

Lectins do not distinguish between heterogenous IgE molecules as defined by differential activity of an IgE-dependent histamine releasing factor

Jörg Kleine-Tebbe, MD, Anne Kagey-Sobotka, PhD, Donald W. MacGlashan, Jr., MD, PhD, Lawrence M. Lichtenstein, MD, PhD, and Susan M. MacDonald, MD *Baltimore, Md.*

It has been suggested that the differential histamine-releasing activity of an IgE-dependent histamine-releasing factor (HRF), which has recently been cloned, is related to carbohydrate differences in the IgE molecule. Lectins are able to recognize specific glycoforms and might therefore be useful in characterizing the proposed heterogeneity of IgE molecules. As one test of this hypothesis, we examined the histamine release potency of several well-characterized lectins on basophils passively sensitized with serum containing IgE molecules that support HRF-induced histamine release (IgE⁺) or serum that does not support release by this stimulus (IgE⁻). Histamine release was induced by challenging basophils with different concentrations of concanavalin A, Lens culinaris (LcH), and Pisum sativum (PSA). Dose-response curves revealed that LcH caused 30% histamine release at 2 µg/ml with IgE⁺ sensitized cells, whereas the same release with IgE⁻ cells required sixfold higher concentrations. Similar values for PSA showed a sevenfold difference. With concanavalin A, the selectivity was reversed in that it was fourfold more active on IgE⁻-sensitized cells. Another pair of sera (IgE⁺ vs IgE⁻) revealed the same result for concanavalin A, but no difference occurred in LcH and PSA induced release between the IgE⁺- and IgE⁻-sensitized cells. These contrasting findings with different pairs of IgE⁺- and IgE⁻-containing sera indicated that the lectins LcH and PSA are not able to discriminate between IgE⁺- and IgE⁻-sensitized cells as does HRF and therefore cannot be used to further define the proposed heterogeneity of IgE. This conclusion was supported by experiments in which basophil preparations from donors possessing natural sensitivity or insensitivity to HRF (having IgE⁺ or IgE⁻ on their surface) were examined for their response to these lectins. No difference was found in the sensitivity of the cells to challenge with LcH or PSA, and the response to concanavalin A was the opposite of that found when passively sensitized cells were used (30% histamine release at 0.9 vs 3.5 µg/ml for IgE⁺ vs IgE⁻ donors, respectively). We conclude that oligosaccharide-specific lectins do not differentiate between HRF-reactive and HRF-nonreactive IgE molecules on basophils. (J Allergy Clin Immunol 1996;98:181-8.)

Key words: Histamine release, HRF, IgE, lectins

Basophil responsiveness to an IgE-dependent histamine-releasing factor (HRF), which has recently been cloned,¹ provided the first evidence for the existence of a distinct type of IgE: individuals

who respond have IgE molecules (called IgE⁺) that are thought to bind HRF, whereas nonresponders do not have this type of IgE (i.e., IgE⁻). Because there is only one gene for the Fc region of human IgE, the events leading to heterogeneity of

From the Department of Medicine (Division of Allergy and Clinical Immunology), The Johns Hopkins University School of Medicine at the Johns Hopkins Asthma and Allergy Center.

Supported by grant AI 07290 from the National Institutes of Health. Dr. Kleine-Tebbe was supported by grant KI 622/2-1 from the Deutsche Forschungsgemeinschaft.

Received for publication Jan. 26, 1995; accepted for publication Oct. 17, 1995.

Reprint requests: Susan M. MacDonald, MD, The Johns Hopkins Asthma and Allergy Center, Allergy and Clinical Immunology Division, Unit Office 2, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-6801.

Copyright © 1996 by Mosby-Year Book, Inc.
0091-6749/96 \$5.00 + 0 1/1/70336

Abbreviations used

Ag30:	Stimulus concentration that induced 30% release of total cellular histamine
Con A:	Concanavalin A
EDTA:	Ethylenediaminetetraacetic acid
HR:	Histamine release
HRF:	Histamine-releasing factor
LcH:	<i>Lens culinaris</i> (lentil lectin)
PAG:	Piperazine-N,N'-bis[2-ethanesulfonic acid] with albumin and glucose
PIPES:	Piperazine-N,N'-bis[2-ethanesulfonic acid]
PSA:	<i>Pisum sativum</i> (pea lectin)

IgE molecules might occur posttranslationally. Therefore it has been suggested that carbohydrate differences may account for the functional heterogeneity of the heavily glycosylated IgE molecule.²

The complete structure of the oligosaccharide components on an IgE myeloma (PS) protein has been obtained.³⁻⁵ Furthermore, the amino acid sequence including the binding sites for N-linked oligosaccharides of another IgE myeloma (ND) has been determined.⁶ Differential glycosylation exists between these two myeloma proteins: ND myeloma IgE has six carbohydrate units per heavy chain, whereas PS myeloma IgE has four.³ Currently, no direct information is available about the glycans of polyclonal human IgE.

