Influence of Temperature on the Inhibition by Colchicine of Allergic Histamine Release* (33786)

DAVID A. LEVY AND JOSEPH A. CARLTON[1]
(Introduced by A. G. Osler)

Department of Radiological Science, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

Human leukocytes have been used extensively for *in vitro* studies of the allergic reaction (1). Pollen antigen reacting with sensitized leukocytes induces the release of histamine from granulocytes. These studies suggest that allergic histamine release is a noncytotoxic, multistep process, the precise mechanism of which remains to be clarified.

It has been suggested that colchicine, an inhibitor of both dividing and nondividing cells, might provide clues to the mechanism of certain cellular functions because of its effect on intracellular microtubular systems (2). Several observations, including reports of the inhibition of leukocyte functions [cited in (3)], suggested its use in *in vitro* studies of the allergic reaction. Colchicine was reported to ameliorate allergic reactions of the urticarial type (4) and, more recently, was shown to inhibit the release of histamine from rat peritoneal mast cells reacted with compound 48/80 (5). In a preliminary report, we noted that colchicine inhibited the antigen-induced release of histamine from human leukocytes (6).

Colchicine-sensitive cytoplasmic microtubules are altered at low temperatures (7–9). Likewise, histamine release from leukocytes is inhibited in the cold (10). In the present report, we describe the influence of temperature on the ability of colchicine to inhibit this reaction.

Methods. Leukocytes were obtained from the blood of persons highly allergic to ragweed and/or grass pollen, as previously described (10). In brief, after sedimentation of the erythrocytes in dextran, leukocytes were separated from platelets and plasma by centrifugation and washed in Tris buffer at room temperature. One ml of cell suspension (in duplicate) was added to colchicine (Sigma Chemical Company) in 0.5 ml of Tris buffer or to the buffer alone. The suspensions were incubated, with occasional mixing, usually for 30 min. When cells were treated with colchicine at 0°, tubes containing the reaction mixtures were transferred to a 37° water bath and the temperature was equilibrated by mixing for at least 3 min. At this time, 0.5 ml of highly purified ragweed pollen antigen E (11) or grass pollen Group I antigen (12) in Tris buffer containing calcium and magnesium was added and the mixtures incubated for 30 min at 37°. The concentration of antigen was selected to induce the release of 40–80% of the histamine from the individual donor's cells (10). Control tubes without antigen or colchicine were included in each experiment.

Histamine was extracted from reaction mixtures, after removal of the cells by centrifugation, and was assayed fluorometrically as described previously (13), with the following modification. We observed that colchicine ($\geq 10^{-4}$ *M*) was extracted with histamine, and because of its strong absorption at 3500 Å, quenched the fluorescence of the histamine-OPT derivative. To avoid this, colchicine was extracted from the supernates of the cell-free, perchloric acid-precipitated reaction mixtures into chloroform prior to extraction of histamine into butanol. Preliminary experiments showed that no 3500 Å-absorbing material was detected in the final extraction product and that less than 5% of the histamine in aqueous solutions, measured either fluorometrically or isotopically (with tritiated histamine), was extracted into chloroform.

Results. Preincubation of leukocytes with colchicine at 37° resulted in a moderate decrease of antigen-induced histamine release,

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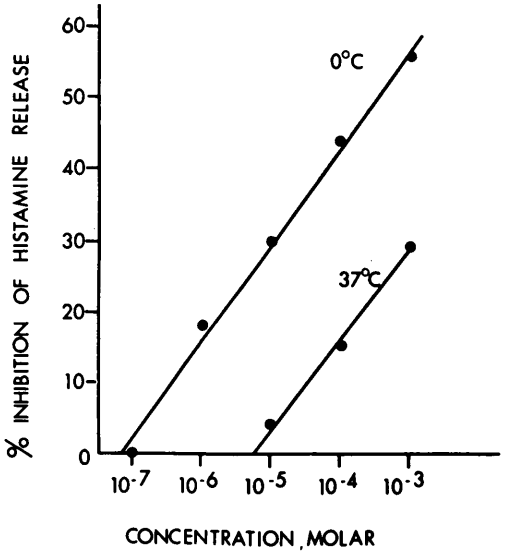

FIG. 1. Influence of temperature on the inhibition by colchicine of antigen-induced histamine release.

whereas the stimultaneous addition of colchicine and antigen to leukocyte suspensions at 37° produced no detectable inhibition. In experiments with cells from eleven allergic donors, preincubation with 10^{-3} *M* colchicine for 30 min resulted in inhibition averaging 30 ± 10%. Colchicine did not change the total cellular histamine content nor did it increase spontaneous histamine release.

