Vol. 138, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1986 Pages 445-454

CSF-1 STIMULATES GLUCOSE UPTAKE IN MURINE BONE MARROW-DERIVED MACROPHAGES

John A. Hamilton, Gino Vairo and Suzanne R. Lingelbach

University of Melbourne, Department of Medicine,
Royal Melbourne Hospital, Parkville, Victoria, 3050, Australia

Received May 20, 1986

^{3}H-2-deoxyglucose was used as an isotopic tracer for the measurement of glucose uptake into quiescent murine bone marrow derived macrophages. A purified colony stimulating factor (CSF-1) was shown to stimulate ^{3}H-2-deoxyglucose uptake in a dose-dependent manner. This stimulation was rapid, with a maximal effect seen at 20-30 minutes after growth factor addition. Both the inhibition by cytochalasin B and also the relative degree of competition by high concentrations of a series of glucose analogues suggest that the basal and CSF-1 stimulated 2-deoxyglucose uptake occur via a carrier facilitated D-glucose transport system.

The data indicate that a purified growth factor can increase the glucose uptake in macrophages, a finding which could be relevant to the survival and/or the proliferative response of this and other haemopoietic cell types.

Colony stimulating factor (CSF-1) is a growth factor which is specific for cells of the mononuclear phagocytic (or macrophage) lineage (1,2). This factor, isolated from mouse L-cell conditioned medium, is a two subunit glycoprotein of 70,000 Mr (3), which mediates its effects on the cells via binding to specific surface receptors (4). Murine bone marrow-derived macrophages (BMM) can be prepared as a relatively homogenous cell population with $\geq$ 95% of these adherent cells expressing CSF-1 binding (5). Cells along the macrophage lineage, including BMM, require a growth factor, such as CSF-1, for their survival, proliferation and differentiation (5).

ABBREVIATIONS: BMM, bone marrow-derived macrophages; CSF-1, macrophage-specific colony stimulating factor (or macrophage growth factor); 2-DOG, 2-deoxy-D-glucose, FBS, fetal bovine serum; HCGF, haemopoietic cell growth factor; LPS, lipopolysaccharide.

0006-291X/86 $1.50

The biochemical events associated with proliferation and differentiation for cells along the various haemopoietic lineages (6) are essentially unknown. We have been studying the interaction of CSF-1 with BMM as a model system for the study of such events. Several studies in other cells have demonstrated that enhanced glucose transport is an early cellular change in the proliferative response to mitogenic stimuli (see, for example, references 7,8). Recently, a haemopoietic precursor cell line (FDC-P2) has been established which depends for its survival and proliferation on a haemopoietic cell growth factor (HCGF) (9). These HCGF-mediated effects appear to involve its action on increasing glucose transport as measured by 2-deoxyglucose (2-DOG) uptake (10,11). It remains to be determined whether normal haemopoietic cells behave in the same way to HCGF and other growth factors. We report here that CSF-1 rapidly stimulates glucose uptake in quiescent BMM in a dose-dependent manner.

MATERIALS AND METHODS

Preparation of bone marrow-derived macrophages

BMM were obtained from precursor cells in bone marrow from CBA or C3H/HeJ mice (Hall Institute, Parkville) as described previously (5,12). Briefly, frozen precursor cells, after a prior incubation in growth medium containing L-cell conditioned medium, are allowed to develop by proliferation and differentiation into the adherent BMM in Linbro (Flow Labs, Australia) 12-well dishes. The BMM are a relatively pure and homogenous population with $\geq$ 95% of the adherent cells binding CSF-1 (5). BMM were generally "starved" of growth factor for approximately 18 hours prior to use for uptake studies by reculturing in growth medium without L-cell conditioned medium.

Such factor-deprived cells have been shown to be in a quiescent G_0/G_1 state of the cell cycle (13,14 unpublished). The protocol typically gave approximately 4 x 10^5 adherent cells per well, measured by nuclei counting (15).

