Biochimica et Biophysica Acta, 983 (1989) 259–263
Elsevier

BBAMEM 74502

Changes in neutral amino acid transport activity in myeloid leukemia cells differentiated by lipopolysaccharide

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(Received 14 February 1989)

Key words: Amino acid transport; Macrophage; Leukemia cell; Differentiation; Lipopolysaccharide; (Mouse)

M1 cells derived from mouse myeloid leukemia have been reported to differentiate to macrophage-like cells upon treatment with substances such as lipopolysaccharide. Previously we found that in mouse peritoneal macrophages most of the neutral amino acids were taken up through a unique Na^+-independent system. In this paper we have investigated the neutral amino acid transport in M1 cells and in those treated with lipopolysaccharide. In M1 cells serine, alanine and proline were taken up mainly by Na^+-dependent transport systems, and leucine was largely transported by a Na^+-independent system. By treating the cells with lipopolysaccharide, the activities of the Na^+-dependent systems markedly decreased, whereas the activity of the Na^+-independent system was little affected. The amino acid concentrations in the cells and the culture medium were measured. As a whole, the intracellular to extracellular distribution ratios for neutral amino acids that are preferred substrates for Na^+-dependent systems were decreased on lipopolysaccharide treatment, whereas those for amino acids that are mainly transported by a Na^+-independent system were slightly increased. From these results we conclude that M1 cells treated with lipopolysaccharide tend to differentiate to macrophage-like cells with respect to the neutral amino acid transport.

Introduction

Transport of amino acids in mammalian cells is carried out by specific systems of mediation acting on discrete groups of substrate molecules [1]. Systems A and ASC are Na^+-dependent neutral amino acid transport systems especially reactive with short, or linear side chain amino acids, and system L is a Na^+-independent system especially reactive with branched chain and aromatic amino acids. The uptake of amino acids seems to be important for cells to function or to grow, and there are many studies on the change of amino acid transport as the cells develop [2–4] or transform to malignant cells [5–7]. M1 is a myeloid cell line established from a spontaneous leukemia of an SL strain mouse [8]. It has been reported that some cell lines derived from myeloid leukemia, including M1 and HL-60 [9], lose the tumorigenic ability and differentiate to mature macrophage-like cells by exposure to various chemicals, e.g., lipopolysaccharide (LPS), glucocorticoides, or a phorbol ester [10–13]. There is, however, little information about the change of amino acid transport activity in these cells when they are treated with such chemicals [14].

We reported previously that in mouse peritoneal macrophages most of neutral amino acids were mainly transported through a common transport system with some resemblance to system L [15]. This system is quite unique, however, in that it accepts almost all neutral amino acids with a similar reactivity.

In the present study, we have investigated the change of neutral amino acid transport activity when M1 cells were treated with LPS. The results demonstrate the decrease in Na^+-dependent transport systems and little change in the Na^+-independent system on LPS treatment.

Materials and Methods

Materials

L-[3-^{3}H]Serine and L-[2,3-^{3}H]alanine were obtained from Amersham International, Amersham, Great Britain. L-[2,3-^{3}H]Proline and 3-*O*-methyl-D-[^{14}C]glucose were from Du Pont-New England Nuclear, Boston, MA and L-[2,3,4,5-^{3}H]leucine was from ICN Corp., Irvine,

Abbreviations: LPS, lipopolysaccharide; BCH, 2-aminobicyclo(2.2.1) heptane-2-carboxylic acid.

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CA. 2-Aminobicyclo(2,2,1)heptane-2-carboxylic acid (BCH) was obtained from Behring Diagnostics, La Jolla, CA. Unlabeled amino acids were from Sigma, St. Louis, MO. Culture media were from GIBCO, Chagrin Falls, OH, and fetal bovine serum was from M.A. Bioproducts, Walkersville, MD. Bacto Lipopolysaccharide was obtained from DIFCO, Detroit, MI.

