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Increase of histidine decarboxylase activity in murine myelomonocytic leukemia cells (WEHI-3B) in parallel to their differentiation into macrophages

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When cells of mouse myelomonocytyc leukemia cell line, WEHI-3B, were cultured in the presence of actinomycin D plus the serum which was obtained from mice injected with bacterial endotoxin, i.e., lipopolysaccharide, their histidine decarboxylase (L-histidine carboxy-lyase, EC 4.1.1.22) (HDC) activity increased about 100-fold with a peak at 48 h. According to the increase in HDC activity, the expression of surface antigens associated with macrophages, such as Mac II, Mac III and Ia^d, increased markedly on WEHI-3B cells as well as their morphological changes to macrophages. Histamine levels in the culture medium increased concomitantly with the increase in the HDC activity in WEHI-3B cells, whereas the histamine contents inside the cells did not increase remarkably. Furthermore, the addition of lipopolysaccharide to the culture medium caused an additional 2-fold increase in the HDC activity of WEHI-3B cells. These results indicate that the increase in HDC activity in WEHI-3B cells may represent an event in the process of the differentiation to macrophages.

Introduction

Although most histamine in the tissues of mice is produced in mast cells, it has been reported that cells other than mast cells, such as enterochromaffin-like cells and neurons, have a histamine-producing enzyme, L-histidine decarboxylase (L-histidine carboxylyase, EC 4.1.1.22) (HDC) and produce histamine [1–4]. These notions were also supported by experiments using WBB6F_1-*W*/*W*^{v} mice (*W*/*W*^{v} mice) which are genetically deficient in mast cells [5] but still contain a significant amount of histamine in some organs, such as the stomach and brain [6]. Our previous paper demonstrated that intravenous injection of staphylococcal enterotoxin A, which is also known to be a T cell mitogen, induced HDC in some organs such as the spleen, lung and liver of *W*/*W*^{v} mice and actually increased their histamine contents, suggesting that HDC was induced in non-mast cells [7]. Subsequently, we tried to identify the cells

Abbreviations: HDC, L-histidine decarboxylase (L-histidine carboxy-lyase, EC 4.1.1.22); post-endotoxin serum, the serum which was obtained from mice injected with bacterial endotoxin, i.e., lipopolysaccharide.

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responsible for the induced HDC and suggested by in vivo studies that these cells might be phagocytic cells of bone marrow origin [8]. Furthermore, we developed the in vitro system of the HDC induction in peritoneal resident cells from W/W^v mice and suggested that macrophages were responsible for the induced HDC in peritoneal resident cells on the basis of the results obtained by various cell fractions [9].

To confirm our idea of being macrophages as the responsible cells for the HDC induction, we used murine macrophage-related cell lines. Cooper et al. [10] reported that cloned myelomonocytic leukemia cells (WEHI-3B) predominantly differentiated to macrophages on their culture in the presence of a low concentration of actinomycin D plus the serum of mice injected with bacterial endotoxin, post-endotoxin serum. Using this system, we found that HDC activity increased in WEHI-3B cells time-dependently during their differentiation to macrophages. The relationships between the increase of HDC activity and the differentiation of WEHI-3B cells to macrophages were discussed.

Materials and Methods

Animals. C57BL/6 mice of 8 to 12 weeks old and pregnant Spraque-Dawley rats (300–400 g) were obtained from Shizuoka Laboratory Animal Center (Hamamatsu, Japan).

Reagents. Mouse recombinant granulocyte-macrophage colony-stimulating factor was a gift from Sumitomo Pharmacol. Co. (Osaka, Japan). The following reagents were purchased: Actinomycin D (Sigma Chemicals, St. Louis, MO, U.S.A.), lipopolysaccharide (*Escherichia coli* 0127: B8; Difco Laboratories, Detroit, IL, U.S.A.) and concanavalin A (Pharmacia, Uppsala, Sweden). All other chemicals were of the highest grade commercially available unless otherwise stated and were used without further purification.

