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Histamine synthesis by mouse T lymphocytes through induced histidine decarboxylase

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SUMMARY

When spleen cells of C57BL/6 mice or mast cell-deficient W/W^v mice were cultured, their histidine decarboxylase (HDC) activity increased with increases in the histamine concentration in the cells and the medium. Addition of concanavalin A (Con A) or *Escherichia coli* lipopolysaccharide (LPS) enhanced the increase. The removal of adherent cells reduced both the control HDC activity and the response to the mitogens. Purified T lymphocytes responded to Con A but not to LPS. Neither Con A nor LPS had any effect on B lymphocytes. Treatment of T cells with anti-Thy-1.2 and complement completely abrogated the induction of HDC. Histamine synthesis dependent on Con A by T cells was stimulated by the addition of conditioned medium from peritoneal adherent cells activated with LPS. The addition of recombinant interleukin-1 (rIL-1) or peritoneal adherent cells fixed with paraformaldehyde significantly enhanced HDC induction dependent on Con A in T cells. These results suggest that histamine is synthesized by T lymphocytes through HDC and that the reaction was enhanced by a soluble factor(s) released from macrophages.

INTRODUCTION

Histamine mediates and modulates inflammation through a variety of immunoregulatory actions (Busse & Sosman, 1977; Hosoda, Ikedo & Saito, 1981; Griswold *et al.*, 1984; Kahn *et al.*, 1985; Nair, Grick & Schwartz, 1986; Nakano, Suzuki, Oh & Yamashita, 1986). The amine is released from mast cells and basophils upon activation of the cells with allergen–reagin complex (Leoutsakos & Pearce, 1986). Histamine is also produced *de novo* by histidine decarboxylase (HDC) in peritoneal-adherent cells with the aid of a soluble factor(s) released from mitogen-activated T cells, B lymphocytes or both (Oh *et al.*, 1988). During this study we also found that the removal of macrophages from the spleen cells did not totally abolish mitogen-dependent histamine biosynthesis; HDC induction still occurred in the culture of the cells remaining. The purpose of this study was to evaluate the role of T cells and B lymphocytes in HDC-dependent histamine biosynthesis. The results obtained indicated that histamine was synthesized by T lymphocytes through HDC and that a soluble factor(s) released from macrophages enhanced the process.

Abbreviations: Con A, concanavalin A; HDC, histidine decarboxylase; LPS, lipopolysaccharide; rIL-1, recombinant interleukin-1.

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MATERIALS AND METHODS

Mice

Mice of the C57BL/6 strain and the WBB6/F$_1$ (W/W^v) strain (Go, Kitamura & Nishimura, 1980) were purchased from the Shizuoka Experimental Animal Cooperative, Shizuoka. Mice of both sexes were used when 2–3 months of age.

Culture of spleen cells

Spleen cells were removed, washed, and suspended in a serum-free synthetic GIT medium (Daigo Nutritive Chemicals Co., Ltd, Osaka). Then 2 ml of suspension containing 2×10^7 cells were put into plastic dishes 3·5 cm in diameter containing 2×10^7 cells. Mast cells were not detected (<0·5%) by toluidine blue staining of the spleen cell suspensions used. Concanavalin A (Con A; Pharmacia Fine Chemicals, Uppsala, Sweden) or lipopolysaccharide (LPS; a Westphal preparation of *Escherichia coli* 055 : B5, Difco Laboratories, Detroit, MI) was added at the dose of 2·5 or 10 μg/ml, respectively. The dishes were incubated at 37° in a humid CO_2 incubator with an atmosphere of 95% air–5% CO_2 for 48 hr. After incubation, the cells and the medium were separated by centrifugation at 250 ***g***. Cell viability was assayed by trypan blue dye exclusion. The cells and the medium were measured for HDC activity and histamine concentration as described elsewhere (Oh *et al.*, 1988).

