Immunology 1988 **65** 443–447

Desensitization of human basophils with suboptimal concentrations of agonist. Evidence for reversible and irreversible desensitization

J. J. PRUZANSKY*† & R. PATTERSON*

*Section of Allergy-Immunology, Department of Medicine and the †Department of Microbiology-Immunology, Northwestern University Medical School, Chicago, Illinois, U.S.A.

Accepted for publication 13 June 1988

SUMMARY

Leucocytes from allergic donors were preincubated with suboptimal concentrations of ragweed or anti-IgE and then challenged with increasing concentrations of the homologous or heterologous agonist. The initial incubation resulted in desensitization, as judged by a reduced reactivity relative to controls preincubated without agonist but challenged similarly. Both homologous and heterologous desensitization were observed and were dose dependent. Evidence was obtained for both a reversible and irreversible component of desensitization, which was also agonist-concentration related. Reversibility occurred to a similar degree either by incubation of suboptimally desensitized cells with optimal concentrations of agonist or by removal of IgE and resensitization. This could implicate IgE–agonist aggregation on the basophil surface as a mechanism of desensitization. Histamine release from desensitized cells was highly correlated with degranulation, suggesting that individual cells were desensitized in an all-or-none manner.

INTRODUCTION

Desensitization of human basophil leucocytes to subsequent challenge by antigen or anti-IgE was first described by Lichtenstein (1971). It occurred if the cells were preincubated for more than 10 min with optimal concentrations of agonist in the absence of Ca^{2+} and Mg^{2+}, which are required for histamine release. May, Schumacher & Williams (1972) observed that immunotherapy of patients with a single clinically relevant allergen but whose leucocytes also demonstrated sensitivity to a different allergen resulted in significantly reduced basophil reactivity to both. On the basis of these results and *in vitro* experiments they concluded that the patients' leucocytes had been desensitized by exposure to subthreshold doses of antigen during therapy. Mendoza & Minagawa (1982) studied the kinetics of desensitization of human leucocytes for histamine release at suboptimal concentrations of anti-IgE or antigen *in vitro*. In this study desensitization with suboptimal concentrations of ragweed or anti-IgE was quantified relative to the activity of the agonist with undesensitized leucocytes. Thus both the desensitizing and challenge concentrations were expressed in terms of their potential activity rather than by weight/volume.

Abbreviations: a-IgE, rabbit anti-human IgE; LS, isotonic saline containing 0·01 M lactic acid, pH 3·9, which was used to remove receptor bound IgE; RW, extract of ragweed pollen; TCM, TS containing Ca^{2+} 3 mM and Mg^{2+} 5 mM; TS, Tris-buffered physiological NaCl, KCl, pH 7·4 at 37°.

Correspondence: Dr J. J. Pruzansky, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611, U.S.A.

This allowed ready comparison of the response to challenge of desensitized cells and the response that would have occurred had they not been desensitized.

MATERIALS AND METHODS

Human leucocytes

Thirty millilitres of blood were obtained from volunteers with informed consent, mixed with 4 ml 5% isotonic dextran and 0·4 ml 10% EDTA and the leucocyte-containing supernatant aspirated. After centrifugation the cells were washed twice in TS. Approximately 50 aliquots were obtained in each experiment so that each aliquot contained the leucocytes derived from about 0·6 ml of blood. Since we have recovered a total of 5×10^5 basophils from most donors there were generally approximately 10^4 basophils per sample, although this could vary among donors.

Desensitization

Leucocytes were incubated with either RW or a-IgE for 45 min at 37° in either TS or TCM. Supernatants were removed and the cells challenged by resuspension in TCM containing RW or a-IgE. After incubation for 45 min at 37° followed by centrifugation, histamine was determined in both the supernatants and the sedimented cells using the automated fluorometric procedure of Siraganian (1974).

Agonists

RW extract and a-IgE were diluted serially three- or 10-fold. The highest concentration of RW extract used was 1:2800,

Table 1. Homologous desensitization of human leucocytes with increasing concentrations of ragweed extract or of anti-IgE

	Challenge			Challenge	
Desensitization ragweed % max*	Ragweed % max	Histamine release % max	Desensitization anti-IgE % max	Anti-IgE % max	Histamine release % max
16	16	5	8	8	4
	34	11		33	17
	64	35		71	33
	91	76		96	58
	100	90		100	75
34	34	11	33	33	13
	64	18		71	21
	91	52		96	29
	100	70		100	44
64	64	11	71	71	13
	91	20		96	15
	100	39		100	21
91	91	13	96	96	8
	100	18		100	6
100	100	11	100	100	6