Cells and cell culture. The myelonomonocytic leukemia cell line, WEHI-3B, was obtained from Dr. Asano (Institute of Medicine, Tokyo University) and macrophage cell lines, P388D1, RAW264.7 and PU5-1.8, were obtained from the American Type Culture Collection. J774.2 was a kind gift from Professor Ito, Department of Anatomy, Tohoku University School of Medicine. Cells of these cell lines were mantained in 50 × 10 mm Petri dishes (Corning, NY, U.S.A.) containing RPMI 1640 (M. A. Bioproducts, Walkerville, MD, U.S.A.) supplemented with 10% (v/v) fetal calf serum (M.A. Bioproducts, Walkerville, MD, U.S.A.) at 37 °C in an atmosphere of 5% CO_2/95% air (v/v). Cultures of these cell lines were passaged every 3 days by collecting loosely adherent cells by a washing with 0.15 M NaCl/0.01 M potassium phosphate buffer (pH 7.4)/0.02% (w/v) EDTA.

Preparations of conditioned media. The post-endotoxin serum was obtained from C57BL/6 mice 3 h after the had been intravenously injected with 5 μg lipopolysaccharide, and stored at −80 °C until use. The content of lipopolysaccharide in the post-endotoxin serum was assayed by the Toxicolor Test (a kind of the Limulus amebocyte lysate assay) (Seikagaku Kogyo Co., Tokyo). The rat-embryo secondary culture condition medium was prepared as follows. Rat embryo cells were seeded at 10^7 cells per 90-mm plastic petri dish in Eagle's minimum essential medium supplemented with 10% (v/v) fetal calf serum. When a monolayer of the cells was formed after 2 to 3 days of culture, the cell sheets were treated with 1% (w/v) trypsin. Cells were washed with Eagle's medium and then cultured at 10^7 cells per dish for another 5 days at 37 °C. The cell suspension was centrifuged at 118 × *g* for 10 min, and the supernatant was preserved as rat-embryo secondary culture condition medium at −80 °C until use. To prepare concanavalin A-stimulated spleen cell-culture medium, spleen cells from C57BL/6 mice were incubated for 3 days at $5 \cdot 10^6$ cells/ml in RPMI 1640 medium containing 10% (v/v) fetal calf serum/2 mM L-glutamine/4 μg/ml concanavalin A. The culture was centrifuged and the supernatant was stored at −80 °C until use.

Detection of surface antigens. WEHI-3B ($1 \cdot 10^6$ per ml) cells cultured with or without actinomycin D plus post-endotoxin serum were washed three times with 0.15 M NaCl/0.01 M potassium phosphate buffer (pH 7.4)/1% (v/v) fetal calf serum and resuspended in saline. To saturate non-specific binding sites, the cells were incubated for 30 min at 4 °C with either 50 μl of 1 mg/ml goat anti-rat IgG, or 40 μl of 1 mg/ml goat anti-mouse IgG.

They were washed three times with the saline, and were further incubated for 30 min at 4°C with 50 μl of anti-Mac II antigen rat monoclonal IgG antibody (10 μg/ml) (Hybritech, San Diego, CA, U.S.A.), or Mac III antigen rat monoclonal antibody (10 μg/ml) (Hybritech, San Diego, CA, U.S.A.), or 20 μl of anti-Ia^d antigen mouse monoclonal IgM antibody (1:100 dilution) (Meiji Institute of Health Science, Tokyo, Japan) as the first antibody and again washed three times with saline. Then the cells were incubated for 30 min at 4°C with either 20 μl of 1 mg/ml fluorescein isothiocyanate-conjugated goat anti-rat (Cappel Lab., Cochrancille, PA, U.S.A.) or anti-mouse (Cappel Lab., Cochrancille, PA, U.S.A.) IgG as the second antibody. After three more washes, the cells were analyzed with an EPICS V cytofluorometric analyzer (Coulter Electronics, Hialeah, FL, U.S.A.).

