The Journal of Immunology

RESEARCH ARTICLE | DECEMBER 01 1987

Colony-stimulating factor-induced monocyte survival and differentiation into macrophages in serum-free cultures.

S Becker; ... et. al

J Immunol (1987) 139 (11): 3703–3709.
https://doi.org/10.4049/jimmunol.139.11.3703

0022-1767/87/13911-3703 $02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 139, 3703–3709, No. 11, December 1, 1987
Printed in U.S.A.

COLONY-STIMULATING FACTOR-INDUCED MONOCYTE SURVIVAL AND DIFFERENTIATION INTO MACROPHAGES IN SERUM-FREE CULTURES[1,2]

SUSANNE BECKER,[3]* M. KIM WARREN,† AND STEPHEN HASKILL‡

*From the * Environmental Monitoring and Services Inc., Chapel Hill, NC 27514; † Department of Cell Biology, Cetus Corporation, Emeryville, CA; and ‡ Department of Obstetrics and Gynecology and Lineberger Cancer Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514*

The role of mononuclear phagocyte-specific colony-stimulating factor (CSF-1) in human monocyte to macrophage differentiation was investigated. The addition of 1000 U/ml of CSF-1 to serum-free monocyte cultures resulted in monocyte survival comparable to that in cultures containing 5% AB serum, whereas cells in serum- and CSF-1-free medium lost their viability in 3 to 5 days. The requirement for CSF-1 coincided with the time (40 to 64 hr of culture) when the major changes in morphology and biochemical function took place in monocytes undergoing differentiation into macrophages. If CSF-1 was removed from the cultures before this time, death of the monocytes resulted. In cultures containing CSF-1, as in serum containing cultures, the lysosomal enzyme acid phosphatase was enhanced 10- to 20-fold by day 4 to 5. Superoxide production in response to phorbol myristic acetate was maintained in CSF-1 cultured monocytes, but declined with time in monocytes cultured in serum. The expression of monocyte-macrophage antigens p150.95 (LeuM5), OKM1, LeuM3, Fc receptors (32.2), and HLA-DR had increased in CSF-1 containing cultures at day 4. When antigen expression was analyzed at day 2 to 3, when cell size and 90° scatter characteristics were still identical to control serum-free cultures, only p150.95, HLA-DR and FcR expression were enhanced by CSF-1. Low amounts of lipopolysaccharide (0.1 ng/ml) were found to enhance monocyte survival in the absence of added CSF-1. Lipopolysaccharide-containing cultures were found to produce CSF-1 (up to 450 U/ml, as detected by radioimmunoassay). Lipopolysaccharide (1 μg/ml), however, did not induce enhanced expression of the maturation-related antigens. Based on these observations we conclude that CSF-1 is enhancing human monocyte survival and is involved in the events leading to the differentiation of monocytes into macrophages.

When blood monocytes are cultured in medium containing human serum (5 to 20%), they undergo a number of morphologic and biochemical changes that have been considered hallmarks of monocyte to macrophage maturation/differentiation (1, 2). Intracellular levels of lysosomal enzymes increase, number and function of complement receptors change, and phagocytosis increases as does also the expression of various antigens of known and unknown functions. All of these changes are paralleled by an increase in cell size and granularity, indicating that size increase is a good marker of macrophage maturation. Attempts to identify the components in serum responsible for inducing these events have been unsuccessful (3). Fetal bovine serum apparently lacks the component because this medium supplement seldom supports macrophage maturation (4, 5).

In several studies (6, 7) mouse bone marrow-derived, peritoneal, and alveolar macrophages were shown to require colony-stimulating factor (CSF-1)[4] for their survival in fetal bovine serum-containing medium. Others (8, 9) have shown that CSF-1 induces differentiation or activation of bone marrow-derived macrophages most likely by inducing type I interferons which in turn induce enhanced Fc receptor function, interleukin 1 production, and Mac 1 expression.

Herein the role of CSF-1 in human monocyte to macrophage maturation was investigated. Monocytes were found to survive and develop maturation characteristics under serum-free conditions in the presence, but not in the absence, of CSF-1, indicating that this factor is a sufficient signal for the induction of the complex events involved in macrophage maturation.

MATERIALS AND METHODS

CSF-1 preparations. Human CSF-1 was produced by the pancreatic carcinoma cell line MIA Paca (10) after stimulation with 50 ng/ml phorbol myristic acetate (PMA) and 10 μM retinoic acid (Sigma Chemical Co., St. Louis, MO) as has been previously described (11). A two-step purification with calcium phosphate and lentil lectin Sepharose resulted in CSF-1 with a specific activity of 5×10^5 U/mg protein. Recombinant CSF-1 was obtained from supernatants of transfected COS cells. Supernatant from COS cells transfected with a plasmid containing no insert served as control in several of the experiments.

Isolation of monocytes. Mononuclear cells were isolated from heparinized venous blood or from "buffy coat" bags provided by the Red Cross Blood Bank (Palo Alto, CA) using Ficoll-Hypaque (LSM,

Received for publication June 18, 1987.
Accepted for publication September 9, 1987.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This study was supported in part by United States Public Health Service Grant HD21546-01 and by United States Environmental Protection Agency Contract 68-02-4179.
[2] The research described in this article has been reviewed by the Health Effects Research Laboratory, United States Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.
[3] Address corresspondence and reprint requests to Susanne Becker, US EPA, Medical Research Building C 224-H, University of North Carolina, Chapel Hill, NC 27514.

