Proc. Natl. Acad. Sci. USA
Vol. 76, No. 5, pp. 2326–2330, May 1979
Cell Biology

Induction of prostaglandin E synthesis in normal and neoplastic macrophages: Role for colony-stimulating factor(s) distinct from effects on myeloid progenitor cell proliferation

(myelopoiesis/subpopulation heterogeneity)

JEFFREY I. KURLAND*, LOUIS M. PELUS, PETER RALPH, RICHARD S. BOCKMAN, AND MALCOLM A. S. MOORE

Laboratories of Developmental Hematopoiesis and Calcium Metabolism, Memorial Sloan-Kettering Institute for Cancer Research, New York, New York 10021

Communicated by Van R. Potter, February 16, 1979

ABSTRACT **The biosynthesis of prostaglandin E (PGE) by normal and neoplastic macrophages is intrinsically linked to their synthesis of, and exposure to, myeloid colony-stimulating factors (CS-factors). The defect in responsiveness to endotoxin lipopolysaccharide (LPS) by macrophages from C3H/HeJ mice extends equally to the synthesis of CS-factor and PGE. However, C3H/HeJ macrophages can be stimulated to synthesize PGE by treatment with agents other than LPS [zymosan, tuberculin purified protein derivative, concanavalin A, poly(I)·poly(C)], which also stimulate CS-factor production, or by the addition of various preparations of soluble CS-factor. In peritoneal wash preparations, constitutive PGE synthesis occurred in rapidly sedimenting macrophage cells, whereas constitutive CS-factor production and inducible PGE synthesis occurred in slower sedimenting adherent cells. A similar functional heterogeneity in CS-factor and PGE production was found in neoplastic macrophage cell lines. The association of elevated CS-factor levels and PGE synthesis by macrophages suggests a role for CS-factor in many of the physiological responses heretofore associated with elevated tissue levels of the E type prostaglandins.**

The growth and maturation of myeloid progenitor cells *in vitro* depends upon a class of regulatory proteins termed colony-stimulating factor(s) [CS-factor(s)] (1, 2). The elaboration of CS-factors by mononuclear phagocytes (3–5), particularly after their exposure *in vitro* to agents—such as bacterial endotoxin (6, 7)—that elevate serum CS-factor levels and stimulate granulocyte and monocyte–macrophage formation *in vivo* (8, 9) has implicated the blood monocyte and tissue macrophage as the primary CS-factor-producing cell population. Clonal proliferation of both granulocyte–macrophage progenitor cells (1) and macrophage precursor cells (10) in semisolid medium is the usual bioassay for the regulatory action of CS-factors.

We recently showed that increasing concentrations of a source of CS-factor progressively stimulated prostaglandin E (PGE) synthesis by human and murine macrophages (11). The endotoxin promotion of CS-factor production was also correlated with increased PGE synthesis by macrophages (12). The present communication demonstrates that PGE synthesis by both normal and neoplastic mononuclear phagocytes may be linked to and regulated by CS-factor production. Furthermore, velocity sedimentation experiments indicate that an intimate relationship between the synthesis of CS-factor and PGE exists in physically separate macrophage subpopulations.

MATERIALS AND METHODS

Cell Cultures. Noninduced peritoneal cells were isolated from 8- to 12-week-old C3HeB/FeJ, C3H/HeJ, and B6D2F$_1$ mice (Jackson Laboratory) by peritoneal lavage and incubated in 35-mm plastic dishes (Lux Scientific, Newbury Park, CA) in McCoy's 5A modified medium containing 15% fetal calf serum (Flow Laboratories, Rockville, MD). After 1.5 hr, nonadherent cells were removed by aspiration, the cell monolayers were washed three times with phosphate-buffered saline, and fresh McCoy's 5A medium supplemented with 15% fetal calf serum was added. At this time, 15–20% of the initial cells remained as adherent esterase-positive macrophages (13). Resuspended cells were incubated alone or with various test compounds for analysis of CS-factor and PGE production. Stimulating agents included lipopolysaccharide endotoxin (LPS) from *Salmonella typhosa* (W0901, Difco), zymosan (ICN, K & K Laboratories, Plainview, NY) prepared as previously described (14), concanavalin A (Con A) (Pharmacia), tuberculin purified protein derivative (PPD) (Merck, Sharp & Dohme), poly(I)·poly(C) (Bionetics, Kensington, MD), semipurified CS-factor from WEHI-3-conditioned medium (15), and CS-factor purified from L-cell-conditioned medium (16).