Cell culture

M1 cells were kindly provided by Dr. K. Onozaki of the Tsukuba University. The cells were subcultured every day with a split ratio of 1 : 2 or 1 : 3 in RPMI 1640 medium containing 5% fetal bovine serum inactivated at 56 °C for 30 min, 50 units/ml penicillin, and 50 μg/ml streptomycin.

Uptake of amino acids

M1 cells were plated at $2.5 \cdot 10^5$ cells/ml in a 35 mm diameter plastic culture dish, or at $1.0 \cdot 10^6$ cells/ml in the dish with 50 μg/ml LPS. M1 cells tended to adhere to the culture vessels. Amino acid uptake was measured in these adhered cells. Amino acid uptake was carried out by techniques described previously [16]. The cells were rinsed three times in warmed PBSG (10 mM phosphate-buffered saline, pH 7.4, containing 0.01% $CaCl_2$, 0.01% $MgCl_2 \cdot 6H_2O$, and 0.1% glucose), and incubated in 0.5 ml of the warmed uptake medium, which was PBSG containing a labeled amino acid, for a given time period at 37 °C. In experiments on the uptake of amino acids in a Na^+-free medium, Na^+ was replaced by choline. The uptake was terminated by washing the dish rapidly three times with ice-cold phosphate-buffered saline, and then the radioactivity in the cells was counted. The rates of the uptake were determined under conditions approaching the initial uptake rates, i.e., by taking the values for the 60-s uptake of serine, alanine, or proline, and the value for the 15-s uptake of leucine.

Determination of amino acid contents in cells and medium

Amino acid contents in the cells were determined as described previously [17]. Amino acids were extracted from the cells with 5% trichloroacetic acid. The acid extract, to which 5 nmol of norleucine was added as an internal standard, was completely evaporated, dissolved in 0.01 M HCl, and analyzed by a Durrum D-500 amino acid analyzer programmed with the standard physiological sample procedures. The steady-state distribution of 3-*O*-methyl-D-glucose was used to estimate the apparent intracellular water volume as described by Kletzien et al. [18]. Amino acid contents in the culture medium were measured as follows: To 0.5 ml of the culture medium, 50 nmol of norleucine was added as an internal standard and this medium was deproteinized by an addition of 0.05 ml of 50% sulfosalicylic acid. After 30 min in an ice bath, the mixture was frozen and stored until the assay. The frozen sample was thawed and then centrifuged at $10000 \times g$ for 20 min. The supernatant solution was taken, its pH adjusted to 2.0 by 1 M LiOH, and 50 μl of the solution assayed by the amino acid analyzer.

Results

Changes in the rate of the uptake of serine and leucine were measured in M1 cells after treatment with LPS (Fig. 1). The activity of serine uptake gradually decreased in the cells cultured with LPS, whereas that of leucine uptake did not change significantly during the culture with LPS. The growth of M1 cells was strongly inhibited by LPS, and the morphological change of the cells began to be observed after two days in culture. To make the cell number nearly equal at the time of the assay, we seeded $2.5 \cdot 10^5$ cells/ml in experiments without LPS and used the cells after 48 h in culture without LPS, but in the case of treatment with LPS we seeded the cells at $1.0 \cdot 10^6$ cells/ml with LPS and used the cells after 72 h in culture in the following experiments.

We investigated the Na^+ dependency for the uptake of serine, alanine, proline and leucine in M1 cells treated with LPS or in the control (not treated with LPS) cells (Table I). In the control cells serine, alanine and proline were mainly transported by the Na^+-dependent route. A similar tendency was observed in LPS-treated M1 cells. The rates of the uptake of these amino acids in the

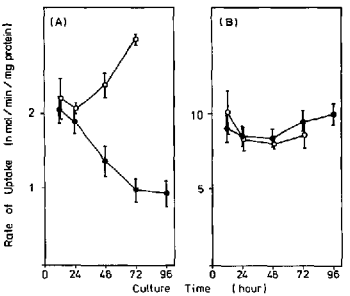

Fig. 1. Changes in the rate of the uptake of serine (A) and leucine (B) in M1 cells after the treatment with LPS. The rate of the uptake of 0.05 mM L-[^{3}H]serine and L-[^{3}H]leucine was measured in LPS-treated cells (●) and in the control (non-treated) cells (○), respectively. The control cells were plated at $2.5 \cdot 10^5$ cells/ml and LPS-treated cells were seeded at $1.0 \cdot 10^6$ cells/ml with 50 μg/ml LPS. Each point represents the means ± S.D. of four determinations from two experiments.