[4] Abbreviations used in this paper: CSF-1, colony-stimulating factor; PMA, phorbol myristic acetate.

Litton Bionetics, Charleston, SC) density separation. Monocytes were isolated either by adherence for 60 min to 24-well plates (Costar, Cambridge, MA) coated with a thin film of AB or autologous serum, or by density separation on Percoll (Pharmacia, Piscataway, NJ) (12). The monocytes that were followed for survival and maturation changes were >80% pure, as determined by morphology and myeloperoxidase staining (13) on cytocentrifuge preparations.

Culture of monocytes. Monocytes were cultured in serum-free medium CPD-24D containing insulin and transferrin (Cetus Corp., Emeryville, CA), either containing various amounts of CSF-1, 5% AB serum (KC Biologicals, Lenexa, KS), or no additives. In some of the experiments (immunofluorescence determination of cell surface markers) the cells were cultured in RPMI 1640 with the same additives. The monocytes were present at 1 to 3×10^5/1.5 ml/well in the 24-well plates and at 3 to 5×10^4/0.2 ml/well or in 96-well plates. At different times after culture initiation the monocytes were detached from the 24-well plates by vigorous pipetting after incubation with versene 1/5000 (GIBCO, Grand Island, NY) for 5 to 10 min and counted to determine total number of surviving monocytes. Viability of the cells was determined by trypan blue exclusion.

Determination of intracellular acid phosphatase. Monocytes were lysed with 0.1 ml 0.02% Triton X-100 in H_2O. Acid phosphatase activity in 4×10^4 cells was determined in a microassay for the enzyme, performed in 96-well microtiter plates. A total of 20 μl of cell lysate were added to 100 μl of 0.1 M citrate buffer pH 4, containing *p*-nitrophenolphosphate (Sigma) as enzyme substrate and the plates were incubated at 37°C. The enzyme reaction was stopped with 100 μl of 0.1 N NaOH at 60 min and the absorbance of released *p*-nitrophenol determined in enzyme-linked immunosorbent assay reader (Biotech, Rockville, MD) at 405 nm. One unit of acid phosphatase was defined as the amount of activity that resulted in 1 μmol of hydrolyzed substrate.

Determination of superoxide production. Superoxide release in response to stimulation with 10 ng/ml of PMA was determined as has been described by Pick and Mizel (14). Monocytes were cultured for various lengths of time in the 96-well plates and the assay for superoxide production was performed directly in the wells. The culture medium was removed and replaced with phosphate-buffered saline, pH 7.6, containing glucose and 18 mg/ml of ferricytochrome C (Sigma), with and without PMA (Sigma), and with and without 300 U/ml superoxide dismutase (Sigma). The amount of protein in each well was determined by a micromodification of the Bio-Rad protein assay (Bio-Rad, Richmond, CA).

Monoclonal antibodies and immunofluorescence. Monocytes were detached from the 24-well plates with Versene 1/5000 and then reacted with monoclonal antibodies to HLA-DR (Becton Dickinson, Mountain View, CA), to complement receptor CR3 (OKM1, Ortho Diagnostics, Raritan, NJ), to monocyte maturation antigen p150.95 (LeuM5, Becton Dickinson) of the LFA-1 adherence protein family, to high affinity FcR (mAb 32.2, kindly provided by Dr. Fanger, Dartmouth, NH), and to a monocyte-macrophage antigen LeuM3 (Becton Dickinson) for 60 min on ice. After two washes in RPMI with 10% human serum the fluorescein isothiocyanate-conjugated goat anti-mouse IgG was added for 60 min, thereafter the cells were washed and fixed with 1% paraformaldehyde. Background fluorescence was determined on monocytes reacted with nonspecific antibodies of the IgG1 and IgG2a subtypes (Becton Dickinson) in the first incubation. Fluorescence intensity, cell size, and 90° light scatter of the monocytes (this cell population was resolved from possible contaminating lymphocytes by size and scatter) were analyzed with a fluorescence-activating cell analyzer (Becton Dickinson). Fluorescence histograms were displayed on log scale and fluorescence intensity of the test population was expressed as mean log channel number of the histogram.

Radioimmunoassay (RIA) for CSF-1. A specific RIA was used as described previously (15). Highly purified (50% pure) CSF-1 from supernatants of MIA PaCa cells was iodinated and used to compete for binding to rabbit antibody against human urinary CSF-1. Partially purified MIA PaCa standard was titered in the murine bone marrow colony assay (15). The murine bone marrow colony assay was also used to measure biologic activity of some monocyte supernatants, with and without antibody to human urinary CSF-1.

