ragweed extract (Table I, columns 2 and 3). None of the donors' leukocytes were appreciably sensitive to ragweed and the cells of only one of the 11 donors could be passively sensitized before the removal of receptor-bound IgE. The remaining leukocytes were treated with 10 ml 3.5S for 2 min at 8°C (the temperature maintained in the laboratory by aliquots of cells suspended in 3.5S that had been refrigerated). The cells were neutralized with 12% tris and washed. Separate samples were incubated for 2 hr at 37°C with a 1/10 dilution of plasma A, B, C, and D, or were left at 25°C for 2 hr. The samples were then washed three times and incubated with or without antigen for 35 min at 37°C . The percentage of cellular histamine released into the supernatant was determined and is recorded in Table I (columns 4–8). After 3.5S treatment, leukocytes of 10 of the 11 donors could be sensitized by plasma A, B, and C. Plasma D (results not shown) obtained from a ragweed-sensitive donor exhibiting 50% leukocyte histamine release failed in eight of eight trials. Controls generally showed reasonably low histamine release when incubated with or without ragweed extract.

Passive sensitization of basophils from atopic donors. A similar experiment was performed with cells of six atopic donors, four of whom were ragweed sensitive and two of whom were mold sensitive (Table II, donors 15 and 16). Attempts to sensitize untreated leukocytes passively did not appear to alter greatly the previous sensitization to ragweed or to sensitize leukocytes of the mold-sensitive donors to ragweed. Basophils of donor 12 did have receptor sites available for further passive sensitization. With 3.5S treatment of the leukocytes, any prior sensitivity to ragweed was lost, which could then be initiated (mold-sensitive donors) or restored (ragweed-sensitive donors) by incubation for 2 hr at 37°C with a 1/10 dilution of plasma A, B, or C (columns 5–7), but not at all by plasma D (not shown). The combined use

TABLE I
Percent histamine release from passively sensitized basophils of nonatopic donors

Cell Donor	Untreated Cells		Acid-treated Cells				
	Unsensitized	Sensitized	Unsensitized ^a	Sensitized ^b			Spontaneous
				A	B	C	
1	5	8	3	42	32	46	0
2	12	36	17	nd ^c	nd	55	11
3	4	15, 8, 7	8	57	44	57	7
4	5	4, 4, 4	4	49	58	48	4
5	4	7, 8, 8	10	13	13	13	6
6	4	8	7	18	23	17	5
7	5	9	4	15	23	16	2
8	6	8, 6	9	27	31	25	4
9	5	5	6	nd	23	nd	nd
10	9	10	11	nd	39	nd	10
11	13	18	15	nd	70	nd	nd

^a Unsensitized acid-treated cells incubated with ragweed.

^b Aliquots were sensitized with plasma of the same three donors A, B, or C.

^c nd = not done.

TABLE II
Percent histamine release from passively sensitized basophils of atopic donors

Cell Donor	Untreated Cells		Acid-treated Cells				
	Unsensitized	Sensitized	Unsensitized ^a	Sensitized ^b			Spontaneous
				A	B	C	
12	29	51	21	63	58	60	18
13	52	52, 46, 53	4	54	42	55	2
14	72	73, 67, 68	7	65	52	62	4
15	4	5	8	30	38	34	3
16	4	4	10	31	27	36	0
17	36	28	6	37	39	41	1

^a Unsensitized acid-treated cells incubated with ragweed.

^b Aliquots were sensitized with plasma of donors A, B, or C.

of 4 mM EDTA and 10 μ g/ml heparin has been reported to enhance but not to induce passive sensitization of leukocytes (11). In a few trials, no enhancement was observed in the passive sensitization of 3.5S- or LS-treated basophils.

Conditions for dissociation of IgE from basophil receptors. Because unbuffered 3.5S appeared to have a pH of 3.9 in the presence of cells and because it is difficult to measure and maintain the pH of unbuffered solutions, a pH 3.9 lactate-buffered salts solution (LS) was tested. In a few preliminary trials, there was close correspondence between results of passive sensitization after treatment with either 3.5S or LS.