A variety of plant lectins have been used to study IgE-binding reactions and their ability to release histamine from basophils and mast cells.⁷⁻⁹ D-mannose-binding lectins, such as concanavalin A (Con A), lentil, and pea lectin, have been shown to bind strongly to IgE⁸ and induce dose-dependent histamine release (HR) from human basophils.^{7,8} We have reported preliminary data, which suggest that lentil lectin, at a concentration of 5 µg/ml, discriminated between IgE⁺ and IgE⁻ molecules.⁹ Therefore in this study we examined the ability of lectins with different carbohydrate specificities, in a full dose-response range, to release histamine in order to find a candidate that might mimic the activity of the IgE-dependent HRF on human basophils and thus facilitate studies of IgE heterogeneity.

METHODS

Materials

The following were purchased: piperazine-N,N'-bis[2-ethanesulfonic acid] (PIPES), ethylenediaminetetra-

acetic acid (EDTA), lactic acid, glucose, dextran, human serum albumin (Sigma Chemical Co., St. Louis, Mo.). All lectins listed in Table I were purchased from Sigma Chemical Company.

Buffers and solutions

PIPES (P) buffer contains 25 mmol/L PIPES, 110 mmol/L NaCl, and 5 mmol/L KCl adjusted to pH 7.4; PAG also contains 0.003% human serum albumin and 0.1% D-glucose. CaCl₂ was present in PIPES with albumin and glucose (PAG) at a 5 mmol/L final concentration for mediator release experiments. Lactic acid (0.01 mol/L) is 90.1 mg/dl lactic acid, 0.82 gm/dl NaCl, 37.3 mg/dl KCl, adjusted to pH 3.9 with 1 N NaOH.

Preparation of peripheral blood basophils

After subjects gave informed consent, peripheral blood was obtained by venipuncture. For all experiments, mixed leukocytes containing basophils were prepared by dextran sedimentation as described previously.¹⁰ In brief, 40 ml of venous blood was mixed with 12.5 ml of 6% dextran and 5 ml of 0.1 mol/L EDTA and allowed to sediment for 90 minutes at room temperature. The leukocyte-containing plasma layer was then removed, and cells were pelleted by centrifugation (150 g, 8 minutes, 4° C). For direct stimulation of basophils without passive sensitization, leukocyte suspensions were washed twice with PAG and resuspended in PAG with 5 mmol/L Ca⁺⁺ for use in the HR assay.

Passive sensitization of lactic acid-treated basophils

Lactic acid treatment was used to dissociate IgE molecules from basophils.¹¹ Leukocyte suspensions were washed once in PAG and once in physiologic saline solution, and the cell pellet was resuspended in 5 ml of ice-cold 0.01 mol/L lactic acid (for 3.5 minutes at room temperature). The cells were next diluted in 45 ml of PAG, washed twice in PAG, and finally resuspended in the appropriate volume (250 to 1000 µl) for passive sensitization, as described previously.¹¹ Acid-treated leukocytes were incubated (37° C, 60 minutes) with serum or myeloma IgE in PAG containing 4 mmol/L EDTA and 10 µg/ml heparin. Unless otherwise stated, the serum was diluted to contain 2000 ng/ml IgE. After passive sensitization, the cell suspensions were washed twice with 1 ml of PAG (3000 rpm, Eppendorf centrifuge; Brinkman Instruments, Inc., Westbury, N.Y.) before being resuspended in PAG and used in the HR assay.

Preparation of HRF and criteria for HRF responsiveness

HRF was prepared from nasal lavages as described by MacDonald et al.² Briefly, nasal lavage fluids were

TABLE I. Lectins used for stimulation of IgE-dependent HR from human basophils

Common name	Taxonomic name	Abbreviation	Monosaccharide specificity
Concanavalin A	<i>Canavalia ensiformis</i>	Con A	α -D-Man, α -D glc
Eel	<i>Anguilla anguilla</i> agglutinin	AAA	α -L-fuc
Coral tree	<i>Erythrina corallodendron</i> agglutinin	ECorA	β -D-gal(1-4)D-glcNAc
Lentil	<i>Lens culinaris</i> agglutinin	LcH	α -D-Man
Limulin	<i>Limulus polyphemus</i> agglutinin	LPA	NeuNAc
Pokeweed mitogen	<i>Phytolacca americana</i>	PWM	(D-glcNAc) ₃
Pea	<i>Pisum sativum</i> agglutinin	PSA	α -D-Man
Furze and gorse	<i>Ulex europaeus</i> agglutinin	UEA II	(D-glcNAc) ₂

Man, Mannose; glc, glucose; fuc, fucose; gal, galactose; glcNAc, N-acetylglucosamine; NeuNAc, neuraminic acid (sialic acid).

obtained from healthy human volunteers after instillation of physiologic saline solution. The pooled samples were partially purified by ion-exchange chromatography and concentrated. HR of 10% of the total cellular histamine content to the partially purified HRF at a dilution of 1:10 was defined as the basis for distinguishing between IgE⁺ ($\geq 10\%$ release) and IgE⁻ ($< 10\%$ release).

