CREATINE KINASE EXPRESSION AND CREATINE PHOSPHATE ACCUMULATION ARE DEVELOPMENTALLY REGULATED DURING DIFFERENTIATION OF MOUSE AND HUMAN MONOCYTES

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Creatine phosphate serves as a reuseable high energy phosphate reservoir in skeletal and cardiac muscle and in the brain. These stores of creatine phosphate are thought to rephosphorylate the ATP hydrolyzed during cellular work (see 1 and 2 for review).

Studies in our laboratory (3) have shown that mononuclear phagocytes obtained from the peritoneal cavities of mice also contain large stores of creatine phosphate. In resident macrophages there are 3 mol of creatine phosphate per mol of ATP. During phagocytosis, these creatine phosphate stores are consumed; presumably they are used to rephosphorylate ADP, thereby maintaining the energy charge and a constant ATP supply.

The reversible transfer of the phosphoryl group of creatine phosphate to ADP is catalyzed by creatine kinase (CK),[1] an enzyme that exists in at least five isozymic forms. All forms of CK are dimers, containing subunits of ~41,000 daltons. In 1965, Dawson et al. (4) showed that these subunits can be separated into the M and B forms. The BB form is found in brain, the MM form is found in skeletal muscle, and the heterodimer MB form is found in cardiac muscle. In addition to these isozymes, Jacobs et al. (5) demonstrated that mitochondria obtained from rat heart and skeletal muscle express a form of CK (mitochondrial CK) whose electrophoretic migration differs from that of the three known cytoplasmic forms. Subsequent reports (6, 7) have established that mitochondria have at least two unique forms of CK.

In this paper, we demonstrate that the expression of CK in mouse and human mononuclear phagocytes is a developmentally regulated process occurring during the in vivo and in vitro differentiation of monocytes into macrophages. The induction of CK during in vitro cultivation occurs independently of the concentration of creatine in the medium. However, the size of the creatine phosphate pool varies directly with the concentration of creatine in the medium.

The isozyme profile of CK in human macrophages differs from that observed in mouse macrophages. Human macrophages express the brain and probably the

[1] *Abbreviations used in this paper:* CK, creatine kinase; PBS, Dulbecco's phosphate-buffered saline.

mitochondrial forms of CK whereas mouse macrophages predominantly express the brain isozyme. These results demonstrate that in both mouse and man, CK is an enzymatic marker for the differentiation of monocytes into macrophages.

Materials and Methods

Human Blood Monocytes.   Human blood leukocytes were isolated from venous blood obtained from normal healthy adult volunteers. The mononuclear leukocyte fraction was separated from heparinized whole blood by Ficoll-Hypaque centrifugation (8). Monocytes were separated from other mononuclear cells by one of two methods. In the first method, adhesion of monocytes to plastic petri dishes was used to separate monocytes from nonadherent cells such as lymphocytes (8). 10 million mononuclear leukocytes (20–30% monocytes, as determined by Wright-Giemsa staining) were plated in 4 ml of RPMI 1640 supplemented with 20% heterologous human serum and streptomycin (0.5 mg/ml) in 60-mm petri dishes. The nonadherent cells, including most of the platelets, were removed by washing the plates several times with RPMI 1640 at 2 and 24 h. Plates washed after 2 h contained ~3 × 10^6 adherent cells, over 90% of which were monocytes. As noted by others (8), ~40% of these adherent cells subsequently detach from the substrate and are lost when the plates are washed after 24 h. The remaining cells (~1–2 × 10^6 cells) form a confluent macrophage monolayer after 7–12 d in culture. In the second method, we used Percoll gradient centrifugation (9) to separate monocytes from other leukocytes. 3 million cells from the monocyte-enriched fraction of the Percoll gradient were plated into each 60-mm petri dish to obtain an adherent cell population that was >95% monocytes. In all cases, monocyte purity was determined after 7 or 14 d in culture by their morphology using phase contrast microscopy and by their capacity to ingest sheep erythrocytes coated with rabbit IgG antibody (3).

