INDUCTION OF CYSTINE TRANSPORT ACTIVITY IN MOUSE PERITONEAL MACROPHAGES

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The transport of amino acids is a cell membrane function that is sensitive to environmental changes and plays an important role in cell activities. We have described in cultured human fibroblasts a Na^+-independent, anionic amino acid transport system highly specific for cystine and glutamate (1, 2). This system is a kind of anion-exchanging agency and cystine is transported in an anionic form in exchange for glutamate (3). Since the intracellular pool of cystine is negligibly small, whereas that of glutamate is very large, the physiologic flows through this system are the entry of cystine and the exit of glutamate, coupled together. In human fibroblasts, cystine is almost exclusively taken up via this route, and therefore, cellular levels of cysteine (reduced form of cystine) and glutathione (GSH)[1] are greatly influenced by the activity of the system (4). This cystine/glutamate exchange system, which is designated as System x_c^- (5), has been found also in rat hepatoma cells (5), rat hepatocytes (6), and various types of cultured cells (7).

Macrophages are actively involved in the inflammatory and immune responses of the host as a result of their extensive endocytic capacity and a wide variety of secretory products. GSH plays an important role in many aspects of the macrophage function; it is a direct precursor of leukotriene C (8) and is used as a cofactor in PG biosynthesis (9). In addition, GSH affords protection against oxidative damage, and this may be of particular importance because macrophages produce large quantities of reactive oxygen metabolites in response to inflammatory stimuli. Thus a detailed study of GSH metabolism in macrophages has been reported (10). However, a role for membrane transport of the precursor amino acids in the GSH metabolism has not been studied in macrophages so far. It is generally believed that cysteine is a rate-limiting precursor for GSH synthesis and that the intracellular GSH level is regulated by availability of cysteine (11). We have undertaken a study on cystine transport in purified macrophage preparations. Our findings concern a drastic induction of the activity of the cystine transport and its effect on intracellular GSH are presented.

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[1] *Abbreviations used in this paper:* GSH, glutathione; BCH, 2-amino(2,2,1)-bicycloheptane-2-carboxylic acid.

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Materials and Methods

Reagents. [3,3′-^{3}H]-L-Cystine, [2,3-^{3}H]-L-glutamate, [2,3-^{3}H]-L-alanine, [4,5-^{3}H]-L-leucine, and [3-^{3}H]-L-serine were obtained from Amersham International, Amersham, United Kingdom. Thioglycollate broth (Brewer's formula) was from Difco Laboratories Inc., Detroit, MI. 2-Amino(2,2,1)bicycloheptane-2-carboxylic acid (BCH) was from Calbiochem-Behring Corp., La Jolla, CA. Other amino acids and GSH reductase were from Sigma Chemical Co., St. Louis, MO. Dibutyl phthalate, GSH, and 5,5′-dithiobis(2-nitrobenzoic acid) were from Wako Chemicals, Ltd., Osaka, Japan. Mineral oil was from Nakarai Chemicals, Ltd., Kyoto, Japan. Culture medium and FCS were from Gibco Laboratories, Grand Island, NY.

Macrophage Cultures. Macrophages were collected by peritoneal lavage from male C57 BL/6N mice, weighing 20–25 g (Doken Co., Ibaraki, Japan), that had received an intraperitoneal injection of 2 ml of 4% thioglycollate broth 4 d previously. In some experiments, macrophages obtained from untreated mice or mice that had been injected with mineral oil or FCS were used. However, unless otherwise stated, macrophages elicited by thioglycollate broth were used throughout. The lavage medium was RPMI 1640 containing 10 U/ml heparin. The cells were washed twice with RPMI 1640, plated at 10^6 per 35-mm culture dish in RPMI 1640 containing 10% FCS, 50 U/ml penicillin, and 50 μg/ml streptomycin, and were incubated at 37°C in 5% CO_2 in air. After 1 h, the medium was changed to a fresh one to remove nonadherent cells.