Neoplastic Cell Lines. The growth characteristics and properties of the various cell lines have been described (17, 18): myelomonocytic leukemia WEHI-3 and macrophage tumors J774, RAW 264, PU5-1.8, and SK2.2(19); T-cell lymphoma EL4, lymphocytic lymphoma RBL3, fibrosarcomas L-929 and HSDM$_1$ (20); and the human adherent SpGcT (21) and monocytic U-937 (22) cell lines.

CS-Factor and PGE Assays. Cell-free supernatants were assayed for CS-factor at concentrations of 10 or 15% (vol/vol) in soft agar cultures of 75,000 mouse bone marrow cells, as described (11). Supernatants were assayed for PGE by radioimmunoassay (Clinical Assays Inc., Cambridge, MA) after organic solvent extraction, silicic acid chromatography, and alkaline hydrolysis (12).

Separation of Peritoneal Cells. The sedimentation velocity method was that described by Miller and Phillips (23). Fractions were examined to determine: the number of cells that adhered to plastic dishes after 1.5-hr incubation in the presence of 15% fetal calf serum, staining for α-napthyl-acetate esterase on etched grids as described (13), cellular morphology by Wright–Geimsa staining of cytocentrifuged preparations, phagocytosis of 0.8-μm latex beads, and 5′-nucleotidase activity (24).

RESULTS

Effects of LPS on the Production of CS-Factor and PGE by Peritoneal Macrophages from C3HeB/FeJ and LPS-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: CS-factor, colony-stimulating factor; PGE, prostaglandin E; LPS, lipopolysaccharide; Con A, concanavalin A; PPD, tuberculin purified protein derivative.

* Present address: Department of Medicine, UCLA School of Medicine, Center for the Health Sciences, Los Angeles, CA 90024.

Nonresponsive C3H/HeJ Mice. C3H/HeJ mice are characteristically nonresponsive to LPS stimulation of CS-factor production both *in vivo* (25) and *in vitro* (26). In the absence of LPS, considerable levels of CSF were detected in 24-hr culture supernatants of peritoneal macrophages from both C3H/HeJ and congenically identical LPS-responsive C3HeB/FeJ mice (Fig. 1). PGE production was observed in unstimulated macrophages from C3HeB/FeJ mice, but was barely detectable in cultures of unstimulated C3H/HeJ macrophages. LPS at 0.10 and 1.0 μg/ml markedly potentiated the elaboration of both CS-factor and PGE from C3HeB/FeJ but not C3H/HeJ macrophages. At a concentration of LPS (10 μg/ml) that was markedly inhibitory to the LPS-responsive C3HeB/FeJ macrophages, small elevations of both CS-factor and PGE were observed in macrophages from C3H/HeJ.

Time Course of LPS-Stimulated CS-Factor and PGE Production. Constitutive CS-factor production by C3HeB/FeJ macrophages cultured in the absence of LPS was observed as early as 3 hr and progressively increased (Fig. 2). Coincidental PGE levels remained low but detectable during the 24 hr of culture. CS-factor production was detected earlier and at significantly greater levels in the presence of LPS at 1.0 μg/ml than in the absence of LPS. Stimulation of CS-factor production was soon followed by the rapid synthesis and accumulation of PGE. In contrast, neither the rate nor the quantity of CS-factor and PGE produced by macrophages from C3H/HeJ mice was affected by exposure to LPS.

Effect of CS-Factor and Agents That Stimulate CS-Factor Production on PGE Synthesis. In contrast to the inability of LPS to elicit CS-factor and PGE production by macrophages from C3H/HeJ mice, exposure to zymosan or Con A promoted the production of both CS-factor and PGE (Table 1). Likewise, the addition of WEHI-3-conditioned medium containing CS-factor (15) to C3HeB/FeJ and C3H/HeJ macrophages stimulated PGE synthesis by both cell populations. PPD and poly(I)·poly(C) each promoted production of both CS-factor and PGE in both macrophage types (not shown). A purified CS-factor preparation from L-cell-conditioned medium (16) was tested and found to dramatically stimulate PGE production by C3H/HeJ macrophages (Fig. 3). Thus, CS-factor is capable of bypassing the inability of LPS to stimulate PGE synthesis by macrophages from C3H/HeJ mice. This result suggests that the stimulatory effects of LPS on PGE synthesis may be mediated or modified by the production or release of CS-factor.

