

The heterogeneity of human IgE exemplified by the passive transfer of D₂O sensitivity

SUSAN M. MACDONALD, JANE M. WHITE, ANNE KAGEY-SOBOTKA,
D. W. MACGLASHAN Jr and L. M. LICHTENSTEIN

The Johns Hopkins University School of Medicine at the Johns Hopkins Asthma and Allergy Center, Division of Clinical Immunology, Baltimore, U.S.A.

Summary

Basophil responsiveness to histamine-releasing factors (HRF) is limited to cells from atopic donors; this response can be transferred passively to non-reactive basophils by IgE molecules from the sera of donors who are intrinsically responsive to HRF [1]. Deuterium oxide (D₂O) also causes mediator release from the basophils of atopic asthmatic subjects [2]. To assess whether basophil responsiveness is IgE dependent, and, if so, whether this release revealed IgE heterogeneity, we tested the ability of sera from HRF responders (IgE+) and non-responders (IgE-) to sensitize basophils to D₂O. Both purified IgE+ and unpurified sera from an HRF responder were passively used to sensitize basophils whose IgE had been removed by lactic acid treatment. As a control, an IgE- myeloma-containing serum was used for passive sensitization. In five experiments, histamine release in the presence of 44% D₂O was $9 \pm 2\%$ and $46 \pm 4\%$ using control IgE- and IgE+ sensitized cells, respectively. The non-responder serum, even at higher IgE levels, did not sensitize the cells for D₂O release. If the IgE receptors on lactic-acid treated cells were first exposed to serum from an IgE- donor, sensitization to D₂O by IgE+ was blocked. The percentage histamine release to D₂O was directly related to both the amount of IgE+ used for passive sensitization and the concentration of D₂O used for release. These experiments further support the concept of IgE heterogeneity and suggest that the occupancy of IgE receptors on the basophil surface 'activate' the cell to make it more responsive to various stimuli.

Clinical and Experimental Allergy, Vol. 21, pp. 133–138. Submitted 20 March 1990; revised 20 August 1990; accepted 28 August 1990

Introduction

A number of investigators have reported that mononuclear cells can generate factors which cause basophil histamine release. We have described a family of factors with such activity derived from other cell types including alveolar macrophages, platelets, endothelial cells and certain cell lines [3–9]. Similar factors are found in biological fluids during late-phase reactions [10]. These latter factors cause selective release from the basophils of atopic individuals [1,11]. Moreover, sensitivity to the histamine-releasing factors (HRF) we have described is due to a distinct type of IgE: those atopic individuals who respond have IgE molecules (called IgE+) which are

thought to bind HRF while non-responders do not have this type of IgE (i.e. IgE-). Sensitivity to HRF can be passively transferred with purified IgE+ from responder sera. This passive sensitization can be blocked by first saturating basophil IgE receptors with IgE from non-responder sera or by removing the IgE from the responder sera by passage over an anti-IgE affinity column [12].

Several years ago we reported that D₂O potentiated IgE-mediated release reactions in human basophils [13]. Subsequently, it was found that a subset of individuals (primarily atopic, asthmatic patients) responded to D₂O by itself, without requiring an additional IgE-related stimulus [2]. The present experiments were undertaken to see if these two unique release reactions manifested by atopic individuals, i.e. the response to HRF and to D₂O, were similar, in that both were dependent upon a special

Correspondence: Dr Susan M. MacDonald, Johns Hopkins Asthma & Allergy Center, 301 Bayview Boulevard, Baltimore, U.S.A.

type of IgE molecule. If such experiments were positive, they would have important theoretical implications regarding the role of IgE in the mechanisms of atopy: if IgE molecules from atopic individuals render cells sensitive to the simple physical change resulting from the exchange of H₂O and D₂O, their role in the release response must be more profound than passively providing the means for cross-linking cell-bound IgE receptors.

Materials and methods

Materials

The serum from a patient with IgE myeloma (HL) was kindly provided by Dr Henry Gewurz (Rush Medical College, Chicago); the serum containing responder IgE was obtained from a clinic patient (MM) after receiving informed consent. Goat anti-human IgE was provided by Dr K. I. Ishizaka, (La Jolla Institute for Allergy and Immunology). Dimeric IgE was obtained from Dr Henry Metzger (NIH) and the details of its preparation and purification have been published previously [14]. Other supplies purchased included piperazine-*N,N'*-bis [2-ethane sulphonic acid] (PIPES), ethylenediamine tetra-acetic acid (EDTA), lactic acid, glucose, human serum albumin (HSA), deuterium oxide (D₂O), heparin, (Sigma, St Louis, U.S.A.; dextran (McGaw, Irvine, U.S.A.); strontium (Fisher, Silver Spring, U.S.A.).