Monocytes prepared by either method were maintained in a humidified CO_2 incubator at 37°C. Percoll-purified monocytes were maintained either in sterile Teflon beakers as suspension cultures or in petri dishes as monolayer cultures for several weeks with no subsequent change of growth medium (10).

Other Human Cell Types.   Three established B lymphocyte cell lines, 8866p, Spoller, and DEL, prepared from mononuclear leukocytes by infection with Epstein-Barr virus, were a kind gift from Dr. Lloyd Mayer and Dr. Henry Kunkel. Erythrocytes were obtained from the pellet of the Ficoll-Hypaque gradient. T lymphocytes were purified from the mononuclear cell fraction of the Ficoll-Hypaque gradients using nylon wool columns (11). Human neutrophils were prepared as described (12). Platelets were isolated from whole blood by centrifugation of the blood at 300 g. The plasma fraction (which contains the platelets) was plated on 60-mm petri dishes in RPMI 1640 supplemented with 20% human serum.

Murine Monocytes and Peritoneal Macrophages.   Mouse peripheral blood monocytes were separated from heparinized mouse blood and other leukocytes using Ficoll-Hypaque centrifugation (13). The Ficoll-Hypaque gradient was prepared by layering 1 part Ficoll-Hypaque of 1.077 density onto 4 parts of Ficoll-Hypaque of density 1.119. Blood was collected from the mouse heart using a heparinized (100 USP units per 1 ml blood) syringe and the blood was immediately diluted 1:4 with Dulbecco's phosphate-buffered saline containing Ca^{++} and Mg^{++} (PBS) (14). 35 ml of the diluted blood was layered onto a 15-ml Ficoll-Hypaque gradient and centrifuged at 500 g in an IEC centrifuge (International Equipment Co., Needham Heights, MA) for 20 min at room temperature. The mononuclear cell fraction located near the 1.077:1.119 interface of the gradient was carefully removed and the cells washed twice in PBS. The monocytes were separated from lymphocytes by adherence on plastic petri dishes. Mononuclear cells were plated at an initial density of 10 million per 60-mm petri dish and maintained in culture in RPMI 1640 medium supplemented with 20% fetal bovine serum and 0.5 mg/ml streptomycin. The cultures were washed with RPMI 1640 after 6 h; ~15–25% of the cells remained adherent. Mouse peritoneal macrophages were obtained from NCS mice (The Rockefeller University). Resident and thioglycollate broth-elicited peritoneal macrophages were col-

lected as described (3) and plated in 35-mm petri dishes at densities of 6 and 2 × 10⁶ cells per dish, respectively. After a 24-h incubation there were ~1.5–1.8 × 10⁶ macrophages per petri dish. The mouse macrophages were maintained in minimal essential medium supplemented with 10% fetal bovine serum and 0.5 mg/ml streptomycin.

ATP and Creatine Phosphate Measurements.   Macrophages or monocytes maintained in 35 or 60-mm petri dishes were washed three times in PBS, scraped with a rubber policeman into 0.4 ml of cold (4°C) 0.04M Tris/borate buffer, pH 9.2. A 200-μl aliquot was removed for CK determination and the remaining sample was boiled for 5 min and centrifuged at 4°C in an Eppendorf microcentrifuge for 5 min at 11,700 rpm. The supernatant was immediately transferred to a test tube and frozen, by submerging the test tube in a −40°C dry ice-acetone bath. Samples were maintained frozen for no longer than 2 wk before measuring ATP and creatine phosphate. ATP and creatine phosphate were measured using a luciferin-luciferase system. ATP was measured in duplicate samples at 4°C in a Packard liquid scintillation counter (Packard Instrument Co., Downers Grove, IL) as described (3, 15). Measured amounts of ATP were run as standards in every assay. Creatine phosphate measurements were performed at 37°C with a Packard Pico-lite luminometer by a modification of previously described methods (3). Briefly, aliquots (30 μl) of extracts were added to 350 μl of assay buffer containing 123 μl of 0.01 M KPO_4 buffer, pH 7.4 (containing 4 mM $MgSO_4$), 123 μl of 0.1 Na arsenate buffer (pH 7.4), 36 μl of 10 mM AMP, 36 μl of reconstituted firefly extract, 23 μl H_2O, and 9 μl of 0.1 mM ADP. The firefly extracts were incubated at 37°C for 10 min before use to hydrolyze any endogenous ATP. Samples were incubated in the Pico-lite luminometer at 37°C for 5 min before recording baseline ATP levels (measured by light intensity). Then, 50 μl of CK (5 mg/ml) was added and the emitted light was recorded. The additional light generated after the addition of CK represents ATP generated from CK-catalyzed transfer of phosphoryl groups from creatine phosphate to ADP (creatine phosphate + ADP → creatine + ATP). The maximum amount of ATP generated under these conditions occurred within 40–60 s. The difference between the maximum and initial light intensity (before and after the addition of CK) was directly proportional to the concentration of creatine phosphate standard added to the incubation mixture over a range of 10^{-11} to 10^{-9} mol of creatine phosphate (Fig. 1). Samples and creatine phosphate standards were measured in duplicate for each experiment.