When leukocytes were reacted at 0° with colchicine, a significantly greater inhibition was observed in all cases. Incubation with 10^{-3} *M* colchicine for 30 min at 0° produced 52 ± 12% inhibition. Figure 1 shows a typical experiment in which the degree of inhibition,

$$\left(1 - \frac{\%\text{ histamine release with colchicine treatment}}{\%\text{ histamine release without colchicine treatment}} \times 100\right)$$

was evaluated as a function of the temperature of incubation and the colchicine concentration. Fifty percent inhibition was obtained with 5×10^{-5} to 1×10^{-3} *M* colchicine (mean 5×10^{-4} *M*) using cells from 8 of the 11 donors. Even with 10^{-6} *M* colchicine, as much as 19% inhibition was observed. In contrast, 50% inhibition was achieved only occasionally when cells were treated at 37°. Individual differences in sensitivity to colchicine could not be eliminated.

Further evidence of the differential effect of temperature on the action of colchicine is shown in Table I. As stated, when colchicine and antigen were added simultaneously to leukocytes at 37°, no inhibition of histamine release was obtained. When antigen was added to cells incubated with colchicine (3×10^{-4} *M*) at 37° for 1, 2 and 3 hr, then 5, 17, and 23% inhibition was observed. As seen in the lower part of the table, 20% inhibition resulted when cells were suspended in ice-cold buffer, added to colchicine (final concentration = 3×10^{-4} *M*) at 0° and then, after being warmed for 4 min, reacted with antigen. If the cells were reacted with colchicine at 0° for just 5 min prior to warming, the level of inhibition was about 80% of that obtained by reaction for 60 min. The reduced responses after preliminary incubation at 0° were not due simply to a reduction of the capacity of leukocytes to respond to antigen. In a series of control experiments, leukocytes that were incubated in buffer at 0° for 30 min responded to antigen added after warming almost as well as did cells that had not been chilled. Also, no histamine release was observed when cells were reacted with antigen at 0°, confirming previous studies (10).

Discussion. Many details of the antigen-induced release of histamine from sensitized human leukocytes are unknown. It is known that the histamine in human granulocytes is

TABLE I. Influence of Temperature on the Time Course of the Colchicine Effect.

Temp (°)	Incubation[a] time (min)	Inhibition (%)
37	0	0
	60	5
	120	17
	180	23
0	0	20
	5	37
	15	42
	30	40
	60	47

[a] The duration of incubation of leukocytes with colchicine prior to incubation with antigen at 37°; colchicine = 3×10^{-4} *M*.

located principally within cytoplasmic granules (14). That the granules must move through the cytoplasm to the plasma membrane prior to the release of histamine is suggested by studies with rat mast cells (15). Whether these granules are discharged by the cells, as apparently happens in histamine release from rat mast cells, has not been determined.

The present data, showing dosage-dependent inhibition of allergic histamine release by colchicine, suggests a possible mechanism for the movement of histamine-containing granules to the cell surface. The effect of colchicine on cells is attributed to changes in microtubules, leading to the disappearance of these organized structures (16). In particular, cytoplasmic microtubules disappear from human polymorphonuclear leukocytes after treatment with colchicine (2.5×10^{-5} to 2.5×10^{-4} *M*) (3). The effect of colchicine on several functions of leukocytes has been linked to this observation.

