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Histamine Release from Human Leukocytes: Modulation by a Cytochalasin B-Sensitive Barrier

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ABSTRACT Cytochalasin B, a metabolic product of several fungi, enhances up to 10-fold the sensitivity and reactivity of human leukocytes to antigen E or anti-IgE-mediated histamine release. The effect of cytochalasin B is a result of its action on the second, antigen-independent, stage of histamine release. These data suggest that normally, antigen-triggered histamine release is modulated by a cytochalasin-sensitive barrier (CSB). This CSB modulation of histamine release can be separated from the modulating effect of cyclic adenosine monophosphate (AMP).

INTRODUCTION

Cytochalasin B, a product of several fungi (1), has been shown to affect intracellular movement of melanin granules (2) and the secretory function of thyroid (3), pituitary (4), and pancreatic beta cells (5), possibly by means of its ability to disrupt microfilaments (6). Ample evidence supports the concept that antigen-induced histamine release from human leukocytes is similar to the function of secretory organs (7). Accordingly, we have investigated the effect of cytochalasin B (CB)[1] on the release of histamine from human leukocytes.

We found that CB enhanced up to 10-fold the sensitivity of human leukocytes to antigen E or anti-IgE antibody-mediated histamine release. In addition, CB effected an increase in reactivity of the leukocytes to antigen E. The response of the cells to CB was rapid, was dose dependent over a range of about 0.2–5.0 μg CB/ml, and was readily reversed by washing the cells before addition of specific antigen. The effect of CB was demonstrable only in the presence of specific antigen or anti-IgE antibody, since CB alone did not trigger histamine release.

On the basis of these observations, we suggest that antigen-mediated histamine release from human leukocytes is modulated by a cytochalasin-sensitive barrier (CSB). Furthermore, the evidence suggests that CSB modulation is distinct from the modulating effect of cyclic adenosine monophosphate (AMP) on histamine release.

METHODS

Human leukocytes obtained from ragweed-sensitive volunteers were collected, separated, and then washed in 0.025 M tris buffer (pH 7.6) containing 0.3% human serum albumin (Tris A)[2] by the method of Lichtenstein and Osler (8).

The washed leukocytes were then suspended in Tris ACM buffer to a concentration of 2.0×10^7/ml. Various reagents diluted in Tris ACM were then added and the mixtures incubated for 60 min at 37°C. The cells were then centrifuged, the supernatant fluids removed, deproteinized with 0.4 N perchloric acid, and analyzed for histamine content by the fluorometric method of Shore, Burkholder, and Cohn (9), as modified by Lichtenstein and Osler (8). Total histamine content was estimated in a portion of the uncentrifuged cell suspension, deproteinized with 0.4 N perchloric acid. Per cent histamine release and total histamine content were calculated as described (8). Dilutions of histamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.) in 0.1 N HCl were used as standard solutions for these calculations.

Ragweed antigen E was obtained from Abbott Laboratories (North Chicago, Ill.) through the courtesy of Dr. F. C. McIntire. Rabbit anti-IgE antibody was kindly provided by Dr. K. Ishizaka (Baltimore, Md.). CB was purchased from Imperial Chemical Industries Ltd. (Macclesfield, Cheshire, England). For most of the experiments reported here, the CB was dissolved in dimethylsulfoxide (DMSO) at a concentration of 1.5 mg/ml. This stock solu-

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[1] *Abbreviations used in this paper:* CB, cytochalasin B; CSB, cytochalasin-sensitive barrier; DMSO, dimethylsulfoxide; SRS-A, slow reacting substance.

[2] Tris A and Tris A buffer with $MgCl_2$ (1×10^{-3} M) and $CaCl_2$ (6×10^{-4} M) (Tris ACM) were prepared as described (8).

TABLE I

Effect of Cytochalasin B on Antigen-Mediated Histamine Release from Human Leukocytes *

Cytochalasin B	Buffer and DMSO (0.3%)	Antigen E	Histamine release
ug/ml		*ng/ml*	%
0	No	0.6	22.0
0	Yes	0.6	19.6
5	No	0.6	54.0
5	No	0.0	0.0
0	Yes	0.0	0.2
0	No	0.0	0.0
5‡	No	0.6	22.0
0‡	No	0.6	20.0

* Leukocytes (2×10^7/ml) incubated as indicated with 1 ml of each reactant and sufficient Tris ACM buffer to make a total volume of 3 ml.