CK Activity and Isozyme Identification.   Tris/borate extracts of cell cultures, prepared as described above, were sonicated at 4°C for 10 s using an Ultrasonic cell disrupter (Heat Systems-Ultrasonic, Inc., Plainview, NY) set at 25% duty cycle and 2.5 output units, and centrifuged in a Sorvall centrifuge (DuPont Instruments, Wilmington, DE) at 10,000 g at 4°C for 60 min. The supernatants were used immediately to measure CK activity and to determine the CK isozymes. No difference was observed in CK activity whether the cells were lysed by Tris-borate sonication or in Triton X-100 (unpublished data). CK activity was measured as described (16) by monitoring the forward reaction of creatine kinase
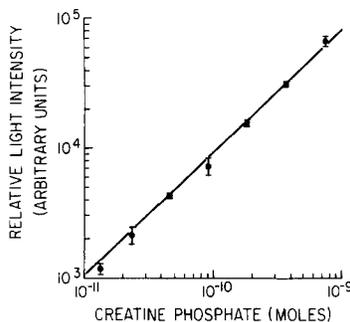

FIGURE 1.   The measurement of creatine phosphate using a bioluminescence assay. Standard concentrations of creatine phosphate (10^{-11}–10^{-9}) were measured as a function of light intensity using a Pico-lite luminometer as described in Materials and Methods.

(creatine phosphate to ATP) in a coupled reaction mixture containing NADP, hexokinase, and glucose 6-phosphate dehydrogenase. The rate of this reaction was followed spectrophotometrically by measuring the reduction of NADP to NADPH. To control for endogenous ATP in the extracts or for enzymatic activities that may catalyze NADP reduction, the rate of NADPH formation was measured in samples from which creatine phosphate was omitted. The values reported represent the difference between the rate of NADPH generated in the presence and absence of added creatine phosphate. One IU of CK is defined (16) as the concentration of enzyme required to convert 1 μmol of creatine phosphate to 1 μmol of creatine in 1 min.

CK isozymes were analyzed by electrophoresis of Tris-borate extracts on cellulose acetate plates at 4°C using the Helena CK isozyme separation procedure (Helena Laboratories, Beaumont, TX) (17). Individual CK isozyme bands on the plates were developed for 30 min at 37°C in the coupled reaction (described above) and the NADPH generated was visualized under UV light. Tracings of the fluorescence bands were recorded using a Helena recording spectrofluorometer (model CCU). For all determinations of human CK isozyme, commerical human CK isozymes were run as standards. For studies of mouse macrophages, standard mouse CK isozymes were separated using mouse skeletal muscle as the source for the MM isozyme, mouse heart as the source for MB isozyme, and mouse brain as the source for the BB isozyme (18).