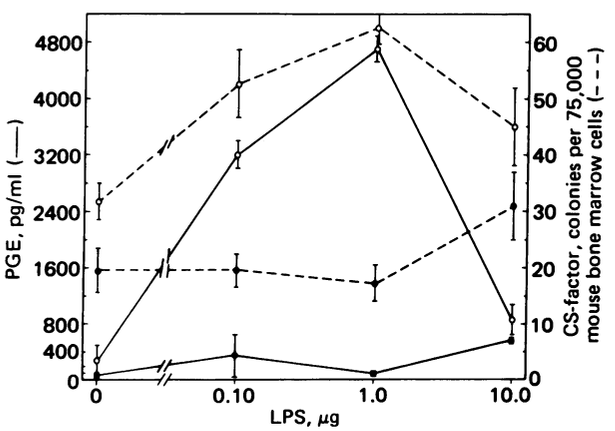

FIG. 1. Effect of LPS on CS-factor and PGE production by macrophages from C3HeB/FeJ and LPS-nonresponsive C3H/HeJ mice. Adherent macrophages from 1 × 10^6 peritoneal cells from C3HeB/FeJ (○) and C3H/HeJ (●) mice were incubated for 24 hr in the absence and presence of various concentrations of LPS, and the supernatant media were assayed for both colony-stimulating activity (colonies at 10% vol/vol) and PGE. Supernatants from triplicate cultures were assayed individually for each point and the results are expressed as mean values ± SEM.

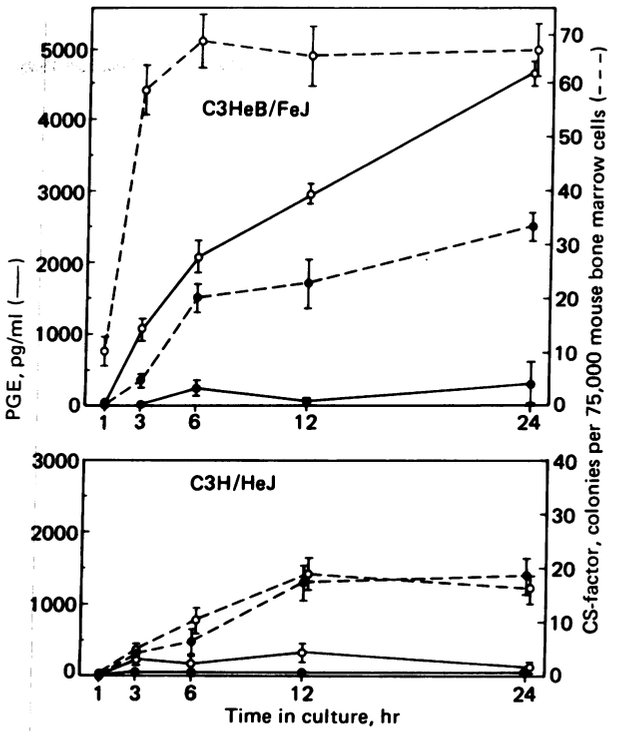

FIG. 2. Kinetics of LPS-stimulated production of CS-factor and PGE production. Supernatant media from triplicate cultures of 1 × 10^6 adherent peritoneal macrophages of C3HeB/FeJ and C3H/HeJ mice containing LPS at 1 μg/ml (○) or control saline diluent (●) were harvested at various times and assayed individually for CS-factor (- - -) and PGE (—).

Characterization of the Peritoneal Macrophage Populations That Produce CS-Factor and PGE Constitutively or after Stimulation. Separation of peritoneal cells by velocity sedimentation indicates two major populations of nucleated

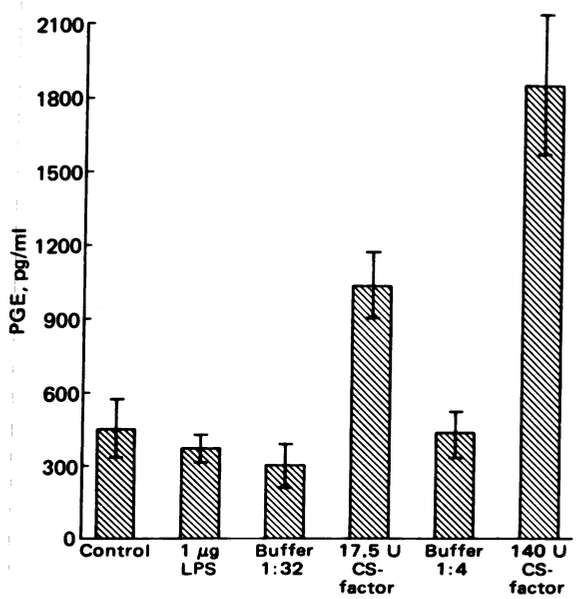

FIG. 3. Effect of purified L-cell CS-factor on PGE synthesis by C3H/HeJ macrophages. Adherent macrophages from 5 × 10^5 C3H/HeJ peritoneal wash cells were incubated for 24 hr in the absence and presence of LPS, purified L-cell CS-factor (U, colony-forming units) and the diluent (Tris buffer) in which the CS-factor was prepared. Supernatants from duplicate cultures were individually assayed for PGE. Error bars are SEM.