Cytoplasmic microtubules are ubiquitous structures whose function remains uncertain. They appear to be involved in the movement of cells and their contents (17), including the movement of cytoplasmic granules (2, 18). They may participate in the secretion of the contents of such granules (19). On the basis of the inhibitory effect of colchicine on histamine release, we proposed that microtubules are involved in the secretion of this amine from its intracellular sites (6). Now we suggest that movement of these granules to the cell membrane requires organized microtubules. The release of catecholamines from granules in the adrenal medulla may require a similar mechanism (20). The intimate relationship of microtubules to the enzymatic reactions trigged by antigen and antibody and to secretory processes in general requires further investigation.

The inhibitory effect of colchicine on histamine release was both more pronounced and more rapid at low temperature. It had been shown that microtubules are in a reversible temperature-sensitive equilibrium, becoming disorganized and disappearing within minutes after cooling cells to 0 or 4° and then reappearing upon rewarming the cells to 37° (8, 9). In view of Malawista's suggestion that the action of colchicine on cell functions involving microtubular structures would be recognized more rapidly if cells were treated when these structures were in the disorganized state (2), our observations were not unexpected. The effect of temperature on microtubule structure also may provide an explanation for the reversible inhibition of allergic histamine release by low temperatures.

The results also suggest an explanation for the previously reported amelioration of urticarial symptoms after intravenous administration of colchicine (4). Since colchicine does not have an antihistaminic action (4), this therapeutic effect could result from inhibition of cellular histamine release.

Summary. Colchicine inhibited the release of histamine from sensitized human leukocytes reacted with pollen antigens. Inhibition was significantly greater when cells were treated with colchicine at 0° prior to their reaction with antigen at 37° than when cells were pretreated at 37°. These observations are discussed in terms of the effect of colchicine and low temperatures on cytoplasmic microtubules. Involvement of these structures in the allergic secretion of histamine is proposed.

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Retarded Immunological Recovery in Sublethally X-Irradiated Mice by Additional Thymic Exposure. Reversal with Injected Marrow Cells* (33787)

WILLIAM E. DAVIS, JR. AND LEONARD J. COLE

Naval Radiological Defense Laboratory, San Francisco, California 94135

The pronounced inhibition of immune responses following sublethal X-irradiation and the requirement of an intact thymus for the subsequent recovery of immune competence are well documented [see reviews by Taliaferro (1), and Miller (2)]. Recent reports have indicated that transplanted cells of the thymus interact synergistically with cells present in the bone marrow to elicit immunocompetence in X-irradiated animals (3–5). *In vitro* observations showed that thymus–marrow cell combinations can reactivate immunological competence of irradiated spleen tissue (6). In the irradiated animal, therefore, recovery of the immune capacity might be dependent not only on recovery of thymic function but also on the recovery of the immunologic stem cells of the marrow. In the present study, the effect of these 2 parameters was investigated by re-exposing the thymus of sublethally whole-body X-irradiated mice to high doses of radiation in order to further delay thymic recovery, and by augmenting the pool of marrow cells in other similarly treated animals. The immunological capability of the mice was then measured at various time intervals. The results show: (i) the residual reticular-epithelial cells of the thymus in sublethally irradiated mice are functionally radiosensitive; (ii) that both the recovery of this thymic function and the supply of marrow-derived immunogenic precursor cells are important determinants of the recovery of immunocompetence in sublethally irradiated mice.

Methods. Adult (3–4 month old) (C57LXA/He)F_1 hybrid mice bred at this Laboratory, were exposed to 500 rad whole-body X-radiation in perforated Lucite tubes placed on a rotating table. The radiation factors were as follows: 250 kVp, 15 mA, 0.5 mm Cu and 1 mm Al filters, 28 rad/min dose rate, and 40 cm TSD. Within 1 hr some of these mice were anesthetized (sodium pentobarbital) and taped into ⅛ in. thick lead boxes (i.d., 7 × 11 × 2 cm) in such a manner that a circular hole in the lead, 1.2 cm in diameter, was located over the thymus. This was done by palpating the first 3–4 segments of the sternum through the hole. The relationship of the irradiated area and the sternum are shown in Fig. 1. The shielded mice were then re-

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