HR assay

Reaction volumes for the HR assay were 0.1 ml, containing approximately 2×10^4 basophils per tube in the presence of 5 mmol/L CaCl₂ and the appropriate stimulus diluted in PAG. After an incubation of 45 minutes at 37° C, 0.9 ml of cold PAG was added to each tube. The tubes were then centrifuged (1000 g, 2.5 minutes, room temperature), and aliquots of the cell-free supernatant were assessed for histamine content by the automated fluorometric assay of Siraganian.¹² The results were based on the mean of duplicate determinations and were expressed as percent HR, by dividing by total cellular histamine after subtracting for spontaneous release of unstimulated basophils (typically, 2% to 5% during the 45-minute assay). Total cellular histamine content was obtained by lysing cells in 2% perchloric acid.

Statistical analysis

The results are expressed as percent HR of duplicate samples. Error bars represent standard error of the mean. The stimulus concentration that induced 30% release of the total cellular histamine (Ag30) is calculated in the Results section by using each individual HR dose-response curve. Geometric means of Ag30 and standard error of the mean, calculated after log-transformation, are given. The statistical significance of different stimulus effects on HR between different groups and differences in the Ag30 levels between different groups of basophils or IgE donors were analyzed by using the Mann-Whitney U test. Values were considered significant at $p < 0.05$.

RESULTS

Single-dose stimulation with mannose-specific lectins after passive sensitization of basophil leukocytes

After passive sensitization of basophils with two different IgE-containing samples (IgE⁺, IgE⁻), the cells were activated by a single dose of various stimuli. Fig. 1 summarizes the results from four different cell donors. Because the IgE concentrations for passive sensitization were matched, no significant difference was found in anti-IgE-mediated HR (aIgE) between the two IgE samples ($51\% \pm 8\%$ vs $42\% \pm 9\%$). Con A, at a dose of 10 μ g/ml, also did not discriminate between IgE⁺ and IgE⁻ containing sera ($33\% \pm 12\%$ vs $37\% \pm 11\%$ HR). *Lens culinaris* (LcH) and *Pisum sativum* (PSA) at 10 μ g/ml induced a better response in the basophils sensitized with IgE⁺ (LcH: $46\% \pm 10\%$ vs $9\% \pm 6\%$); however, a significant difference was only found for PSA ($41\% \pm 7\%$ vs $3\% \pm 0.5\%$ HR, $p < 0.05$). In these single-dose experiments, in which passively sensitized cells were used, LcH and PSA showed a profile of HR similar to the HRF preparation, which caused release only from the IgE⁺-sensitized basophils ($20\% \pm 0.7\%$ vs $1.5\% \pm 0.9\%$, $p < 0.05$).

Dose-response curves of mannose-specific lectins after passive sensitization

To obtain more detailed information, basophils were triggered with several concentrations of the same lectins after passive sensitization with sera containing IgE⁺ or IgE⁻ or with buffer as a negative control. Fig. 2 shows the dose-dependent effects after lectin stimulation in a single experiment. Although lower doses of LcH and PSA caused HR from IgE⁺-sensitized basophils, Con A stimulation occurred at lower concentrations in IgE⁻-sensitized basophils. These results were con-