RESULTS

Effects of CSF-1 on human monocyte survival under serum-free conditions. Monocytes cultured for 2, 4, and 6 days in the presence and absence of 1000 U/ml partially purified CSF-1 (from MIA PaCa) or in the presence of 5% human AB serum were analyzed for cell number and viability (Fig. 1). At day 2 cell recovery and viability

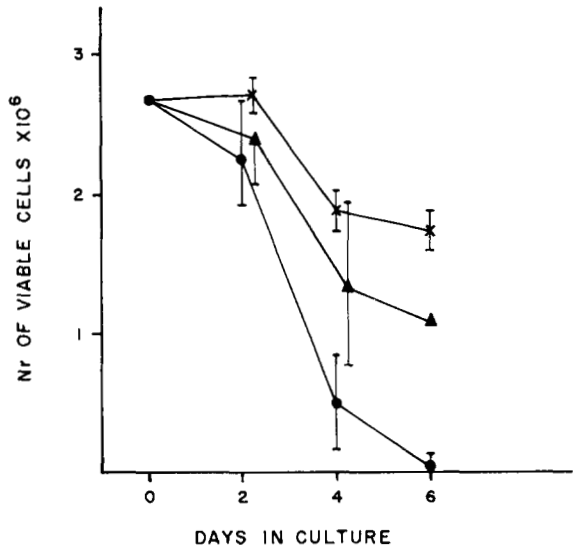

Figure 1. Survival of monocytes in serum-free medium containing CSF-1 and in 5% human AB serum. Monocytes were cultured for various lengths of time in 5% AB serum (▲), 1000 U/ml CSF-1 (X), and control serum-free cultures (●). The *points* represent mean ± SD of three experiments.

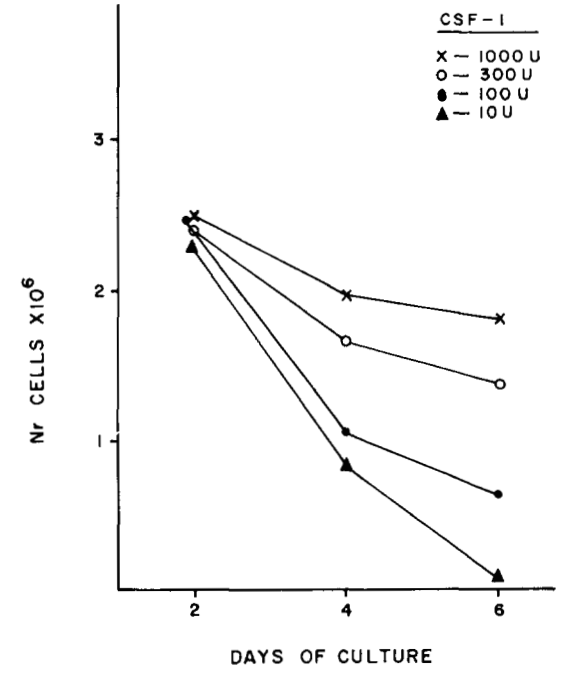

Figure 2. Determination of rCSF-1 concentration for optimal survival of monocytes in serum-free cultures. Monocytes were cultured in serum-free medium with 1000 U/ml (X), 300 U/ml (O), 100 U/ml (●), and 10 U/ml (▲) of CSF-1 added. Survival was determined by total cell count of trypan blue excluding monocytes.

was similar under all three culture conditions. After 4 days the survival of initially plated cells was 18% in serum-free cultures and 64% in cultures containing 1000 U/ml CSF-1 (mean ± SD of three experiments). At day 6 all monocytes were dead in the control cultures while recovery of viable cells in CSF-1 was 61%. In these experiments viability in 5% AB serum was lower than in CSF-1.

Optimal dose of CSF-1 for monocyte survival and maturation. Monocytes were cultured in medium containing various concentrations of rCSF-1 (Fig. 2). The optimal dose of CSF-1 was determined to be 1000 U/ml,

while lower doses resulted in decreased viability in a dose-dependent manner. Levels of CSF-1 of more than 1000 U/ml did not result in enhanced viability, neither with rCSF nor conventional MIA PaCa CSF-1 (not shown). Cells surviving in the cultures underwent morphologic changes characteristic of monocyte to macrophage maturation such as increase in size (Coulter volume), in granularity (90% light scatter), and adherence. These changes occurred at a later time (4 to 6 days) than in human serum-containing cultures (3 to 4 days) (data not shown).

Determination of time when CSF-1 is required for survival and maturation. A total of 1000 U of CSF-1 was added to serum-free cultures, or removed from cultures of monocytes stimulated with CSF-1 since day 0, at various times, to determine the temporal requirements of CSF-1 for induction of survival and maturation-related changes in morphology. Figure 3 shows a representative experiment in which CSF-1 was found to be required between days 2 and 3 of culture. This preceded the time when the major changes in enzyme activity as well as monocyte morphology took place in cultures with CSF-1 present throughout the time course. In different experiments the actual time point CSF-1 was required varied between 2 and 4 days in different monocyte preparations, but always preceded the time when CSF-1-containing cultures showed maturation changes.

Enhancement of acid hydrolase activity in monocytes cultured in CSF-1. Enhanced lysosomal enzyme levels accompany maturation of monocytes into macrophages (1, 2). To evaluate the effects of CSF-1 on this marker of maturation, acid phosphatase activity was monitored at various times in monocytes cultured in CSF-1 and in 5% AB serum. In Figure 4, acid phosphatase levels in CSF-1 cultured monocytes were compared with those in monocytes maturing into macrophages in the presence of 5% AB serum. Cultures containing 5% AB serum developed higher levels of activity than those containing CSF-1 on a per cell basis, whereas activity on per milligram protein basis was higher in the cultures maturing in the presence of CSF-1. Monocytes cultured serum-free were also analyzed for acid phosphatase activity at day 2 and showed low activity similar to cells cultured in CSF-1 and AB serum. Cells at the later time points were not tested because at day 4 less than 30% of the cells were viable and at day 7 viability was less than 5%.