Uptake of Amino Acid. Amino acid uptake was measured by techniques described previously (1). After culture of the cells in a 35-mm diameter dish, the cells were rinsed three times in warmed 10 mM PBS containing 0.01% $CaCl_2$, 0.01% $MgCl_2$ $6H_2O$ and 0.1% glucose. The cells were then incubated in 0.5 ml of the warmed uptake medium for specified time periods at 37°C. The uptake medium consisted of the same PBS used to rinse the cells and labeled amino acid (1 μCi/0.5 ml). The uptake was terminated by rapidly rinsing the dishes three times with ice-cold PBS, and the radioactivity in the cells was determined as described before (1). The rates of uptake were determined under conditions approaching initial rates, i.e., by taking the values for the 2-min uptake of cystine or glutamate, and for the 1-min uptake of alanine, serine, or leucine.

Amino acid uptake by suspended or unattached cells was measured according to the method of Novogrodsky et al. (12). The peritoneal cells were washed twice and suspended at 1.2×10^6 cells per 0.1 ml in warmed PBS containing $CaCl_2$, $MgCl_2$, and glucose. Uptake was started by addition of 0.02 ml of [^{3}H]-L-cystine solution (0.3 mM and 0.5 μCi) to 0.1 ml of the cell suspension. The suspension was incubated at 37°C for 2 min with shaking. Then 0.1 ml of the suspension was removed and layered on a mixture (0.2 ml) of mineral oil and dibutyl phthalate (15:85 by volume) in micro tubes. Cells were rapidly separated from the medium by centrifugation for 0.3 min in Microfuge B (Beckman Instruments, Inc., Palo Alto, CA) and the radioactivity in the cell pellet was measured.

Assay of Glutamate and GSH in the Cells and Thiol Concentration in the Medium. Glutamate content in the cells was measured as described previously (3). Amino acids were extracted from 5×10^6 cells plated in two 60-mm diameter dishes, and analyzed by a Li^+ citrate-equilibrated column of a D-500 amino acid analyzer (Durrum Instrument Corp., Palo Alto, CA) programmed with the standard physiological sample procedure. GSH content in the cells was measured by enzymatic method described previously (4), which is based on the catalytic action of GSH in the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) by the GSH reductase system (13). Thiol concentration in the culture medium was measured with 5,5′-dithiobis(2-nitrobenzoic acid) as described previously (14).

Results

Induction of the Activity of Cystine Uptake during Culture. Peritoneal macrophages were isolated from thioglycollate-treated mice and the uptake of cystine by these cells was investigated before and after culture. Fig. 1 illustrates the time-dependent changes in the rate of the uptake of cystine during culture. The rate

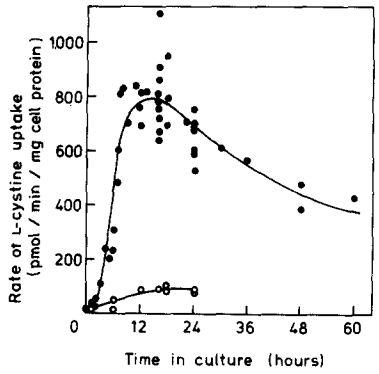

FIGURE 1. Changes in the rates of the uptake of L-cystine by macrophages during culture. Macrophages, elicited by thioglycollate broth, were cultured and at the times indicated, the rates of the uptake of L-cystine (0.05 mM) were measured. (●) Macrophages were cultured in the normal medium; (○) macrophages were cultured in the medium containing cycloheximide (0.5 μg/ml). Cumulative data of nine separate experiments are presented. Each point represents a mean value of duplicate assays.