‡ Incubated 15 min, 37°C, then washed twice with Tris A buffer; resuspended in Tris ACM before adding antigen E.

tion was stored at − 50°C. Immediately before use, the stock CB was diluted 1/300 or more in Tris ACM buffer. Preliminary experiments showed that 0.3% DMSO alone in Tris ACM had no effect on the release or assay of histamine (see Table I). In addition, some experiments were performed using stock CB (50 μg/ml) diluted 1/10,000 in Tris ACM buffer; i.e., final DMSO 0.01%.

For experiments designed to determine whether CB affected the first or second stage of histamine release, human leukocytes were suspended in Tris A buffer. Following the first-stage reaction, the cells were washed twice in Tris A at 0°C and then resuspended in Tris ACM. For each stage, various reagents were dissolved in the appropriate buffer. This procedure was described in detail by Lichtenstein (10).

RESULTS

Kinetics of effect of CB on antigen E-mediated histamine release. The results of preliminary experiments showed the maximal effect of CB on antigen E-mediated histamine release was completed in a preincubation period of 10–20 min. Accordingly, in all the subsequent experiments, the cell suspensions were incubated with CB for 15 min before antigen was added. Since other experiments indicated that the effect of CB on histamine release was reversed by washing the cells before addition of antigen E (Table I), it was not possible to separate the kinetics of the CB effect from the kinetics of histamine release.

Effect of CB concentration on antigen E-mediated histamine release. 1-ml portions of a washed leukocyte suspension were exposed for 15 min at 37°C to 1.0 ml of Tris ACM containing CB at concentrations ranging from 0.167 to 5 μg/ml. Antigen E (0.6 ng/ml) was then added and the mixtures incubated for 60 min at 37°C. Controls consisted of leukocytes exposed to CB alone, antigen E alone, and buffer alone. The results of this experiment given in Fig. 1 show that the augmentation of antigen-mediated histamine release was a function of CB concentration. At a concentration of CB in excess of 2–3 μg/ml, there was no additional augmentation of histamine release. CB at a concentration of 50 μg/ml augmented antigen-induced histamine release, but to a lesser extent than smaller doses. For example, CB at 50 μg/ml increased antigen-mediated release (antigen E, 0.5 μg/ml) to 45% from a control of 35%, whereas exposure to CB at 5–16 μg/ml resulted in 70% release. Accordingly, in subsequent experiments, CB was used at a concentration (5 μg/ml) that resulted in maximum augmentation of histamine release.

Effect of CB on sensitivity of leukocytes to antigen E-mediated histamine release. Washed leukocytes were preincubated in Tris ACM containing CB (5 μg/ml) or in Tris ACM alone, then exposed to varying concentrations of antigen E. The mixtures were then incubated for 60 min at 37°C and histamine release determined in the usual manner.

The results of a typical experiment are shown in Fig. 2*a*. In this experiment, CB effected an eightfold increase in sensitivity of the cells to antigen E-mediated release. Over a 3 month period this experiment was repeated five times using white cells from the same donor. In each instance CB increased the sensitivity to antigen-mediated histamine release eight- to ninefold. With one exception (Fig. 2*b*), exposure of the cells of 11 other individuals to CB resulted in a 5- to 10-fold increase in sensitivity to antigen E. A. similar aug-

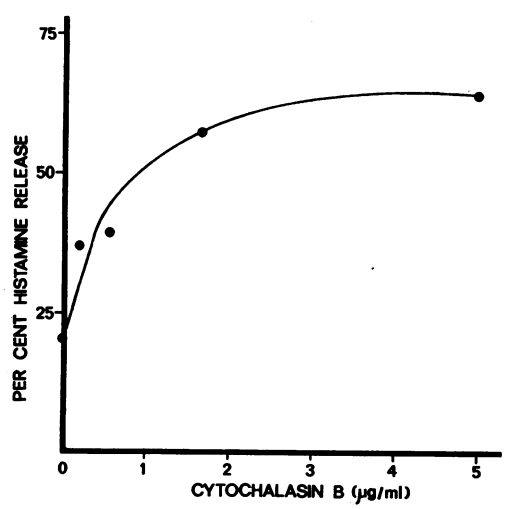

FIGURE 1 Effect of concentration of CB on antigen E-mediated histamine release. Histamine release 0.3% in presence of CB (5 μg/ml) alone; antigen E alone resulted in 20.4% histamine release.