To characterize the pH dependence of IgE dissociation, washed leukocytes were treated for 2 min in an ice bath with LS that had been adjusted to pH of 3.7 to 4.5. For comparison, a 0.05 M acetate (pH 3.7)-containing NaCl solution (4) and a 0.05 acetate (pH 4.0)-containing NaCl solution (5) were used. The washed cells were then sensitized for 2 hr at 37°C with 10% plasma from one highly sensitive donor. After washing three times and challenging with allergen, histamine release was determined (Table III). With the use of the 0.01 M lactate-buffered salts, a relatively broad range of pH from 3.7 to 4.2 seemed optimal. Neither 0.05 M acetate solution was effective under the stated conditions. With either solution, it was noted that residual erythrocytes in the leukocyte preparations were lysed during the treatment or during washes. Basophils were not lysed, however; total histamine was similar to that of lactate-treated cells. No hemolysis was seen with any of the lactate solutions.

The results of exposure of leukocytes to acidified saline, for two periods of time at two temperatures, on subsequent passive sensitization are shown in Table IV. The lactate buffer was equally effective in 2- or 10-min treatments at either 0°C or 25°C. At 37°C, however, even a 1-min exposure was deleterious to the cells (not shown). Treatment with acetate-buffered saline resulted in impaired passive transfer.

The comparative effects of time of exposure and of buffer

TABLE III
Effect of pH of pretreatment on passive sensitization of basophils

Buffer	pH	Molarity	Treatment	Histamine Release (%)
Lactate	3.7	0.01 ^a	2 min, 0°C	72
	3.8			75
	3.9			79
	4.0			79
	4.1			80
	4.2			77
	4.3			41
	4.5			24
Acetate	3.7	0.05 ^b	10 min, 0°C	17
Acetate	4.0	0.05 ^c	20 min, 25°C	11

^a Total concentration of lactate and lactic acid; at pH 3.9 both were about 0.005 M.

^b Total concentration of acetate and acetic acid; approximately 92% acetic acid; prepared as in Reference 4.

^c Total concentration of acetate and acetic acid; approximately 85% acetic acid; prepared as in Reference 5.

TABLE IV
Effect of time and temperature of pretreatment on passive sensitization of basophils

Buffer	Time (min)	Temperature (°C)	Histamine Release (%)
Lactate ^a	2	0	65
	2	25	63
	10	0	66
	10	25	60
Acetate ^b	2	0	23
	10	0	21
	10	25	23

^a pH 3.9; 0.01 M; see footnotes Table III.

^b pH 3.7, 0.05 M.

composition on the extent of dissociation of IgE from basophil receptors was assessed (Table V). In two typical experiments, LS- and pH 3.7 acetate-buffered saline were compared with the use of 2- to 10-min exposures. In none of these nor in nine other experiments did 0.05 M acetate-buffered saline result in functional cells in histamine release. The pH 4.0 acetate was also tested after two separate 10-min incubations rather than after a single 20-min incubation and similar results were achieved. Despite the great differences in histamine release, similar amounts of IgE were eluted. Although in the 11 trials lactated saline was usually superior to pH 3.7 acetate-saline in dissociation of IgE, the acetate solution was occasionally somewhat better. The maximal yield of IgE per basophil was 4×10^5 in one donor and was below 3×10^4 at the other extreme.

A second treatment with 0.05 M acetate-saline, pH 3.7, was reported to dissociate no additional IgE (4). This has also been found to be true for LS in this investigation. Although such results were considered to indicate complete removal of IgE (4), it is quite possible that IgE-receptor bonds of higher affinity were not broken. Preliminary evidence for this possibility has been obtained, but it has not been studied sufficiently to report.

As a consequence of these results in the dissociation of IgE, a standard procedure for further study was adopted. A 5-min exposure to LS at 0°C seemed to be near the middle of the optimal pH and time range while reducing the chance of harmful metabolic changes.