2-DOG uptake

^{3}H-2-deoxy-D-glucose (^{3}H-2-DOG) uptake into quiescent BMM was performed in a HEPES buffer (0.1mM 2-DOG, 135mM NaCl, 5mM KCl, 0.8mM $MgCl_2$, 1.8mM $CaCl_2$, 20mM HEPES, pH 7.4). Uptake measurements were initiated by adding ^{3}H-2-DOG (30-60 Ci/mmole, Amersham, Australia, 1 μ Ci/culture). Incubations of the 1ml cultures were in a 37°C shaking water bath. Uptake was stopped by washing the monolayers 4 times with warm HEPES buffer containing 5.5mM D-glucose and the cells solubilized in 1 ml of 0.2M NaOH. Incorporated radioactivity in 0.5ml of solubilized cells was measured by scintillation counting. Cellular protein was measured by a modified Lowry method using the BCA reagent from Pierce Chemical Company, Illinois, U.S.A.

Sources of CSF-1

For BMM cultures : Serum containing L-cell conditioned medium for BMM growth was prepared principally as described (16) but with the following alterations. Mouse L-cells (L-929 strain) were grown in 300 ml Dulbecco's modified minimum essential medium (DMEM) supplemented with 0.1g/l neomycin sulfate, 10% heat -inactivated fetal bovine serum (FBS) in 850 cm^2 Corning plastic roller bottles.

For uptake measurements : CSF-1 derived from serum-free L-cell conditioned medium was purified to homogeneity as described (17). Briefly the purification stages were: concentrated serum free L-cell conditioned medium (stage 1), calcium phosphate gel absorption (stage 2), DEAE-sepharose chromatography (Stage 3), Ultrogel AcA-44 chromatgraphy (stage 4), HPLC size exclusion

chromatography (Stage 5), HPLC reverse phase chromatography (Stage 6) and rechromatography on reverse phase HPLC (Stage 7). CSF-1 bioactivity was measured using C57Bℓ bone marrow cells in semi-solid agar medium according to ref. 6.

REAGENTS

The following reagents were obtained commercially : FBS (Flow laboratories, Australia), L-glucose, 3-O-methylglucose, 6-deoxyglucose, D-xylose and cytochalasin B (Sigma Chemical Company, St. Louis, Missouri) and D-glucose (Ajax, Australia). All other reagents were of analytical grade. All practical precautions for minimizing endotoxin contamination were taken. Solutions were routinely made up in pyrogen-free water (Abbott Hospital Products) and endotoxin levels were routinely monitored by limulus lysate tests (Commonwealth Serum Laboratories).

RESULTS

For BMM in the absence of CSF-1 the uptake of 2-DOG (0.1 mM) was linear for at least 30 minutes (Fig. 1). Incubation of BMM

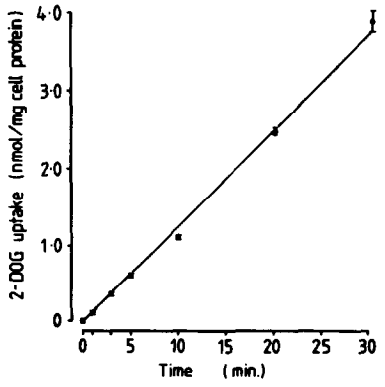

Figure 1. Glucose uptake in untreated BMM

Quiescent BMM from CBA mice were washed twice with HEPES buffer and then pulsed with ^{3}H-2-DOG for varying intervals. The zero time point was determined by pretreating the cells with phloretin (0.3mM) and immediately harvesting. This background value was subtracted from all of the experimental points. Each point represents the mean of triplicate cultures (± S.E.M.).