Superoxide production in cultures maturing in the presence of CSF-1. Superoxide production in AB serum-containing cultures declines as monocytes mature into macrophages (19). In serum-free cultures containing CSF-1 the ability to reduce ferricytochrome *c* in response to PMA was maintained on per cell basis (Fig. 5*A*) for the 6 days they were studied. When activity per milligram of protein was calculated (Fig. 5*B*), there was a gradual decrease in activity due to gradual increase in protein per cell.

Development of macrophage membrane markers in CSF-1 containing cultures. Monocytes cultured for 3 days in 1000 U/ml of CSF-1 were analyzed for expression of HLA-DR, p150.95, CR3, FcR1, and LeuM3. At this time point no apparent increase in cell size was found in the CSF-1 cultures compared with control serum-free cultures, whereas AB serum-containing cultures had increased their size 1.8-fold. In response to CSF-1, HLA-DR was the only antigen showing a considerable increase in expression (2.3-fold) although LeuM5 and 32.2 showed a clear shift in the histogram display of fluorescence intensity but a smaller difference in mean fluorescence (Fig. 6 *A* to *C*; Table I). When the cells were analyzed at day 4 (Table I) and the monocytes cultured in CSF-1 had increased in size, the expression of OKM1 and LeuM3 was also increased.

CSF-1 production and enhanced monocyte survival

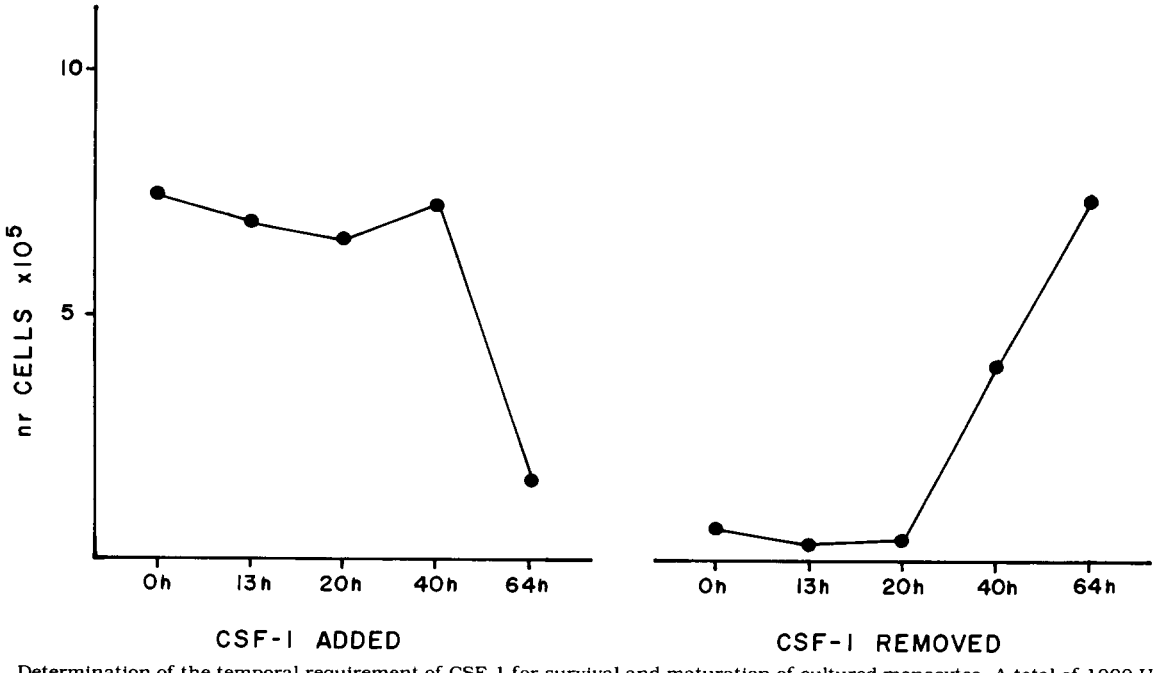

Figure 3. Determination of the temporal requirement of CSF-1 for survival and maturation of cultured monocytes. A total of 1000 U/ml of rCSF-1 was added to, or CSF-1 (added at day 0) was removed from, monocyte cultures at various times. Total number of surviving monocytes was determined at day 6 of culture.