TABLE I

Enhancement of L-Cystine Uptake during Culture in Resident or Elicited Macrophages

Macrophages	Rate of uptake of L-cystine (pmol/min/mg cell protein)	
	Cultured for 1 h	Cultured for 16 h
Resident	5 ± 2	3,580 ± 980
Elicited by FCS	25 ± 3	1,120 ± 220
Elicited by mineral oil	33 ± 7	1,790 ± 880

Macrophages were collected by peritoneal lavage from untreated mice or from mice that had received, 4 d previously, an intraperitoneal injection of 1 ml each of FCS or mineral oil. They were cultured for 1 or 16 h and the rates of uptake of L-cystine (0.05 mM) were measured. Values are means ± SD of six determinations in three separate experiments.

of the uptake, which was determined under conditions approaching the initial rate (2 min uptake), was very low before culture (19 ± 11 pmol/min/mg cell protein) or in the early time in culture (34 ± 13 pmol/min/mg cell protein, after 2 h in culture). However, it increased greatly and reached maximum after 16 h (797 ± 134 pmol/min/mg cell protein). It should be noted that cells before culture (i.e., time 0 in Fig. 1) contained all peritoneal exudate cells, whereas cells after culture contained adherent cells only. Cycloheximide potently inhibited the increase, suggesting that the increase in the rate of the uptake requires protein synthesis. Increases in the rate of cystine uptake during culture in resident macrophages or macrophages elicited by other irritants were also examined and the results are summarized in Table I. Obviously, the rate of the uptake of cystine was greatly enhanced during culture in any macrophage preparation. Resident macrophages responded the best, but because only a few resident macrophages were obtained from mice, the following experiments were done using macrophages elicited by thioglycollate broth.

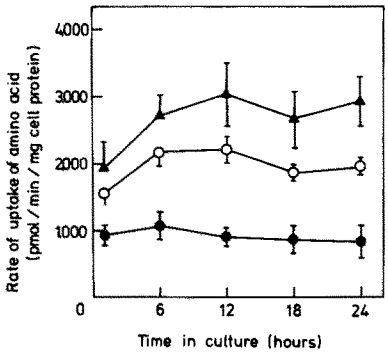

FIGURE 2. Changes in the rates of the uptake of L-alanine, L-leucine, and L-serine by macrophages during culture. Macrophages, elicited by thioglycollate broth, were cultured and at the times indicated, the rates of the uptake of L-alanine (○), L-leucine (●), and L-serine (▲) were measured. Amino acid concentration in the uptake medium was 0.05 mM. Data are shown as means ± SD of six determinations in three separate experiments.

Fig. 2 shows changes in the rates of uptake of some neutral amino acids during culture. In contrast to the uptake of cystine, no marked changes in the rates of the uptake of serine, alanine, and leucine were observed, although the uptake of serine and alanine was significantly enhanced during the first 6 h in culture.

Characterization of the Induced Activity of the Cystine Uptake. The rates of the uptake of cystine by macrophages cultured for 16 h were measured in the medium containing or not containing Na^+. In the latter case, Na^+ in the uptake medium was replaced by choline. The results clearly showed that within an experimental error there was no difference between the rates of the uptake of cystine in Na^+-containing and Na^+-free medium. The uptake of cystine was almost entirely Na^+-independent. Then the rates of the uptake of cystine were measured in the presence of the excess of an amino acid (Table II). This experiment affords information about the ability of the amino acid to interfere with the cystine uptake. It was found that the cystine uptake was strongly inhibited by glutamate, homocysteate, α-aminoadipate, and α-aminopimelate. These are anionic amino acids with relatively long side chains. Aspartate and cysteate, which have short side chains, inhibited the cystine uptake to a lesser extent. Imino acids and cationic amino acids had very little effect on the cystine uptake, whereas most of neutral amino acids inhibited the uptake by ~40%. α-(Methylamino)isobutyrate and BCH are the model substrates for the amino acid transport System A and L, respectively (15).

The rates of the uptake of cystine at the various concentrations were measured in the absence and in the presence of glutamate and the results are shown as double reciprocal plots (Fig. 3). The plots were linear and had a crossing point near the ordinate, showing that the uptake of cystine apparently follows saturation kinetics and that the inhibition of the cystine uptake by glutamate is competitive. The K_m for cystine and the K_i for glutamate were estimated to be ~0.04 mM and 0.1 mM, respectively.

