GLUTAMINE PROMOTES COLONY FORMATION IN BONE MARROW AND HL-60 CELLS; ACCELERATES MYELOID DIFFERENTIATION IN INDUCED HL-60 CELLS

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SUMMARY

Several studies indicate that glutamine is a critical requirement for growth of cultured cells. The present studies describe the effect of deprivation of glucose or glutamine on mouse bone marrow cell or HL-60 cell colony formation in soft agar. The mouse bone marrow cells were induced to undergo granulocyte/macrophage type differentiation by colony-stimulating factor. Glutamine, but not glucose, was found to be an indispensable metabolite for the cloning of HL-60 cells or differentiated mouse bone marrow cells. In addition, the effect of glucose or glutamine on the rate of differentiation of dimethylsulfoxide (DMSO)-induced HL-60 cells in liquid culture was studied. Glutamine was found to be superior to glucose in its ability to support the proliferation and myeloid differentiation of HL-60 cells. When an optimal concentration of DMSO was used, the rate of differentiation of induced HL-60 cells was found to be a function of the concentration of glutamine. In addition to these studies glutamine utilization and product formation was studied in induced and uninduced HL-60 cells after 60 min incubation with 1 mM initial glutamine concentration. The fractional distribution of the glutamine carbon into its metabolic products remained unchanged in induced versus uninduced HL-60 cells. However, the rate of utilization of glutamine and product formation by terminally differentiated HL-60 cells was less than the rate of utilization of glutamine by undifferentiated HL-60 cells. The data do not explain the role of glutamine in the complex process of differentiation but establish the critical requirements for glutamine, but not glucose, in myelopoiesis.

Key words: glutamine; glucose; colony-stimulating factor; colony formation; glutamine metabolism; dimethylsulfoxide-induced and uninduced HL-60 cells.

INTRODUCTION

Several studies on various cell strains indicate that glutamine is a critical requirement for cell growth (proliferation) in vitro (1-4). The critical need for glutamine, but not glucose, for cell growth has been attributed to a number of factors; among these being the role of glutamine as an energy source (5,6) and as a precursor to nonessential amino acids (7,8) and to nucleotides (9,10). However, nothing is known about the need for glucose or glutamine in differentiating cells; therefore we directed these studies in evaluating the need for glutamine in differentiating cells. Special interest was focused on the need for glucose or glutamine in colony-stimulating factor (CSF) induced bone marrow cell differentiation (granulocyte/macrophage) (11) and dimethylsulfoxide (DMSO)-induced human leukemic HL-60 cell differentiation (myeloid) (12). In addition, the fractional yield of glutamine carbon into glutamate, glucose, and lactate was studied in induced and uninduced HL-60 cells.

MATERIALS

L-Glutamine, D-glucose, DEAE dextran, L-asparagine, Wright's stain, and DMSO were purchased from Sigma Chemical Co., St. Louis, MO. Dulbecco's modified (eagle) medium (DME) without glutamine or glucose was purchased from
K. C. Biologicals, Lenaxa, KA. Uniformly labeled L-glutamine-\(^{14}\)C was purchased from New England Nuclear Corp., Boston, MA. C57/BL Mice, for bone marrow studies, were purchased from Jackson Laboratories, Bar Harbor, ME. Fetal bovine serum (FBS) and horse serum (HS) were purchased from Flow Laboratories, Rockville, MD, and GIBCO, Grand Island, NY. Bacto agar was purchased from Difco Laboratories, Detroit, MI. Source of CSF was conditioned medium from MIA PaCa-2 cells (13).