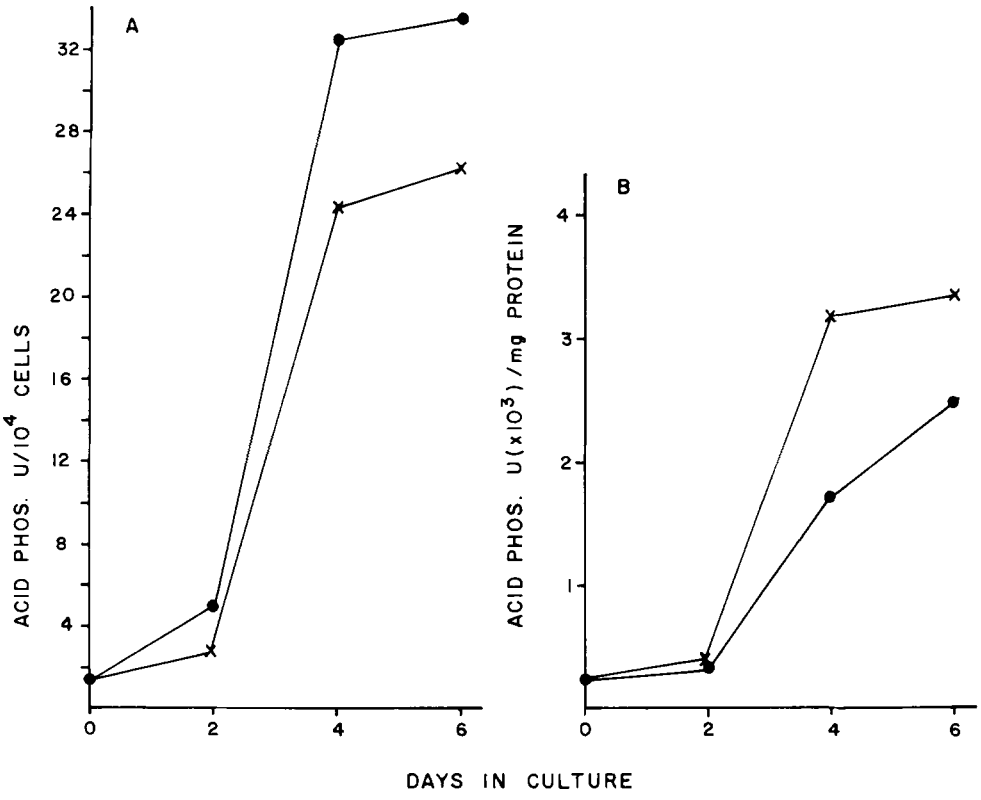

Figure 4. Acid phosphatase activity in monocytes cultured in purified CSF-1 for different lengths of time. Acid phosphatase activity was analyzed either on per cell basis (*A*) or per milligrams of protein (*B*). (●) monocytes cultured in 5% AB serum, (X) monocytes cultured in 1000 U/ml CSF-1. The *points* are means of triplicate cultures with a SD <5%.

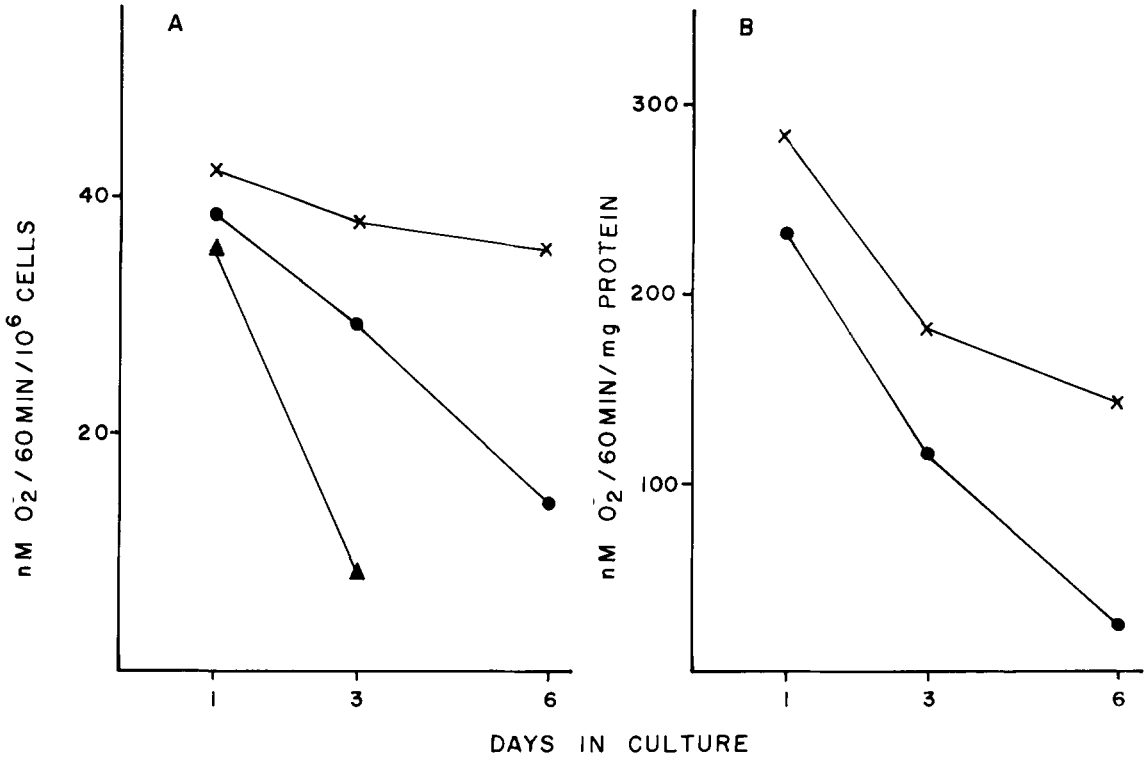

Figure 5. Superoxide production by monocytes cultured in purified CSF-1 under serum-free conditions. Monocytes were tested for O_2^- production in response to PMA after different times of culture, (●) monocytes cultured in 5% AB serum, (X) monocytes cultured in 1000 U/ml CSF-1, and (▲) with no additives.

by lipopolysaccharide (LPS). The experiments evaluating CSF-1-induced survival and maturation were done in medium that was tested to be LPS-free to the sensitivity of 0.05 ng/ml. The role of possible LPS contamination in monocyte survival was then investigated. When various concentrations of LPS were added to the cultures, survival was enhanced with as little as 0.1 ng LPS/ml. The supernatants of LPS-stimulated monocytes were found to contain CSF-1 by RIA (Table II). Bioactivity of CSF-1 in the LPS-stimulated monocyte supernatant was confirmed in mouse bone marrow colony assay. Addition of specific antibody to CSF-1 eliminated colony formation,