Purification of T and B lymphocytes

The spleen cells were first passed through a Sephadex G-10

column to remove macrophages (Ly & Mishell, 1974). The eluate was termed T+B lymphocytes. Macrophages were not detected in the T+B-cell fraction as judged by non-specific esterase staining. Some cultures of T+B lymphocytes were treated with cycloheximide (1 μg/ml; Wako Pure Chemical Industries Ltd, Osaka). T lymphocytes were obtained by putting cells that had passed through Sephadex G-10 onto a nylon-wool column (Julius, Simpson & Herzenberg, 1973). Contamination by Ig-bearing cells of the T-cell preparations was below 2%. Some T-cell fractions were treated with anti-Thy-1.2 (final dilution, 1:1500; Cedarlane Laboratories, Ontario, Canada) and complement (low tox M rabbit complement, final dilution 1:5; Cedarlane). B lymphocytes were purified by sequential separation on a Sephadex G-10 column and anti-Thy-1.2 and complement (Vogel, Hilfiker & Caufield, 1983). No Thy-1-positive cell was detected in the B-cell preparations (<0·1%). Each cell fraction was suspended in 2 ml of serum-free GIT medium at the concentration of 1×10^7 cells/ml and put into plastic dishes 3·5 cm in diameter.

Culture of T lymphocytes in the presence of IL-1, fixed peritoneal-adherent cells, or conditioned medium of peritoneal-adherent cells stimulated with LPS

In one experiment, recombinant human interleukin 1 (rIL-1; the generous gift of Otsuka Pharmaceutical Co., Ltd, Tokushima) was added to the culture of T lymphocytes at concentrations of 10–1000 U/ml. Conditioned medium of peritoneal-adherent cells was prepared as follows. Peritoneal-adherent cells were obtained from peritoneal exudates elicited with glycogen and purified as described earlier (Oh *et al.*, 1988). The cells (1×10^6) were put into dishes 3·5 cm in diameter each containing 2 ml of a serum-free GIT medium in the presence of 10 μg/ml LPS. The dishes were incubated for 24 hr at 37°. After incubation, the cells and the medium were collected separately. The cells were fixed with paraformaldehyde (Hurme, 1987). The medium was filtered through a membrane filter with a pore size of 0·45 μm, diluted to 50% with fresh GIT medium, and used as the conditioned medium. To dishes containing purified T lymphocytes (2×10^7) were added about 1×10^6 fixed adherent cells, 2 ml of the 50% conditioned medium, or GIT medium alone. Incubation was for 48 hr at 37° in a humid CO_2 incubator. After incubation, the cells and the medium were obtained separately by centrifugation at 250 *g* and assayed for both HDC activity and histamine concentration as described previously (Oh *et al.*, 1988). The histamine level and HDC activity, that had been originally contained in the conditioned medium of LPS-activated macrophages were subtracted from each value obtained from the culture of T cells in the presence of the conditioned medium.

Statistical analysis

The multiple range test after analysis of variance or Student's *t*-test was used as appropriate in each experiment (Dowdy & Wearden, 1983).

RESULTS

Effects of removal of macrophages from spleen cells on HDC activity and histamine biosynthesis

The culture of mouse spleen cells led to a spontaneous increase in the activity of HDC in the cells and the medium (Fig. 1). There was a concomitant increase in the histamine level in the medium. The addition of Con A greatly augmented the spontaneous increase of HDC activity in the medium with a concomitant increase in its histamine concentration. There was also an increase in the HDC activity and the histamine level in the cells, but it was far less than that observed in the medium. These results confirmed earlier ones that the enzyme was released from the cells shortly after its synthesis (Oh *et al.*, 1988). Therefore, the HDC activity and the histamine level in the medium alone were estimated in the later studies. The addition of LPS also enhanced the spontaneous increase in the HDC activity and the histamine concentration in the medium. However, the degree of increase was less than that of the culture treated with Con A. Removal of macrophages from the spleen cells by the use of a Sephadex G-10 column reduced both the control HDC activity in the remaining cells and their response to the mitogens. The accumulation of histamine in the medium was also retarded in the culture of the macrophage-depleted spleen cells. However, the remaining T+B cells still responded to Con A, leading to a significant increase in HDC-dependent histamine biosynthesis. Similar results were obtained with the culture of spleen cells of W/W^v mice, which are genetically deficient in mast cells (Fig. 2). The treatment of T+B lymphocytes with cycloheximide completely abrogated both spontaneous and Con A-dependent increases in the HDC activity and the histamine level in the