Buffers

PIPES (P) buffer was used, containing 25 mM PIPES, 110 mM NaCl, and 5 mM KCl adjusted to pH 7.4, PA also contained 0.003% HSA; PAG additionally contained 0.1% D-glucose, and PAGCM included 1 mM CaCl₂ and 1 mM MgCl₂; D₂O-PAGCM is brought to volume with 100% D₂O instead of water, making the final concentration in all of the working buffers 88% D₂O. Lactic acid contained 90.1 mg% lactic acid, 0.82 g% NaCl, 37.3 mg% KCl, adjusted to pH 3.9 with 10 N NaOH.

Preparation of peripheral blood basophils

After informed consent, peripheral blood was obtained by venepuncture. Donors were healthy laboratory volunteers whose basophils released histamine to 0.1 µg/ml anti-IgE. Mixed leucocytes containing basophils were prepared by dextran sedimentation as previously described [15]. Briefly, 40 ml of venous blood were mixed with 12.5 ml of 6% clinical dextran and 5 ml of 0.1 M EDTA and allowed to sediment for 90 min at room temperature. The leucocyte-containing plasma layer was then removed and cells were pelleted by centrifugation (150 × g for 8 min), washed once with PAG and once with

unbuffered normal saline. The cell pellet was then divided for lactic acid elution of basophil IgE. It should be noted that only donors whose basophils could be passively sensitized to yield D₂O responsiveness were employed in these experiments.

Lactic-acid elution of basophil IgE

The method of Pruzansky *et al.* [16] was used to dissociate IgE from basophils. Leucocytes were washed once in PAG, once in unbuffered normal saline and the cell pellet was resuspended in 5 ml of 0.01 M lactic acid, pH 3.9, incubated (23°C, 3.5 min), then diluted in 30 ml of PAG, centrifuged and washed twice in PAG. The cells were resuspended in the appropriate volume of PAG for passive sensitization.

Passive sensitization of lactic-acid treated basophils

Passive sensitization was accomplished by the method of Levy and Osler [17]. Acid-treated leucocytes were incubated (37°C, 90 min) with serum or various sources of purified IgE in PAG containing 4 mM EDTA and 10 mg/ml heparin. In most experiments the serum (MM) was diluted to contain 1000 ng/ml IgE while the IgE myeloma-containing serum (HL) was diluted to contain 5000 ng/ml. Following incubation, the cells were washed twice in PAG and resuspended in the appropriate volume of PAGCM for use in the histamine release assay.

Purification of IgE

The IgE from serum MM was purified based on the method of Lohman and Halonen [18] followed by passage over an IgE affinity column. An estimation of molecular weight (<300 kD) was obtained by high performance liquid chromatography (HPLC) using a TSK 3000 gel filtration column (Altex, Berkeley, CA) connected in series to a TSK 4000 column run in phosphate-buffered saline.

Histamine release assay

Reaction volumes for the histamine release assay were 0.1 ml. Mixed leucocytes were incubated in PAGCM with and without D₂O at desired percentages. Cell concentrations were adjusted so that there were approximately 2×10^4 basophils per tube (i.e. a total of 20 ng histamine per tube). Based on kinetic experiments, an incubation period of 45 min at 37°C was chosen for the release reaction. At the end of the incubation, 0.9 ml of cold PAG were added to each tube. The tubes were then centrifuged (1000 × g for 2.5 min at 23°C) and aliquots of the cell-free

