Histamine Release Induced by Human Leukocyte Lysates: Implication of a Specific, Complement-Independent, Noncytotoxic Reaction

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The characteristics of histamine release induced by human leukocyte lysates were determined. Intact human leukocytes released histamine during incubation with leukocyte lysates. Maximal release occurred under physiological conditions of temperature and pH, and both Ca\(^{2+}\) and Mg\(^{2+}\) were required. The addition of ethylenediaminetetraacetic acid abruptly inhibited release. Complement was not required, and serum actually inhibited release. Histamine release was maximal at lysate concentrations of 0.25 to 1.0 \(\mu\)g of protein per ml, and it was reduced at either higher or lower concentrations. Leukocytes continued to exclude trypan blue and maintained chemotactic activity after histamine release. Leukocytes released histamine independently of cellular protein or enzymes. Histamine release induced by leukocyte lysates was interrupted by subsequent removal of the lysate. The data suggest that this histamine release occurs by a specific, complement-independent, noncytotoxic process which resembles, but is not identical with, antigen-induced histamine release. The system has several unique characteristics which suggest that it is a novel form of inflammatory histamine release. This system should provide a useful in vitro model for studies of cellular processes in human inflammation.

Histamine is an important mediator of inflammation because it is responsible for the early stages of vasodilation and increased vascular permeability which characterize the acute inflammatory response. Accordingly, the mechanisms of histamine release have received considerable attention. Many studies have been done on histamine release induced by exogenous substances such as tissue extracts (1, 2) or antigens (8, 13, 17). However, there are few studies on endogenous mechanisms of histamine release, and these have been concerned with animal systems (14).

Our recent studies showed that lysates of human leukocytes release histamine from intact human leukocytes in vitro (7). Our previous studies, using the intact dog as a model, also demonstrated that leukocyte lysates release histamine in vivo (6). All the components of the leukocyte lysate system are normally present in inflammatory sites in vivo. Therefore, histamine release induced by human leukocyte lysates can provide a useful in vitro model for studies of endogenous mechanisms of histamine release at the cellular level.

The purpose of the present study was to analyze the characteristics of histamine release induced by human leukocyte lysates. Our results indicate that this system of histamine release involves a specific, nontoxic, complement-independent reaction which requires divalent cations and proceeds optimally under physiological conditions of temperature and pH. Human leukocyte lysate-induced histamine release is similar to but not identical with several different histamine release systems. The overall characteristics of the leukocyte lysate system suggest that it represents a new type of inflammatory histamine release.

MATERIALS AND METHODS

Preparation of leukocytes and leukocyte lysates. The leukocytes used in this study were prepared from the peripheral blood of normal human donors as
previously described (8). Leukocytes were washed and suspended in tris(hydroxymethyl)aminomethane (Tris) A (isotonic saline solution containing human serum albumin) (10), and lysates were prepared by sonic treatment as previously described (7). The leukocyte suspensions in the preparation of the lysates had 5 to 10 platelets per leukocyte, but removal of the platelets by Tris-NH4Cl lysis had no effect on the lysate histamine-releasing activity. For histamine release assays, the leukocytes were washed in Tris A and suspended in Tris ACM (Tris A supplemented with Ca2+ and Mg2+) (10).

Histamine release assay. Histamine release was determined as previously described (7, 10). Three types of histamine release assay were used in these studies as follows. Standard conditions: A sample (1 ml) of a suspension of leukocytes at 105 cells/ml was incubated with 2.5 ml of buffer and 0.5 ml of leukocyte lysate diluted 1:3,000. After 60 min at 38°C, the cells were removed by centrifugation and the histamine released into the supernatant fluids was measured. The final concentration of lysate, 0.25 to 1.0 µg of protein per ml, provided maximal histamine release. Time course: Leukocytes were incubated as above, but samples were withdrawn at intervals and placed on ice to stop the reaction. The leukocytes were removed by centrifugation in the cold, and the histamine released into the supernatant fluids was measured. Dose response: Leukocytes were incubated in buffer with serial dilutions (100 to 100,000 fold) or leukocyte lysate at 38°C for 60 min. The leukocytes were removed by centrifugation, and the supernatant histamine was measured.