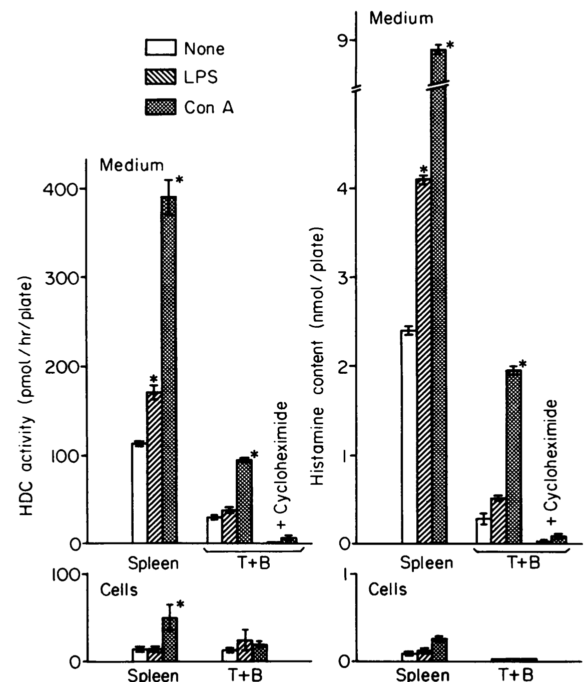

Figure 1. Effects of Con A and LPS on the activity of HDC and the histamine content in the cell and the medium of cultured spleen cells of C57BL/6 mice. Spleen cells and macrophage-depleted spleen cells were cultured in a volume of 2 ml containing 2×10^7 cells in the presence or absence of Con A (2·5 μg/ml), LPS (10 μg/ml), or cycloheximide (1 μg/ml) at 37° for 48 hr. The HDC activity and the histamine level in the cell and the medium were assayed separately. Each value represents the mean ± SEM (bars) of the three experiments. Statistically different from the unstimulated group: * $P < 0·05$.

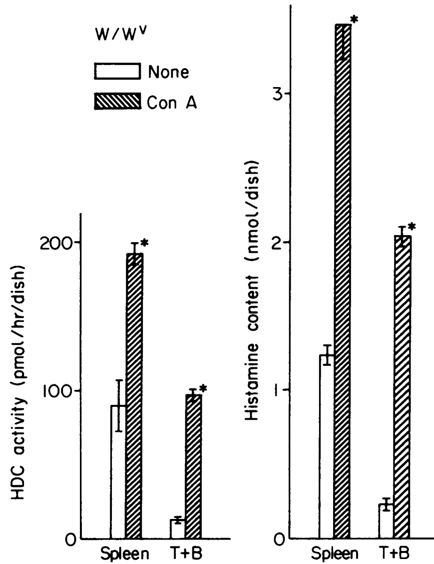

Figure 2. Effects of Con A on the HDC activity and the histamine level in the culture of spleen cells of W/W^v mice genetically deficient with mast cells. Spleen cells and macrophage-depleted spleen cells of the mice were cultured at the concentration of 1×10^7 cells/ml in the presence or absence of Con A (2·5 μg/ml) for 48 hr at 37°. After incubation, the medium was assayed for the HDC activity and the histamine level. Each value represents the mean ± SEM of three experiments. Statistically different from the unstimulated group: * $P < 0.01$.

