INFECTION AND IMMUNITY, May 1989, p. 1512–1516
0019-9567/89/051512-05$02.00/0

Vol. 57, No. 5

Stimulation of Macrophage Phagocytic but Not Bactericidal Activity by Colony-Stimulating Factor 1

C. CHEERS,[1]* M. HILL,[1] A. M. HAIGH,[1] AND E. R. STANLEY[2]

Department of Microbiology, University of Melbourne, Parkville, Victoria 3052, Australia,[1] and Department of Developmental Biology and Cancer, Albert Einstein School of Medicine, Bronx, New York 10461[2]

Received 3 October 1988/Accepted 6 February 1989

The ability of mouse peritoneal cells to phagocytose and lyse *Listeria monocytogenes* was measured after the cells were incubated with purified murine macrophage-specific colony-stimulating factor (CSF-1). Activation of combined phagocytic and bacteriolytic ability required 24 h, with an optimal dose of 1,000 U of CSF-1 per ml. No activation was achieved with a shorter period of incubation, known to be sufficient for GM-CSF to stimulate phagocytosis by granulocytes, and there was no advantage in longer exposure. After 24 h in 1,000 U of CSF-1, macrophages showed visibly increased spreading on the plastic petri dish. Activated cells examined microscopically showed an increase in the number of phagocytic cells and in the numbers of bacteria per phagocytic cell. This increased phagocytic ability was evident also in the increase in the amount of radioactivity associated with the cells following a 30-min incubation with radiolabeled bacteria. When these cells were carefully washed, the percentage of this initial uptake released during the next 2 h was not increased by pretreatment of the cells with CSF-1, showing that the effect of this growth factor was on phagocytosis of the bacteria not on the killing mechanisms per se.

The colony-stimulating factors (CSFs) comprise a group of growth factors responsible for the proliferation and maturation of haemopoietic precursor cells into end cells (13). There are four CSFs in the mouse which promote the formation of granulocytes and macrophages. CSF-1 (or M-CSF) stimulates the formation of macrophages, G-CSF stimulates the formation of granulocytes, and GM-CSF stimulates the production of both. Interleukin-3 (or multi-CSF) stimulates formation not only of granulocytes and macrophages but also of megakaryocytes, eosinophils, and mast cells. Of these four, CSF-1 and G-CSF predominate in the serum within 24 h of infection of mice with the intracellular bacterium *Listeria monocytogenes* (4). Only small amounts of GM-CSF were detected, and no interleukin-3 was detected. Because CSF-1 can be measured by radioimmunoassay, it can be detected in tissue extracts which might be toxic in biological assays. Following infection, CSF-1 increased dramatically in the spleen and liver (major sites of infection), lungs, and salivary gland (6).

In recent years there have been increasing numbers of reports of CSFs exerting effects on end cells, both human and mouse (13). CSF-1 stimulates the survival as well as the proliferation and differentiation of mononuclear phagocytes (18). It has also been shown to enhance antitumor activity (20) and killing of *Candida albicans* (10) and to protect macrophages from lytic vesicular stomatitis infection (11). Since CSF-1 is the major CSF induced by *Listeria* infection and appears very early in infection (6), and since that organism resides mainly in macrophages, it was of considerable interest to test whether CSF-1 could activate macrophages to kill *Listeria* organisms with increased efficiency.

The radiolabel release assay chosen to measure lysis of *Listeria* organisms has been shown convincingly to reflect actual killing of the bacteria (7) and has great advantages over assays involving counts of viable bacteria. It is much more sensitive, does not require the use of antibiotics to control extracellular growth of the bacteria, and can be accomplished in a short incubation time.