Total leukocyte histamine was determined after lysis in perchloric acid, and percentage of histamine release was determined based on these controls. Spontaneous histamine release in the absence of leukocyte lysates was always less than 10% of the total histamine.

Assay of chemotactic activity. Chemotactic activity of leukocytes was assessed by using a modification of the technique of Cornely (4). Leukocytes were suspended at 106 cells/ml in Tris ACM with 5% heat-inactivated autologous serum. Samples (0.5 ml) of leukocyte suspensions were placed in the chemotaxis chambers on one side of membrane filters (3-µm pore size, Millipore Corp.). The chemotactic substance was Escherichia coli 014 culture filtrate which had been dialyzed, lyophilized, and suspended at 2.0 mg (dry wt)/ml in Tris ACM plus 5% heat-inactivated serum. The chambers were incubated at 38°C for 3 h, and the filter membranes were fixed and stained with Ehrlich hematoxylin. The leukocytes which had migrated the entire thickness of the membrane were counted in five random fields under 400x magnification, and the average count per field was calculated.

Protein and enzyme assays. Protein concentrations were determined by the technique of Lowry et al. (11) by using crystalline lysozyme as a standard. Muramidase, acid phosphatase, and cathepsin activities were determined by the methods of Cohn and Hirsch (3).

RESULTS

Effect of temperature on histamine release. Time course studies of leukocyte lysate-induced histamine release were performed at various temperatures from 0°C to 46°C. The rate and total quantity of histamine release were maximal at 38°C to 39°C, and both parameters of release were reduced during incubation at 4°C or 46°C (Fig. 1). No histamine release occurred after incubation at 0°C, but incubation at 20°C permitted about 50% of the release obtained at 38°C. Preincubation of the leukocytes at 46°C for 15 min did not reduce their ability to release histamine during subsequent incubation with lysate at 38°C (Fig. 2). Therefore, the histamine release process in our system is inhibited at 46°C, but the cells are not rendered less responsive by preexposure to this temperature.

Effect of pH on histamine release. Leukocytes were incubated under standard conditions in Tris ACM adjusted to pH values ranging from 7.0 to 8.0 by altering the relative concentrations of Tris and hydrochloric acid. Leukocyte lysate-induced histamine release was clearly pH dependent (Fig. 3). The pH optimum was 7.5, and release was inhibited by more than 60% at pH 7.0 or 8.0.

Effect of Ca2+ and Mg2+ on histamine release. Leukocytes were incubated with lysates diluted 1:3,000 (standard conditions) in buffers containing various concentrations of Ca2+, Mg2+, or combinations of these cations. Maximal histamine release occurred in the presence of 5 x 10^-4 M Ca2+ and 5 x 10^-2 M Mg2+ (Table 1). However, a low level of hista-
Effect of preincubating leukocytes at 46°C for 15 min on subsequent histamine release induced by human leukocyte lysates. Leukocytes were incubated in buffer at 46°C for 15 min and then challenged with leukocyte lysate at 38°C, and the percentage of histamine release was determined.

Leukocyte lysates, but, because the lysates were diluted 1:24,000 in the final incubation mixtures, this possibility seems unlikely. The addition of Mg²⁺ alone to such cells did not improve histamine release. The addition of Ca²⁺ alone increased release to about two-thirds of that obtained under optimal conditions. Therefore, both Ca²⁺ and Mg²⁺ are required for maximal histamine release, although Ca²⁺ appears to be more important.

The role of divalent cations in our system was further investigated by examining the effect of EDTA on histamine release. When EDTA (0.01 M final concentration) was added to leukocytes plus lysate after 15 min of incubation, histamine release stopped abruptly (Fig. 4). This result indicates that divalent cations are required not only for the initiation of release, but also for continued release after initiation.

**Effect of serum and complement on histamine release.** The role of complement in leukocyte lysate-induced histamine release was assessed by incubating leukocytes plus leukocyte lysates in the presence or absence of 10% normal or heat-inactivated autologous serum. At this concentration, approximately 50% inhibition of histamine release occurred with either fresh or heated serum (Fig. 5). In addition, leukocytes were washed twice in buffer plus 0.05 M EDTA to inactivate cell-bound complement, and this had no effect on subsequent histamine release. These results suggest that complement is not required for leukocyte lysate-induced histamine release.