Miscellaneous Techniques. Protein determinations were made using the method of Lowry et al. (19) on sonicated Tris-borate extracts (20 μl) dissolved in 100 μl 1 N NaOH and neutralized with 100 μl of 1 N HCl. The concentration of Tris in these extracts did not interfere with protein determinations. All cultures used in these studies contained >95% viable cells as determined by trypan blue exclusion (20). Dialyzed human serum was prepared by dialysis for 3 d against 100 vol of 0.9% NaCl, which was replaced daily with fresh saline. Creatine concentrations in serum were ~1–2 × 10^{-5} M and were measured as described (21). All experiments were repeated at least three times and, in most cases, duplicate samples were used for each experiment.

Materials. Brewer's thioglycollate broth was purchased in powder form from Difco Laboratories, Detroit, MI and prepared as described on the package. RPMI 1640, Eagle's minimal essential medium, and fetal bovine serum were purchased from Gibco Laboratories, Grand Island, NY. ATP, firefly lanterns, creatine phosphate, creatine, hexokinase, and glucose 6-phosphate dehydrogenase were purchased from Sigma Chemical Co., St. Louis, MO. CK was purchased from Boehringer Mannheim Biochemicals, Indianapolis IN. Heparin (Lipo-Hepin, 5,000 USP units per ml) was purchased from Riker Laboratories, Northridge, CA. Percoll and Ficoll-Hypaque were obtained from Pharmacia Fine Chemicals, Piscataway, NJ. All materials used for the electrophoretic separation of CK isozymes were purchased from Helena Laboratories. Human and rabbit CK isozyme standards were obtained from Roche Diagnostics, Nutley, NJ and Helena Laboratories, respectively. Rabbit antiserum directed against human CK-BB was purchased from Organon Diagnostics, West Orange, NJ and rabbit anti-human MM antiserum was a kind gift of Dr. Robert Roberts, Washington University, St. Louis, MO. Goat antiserum directed against human CK-MM was purchased from Merck AG, Darmstadt, Federal Republic of Germany. Human serum for culturing human monocytes was obtained from healthy volunteers with type A blood.

Results

Creatine Phosphate and CK Activity in Mouse Monocytes and Peritoneal Macrophages. Mouse monocytes maintained in culture for 24 h contain no detectable creatine phosphate or CK activity (Table I). Mouse macrophages obtained from the peritoneal cavities of normal mice and maintained in culture for 24 h express 70 mU of CK per mg protein and contain about 2.2 mol of creatine phosphate per mol of ATP (Table I) (3). Mouse monocytes maintained in culture for 7 d appear morphologically like peritoneal macrophages and express significant CK

TABLE I

ATP, Creatine Phosphate, and CK in Mouse Monocytes and Macrophages*

	Time in culture	ATP	Creatine phosphate	CK	Protein
		nmol/mg		*mU/mg*	*mg*
Mouse monocytes	24 h	10.0	<0.3[‡]	<3.0	0.23
	7 d	ND[§]	ND	120	0.4
Mouse peritoneal macrophages					
Resident	24 h	2.6	8.0	70	0.14
Thioglycollate elicited	2 h	11.9	35	370	0.19
	24 h	8.8	47	350	0.22
	7 d	2.5	55.0	1100	0.40

* Prepared and measured as described in Materials and Methods.

‡ Low concentrations of creatine phosphate may be due to contaminating platelets and not monocytes present in the cell preparation.

§ Not determined.

activity (120 mU/mg protein; Table I). Since peritoneal macrophages are derived from blood monocytes (22), these results suggest that the expression of CK in mouse macrophages is a developmentally controlled process induced during both in vivo and in vitro differentiation of monocytes.

Enhanced expression of CK is also observed during in vitro cultivation of inflammatory macrophages. Inflammatory macrophages, elicited by the intra-peritoneal injection of thioglycollate broth and maintained in culture for 2 and 24 h, contain 3 and 5 mol of creatine phosphate per mol of ATP and express 350 and 370 mU of CK per mg protein, respectively (Table I). Thioglycollate-elicited macrophages maintained in culture for 7 d show a threefold increase in the specific activity of CK (1,100 mU/mg) and an ~20% increase in creatine phosphate (Table I) when compared with the same cells cultured for 24 h or less.

