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Effect of Acute Nutritional Deprivation on Macrophage Colony-Stimulating Factor and Macrophage Progenitor Cells in Mice

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The effect of short-term nutritional deprivation on host defenses and on the parameters of macrophage production was determined in outbred mice. Confirming previous data from this laboratory, initial experiments demonstrated that starved mice were relatively resistant to infection by *Listeria monocytogenes* as determined by spleen and liver bacterial counts. The number of macrophage progenitor cells in bone marrow rose slightly during a 72-h starvation period and returned to normal during refeeding. By contrast, the number of progenitor cells in spleens fell to 12% of the base line during starvation. The concentration of the macrophage colony-stimulating factor in serum decreased during starvation and returned to normal during refeeding. Additional experiments were performed to determine whether starved mice had increased parameters of macrophage production during listerial infection. The number of progenitor cells in the bone marrow and spleens of starved mice had increased compared with that of fed mice early in infection. Macrophage colony-stimulating factor levels in starved mice rose early and remained elevated during infection but were not as high as in fed mice. These data document the changes in the parameters of monocyte production during starvation and suggest that the number of macrophage progenitor cells may be related to increased resistance to *L. monocytogenes*.

Previous studies from this laboratory demonstrated that short-term starvation altered cell-mediated immune host defenses in mice. Starvation depleted thymic and splenic cellularity but, surprisingly, enhanced resistance to challenges of *Listeria monocytogenes* (15). The mechanism for the increased resistance was operative during the first 48 to 72 h of infection (11), suggesting that cells of the monocyte-macrophage lineage were responsible for protection. Further experiments showed that the protective effect was sensitive to low-dose radiation, which indicated that a proliferating cell population was important in the enhanced resistance (10).

Bone marrow-derived monocytes are the cells primarily responsible for limiting early multiplication of *Listeria* spp. in the liver and spleen. Evidence for this concept was originally generated by North (5) and more recently by studies with mice genetically resistant to *L. monocytogenes* (3). Fixed tissue macrophages play a role in the early clearance of bacteria from the bloodstream and in initial killing, but these phagocytes are less important in controlling the infection than are infiltrating, bone marrow-derived monocytes. Because of the importance of monocytes in host defenses, we hypothesized that the protective mechanism in starved mice was associated with an increased capacity to generate and mobilize protective monocytes. Experiments were designed, therefore, to measure the parameters regulating monocyte production in starved mice. These parameters included the serum macrophage colony-stimulating factor (M-CSF) and the macrophage CFU progenitor cells (CFUm) in bone marrow and the spleen.

MATERIALS AND METHODS

Mice. Female CFW mice were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Mice were housed in well-ventilated rooms kept between 22 and 23°C. The mice used were between 6 and 10 weeks of age and were fed no. 5001 rodent laboratory chow (Ralston Purina Co.) containing, by weight, 23.4% protein, 4.5% fat, 5% fiber, 7.3% ash, 50% utilizable carbohydrate, and vitamins. Animals were inoculated in a lateral tail vein with a suspension of 10^4 or 10^5 *L. monocytogenes* as described previously to establish infection (13). The number of viable *L. monocytogenes* was confirmed by culture on tryptic soy agar.

Number of organ bacterial counts. The number of *L. monocytogenes* in spleens and livers was determined at various times after inoculation by methods described previously (12).

Food deprivation. Groups of 5 to 10 mice were starved by placing them in clean cages without food for 72 h. Water was given ad libitum. This period of starvation resulted in approximately a 25% weight loss but no mortality (15).

Measurement of CFUm in bone marrow and spleens. CFUm were quantitated by a colony-forming assay (6, 13). Briefly, bone marrow cells were obtained by flushing each femur with 2.0 ml of McCoy 5A medium with a no. 23-gauge needle. A suspension of spleen cells was prepared by forcing spleens through a stainless steel mesh. The cell suspensions from three mice were combined, aspirated through an 18-gauge needle to break up clumps of cells, and counted in a hemacytometer. The cells were then brought to a final concentration of 10^5 cells per ml (bone marrow) or 10^6 cells per ml (spleen) in supplemented McCoy 5A medium containing 15% fetal calf serum, amino acids, penicillin (100 U/ml), streptomycin (100 μg/ml), amphotericin (0.25 μg/ml), and 0.3% agar. Various dilutions of L-cell-conditioned medium as a source of M-CSF were added. The cell suspensions were then placed in tissue culture dishes (35 by 10 mm) and incubated for 7 days at 37°C in a humidified atmosphere containing 7.5% CO_2. After 7 days, the number of macrophage colonies in each plate was counted with a dissecting