Table 1. Production of CS-factor and PGE by LPS-responsive and unresponsive macrophages

Macrophage	CS-factor, colonies				PGE, pg/ml				
	Control	+LPS	+Zymosan	+Con A	Control	+LPS	+Zymosan	+Con A	+CS-factor
$B6D2F_1$	21 ± 4	42 ± 4	54 ± 2	45 ± 3	105 ± 53	2556 ± 59	2804 ± 62	1393 ± 511	ND
C3HeB/FeJ	26 ± 6	74 ± 9	ND	47 ± 6	200 ± 30	1905 ± 182	5822 ± 162	1679 ± 659	3067 ± 443
C3H/HeJ	18 ± 2	16 ± 2	50 ± 2	43 ± 4	369 ± 62	429 ± 44	2084 ± 110	2030 ± 228	3157 ± 41

Supernatant media from duplicate 1-ml cultures of 5×10^5 (BDF_1) or 1×10^6 (C3H) peritoneal macrophages containing diluent medium (control), 1 μg of LPS, 25 μg of zymosan or 10 μg (C3H) or 25 μg (BDF_1) of Con A assayed for CS-factor at 10% vol/vol and for PGE by radioimmunoassay. Results are expressed as mean values ± SEM. ND, not determined.

cells (Fig. 4*A*). The first had a sedimentation velocity of 3.6 mm/hr and consisted of small lymphocytes. The second had a sedimentation velocity of 6.3 mm/hr and consisted of large mononuclear cells and macrophages that adhered to plastic, stained positive for α-napthyl-acetate esterase, and actively phagocytized latex beads. Fig. 4*B* indicates that the peak constitutive CS-factor-producing cells had a sedimentation velocity of 5.9 mm/hr, whereas the peak population that constitutively synthesized PGE sedimented at 7.8 mm/hr.

After incubation with either LPS or CS-factor, peak PGE synthesis shifted to a population of cells sedimenting at 6.3 mm/hr (Fig. 4*C*), corresponding to constitutive CS-factor-producing cells. Thus, there exist at least two distinct populations of adherent, phagocytic, and esterase-positive mononuclear cells: large cells synthesizing PGE and smaller cells producing CS-factor and being responsible for most of the PGE stimulated by LPS and CS-factor.

Rapidly sedimenting cells that constitutively synthesized PGE were stimulated to synthesize only 3- and 4-fold additional PGE by LPS and CS-factor, respectively. In contrast, LPS and CS-factor stimulated PGE synthesis 25- and 100-fold, respectively, from macrophages with a sedimentation rate of 6.3 mm/hr. Furthermore, both LPS and CS-factor induced new PGE synthesis in a population of smaller cells that were otherwise unable to produce detectable levels of PGE. This population of inducible PGE-synthesizing cells, which sedimented at 4.7 mm/hr, was also stimulated to produce CS-factor.

Ectoenzyme 5′-nucleotidase activity was detected in adherent cell populations with sedimentation velocities of 9.61 to 5.07 mm/hr. Macrophage populations with modal velocities of 9.61 to 6.86 had mean (±SEM) nucleotidase activity of 143 ± 11 nmol/30 min per 10^6 cells (range 126–175 nmol/30 min per 10^6 cells). Cell populations sedimenting at 6.25–5.07 mm/hr had mean nucleotidase activities of 69 ± 6.7 nmol/30 min per 10^6 cells (range 51–80 nmol/30 min per 10^6 cells).

CS-Factor and PGE Synthesis by Macrophage and Nonmacrophage Cell Lines. We examined whether the biosynthetic link between CS-factor and PGE extended also to neoplastic macrophages (Table 2). The murine cell lines WEHI-3, SK2.2, and PU5-1.8, and the human cell line SpGcT, constitutively elaborated both CS-factor and PGE. Incubation with LPS augmented both CS-factor and PGE production in the murine macrophage lines. The RAW264 and J774 macrophage tumors did not constitutively produce either CS-factor or PGE. However, new synthesis of both CS-factor and PGE could be induced in these cells by LPS. Furthermore, the addition of a source of CS-factor itself stimulated WEHI-3 and induced both RAW 264 and J774 to synthesize new PGE at levels very similar to the level induced by LPS. Agents other than LPS—such as poly(I)·poly(C), zymosan, and PPD—that induced CS-factor production (18) also induced the new synthesis of PGE in RAW264 (Table 3). Poly(I)·poly(C) stimulated CS-factor and PGE synthesis by normal peritoneal macrophages (Table 3). The human phagocytic cell line U-937 was the only macrophage-related cell line that did not produce either CS-factor or PGE, and which could not be induced to do so with LPS.