The pH profiles for the cystine uptake and its inhibition by glutamate are shown in Fig. 4. The cystine uptake showed a marked dependence on pH; it was strongly enhanced upon elevating the pH of the uptake medium. Glutamate was

TABLE II
Comparative Inhibitory Action of Various Amino Acids on the Uptake of L-Cystine

Amino acid added (2.5 mM)	Percent inhibition of uptake of L-cystine (0.05 mM)
L-Proline	7.8 ± 3.8
L-Hydroxyproline	4.4 ± 4.7
L-Lysine	2.4 ± 10.0
L-Arginine	4.1 ± 10.6
Glycine	30.7 ± 7.2
L-Alanine	44.5 ± 5.8
L-Serine	47.5 ± 2.9
L-Threonine	37.0 ± 4.6
L-Asparagine	42.2 ± 4.3
L-Glutamine	47.4 ± 2.9
L-Methionine	45.5 ± 4.6
L-Valine	45.6 ± 5.2
L-Leucine	42.4 ± 4.0
L-Isoleucine	42.8 ± 2.4
L-Phenylalanine	41.3 ± 1.5
L-Tyrosine	41.1 ± 1.8
L-Tryptophan	42.3 ± 0.5
L-Histidine	47.3 ± 4.2
L-Aspartate	35.0 ± 4.2
L-Glutamate	92.1 ± 1.1
L-Cysteate	47.4 ± 6.7
L-Homocysteate	92.3 ± 1.7
D,L-α-Aminoadipate	92.0 ± 1.1
D,L-α-Aminopimelate	84.0 ± 0.2
α-(Methylamino)isobutyrate	4.0 ± 12.6
BCH	40.7 ± 11.3

Macrophages were cultured for 16 h and the rates of the uptake of L-cystine (0.05 mM) were measured in the presence of the amino acid. Values are means ± SD of two to four separate experiments with duplicate assays for each.

a potent inhibitor for the cystine uptake throughout the pH range examined; the inhibition by glutamate was not influenced by pH.

Uptake of Glutamate. Since the results described above suggest a transport interaction between cystine and glutamate, we investigated the uptake of glutamate in macrophages. Fig. 5 shows changes in the rates of the uptake of glutamate during culture. The activity of the glutamate uptake was very low during the early time in culture, but it was greatly enhanced during culture in a similar manner to that of cystine. The induced activity of the glutamate uptake was mainly Na^+-independent and was inhibited more potently by cystine than by aspartate (Table III).

The pH profiles for the uptake of glutamate and its inhibition by cystine are illustrated in Fig. 6. The uptake of glutamate was almost insensitive to pH in the medium, whereas the inhibition of the uptake by cystine clearly depended on pH.

In cystine/glutamate exchange system, cystine or glutamate is taken up in

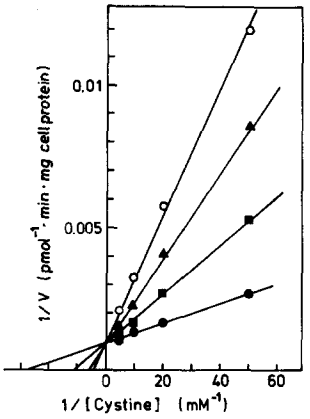

FIGURE 3. Double reciprocal plots of the inhibition of the L-cystine uptake by L-glutamate. Macrophages cultured for 16 h were used. The rates of the uptake of L-cystine at various concentrations were measured in the absence or in the presence of L-glutamate. Concentration of L-glutamate: (●) 0 mM; (■) 0.1 mM; (▲) 0.2 mM; (○) 0.5 mM.

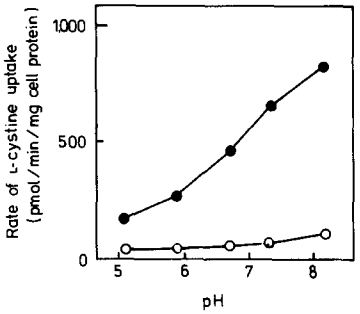

FIGURE 4. pH profile of the uptake of L-cystine. Macrophages cultured for 16 h were used. The rates of the uptake of L-cystine (0.05 mM) were measured in the absence (●) or in the presence (○) of 2.5 mM L-glutamate at various pH. The data are from one experiment typical of three similar experiments.

exchange for the efflux of glutamate from the cell, and therefore the rates of uptake of radiolabeled cystine or glutamate are affected by the intracellular concentration of glutamate (3). We measured the content of glutamate in macrophages with the amino acid analyzer. It was 32.9 ± 3.9 nmol/mg cell protein (mean ± SD of six experiments) for macrophages cultured for 1 h, and 34.2 ± 2.5 nmol/mg cell protein for macrophages cultured for 16 h. Thus, a possibility that the uptake of cystine or glutamate during early time in culture is depressed because of a low intracellular content of glutamate was excluded.