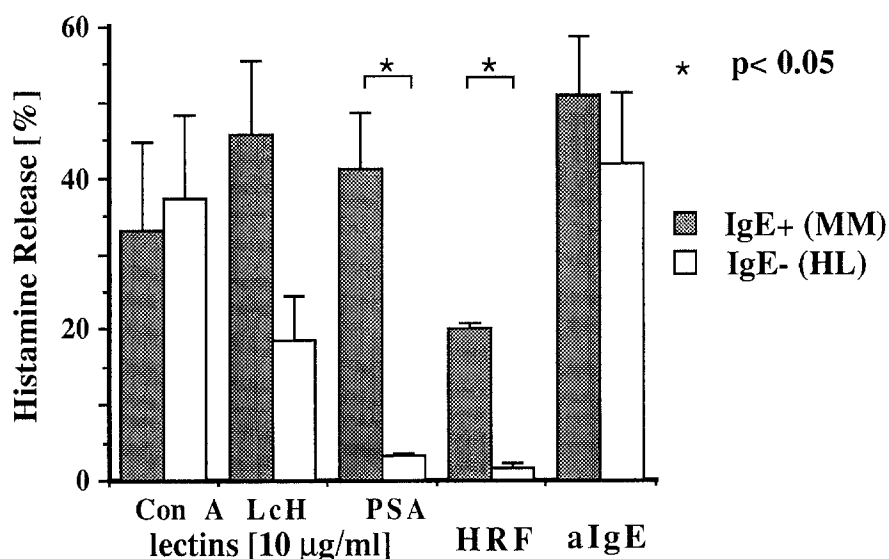


FIG. 1. HR in response to various stimuli after passive sensitization of lactic acid-treated basophils from different donors ($n = 4$) with $2 \mu\text{g/ml}$ total IgE from IgE⁺- or IgE⁻-containing sera. The mediator response was expressed as a percentage of the total cellular histamine content. The IgE serum samples (IgE⁺, MM vs IgE⁻, HL) were defined by their ability to react with HRF (IgE⁺, $\geq 10\%$ HR; IgE⁻, $< 10\%$ HR to a 1:10 dilution of a partially purified HRF preparation). The following lectins were used as stimuli: Con A, LcH, and PSA.

sistent with our findings from the previous experiment (Fig. 1): no difference was found in Con A-induced release at $10 \mu\text{g/ml}$, but differences in HR were revealed when LcH and PSA were used at this concentration. This experiment was repeated with passively sensitized basophils from different donors, and the dose of each lectin causing 30% release of the total histamine was calculated and is shown in Fig. 3, A. Con A binds preferentially to the IgE⁻-sensitized basophils (Ag30 geometric means with IgE⁺ vs IgE⁻: 2.4 vs $0.67 \mu\text{g/ml}$, $p < 0.05$), whereas LcH and PSA bind better to the IgE⁺-treated specimens (LcH-Ag30 in IgE⁺ vs IgE⁻: 2.3 vs $10.2 \mu\text{g/ml}$, $p < 0.05$; PSA-Ag30 in IgE⁺ vs IgE⁻: 5.3 vs $23.3 \mu\text{g/ml}$, $p < 0.05$).

When we characterized another pair of IgE⁺- and IgE⁻-containing samples, shown in Fig. 3, B, again Con A was significantly more active on the IgE⁻-treated cells ($p < 0.05$), but no difference was found between IgE⁺- and IgE⁻-sensitized cells when LcH and PSA were used. These results contrasted with those obtained when two different pairs of IgE⁺ and IgE⁻ sera were used for passive sensitization, indicating that LcH and PSA do not discriminate consistently between IgE⁺- and IgE⁻-sensitized basophils and do not mimic the differential pattern of HRF-induced release consistently. Therefore these lectins are not useful to

further define the proposed heterogeneity of IgE.

Dose-related stimulation of basophils from a large group of donors, known to be naturally sensitized with IgE⁺ or IgE⁻, with mannose-specific lectins

The data shown in Fig. 3 indicate that differences between IgE⁺- and IgE⁻-sensitized basophils can be found in response to Con A. Therefore we examined two larger groups of basophil donors, known to be naturally sensitized either with IgE⁺ or IgE⁻ molecules. The dose-response curves obtained after basophil activation by Con A (Fig. 4) differed from our previous findings. Lower Con A concentrations (2.5-fold) were sufficient for 30% HR with IgE⁺-sensitized basophils and not for IgE⁻-containing basophils as demonstrated before. Similarly, basophils from HRF responders (IgE⁺) were more sensitive to PSA than were cells from nonresponders (IgE⁻), whereas no differential sensitivity occurred when LcH was the stimulus. For further analysis, we compared the lectin doses causing 30% HR from each individual curve (Fig. 5). The overall response to the lectins varied over a wide range, approximately 1 to 2 logs. Furthermore, the lectins showed different binding capabilities: Con A required lower concentrations than LcH, and LcH required slightly lower doses

