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# Histamine Release from Human Leucocytes by Concanavalin A and Other Mitogens (38410)

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(Introduced by Stephan E. Mergenhagen)

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In immediate hypersensitivity reactions histamine is secreted by basophiles or mast cells as a result of the reaction of specific reaginic antibody (IgE) on their surface with appropriate antigen (1). A variety of other agents and mechanisms may also cause histamine release from these cells. For example, lysates of purified suspensions of platelets, lymphocytes, and granulocytes release histamine from human leukocytes (2). Lecithinase A from snake venom may cause a "melting" of basophile granules (3). We recently reported that the mitogenic proteins concanavalin A (Con A) and phytohemagglutinin (PHA) released histamine from normal hamster mast cells by a direct, noncytolytic interaction (4). The present paper describes histamine release from human basophiles by Con A, PHA, and an extract of walnuts which is also mitogenic for lymphocytes and investigates the relationship of Con A-induced histamine release to IgE-mediated release.

*Materials and Methods.* Human peripheral blood leukocytes were collected from adult atopic subjects having a history of clinical symptoms and positive skin tests to one or more of the allergens used in this study and from healthy adult "controls" that were not reactive to the allergens used. Venous blood was mixed with dextrose–dextran-EDTA solution, the erythrocytes sedimented, and the supernatant cells washed as described by Lichtenstein *et al.* (5).

Concanavalin A was purchased from Calbiochem, San Diego, CA. Phytohemagglutinin was obtained from Dr. G. Hitchings, Burroughs-Wellcome, Tuckahoe, NY. A saline extract of domestic, shelled black walnuts was prepared by Dr. Ann Sandberg from 100-g nuts by blending with 200 ml phosphate-buffered saline, pH 7.2 (PBS) followed by centrifugation at 600*g* for 8 min at 4°. The aqueous layer was recovered, recentrifuged, and filtered through Whatman No. 42 and 0.45-$\mu$m Millipore filters consecutively. Mouse, guinea pig, and rabbit dander allergens were purchased from Hollister-Stier Labs, Yeadon, PA and dialyzed versus 0.02 *M* PBS. Purified antigen E was purchased from Worthington Biochemicals Corp., Freehold, NJ. Rabbit antiserum to human IgE ($\epsilon$ chain) was purchased from Behring Diagnostics (Somerville, NJ). The diluent for all materials was *tris*-ACM buffer (5).

Reaction mixtures contained 1 ml washed leukocytes and 0.1 ml of diluted mitogen or antigen and were incubated in triplicate at 37° for 1 hr in 12 × 75-mm polypropylene tubes. After incubation, cells were sedimented by centrifugation at 400*g* for 15 min and the supernatant fluids decanted and assayed for histamine content. Histamine was measured fluorometrically by a small-volume method (5, 6). The available histamine for each individual was determined after boiling cell suspensions for 5 min. Results were recorded as the mean percent of the available histamine ± 1 SE.

*Results.* Optimal histamine release by leukocytes from one atopic and three control subjects was achieved using from 1 to 2.5 $\mu$g Con A and from 5 to 10 $\mu$g PHA (Fig. 1). Concanavalin A released from 26 to 70% of the available histamine and PHA from 2 to 44%. Concanavalin A demonstrated a sharper optimum dose response than PHA. At superoptimal doses of the mitogens, histamine release was inhibited. Leukocytes from allergic individual KK responded to Con A in a manner similar to cells from the