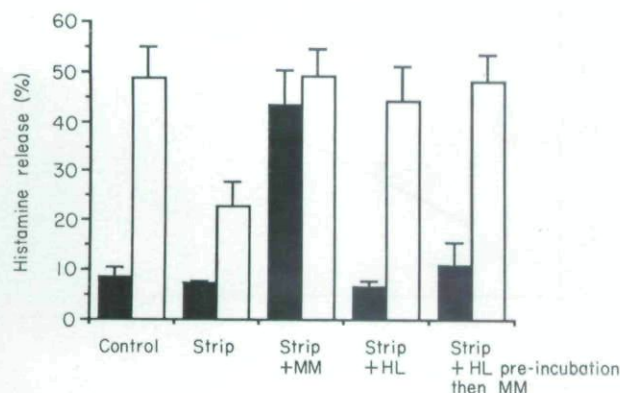


Fig. 1. The effect of passive sensitization of lactic acid-treated basophils with responder (MM) or non-responder (HL) serum. In five experiments, cells were incubated with buffer (control) or lactic acid (strip). Lactic-acid treated cells were then passively sensitized with MM serum, diluted to contain 1000 ng IgE/ml or with HL, diluted to contain 5000 ng IgE/ml. Lactic-acid treated cells were also sensitized with a mixture of HL and MM (strip + HL pre-incubation + MM). The cells were washed and challenged in duplicate with either D₂O (■, 44%) or anti-IgE (□, 0.1 µg/ml). The results are expressed as mean ± s.e. ($n = 5$).

supernatants assessed for histamine content by the automated fluorometric assay of Siraganian [19]. Results were based on the mean of duplicate determinations and were expressed as percentage histamine release by dividing by total cellular histamine after subtracting the spontaneous release of unstimulated basophils (typically, 2–5% during the 45 min assay). Total cellular histamine was obtained by lysing cells in 2% perchloric acid.

Results

Figure 1 provides the basic data which establish that sensitivity to D₂O can be transferred by IgE+ molecules. The mean result of five experiments using the cells of three different donors is shown. The choice of the sera used in these experiments was based on our earlier work with HRF: we selected one positive serum (MM; IgE+) which transfers sensitivity to HRF and one control serum (HL; IgE–) from a patient with an IgE myeloma which we know does not transfer HRF sensitivity. There was minimal release to D₂O (<10%) by the control cells. When the cells were 'stripped' of IgE by lactic acid treatment, a similar, minimal release was noted. However, when these cells were passively sensitized with MM (IgE+), D₂O alone caused $43 \pm 7\%$ histamine release. These experiments also show that when passive sensitization was executed with HL (IgE–), even at extremely high IgE concentrations, no response to D₂O was seen ($P < 0.05$). More importantly, however, these experiments also demonstrate that the sensitivity to D₂O

depends upon IgE interacting with cell surface IgE receptors, because if the cells were pre-treated with a high concentration of IgE– (HL) before being passively sensitized with IgE+ (MM), the response to D₂O was completely abolished. Here we have used selected prototype sera to define another parameter of IgE heterogeneity. We found, however, that normal (non-myeloma) IgE-containing sera were also ineffective (data not shown). Note that the response to anti-IgE was only slightly decreased by lactic acid treatment (which removes approximately 90% of the IgE) and that this response shows no selectivity for the type of IgE placed upon the basophil. The limited decrease in histamine release by anti-IgE after lactic acid treatment of the cells is not surprising because only 1000–3000 molecules of IgE per basophil are needed for half maximal release to anti-IgE [20].

Figure 2 shows the IgE and D₂O dose dependency of this release reaction. We have previously established that there is a linear relationship between the IgE concentration used for passive sensitization and the number of IgE molecules found on the basophil cell surface IgE receptors [21]. This is reflected in the response, in terms of histamine release, shown in Figure 2a: as little as 60 ng of IgE+ sensitizes the cells for >10% histamine release. Similar dose-response curves were observed in five other experiments. The basophils of individuals who respond to D₂O do so in a dose-related fashion. This was also observed after passive sensitization with responder IgE+ (Fig. 2b). Similar results were obtained in three experiments.

The kinetics of IgE-mediated histamine release from basophils are quite characteristic. Release caused by peptide secretagogues (C5a, f-met-leu-phe) is over in less than 3 min while that caused by ionophores or phorbol esters (TPA) is still continuing at 60 min. Antigen or anti-IgE-induced release is >50% complete by 10 min and is largely over by 30 min. Figure 3 examines the kinetics of D₂O-induced release from basophils sensitized with IgE+ as compared to that resulting from challenge with anti-IgE. The rates are very similar, suggesting that IgE cross-links are responsible for both events. The Ca²⁺ and temperature dependence of both release reactions are the same, with a maximal response at 1 mM and 37°C, respectively (data not shown).