In contrast to the findings with the monocyte-macrophage cell lines, neither the murine T-cell lymphoma EL4 nor the lymphocytic lymphoma RBL-3 elaborated any PGE (Table 2). Although EL4 was effectively induced by Con A but not by LPS to elaborate CS-factor (18), such treatment failed to induce concomitant PGE synthesis. RBL-3, on the other hand, con-

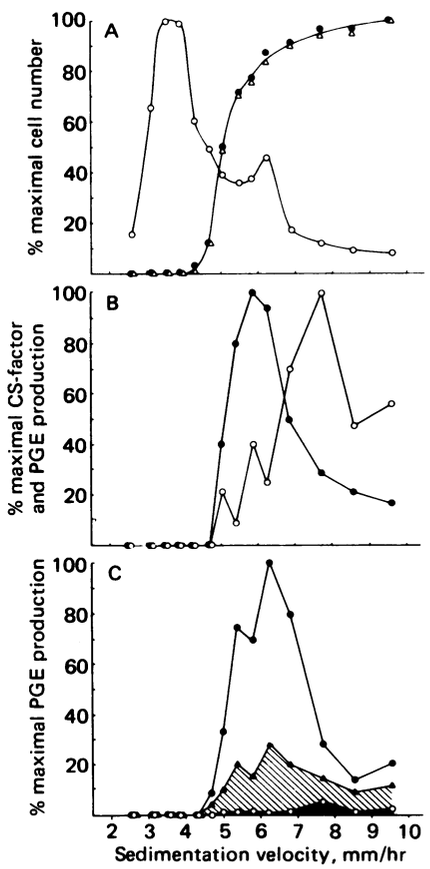

FIG. 4. Sedimentation velocity separation of the constitutive and stimulated CS-factor- and PGE-producing peritoneal macrophages. (*A*) Individual and pooled fractions of peritoneal wash cells separated by sedimentation velocity were measured for total nucleated cells (○), plastic-adherent and α-naphthyl-acetate esterase-positive cells (●), and adherent cells that phagocytized 0.8-μm latex beads (△). Results are normalized to the respective peak values (nucleated cells, 17×10^6; adherent esterase-positive, 98.8%; phagocytic, 97.2%). (*B*) Constitutive CS-factor (●) and PGE (○) were assayed from duplicate cultures containing the adherent cells from 8×10^5 sedimentation velocity-separated peritoneal wash cells after 24 hr of incubation in the absence of stimulants. Results are normalized to the peak levels of CS-factor (169 ± 4 colonies per 75,000 mouse bone marrow cells stimulated by 15% vol/vol supernatant) and PGE (1147 ± 37 pg/ml). (*C*) The same macrophage fractions were incubated in the presence of 1 μg of LPS (▲) and 10 μl of CS-factor (200-fold-concentrated serum-free WEHI-3-conditioned medium) (●) for 24 hr and the supernatant media were assayed for PGE. The darkened and hatched areas represent, respectively, the nonstimulated (constitutive) and LPS-stimulated PGE production normalized to the peak CS-factor-stimulated level of PGE (15,585 ± 190 pg/ml).

Table 2. Production of CS-factor and PGE by macrophage and nonmacrophage cell lines

Cell line	CS-factor, colonies			PGE, pg/ml			
	Control	+LPS	+Con A	Control	+LPS	+Con A	+CSF
Murine monocyte-macrophage							
WEHI-3	84 ± 4	161 ± 18	ND	173 ± 91	1040 ± 85	ND	863 ± 35
SK 2.2	16 ± 3	91 ± 9	ND	296 ± 13	3462 ± 149	ND	ND
PU5.18	12 ± 2	94 ± 12	ND	283 ± 37	2385 ± 312	ND	ND
RAW 264	0	111 ± 1	ND	0	305 ± 25	ND	393 ± 25
J774	0	128 ± 10	ND	0	1033 ± 61	ND	1670 ± 88
Murine lymphocyte							
EL4	0	0	106 ± 8	0	0	0	0
RBL3	152 ± 12	154 ± 10	139 ± 6	0	0	0	0
Murine fibroblast							
L-929	76 ± 4	90 ± 10	ND	0	0	0	0
$HSDM_1$	0	0	ND	5050 ± 280	4252 ± 80	ND	4575 ± 360
Human							
SpGct	45 ± 5* 128 ± 12†	ND	ND	3129 ± 101	ND	ND	ND
U-937	0*†	0	ND	0	0	ND	ND