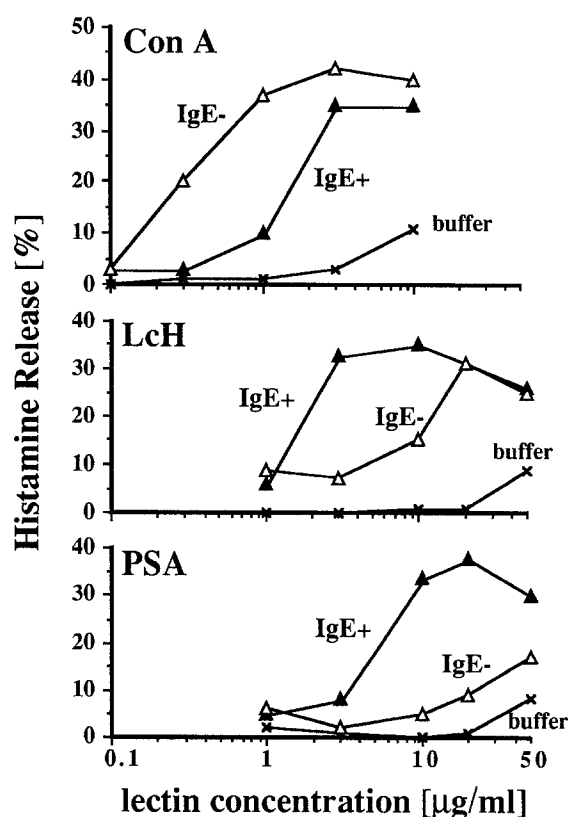


FIG. 2. Lectin-induced HR after passive sensitization (2 μg IgE/ml) of basophils from one donor. After lactic acid treatment, basophils were passively sensitized with IgE⁺ from one donor, IgE⁻ from a different donor, or buffer control.

than PSA. Geometric means of the lectin Ag30 doses show a significant difference between IgE⁺ and IgE⁻ with Con A (0.9 ± 1.3 μg/ml vs 2.3 ± 1.2 μg/ml, $p < 0.05$) and with PSA (3.8 ± 1.3 μg/ml vs 9.7 ± 1.2 μg/ml, $p < 0.05$), but not for LcH (2 ± 1.2 μg/ml vs 2.9 ± 1.2 μg/ml). However, the results with Con A were the reverse of those presented above for experiments in which passive sensitization was used. Thus Con A cannot distinguish consistently between IgE⁺ and IgE⁻ on basophils and appears to be of little use for studying the IgE heterogeneity defined by HRF.

Dose-related stimulation of basophils from different donors with lectins of other specificities

A panel of lectins with different specificities (Table I) was tested on basophils from donors known to be sensitized with either IgE⁺ or IgE⁻ molecules. With the HRF preparation, dose-de-

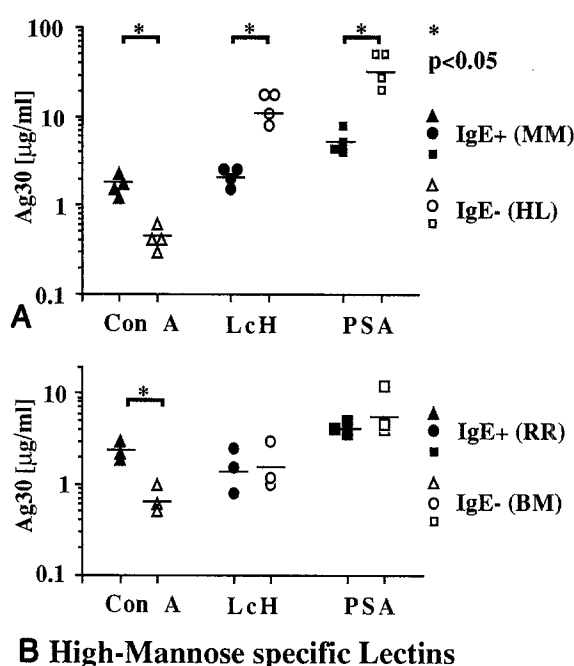


FIG. 3. Cellular sensitivity (Ag30, stimulus concentration for 30% HR) of passively sensitized basophils from different donors to mannose-specific lectins. **A**, Donors MM and HL. **B**, Donors RR and BM. Basophils were sensitized with two different pairs of IgE samples, known to be IgE⁺ (filled symbols) or IgE⁻ (open symbols). Horizontal bars represent geometric means.

pendent HR only occurred with the IgE⁺ donor cells (Fig. 6). In contrast, none of the lectins could distinguish between the two groups of basophil donors in the same manner as HRF. More specifically, eel lectin activated only two of four IgE⁺ donor cells, and coral tree lectin stimulated only two of six IgE⁻ donor cells. Some of the donor cells from both groups (IgE⁺ and IgE⁻) could be fully activated by pokeweed mitogen and *Ulex europaeus* agglutinin, whereas limulin lectin was a weak stimulus in only two of six IgE⁻ donors. Interestingly, the cells of two IgE⁻ donors were extremely sensitive to coral tree lectin, pokeweed mitogen, and *U. europaeus* agglutinin because the rising part of the dose-response curves was not obtained with the lectin concentrations in the microgram-per-milliliter range used for activation. Repeated experiments showed almost identical results for these cell donors. Thus the responses to the various non-mannose-specific lectins used were not only quite heterogeneous within each donor group (IgE⁺ and IgE⁻) but caused release from both cell sources and thus did not mimic HRF-induced release (Fig. 6).