MATERIALS AND METHODS

Production of CSF. Two preparations of CSF-1 of different degrees of purity were used. Their production is described in detail by Stanley (16). A semipurified preparation, known as stage 1 L-cell CSF-1, was made from serum-free L-cell-conditioned medium by batch calcium phosphate gel chromatography and used in preliminary experiments to establish optimal conditions for the effects on macrophage activity. Highly purified stage 4 L-cell CSF-1 was prepared by subjecting stage 1 material to DEAE-cellulose chromatography, followed by gel filtration and affinity chromatography on concanavalin A-sepharose. The purity of this preparation was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis and was found to constitute a single band. The concentration of CSF-1 in the preparations was measured by radioimmunoassay (16).

Mice. Inbred CBA/H mice were bred and maintained under conventional conditions in the Microbiology Animal Breeding Unit, University of Melbourne. The 50% lethal dose of *L. monocytogenes* for these mice is 2×10^3 intravenously, and they are genetically susceptible to the infection (5).

Preparation of cells. Mice were killed by an overdose of fluothane anaesthetic, and their resident cells were washed from the peritoneal cavity with 5 ml of RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) containing 10% fetal calf serum (FCS) and 10 U of heparin per ml. The cells were centrifuged at $800 \times g$ for 7 min through a cushion of FCS, and 2×10^6 cells in 1 ml of RPMI 1640–10% FCS were cultured in 35-mm Falcon tissue culture dishes (Becton Dickinson Labware, Oxnard, Calif.). The dishes had been coated with gelatin by adding 1 ml of sterile 1% gelatin per dish, removing the surplus after 2 h at 4°C, and drying at 37°C for 24 h. When appropriate, either stage 1 or stage 4 CSF-1 was incorporated in the medium and the cells were incubated at 37°C in 5% CO_2 in a humidified incubator. After

* Corresponding author.

various times of incubation, the cells were harvested by replacing the medium with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-buffered Dulbecco modified Eagle medium (HEPES-DMEM; GIBCO) and incubating the dishes at 4°C for 1 h. The released cells were washed off, and the remaining cells were exposed at room temperature for 15 min to 0.2% EDTA in phosphate-buffered saline free of Ca^{2+} and Mg^{2+}. The plates were again washed and finally scraped to remove the last of the cells. Cells from the three washings were pooled, centrifuged, and suspended in HEPES-DMEM. The viability of the cells recovered by these gentle means was greater than 95%.

Bactericidal assay. This assay was adapted from that described by Davies (7). *L. monocytogenes* organisms were radiolabeled by 16 h of incubation at 26°C of 10^7 organisms plus 10 μCi of [^{3}H]thymidine ([^{3}H]TdR) (25 Ci/mmol) in 10 ml of Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy broth. Labeled bacteria were washed free of unincorporated [^{3}H]TdR by centrifuging at 4,000 × *g* for 15 min at 4°C and suspending in HEPES-DMEM four times. The incorporated [^{3}H]TdR counts per ml were adjusted to 100,000 cpm by resuspending the bacteria in the appropriate volume of HEPES-DMEM. Bacterial numbers were checked retrospectively and varied between 5×10^7 and 2×10^8/ml. To a 1.5-ml Ependorf centrifuge tube was added 0.25 ml each of cells (4×10^6/ml) and bacteria. In some experiments, after a 30-min period for initial phagocytosis, cells were washed free of nonassociated bacteria by centrifuging at 100 × *g* for 10 min at 4°C through a cushion of FCS. They were then resuspended, sampled for the amount of cell-associated radioactivity, and further incubated for 2.5 h. The tubes were removed to ice, and 20 μl of FCS and 0.5 ml of 20% trichloracetic acid were added. After equilibration for at least 1 h at 4°C, the tubes were centrifuged in a Beckman Microfuge at 10,000 × *g* for 5 min. Soluble label released by digestion of the bacteria following phagocytosis was measured by sampling 0.5 ml of the supernatant and counting in 5 ml of scintillant (EP Ready Solv; Beckman Instruments, Inc., Fullerton, Calif.) using a liquid scintillation counter (MINIMAX β TRI-CARB 4000; Packard Instrument Co., Inc., Rockville, Md.). Spontaneous release of [^{3}H]TdR by bacteria alone was always measured. In other experiments, the total effect of continued phagocytosis and bacterial digestion on the release of soluble ^{3}H was measured by omitting the washing step in order to assess the overall bacteriolytic activity of the cells.