There are several trivial explanations for the data described above. The first possibility is that the serum which provides D₂O sensitivity contains functional IgE dimers which are providing the cells with a limited signal for release; D₂O could, therefore, merely be augmenting this stimulus. The character of the dimers could be simple physical aggregates or they could be provided by circulating IgE–anti-IgE complexes. We explored the possibility

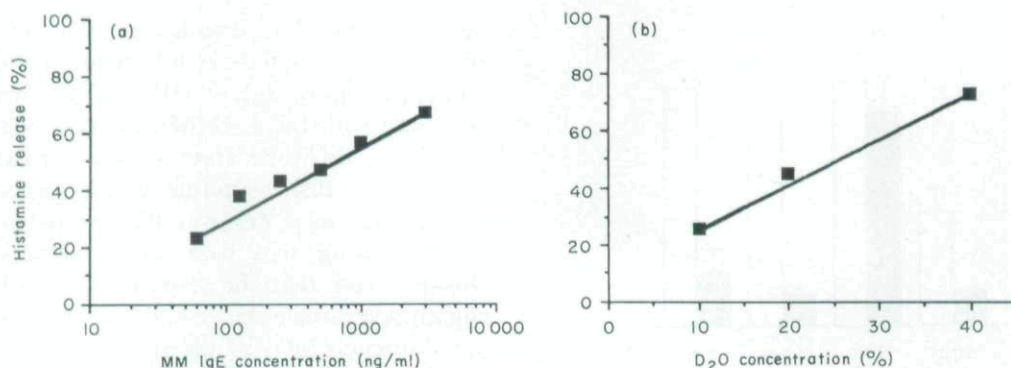


Fig. 2. (a) The effect of the concentration of IgE used for passive sensitization of basophils on subsequent release by D₂O. Cells were treated with lactic acid and then passively sensitized with MM serum diluted to contain from 60 to 3000 ng IgE/ml. The cells were washed and challenged with D₂O (44%). The results of one of six such experiments are shown. (b) The dose dependency of D₂O-induced histamine release is shown. After treatment with lactic acid, cells were passively sensitized with MM serum (1000 ng IgE/ml) washed and then challenged with several concentrations of D₂O. The results of one of three similar experiments are shown.

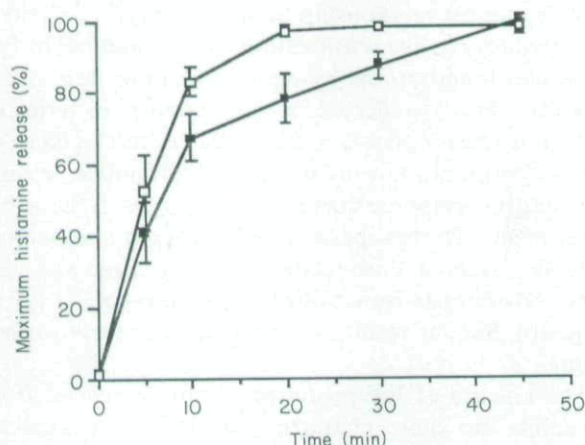


Fig. 3. The kinetics of basophil histamine release by 44% D₂O (■) and anti-IgE (□) (0.1 µg/ml) are shown. With both stimuli, release is slow, with 50% of maximal release occurring at about 5 min. The mean (± s.e.) of three experiments is shown (n = 3).

that dimers were the stimulus in two ways. The first involved the use of a highly purified preparation of MM IgE+. This purified IgE was subjected to size analysis on HPLC, and the IgE concentration was determined by radioimmunoassay. Carrying out passive sensitization with one preparation of purified IgE+, by HPLC, which had a molecular weight of < 300 kD resulted in precisely the same release as that observed after passive sensitization with the unfractionated serum of MM (Fig. 4). In three experiments, sensitization with 1000 ng of affinity-purified IgE caused $32 \pm 7\%$ histamine release while the same concentration of IgE in whole serum led $35 \pm 10\%$ release. Thus, purified monomeric IgE induced sensitivity to D₂O.