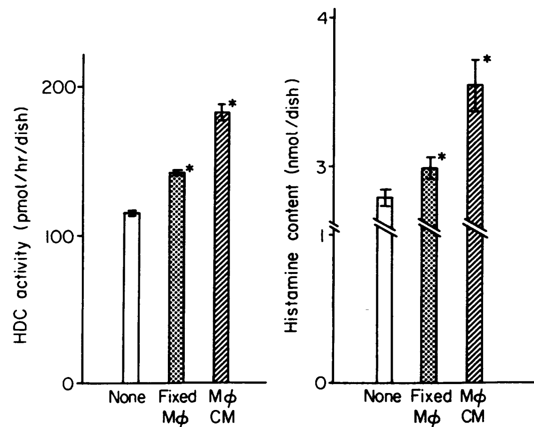

Figure 4. Effects of fixed macrophages (Mφ) and conditioned medium of macrophages on the HDC activity and the histamine level in the culture of T lymphocytes. T lymphocytes (2×10^7 cells/plate) were cultured for 48 hr in the presence of peritoneal-adherent cells activated with LPS and fixed with paraformaldehyde (about 1×10^6 cells/plate) or 50% conditioned medium of the peritoneal adherent cells activated with LPS as described in the Materials and Methods. The HDC activity and the histamine level in the medium were assayed. Each value represents the mean ± SEM of three experiments. Statistical differences between the culture with Con A alone added and the culture with Con A and fixed macrophages or macrophage-conditioned medium: * $P < 0.05$.

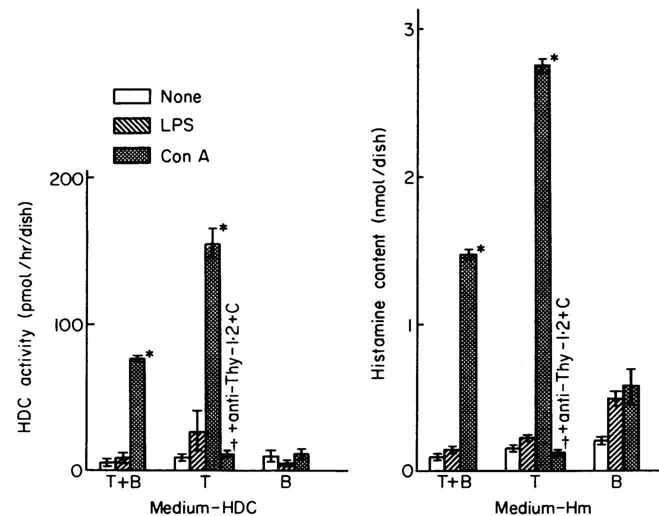

Figure 3. Effects of Con A and LPS on the HDC activity and the histamine level in the medium of cultured mouse splenic T and B lymphocytes. The conditions of culture were essentially the same as those described in the legend of Fig. 1. Some T-cell fractions were treated with anti-Thy-1.2 and complement, and cultured. Values are the mean ± SEM (bars) of three experiments. Statistically different from the unstimulated group: * $P < 0.05$. Statistically different from the group treated with Con A alone; † $P < 0.001$.