When microscopic assessment of the rate of phagocytosis was required, a similar system was used, except that the *Listeria* organisms were not radiolabeled, and 10 μl was sampled from the bacteria-cell mixture at the required time intervals. The sample was placed onto 200 μl of cold FCS in a cytocentrifuge cup (Cytospin 2; Shandon Southern Products Ltd., Astmoor, United Kingdom) and centrifuged at 100 × *g* at 4°C for 5 min. The slides were fixed and stained by Diff Quick (Lab-Aids, Narrabeen, New South Wales, Australia). For each slide, 100 cells in randomly chosen fields were counted; the person counting had no knowledge of the experimental group to which the cells belonged.

Assay for lipopolysaccharide. All reagents used were assayed for contamination with lipopolysaccharide by using coagulation of *Limulus* amaebocyte lysate (Microbiological Associates of Cape Cod, Inc., Woods Hole, Mass.). The test was capable of detecting as little as 0.5 ng of LPS per ml, and all reagents used passed this test.

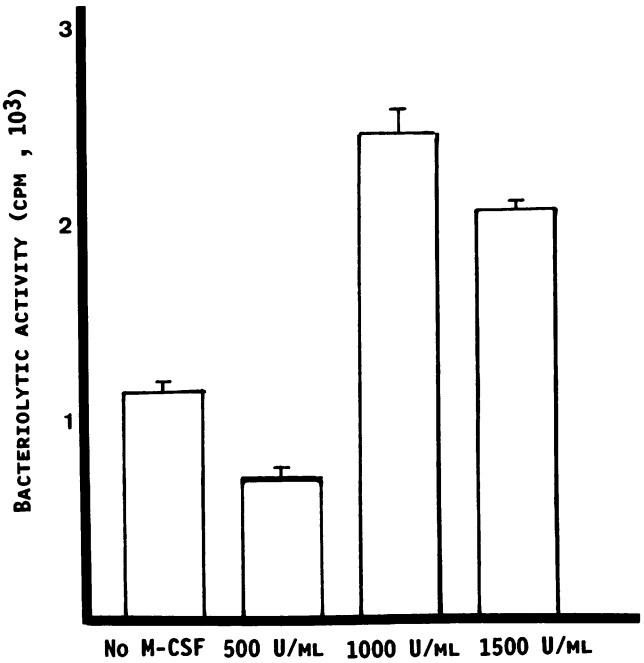

FIG. 1. Stimulation of overall bacteriolytic activity of peritoneal cells incubated for 24 h with various doses of CSF-1. Recovered cells were pooled within groups and incubated for 2.5 h with [^{3}H]TdR-labeled bacteria. The values represent mean ± the standard deviation of groups of four assay tubes. Spontaneous release of [^{3}H]TdR by bacteria in the absence of cells has been subtracted.

RESULTS

A number of preliminary experiments using stage 1 purified L-cell-conditioned medium of known CSF-1 concentration and measuring the overall effect of phagocytosis and digestion (see Materials and Methods) were performed to establish optimal conditions. Key typical experiments are presented. Although labeling efficiency varied somewhat, a useful rule of thumb is that 1,000 cpm represent 10^6 bacteria. The experiments showed that 2×10^6 cells per dish gave optimal stimulation, while at higher concentrations the cells, under microscopic examination, showed signs of physical overcrowding. The optimal concentration of CSF-1 was determined by incubating 2×10^6 peritoneal cells with various concentrations of CSF-1 for 24 h and then testing the bacteriolytic activity of the cells (Fig. 1). Maximum activation occurred at 1,000 U/ml. At this concentration, the cells were visibly spreading on the petri dish after the 24-h incubation. No further activation of the cells was observed if they were incubated for 48 h or 72 h with CSF-1 (data not shown). Sometimes the level of activity remained the same, sometimes it declined with time. The bacteriolytic activity of peritoneal cells incubated with highly pure stage 4 CSF-1 under the conditions established here was indistinguishable (756 cpm ± 73 [standard deviation]) from the activity of cells incubated with stage 1 CSF-1 (853 ± 99; control [i.e., no treatment], 295 ± 79).