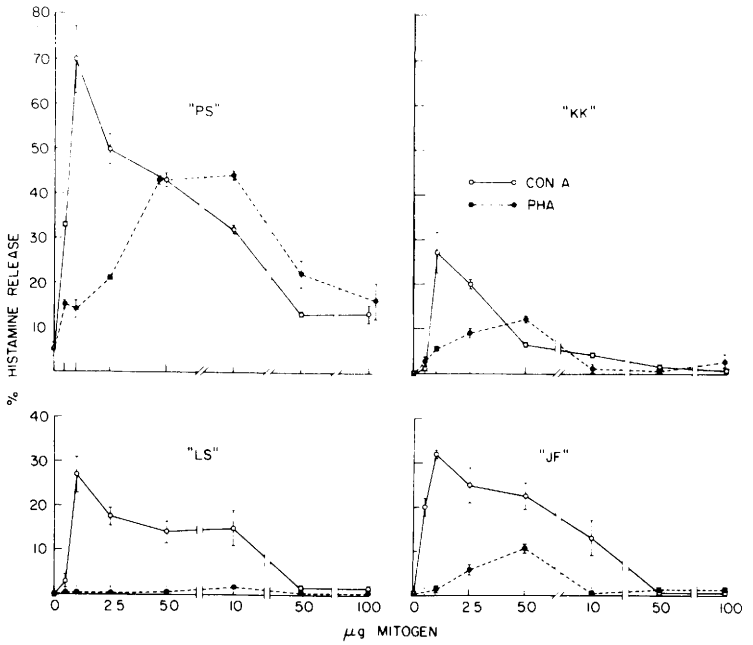


FIG. 1. Histamine release from human basophiles by concanavalin A (Con A) and phytohemagglutinin (PHA) after incubation for 1 hr at 37°. Subjects PS, LS, and JF had no history of allergy, while KK was sensitive to ragweed pollen.

control subjects PS, LS, and JF.

Figure 2 compares the amount of histamine released by Con A with the amount released by increasing amounts of ragweed antigen E and rat dander allergen by leukocytes from three atopic and one control subject. Subject GL was allergic to ragweed; SD to rat dander; BH to both ragweed and rat dander; while BM was a control. The leukocytes from all the subjects showed histamine release with Con A. In contrast, leukocytes from the three allergic subjects released histamine only with the specific allergen to which they were sensitized. With excessive doses of both mitogen and specific allergens, histamine release from leukocytes was usually inhibited.

The histamine release by leukocytes from 12 allergic and 12 control subjects by mitogenic extracts from black walnuts, Con A, PHA, and extracted allergens from mouse, guinea pig, rat, and rabbit dander or antigen E were compared (Table I). Leukocytes from subjects allergic to animal dander and/or ragweed allergen released histamine with Con A and PHA to approximately the same extent as the control group. However, histamine release by leukocytes from allergic subjects was significantly greater when exposed to walnut extract, animal dander and antigen E. Leukocytes from two subjects with documented allergy to walnuts released significantly more histamine (39 and 86%) than the mean release obtained by walnut extract with subjects allergic to danders (25%) and control subjects (7.5%).

Histamine release from control human leukocytes by Con A was compared with release by antiserum to human IgE to find if cells showing low release with Con A appeared to be lacking in IgE. In general, sensitivity to release by Con A was accompanied by a release caused by anti-IgE with a coefficient of correlation of 0.76 ($P < 0.05$) (Fig. 3). Two subjects were found who released histamine poorly with Con A. When they were tested for release by antiserum to IgE, a similar lack of reactivity was observed. At the same time an individual with unusually high release to both reagents was found (Fig. 3).

Mitogens bind receptors on a wide variety of cell types. We have previously found that Con A and PHA cause a noncytolytic release of histamine from hamster mast cells at the same doses at which lymphocytes are activated (4). In the present paper we attempt to relate mitogen-induced histamine release from human basophiles