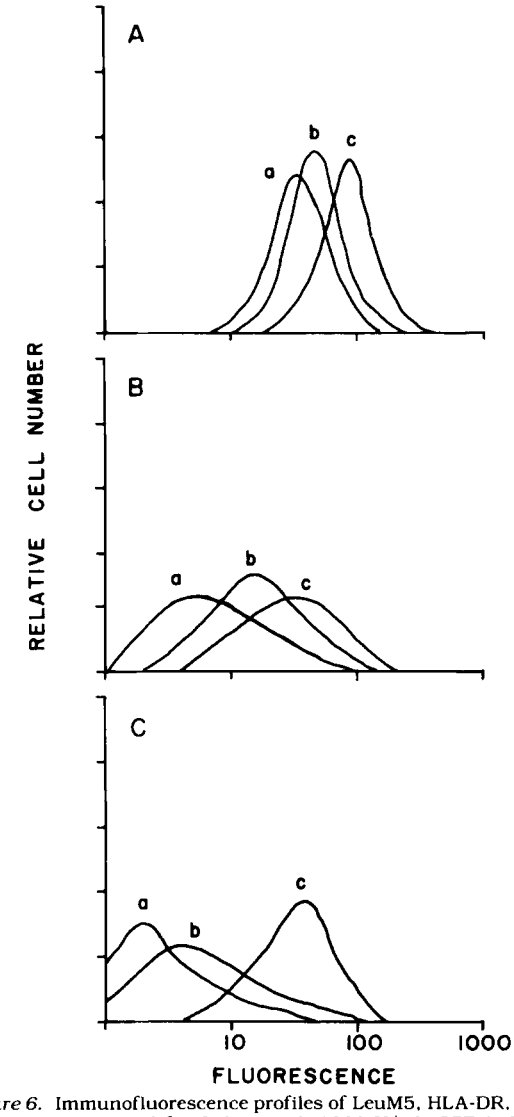

Figure 6. Immunofluorescence profiles of LeuM5, HLA-DR, and 32.2. Monocytes were treated for 3 days with 1000 U/ml rCSF-1 (*b*), 5% AB serum (*c*), and no additive (*a*). *A* shows expression of LeuM5, *B* shows HLA-DR, and *C* shows expression of 32.2. The fluorescence histograms are shown on a log scale.

TABLE I

CSF-1-induced marker expression on monocytes in serum-free cultures

Treatment	Mean Fluorescence Channel No.			
	HLA-DR	32.2	LeuM5	OKM1
Day 3				
None	7	5	42	26
CSF-1 1000 (U/ml)	16	9	52	27
5% AB[a]	35	44	100	34
	LeuM5	OKM1	LeuM3	Size
Day 4				
None[b]	39	20	19	44
CSF-1 1000 (U/ml)	78	60	32	64
5% AB	130	135	61	140

[a] The monocytes in AB serum had increased their volume 1.8-fold.
[b] Control cells (in serum-free medium with no additives) were 46% viable when analyzed. Only viable cells were analyzed for fluorescence.

confirming the presence of CSF-1 in the supernatants (data not shown). Monocytes stimulated with LPS did not, however, undergo antigenic changes indicative of monocyte to macrophage maturation, as defined by changes occurring in 5% autologous serum. HLA-DR and LeuM5 levels were less than those of serum-free control monocytes (Table III).

TABLE II

LPS induces CSF-1 production and enhanced survival of monocytes[a]

LPS (ng/ml)	CSF-1 (U/ml)	Survival (%)
100	444	83
10	168	85
1	267	87
0.1	169	85
	31	21

[a] Supernatants for determination of units of CSF-1 were harvested at 72 hr. Viability of the monocytes was determined at day 5.

TABLE III

LPS-stimulated monocytes do not undergo antigenic changes indicative of monocyte to macrophage maturation[a]

Treatment	Mean Fluorescence Channel No.		
	HLA-DR	LeuM5	OKM1
	11	38	20
LPS (1 μg/ml)	7	25	59
CSF-1 (1000 U/ml)	26	78	60
5% AB	53	135	130

[a] The flow cytometry experiments were performed at day 4. The control cells (no additives to serum-free medium) were 75% viable at the time of assay.

DISCUSSION

The expression of monocyte-macrophage maturation-related changes and functions are under the influence of a wide variety of biologic signals (15–17). The basic changes occurring during in vitro adherent culture of monocytes, in medium containing human serum (5 to 20%), are believed to represent events taking place in vivo as the monocytes migrate out of the blood vessels and differentiate into tissue macrophages in the absence of a specific modifying stimulus. The actual signal in serum that allows the maturation changes to take place has not been defined, but was shown to co-isolate with serum albumin in a study by Musson (3). Herein we have identified CSF-1 as a factor that by itself can induce monocytes to mature into macrophages in the absence of any serum proteins or lipids. Previous studies by Stanley and co-workers (6, 7) in which highly purified L cell-derived CSF-1 was used to study macrophage survival indicated that this factor was necessary to specifically stimulate macrophage colony formation in bone marrow cultures as well as to maintain viability and protein synthesis in various mouse macrophages including peritoneal, alveolar, and bone marrow-derived macrophages. Our studies on the effect of CSF-1 on human monocytes emphasize not only survival but also macrophage differentiation-related events. After establishing that CSF-1 greatly enhanced the survival of monocytes under serum-free conditions, we chose to monitor the activity of the lysosomal enzyme acid phosphatase in monocytes cultured with CSF-1. We found that cells cultured in this factor showed a similar increase in activity of this lysosomal enzyme as monocytes cultured in 5% human serum. This suggested that CSF-1 also was capable of inducing changes representative of maturation in monocytes. Interestingly the requirement for CSF-1 in serum-free cultures was found to coincide with the time when obvious morphologic maturation changes took place in serum-containing cultures. Previous studies by Warren and Ralph (18) found that 2 to 3 days of monocyte-CSF-