As certain other systems, involving activation of granulocytic cells with CSFs, require only a short period of incubation (8, 16, 19, 22, 26), macrophages were preincubated with 1,000 U of CSF-1 per ml in the bacteriolytic assay tube for 1 h at 37°C before the addition of *L. monocytogenes*. The CSF 1 remained present during the 2.5 h of the bacteriolytic assay, but there was no significant stimulation of bacteri-

TABLE 1. Separate determination of phagocytic activity and bacteriolysis of CSF-1-treated and untreated cells

Treatment	Uptake of [^{3}H]TdR-labeled bacteria (cpm ± SD/10^6 cells)[a]	Soluble label released[b] (cpm ± SD/10^6 cells)	% Release of soluble ^{3}H
Normal peritoneal cells incubated in vitro[c]			
Untreated	1,539 ± 281	246 ± 30	16
Stage 4 pure CSF-1	3,378 ± 707	371 ± 21	11
Peritoneal cells assayed immediately after harvesting[d]			
Normal mice	1,110 ± 240	90 ± 8	8
Listeria-infected mice	1,930 ± 110	335 ± 40	21

[a] Cells were incubated with bacteria for 30 min and then washed free of unassociated bacteria as described in Materials and Methods. The difference in uptake was significant ($P < 0.01$, Student's t test).
[b] The cells were further incubated for 2 h, the mixture was trichloroacetic acid precipitated, and the released soluble label was measured.
[c] Cells were preincubated with 1,000 U of CSF-1/ml or without CSF-1 for 24 h before harvesting and assay.
[d] Mice infected intravenously 8 days before assay with 10^3 *L. monocytogenes* or uninfected mice were used as donors of cells which were assayed immediately upon harvesting.

olytic activity, although positive controls exposed to CSF-1 for 24 h were activated (overall bacteriolytic activities: 3.5 h with CSF-1, 505 ± 96; 3.5 h without CSF-1, 651 ± 77; 24 h with CSF-1, 1,159 ± 43; 24 h without CSF-1, 314 ± 40).

To determine separately phagocytosis and bacteriolytic activity, peritoneal cells were incubated for 24 h with pure stage 4 CSF-1 and allowed to phagocytose radiolabeled *L. monocytogenes* for 30 min. The cells were washed as described in Materials and Methods, and cell-associated radioactivity was sampled (Table 1). The cells which had been incubated with CSF-1 showed double the uptake of radioactivity observed with untreated cells. When the cells and their associated bacteria were incubated for a further 2 h, the percentage of cell-associated radioactivity released was comparable for the CSF-1-treated and untreated cells. Thus, the efficiency of the CSF-1-treated cells in the earlier experiments is attributable to increased phagocytosis rather than activation of their killing mechanisms. The finding that the phagocytic activity but not the bacteriolytic activity per se was increased was shown in repeated experiments and was in contrast to results using the same assay on cells harvested from *L. monocytogenes*-infected mice, in which both the phagocytic and bacteriolytic activity were increased (Table 1).

The effect of pure stage 4 CSF-1 on phagocytic activity was further analyzed microscopically as described in Materials and Methods. The percentage of phagocytic cells and the mean number of bacteria per phagocytic cell are shown in Table 2, and the distribution of the numbers of bacteria per cell is shown in Fig. 2. It may be seen that when cells were incubated with CSF-1, a greater percentage were phagocytic and there were more bacteria per phagocytic cell (Table 1). As time passed, the numbers of cells containing bacteria dropped slightly, presumably due to digestion of bacteria in the less actively phagocytic cells. Among the more actively phagocytic cells, the numbers of bacteria per cell rose. A phagocytic index may be calculated by multiplying the percentage of cells which are phagocytic by the mean number of bacteria per phagocytic cell. This clearly indicates the superiority of the cells incubated in CSF-1.