TABLE I

Effect of LPS on the uptake of serine, alanine, proline and leucine

Cells were plated at $2.5 \cdot 10^5$ cells/ml or at $1.0 \cdot 10^6$ cells/ml with 50 μg/ml LPS. The uptake of 0.05 mM [^{3}H]serine, [^{3}H]alanine, [^{3}H]proline and [^{3}H]leucine was measured in a Na^+-containing or a Na^+-free medium (pH 7.4). The results are the means ± S.D. of at least four determinations from two experiments.

Amino acid	LPS	Amino acid uptake (nmol/min per mg protein)		Na^+ dependency (%)
		$+Na^+$	$-Na^+$	
Ser	−	2.48 ± 0.40	0.08 ± 0.01	96.8
	+	0.97 ± 0.14	0.11 ± 0.08	88.7
Ala	−	3.87 ± 0.65	0.13 ± 0.02	96.6
	+	1.31 ± 0.37	0.19 ± 0.10	85.5
Pro	−	1.02 ± 0.12	0.06 ± 0.03	94.1
	+	0.27 ± 0.04	0.08 ± 0.04	70.4
Leu	−	7.59 ± 0.57	5.68 ± 0.44	25.2
	+	8.87 ± 0.79	5.95 ± 0.56	32.9

cells treated with LPS, however, decreased to less than a half of those of the control cells. On the other hand the uptake of leucine was similar in LPS-treated cells and in control cells with respect to the Na^+ dependency and the rate of the uptake.

Rather arbitrarily, we divided the total uptake of the amino acid into systems A, ASC, and L components using the methods described by Shotwell et al. [19]. We have designated the part of the total uptake of a test amino acid in a Na^+-containing buffer which is inhibited by 2-methylaminoisobutyric acid as system A transport. The remaining Na^+-dependent, 2-methylaminoisobutyric acid-noninhibitable uptake has conventionally been attributed to system ASC transport. System L uptake has been defined as the uptake of an amino acid in Na^+-free buffer which is subject to inhibition by BCH. The Na^+-independent, BCH-insensitive uptake of an amino acid then remains as a measure of the nonsaturable component. The Na^+-dependent part of the uptake of serine, alanine, and proline was, according to these definitions, mainly by system A, with its activity sharply decreased by treating the cells with LPS (Fig. 2). The activity of system ASC was also influenced by the treatment but to a lesser extent. The activity of system L did not change at all on treatment with LPS.

We measured the initial rate of the uptake of serine at 0.025–2 mM in both LPS-treated and non-treated cells, and the Michaelis-Menten parameters were determined by graphing the data as a double-reciprocal plot (Fig. 3). An apparent K_m value obtained in LPS-treated cells was 0.77 mM, which was quite similar to the value obtained in the control cells. However, the V_{max} value of LPS-treated cells was 15.5 nmol/min per mg of cell protein, about half that of the control cells. This decline suggests that the decrease in the activity of