1 co-culture was required before this factor was depleted from the medium and the activation effects of CSF-1, such as enhanced tumor necrosis factor and interferon production, could be measured. Other findings that fit the relatively long culture time required before CSF-1 exerts its effects are data on loss and reexpression of c-fms mRNA, the putative CSF-1 receptor, after adherence of monocytes and subsequent culture for 24 to 48 hr.[5]

In contrast to the increase in lysosomal enzyme levels, the ability of cultured monocytes to produce reactive oxygen species, O_2^- and H_2O_2, decreases as the cells mature into macrophages in serum-containing medium (19). When the cells differentiate in the presence of CSF-1 this activity is maintained on a per cell basis, and the cells produce similar levels of O_2^- at day 5 of culture as on the first day. This suggests that tissue macrophages produce low levels of reactive oxygen, possibly due to modulating factors in their in vivo environment. For example, the alveolar macrophages spontaneously produce some H_2O_2 and are poorly stimulated by PMA (20). This could be due to the constant phagocytic stimulation of the macrophages in their environment. Mouse macrophages have been shown to have enhanced superoxide production when cultured for 48 hr with CSF-1 (21) whereas human monocytes cultured in 5% serum in the presence of CSF-1 did not show enhanced production of superoxide (data not shown); however, the experiments were done with monocytes cultured for 48 hr at which time CSF-1 might not have affected the monocytes due to lack of CSF-1 receptor expression. However, Nathan et al. (22) also found no effect of CSF-1 on H_2O_2 production by human monocytes.

Various monoclonal antibodies have been developed against surface antigens of human monocytes and macrophages. In general the antigens detected by the antibodies are expressed at a lower amount on monocytes than on macrophages due to their smaller size and surface area. Some antigens are therefore directly related to cell size whereas others such as HLA-DR and Fc receptors can be modulated by different biologic factors (16, 17). The five antigens we have investigated in this study, HLA-DR, OKM1, LeuM5, 32.2, and LeuM3 are all expressed on monocytes and to a higher degree on monocyte-derived macrophages. Herein we have found that the antigens HLA-DR and FcR1 (32.2) were induced to higher levels of expression (surface density) by CSF-1 before an actual size increase in the cells took place, suggesting that these antigens were regulated by this factor. In contrast, OKM1 and LeuM3 expression was not affected until the cells actually increased in size.

CSF-1 appears to be an autoregulatory molecule since the factor is produced by monocytes[5] (23). Adherence of monocytes has been shown to induce CSF-1 message in these cells, without release of significant levels of CSF-1 into the culture supernatants. A second signal provided by LPS or PMA leads to the marked release of CSF-1 into the media.[5] During the course of our experiments it became obvious that endogenously produced CSF-1 could possibly affect the survival of monocytes, and therefore, it was crucial to perform the experiments in endotoxin-free medium. We found that as low levels of LPS as 0.1 ng/ml triggered the production of >300 U of CSF/10^6 cells in some individuals. LPS-stimulated monocytes survived for more than 6 days but did not develop maturation characteristics as determined by monoclonal antibodies and morphology. This could be due to the multitude of other competing factors that are induced by LPS which regulate antigen expression and maturation changes. Cline et al. (24) previously showed that colony-stimulating activity was released after triggering of adherent cells with LPS, although in their studies the nature of the colony-stimulating factor was not determined. In our studies blood monocytes from some donors survived under serum-free, LPS-free conditions but when the supernatants were tested they were found to contain >100 U CSF-1, suggesting that the cells were already stimulated when they were obtained. Of course it is not clear from these experiments that the CSF-1 is the only cause of enhanced survival after LPS treatment, because a wide variety of regulatory factors is induced, but it is reasonable to speculate that CSF-1 is at least partly responsible for the effect, based on the role it has as a survival- and maturation-enhancing molecule in serum-free monocyte cultures.

Acknowledgments. We thank P. Shadle and J. Csejtey of Protein Chemistry for purification of rCSF-1, and the Assay Laboratory, Cetus Corp., Emeryville, CA, for CSF-1, RIA, and endotoxin assays. We also thank Velva Milholland for analyzing the immunofluorescence assays, Peter Ralph and Hillel Koren for comments on the manuscript, and Linda McAlister for manuscript preparation.