DISCUSSION

We have shown that CSF-1 can activate the phagocytosis by macrophages of *L. monocytogenes* and thereby increase their overall bacteriolytic efficiency. *L. monocytogenes* are intracellular bacteria which largely survive within normal macrophages. Variation in the resistances of different mouse strains is reflected in differences in the bactericidal numbers in the liver and spleen 24 to 48 h postinfection (5). Resistance is related to the earlier appearance of a highly bactericidal population of monocytes at the site of infection (21) and to higher numbers of colony-forming cells in the bone marrow and spleen (22) which would supply those monocytes. Both genetically resistant and susceptible mice show a rise in CSF production, particularly CSF-1, by 24 h postinfection, with susceptible mice actually producing more CSF at the same dose of bacteria (4, 6, 22), probably because they rapidly acquire a heavier bacterial load.

Since specific acquired immunity is not evident until 3 to 4 days postinfection (5), these critical events occur before one would expect the macrophage-activating factor, gamma interferon, to be secreted by *Listeria*-specific T lymphocytes. While it has been shown that gamma interferon is essential to

TABLE 2. Phagocytosis by cells under the influence of CSF-1[a]

Phagocytic period (min)	% Cells phagocytic		Mean number of bacteria/phagocytic cell (no. of cells counted)		Phagocytic index[b]	
	With CSF	Without CSF	With CSF	Without CSF	With CSF	Without CSF
15	67 ± 4[c]	35 ± 3	6.9 ± 8.4[d] (200)	4.1 ± 4.4 (96)	462	144
30	62 ± 3[d]	40 ± 5	7.9 ± 9.9[d] (186)	3.4 ± 4.3 (119)	489	136
60	59 ± 11[d]	23 ± 3	15.6 ± 13.8[c] (177)	7.5 ± 10 (67)	920	172
150	53 ± 4	31 ± 10	21.1 ± 16.7[c] (158)	9.9 ± 9.3 (92)	1,118	307

[a] Cells were preincubated in 1,000 U of stage 4 CSF-1 per ml for 24 h. They were harvested and mixed with *L. monocytogenes* at 37°C for the times shown, and a sample was cytocentrifuged for microscopic examination.
[b] Product of percent cells phagocytic and mean number of bacteria per phagocytic cell.
[c] Significant difference with and without CSF-1 ($P < 0.001$, Student's t test).
[d] Significant difference with and without CSF-1 ($P < 0.01$, Student's t test).

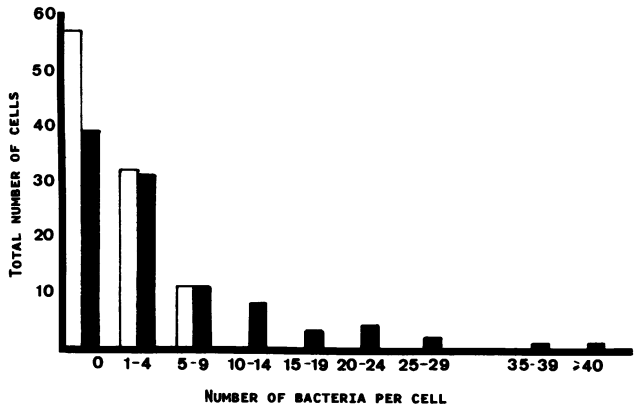

FIG. 2. Number of bacteria per cell after 30 min of phagocytosis by cells preincubated for 24 h in 1,000 U of M-CSF per ml (■) or without CSF-1 (▭). At least 100 cells on each of three slides were counted per group.

recovery from *Listeria* infection (3), peak production does not occur until 6 days postinfection (9, 14). The present study provides evidence for an earlier mechanism of macrophage activation, potentially effective in both resistant and susceptible mouse strains. It is important to note here that although the genetically susceptible mice lack the early inflammatory response which gives the advantage to the resistant strains, ablation of their resident macrophages by silica or carrageenin markedly exacerbates the infection in either susceptible or resistant mice (C. Cheers and J. Macgeorge, unpublished data). Thus, even susceptible mice have an effective resident macrophage population, and further activation of those cells could only be of advantage.