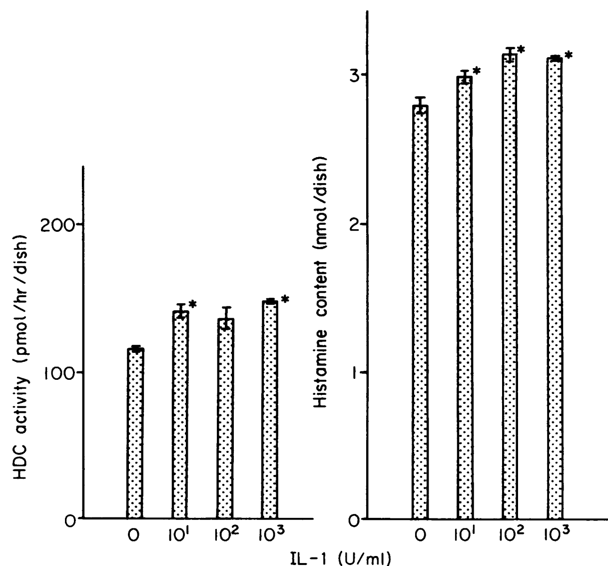

Figure 5. Effects of human rIL-1 on the HDC activity and the histamine level in the culture medium of mouse T lymphocytes as a function of dose. T cells were purified by sequential separation with a Sephadex G-10 column and a nylon-wool column. The cells (2×10^7 cells/plate) were cultured for 48 hr in the presence of Con A (2·5 μg/ml) with or without varying doses of human rIL-1. The HDC activity and histamine level in the culture medium were assayed. Each value represents the mean ± SEM of three experiments. Statistical differences between the culture with Con A alone added and that with Con A and IL-1 added: $P < 0.05$.

culture. Unlike Con A, LPS had little effect on the HDC activity and the histamine level in the culture of macrophage-depleted spleen cells of C57BL/6 mice.

Mitogen-dependent histamine synthesis by purified T cells and B lymphocytes

The results obtained in the experiments above indicated that macrophages may be important in mitogen-dependent histamine biosynthesis by spleen cells. There were two possible roles for the macrophages. First, macrophages *per se* may synthesize histamine, as suggested previously (Oh *et al.*, 1988). Second, macrophages may act as accessory cells in histamine synthesis by T cells or B lymphocytes. The next study was done to examine the second possibility. Mitogen-mediated histamine biosynthesis was studied with purified T cells and B lymphocytes. The addition of Con A to the culture of T lymphocytes provoked a similar and somewhat larger extent of increase in HDC activity and histamine concentration than that observed with T + B cells (Fig. 3). Treatment of these cells with anti-Thy-1.2 and complement totally abrogated the reactions. LPS had no appreciable effects on HDC activity and histamine biosynthesis by T cells. There was no notable change in HDC activity or the histamine level in the culture of B lymphocytes.

Effects of conditioned medium of LPS-activated peritoneal-adherent cells on histamine biosynthesis by T lymphocytes

The results of the above studies suggest that histamine was synthesized by T lymphocytes and that macrophages enhanced the reaction. Macrophages secrete various soluble factors, such as IL-1, that activate T cells (Decker, Lohman-Mattheus & Gifford, 1987). Therefore, the next study was done to examine the possible role of macrophage-derived factor(s) on histamine synthesis by T lymphocytes. The addition of conditioned medium of peritoneal-adherent cells stimulated with LPS significantly increased both the HDC activity and the histamine concentration in the culture medium of T cells (Fig. 4).

Effects of rIL-1 or peritoneal-adherent cells activated with LPS and fixed with paraformaldehyde and on histamine biosynthesis dependent on Con A by T lymphocytes

We evaluated the possible roles of soluble and membrane-associated IL-1 on HDC-dependent histamine synthesis by T cells. We used human rIL-1, because mouse IL-1 was not available and because these two molecular species seem not to be different in their stimulation of mouse T cells (Gery, Gershon & Waksman, 1972; Mortensen *et al.*, 1988). Large amounts of IL-1 are expressed on the surface of macrophages after activation of the cells with LPS (Hurme, 1987). The membrane-associated IL-1 may be more important than soluble IL-1 in the activation of T cells (Hurme, 1987). To examine the role of membrane-associated IL-1, LPS-activated macrophages were fixed with paraformaldehyde and added to the culture of T lymphocytes. The addition of IL-1 caused a slight but significant increase in the HDC activity and histamine level in the culture of T cells at its doses of 10–1000 U/ml (Fig. 4). Co-culture of T lymphocytes with peritoneal-adherent cells fixed with paraformaldehyde also significantly enhanced Con A-dependent HDC induction and the accumulation of histamine in the culture of T cells (Fig. 5).