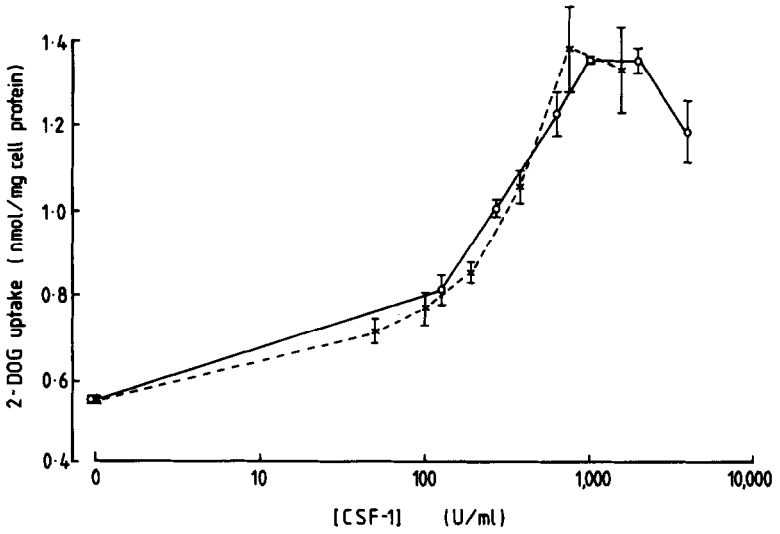

Figure 2. Effect of CSF-1 dose on glucose uptake in BMM

Quiescent BMM from C3H/HeJ mice were treated with various doses of partially purified (stage 2) CSF-1 (x---x) or pure (stage 7) CSF-1 (0—0) for 1 hour in HEPES buffer and then pulsed with ^{3}H-2-DOG for 10 minutes (Materials and Methods). CSF-1 dilutions were made in HEPES buffer containing 15% FBS, leading to a final FBS concentration in the cultures of 1.5%. Each point represents the mean of triplicate cultures (±S.E.M.).

with both partially purified (stage 2) and pure (stage 7) CSF-1 stimulated 2-DOG uptake in a dose-dependent manner (Fig. 2). Comparison of the dose response curves showed that there was little difference in the ability of the two CSF-1 preparations to increase 2-DOG uptake, indicating that purified CSF-1 was active and that the activity in the partially purified L-cell conditioned medium was due to the CSF-1. The experiment presented in Fig. 2 was carried out with BMM from the lipopolysaccharide (LPS)-hyporesponsive mouse strain, C3H/HeJ (18), suggesting that the increased 2-DOG uptake was not caused by endotoxin (LPS) contamination in the samples.

Figure 3 shows the time course of stimulation of the 2-DOG uptake by CSF-1. (For the remaining experiments presented, the partially purified CSF-1 sample was used.) Quiescent BMM were pulsed for 3 minutes at varying intervals after addition of the

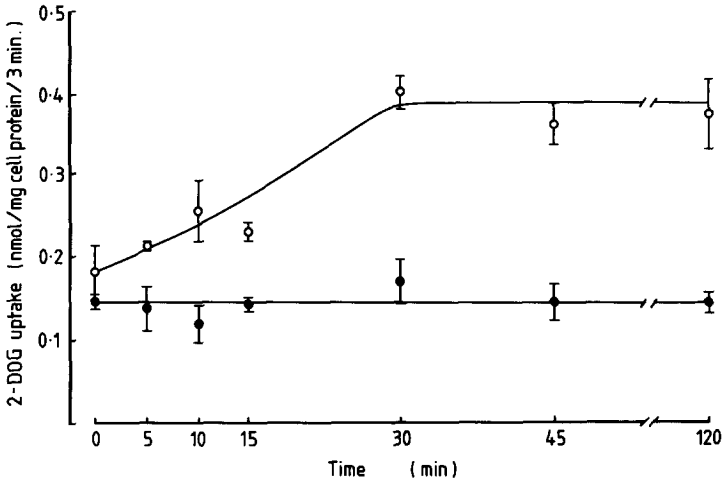

Figure 3. Kinetics of stimulation of BMM glucose uptake by CSF-1

Quiescent BMM from CBA mice were washed twice with HEPES buffer and pulsed in the same buffer for 3 minutes with ^{3}H-2-DOG at varying intervals after the addition of partially purified CSF-1 (stage 2, 1000 U/ml). (O-O), + CSF-1; (●-●), no CSF-1. Each point represents the mean of triplicate cultures ± S.E.M.