CSF-1 activated the phagocytic potential of macrophages but not their bactericidal capacity per se. The cells exposed to CSF-1, when compared with untreated cells, did not digest an increased percentage of the cell-associated radioactivity acquired during an initial 30-min period of phagocytosis. Total bacteriolytic effect was increased during continual phagocytosis because more bacteria were exposed to killing by the highly phagocytic CSF-1-treated cells. It is of interest that no increased activity of the macrophages occurred at a low dose of CSF-1 (500 U/ml) which is sufficient to maintain viability of cells over a period of 24 h or more (17). Therefore, this does not seem to be an effect of cell viability. Tushinski et al. (18) and Ampel et al. (1) have observed increased spreading on surfaces, as did we at concentrations of CSF-1 which also activated macrophage phagocytic activity. Spreading, like increased phagocytosis, was not observed before 24 h, although effects of CSF-1 on membrane ruffling and the appearance of vacuoles are reported to occur within minutes (17). Thus, the increased phagocytosis requires more than increased membrane activity. Since there was no anti-*Listeria* antibody (or even FCS) in the test system which we used, the enhanced expression of Fc receptors observed by Magee et al. (12) is not relevant to our system. While the increased surface area of the treated macrophages may allow increased contact with the particles to be phagocytosed, one would instinctively assume, although without proof, a more sophisticated mechanism for increased phagocytosis than this. Others have described increased oxygen reduction by macrophages under the influence of CSF-1 (19), and this might have been expected to result in higher bacterial killing in addition to that caused by higher rates of phagocytosis. However, the role of oxygen reduction products in the killing of *L. monocytogenes* by macrophages is controversial (2, 8, 15), and it is difficult to critically assess the role of these products unless the killing effect can be clearly reversed by their scavengers. Furthermore, we have found that some transgenic mice expressing high levels of GM-CSF also generate increased O_2 reduction products without increased killing of *L. monocytogenes* other than that attributable to their high rate of phagocytosis (C. Cheers, H. T. T. Tran, and D. Metcalf, unpublished data).

It does seem clear from the present study that, through its effect on phagocytosis, CSF-1 is likely to contribute to early resistance to infection.

ACKNOWLEDGMENTS

This work was supported by grants from the Medical Research Committee of Melbourne University and the Australian National Health and Medical Research Council to CC and by Public Health Service grants (CA 26504 and CA 32551) to ERS from the National Institutes of Health.

The expert technical assistance of Kaylene Sellick is gratefully acknowledged.