To examine the induction of CK during monocyte differentiation in greater detail, we measured CK expression in human monocytes and in monocyte-derived macrophages. Human monocytes were chosen for this study because these cells can be obtained in large numbers from venous blood and are known to differentiate in culture (8, 22, 23).

CK and Creatine Phosphate in Cultured Human Monocytes and in Monocyte-derived Macrophages. Human monocytes maintained in culture for 24 or 48 h contain <3 mU of CK per mg protein (the lower limit of detection in this assay) and undetectable levels of creatine phosphate. The ATP content of these cells was ~4 nmol per mg protein. An increase in monocyte CK activity was first detected on the 5th d of culture. By 7 and 14 d, these adherent cell cultures contained 20 and 100 mU of CK per mg protein, respectively (Fig. 2). Creatine kinase activity did not usually increase after 21 d in culture. Thus, in human monocyte-derived macrophages, CK is developmentally regulated; that is, its expression is dependent on the length of in vitro cultivation.

To examine whether cell adherence is required for CK expression, monocytes purified by Percoll centrifugation were maintained either as adherent cells in
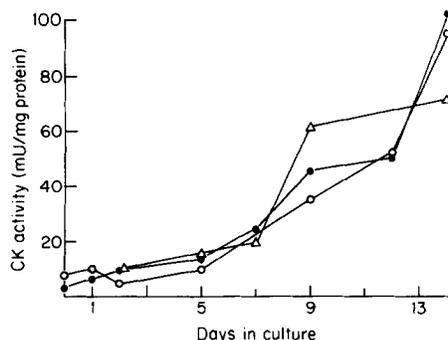
FIGURE 2.   CK activity of human monocytes maintained in culture. Human monocytes were isolated by Ficoll-Hypaque centrifugation and selective adherence to plastic substrate or by Percoll gradient centrifugation as described in Materials and Methods. Percoll-purified monocytes were maintained either as suspension cultures in Teflon beakers or as adherent cultures in petri dishes. At the indicated times, cultures were harvested, extracts prepared, and CK activity measured as described in Materials and Methods. The results presented are of a representative experiment. The experiment was repeated three times with similar results. (△) Percoll-purified monocyte cultures maintained as suspension cultures in Teflon, (○) Percoll-purified cells maintained as adherent cultures, (●) monocytes prepared by Ficoll-Hypaque centrifugation and selective adherence.

Petri dishes or as suspension cultures in Teflon beakers. The rate and extent of CK induction was the same in these cells as in monocytes maintained in monolayer cultures (Fig. 2). These experiments show that the expression of CK is independent of cell adherence to the plastic substrate.

Dialyzed human serum contains $<10^{-6}$ M creatine. Human macrophages, maintained for 14 d in medium supplemented with 20% dialyzed human serum, contained normal amounts of CK (95 mU/mg protein), but no creatine phosphate. Addition of 10^{-4} M guanidinoacetic acid (the metabolic precursor to creatine) to the medium did not promote creatine phosphate accumulation, even though the cells contained the expected concentration of CK (100 mU/mg). These experiments demonstrate that monocyte-derived macrophages, like brain and muscle cells (1), do not contain the appropriate enzymes to synthesize creatine from its known precursors (glycine and guanidinoacetic acid) and must therefore obtain creatine from serum.

The specific activity of CK in macrophages maintained in medium containing high concentrations of creatine (10^{-3} M) was identical to that found in macrophages maintained in medium containing low concentrations (2×10^{-6} M) of creatine (data not shown). Thus, CK induction is independent of the presence or amount of creatine present in the medium or of creatine phosphate in the cells.