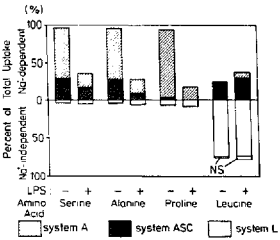

Fig. 2. Division of the total uptake of some neutral amino acids into contributions of individual transport systems. The total uptake of 0.05 mM [^{3}H]serine, [^{3}H]alanine, [^{3}H]proline and [^{3}H]leucine was divided into its transport system contributions as follows. System A, the part of the total uptake in the Na^+-containing medium which is inhibited by 2.5 mM 2-methylaminoisobutyric acid. System ASC, the part of the Na^+-dependent uptake which is not inhibited by 2.5 mM 2-methylaminoisobutyric acid. System L, the part of the Na^+-independent uptake which is inhibited by 2.5 mM BCH. Nonsaturable (NS), the part of the Na^+-independent uptake which is not inhibited by 2.5 mM BCH. Each component is represented as the percent of the total uptake in the control cells. The result is the means of four determinations from two experiments.

serine uptake in LPS-treated cells results from the decrease in the number of transport carriers for serine.

Changes of amino acid concentrations in the cells and in the culture medium were also investigated in the cells treated with LPS (Table II). The intracellular to extracellular distribution ratios for amino acids are also given in the table. The distribution ratios for some amino acids that were mainly transported by Na^+-de-

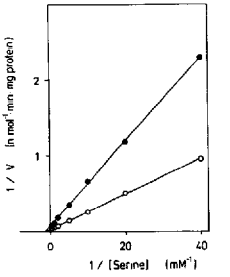

Fig. 3. Double-reciprocal plots of the rates of the uptake of serine in LPS-treated and non-treated M1 cells. The uptake of L-[^{3}H]serine at 0.025, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mM was measured in LPS-treated cells (●) and in the control cells (○). The data points are the means of four determinations from two experiments.

TABLE II

Amino acid concentrations in M1 cells and in the culture medium

Cells were plated at $2.5 \cdot 10^5$ cells/ml or at $1.0 \cdot 10^6$ cells/ml with 50 μg/ml LPS. The amino acids in the cells and in the medium were determined after 2 days for control cells and after 3 days for LPS-treated cells. The value of the apparent intracellular water volume for the control cells and LPS-treated cells were 5.5 μl/mg of cell protein and 5.3 μl/mg of cell protein, respectively. The results are the means of four determinations from two experiments.

Amino acid	− LPS			+ LPS		
	cell (mM)	medium (mM)	cell/ medium	cell (mM)	medium (mM)	cell/ medium
Asp	5.00	0.166	30.1	0.74	0.173	4.28
Thr	0.77	0.073	10.5	1.29	0.112	11.5
Ser	1.44	0.053	27.2	1.68	0.132	12.7
Asn	8.22	0.289	28.4	4.10	0.373	11.0
Glu	11.8	0.403	29.3	12.5	1.02	12.3
Gln	1.24	0.151	8.21	0.24	0.078	3.08
Pro	2.11	0.099	21.3	2.99	0.156	19.2
Gly	4.45	0.283	15.7	7.29	0.339	21.5
Ala	9.04	0.312	29.0	7.23	0.476	15.2
Val	0.17	0.064	2.66	0.34	0.103	3.30
Met	n.d. [a]	0.041	–	n.d. [a]	0.060	–
Iso	0.64	0.194	3.30	1.06	0.221	4.80
Leu	0.53	0.164	3.23	1.03	0.212	4.86
Tyr	0.20	0.054	3.70	0.37	0.076	4.87
Phe	0.12	0.034	3.53	0.28	0.056	5.00
Orn	0.41	0.181	2.27	0.39	0.218	1.79
Lys	0.15	0.071	2.11	0.30	0.143	2.10
His	0.21	0.050	4.20	0.31	0.068	4.56
Arg	1.77	0.612	2.89	1.38	0.649	2.13

[a] n.d., non detected.

pendent manner, e.g., serine, alanine and glutamine, decreased on LPS treatment. On the other hand the distribution ratios for the amino acids that were largely taken up by a Na^+-independent route, e.g., leucine, valine and phenylalanine, were slightly increased on LPS treatment. The distribution ratios for basic amino acids and some neutral amino acids were little changed on treatment with LPS, whereas those for acidic amino acids, i.e., glutamate and aspartate decreased markedly.