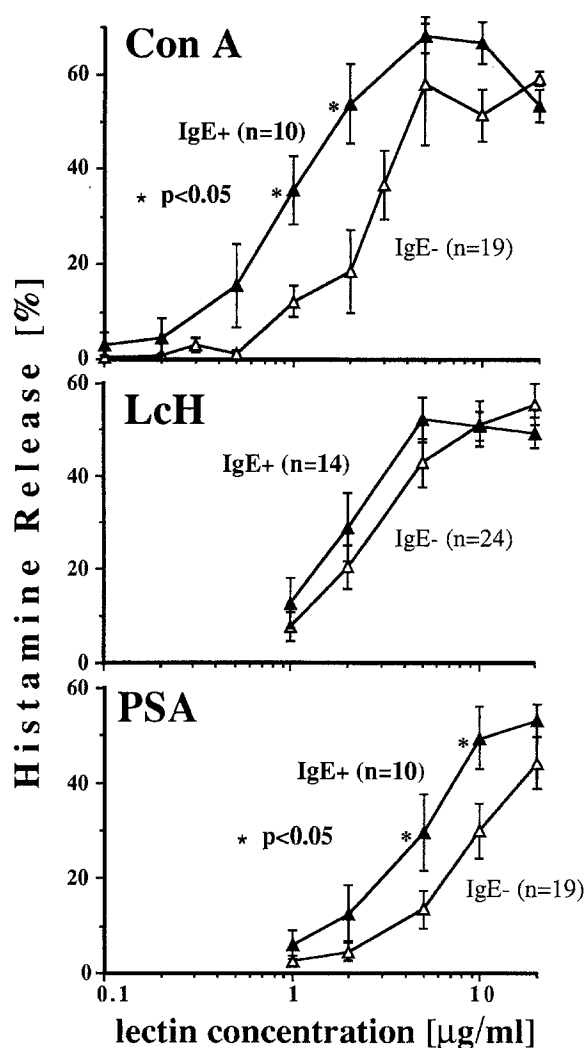


FIG. 4. Dose-dependent lectin-induced HR in a large population of basophil donors, known to be naturally sensitized with either IgE⁺ (filled symbols) or IgE⁻ (open symbols).

DISCUSSION

We chose to use lectins in an attempt to understand the interaction between HRF and IgE for several reasons. Lectins have been reported to release histamine from basophils in an IgE-dependent manner and with similar kinetics,⁷ and direct interaction of these carbohydrate-binding proteins with the IgE molecule has been demonstrated.⁸ Competitive inhibition of the binding reactions by specific sugars indicates that human IgE interacts with these lectins through its carbohydrate moieties.⁸ Furthermore, the IgE-binding protein, an endogenous soluble lectin with S-type carbohydrate-recognition activity and β -galactoside specificity, recognizes different glycosylated forms of human IgE.¹³

Two findings confirm the concept that the re-

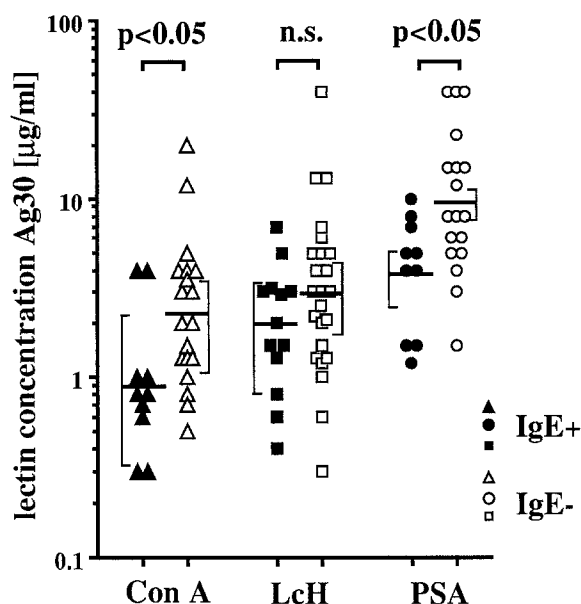


FIG. 5. Lectin-induced HR from naturally sensitized basophils of IgE⁺ and IgE⁻ donors. From each individual dose-response curve, summarized in Fig. 4, the lectin concentration for 30% HR was calculated. The resulting cellular sensitivity (Ag30, y axis) of the basophils to the lectins is shown. Geometric means and standard error of the mean (after log-transformation) were calculated. *n.s.*, Not significant.

sponse to the lectins we used is indeed based on an interaction with the IgE molecule⁸ and not due to an intrinsic property of the basophil in different preparations. First, when different basophil donors were used for passive sensitization, almost identical lectin-induced dose-response curves were obtained with the same serum. Second, minimal release occurred at the highest lectin concentration after lactic acid removal of the IgE from the basophils without subsequent passive sensitization (Fig. 2). The small amount of HR seen is most likely related to incomplete removal of the IgE from the basophils by lactic acid, although direct interaction of the lectins with the Fc ϵ RI, which has been shown to contain N-linked oligosaccharides in rat basophilic leukemia cells,^{14, 15} is a possibility.