**Dose response relationship of histamine**

![Graph showing dose response relationship of histamine](image)

**Table 1. Effect of divalent cations on histamine release**

<table>
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<th>Concentrations (M)</th>
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<th>Magnesium</th>
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<td>5 x 10⁻⁴</td>
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*a Percent histamine release induced by leukocyte lysate in the presence of various concentrations of calcium and magnesium.*
for this inhibition were explored: (i) histamine is released but rendered unavailable for assay or (ii) the cells are unable to release histamine and it remains cell-associated. Time course experiments were conducted by using a 1:10 dilution of lysate which did not induce histamine release, and a 1:1,000 dilution of lysate which produced near maximal release. Free and cell-associated histamine was measured for each sample. In the incubation with high lysate concentration, essentially 100% of the histamine remained cell-associated (Fig. 6). In contrast, the incubation with lysate diluted 1:1,000 had a progressive rise in free histamine accompanied by a decrease in cell-associated histamine. This indicates that histamine release is inhibited at high lysate concentrations.

Effect of histamine release on leukocyte viability. Dose response studies were performed, and the leukocytes were mixed with 1% trypan blue to assess the percentage of viability. An average of 94% of the leukocytes excluded the dye before incubation with lysate. After release of more than 50% of the total available histamine in the presence of lysate, trypan blue exclusion remained 94%. Therefore, leukocyte viability, as determined by trypan blue exclusion, was not reduced after histamine release.

The chemotactic activity of leukocytes after histamine release was also examined as a measurement of viability. Time course studies were carried out, and chemotactic activity and histamine release were determined for each sample. Control leukocytes were incubated in the absence of leukocyte lysate. There was no significant difference between the chemotactic activ-

Fig. 4. Effect of EDTA on the time course of histamine release induced by human leukocyte lysate. Duplicate leukocyte suspensions were incubated with leukocyte lysate, and samples were withdrawn at intervals for histamine release assays. EDTA (0.01 M final concentration) was added to one incubation mixture after 15 min.

Fig. 5. Effect of serum on histamine release induced by human leukocyte lysate. Leukocytes were challenged with leukocyte lysate in the presence or absence of 10% autologous serum, and the percentage of histamine release was determined.

release. As demonstrated in a previous publication (8), leukocyte lysate-induced histamine release is maximal at lysate concentrations of 0.25 to 1.0 μg of protein per ml and is reduced at either higher or lower concentrations (Fig. 5). The impaired release at low lysate concentration was presumably a dilution effect, but the reasons for inhibition at high lysate concentrations were not clear. Two possible explanations

Fig. 6. Effect of leukocyte lysate concentration on the release of histamine from human leukocytes. Amount of free and cell-associated histamine was determined after incubation of leukocytes in the presence of high or low lysate concentrations.
ity of leukocytes which had released histamine and the controls which had not (Fig. 7).

These studies suggest that viability of leukocytes is not reduced after histamine release induced by human leukocyte lysates. However, basophil leukocytes are probably responsible for the histamine release in this system, and they compose only about 1% of the leukocyte population. Therefore, a selective toxic effect on basophils cannot be ruled out by these studies.

Effect of removal of leukocyte lysate after induction of histamine release. Time course experiments were performed by using duplicate mixtures of leukocytes and lysate. After 15 min of incubation in the presence of lysate, the cells were removed from both incubation mixtures by centrifugation. One cell button was suspended in buffer without lysate while the other was suspended in buffer plus fresh lysate. The incubations were then continued. In this experiment, 16.5% of the total available histamine was released after 15 min of incubation (Fig. 8). The leukocytes suspended in buffer without lysate had very little additional histamine release. However, the cells suspended in the presence of lysate released histamine normally. Therefore, histamine release in this system is not all-or-none as in a lytic reaction; it is a gradual process which requires continued presence of the inducer for continued release.

Specificity of histamine release. Leukocytes were incubated with lysate in dose response experiments, and the percentages of histamine, protein, acid phosphatase, cathespin, and muramidase released into the supernatant fluids were determined. The total quantities of each enzyme in the leukocytes were assayed on sonically treated suspensions which had been incubated in the absence of lysate. In these experiments, more than 50% of the available histamine was released in the presence of lysate, whereas less than 5% histamine release occurred in the absence of lysate. In contrast, the release of cellular protein and enzymes averaged 14% in the presence or absence of lysate. Therefore, histamine release appears to be a specific process in our system and is not accompanied by loss of other cellular constituents.