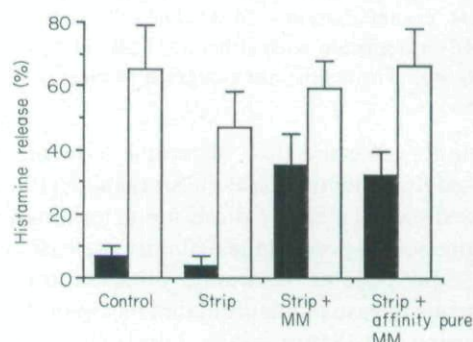


Fig. 4. The ability of purified IgE from a responder serum to confer D₂O sensitivity to basophils is shown. Cells were incubated with buffer (control) or lactic acid (strip). Lactic-acid treated cells were then sensitized with either MM serum (1000 ng IgE/ml) or with IgE (1000 ng IgE/ml) purified from MM serum. The cells were washed and challenged with D₂O (■, 44%) or anti-IgE (□, 0.1 µg/ml). The mean (± s.e.) percentage histamine release from three experiments is shown (n = 3).

The possibility that IgE aggregates were leading to the histamine release was also explored by comparing release with responder serum and D₂O to that induced by a chemically linked IgE dimer which had been studied previously [14] (Fig. 5). IgE was removed from the basophils and they were passively sensitized with increasing concentrations of IgE+ or the chemically linked IgE dimer. The cells were then allowed to undergo histamine release in the presence or absence of D₂O. Dimeric IgE was quite active on lactic-acid treated cells even in the absence of D₂O and this reagent caused an enhancement of release. In contrast, cells treated with equal concentrations of responder IgE+ released essentially no histamine

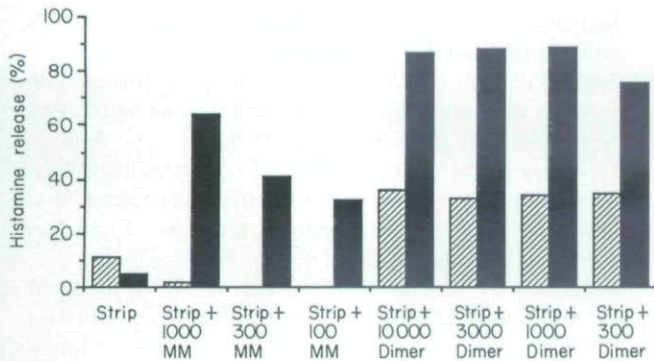


Fig. 5. The effect of passive sensitization of basophils with monomeric and dimeric IgE. Cells were treated with lactic acid (strip) and portions were then passively sensitized with monomeric IgE (MM) or with covalently cross-linked IgE molecules (dimer). The various concentrations (ng/ml) of each IgE source used are indicated by the number of the lower part of the figure. The cells were then washed and allowed to release histamine in the absence (▨) or presence (■) of 44% of D₂O. Note the lack of release by MM serum in the absence of D₂O, while significant release by the IgE dimer occurs under these conditions. Data from one of two similar experiments are shown.

in the absence of D₂O; when the latter was present, however, release was dramatically increased to the level caused by the dimers. This marked discrepancy between the results obtained with IgE+ and dimeric IgE suggest that the former is not acting as a dimer to cause release.

We investigated a second possibility because of reports of circulating anti-IgE antibodies in some disease states, including atopy [22]; some of these antibodies have the ability to release histamine from basophils [23]. Although the experiments with highly purified responder IgE argue strongly against this possibility, we carried out the following experiment to examine this question. The IgE+ serum was divided into two aliquots and one was passed over an IgE immunosorbent. Both were then used to sensitize passively the same set of basophils which were subsequently exposed to D₂O. After passage over the IgE column, 83 ± 8% of the activity was retained, indicating that the histamine release activity was not due to an anti-IgE in the serum used for passive sensitization (data not shown).

Discussion

We have reported data which demonstrate the unexpected transfer of basophil sensitivity to D₂O by certain molecules. This observation is important for two reasons. First, it provides another parameter by which IgE heterogeneity can be demonstrated. These data, together with the results reported with respect to HRF, suggest

that the atopic state is not only a question of having IgE antibodies to certain antigens, but that it also involves a qualitatively different IgE [12]. This might explain why some individuals who have IgE antibody and positive skin tests fail to respond with clinical symptoms at the time of exposure to appropriate antigens. The pathogenetic significance of these qualitative differences in IgE molecules will be addressed further using HRF. However, clinical relevance was suggested in preliminary experiments in which it was demonstrated that the response of an individual's basophils to HRF (i.e. the presence of IgE+) correlated significantly with the intensity of the late-phase reaction after antigen challenge of the upper airways [24].