The results obtained when HRF-reactive and nonreactive IgE-containing sera (IgE⁺ vs IgE⁻) were used for passive sensitization of basophils and single doses of mannose-specific lectins to stimulate HR confirmed our preliminary findings.⁹ When the same source of IgE⁺ and IgE⁻ molecules was used, LcH and PSA at a dose of 10 μ g/ml discriminated between IgE⁺- and IgE⁻-sensitized basophils. However, the results with LcH did not reach statistical significance ($p = 0.06$), probably

because of the small number of experiments ($n = 4$). Full dose-response curves of Con A-, LcH-, and PSA-induced release after passive sensitization demonstrated differences between IgE^+ and IgE^- with all three lectins. It became obvious that the differences found in the single-dose experiments were a direct result of their position on the lectin-induced dose-response curves in both groups (i.e., no difference could be detected with 10 $\mu\text{g/ml}$ Con A despite a difference in the Con A dose-response curves obtained with IgE^- and IgE^+ , Fig. 2). When another pair of IgE^+ and IgE^- sera was used, a different pattern was obtained. Although Con A produced similar results, LcH and PSA did not discriminate between IgE^+ - and IgE^- -sensitized basophils, indicating that these two lectins do not consistently interact with specific IgE molecules.

To understand the variable responses of the lectins used, we examined a larger population of donors with basophils known to be either reactive or nonreactive to HRF. The sensitivity of IgE^+ -sensitized basophils (HRF-reactive) to lower concentrations of Con A and PSA (Figs. 4 and 5), but not of LcH, contrasted with our previous findings. Several reasons for these conflicting results may exist. During passive sensitization, the individual cellular sensitivity of the basophil donors was controlled for by the fact that the same cells were used to examine the response after both IgE^+ and IgE^- sensitization. In addition, IgE concentrations from different sera were equally matched, although our protocol did not allow control of the precise number of IgE molecules bound to the basophils. In contrast, direct stimulation of basophils from IgE^+ and IgE^- donors did not allow any control of their intrinsic cellular sensitivity or the total IgE level and the number of occupied Fc ϵ RI receptors on the basophils, which are known to be correlated.¹⁶ Because the lectin response depends not only on the quality and quantity of glycan moieties on the IgE molecules but also on the intrinsic basophil sensitivity¹⁷ and on the number of IgE antibodies per cell, several additional sources of variability were introduced in these experiments. Given the multiple influences on the response to lectins, it is surprising that a difference between donor groups was observed for Con A. Because the difference in sensitivity was the reverse of that with the passive sensitization experiments, lectins do not mimic HRF-induced release. The combination of these two sets of data and those from Fig. 6 indicate that lectins are unable to consistently distinguish between IgE^+ and IgE^- .