Cell counts. Suspended cells were counted in a standard hemocytometer. The proportion of viable cells was estimated by the trypan blue exclusion test. In some experiments, cytocentrifuged preparations of WEHI-3B cells were stained with May-Grünwald-Giemsa stain for differential counts.

Assays of HDC activity and histamine contents. For HDC assay, the cultured cells were homogenized in 0.5 ml of cold solution A (0.1 M potassium phosphate buffer (pH 6.8), 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5′-phosphate, 1% poly(ethylene glycol) (average molecular weight 300, (w/w)), and 0.5 μg/ml each of leupeptin and chemostatin) in a Polytron homogenizer (Kinematica, Lucern, Switzerland) operated at the maximum setting for two 10-s periods in an ice bath. The homogenate was centrifuged at 10000 × *g* for 20 min and the supernatant was dialyzed three times against 100 vol. of solution A. HDC activity was assayed as described previously [11]. Briefly, the dialysate was incubated with 0.25 mM L-histidine for 15 h, and histamine was separated from histidine on a short column of Amberlite CG-50, and measured fluorometrically by the *o*-phthalaldehyde method [12] as described by Watanabe et al. [11]. For histamine analysis, WEHI-3B cells were homogenized with 1.0 ml of 3% perchloric acid, and the homogenate was centrifuged as described above. The supernatants and cell-free culture media containing 3% perchloric acid were subjected to histamine analysis. Histamine was measured fluorometrically by the *o*-phthalaldehyde method [12] in an HPLC system as described by Yamatodani et al. [6,13].

Results

Time-course of HDC activity in WEHI-3B cells, and changes of histamine contents in cells and culture medium

HDC activity was very low in WEHI-3B cells during culture without actinomycin D and post-endotoxin serum, but increased very much in their presence (Table I). WEHI-3B cells cultured with actinomycin D or post-endotoxin serum alone showed lower HDC activities, as shown in Table I. Fig. 1 shows HDC activity in WEHI-3B cells during their culture in the presence of actinomycin D and post-endotoxin serum. The HDC activity increased gradually during culture with them; the maximum HDC activity was found at 48 h of culture. Beyond 48 h, the extent of HDC activity decreased or plateaued between 48 and 72 h. In accord with the increase in enzyme activity, the histamine content in culture medium increased, but that in cells did not increase remarkably. The cell number of WEHI-3B cells during the culture with actinomycin D plus post-endotoxin serum almost doubled in 24 h and then reached a plateau of about half the maximum population without actinomycin D plus post-endotoxin serum.

TABLE I

EFFECTS OF ACTINOMYCIN D AND POST-ENDOTOXIN SERUM ON HDC ACTIVITY IN WEHI-3B CELLS

WEHI-3B cells ($4 \cdot 10^6$) were cultured in 10 ml medium containing 5 ng/ml actinomycin D, 2% (v/v) post-endotoxin serum, or actinomycin D plus post-endotoxin serum. After 2 days culture, cells were harvested and HDC activity was assayed as described in Materials and Methods.

Addition to culture	HDC activity (pmol/min per 10^7 cells)
None	0.01
Actinomycin D	0.18
Post-endotoxin serum	0.06
Actinomycin D + post-endotoxin serum	1.13

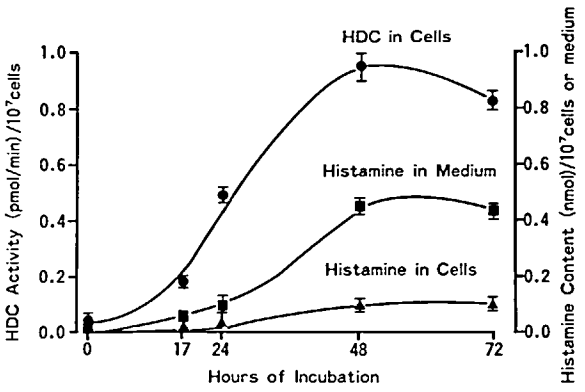

Fig. 1. Time-course of HDC activity in WEHI-3B cells and histamine content in the cells and medium during culture. WEHI-3B cells ($4 \cdot 10^6$) in 10 ml of the medium were cultured in the presence of 5 ng/ml actinomycin D plus 2% (v/v) post-endotoxin serum in 90×10 ml plastic Petri dishes at 37°C with 5% (v/v) CO_2/95% (v/v) air. The cells were harvested at various times and HDC activity was assayed, and histamine in the cells and culture medium was measured as described in Materials and Methods.