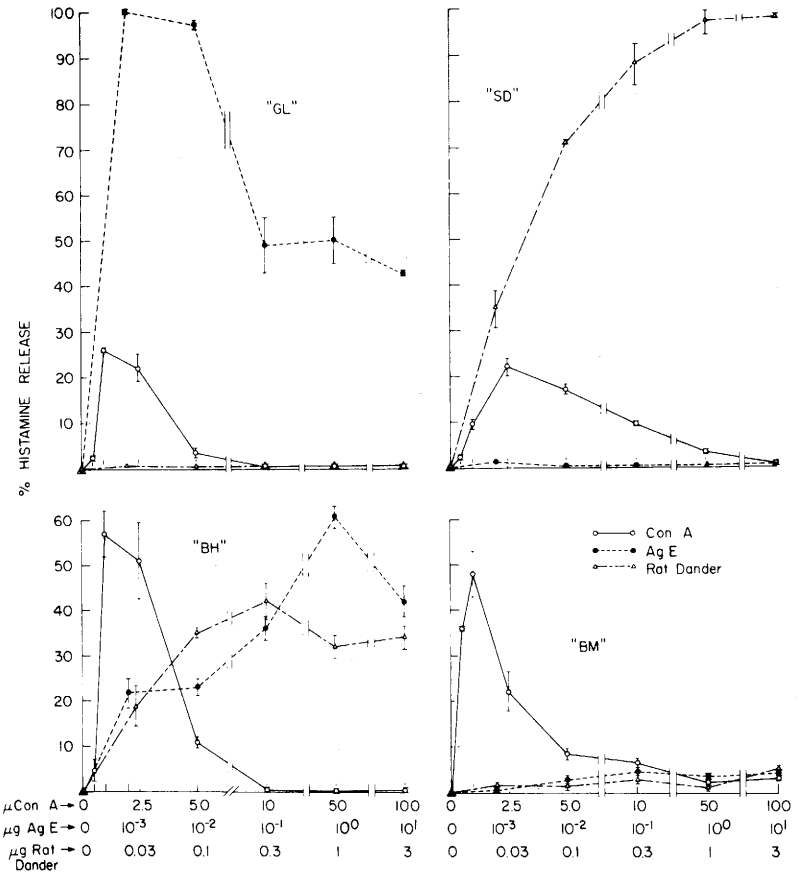


FIG. 2. Histamine release by increasing doses of concanavalin A (Con A) compared with release by ragweed allergen (AgE) and rat dander. Subjects GL, SD, and BH were allergic, and BM was a control.

with the allergic state of the subjects tested. The mechanisms by which lectin mitogens release histamine from basophiles appear to be completely independent of the conventional IgE antibody-mediated allergic reactions. In contrast with walnut extract there was little difference in histamine release by Con A or PHA from leukocytes of patients known to be allergic to dander and pollen when compared with "controls" that lack cutaneous reactions to these allergens. None of the individuals in either group had any history of clinical allergy to Con A or PHA. This suggests that histamine release by lectins is independent of clinical allergy and probably not mediated by IgE anti-lectin antibodies.

Keller recently reported that mast cells from rats that had been sensitized to the nematode *Nippostrongylus brasiliensis* released histamine rapidly with Con A, but not PHA, while cells from unsensitized animals failed to release histamine (8). He suggested that Con A reacted in a manner similar to specific allergen by cross-linking the Fc regions of IgE immunoglobulin on mast cells. We have previously observed that the histamine release of hamster mast cells by Con A was competitively inhibited by methyl-α-D-mannopyranoside (4), which further suggests that Con A interacts with a carbohydrate rather than an antibody component. Concanavalin A also did not generate any lymphokines which induced histamine to be released (4). Furthermore, Siraganian and Siraganian (unpublished data) have demonstrated a direct correlation between histamine-releasing sensitivity of human leukocytes to Con A and anti-IgE and have suggested that histamine release by Con A may be due to the lectin reacting with the carbohydrate component of cell-bound IgE. Similarly, we

TABLE I. Histamine Release from Allergic and Control Leucocytes by Mitogens and Allergens.