REFERENCES

1. **Musson, R. A., H. Shafran, and P. M. Henson.** 1980. Intracellular levels and stimulated release of lysosomal enzymes from human peripheral blood monocytes and monocyte-derived macrophages. *J. Reticuloendothel. Soc. 28:249.*
2. **Haskill, S., and S. Becker.** 1982. Flow cytometric analysis of macrophage heterogeneity and differentiation. *J. Reticuloendothel. Soc. 32:273.*
3. **Musson, R. A.** 1983. Human serum induces maturation of human monocytes in vitro, changes in cytolytic activity, intracellular lysosomal enzymes, and nonspecific esterase activity. *Am. J. Pathol. 111:331.*
4. **Johnson, W. D., B. Mei, and Z. A. Cohn.** 1977. The separation, long-term cultivation, and maturation of the human monocyte. *J. Exp. Med. 146:1613.*
5. **Hammarstrom, J.** 1979. Human macrophage differentiation in vivo and in vitro. A comparison of human peritoneal macrophages and monocytes. *Acta Pathol. Microbiol. Immunol. Scand. [C] 87:113.*
6. **Tushinski, R. J., I. T. Oliver, L. J., Guilbert, P. W. Tynan, J. R. Warner, and E. R. Stanley.** 1982. Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. *Cell 28:71.*
7. **Das, S. K., and E. R. Stanley.** 1982. Structure-function studies of a colony stimulating factor (CSF-1). *J. Biol. 257:13679.*
8. **Warren, M. K., and S. N. Vogel.** 1985. Bone marrow-derived macrophages: development and regulation of differentiation markers by colony-stimulating factor and interferons. *J. Immunol. 134:982.*
9. **Moore, R. N., F. J. Pitruzzello, H. S. Larsen, and B. T. Rouse.** 1984. Feedback regulation of colony-stimulating factor (CSF-1)-induced macrophage proliferation by endogenous E prostaglandins and interferon-α/β. *J. Immunol. 133:541.*
10. **Csejtey, J., and A. Boosman.** 1986. Purification of human macrophage colony stimulating factor (CSF-1) from medium conditioned by pancreative carcinoma cells. *Biochem. Biophys. Res. Commun. 138:238.*
11. **Ralph, P., M. K. Warren, M. T. Lee, J. Csejtey, J. F. Weaver, H. E. Broxmeyer, D. E. Williams, E. R. Stanley, and E. S. Kawasaki.** 1986. Inducible production of human macrophage growth factor, CSF-1. *Blood 68:633.*
12. **Colotta, F., G. Peri, A. Villa, and A. Mantovani.** 1984. Rapid killing of actinomycin D-treated tumor cells by human mononuclear cells. I. Effectors belong to the monocyte-macrophage lineage. *J. Immunol.*

[5] **Haskill, S., K. Warren, S. Becker, M. Ladner, C. Johnson, D. Eierman, P. Ralph, and D. Mark.** 1987. Adherence induces CSF-1 gene expression in monocytes: autocrine regulation of maturation? *Submitted for publication.*

132:936.

13. **Kaplow, L. S.** 1965. Simplified myeloperoxidase stain using benzidine dihydrochloride. *Blood 26:215.*
14. **Pick, E., and D. Mizel.** 1981. Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an automatic enzyme immunoassay reader. *J. Immunol. Methods 46:211.*
15. **Stanley, E. R.** 1985. The macrophage colony-stimulating factor, CSF-1. *Methods Enzymol. 116:564.*
16. **Guyre, P. M., P. M. Morganelli, and R. Miller.** 1983. Recombinant immune interferon increases immunoglobulin G Fc receptors on cultured human mononuclear phagocytes. *J. Clin. Invest. 72:393.*
17. **Gerrard, T. L., T. R. Cupps, C. H. Jurgensen, and A. S. Fauci.** 1984. Hydrocortisone-mediated inhibition of monocyte antigen presentation: dissociation of inhibitory effect and expression of DR antigens. *Cell. Immunol. 85:330.*
18. **Warren, M. K., and P. Ralph.** 1986. Macrophage growth factor CSF-1 stimulates human monocyte production of interferon, tumor necrosis factor, and colony stimulating activity. *J. Immunol. 137:2281.*
19. **Nakagawara, A., C. F. Nathan, and Z. A. Cohn.** 1981. Hydrogen peroxide metabolism in human monocytes during differentiation in vitro. *J. Clin. Invest. 68:1243.*
20. **Murray, H. W., R. A. Gellene, D. M. Libby, C. D. Rothermel, and B. Y. Rubin.** 1985. Activation of tissue macrophages from AIDS patients: in vitro response of AIDS alveolar macrophages to lymphokines and interferon-γ. *J. Immunol. 135:2374.*
21. **Wing, E. J., N. M. Ampel, A. Waheed, and R. K. Shadduck.** 1985. Macrophage colony-stimulating factor (M-CSF) enhances the capacity of murine macrophages to secrete oxygen reduction products. *J. Immunol. 135:2052.*
22. **Nathan, C. F., T. J. Prendergast, M. E. Wiebe, E. R. Stanley, E. Platzer, H. G. Remold, K. Welte, B. Y. Rubin, and H. W. Murray.** 1984. Activation of human macrophages: comparison of other cytokines with interferon-γ. *J. Exp. Med. 160:600.*
23. **Horiguchi, J., M. K. Warren, P. Ralph, and D. Kufe.** 1986. Expression of the macrophage specific colony-stimulating factor (CSF-1) during human monocytic differentiation. *Biochem. Biophys. Res. Commun. 141:924.*
24. **Cline, M. J., B. Rothman, and D. W. Golde.** 1974. Effect of endotoxin on the production of colony-stimulating factor by human monocytes and macrophages. *J. Cell. Physiol. 84:193.*