Cells (3×10^5) were incubated in 1 ml of growth medium for 48 hr in the absence and presence of 1 μg of LPS, 25 μg of Con A, or 50 μl of CS-factor (200-fold concentrated WEHI-3-conditioned medium) and assayed for CS-factor (colonies stimulated by 15% vol/vol supernatants) and PGE. Results are means ± SEM. Supernatants of SpGcT cell line were provided by J. DiPersio. The human cell line supernatants were assayed for both human-active CS-factor (*) (with 2×10^5 nonadherent human bone marrow cells) and mouse-active CS-factor (†). ND, not determined.

stitutively produced CS-factor in the absence of LPS and Con A, but, like EL4, failed to synthesize any detectable PGE. Furthermore, the addition of the same source of CS-factor that induced PGE synthesis by both RAW264 and J774 had no effect on the lymphocytic cell lines.

A similar dissociation between the production of CS-factor and PGE in lymphoid cells was observed in murine fibroblast cell lines. L-929 constitutively produced CS-factor in the absence of LPS, but failed to demonstrate any detectable PGE with or without LPS or CS-factor. In direct contrast, the $HSDM_1$ fibrosarcoma was a potent constitutive producer of PGE but failed to elaborate detectable CS-factor. The levels of PGE synthesized by $HSDM_1$ were not influenced by LPS or CS-factor, but were strikingly sensitive to the cyclooxygenase inhibitor indomethacin (unpublished). $HSDM_1$ did not elaborate any detectable CS-factor, even in the presence of indomethacin, and could not be induced to produce CS-factor after treatment with LPS (Table 2). Thus, the ability to produce both CS-factor and PGE extends only to populations of normal or neoplastic macrophages in which the capacity to synthesize CS-factor is a constitutive, stimulated, or inducible function.

DISCUSSION

A close biosynthetic relationship exists between the production of CS-factors and PGE by both normal and neoplastic macrophages. Promotion of additional CS-factor production leads to stimulation of macrophage PGE synthesis. Likewise, short-term exposure of macrophages either to conditioned medium containing CS-factor (27) or to purified CS-factor resulted in the stimulation of new PGE synthesis. The LPS insensitivity of C3H/HeJ macrophages for CS-factor and PGE production did not represent two separate defects, but rather suggests that the stimulation of the macrophage's biosynthetic and secretory capacity for CS-factor and PGE are inherently linked. The fact that other agents that promote CS-factor production, or pure CS-factor itself, could stimulate PGE synthesis, indicates that the effect of LPS on PGE synthesis is mediated by a mechanism involving CS-factor production. On the other hand, CS-factor production is not dependent upon PGE, because the former occurs in the absence of PGE synthesis, as seen with murine or human mononuclear phagocytes treated with indomethacin (11, 27). There is no agent known that exclusively inhibits CS-factor production. A granulocyte-derived inhibitor of monocyte-macrophage CS-factor production, recently identified by Broxmeyer *et al.* to be lactoferrin (28), effectively reduces constitutive CS-factor and PGE synthesis (27). Both effects can be overcome by simultaneous stimulation with LPS (ref. 28; unpublished data).

The human cell line U-937 may at first be considered an exception to the biosynthetic link between CS-factor and PGE. Despite possessing some properties of monocytes, such as endogenous pyrogen (unpublished observations), peroxidase, receptors for Fc and complement, lysozyme, and being weakly phagocytic (22), U-937 was unable to produce CS-factor or PGE constitutively or with LPS. This supports our interpretation that PGE synthesis in macrophages depends on the capacity to actively synthesize CS-factor, and the noninducibility of U-937 may, therefore, reflect the relative immaturity of this cell line. Thus, the heterogeneity in the macrophage cell lines may represent a progressive sequence from U-937 to the inducible lines, RAW 264 and J774, followed by the constitutive CS-factor- and PGE-producing macrophage tumors, WEHI-3,