CSF-1 sample. There was a significant increase in the rate of 2-DOG uptake within 5 minutes after growth factor addition, rising sharply to a plateau within 30 minutes until 120 minutes, at which time the experiment was terminated. In other experiments, the plateau in the CSF-1 treated cultures was reached within 20 minutes and incubation in the low glucose medium resulted in a gradual increase in the basal 2-DOG uptake to a new steady state level over the 120 minute period. The maximal enhancement of 2-DOG uptake under optimal conditions never exceeded 3-fold. The experiment presented in Fig. 3 was carried out with BMM from the CBA mouse strain. Polymyxin B (1 µg/ml), a polypeptide antibiotic which binds to LPS, did not block the CSF-1 effect in this strain yet did so when LPS (100 ng/ml, E. coli, 0111:B4, Difco Labs., Detroit) was tested by itself (data not shown), again suggesting that the increased 2-DOG uptake in the CSF-1 samples was not due to endotoxin contamination.

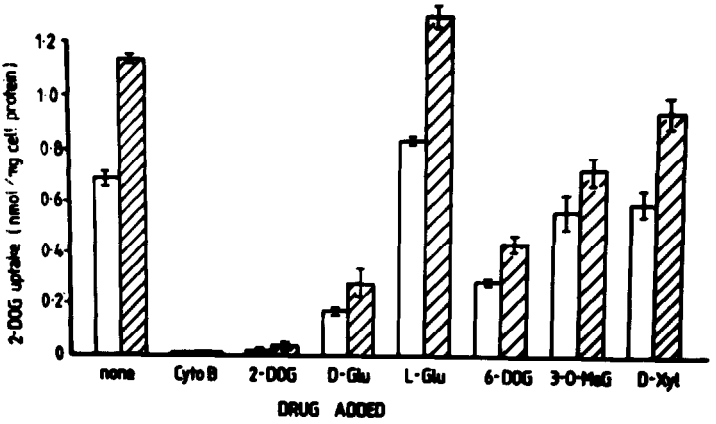

Figure 4. Specificity of the glucose uptake system in untreated and CSF-1 treated BMM

Same experiment protocol as for figure 2 except that partially purified CSF-1 (stage 2, 1000 U/ml) was used and the competing drugs were added 15 minutes before the 10 minute pulse with ^{3}H-2-DOG. BMM were from CBA mice. Cytochalasin B (Cyt) was at a final concentration of 50 μM while the following sugars were at 10mM: 2-DOG, D-glucose (D-Glu),L-glucose (L-Glu), 6-deoxy-D-glucose (6-DOG), 3-O-methylglucose (3-O-MeG), and D-xylose (D-Xyl). Each point represents the mean of triplicate cultures (±S.E.M.)

2-DOG can be used as a measure of glucose transport because it is transported by the glucose carrier (19). However, while it is non-utlilizable by incorporation into cellular material, it can be phosphorylated by cytoplasmic kinases. For this reason, 3-O-methyl glucose, a glucose analogue which is not phosphorylated intracellularly, is often used. However, we found that this analogue is taken up rather poorly in unstimulated and in CSF-1-stimulated BMMs (data not shown). Others have found that this molecule is transported poorly by macrophages (20).

Cytochalasin B, which is known to inhibit glucose uptake by its effect on the glucose carrier itself (21), completely inhibited the basal and CSF-1 induced 2-DOG uptake (Fig. 4). Similar inhibition was also caused by phloretin (0.3mM), another inhibitor of glucose transport (data not shown). Also, carrier-facilitated transport of 2-DOG is competed for by D-glucose but not by L-glucose. In Fig. 4, it can be seen that both the basal

and CSF-1 induced ^{3}H-2-DOG incorporation was lowered by high concentrations of 2-DOG and D-glucose, but not by L-glucose. 3-O-methyl-D-glucose, 6-deoxy-D-glucose and D-xylose have also been shown to have some specificity with the D-glucose transport system (22) and are effective to varying degrees in competing with the 2-DOG uptake in BMM (Fig. 4). The findings in Fig. 4 are consistent with the basal and CSF-1-enhanced 2-DOG uptake as being through the carrier-facilitated D-glucose transport system.