Changes in GSH Contents in the Cells and Thiol Concentrations in the Medium during Culture. In most mammalian cells an important metabolite of cystine taken up is GSH. Therefore, we examined the GSH content of macrophages as function of culture time (Fig. 7). Freshly isolated macrophages contained 12–17 nmol of GSH per mg of cell protein. When the cells were cultured for 14 h, intracellular GSH rose to a steady-state level of ~26 nmol per mg cell protein, which was maintained thereafter. The increase in intracellular GSH during culture was suppressed by homocysteate, which is a potent inhibitor of the cystine

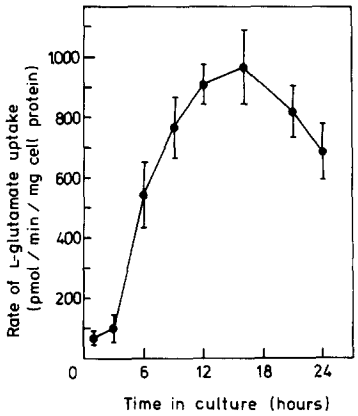

FIGURE 5. Changes in the rates of the uptake of L-glutamate by macrophages during culture. Macrophages were cultured and at the times indicated, the rates of the uptake of L-glutamate (0.05 mM) were measured. Data are shown as means ± SD of six determinations in three separate experiments.

TABLE III

Na^+ Dependency and Inhibition by L-Cystine or L-Aspartate of the Uptake of L-Glutamate

Uptake medium	Rate of uptake (pmol/min/mg cell protein) of L-glutamate (0.05 mM)	Percent inhibition by the amino acid
Na^+-containing medium	851 ± 241	
Plus 0.5 mM L-cystine	223 ± 24	73.8
Plus 2.5 mM L-aspartate	565 ± 77	33.6
Na^+-free medium	763 ± 101	
Plus 0.5 mM L-cystine	171 ± 36	77.6
Plus 2.5 mM L-aspartate	687 ± 26	10.0

Macrophages were cultured for 16 h and the rates of the uptake of L-glutamate (0.05 mM) were measured in Na^+-containing and Na^+-free (Na^+ was replaced by choline) medium. Values are means ± SD of four determinations in two separate experiments.

uptake (Table II). Furthermore, the intracellular GSH level decreased when the cells were cultured in cystine-deficient medium (Fig. 7), even though the activity of the cystine uptake was normally induced in macrophages cultured in homocysteate-added or in cystine-deficient medium (data not shown). These results suggest that the increase in GSH content is accounted for by the enhancement of the cystine uptake during culture.

Another aspect of the metabolism of cystine taken up by the cell is the formation of thiol groups in the culture medium. The thiol content of a stock culture medium is usually very low, because thiol groups of cysteine readily autoxidize and those of serum proteins are masked by mixed disulfide formation with cystine present in the medium. Such cells as human fibroblasts actively take up cystine, reduce it intracellularly, and release a reduced product, cysteine, into the medium (4, 14). As a consequence, thiol groups, which are easily measurable

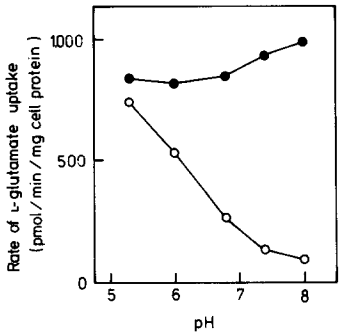

FIGURE 6. pH profile of the uptake of L-glutamate. Macrophages cultured for 16 h were used. The rates of the uptake of L-glutamate (0.05 mM) were measured in the absence (●) or in the presence (○) of 0.5 mM L-cystine at various pH. The data are from one experiment typical of three similar experiments.