Table 3. CS-factor and PGE stimulation in RAW 264 and peritoneal macrophages

Macrophage	CS-factor, colonies				PGE, pg/ml			
	Control	poly(I)·poly(C)	Zymosan	PPD	Control	poly(I)·poly(C)	Zymosan	PPD
RAW 264	0	42 ± 3	36 ± 3	28 ± 1	0	219 ± 27	582 ± 36	381 ± 58
$B6D2F_1$	13 ± 1	37 ± 4	ND	ND	62 ± 26	641 ± 45	ND	ND

Normal $B6D2F_1$ peritoneal and RAW 264 macrophages (5×10^5) were incubated in 1 ml for 48 hr in the absence or presence of 10 μg of poly(I)·poly(C), 25 μg of zymosan, or 5 μg of PPD, and the supernatants from triplicate cultures were assayed individually for CS-factor and PGE. Results are expressed as mean numbers of colonies stimulated by 10% vol/vol supernatant and amounts of PGE, ± SEM. ND, not determined.

SK2.2, and PU5-1.8, which could be stimulated to synthesize additional CS-factor and PGE. There was no association between CS-factor and PGE in any of the lymphocyte or fibroblast cell lines tested, whether they were constitutive or inducible CS-factor producers or producers of PGE.

Subpopulations of normal peritoneal macrophages also differed in their capacity to produce CS-factor and PGE constitutively or inducibly. The PGE-producing cells comprised the more rapidly sedimenting cells and CS-factor production occurred in the slower sedimenting cells. Both CS-factor and LPS promoted PGE synthesis, predominantly in subpopulations of slowly sedimenting constitutive CS-factor producers and also in the very slowly sedimenting adherent cells, which did not constitutively produce either activity. In comparison, LPS and CS-factor only moderately augmented the synthesis of PGE among the rapidly sedimenting macrophages. It is of interest to note that peak constitutive PGE production occurred in cells having high 5′-nucleotidase activity, whereas peak constitutive CS-factor production occurred in cells having intermediate nucleotidase activity. The exact nature of association of ectoenzyme activity and CS-factor and PGE synthesis is unknown. Recent evidence (29) demonstrates that 5′-nucleotidase activity is high in unstimulated resident macrophage populations and low or absent in elicited populations, suggesting that this activity may reflect cell maturity or functional state of activation.

While studies have been performed with a variety of CS-factor preparations, it should not be assumed that all CS-factors possess the capacity to stimulate PGE synthesis by macrophages. Unfractionated and semipurified WEHI-3-conditioned media having colony-stimulating activity (15, 27), unfractionated L-929- and RBL-3-conditioned media (unpublished observations), and purified CS-factor from L cells (16) all stimulated PGE synthesis by macrophages. In contrast, no PGE synthesis could be stimulated by various conditioned media or fractions of WEHI-3 medium that did not demonstrate detectable CS-factor (27). It is of interest, however, that CS-factor purified from mouse lung-conditioned medium (30) did not demonstrate PGE-stimulating activity despite promotion of colony formation (unpublished observations). This last CS-factor does not crossreact immunologically with purified L-cell CS-factor (31), suggesting that heterogeneity exists among species of CS-factor.

There are several possible mechanisms by which CS-factor may promote new PGE synthesis. CS-factor may activate phospholipase to liberate arachidonate from membrane-bound phospholipids or may activate cyclooxygenase directly to convert arachidonate to prostaglandins G_2 and H_2, bioactive precursors of PGE_2. With increasing knowledge of the structures and functions of the prostaglandin intermediates and other members of the arachidonic acid pathway, the modulatory role of CS-factor on the biosynthesis of these compounds may lead to new insights into their regulatory functions.

We have previously shown that the CS-factor and PGE produced by mononuclear phagocytes control the formation of granulocytes and monocyte-macrophages *in vitro* by their opposing positive and negative influence on the proliferation of the myeloid progenitor cell (11). The present report documents that the synthesis of the inhibitor PGE is inherently linked with the ability of the same macrophage population to be stimulated or induced to synthesize the opposing activity, CS-factor, substantiating the sensitivity of the effector limb in the control of myelopoiesis *in vitro*. If the mononuclear phagocyte is a major contributor to tissue levels of PGE under a variety of conditions, the role of CS-factor in stimulating PGE synthesis suggests a more universal action for myelopoietic colony-stimulating factors than heretofore realized.