Another feature associated with differentiation of monocytes into macrophages in culture is the development of a creatine phosphate reservoir (Fig. 3). Monocytes maintained in medium supplemented with 1.0 mM creatine contain two- to threefold the amount of creatine phosphate and approximately the same amount of ATP as monocytes maintained in medium containing 2×10^{-6} M creatine (Fig. 3). Thus, the size of the creatine phosphate pool, unlike the specific activity of CK, depends on the creatine concentration in the medium.
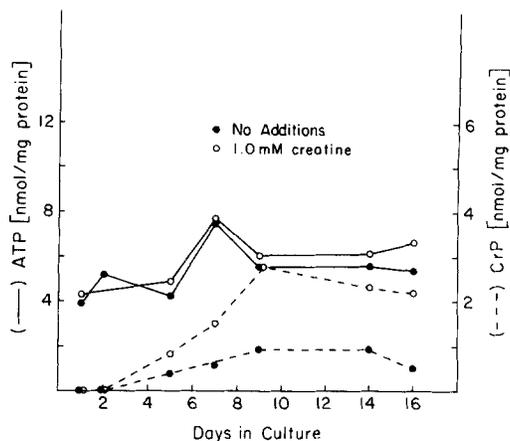
FIGURE 3.   ATP and creatine phosphate levels in monocyte cultures. Human monocytes were isolated by Ficoll-Hypaque gradient centrifugation and selective adherence as described in Materials and Methods and were maintained as adherent cultures in the absence (●) or presence (O) of an additional 1.0 mM creatine. ATP and creatine phosphate were measured at the indicated times. Each point represents the average of duplicate samples. The standard deviation from the average of the duplicate samples was <15%.

Isozyme Pattern of CK in Human and Murine Macrophages.   Electrophoresis of CK from human monocytes maintained in culture for 7 or 14 d reveals three bands that possess CK activity (Fig. 4a). The anodal band corresponds to the brain type of CK (BB), based on its electrophoretic mobility and the capacity of antiserum to the BB isozyme to inhibit its activity (Fig. 4a). Control experiments (Fig. 4b) demonstrate that the anti-BB antiserum inhibits the BB and MB isozymes but not the MM isozyme.

The isozyme that migrates further towards the cathode than the MM standard is not inhibited by either anti-BB or anti-MM antisera; based on its mobility, it appears to be a mitochondrial form of CK (4–6).

The identity of the isozyme that has an electrophoretic mobility corresponding to the MM isozyme is less clear. Goat and rabbit anti-human MM antisera do not abolish its activity (Fig. 4), while both antisera inhibit the activity of standard human MM isozyme. Furthermore, the intensity of this band relative to the BB band varies with macrophages from different donors.

The isozyme pattern of human macrophages differs from that observed in tissue macrophages obtained from mice. Resident and thioglycollate-elicited macrophages from mice contain one major band, the BB isozyme of CK (Fig. 5). There are also traces of enzyme activity whose electrophoretic mobility is similar to the MM isozyme.

Creatine Phosphate and CK in Other Blood Cells.   We have found that human platelets, freshly isolated from whole blood (Fig. 5 and Table II), contain both CK and creatine phosphate. In contrast, freshly isolated human blood T and B lymphocytes (from which the monocytes have been removed by a Percoll centrifugation step), erythrocytes and polymorphonuclear leukocytes contain neither CK nor creatine phosphate. B lymphocytes transformed by Epstein-Barr virus
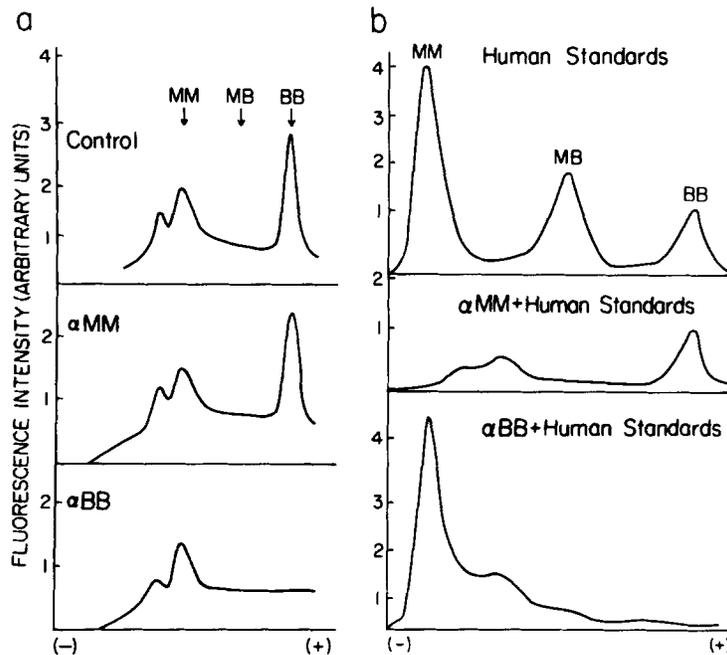
FIGURE 4. (a) Electrophoretic analysis of CK isozymes in human macrophages. Human macrophage extracts were prepared from 14-d-old cultures as described in Materials and Methods. Extracts were preincubated for 30 min at 37°C with a 1:100 dilution of nonimmune sheep serum (top), sheep anti-MM antiserum (middle), or anti-BB antiserum (bottom). Electrophoresis was done as described in Materials and Methods. The MM, MB, and BB indicator marks are the relative positions where standard MM, MB, and BB isozymes migrate on the cellulose acetate plates. (b) Electrophoretic patterns of human CK standards incubated in the presence or absence of antisera directed against either the MM or BB isozyme. Human CK standards were preincubated for 30 min at 37°C in a 1:100 dilution of nonimmune sheep serum (top), sheep anti-MM antisera (middle), or sheep anti-BB antisera (bottom). The antisera-CK mixtures were then loaded on cellulose acetate plates and electrophoresed as described in Materials and Methods.