| Mitogen/Allergen | Amount ($\mu$g/ml cells) | Percent histamine release[a] | |
|---|---|---|---|
| | | Allergic WBC[b] | Control WBC |
| *tris*-ACM (control) | — | 5.7 ± 2.2 | 2.1 ± 0.6 |
| Con A | 2.5 | 20 ± 3.1 | 24 ± 6.8 |
| PHA | 5 | 6.5 ± 1.8 | 12 ± 8.0 |
| Walnut extract | 39 | 25 ± 6.8 | 7.5 ± 2.4 |
| Mouse dander | 11 | 41 ± 7.0 | 6.2 ± 1.7 |
| Guinea pig dander | 15 | 32 ± 8.4 | 3.6 ± 1.1 |
| Rabbit dander | 16 | 43 ± 5.7 | 11 ± 4.4 |
| Rat dander | 9 | 46 ± 7.3 | 4.3 ± 0.2 |
| Antigen E | 0.1 | 34 ± 9.7 | 2.3 ± 0.8 |

[a] Percent histamine release given as mean ± 1 SE of 12 individuals in each group. Values given below dashed line were corrected for background (control) histamine release by basophiles incubated in the absence of mitogen or antigen.
[b] Individuals with known allergies to animal danders and/or ragweed allergen.

find that many individuals who showed moderate or high release of histamine with Con A also did so in response to antiserum to IgE, and conversely that two subjects whose leukocytes failed to release histamine with Con A also failed to do so with anti-IgE. Because allergic subjects generally have more IgE on their basophiles than nonallergic donors (1), it seems possible that most of our controls which reacted to Con A and anti-IgE may be allergic to allergens other than those tested.

The mechanism of PHA-induced release of histamine is unknown. However, both Con A and PHA presumably release histamine in the absence of any known prior immunization and independently of specific IgE antibody directed against the lectins. Our data indicate PHA to be quantitatively less effective than Con A. Phytohemagglutinin may therefore be releasing histamine by binding carbohydrates on IgE, but not as effectively as Con A, or alternatively it may be reacting with other receptors on the surface of the basophile.

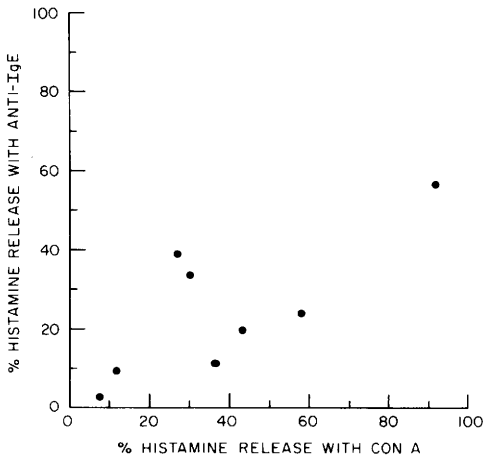


FIG. 3. Comparison of histamine release by human leukocytes from nonallergic subjects with Con A and antiserum to IgE.

Two individuals with documented allergy to walnuts exhibited significantly higher histamine release with this mitogenic extract than either the dander/ragweed-allergic or control subjects. We do not know why the walnut extract caused more histamine release from basophiles of allergic than from normal individuals. However, it is noteworthy that release by walnut extract did not correlate with release caused by Con A or anti-IgE. Histamine release by walnut mitogen may be due to a lower threshold for histamine secretion from basophiles of allergic subjects by a nonspecific component in the extract. Alternatively, there may be a relationship between dander/ragweed allergy and histamine release by walnut extract, possibly based on a cross reaction between walnut extract and IgE antibody to danders.

*Summary.* The lectins Con A and PHA and a mitogenic extract of walnuts released histamine from human leukocytes. Subjects allergic to animal dander and ragweed pollen showed histamine release with Con A and PHA to a degree similar to the control group, suggesting that histamine secretion by lectins was independent of

clinical allergy. However, there was a direct correlation between the degree of histamine released from leukocytes by Con A and antiserum to IgE. Concanavalin A may react with a carbohydrate receptor distinct from the antigen binding site of IgE present on the surface of basophiles.

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