* Leucocytes were desensitized 45 min at 37° without Ca^{2+}, Mg^{2+}, washed and challenged in Ca^{2+}, Mg^{2+} buffer. Agonist concentrations were normalized with respect to maximal histamine releasing capacity for non-desensitized cells. Maximum for ragweed challenge was 56% and for anti-IgE was 48%. Agonists were diluted three-fold.

which represents the soluble extract obtainable when 1 gram of defatted ragweed pollen is extracted by 2800 ml of fluid. The highest concentration of anti-IgE used was 200 IU per ml. In each experiment control aliquots were preincubated in TS alone for 45 min at 37° and then challenged with the agonist dilutions in TCM. The same agonist concentrations were used in both desensitization and challenge of the experimental aliquots of cells. The concentrations were expressed in terms of the histamine releasing capacity of each dilution in the controls relative to the maximal control histamine released. This allowed comparison of the activity of desensitized and undesensitized leucocytes within and between experiments.

Degranulation

In some experiments the supernatants from each aliquot were used to determine histamine but the sedimented cells were used to determine percentage degranulation, as described previously (Pruzansky, Zeiss & Patterson, 1980). Briefly, the cells were suspended in 0·2 ml 0·1% EDTA, reacted 1 min with 0·2 ml alcian blue and then with 0·03 ml 1 N HCl following the procedure of Gilbert & Ornstein (1975). Cells from undesensitized and unchallenged controls were then counted at 400× magnification until approximately 300 basophils were identified. The same volume of the experimental cell samples was also counted. Basophil concentrations in each experimental aliquot were subtracted from the controls to obtain the concentration of degranulated cells. Correlation between the release and degranulation was calculated by the method of least squares.

Resensitization

In some experiments desensitized leucocytes were incubated with LS for 5 min at 0° to remove resident IgE. They were then washed and reincubated for 60 min at 37° with 0·1 ml of plasma from a donor sensitive to a different allergen than the cell donor. After washing, the leucocytes were challenged with the second allergen. The response of the cells compared to controls that were treated with LS and plasma but which had not been desensitized allowed calculation of the percentage resensitization, which could also be considered to be the percentage reversibility.

RESULTS

Homologous desensitization

Leucocytes were incubated at 37° for 45 min with increasing concentrations of either RW or a-IgE in TS. They were then centrifuged, washed and challenged with increasing concentrations of the homologous agonist in TCM at 37° for 45 min. Control aliquots were standardized by incubating first for 45 min at 37° in TS alone and then in TCM containing the same range of concentrations of RW and a-IgE used in desensitization. In order to compare the activity of different agonists, the concentrations were expressed as the histamine released by each standard dilution relative to the maximum histamine released after correction for non-specific release. The data for a typical experiment are shown in Table 1. After preincubation with RW in TS at the lowest concentration, 19% of maximum potential

Table 2. Heterologous desensitization of human leucocytes with increasing concentrations of ragweed extract or of anti-IgE

Challenge			Challenge		
Desensitization* ragweed % max	Anti-IgE % max	Histamine release % max	Desensitization anti-IgE % max	Ragweed % max	Histamine release % max
19	9	5	9	19	16
	20	7		33	19
	47	20		56	46
	75	36		84	73
	100	63		100	92
	100	55		100	98
33	20	10	20	33	19
	47	22		56	39
	75	44		84	64
	100	62		100	87
	100	58		100	95
56	47	12	47	56	25
	75	27		84	44
	100	29		100	71
	100	48		100	84
84	75	17	75	84	24
	100	29		100	40
	100	38		100	56
100	100	15	100	100	10
	100	19			18
100	100	15	100	100	6

* Protocol as for Table 1. Maximum histamine release with ragweed challenge was 67·5% and with anti-IgE challenge was 58·5%.

Table 3. Comparison of homologous desensitization in the presence or absence of Ca^{2+} and Mg^{2+}

Desensitization* with Ca^{2+}, Mg^{2+} ragweed (histamine release) % max	Challenge		Desensitization without Ca^{2+}, Mg^{2+} ragweed % max	Challenge	
	Ragweed % max	Histamine release % max		Ragweed % max	Histamine release % max
23	23	0	23	23	3
	53	0		53	9
	63	0		63	20
	91	25		91	76
	100	44		100	91
53	53	0	53	53	0
	63	0		63	13
	91	9		91	71
	100	27		100	87
63	63	0	63	63	4
	91	0		91	60
	100	15		100	89
91	91	0	91	91	29
	100	0		100	49
100	100	0	100	100	13

* Except for the presence of Ca^{2+} and Mg^{2+} resulting in histamine release during 'desensitization' in this half of the experiment and 10-fold ragweed dilutions, the protocol is as in Table 1. Maximal histamine release was 79%.