Morphological changes in WEHI-3B cells cultured in the presence of actinomycin D plus post-endotoxin serum

Fig. 2 shows the comparative morphology of WEHI-3B cells cultured with or without actinomycin D plus post-endotoxin serum. The cells cultured for 2 days with actinomycin D plus post-endotoxin serum were large and heterogenous with numerous vacuoles in the cytoplasm, typical features for macrophages (b). On the other hand, the cells cultured without actinomycin D plus post-endotoxin serum showed a typical morphology of myelomonocytes with a large normal nucleus (a). These observations were consistent with the results obtained by other workers [10,14].

Changes of surface antigens on WEHI-3B cells cultured with actinomycin D plus post-endotoxin serum

Using indirect immunofluorescence and flow cytometric analyses, we examined the expression of cell surface antigens in WEHI-3B cells, such as Mac II, Mac III and Ia^d antigens which are associated with the differentiation into macrophages. As shown in Fig. 3, WEHI-3B cells showed minimum reactivity with anti-Mac II or Mac III, but did react significantly with anti-Ia^d antigen. In contrast, the expression of these three antigens was markedly increased on WEHI-3B cells during the 48 h incubation in the presence of actinomycin D plus post-endotoxin serum. Kinetic analyses of the expression of those antigens showed that the maximum expressions on WEHI-3B cells were observed at 24 h of culture (data not shown). Quantitative comparison of cells cultured with actinomycin D plus post-endotoxin serum vs. those without this addition showed a 3–4-fold increase in Mac II and Mac III and a 5-fold increase in Ia^d antigen. From fluorescence microscopic observations it appeared that about 80% of the cells at 48 h of culture was positive to Mac II and Mac II antigens, and almost all the cells were positive to Ia^d antigen.

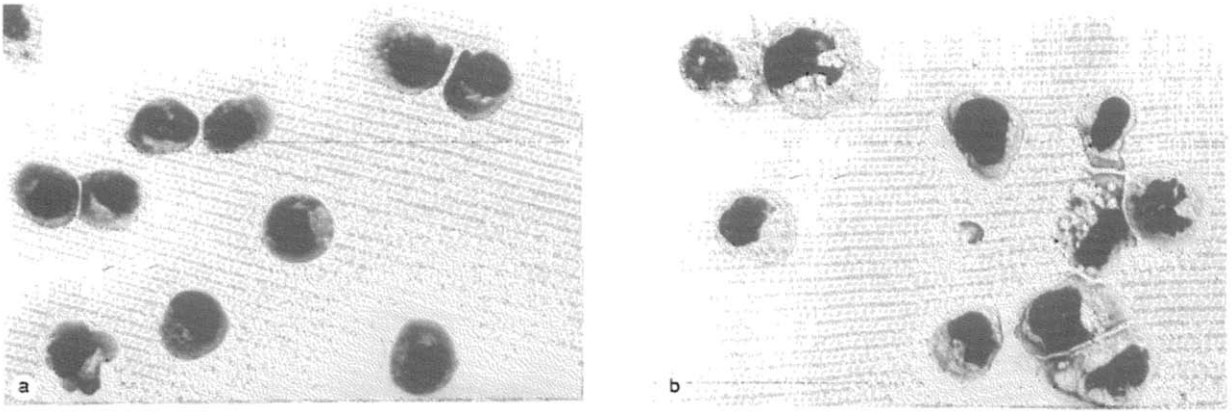

Fig. 2. Morphological changes of WEHI-3B cells cultured in the presence of actinomycin D plus post endotoxin serum. The cells at 0 (a) and 48 (b) h of culture. Wright-Giemsa staining (×400).