DISCUSSION

We have provided data which suggests that BMM possess a basal and stereospecific glucose transport system that is stimulated rapidly by CSF-1 in a dose-dependent manner. The monosaccharide transport systems of alveolar and peritoneal macrophages have been described previously and have characteristics consistent with those of a facilitated diffusion system (15,20). It is well recognised that quiescent cells stimulated to growth increase their uptake of glucose through a transport system so that more glucose is made available to meet the additional energy requirements attendant with that process (7,8,19). Recently evidence has shown that the HCGF-dependent survival of a number of haematopoietic precursor cell lines is dependent on an increase in energy metabolism resulting from the action of that particular growth factor (9-11) on glucose transport as measured by 2-DOG uptake. Our results are the first to indicate for a normal hemopoietic cell type that a purified growth factor can cause an increase in glucose uptake.

As mentioned, BMMs depend on a growth factor such as CSF-1 for survival and, in addition, BMMs and progenitor cells require the continual presence of CSF-1 for proliferation (5). It could be that for all cells along the monocyte-macrophage lineage, CSF-

1 mediates its survival effect on enhanced glucose uptake. Further experiments are in progress to answer this question and to determine the relevance or otherwise of our findings to the mitogenic effect of CSF-1 on BMM. By implication the situation could be the same for the other hemopoietic lineages which are dependent on different specific growth factors (CSFs) (6).

ACKNOWLEDGEMENTS

This work was supported by grants from the National Health and Medical Research Council of Australia, the Anti-Cancer Council of Victoria, the Utah Foundation, the Clive and Vera Ramaciotti Foundations and the Jack Brockhoff Foundation. Drs. D. Metcalf and A. Burgess are thanked for CSF-1 bioassays, Ms. K. Shanks and Mrs L. Williamson for typing of the manuscript.

REFERENCES

1. Stanley, E.R., Chen, D-M, Lin, H-S. (1978) Nature 274:168-170.
2. Stanley, E.R., Guilbert, L.J. (1981) J. Immunol. Methods 42:253-284.
3. Stanley, E.R., Heard, P.M. (1977) J. Biol. Chem. 252:4305-4312.
4. Morgan, C.J., Stanley, E.R. (1984) Biochem. Biophys. Res. Comm. 119:35-41.
5. Tushinski, R.J., Oliver, I.T., Guilbert, L.J., Tynan, P.W., Warner, J.R., Stanley, E.R. (1982) Cell. 28:71-81.
6. Metcalf, D. (1984) The hemopoietic colony stimulating factors. Elsevier. Amsterdam, New York, Oxford.
7. Bose, S.K., Zlotnick, B.J. (1973) Proc. Natl. Acad. Sci. USA. 70:2374-2378.
8. Dubrow, R., Pardee, A.B., Pollack, R. (1978) J. Cell. Physiol. 95:203-212.
9. Bazill, G.W., Haynes, M., Garland, J., Dexter, T.M. (1983) Biochem. J. 210:747-759.
10. Whetton, A.D., Bazill, G.W., Dexter, T.M. (1984) EMBO J. 3:409-413.
11. Dexter, T.M., Whetton, A.D., Bazill, G.W. (1984) Differentiation 27:163-167.
12. Vairo, G., Hamilton, J.A. (1985) Biochem. Biophys. Res. Comm. 132:430-437.
13. Pluznick, D.H., Cunningham, R.E., Naguchi, P.D. (1984) Proc. Natl. Acad. Sci. USA. 81:7451-7455.
14. Tushinski, R.J., Stanley, E.R. (1985) J. Cell. Physiol. 122:221-228.

15. Norton, J.M., Munck, A. (1980) J. Immunol. 125:252-258.
16. Hume, D.A., Gordon, S. (1983) J. Cell. Physiol. 117:189-194.
17. Burgess, A.W., Metcalf, D., Kozka, I.J., Simpson, R.J., Vairo, G., Hamilton, J.A., Nice, E.C. (1985) J. Biol. Chem. 260:16004-16011.
18. Guilbert, L.J., Stanley, E.R. (1984) J. Immunol. Methods. 73:17-28.
19. Wohlhueter, R.M., Plagemann, P.G.W. (1980) Int. Rev. Cytol. 64: 171-240.
20. Mukkada, A.J., Bonventre, P.F. (1975) J. Reticuloendothel. Soc. 17:20-29.
21. Salter, D.W., Weber, M.J. (1974) J. Biol. Chem. 254:3554-3561.
22. Romano, A.H., Connell, N.D. (1982) J. Cell Physiol. 111:77-82.