Passive sensitization of human basophils. Having established standard conditions for elution of basophil-bound IgE, a series of experiments were performed to optimize resensitization of LS-treated cells. In previous experiments, a 1/10 dilution of human plasma was used as had been done by other investigators (2). Because it seemed possible that less potent plasma might benefit from increasing the concentration, several preliminary trials were made. It was found that undiluted plasma caused no appreciable nonspecific release of histamine after 2 hr at 37°C and therefore more concentrated plasma could be tried. Undiluted plasma D, referred to above as being ineffective at 1/10 dilution, was able to sensitize cells of two donors to a significant but modest degree (36 and 29% histamine release) after but not before acid treatment, but was only marginally active with leukocytes of another donor.

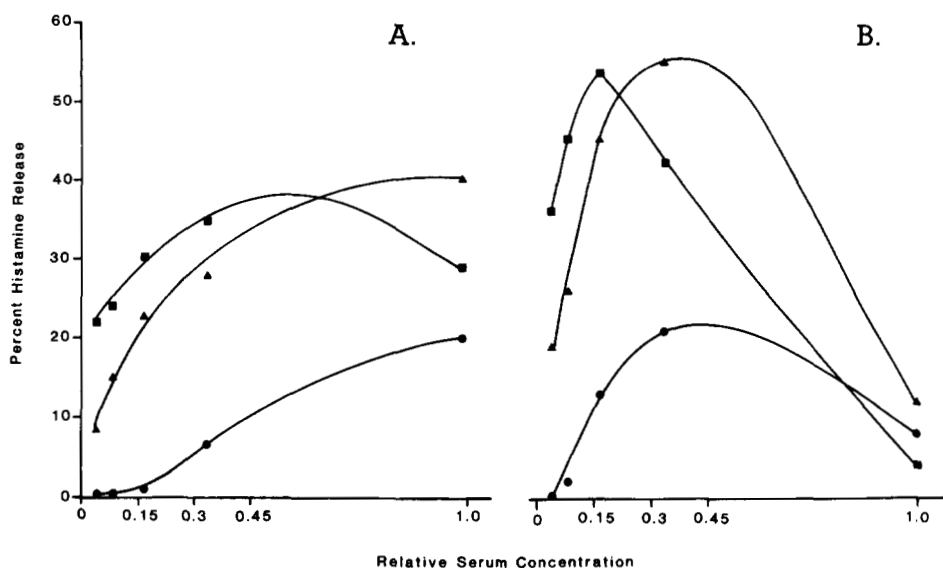
Expected plasma dose-response curves were seen in passive sensitization with diluted plasma. With undiluted plasma, inhibition was observed with basophils from one individual with the use of almost any plasma and in several individuals only with particular plasmas. Figure 1 illustrates the differential dose response of LS-treated leukocytes from two donors using the same three plasma samples for passive sensitization. With leukocytes from donor A, only the most potent plasma was somewhat inhibitory when used undiluted. In contrast, all three undiluted plasma samples were highly inhibitory for donor B cells, as

TABLE V
Effect of time and temperature of pretreatment of basophils on the dissociation of IgE and histamine release after passive sensitization

Buffer	Time (min)	Temperature (°C)	Histamine Release (%)	IgE Molecules per Basophil
Lactate ^a	5	0	78	1.9×10^5
	10	0	81	1.7×10^5
Acetate ^b	5	0	17	1.8×10^5
	10	0	16	1.3×10^5
Acetate ^c	20	25	16	1.3×10^5
Lactate ^a	2	0	41	9.3×10^4
	10	0	56	7.0×10^4
Acetate ^b	2	0	19	1.1×10^5
	10	0	22	7.0×10^4

^{a-c} See footnotes a-c in Table III.