DISCUSSION

Previous studies have revealed two basic categories of histamine release reaction. Cytotoxic reactions have been implicated in anaphylactic histamine release from rabbit platelets (A. G. Osler, Fed. Proc., p. 1729–1736, 1969) and in platelet histamine release induced by antigen-antibody complexes (5). In these systems, histamine release occurs by cell lysis and it is characterized by a requirement for serum complement.

The second category of histamine release, involving noncytotoxic reactions, has been implicated for immunological systems including
anaphylactic (13, 17) and allergic histamine release (8). Similar nontoxic mechanisms are involved in histamine release induced by cationic protein (14) and by compound 48/80 (A. R. Johnson and N. C. Moran, Fed. Proc., p. 1716–1720, 1969). This type of histamine release resembles a secretory process (A. G. Osler, Fed. Proc., p. 1729–1736, 1969) and is temperature, pH, and cation dependent (17). Complement is not required, and the cells are not damaged during the release reaction.

The present study indicates that histamine release induced by human leukocyte lysates involves a specific, nontoxic, complement-independent reaction which requires divalent cations and proceeds optimally under physiological conditions. Therefore, this system falls into the complement-independent, non-cytotoxic category, and it has several characteristics in common with other histamine release systems of this type.

In our previous studies, we reported the complex dose response relationship observed in leukocyte lysate-induced histamine release (7). This relationship is characterized by histamine release values which increase with increasing concentrations of lysate until a maximum is reached at 0.25 to 1.0 μg of lysate protein per ml. Greater concentrations result in progressively reduced release values (see Fig. 5). The reduced release at low lysate concentrations can be explained as a dilution phenomenon, but that at higher concentrations is more difficult to explain. The studies on the dose response relationship reported in this investigation indicate that histamine release is actually inhibited at high lysate concentrations. Histamine remains in association with the leukocytes in the presence of high lysate concentrations and therefore is not released and destroyed.

Several histamine release systems, involving antigen-antibody reactions on cell surfaces, have dose response relationships similar to that of our system (8, 17). However, nonimmunological histamine release processes, such as cationic protein- or compound 48/80-induced histamine release, have simple dose response curves (A. R. Johnson and N. C. Moran, Fed. Proc., p. 1717–1720, 1969; 14). This indicates that membrane binding phenomena, perhaps immunological in nature, may be involved in leukocyte lysate-induced histamine release, but a definitive answer to this question must await purification and characterization of the histamine-releasing agent in human leukocyte lysates.

Although histamine release induced by human leukocyte lysates is similar to anaphylactic and allergic histamine release, the present studies also reveal several unique characteristics. Preheating the leukocytes at 46 C for 15 min before incubation with lysate had no effect on histamine release in our system. In the anaphylactic and allergic systems, histamine release is irreversibly inhibited by exposure of the cells to 45 C for 5 min before incubation with antigen (8, 17).

Lichtenstein (9) has presented evidence that allergic histamine release will proceed normally in the absence of inducer after a short period of activation in the presence of antigen. In our system, histamine release was inhibited by removal of the inducer even after the release process was well established. These studies suggest that, although leukocyte lysate and antigenic histamine release are similar in general, the actual cellular mechanisms involved may differ.

The role of histamine in the vasodilation and increased vascular permeability which characterize the acute inflammatory response has been well established (18). Our previous studies indicated that human leukocytes contain factors which release histamine from intact leukocytes in vitro (7). These factors may play an important role in acute inflammation by releasing stores of histamine from circulating basophils and tissue mast cells. Human leukocyte histamine-releasing factors may be particularly important because they provide an endogenous mechanism for histamine release. Most other histamine release systems require an exogenous inducer such as antigen. However, in the leukocyte lysate system the histamine-releasing factors are present in normal human leukocytes. Since leukocytes appear early at the sites of most injuries, these factors could be part of a built-in system for development of the acute inflammatory response. Therefore, the human leukocyte lysate histamine release system may provide a useful in vitro model for an analysis of human inflammation at the cellular level.

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LITERATURE CITED