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INDUCTION OF DIFFERENTIATION OF CULTURED MOUSE MYELOID LEUKEMIC CELLS BY ARGINASE

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SUMMARY

Mouse myeloid leukemic cells (Ml) could be induced by a factor in ascitic fluid to phagocytize, migrate in agar, and change into forms that were morphologically similar to macrophages and granulocytes. Arginase also induced these differentiation-associated properties of the cells. The Ml cells did not differentiate in culture medium containing arginine, but they differentiated into macrophages and granulocytes during culture in arginine-deficient culture medium. Therefore, the effect of arginase may be attribute to arginase-mediated arginine depletion.

INTRODUCTION

Ml cells, established from an SL strain mouse, are myeloid leukemic cells that can be induced to differentiate into forms that are functionally and morphologically similar to macrophages and granulocytes by various inducers, including conditioned medium from various cell cultures (1-3), ascitic fluid of mice and rats bearing various tumors (4), and glucocorticoid hormones (5,6). On treatment with these inducers, Ml cells differentiate morphologically into cells like macrophages and granulocytes with phagocytic and locomotive activities and lysosomal enzymes (7-9).

We previously reported that peritoneal macrophages and granulocytes in ascites were responsible for the production of factors inducing differentiation of Ml cells, and that the factors were present in conditioned medium from macrophages and granulocytes (4). Recently it was reported that the cytotoxic activity of activated macrophages on neoplastic cells was associated with the induction and release of arginase by these macrophages (10). Therefore, in this paper we examined the effect of arginase on induction of differentiation of Ml cells.

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Table 1. Induction of differentiation-associated properties in Ml (B-9) cells by ascitic fluid or arginase

	Inducer		
	None	Arginase (8 U/ml)	Ascitic fluid (20%)
Cell growth (% of control)	100	54	140
Phagocytosis (% of phagocytic cells)	2.4	50.0	69.1
Locomotive activity (% of dispersed colonies)	0	56.0	70.0
Morphological changes			
Myeloblastic cells (%)	94	42	43
Cells in intermediate stages (%)	6	31	37
Mature macrophages or granulocytes (%)	0	27	20

Phagocytosis was determined 3 days after seeding with arginase. For assay of locomotive activity, arginase was poured onto 10-day-old colonies and the percentage of dispersed colonies was calculated. Ascitic fluid was obtained from ascites of rats with hepatoma AH-130 (13).

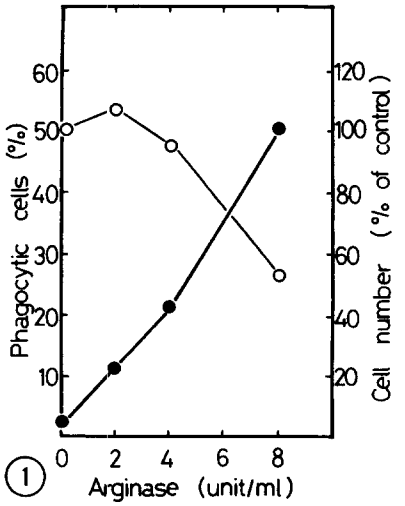
MATERIALS AND METHODS

Cell line and cell culture; Ml clone S-1 and B-9 can be induced to differentiate by various inducers (11). The cells were cultured in suspension in Eagle's minimum essential medium (MEM) containing twice the normal concentrations of amino acids and vitamins, and supplemented with 10% undialyzed calf serum (13). Arginase from bovine liver (Sigma, Chemical Co.) was added to this medium. Arginine-free Eagle's MEM (Nissui Seiyaku, Japan) with 10% undialyzed calf serum was used as "arginine-deficient" medium, although the added serum contained some arginine. L-Arginine (126 μg/ml, Sigma, Chemical Co.) was added to this medium when required.

Assay of the properties of differentiated cells; Phagocytic and locomotive activities were assayed by the procedures reported previously (12). For assay of phagocytic activity, cells were incubated for 4 h with a suspension of polystyrene latex particles (2 μl/ml serum-free medium, average diameter 1 μm, Dow Chemical Co.), and then the percentage of phagocytic cells in the total cells was calculated. The percentages of cells that were morphologically similar to granulocytes and macrophages were determined in smears treated with May-Grünwald-Giemsa stain (13).

RESULTS

Phagocytic activity, locomotive activity and morphological changes, which are differentiation-associated properties of Ml cells, were induced as well by



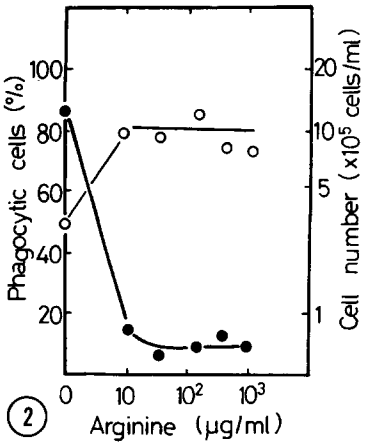


Fig.1. Effect of arginase on induction of phagocytic activity (●) and growth (○) of Ml cells. Ml (B-9) cells were incubated with or without various concentrations of arginase for 3 days. One unit (U) of arginase converts 1 μMole of L-arginine to ornithine and urea per minute at pH 9.5 at 37°C.