LITERATURE CITED

1. **Ampel, N. M., E. J. Wing, A. Waheed, and R. K. Shadduck.** 1986. Stimulatory effects of purified macrophage colony stimulating factor on murine resident peritoneal macrophages. Cell. Immunol. **97**:344–356.
2. **Bortolussi, R., C. M. J. E. Vandenbroucke-Grauls, B. S. van Asbeck, and J. Verhoef.** 1987. Relationship of bacterial growth phase to killing of *Listeria monocytogenes* by oxidative agents generated by neutrophils and enzyme systems. Infect. Immun. **55**:3197–3203.
3. **Buchmeier, N. A., and R. D. Schreiber.** 1985. Requirement for endogenous interferon γ production for resolution of *Listeria monocytogenes* infection. Proc. Natl. Acad. Sci. USA **82**: 7404–7408.
4. **Cheers, C., A. M. Haigh, A. Kelso, D. Metcalf, E. R. Stanley, and A. M. Young.** 1988. Production of colony stimulating factors (CSFs) during infection with an intracellular bacterium: separate determinations of macrophage-, granulocyte-, granulocyte-, and multi-CSFs. Infect. Immun. **56**:247–251.
5. **Cheers, C., I. F. C. McKenzie, H. Pavlov, C. Waid, and J. York.** 1978. Resistance and susceptibility of mice to bacterial infection. Course of listeriosis in resistant or susceptible mice. Infect. Immun. **19**:763–770.
6. **Cheers, C., and E. R. Stanley.** 1988. Macrophage production during murine listeriosis: CSF-1 and CSF-1 binding cells in genetically resistant and susceptible mice. Infect. Immun. **56**: 2972–2978.
7. **Davies, W. A.** 1982. Kinetics of killing *Listeria monocytogenes* by macrophages: correlation of ^{3}H-DNA release from labelled bacteria and changes in numbers of viable organisms by mathematical model. RES J. Reticuloendothel. Soc. **32**:461–476.
8. **Godfrey, R. W., and M. S. Wilder.** 1984. Relationship between oxidative metabolism, macrophage activation and anti-listerial activity. J. Leucocyte Biol. **36**:533–543.
9. **Havell, E. A., G. L. Spitalny, and P. J. Patel.** 1982. Enhanced production of murine interferon γ by T cells generated in response to bacterial infection. J. Exp. Med. **156**:112–127.
10. **Karbassi, A., J. M. Becker, J. S. Foster, and R. N. Moore.** 1987. Enhanced killing of *Candida albicans* by murine macrophages treated with macrophage colony stimulating factor: evidence for augmented expression of mannose receptors. J. Immunol. **139**: 417–420.
11. **Lee, M. T., and M. K. Warren.** 1987. CSF-1 induced resistance to viral infection in murine macrophages. J. Immunol. **138**: 3019–3022.
12. **Magee, D. M., E. J. Wing, E. M. Ampel, A. Waheed, and R. K. Shadduck.** 1987. Macrophage colony stimulating factor enhances the expression of Fc receptors on murine peritoneal

macrophages. Immunology **62**:373–378.
13. **Metcalf, D.** 1986. The molecular biology and functions of the granulocyte-macrophage colony stimulating factors. Blood **67**: 257–267.
14. **Nakane, A., and T. Minagarva.** 1984. The significance of alpha/beta interferons and gamma interferon produced in mice infected with *Listeria monocytogenes*. Cell. Immunol. **88**:29–40.
15. **Saito, H., H. Tomioka, Y. Yamada, and J. Jidoi.** 1986. Oxidative and phagocytic functions of macrophages during infections induced in mice by *Mycobacterium intracellulare* and *Listeria monocytogenes*. J. Gen. Microbiol. **132**:1117–1125.
16. **Stanley, E. R.** 1985. Macrophage colony stimulating factor. Methods Enzymol. **116**:564–587.
17. **Stanley, E. R., and P. T. Jubinsky.** 1984. Factors affecting the growth and differentiation of haemopoietic cells in culture. Clin. Haematol. **13**:329–348.
18. **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**:71–81.
19. **Wing, E. J., N. M. Ampel, A. Waheed, and R. K. Shadduck.** 1985. Macrophage colony-stimulating factor (M-CSF) enhances the capacity of murine macrophage to secrete oxygen reduction products. J. Immunol. **135**:2052–2056.
20. **Wing, E. J., A. Waheed, R. K. Shadduck, and L. S. Nagle.** 1982. Effect of colony stimulating factor on murine macrophages. Induction of anti-tumor activity. J. Clin. Invest. **69**:270–276.
21. **Wood, P. R., V. Spanidis, K. Frangos, and C. Cheers.** 1986. The *in vitro* bactericidal activity of peritoneal and spleen cells from *Listeria* resistant and susceptible mouse strains. Cell. Immunol. **99**:160–169.
22. **Young, A. M., and C. Cheers.** 1986. Colony forming cells and colony-stimulating activity during listeriosis in genetically resistant or susceptible mice. Cell. Immunol. **97**:227–237.