Figure 1. Dose-response curves for the re-sensitization of LS-treated leukocytes from two donors (A and B) using the same three plasma samples undiluted and in dilutions of 1/3, 1/6, and 1/24. Plasma 1 (●), plasma 2 (■), plasma 3 (▲) were incubated with treated leukocytes for 1.5 hr at 37°C, washed three times, and incubated for 35 min at 37°C with the predetermined optimal dose of specific allergen.



were 11 of 12 other samples. For most donors, only certain plasma and cell combinations exhibited high plasma dose inhibition, but did so repeatedly. It is therefore necessary to determine the optimal plasma concentration for each cell donor.

In all previous experiments, a standard sensitization time of 2 hr at 37°C was employed (2). Other modalities were studied to guide further development of this procedure. In Table VI, the results of several times and temperatures of sensitization of LS-treated leukocytes by 16% plasma of a very sensitive donor are shown. In this instance, optimal sensitization only occurred after 4 hr at 0°C or 24°C. At 37°C sensitization appeared to be complete by 1 hr and declined markedly thereafter. In two other experiments, the same general pattern was observed, indicating a slower rate of sensitization at 0°C and 24°C compared with 37°C, but a significantly reduced sensitization in 4 hr at 37°C. In these instances, incubation for either 1 or 2 hr at 37°C was optimal.

The quantity of IgE taken up by acid-treated basophils during passive sensitization for 2 hr at 37°C with a potent undiluted plasma was determined. First the quantity of IgE eluted from a sample of leukocytes by LS was determined. In several control experiments, no further IgE was eluted by a second incubation with LS. The treated cells were then incubated with the undiluted plasma and washed three times, and a second LS elution was performed. The initial dissociation revealed that, on the average, there were 2.8×10^5 IgE molecules per basophil before acid treatment. The second elution showed that 1.1×10^5 IgE molecules had reassociated with each basophil after acid treatment and incubation with plasma. In other experiments, resensitization approached 100% of the initial basophil-bound IgE.

TABLE VI

Effects of time and temperature on passive sensitization of LS-treated leukocytes

Sensitization ^a		Histamine Release (% ± SEM)
Temperature (°C)	Time (Hr)	
0	1	44 ± 2.1
0	2	43.5 ± 0.5
0	4	62.5 ± 2.5
24	1	45.5 ± 1.5
24	2	48 ± 1.0
24	4	58.5 ± 2.5
37	1	56.5 ± 2.5
37	2	41.5 ± 0.5
37	4	15.7 ± 1.5

^a Each sample of cells was sensitized with 16% plasma from a single donor.

DISCUSSION

The observation of Levy and Osler (2) that passive sensitization for histamine release is only possible with leukocytes from a small percentage of donors has been confirmed in these and in previous experiments in this laboratory. The most probable reasons for the usual failure of passive transfer of sensitivity are the occupancy of most receptors by endogenous IgE and a slow rate of dissociation of the occupant molecules. In the vicinity of pH 4, a large number of IgE molecules could be dissociated from well-washed leukocytes by several buffered or unbuffered isotonic salt solutions. The quantity of IgE recovered was quite similar in most cases. Included in these solutions is 0.05 M acetate-buffered saline, pH 3.7, which is the standard used to elute and quantify the IgE from washed human leukocytes that appears to be derived mostly if not entirely from the basophils (4). In our experiments, two buffered salines containing 0.05 M acetic acid/acetate were effective in the dissociation of IgE, but cells obtained after treatment with either could not be passively sensitized for histamine release. Because IgE has been shown to reassociate with acid-treated cells (3, 5), it is likely that the treatment adversely affected the cells rather than their subsequent binding of IgE. Even after only 2 min of exposure to the pH 3.7 acetate buffer at 0°C, which effectively removed cellular IgE, the cells could no longer be passively sensitized. It has not been determined whether acetic acid/acetate was specifically deleterious to basophils or whether the concentration was excessive. What is clear is that merely short exposure to acid pH is not demonstrably harmful to the cells, because unbuffered 3.5S or LS did not impair subsequent sensitization.