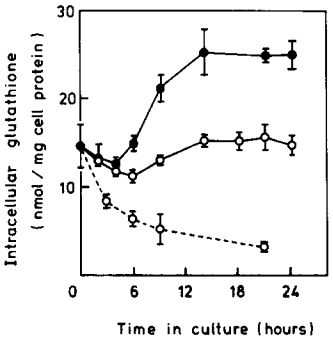

FIGURE 7. Changes in the intracellular GSH levels of macrophages during culture. Macrophages were cultured and at the times indicated, the intracellular GSH levels were determined. (—●—) Cultured in normal medium; (—○—) cultured in the medium containing 2.5 mM L-homocysteate; (--○--) cultured in cystine-free medium. Data are shown as means ± SD of six determinations in three separate experiments.

with 5,5′-dithiobis(2-nitrobenzoic acid), are generated in the medium. Since macrophages acquire ability to take up cystine during culture, we examined thiol concentrations in the medium of macrophage culture as a function of the culture time (Fig. 8). As shown in the figure, macrophages produced thiol groups in the culture medium concurrently with the induction of the activity of the cystine uptake, and the production was completely blocked in the presence of homocysteate. Probably macrophages generate thiol groups as the result of induction of the activity of the cystine uptake.

Factors that may Contribute to the Induction of the Cystine Transport Activity. It is important to note that macrophages are very sensitive to changes in their environment and therefore they are modified considerably from their native state in vivo. Freshly explanted macrophages adhere avidly to plastic vessels coated for tissue culture. To investigate the effect of the adhesion on the induction of the cystine transport activity, we cultured thioglycollate-elicited peritoneal cells in uncoated plastic vessels with gentle rotation (two rotations per min) for 16 h. Most of the cells remained unattached, but some cells died during the culture. The rate of the cystine uptake of these cells (i.e., suspension-cultured

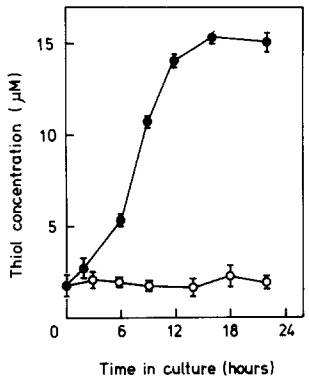

FIGURE 8. Changes in thiol concentrations in the medium during culture of macrophages. Macrophages were cultured and at the times indicated, the thiol concentrations in the medium were determined. (●) Cultured in normal medium; (○) cultured in the medium containing 2.5 mM L-homocysteate. Data are shown as means ± SD of four determinations in two experiments.

cells) was measured by the oil-layer technique described in Materials and Methods. The rate was expressed as nanomoles cystine taken up per minute per milligram cell protein and it was about one-third that of the control cells, i.e., cells cultured normally for 16 h. Since the suspension-cultured cells contain dead cells and nonadhesive cells, the value expressed on the basis of cell protein may not represent the actual rate of the uptake by viable macrophages. When the suspension-cultured cells were plated in a tissue culture dish and incubated for 1 h, macrophage-like cells readily adhered to the dish and thus separated from dead cells or nonadhesive cells. The rate of the cystine uptake of these attached cells was ~60% as high as that of the control cells, and the uptake was strongly inhibited by glutamate. Therefore, it was concluded that although the cell adhesion might stimulate the induction of the cystine transport activity, it was not an essential factor for the induction.