The second reason that these results are exciting is their implications as to the mechanism of IgE-mediated mediator release. The fact that basophils sensitized with appropriate IgE molecules can respond merely as a result of replacing 10% of the H₂O with D₂O suggests that the mechanisms which lead to the release cascade must be very finely controlled. The biochemical basis by which D₂O enhances IgE-mediated histamine release or, in this instance, causes release itself, is not known. Early work suggested that D₂O interacted with microtubules to promote release [25]; more sophisticated work indicated that antigen activation leads to IgE receptor immobilization by attachment to the cytoskeleton [26]. D₂O potentiates the latter effect. Other work by Beaven *et al.* [27] suggested that, in RBL cells, D₂O caused increased phosphatidyl inositol (PI) turnover; it is widely believed that PI metabolism is one of the early events in signal transduction caused by a variety of stimuli [28]. None of these potential mechanisms by which D₂O acts explains why qualitatively different molecules allow cells to respond to D₂O. It seems most likely that our results indicate that there are random and transient collisions of cell surface IgE receptors and that it is these which are being influenced by the type of IgE molecule and amplified by the presence of D₂O.

Another way of stating this hypothesis is that the occupancy of the IgE receptor by IgE+ molecules 'activates' the cells such that D₂O can cause release. We have recently reported evidence which supports this hypothesis. Human basophils are rendered responsive to human IL-3 by IgE+ and not by IgE-. The release, however, is not due to an IgE+/IL-3 interaction. The same sensitivity to IL-3 is induced by sub-threshold (for histamine release) cross-linking of basophil IgE- by anti-IgE [29]. Thus, in this instance, IgE+ activates basophils in a similar fashion to a minimal cross-linking of IgE- molecules. We think the release induced by IgE+ sensitization followed by D₂O exposure is similarly due to cell activation by IgE+ molecules.

Acknowledgments

Publication No. 029 from John Hopkins Asthma and Allergy Center, 301 Bayview Boulevard, Baltimore, MD 21224. Supported by grants AI07290, and AI20253 from the National Institutes of Health, Bethesda, MD. SMM is a RJR/Nabisco Scholar in Pulmonary Medicine and supported by a Physicians Scientist Award 1K11AI00804, National Institutes of Health, Bethesda, Maryland. LML is the recipient of a Pfizer Biomedical Research Award. The authors would like to thank Ms Bonnie Hebden for excellent secretarial assistance in the preparation of the manuscript.