DISCUSSION

It is unlikely that the increase in the histamine level in the medium of cultured spleen cells was due to the release of preformed histamine stored in mast cells, because (i) the increase in the histamine level was due to HDC-dependent *de novo* synthesis of the amine; (ii) histamine was also produced by the spleen cells of W/W^v mice which are genetically deficient in mast cells. However, one can not totally neglect the possibility that mast cells are responsible, in part, for the Con A-dependent HDC induction; the degree of HDC induction was less in the spleen cells from W/W^v mice than that in the cells from C57BL/6 mice (Figs 1 and 2), in which some (< 0·5%) mast cells/basophils were still contained.

The results obtained here suggest that macrophages are important in histamine synthesis by spleen cells. Macrophages may act biphasically. First, the cells may produce histamine by themselves with the aid of a soluble factor(s) released from mitogen-activated T cells and B lymphocytes, as described elsewhere (Oh *et al.*, 1988). Second, macrophages may enhance histamine synthesis by T lymphocytes. Macrophages synthesize histamine at a rate 10–20 times that of T cells (Oh *et al.*, 1988). Therefore, histamine synthesis by T-cell preparations may be due to contamination with macrophages. However, this is unlikely, as the population of macrophages in the T-cell fractions was less than 1/200 that of T cells.

Dy and his group also showed that spleen cells produced histamine in response to T-cell mitogens (Dy & Lebel, 1983; Schneider *et al.*, 1987). They hypothesized that histamine was produced by mast cell progenitors and that lymphokines such as IL-3 and granulocyte–macrophage colony-stimulating factor activate the reaction. The latter view was a conclusion from the finding that medium conditioned with spleen cells activated the histamine synthesis by bone marrow cells. Medium conditioned with spleen cells has a large amount of HDC, which is synthesized and 'released' into the medium, as shown in Fig. 1. Therefore, histamine production in the culture of bone marrow cells in the presence of conditioned medium of spleen cells (Dy & Lebel, 1983) may be due, in part, to HDC that was originally in the medium. In this study, the HDC activity that had been in the conditioned medium of macrophages originally was subtracted from each value obtained with the culture of T cells. In addition, Dy & Lebel (1983) found no increase or a slight increase in histamine production by spleen cells after B-cell mitogens. LPS activates macrophages, leading to HDC-dependent histamine biosynthesis (Oh *et al.*, 1988). Therefore, the findings by Dy & Lebel (1983) may reflect different processes from those studied here (Oh *et al.*, 1988).

This study showed that macrophages enhanced HDC-dependent histamine synthesis by T lymphocytes. Free and membrane-bound IL-1 may bring about the action of macrophages. Another soluble factor(s) may also participate in macrophage-dependent histamine synthesis by T cells. Histamine synthesis by T cells did not recover, even in the presence of the macrophage-conditioned medium, to the level of that elicited by whole spleen cells. This may indicate that the presence of living macrophages is necessary for T cells to be fully activated.

Histamine has a variety of immunoregulatory actions (Barsoum *et al.*, 1984). However, almost all of the reports of these actions were from studies on the effects of histamine added

exogenously to each immune system. Our suggestion that histamine is produced by a macrophage–T-lymphocyte system may explain the reason for previous unexplained dealing with the immunoregulatory actions of this amine. For example, the histamine H_2-antagonist cimetidine alone, in the absence of any exogenous histamine, enhances lymphocyte blastogenesis (Ogden & Hill, 1980; Gifford, Hatfield & Schmidke, 1980). The addition of Con A to the culture of human peripheral blood leucocytes activates suppressor T lymphocytes, and the process is abrogated by histamine-type 2 antagonists (Schnaper, Aune & Roby, 1987). No persuasive explanation has been given of these immunoregulatory actions of histamine-type 2 antagonists *per se*. Histamine, that is produced in the macrophage–T-cell system, may mediate the immune reactions. Thus, histamine-type 2 antagonist may block the actions of endogenous histamine.

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