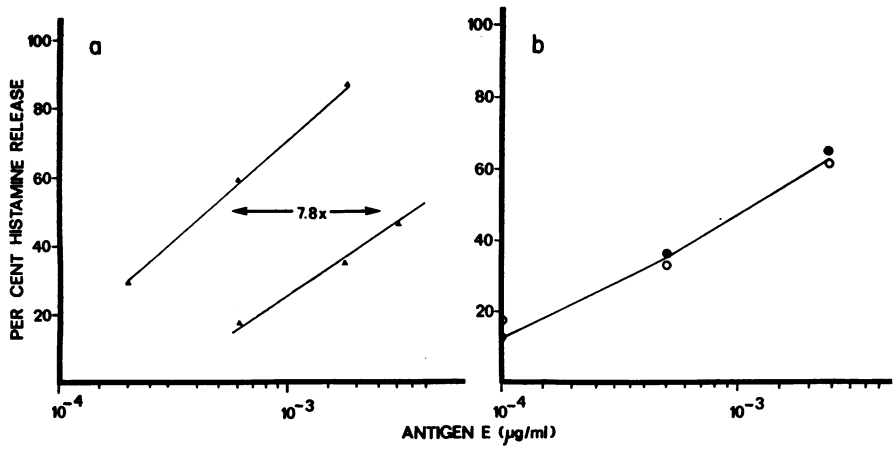

FIGURE 2 (*a*) Effect of CB on leukocyte sensitivity to antigen E: (▲), cells exposed to CB (5 μg/ml) 15 min at 37°C before adding antigen E; (△), cells incubated in buffer 15 min at 37°C before antigen E.) (*b*) Response to CB of leukocytes from severe asthmatic patient: (●), CB (5 μg/ml); (⊙), buffer control.

mentation of histamine release by cytochalasin was detected when leukocytes were exposed to anti-IgE antibody (see Table II). Leukocytes obtained from one patient with *severe* asthma and ragweed sensitivity failed to respond to CB (see Fig. 2*b*).

Effect of CB on leukocyte reactivity. Levy, Lichtenstein, Goldstein, and Ishizaka (11) have distinguished between the sensitivity of leukocytes to antigen (the amount of antigen required to release 50% of the total histamine) and the reactivity of the cells (maximum antigen-induced histamine release). The following experiment was performed to determine the effect of cytochalasin on cell reactivity. Poorly reactive leukocytes (maximum 50% histamine release) were exposed to CB (5 μg/ml) or to buffer for 15 min, then to varying concentrations of Antigen E. CB and buffer controls were included as usual. The results in Fig. 3 show that CB increases the reactivity of leukocytes to antigen-mediated histamine release.

Two-stage histamine release: effect of CB. Histamine release can be separated into two stages: a calcium-independent, cyclic AMP-sensitive activation stage, and a calcium-dependent, antigen-independent release stage (7). An experiment was designed, therefore, to determine whether CB augments histamine release by an effect on the activation mechanism or by an effect on the release mechanism. Leukocytes suspended in Tris A were exposed to 5 μg/ml CB (dissolved in Tris A), then to antigen E (10 ng/ml, or to buffer alone for 2.5 min at 37°C. The cells were then washed twice at 0°C with Tris A buffer and resuspended in

TABLE II
Effect of Cytochalasin B on Anti-IgE-Induced Histamine Release

Patient	% Histamine release from leukocytes exposed to		
	CB* alone	Anti-IgE‡	CB* and Anti-IgE‡
K. C.	< 1.0	3.0	41.5
N. S.	0.0	32.0	63.2
H. C.	0.0	9.5	56.1
M. W.	0.0	1.1	10.0
L. F.	< 1.0	28.0	79.5

* CB used at a concentration of 5 ug/ml.
‡ Rabbit anti-IgE antibody used at a concentration of 0.5 ug antibody protein/ml.

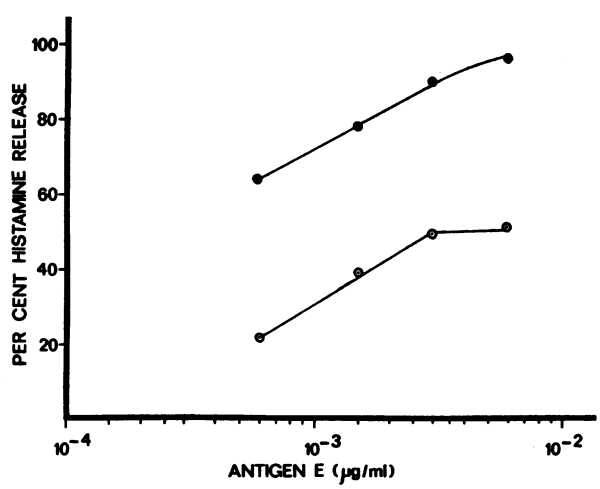

FIGURE 3 Effect of CB on leukocyte reactivity: (●), cells exposed to CB (5 μg/ml) and (⊙), buffer control; then to varying concentrations of antigen E.