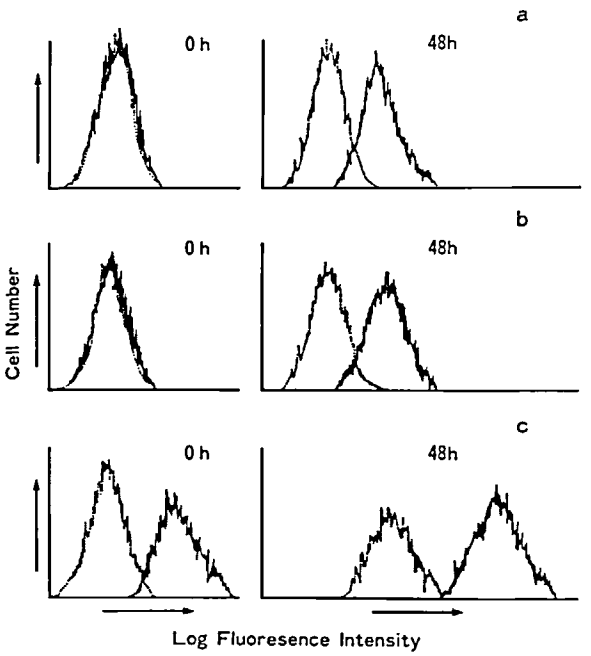

Fig. 3. Flow cytometric analysis of Mac II, Mac III and Ia^d antigen expression in WEHI-3B cells cultured in the presence of actinomycin D plus post-endotoxin serum. The cells were cultured for 0 or 48 h. The cells treated with the second antibody unconjugated with fluorescein isothiocyanate were shown in (dash line). Cells were stained with anti-Mac II (a), anti-Mac III (b) or anti-Ia^d (c) monoclonal antibody (solid line). For details, see Materials and Methods.

Effects of reagents stimulating the differentiation of WEHI-3B on HDC activity

It is well-known that a high level of granulocyte-macrophage colony-stimulating factor is induced in post-endotoxin serum of mice [15]. We used the mouse recombinant granulocyte-macrophage colony-stimulating factor instead of post-endotoxin serum in the culture of WEHI-3B, and examined its effect on the HDC activity. As shown in Table II, the recombinant factor was also effective for the increase in HDC activity in WEHI-3B cells and for the production of histamine in the culture medium: Almost 250 units per ml of the factor was enough for the increase in HDC activity.

Enhancement of HDC activity in WEHI-3B cells by lipopolysaccharide

As shown in Table III, HDC activity in WEHI-3B increased about 100-fold when the cells were cultured with actinomycin D plus post-endotoxin serum containing a very low amount of lipopolysaccharide (less than 0.1 ng/ml). The addition of lipopolysaccharide to the culture media caused an additional 2-fold increase in HDC activity in WEHI-3B cells after 2 days when compared to that of cells cultured without lipopolysaccharide (with actinomycin D plus post-endotoxin serum only) (Table III). It is well-known that many factors for the differentiation of myelomonocytic leukemia cells are contained in the rat-embryo secondary culture condition medium or the concanavalin A-stimulated spleen cell culture medium. The replacement of actinomycin D plus post-endotoxin serum by the two media in the culture of WEHI-3B cells caused an enhancement of HDC activity similar to lipopolysaccharide, although either medium only did not remarkably increase HDC activity.

HDC activities of other macrophage-related cell lines, P388D1, J774.2, RAW 264.7 and PU5-1.8

Mature macrophage cell lines, such as P388D1, J774.2, RAW 264.7 and PU5-1.8, were employed to examine their ability for the HDC induction.

TABLE II

EFFECTS OF POST-ENDOTOXIN SERUM AND RECOMBINANT MOUSE GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR ON HDC ACTIVITY IN WEHI-3B CELLS

WEHI-3B cells ($4 \cdot 10^6$) were cultured in the presence of 5 ng/ml actinomycin D plus 2% (v/v) post-endotoxin serum or various amounts of the recombinant factor. After 2 days culture, cells were harvested and HDC activity in the cells and histamine contents in culture media were assayed as described in Materials and Methods.