Removal of IgE from its receptors consistent with functional integrity of the basophils has been accomplished with the blood of both atopic and nonatopic donors. Before treatment, the cells of only one of 11 nonatopic donors could be passively sensitized; after treatment, cells of 10 of the 11 responded. Of the six atopic donors, leukocytes of one donor were appreciably sensitized before acid exposure, whereas the sensitivities of the rest were not significantly changed. Acid treatment of leukocytes abrogated any prior sensitivity to ragweed and presumably other allergens (cells of one donor were somewhat unstable), but rendered them responsive for passive sensitization of ragweed and presumably other allergens as well. In subsequent experiments, sensitization to other allergens has been readily accomplished after acid treatment under the same conditions.

It appears that cells from different donors have different potentials for sensitization after acid treatment. Cells of each donor were sensitized to a very similar extent by any of the three potent plasma samples, but exhibited differential activity from donor to donor, which was maintained over a 12-mo period. In addition, maximal *in vivo* and *in vitro* leukocyte sensitivities were quite similar for each of three of the ragweed-sensitive donors. The data taken together suggest that cells from each individual have a predetermined potential for sensitization. If the plasma or serum has an appropriate concentration of IgE antibody in relation to total IgE and the time and other conditions of sensitization are optimal, that potential will be reached. Because histamine release by individual cells appears to be an all or none response (12), the existence of leukocytes with low potential implies that a large proportion of the basophils are incapable of sensitization, of histamine release, or of both. Inadequate sensitization, if it occurs, could be due to insufficient concentration, to improper configuration of IgE, or to low affinity of IgE receptors for IgE. Deficits in metabolic or other functional capacity might also explain the inactivity of individual cells; however, the actual reasons for the wide variation in cellular potential of individual donors are not understood.

The parameters of sensitization of treated leukocytes have also been examined. With very receptive leukocytes and potent plasma, maximal sensitization was seen by 1 hr at 37°C and by 2 to 4 hr at 0°C and 24°C. Incubation of the cells for 4 hr at 37°C with this plasma unexpectedly produced a reduction in the degree of sensitization.

Somewhat similar, anomalous results were obtained when the highly receptive leukocytes from the same donor (donor B, Fig. 1B) were shown to be less reactive after incubation with three different undiluted plasma samples compared with a three- to sixfold dilution of the same plasma. Because the same plasma samples were at least as effective undiluted as diluted with the cells of other donors, it is unlikely that an inhibitor of sensitization or of relevant cellular function is present in the plasma. Further analysis of the kinetics of binding of IgE in parallel with passive sensitization and histamine release is needed to delineate the factors that led to such unexpected results.

Another seemingly incongruous observation was that leukocytes from the donor of plasma D (patient 13, Table II) released 52% of their histamine before sensitization, but plasma D was unable to passively sensitize leukocytes of donor D or of any other donor *in vitro*. This means that plasma D under *in vivo* conditions was capable of sensitizing donor D leukocytes. This plasma had low total IgE and specific IgE to ragweed antigen E below 5 ng/ml. It is more surprising then that the donor's leukocytes were sensitized *in vivo* than that the plasma would not passively transfer sensitivity under *in vitro* conditions to autologous or homologous basophils. Here again, further analysis of the relationship of plasma-specific and total IgE and the kinetic and equilibrium parameters of their binding to basophil

receptors is necessary to understand the natural history of allergic sensitization.

The ability to dissociate IgE from receptors on human basophils with retention of cellular reactivity suggests several further applications. Human lung mast cells have recently been isolated, purified, and then sensitized (13, 14). A mild acid treatment might well prove useful in improving the process of sensitization. In addition, more meaningful kinetic and equilibrium data should be obtainable from basophils with many open receptors than from those obtained before treatment or those obtained after treatment with impairment of their functional integrity. There is also the possibility of a separate examination of cellular and plasma components in clinical allergic disease states. Comparative cellular potential to accept sensitization by standard sera and then to release mediators may be studied in parallel with the ability of the plasma to sensitize standard cell preparations for mediator release. Thus, the dissociation of IgE from human basophils could lead to a better understanding of certain aspects of IgE-mediated processes.

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