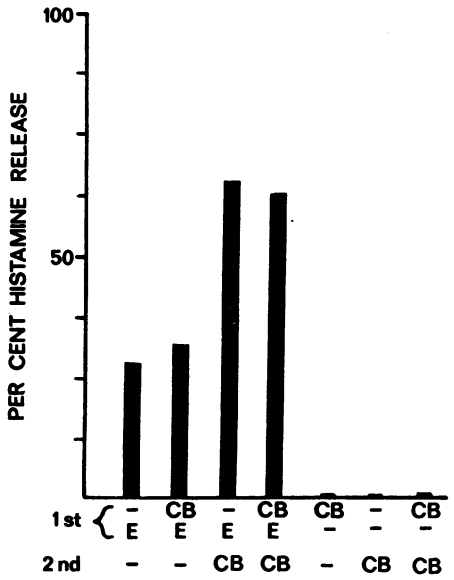

FIGURE 4 Two-stage histamine release: effect of CB. Cells exposed to reactants (antigen E = E and cytochalasin B = CB) in first and/or second stage of histamine release as indicated below each solid bar. (—) indicates buffer alone.

cold Tris ACM. One portion of each of the different mixtures was incubated for 60 min at 37°C with CB (5 μg/ml in Tris ACM); another portion of each was incubated in buffer alone. The results of this experiment shown in Fig. 4 indicate that CB augments histamine release primarily by its action on the second stage with only an insignificant effect on the first stage of histamine release.

DISCUSSION

Studies of in vitro correlates of immediate hypersensitivity have permitted a detailed examination of the mechanism of histamine release (7, 8, 12, 13). In particular, it has been possible to separate the factors that initiate release from factors that modulate the amount of histamine released. For example, the release of histamine and slow reacting substance (SRS-A) initiated by interaction of homocytotropic antibody with its corresponding antigen, can be inhibited by beta adrenergic stimulators and/or prostaglandins (7, 14). The modulating effect of these agents is a result of their effect on the cyclic AMP system.

The experiments presented in this report suggest that a modulator of histamine release, distinct from the cyclic AMP system, can be detected with the use of CB. We propose that CB, a product of the fungus *Helminthosporium dematioideum*, disrupts reversibly a normal intracellular barrier (CSB) to histamine release. Furthermore, the integrity of this barrier may determine the severity of the clinical response to specific allergens. These conclusions are based on the following evidence: (*a*) CB increases the sensitivity and reactivity of human leukocytes to specific antigen or to anti-IgE antibody-mediated histamine release; (*b*) CB has no effect on histamine release by itself; i.e., CB only modulates but cannot initiate histamine release; (*c*) CB modulates in the second stage of histamine release. In contrast, the second stage of release is relatively insensitive to agents that act via changes in cyclic AMP levels (7), indicating that modulation by the CSB occurs at a later stage in the sequence of events leading to histamine release. Moreover, CB does not significantly affect the first or cyclic AMP-sensitive stage of histamine release. Finally, the observation that cells of a patient with severe clinical allergic symptoms failed to respond to CB, suggests that in some individuals the CSB may be relatively deficient and that this defect may account, at least in part, for the degree of symptomatology. Additional studies will be required to substantiate this hypothesis and to ascertain whether the integrity of the CSB is a function of genetic and/or environmental factors.

Cytochalasin B is thought to affect function of secretory cells by disrupting the microfilamentous "cell web." This, in turn, is believed to interfere with the process of emiocytosis; i.e., the process of migration to and fusion of secretory granules with the cell membrane (5). Recent studies (15) suggest that the release of histamine from primate cells is not a consequence of emiocytosis of histamine granules. Therefore, at the present time, it is not possible to identify a morphologic equivalent of the CSB in the human basophil.

Recently Orr, Allison, and Hall (16) have suggested that CB inhibits the release of histamine from rat mast cells. Differences in the species and the cell type employed make it difficult to compare our results with theirs (16).

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