Addition to culture	HDC activity (pmol/min per 10^7 cells)	Histamine content (pmol/ medium)
None	0.01	28
Post-endotoxin serum	0.71	2040
Granulocyte macrophage colony-stimulating factor		
(250 units)	0.42	1180
(500 units)	0.43	1320
(1000 units)	0.53	n.d. [a]

[a] n.d., not determined.

TABLE III

ENHANCEMENT OF HDC ACTIVITY IN WEHI-3B CELLS BY LIPOPOLYSACCHARIDE

WEHI-3B cells ($4 \cdot 10^6$) were cultured in 10 ml of medium containing 5 ng/m actinomycin D plus 2% (v/v) post-endotoxin serum, 10% (v/v) concanavalin A-stimulated spleen cell-culture medium. After 2 days culture, 1 μg/ml lipopolysaccharide was added to some dishes and cells were incubated for 6 h at 37°C. HDC activity was assayed as described in Table I. Values (mean ± S.E.) were obtained from the three separated dishes.

Addition to culture	Lipopoly-saccharide	HDC activity (pmol/min per 10^7 cells)
None	−	0.01 ± 0.02
	+	0.13 ± 0.05
Actinomycin D + post-endotoxin serum	−	1.51 ± 0.28
	+	2.99 ± 0.64
Rat-embryo secondary culture condition medium	−	0.09 ± 0.15
	+	2.96 ± 1.52
Concanavalin A-stimulated spleen cell-culture medium	−	0.08 ± 0.15
	+	2.81 ± 1.55

TABLE IV

HDC ACTIVITY IN VARIOUS KINDS OF MACROPHAGE CELL LINES

$(3–4) \cdot 10^6$ cells of each macrophage cell line were cultured in 10 ml of medium or medium containing 10% (v/v) concanavalin A-stimulated spleen cell-culture medium plus 1 μg/ml lipopolysaccharide for 2 days. HDC activity was assayed as described in Table I. Values (mean ± S.E.) were obtained from the three separated dishes.

Cell line	Concanavalin A-stimulated spleen cell-culture medium plus lipopolysaccharide	HDC activity (pmol/min per 10^7 cells)
P388D1	−	0.01
	+	0.19 ± 0.05
J774.2	−	0.00
	+	0.03 ± 0.01
RAW264.7	−	0.00
	+	0.05 ± 0.01
Pu5-1.8	−	0.00
	+	0.14 ± 0.10

Table IV shows HDC activity in various kinds of macrophage cells cultured with concanavalin A-stimulated spleen cell-culture medium plus lypopolysaccharide or without those reagents. However, little HDC activity was observed in these macrophage cell lines, even after culture with the reagents, although P388D1 cells showed the highest HDC activity after the culture, but only 6% of that of WEHI-3B cells.

Discussion

Our previous studies suggested that murine peritoneal macrophages have an ability to induce HDC [7–9]. This suggestion was mainly based on the following experiments, When peritoneal resident cells were incubated in vitro, HDC was remarkably induced with a peak at several hours. A similar pattern of HDC induction was observed even in the peritoneal resident cells which were derived from genetically mast-cell-deficient W/W^v mice [5]. Cells showing high HDC activity were phagocytic and plastic adherent. In the present work, murine myelomonocytic cell line WEHI-3B was used to confirm our previous suggestion. The present results showed that a high activity of HDC was found when WEHI-3B cells were cultured with actinomycin D plus post-endotoxin serum, reaching its maximum after 2 days of culture. Under these culture conditions, WEHI-3B cells differentiated predominantly into macrophages with morphological features such as many vacuoles inside cells and large heterogenous shapes, confirming the results reported by Cooper et al. [10] and Gonda and Metcalf [14]. In addition to these morphological changes, we found in the present work that macrophage-associated surface antigens, such as Mac II and Mac III, expressed newly on more than 80% of WEHI-3B cells at 48 h culture. The expression of Ia^d antigen, which is also known to be expressed on activated macrophages, increased time-dependently on WEHI-3B cells after culture with actinomycin D plus post-endotoxin serum. These changes of surface antigen expressions on WEHI-3B cells cultured with actinomycin D plus post-endotoxin serum are consistent with the results reported by Cooper et al. [10] in that the PAGE patterns of protein components of the extracts of