Fig.2. Induction of phagocytic activity of Ml cells cultured in arginine-deficient medium. Ml (B-9) cells were incubated with or without various concentrations of arginine in the presence of 10% undialyzed calf serum. Phagocytic cells (●) and the cell number (○) were counted 2 days later.

treatment of the cells for 3 days with bovine liver arginase as by treatment with ascitic fluid containing inducer (Table 1). Figure 1 shows the dose-response curve of arginase (0-8 units/ml) for induction of phagocytic activity. Growth of the cells was significantly inhibited by 8 units/ml of arginase, but differentiation of the cells could be induced without inhibition of cell growth by 4 units/ml of arginase.

Ml cells also differentiated when cultured for 3 days in arginine-deficient conditions (Table 2), but not in methionine or leucine-deficient conditions (data not shown). As shown in Fig.2, phagocytic activity could be induced by culturing the cells for 2 days in arginine-deficient medium, but not by culture in medium containing arginine. These results indicate that the differenti-

Table 2. Induction of differentiation-associated properties in Ml (B-9) cells by arginine-deficient conditions

	Arginine (126 μg/ml)	
	+	−
Cell growth (% of control)	100	19
Phagocytosis (% of phagocytic cells)	2.4	69.5
Morphological changes		
Myeloblastic cells (%)	97	22
Cells in intermediate stages (%)	3	37
Mature macrophages or granulocytes (%)	0	41

Induction of differentiation was determined 3 days after removal of arginine from the culture medium in the presence of 10% undialyzed calf serum, as described in Materials and Methods.

ation-inducing activity of arginase may be due to arginase-mediated arginine depletion.

The induction of phagocytic activity by inducer in ascitic fluid was also significantly inhibited by addition of excess arginine (Fig.3). The induction of phagocytic activity of Ml cells by ascitic fluid (20%, v/v) was inhibited 50% by addition of 100-200 μg/ml of arginine. The same concentrations of lysine or leucine did not inhibit induction of phagocytic activity, although higher concentrations of lysine (2.5-10 mg/ml) caused almost 50% inhibition (data not shown).

DISCUSSION

In this work we found that bovine liver arginase induced Ml cells to differentiate into macrophages and granulocytes (Table 1). In rats with hepatoma AH-130, arginase activity (1-3 units/ml) was detected in the ascitic fluid which has differentiation-inducing activity. Moreover, excess arginine inhibited the induction of differentiation of Ml cells by the ascitic fluid (Fig.3). These results suggest that the arginase activity in the ascitic fluid is partly responsible for its inducer activity. However, the arginase activity in ascitic

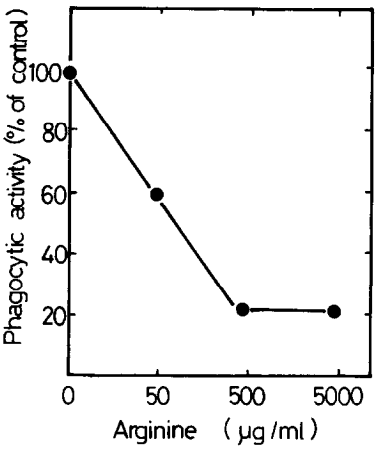


Fig.3. Effect of L-arginine on phagocytic activity of Ml cells induced by ascitic fluid. Ml (S-1) cells were incubated with arginine and 20% ascitic fluid, and activity was assayed 3 days later. The phagocytic activity of control cells was 35.5%. Phagocytic activity is shown as a percentage of that of control cells.

fluid is probably only partly responsible for induction of differentiation of Ml cells, since 20% ascitic fluid was effective for induction although its arginase activity is low. Moreover, no arginase activity was detected in conditioned medium of SL strain mouse embryo cells, which is known to be an inducer of Ml cells (data not shown).

Cell growth was significantly inhibited by treating the Ml cells with arginase or culturing them in arginine-deficient medium (Tables 1,2). The Ml cells, however, could not be induced to differentiate simply by inhibition of thier growth by treatment with 5-fluorodeoxyuridine (data not shown).

As described above, leukemic Ml cells were induced to differentiate into macrophages and granulocytes by treatment with arginase or by culture in arginine-deficient medium. Malignant cells are reported to require a higher concentration of arginine in the medium than their normal counterparts (14). A lymphoblastoid cell line (Mono-1-207) established from the peripheral blood of a patient with acute myelomonocytic leukemia exhibited myeloid cell properties

when cultured in arginine-deficient medium (15). These results suggest that arginine-depletion may be responsible for induction of differentiation of myeloid cells.

The present work suggests that it may be possible to use arginase in therapy of myeloid leukemia as L-asparaginase is used in therapy of lymphoma (16).

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