**METHODS**

**Cell culture.** Human leukemic (promyelocytic) HL-60 cells were passaged twice weekly in regular DME media supplemented with 15%, heat-inactivated FBS, without antibiotics. They were maintained in continuous suspension culture in T flasks in 5% CO\(_2\). Cell growth was monitored in a particle data counter. Cell viability was accessed with the trypan blue dye exclusion test. For liquid culture experiments, cells were centrifuged and growth medium was removed; cells were washed twice with sterile saline and resuspended in DME media without glucose or glutamine, supplemented with 15% heat-inactivated FBS. All liquid culture experiments were started at an initial concentration of \(1 \times 10^5\) cells/ml. Known concentrations of glucose or glutamine were added back to this medium. For the study of differentiation of HL-60 cells, 1.3% DMSO was used. All experiments were set up in 35 X 10-mm tissue culture dishes.

**Morphology.** Morphological changes of the HL-60 cells were evaluated by differential counts under light microscopy of slides stained with Wright’s stain. A minimum of 200 cells were counted for each experimental point.

**Soft agar assay.** The DME media without glucose or glutamine was brought to a boil with 0.6% (wt/vol) agar. It was then mixed with an equal volume of DME medium, without glucose or glutamine, containing 20% (vol/vol) FBS and 20% (vol/vol) HS, to give a final agar concentration of 0.3% and serum concentration of 20%; the final HL-60 cell concentration was 500 cells/ml. Pen strep was used at a concentration of 100 U/ml and DEAE dextran at 75 \(\mu\)g/ml. Bone marrow cells were obtained from the femurs of C57/BL mice and counts were done on a hemocytometer after lysis of red blood cells with a 3% acetic acid solution. The final concentration of bone marrow cells was \(1.0 \times 10^5\) cells/ml. Fixed known concentrations of glucose or glutamine were pipetted directly to 35 X 10-mm tissue culture dishes. Standard soft agar assay conditions employ 4 mM L-glutamine, 5.55 mM D-glucose, and 0.132 mM L-asparagine (14). For bone marrow experiments, 50 \(\mu\)l of CSF, approximately 100 U (as assayed on mouse bone marrow), was also added to each dish. Total sample volume in each dish was adjusted to 200 \(\mu\)l with sterile water. One milliliter of agar solution containing appropriate cells was then pipetted into each dish. Plates were then incubated at 37\(\degree\) C, 10% CO\(_2\), and full humidity. Bone marrow plates were counted on Day 5. The HL-60 cell plates were counted on Day 7. Colonies were defined as aggregates of greater than 30 cells and counted with a dissecting microscope.

**Uptake and metabolism of glutamine.** The rate of utilization of glutamine and its conversion into metabolic products by induced and uninduced HL-60 cells was assessed at 1 mM initial glutamine concentration. Cells were cultured in regular DME media (± DMSO), harvested from T flasks on Day 7 by centrifugation, washed three times in phosphate buffered saline (PBS) and resuspended to 1.0 \(\times 10^7\) cells in less than 1 ml. The reaction mixture in a final volume of 1.0 ml contained 1.0 \(\times 10^7\) cells, L-glutamine-\(^{14}\)C (sp act 2.0 \(\mu\)Ci/1 \(\mu\)mole glutamine), pH 7.45. As accessed by thin layer chromatography (TLC), L-gln-\(^{14}\)C was greater than 98% pure. The reaction mixture was incubated at 37\(\degree\) C, kept air-tight after briefly flushing with 5% CO\(_2\), balance air. At time intervals starting from 1 to 60 min, small aliquots were taken, immediately sonicated and 15 \(\mu\)l spotted on TLC. The viability of cells at the end of 60 min was greater than 95%. The chromatogram was developed exactly as described before (15). In this system, glutamine has a \(R_f\) of 0.36; glutamate, 0.44; glucose, 0.65; and lactic acid, 0.77. Glutamate was also measured enzymatically (16). The rate of utilization of glutamine, 1 mM by 1.0 \(\times 10^7\) cells/ml, was linear up to 1 h.

**RESULTS**

**Growth of HL-60 cells or differentiating HL-60 cells in DME medium containing glutamine or glucose.** The rate of proliferation of uninduced HL-60 cells in DME medium containing either glutamine or glucose is shown in Fig. 1. The doubling time of uninduced HL-60 cells was