In summary, we have demonstrated differential

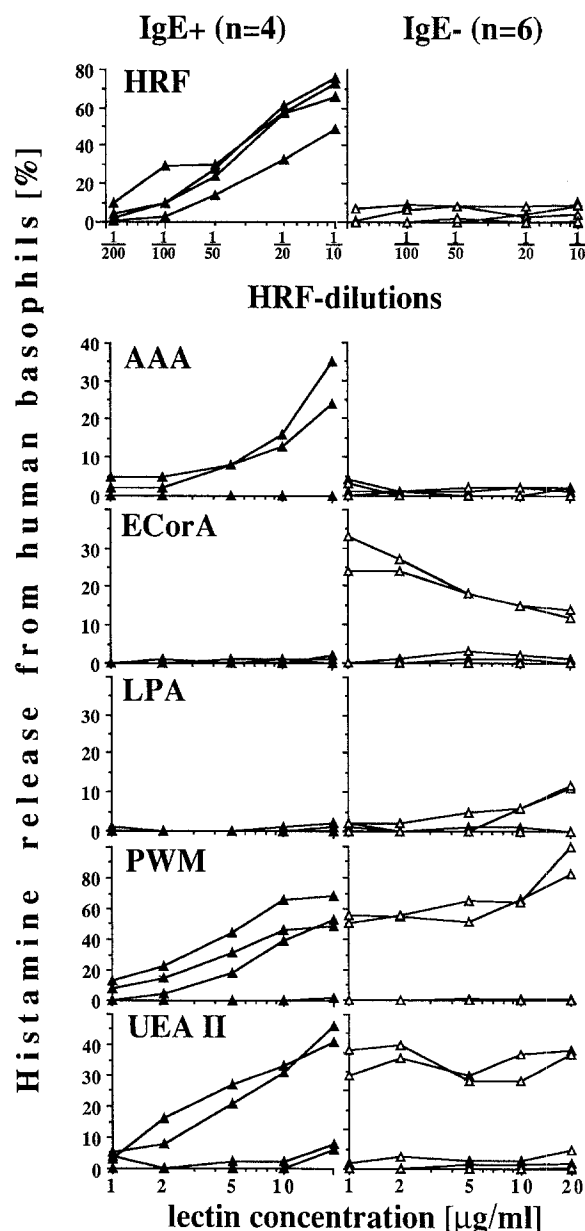


FIG. 6. Dose-dependent HR after stimulation of naturally sensitized basophils from IgE^+ and IgE^- donors with HRF and a panel of non-mannose-specific lectins. Each curve represents a single experiment. See Table I for lectins used. AAA, Eel lectin; ECor A, Coral tree lectin; LPA, limulin lectin; PWM, pokeweed mitogen; UEA II, *Ulex europaeus* agglutinin.

activities of various lectins in inducing IgE-dependent HR from the basophil leukocytes and the sera from IgE^+ and IgE^- donors. Despite the fact that mannose-specific lectins, Con A and PSA, were two to three times more active on IgE^+ -sensitized basophils from a large population of donors, these results should be interpreted with caution, because various intrinsic sensitivities of the basophils could

also account for these differences.¹⁷ Lectins of other specificities did not discriminate between IgE⁺- and IgE⁻-sensitized cells and induced variable responses among different donors. Therefore we conclude that lectins cannot convincingly distinguish between HRF-reactive and HRF-nonreactive basophils and are not useful tools for defining the proposed heterogeneity of IgE molecules.²

REFERENCES

- MacDonald SM, Rafnar T, Langdon J, Lichtenstein LM. Molecular identification of the IgE-dependent histamine releasing factor. *Science* 1995;269:688-90.
- MacDonald SM, Lichtenstein LM, Proud D, et al. Studies of IgE-dependent histamine releasing factors: heterogeneity of IgE. *J Immunol* 1987;139:506-12.
- Baenziger J, Kornfeld S. Structure of the carbohydrate units of IgE immunoglobulin. I. Over-all composition, glycopeptide isolation, and structure of the high mannose oligosaccharide unit. *J Biol Chem* 1974;249:1889-96.
- Baenziger J, Kornfeld S. Structure of the carbohydrate units of IgE immunoglobulin. II. Sequence of the sialic acid-containing glycopeptides. *J Biol Chem* 1974;249:1897-903.
- Rearick JJ, Kulczycki A Jr, Kornfeld S. Structural studies of oligosaccharides of rat IgE and reexamination of the high-mannose oligosaccharide of human IgE. *Arch Biochem Biophys* 1983;220:95-105.
- Dorrington KJ, Bennich HH. Structure-function relationships in human immunoglobulin E. *Immunol Rev* 1978;41:3-25.
- Siraganian RP, Siraganian PA. Mechanism of action of concanavalin A on human basophils. *J Immunol* 1975;114:886-93.
- Shibasaki M, Sumazaki R, Isoyama S, Takita H. Interaction of lectins with human IgE: IgE-binding property and histamine-releasing activity of twelve plant lectins. *Int Arch Allergy Immunol* 1992;98:18-25.
- MacDonald SM, White JM, Kagey-Sobotka A, Lichtenstein LM. Is glycosylation the basis of IgE heterogeneity? [Abstract]. *Clin Res* 1988;36:602A.
- Schleimer RP, MacGlashan DW, Gillespie E, Lichtenstein LM. Inhibition of basophil histamine release by antiinflammatory steroids. II. Studies on the mechanism of action. *J Immunol* 1982;129:1632-5.
- Pruzansky JJ, Grammer LC, Patterson R, Roberts M. Dissociation of IgE from receptors on human basophils. I. Enhanced passive sensitization for histamine release. *J Immunol* 1983;131:1949-53.
- Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975;7:283-90.
- Robertson MW, Liu FT. Heterogeneous IgE glycoforms characterized by differential recognition of an endogenous lectin (IgE-binding protein). *J Immunol* 1991;147:3024-30.
- Kulczycki A, McNearney TA, Parker CW. The rat basophilic leukemia cell receptor for IgE. I. Characterization as a glycoprotein. *J Immunol* 1976;117:661-5.
- LaCroix EL, Froese A. The N-linked oligosaccharides of the Fcε receptors of rat basophilic leukemia cells. *Mol Immunology* 1993;30:321-30.
- Conroy MC, Adkinson NF, Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977;118:1317-21.
- MacGlashan DW Jr. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J Allergy Clin Immunol* 1993;91:605-15.