Another important environmental change as cells go from the peritoneal cavity to the culture system is the increase in oxygen tension. We cultured peritoneal macrophages in an atmosphere of 20% air, 75% N_2, and 5% CO_2 for 16 h. Macrophages under these conditions attached to the culture dish and extended in a similar manner to those cultured normally (95% air/5% CO_2). Interestingly, the rate of the cystine uptake of these hypoxically cultured cells was much reduced, about one-third that of normally cultured cells. This suggests that oxygen tension is an important factor that regulates the induction of the cystine transport activity. Hence a question that arises is whether the cystine transport activity of peritoneal macrophages is induced in vivo. We investigated the in vivo induction of the cystine transport activity in macrophages given phagocytic stimuli. Thioglycollate-elicited macrophages were exposed to zymosan (2.5 mg per mouse) in vivo for 4 h. Then the cells were collected and plated in a culture dish. After 1 h, the rate of the uptake of cystine at 0.05 mM was measured. It was 163 ± 41 pmol/min/mg cell protein (mean of six experiments) and the uptake was strongly inhibited by 2.5 mM glutamate. Since the control cells not exposed to zymosan never exhibit such activity under these conditions, it is

evident that the induction of the cystine transport activity takes place in vivo, although the activity induced in vivo may not be so high as that induced in vitro.

Discussion

Cystine is one of the largest amino acids and its passage across membrane is thought to be carrier mediated (16). Characteristics of the cystine uptake by macrophages in culture are quite similar to those of cultured fibroblasts (1, 2). The uptake was Na^+-independent and inhibited competitively by glutamate. The uptake of glutamate was also enhanced during culture in a similar manner to that of cystine, and cystine potently inhibited the uptake of glutamate. The uptake of cystine was pH-sensitive whereas that of glutamate was not, and cystine inhibited the glutamate uptake in a pH-dependent manner. These pH profiles closely resemble those found in fibroblasts. The remarkable difference between the pH dependency of the uptake of cystine and glutamate has been ascribed to different ionic states of cystine and glutamate (2). The amino group of glutamate is almost fully protonated within the pH range studied here, whereas the amino groups of cystine are not always protonated. The relative concentration of an anionic form of cystine, i.e., an ionic form with only one amino group protonated and two carboxyl groups dissociated, increases with an increase in pH and reaches maximum at pH 8.3. The pH profiles shown here are accounted for by assuming that this anionic form of cystine is transportable by the system studied here. These results indicate that in cultured macrophages, as in fibroblasts, cystine and glutamate share a transport system, previously designated as System x_c^-, and that most of these amino acids are transported via this system.

The present study showed that the activity of System x_c^- of macrophages is induced during culture and the rate of the uptake of cystine at 0.05 mM reached about 800 pmol/min/mg cell protein after 16 h in culture. This rate is very high and comparable to that of fetal human fibroblasts, which have the highest activity of System x_c^- among various cultured cells so far studied. Isolated rat hepatocytes (essentially free of Kupffer cells) have shown a somewhat similar influence of culture time on cystine uptake (6). The System x_c^- activity of freshly prepared hepatocytes was undetectable, but it was induced within a day in culture in the presence of insulin and dexamethasone. However, the induced activity of the hepatocytes was rather low, one order of magnitude lower than that of macrophages shown here. Mouse splenic lymphocytes have also been tested for the System x_c^- activity (Ishii, T., unpublished data), but no detectable activity was found during 48 h in culture, regardless of the presence of mitogen. Therefore, although our information is limited at present, it is likely that the drastic increase in the activity of System x_c^- is a characteristic of macrophages cultured in vitro.

It is noted that many neutral amino acids moderately inhibited the uptake of cystine (Table II). The rates of the uptake of cystine in the present experiments were determined by taking the values for the 2-min uptake, where the relation between the amount of cystine taken up and the time was ordinarily linear. However, the relation was not linear in the presence of the neutral amino acid in excess; the rate of the uptake decreased gradually after about 0.5 min (data not shown). Therefore, if the uptake is determined by taking the value for the uptake of the earlier time, the apparent inhibition by the neutral amino acid will

be smaller. Presumably, these neutral amino acids interfere with the cystine uptake indirectly. Since the neutral amino acids were added in large quantity (2.5 mM) in comparison with cystine (0.05 mM), it would be very actively taken up by the cells via neutral amino acid transport systems. It seems likely that this transport process interferes with the transport by System x_c^- for some reason, e.g., wasting transport energy. In contrast to neutral amino acids, the inhibition of the uptake of cystine by glutamate or homocysteate was immediate; they were fully inhibitory even at 0.5-min uptake (data not shown).