WEHI-3B cells cultured under the same conditions were more similar to those from peritoneal macrophages rather than to those from peritoneal polymorphs. On the other hand, Mac II, Mac III and Ia^d surface antigens were not detected on mast cells developed from bone-marrow cells by IL-3 cultivation [16]. So far no evidence has been presented that WEHI-3B cells differentiate to mast cells or basophils which are known to be histamine-producing cells. Therefore, this work represents the first evidence that the murine cell line which is not related to mast cells or precursors of mast cells has an ability to produce histamine. No increase in intracellular pool of histamine during the culture of WEHI-3B cells was observed, and the histamine produced was quickly released out of the cells to medium instead of storage inside the cells (Fig. 1). These results are similar to our previous results in peritoneal resident cells [9] and also support the idea that mast cells are not involved in the present case, because mast cells have the ability to store histamine in the cells.

As described in our previous paper, HDC was induced in peritoneal resident cells [9]. The maximum activity of the induced HDC in peritoneal resident cells was similar to that of WEHI-3B cells. (Compare HDC activities reported in Table III of Ref. 9 and Table III in the present paper.) However, the time-course of the increase in HDC activity in WEHI-3B cells by the cultivation with actinomycin D plus post-endotoxin serum was different from that of the previous case in peritoneal resident cells. In the latter case, maximum HDC activity was observed at several hours of in vitro culture, and the activity decreased to the original low level at 24 h of culture. As described above, in the present paper the maximum activity occurs during 24–48 h of culture. These differences may be due to the different types of cell, or different methods of induction, or involvement of different regulatory mechanisms. The longer time required for HDC induction is similar to results reported by Dy et al. [17]; 24–48 h was necessary for the induction by a lymphokine. Recently, Schneider et al. [18] reported that IL-3 or granulocyte-macrophage colony-stimulating factors induced de novo synthesis of HDC in hemopoietic progenitor cells [18]. In the present work, colony-stimulating factor may be involved in the increase in HDC activity in WEHI-3B cells, but the involvement of IL-3 was excluded since WEHI-3B cells do not produce IL-3 (J. Abe, Research Laboratories, Chugai Pharmaceutical Co., personal communication). As the present results showed that rat-embryo secondary culture or the concanavalin A-stimulated spleen cell-culture condition medium and lipopolysaccharide also affected the increase in HDC activity in WEHI-3B cells, it was suggested that cytokines or lymphokines other than colony-stimulating factor were also involved in the HDC induction. Actinomycin D is known to induce macrophage differentiation of other myelomonocytic leukemia cell line, M1 [19]. Actinomycin D (5 ng/ml) in the culture medium of the present work may function as another factor causing an increase in HDC activity.

Little increase of HDC activity was observed in other mature macrophages cell lines such as J774.2, P388D1, RAW 264.7 and PU5-1.8 cultured with concanavalin A-stimulated spleen cell-culture condition medium and lipopolysaccharide. From these results, it was suggested that mature macrophage cell lines have not an ability to induce HDC.

There are many reports that histamine may function as a modulater of differentiation of immune and hematopoietic cells through the elevation of intracellular concentrations of cyclic AMP [20,21], and that human monocytic-like cell lines such as HL-60 and U937, guinea pig alveolar macrophage and murine macrophage cell lines had receptors for histamine [21–26]. The present study supports the idea that myelomonocytic cells appeared to have HDC activity during the differentiation into mature macrophages. Thus, the possibility that histamine produced by myelomonocytic cells is involved in the differentiation as autocrine phenomenon is inferred. Studies are now in progress on isolated mouse peripheral monocytes to confirm this idea.

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