and maintained as continuous cell lines do not express CK and have no detectable creatine phosphate.

Discussion

In vitro cultivation of monocytes results in morphological and biochemical changes that mimic, in part, differentiation in vivo. Changes that occur during in vitro cultivation of human monocytes include increases in cell size (8), the loss of myeloperoxidase staining (23), decreased capacity to release reactive oxygen intermediates (23), increased capacity to ingest antibody-coated particles (10), and qualitative changes in the activities of the C3b and C3bi receptors (10).

This paper identifies CK as a new marker for the differentiation of monocytes into macrophages. Our results demonstrate that the expression of CK and the accumulation of a creatine phosphate reservoir is a developmentally regulated process in cultured human and mouse monocytes. Our finding that mouse monocytes lack CK and creatine phosphate while tissue macrophages contain both CK and creatine phosphate provides direct evidence that the expression of
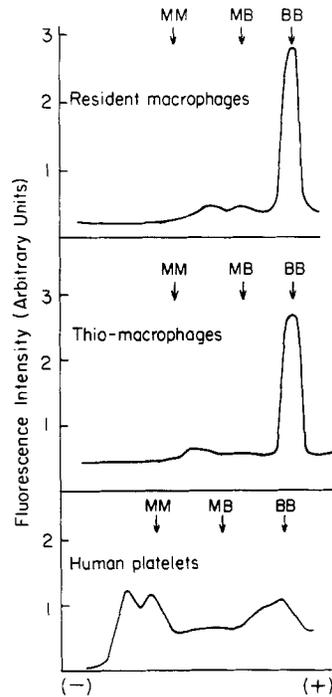
FIGURE 5.   CK isozymes in mouse thioglycollate-elicited macrophages (thio-macrophages) and human platelets. Tris/borate extracts were prepared (as described in Materials and Methods) from mouse resident macrophages (*top*) and thioglycollate-elicited macrophages (*middle*) maintained in culture for 1 d, and freshly isolated human platelets (*bottom*). CK isozymes were separated by electrophoresis on cellulose acetate plates as described in Materials and Methods.

TABLE II

*ATP, Creatine Phosphate, and CK in Platelets, Erythrocytes, Lymphocytes, and Polymorphonuclear Leukocytes**

	Time in culture	ATP	Creatine phosphate	CK
		nmol/mg		*mU/mg*
Human platelets (4.7 × 10⁶ cells)	3 h	2.1	0.8	ND
Lymphocytes (B and T cells) (5 × 10⁶)	3 h	30	<1.0‡	<2.0
Polymorphonuclear leukocytes (3 × 10⁶ cells)	3 h	17.2	0.9	<5.0
Erythrocytes (1.8 × 10⁸ cells)	3 h	25.9	<0.01	<3.0
B lymphocytes (8866p) (4 × 10⁶)	7 d	8.0	<0.01	<2.0

* Prepared and measured as described in Materials and Methods.
‡ Low concentrations of creatine phosphate may be due to contaminating platelets and not monocytes present in the cell preparation.