Table 4. Estimation of the extent of irreversible desensitization by ragweed or anti-IgE

Desensitization* ragweed % max	Challenge† histamine release % max	Resensitization‡ histamine release % max	Desensitization* anti-IgE % max	Challenge† histamine release % max	Resensitization‡ histamine release % max
7	100	80	0	98	87
11	97	80	10	95	87
25	83	72	26	45	46
61	54	36	57	24	26
95	19	18	93	7	5
100	11	23	100	5	8

* Desensitization was performed as in Table 1 using three-fold dilutions of either agonist.

† All challenges were with concentrations of agonists producing maximal histamine release with undesensitized cells.

‡ Aliquots of desensitized cells were treated with LS to remove resident IgE, resensitized with plasma from a mold-sensitive donor and challenged with a maximally reactive concentration of aspergillus antigen. Maximum release with ragweed was 57%; with anti-IgE was 42%; with aspergillus was 46%.

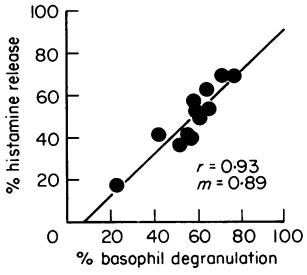

Figure 1. Correlation between percentage histamine release and percentage degranulation in leucocytes desensitized by preincubation with, and then challenged with, varying concentrations of RW.

activity, rechallenge in TCM (column 3) resulted in a considerable reduction in histamine release compared with the expected control release (column 2). However, as the challenge concentration increased the deficit narrowed so that at the optimal challenge concentration of RW only minor irreversible desensitization seemed to have occurred. As the desensitizing concentration increased a similar pattern of histamine release was observed but reversibility at optimal RW concentration decreased. With a-IgE of approximately comparable activity, reversibility was less complete than with RW. In other experiments, similar results were obtained with cells from the same donor and with leucocytes from another donor.

Heterologous desensitization

The effect of preincubation with RW or a-IgE in TS on the subsequent activity of leucocytes to the heterologous agonist was then studied. As seen in Table 2, evidence for both reversible and irreversible desensitization was again observed. Although in this experiment preincubation with ragweed was more inhibitory than preincubation with a-IgE at an equivalent dose, the reverse was true with cells from another donor.

Desensitization in the course of histamine release

If leucocytes had been preincubated in TCM instead of TS, histamine release would have occurred proportionate to the concentration of the stimulus. Nevertheless, it seemed of interest to see whether cells incubated initially with suboptimal concentrations of agonist in TCM would also show evidence for desensitization to challenge with higher concentrations of agonist. Accordingly, leucocytes were preincubated in TCM at 37° for 45 min with RW at increasing concentrations; histamine was determined in the supernatants and the cells were resuspended and reincubated with RW in TCM at 37° for 45 min. The results are shown in Table 3. Column 1 shows the histamine release which by definition is the relative concentration of RW used in 'desensitization.' Histamine release observed after rechallenge (column 3) did not equal that expected (column 2), even when corrected for (added to) that which was released during the preincubation. Again, it should be noted that higher challenge concentrations in equally desensitized aliquots resulted in apparent reversibility. For comparison, the right-hand side of Table 3 depicts the results obtained with aliquots from the same blood donor preincubated concurrently with RW in TS and then challenged. In two other experiments desensitization with RW or a-IgE in TCM produced similar results.

Assessment of reversibility of desensitization

In the previous sections, the data suggested that there were both reversible and irreversible desensitization, which were indicated by the ability or inability, respectively, of optimal concentrations of agonist to effect maximal histamine release. Since the desensitization observed was agonist concentration related, it seemed likely that reversible desensitization involved the configuration of receptor-bound IgE and its ligand. If this was the basis for reversible desensitization, another measure of reversibility might also be determined by removing membrane-bound IgE from desensitized cells and resensitizing them with IgE of a specificity different from that of the original donor cells. The ability of desensitized cells to respond to optimal challenge, compared with undesensitized cells otherwise treated identically, would indicate the degree of reversibility. Such an

experiment is shown in Table 4. As before, desensitization was produced by incubation with increasing concentrations of RW or a-IgE. Some aliquots were challenged in TCM with a single optimal concentration of agonist (columns 2 and 4) while others similarly pretreated were suspended in LS at 0° for 5 min to remove membrane IgE and then resensitized with plasma from a mold-sensitive donor under conditions previously determined to effect optimal sensitization. Control aliquots were not desensitized but were also LS treated and resensitized. All of the passively sensitized samples, except spontaneous controls, were then challenged with the optimal concentration of aspergillus extract, to which the plasma donor was allergically sensitive (columns 3 and 6). The pattern of reactivity of desensitized leucocytes subjected to both procedures was quite similar. Three other experiments with desensitized cells using IgE removal and resensitization demonstrated reversible and irreversible desensitization that was related directly to the pretreatment dose.