We thank Drs. N. Williams and R. R. Eger for concentrated preparations of WEHI-3 CS-factor, R. Shadduck for purified L-cell CS-factor, A. Burgess for purified CS-factor from lung-conditioned medium, and J. DiPersio for samples of SpGcT cell supernatants. The expert technical assistance of Mr. R. Ferguson and Ms. E. Valera is gratefully acknowledged. This work was supported by Grants CA-19052 from the National Cancer Institute, PCM75-19734 from the National Science Foundation, PDT-60 from the American Cancer Society, National Research Service 1F-32CA05904 (to L.M.P.) from the National Cancer Institute, and the Gar Reichman Foundation.

1. Metcalf, D. & Moore, M. A. S. (1971) *Haemopoietic Cells* (North-Holland, Amsterdam).
2. Stanley, E. R., Cifone, M., Heard, P. M. & Defendi, V. (1976) *J. Exp. Med.* **143,** 631–647.
3. Moore, M. A. S. & Williams, N. (1972) *J. Cell. Physiol.* **80,** 195–206.
4. Chervenick, P. A. & LoBuglio, A. F. (1972) *Science* **178,** 164–166.
5. Golde, D. W. & Cline, M. J. (1972) *J. Clin. Invest.* **51,** 2981–2983.
6. Eaves, A. C. & Bruce, W. R. (1974) *Cell Tissue Kinet.* **7,** 19–30.
7. Cline, M. J., Rothman, B. & Golde, D. W. (1974) *J. Cell Physiol.* **84,** 193–196.
8. Quesenberry, P. J., Morley, A. A., Rickard, K. A., Garrity, M., Howard, D. & Stohlman, F. (1972) *N. Engl. J. Med.* **286,** 227–232.
9. Broxmeyer, H. E. (1978) *Blood* **51,** 889–901.
10. Lin, H. & Stewart, C. C. (1973) *Nature (London) New Biol.* **243,** 176–177.
11. Kurland, J. I., Bockman, R. S., Broxmeyer, H. E. & Moore, M. A. S. (1978) *Science* **199,** 552–553.
12. Kurland, J. I. & Bockman, R. S. (1978) *J. Exp. Med.* **147,** 952–957.
13. Kurland, J. I., Kincade, P. W. & Moore, M. A. S. (1977) *J. Exp. Med.* **146,** 1420–1435.
14. Humes, J. L., Bonney, R. J., Pelus, L. M., Dahlgren, M. E., Sadowski, S. J., Kuehl, F. A. & Davies, P. (1977) *Nature (London)* **289,** 149–150.
15. Williams, N., Eger, R. R., Moore, M. A. S. & Mendelsohn, N. (1978) *Differentiation* **11,** 59–63.
16. Shadduck, R. K. & Waheed, A. (1977) *Blood* **50,** Suppl. 1, 159.
17. Ralph, P. & Nakoinz, I. (1977) *J. Immunol.* **119,** 950–954.
18. Ralph, P., Broxmeyer, H. E., Moore, M. A. S. & Nakoinz, I. (1978) *Cancer Res.* **38,** 1414–1419.
19. Moore, M. A. S. & Dexter, T. M. (1978) *Transplant. Proc.* **10,** 83–90.
20. Tashjian, A. H., Voelkel, E. F., Levine, L. & Goldhaver, P. (1972) *J. Exp. Med.* **136,** 1329–1343.
21. DiPersio, J. F., Brennan, J. K., Lichtman, M. A. & Speiser, B. L. (1978) *Blood* **51,** 507–519.
22. Sundstrom, C. & Nilsson, K. (1976) *Int. J. Cancer* **17,** 565–577.
23. Miller, R. G. & Phillips, R. A. (1969) *J. Cell Physiol.* **73,** 191–201.
24. Avruch, J. & Hoelzl-Wallach, D. F. (1971) *Biochim. Biophys. Acta* **233,** 334–347.
25. Apte, R. N. & Pluznik, D. H. (1976) *J. Cell Physiol.* **89,** 313–320.
26. Russo, M. & Lutton, J. D. (1977) *J. Cell Physiol.* **92,** 303–308.
27. Kurland, J. I., Broxmeyer, H. E., Pelus, L. M., Bockman, R. S. & Moore, M. A. S. (1978) *Blood* **52,** 388–407.
28. Broxmeyer, H. E., Smithyman, A., Eger, R. R., Meyers, P. A. & DeSousa, M. (1978) *J. Exp. Med.* **148,** 1052–1067.
29. Edelson, P. J. & Cohn, Z. A. (1976) *J. Exp. Med.* **144,** 1581–1595.
30. Burgess, A. W., Camakaris, J. & Metcalf, D. (1977) *J. Biol. Chem.* **252,** 1998–2003.
31. Stanley, E. R. (1978) *J. Supramol. Struct.*, Suppl. 2, 175.