References

- MacDonald SM, Kagey-Sobotka A, Proud D, Naclerio RM, Lichtenstein LM. Histamine releasing factors: release mechanism and responding population. *J Allergy Clin Immunol* 1987; 79:248(abstr).
- Tung R, Lichtenstein LM. In vitro histamine release from basophils of asthmatic and atopic individuals in D₂O. *J Immunol* 1982; 128:2067-72.
- Thuesen DO, Speck LS, Lett-Brown MA, Grant JA. Histamine releasing activity (HRA). II. Interaction with basophils and physicochemical characterization. *J Immunol* 1979; 123:633-9.
- Kaplan AP, Haak-Frendscho M, Fauci A, Dinarello C, Halbert E. A histamine-releasing factor from activated human mononuclear cells. *J Immunol* 1985; 135:2027-32.
- Alam R, Rozniecki J, Salmaj K. A mononuclear cell derived histamine releasing factor (HRF) in asthmatic patients. Histamine release from basophils in vitro. *Ann Allergy* 1984; 53:66-9.
- Schulman ES, Liu MC, Proud D, MacGlashan Jr DW, Lichtenstein LM, Plaut M. Human lung macrophages induce histamine release from basophils and mast cells. *Am Rev Respir Dis* 1985; 131:230-5.
- Orchard MA, Kagey-Sobotka A, Proud D, Lichtenstein LM. Basophil histamine release induced by a substance from stimulated human platelets. *J Immunol* 1986; 136:2240-4.
- Schleimer RP, Derse C, Landy S, Lichtenstein LM. Vascular endothelial cell (VEC) supernatants (SUP) cause histamine release (HR) from human basophils. *Fed Proc* 1986; 45:1105(abstr).
- Liu MC, Proud D, Lichtenstein LM, *et al.* Human lung macrophage-derived histamine releasing activity is due to an IgE-binding factor(s). *J Immunol* 1986; 136:2588-95.
- Warner JA, Pienkowski MM, Plaut M, Norman PS, Lichtenstein LM. Identification of a histamine releasing factor in the late phase of cutaneous IgE mediated reactions. *J Immunol* 1986; 136:2583-7.
- Fisher RH, Kagey-Sobotka A, Proud D, Orchard MA, Lichtenstein LM. Platelet-basophil interactions: Clinical correlates. *J Allergy Clin Immunol*, 1987; 79:196(abstr).
- MacDonald SM, Lichtenstein LM, Proud D, *et al.* Studies of IgE-dependent histamine releasing factors: Heterogeneity of IgE. *J Immunol* 1987; 139:506-12.
- Gillespie E, Lichtenstein LM. Histamine release from human leukocytes: Studies with deuterium oxide, colchicine and cytochalasin B. *J Clin Invest* 1972; 51:2941-7.
- Segal DM, Taurog J, Metzger H. Dimeric immunoglobulin E serves as a unit signal for mass cell degranulation. *Proc Natl Acad Sci U.S.A.* 1977; 74:2993-7.
- Lichtenstein LM, Osler AG. Studies on the mechanisms of hypersensitivity phenomena. IX. Histamine release from human leukocytes by ragweed pollen antigen. *J Exp Med* 1964; 120:507-30.
- Pruzansky JJ, Grammer LC, Patterson R, Roberts M. Dissociation of IgE from receptors on human basophils. I. Enhanced passive sensitization for human release. *J Immunol* 1983; 131:1949-53.
- Levy DA, Osler AG. Studies on the mechanism of hypersensitivity phenomena XIV. Passive sensitization in vitro of human leukocytes to ragweed pollen antigen. *J Immunol* 1966; 97:203-12.
- Lohman IC, Halonen M. Purification of rabbit serum IgE. *Fed Proc* 1986; 45:388(abstr).
- Siraganian RP. An automated continuous-flow system for the extract and fluorometric analysis of histamine. *Anal Biochem* 1974; 57:383-94.
- Dembo M, Goldstein B, Sobotka AK, Lichtenstein LM. Degranulation of human basophils: quantitative analysis of histamine release and desensitization due to a bivalent penicilloyl hapten. *J Immunol* 1979; 123:1864-72.
- MacGlashan Jr DW, Mogowski M, Lichtenstein LM. Studies of antigen binding on human basophils: II. Continued expression of antigen specific IgE during antigen induced desensitization. *J Immunol* 1983; 130:2237-42.
- Inganäs M, Johansson SGO, Bennich H. Anti-IgE antibodies in human sera: occurrence and specificity. *Int Arch Allergy Appl Immunol* 1981; 65:51-61.
- Johansson SGO. Anti-IgE antibodies in human serum. *J Allergy Clin Immunol* 1986; 77:555-7.
- Iliopoulos O, Proud D, Lichtenstein LM, *et al.* Relationship between early (ER), late (LPR) and rechallenge (RCR) responses to nasal challenge. *J Allergy Clin Immunol* 1987; 79:253(abstr).
- Gillespie E, Lichtenstein LM. Heavy water enhances IgE-mediated histamine release from human leukocytes: evidence for microtubule involvement. *Proc Soc Exp Biol Med* 1972; 140:1228-30.
- Millard P, Gross D, Webb W, Fewtrell C. The nature and origin of changes in free calcium in individual RBL cells. *Fed Proc* 1987; 46:928(abstr).
- Maeyama R, Hohman JJ, Metzger H, Beaven MA. Quantitative relationships between aggregation of IgE receptors, generation of intracellular signals and histamine secretion in rat basophilic leukemia (2H3) cells. *J Biol Chem* 1986; 261:2583-92.
- Nishizuka Y. Turnover of inositol phospholipids and signal transduction. *Science* 1984; 225:1365-70.
- MacDonald SM, Schleimer RP, Kagey-Sobotka A, Gillis S, Lichtenstein LM. Recombinant interleukin-3 induces histamine release from human basophils. *J Immunol* 1989; 142:3527-32.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.