Relationship between degranulation and histamine release in desensitized leucocytes

Donor cells were desensitized as before using suboptimal to optimal concentrations of ragweed extract and then challenged with varying concentrations of ragweed. The dose response of undesensitized controls was determined as before to characterize the relative activity of the desensitizing and challenge concentrations of ragweed used. Both histamine release and degranulation were measured concurrently. Figure 1 is a plot of percentage histamine release as a function of percentage degranulation. A correlation coefficient of 0·93 and a slope of 0·89 were found. In another experiment, similar results were obtained with a correlation coefficient of 0·91 and a slope of 0·97.

DISCUSSION

The data obtained in this study demonstrate that preincubation of leucocytes with two typical IgE cross-linking agents at suboptimal concentrations either in the presence or absence of Ca^{2+} and Mg^{2+} resulted in both a reversible and an irreversible component of desensitization to subsequent challenge. Desensitization was considered to have occurred when cells pretreated with agonist released less histamine than undesensitized controls challenged identically. Reversibility at each level of desensitization was evidenced by decreased desensitization as the challenge concentration increased. It was inversely related to the desensitizing concentration of agonist so that desensitization with the optimally active dose of agonist was essentially irreversible. Reversibility was also apparent from experiments removing membrane-bound IgE and resensitizing to another agonist. Both challenge of desensitized leucocytes with optimal concentrations of agonist and resensitization of desensitized cells from which IgE had been removed indicated similar quantitative degrees of reversibility. These observations suggested the formation of inactive IgE–agonist complexes on the basophil membrane. The nature of the inactive complexes and the manner in which they effected desensitization is not known. Perhaps those responsible for reversible desensitization were small enough that they only hindered but did not prevent subsequent bridging. Their formation may have reduced the concentration of unbound IgE available for cross-linking or may have caused steric hindrance which could be overcome by increasing the challenge concentration or by their removal at low pH. Irreversibility might have been associated with larger complexes that could not be removed. Large, inactive and difficult to elute complexes have been reported during desensitization of human basophils (MacGlashan, Mogowski & Lichtenstein, 1983).

Individual leucocytes desensitized at various concentrations of antigen seemed to respond to challenge in an all-or-none manner similar to the response of undesensitized cells (Pruzansky *et al.*, 1980) and to cells passively sensitized with suboptimal concentrations of specific IgE (Pruzansky & Patterson, 1987). This conclusion was based on the tight correlation between percentage histamine release and percentage degranulated cells in all three instances. While it is clear that membrane receptors are mobile, at any instant there is a fixed steric relationship between them, which on a statistical basis could vary from cell to cell. Activation or desensitization of individual cells could have resulted from the heterogeneous distribution of the IgE receptors themselves or of the IgE molecules of a particular specificity. Variable degrees of clustering might account for the fact that under optimal conditions some cells are activated before others (Pruzansky *et al.*, 1980) and for the formation of increasingly larger complexes that may be associated with the reversible or irreversible desensitization described in this study.

ACKNOWLEDGMENTS

We thank J. Lipsey for her expert secretarial assistance. This work was supported by the National Institutes of Health Grant AI-11403 and the Ernest S. Bazley Grant.

REFERENCES

GILBERT H.S. & ORNSTEIN L. (1975) Basophil counting with a new staining method using alcian blue. *Blood*, **40**, 279.

LICHTENSTEIN L.M. (1971) The immediate allergic response: *in vitro* separation of antigen activation, decay and histamine release. *J. Immunol.* **107**, 1122.

MACGLASHAN D., MOGOWSKI M. & LICHTENSTEIN L. (1983) Studies of antigen binding on human basophils II. Continued expression of antigen-specific IgE during antigen-induced desensitization. *J. Immunol.* **130**, 2337.

MAY C.D., SCHUMACHER M.J. & WILLIAMS C.S. (1972) Significance of concordant fluctuation and loss of leucocyte sensitivity to two allergens during injection therapy with nonspecific desensitization. *J. Allergy Clin. Immunol.* **50**, 99.

MENDOZA G.R. & MINAGAWA K. (1982) Subthreshold and suboptimal desensitization of human basophils. Kinetics of decay of releasability. *Int. Arch. Allergy Immunol.* **68**, 101.

PRUZANSKY J.J. & PATTERSON R. (1988) Limiting concentrations of human basophil bound IgE antibody required for histamine release. *Immunology*, **64**, 307.

PRUZANSKY J.J., ZEISS C.R. & PATTERSON R. (1980) A linear correlation between histamine release and degranulation of human basophils by specific antigen or the ionophore A23187. *Immunology*, **40**, 411.

SIRAGANIAN R.P. (1974) An automated continuous flow system for the extraction and fluorometric analysis of histamine. *Anal. Biochem.* **57**, 383.