Discussion

Previously we reported that in mouse peritoneal macrophages most of the neutral amino acids, including serine and alanine, were mainly taken up by a Na^+-independent system with a unique substrate specificity [15]. This transport system is different from the Na^+-independent system ase found in erythrocytes [20,21] and has never been reported in cells other than macrophages. M1 cells are known to differentiate to macrophage-like cells on LPS treatment and it seemed of interest to investigate whether M1 cells treated with LPS might have a transport system similar to that observed in macrophages. It has been reported that such neutral amino acids as alanine and serine are largely transported by Na^+-dependent systems in a variety of cell types including Ehrlich ascites cells [22,23], Chinese hamster ovary cells [19], human fibroblasts [24], and rat hepatocytes [25]. Similar transport systems can be seen in leukocytes including leukemic lymphocytes and granulocytes [26], normal leukocytes [27], peripheral lymphocytes [28], lymphocytes derived from the thymus or spleen [29], and murine P388 leukemia cells [30]. The present experiments demonstrated that M1 cells per se had transport systems for neutral amino acids similar to those of the above cells but did not have the system characteristic of macrophages.

By treating M1 cells with LPS, the activities of systems A and ASC decreased,while that of system L did not change at all. The activity of system A increases in 3T3 cells [5] and BHK21 cells [6] when they are transformed by certain viruses. In virus-transformed chicken embryo fibroblasts the activity of system A increases by transformation with unchanged activity of system L [7]. In the present study the change of neutral amino acid transport of malignant cells during differentiation to cells which have a character similar to that of normal cells was demonstrated. From these results, it is likely that only the activities of Na^+-dependent systems change when the normal cells transform to malignant cells or when the malignant cells differentiate to the apparently normal cells. The property of the Na^+-independent transport system observed in mouse peritoneal macrophages is clearly different from the Na^+-independent system of M1 cells treated with LPS in that alanine and serine are the preferred substrates of the system in peritoneal macrophages. Considering the process of the differentiation of M1 cells to macrophage-like cells by LPS treatment from the view of the neutral amino acid transport, LPS causes a lowering the activities of Na^+-dependent systems, but does not cause the qualitative change of the Na^+-independent system into one analogous to the macrophage system.

When B-lymphocytes from chronic lymphocytic leukemia, which showed a specific diminution of system L, had been treated with a phorbol ester to differentiate to cells that had a function similar to normal lymphocytes, the activity of system L of the leukemia B-lymphocytes was restored to the level comparable to normal cells [10]. The change in the transport system in these cells differs from that observed in M1 cells here. Considering the results for leukemia lymphocytes and for M1 cells, it is suggested that restoration of the normal cell functions in malignant cells may generally accompany the change in amino acid transport.

The present paper showed the cell-to-medium distribution ratios for amino acids in the culture of both malignant cells and those differentiated to apparently normal cells. The distribution ratios in M1 cells are much higher than those in the peritoneal macrophages

[15]. Presumably this reflects the difference between the transport systems of M1 and those of the macrophages. Changes in the distribution ratios for some neutral amino acids by LPS treatment reflected the change of the amino acid transport activity. However, the ratios for other neutral amino acids did not conform to the change in the activity of the transport systems. The metabolic rates of each amino acid in the cells may cause this inconsistency between the change in distribution ratios and the change in the transport activity.

The macrophage-specific system with a broad substrate specificity was not found in LPS-treated M1 cells, although Na^+-dependent systems of M1 cells largely disappeared on LPS treatment. Other factors in addition to LPS might be necessary to induce the macrophage-specific system in M1 cells. These and other myeloid leukemia cell lines seem to provide excellent experimental models for the investigation of the role of amino acid transport in the differentiation, malignant transformation and cell growth.

Acknowledgement

We are grateful to Dr. Kikuo Onozaki for helpful comments. This study was supported by a grant for scientific research from the Ministry of Education, Science and Culture of Japan (63570108).

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