Changes in macrophage GSH levels that occur as a function of culture time have been reported by Rouzer et al. (17). Resident peritoneal macrophages isolated by adherence for 1 h contained 16.0 pmol GSH per μg of cell protein, and those cultured for a further 17 h contained 34.7 pmol GSH per μg of cell protein. These values compared favorably with the values presented here. The same authors have studied GSH turnover in cultured macrophages (10). The half-life of intracellular GSH was 1.9 h in experiments using an inhibitor of GSH synthesis, and similar rates of GSH depletion (half-life, 2.1 h) were obtained for macrophages incubated in the medium devoid of cyst(e)ine. These results indicate that the turnover of GSH in macrophages is very rapid and that exogenous cyst(e)ine is required for GSH production. Cysteine easily autoxidizes to cystine in the culture medium (18). Regardless of whether the medium had originally contained cysteine or cystine, owing to autoxidation essentially no cysteine was present in the stock culture medium at the time of use. Therefore, macrophages in culture must take up cystine for GSH synthesis. It is, thus, reasonable that the induction of the activity of the cystine uptake causes the increase in the intracellular level of GSH.

Macrophages in culture produced thiol groups in the medium (Fig. 8). It is very probable that macrophages take up cystine, reduce it intracellularly to form cysteine, and release cysteine into the medium, as is the case with fibroblasts (4, 14). Since cysteine in the medium is rapidly oxidized to cystine with half-life of less than 1 h (14), a large part of cystine taken up seems to be consumed in an apparently futile cycle of oxido reduction of cyst(e)ine. However, the formation of thiol groups in the culture medium is of particular importance to the interaction of macrophages and lymphoid cells. Lymphoma cell lines and lymphocytes are deficient in the uptake of cystine (19, 20). These cells cannot survive by themselves in vitro, but they do survive when macrophages or other feeder cells are cocultured, or 2-ME is supplemented (21, 22). The common function of macrophages, feeder cells, and 2-ME is to provide cysteine to the lymphoid cells (19, 23). Thus the induction of the activity of System x_c^- in macrophages may have a physiological significance in macrophage/lymphocyte interactions.

The mechanism by which the activity of System x_c^- is induced in vitro remains to be challenged. The present study demonstrated that oxygen takes part in the induction. This seems of interest because oxygen plays an important role in macrophage function, especially in phagocytosis. However, further studies to elucidate these points are required before a definitive statement can be made about the physiological importance of System x_c^-, the cystine/glutamate exchange system.

Summary

Uptake of cystine was investigated in mouse peritoneal macrophages. The rates of the uptake of cystine in resident macrophages or macrophages elicited by some irritants were very low, but a drastic increase was observed when the cells were cultured in vitro. This increase was time-dependent and required protein synthesis. In macrophages elicited by thioglycollate broth, the rate of the uptake of cystine increased by about 40-fold after 16 h in culture. Contrary to the uptake of cystine, the rates of uptake of some neutral amino acids did not change markedly during culture. We characterized the induced activity of the cystine uptake in macrophages elicited by thioglycollate broth. Cystine was taken up in an Na^+-independent and pH-sensitive manner, and the uptake was potently inhibited by extracellular glutamate and the analogous anionic amino acids, but not by aspartate. The activity of the glutamate uptake was also induced during the culture in a way similar to that of cystine uptake, and the uptake of glutamate was potently inhibited by cystine. From these results we concluded that the uptake of cystine and glutamate in macrophages was mostly mediated by a single transport system similar to the ones previously reported in human fibroblasts (1) and some other cells (5–7). As a consequence of the induction of the activity of the cystine uptake, glutathione levels in macrophages doubled during culture, and a thiol compound, presumably cysteine, was released into the culture medium and accumulated there. When the macrophages were cultured hypoxically, the induction of the cystine uptake activity was markedly depressed, suggesting an involvement of oxygen in the induction.

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