CK by macrophages is not an artifact of in vitro cultivation. Furthermore, preliminary studies indicate that CK is also expressed in macrophages from human tissue. Alveolar macrophages obtained by bronchial lavage from a single patient contained significant CK activity (70 mU/mg protein).

Neither mouse peritoneal macrophages nor human monocyte-derived macro-

phages are able to synthesize creatine from its known precursors, guanidinoacetic acid and glycine. Therefore, maintenance of a creatine phosphate pool is dependent upon a continuous supply of creatine in the medium. This is also evident from our observation that murine peritoneal macrophages decrease their creatine phosphate stores by 90% when maintained in culture for several days in medium lacking creatine (Loike and Silverstein, manuscript in preparation).

Five isozymes of CK have been described (4, 6). We demonstrate that human monocyte-derived macrophages express at least two and possibly three of these isozymes. We have identified one of these isozymes as the brain form of CK. Further work is required to establish the identity of the second isozyme, which we believe is the mitochondrial form of CK. The identity of the CK isozyme whose electrophoretic mobility is similar to the MM isozyme remains unclear, since activity of this isozyme is not inhibited by the two anti-MM antisera that we have tested.

Two important similarities between macrophages and muscle cells (23–25) emerge from these studies. In both cell types, creatine cannot be synthesized from its known precursors and must be obtained from extracellular sources, and, CK expression accompanies cellular differentiation.

What is the role of creatine phosphate in human and mouse macrophages? We have previously shown (3) that during the ingestion of antibody-coated sheep erythrocytes by mouse macrophages, creatine phosphate turnover increases dramatically. Yet all experimental evidence indicates that ATP and not creatine phosphate fuels phagocytosis. Mouse macrophages depleted of >90% of their creatine phosphate stores continue to phagocytose. Studies with human phagocytes confirm that the presence of creatine phosphate is not required for phagocytosis. Human monocytes and polymorphonuclear leukocytes lack creatine phosphate and CK and are able to phagocytose a wide variety of particles. Thus, it appears that, in macrophages, the development of a creatine phosphate pool is one of perhaps many mechanisms for maintaining constant ATP levels in the face of sporadic demands for chemical energy during cellular work.

Summary

We have studied the expression of creatine kinase (CK) and the accumulation of creatine phosphate during the differentiation of human and mouse peripheral blood monocytes. Mouse monocytes cultured for 24 h do not contain detectable levels of CK and creatine phosphate. However, resident tissue macrophages and inflammatory elicited macrophages obtained from the peritoneal cavities of mice have 70 and 300 mU per mg protein of CK activity and contain 3 and 6 mol of creatine phosphate per mol of ATP, respectively. The major isozyme of CK in these cells has been identified as the brain form. These findings suggest that the differentiation of monocytes into macrophages is associated with the expression of CK and the accumulation of creatine phosphate. We have found a similar pattern in human monocytes. Human blood monocytes, maintained in culture for 24 or 48 h, do not contain detectable levels of CK or creatine phosphate. Monocyte-derived macrophages (monocytes maintained in tissue cultures for 1 to 2 wk) have up to 100 mU per mg protein of CK activity and contain 0.5 mol of creatine phosphate per mol of ATP. Human macrophages express multiple

isozymes of CK including the brain (BB) and possibly the mitochondrial forms of this enzyme. Thus, the expression of CK and the accumulation of creatine phosphate in human monocytes is induced by their in vitro cultivation. The induction of CK during in vitro cultivation occurs independently of the concentration of creatine in the medium. However, the size of the creatine phosphate pool varies with respect to extracellular creatine concentration. Creatine phosphate and CK are not detectable in freshly isolated human lymphocytes, polymorphonuclear leukocytes or erythrocytes, but are found in freshly isolated human platelets.

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