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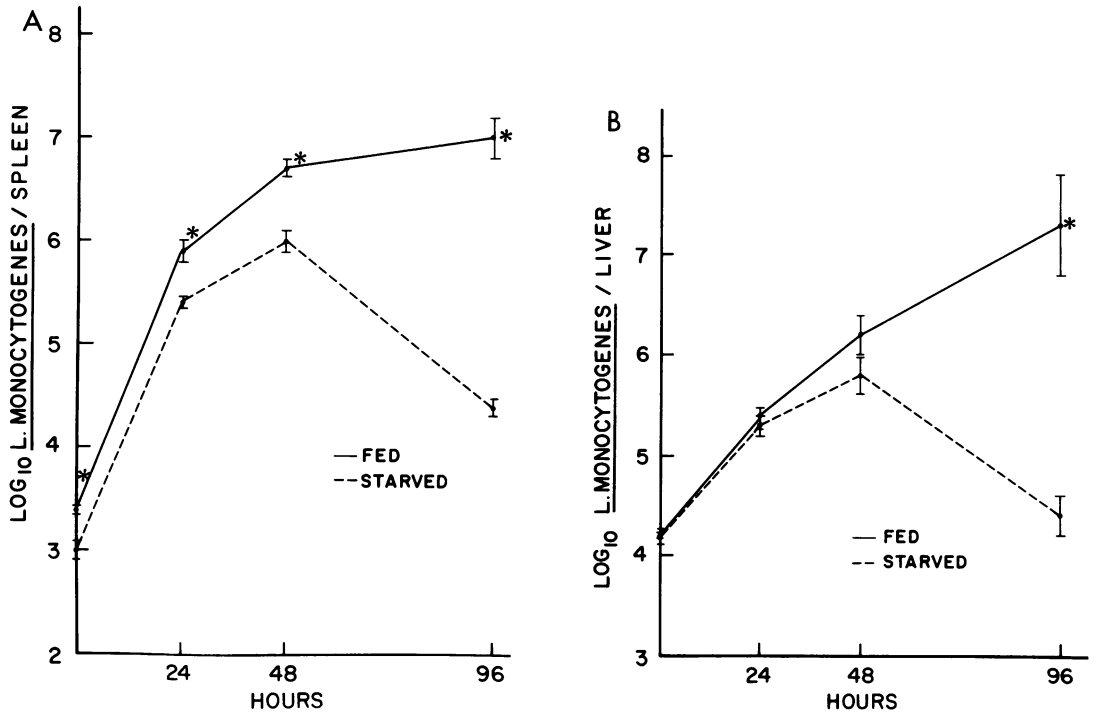

FIG. 1. Number of *L. monocytogenes* in spleens (A) and livers (B) of starved and fed mice. Mice were fed or starved for 72 h and inoculated with 10^5 *L. monocytogenes*. The number of listerial bacteria per organ is expressed as the mean (log 10) ± 1 standard error of the mean for five mice and is shown at various times after inoculation. An asterisk signifies $P < 0.05$.

microscope. Colonies with greater than 50 cells were scored. Cells primarily of the macrophage lineage are present in these colonies after 7 days of culture when L-cell-conditioned media are used as sources of colony-stimulating factor (7). The number of CFUm per femur or spleen was calculated from the mean number of CFUm per plate and the total number of cells per organ. Results are expressed as a percentage of values obtained from fed, uninfected control mice.

M-CSF levels. M-CSF levels in serum samples were measured by a double-antibody radioimmunoassay (2). Serum samples were obtained by bleeding mice from the retro-orbital venous plexus at various times after inoculation. Samples (0.01 ml) were assayed in duplicate.

RESULTS

Number of *L. monocytogenes* in spleens and livers of starved and fed mice. Initial experiments were performed to confirm previous reports from this laboratory that starved mice were protected against listerial infection compared with the findings for fed mice. Mice were fed or starved for 72 h and then inoculated intravenously with 10^5 *L. monocytogenes*. The number of these organisms in the spleens of starved mice was less than that in fed mice at 24, 48, and 96 h after inoculation ($P < 0.05$) (Fig. 1). The difference between liver counts was significant after 96 h ($P < 0.05$).

Effect of starvation on the number of CFUm in bone marrow. Experiments were designed to measure the number of CFUm in bone marrow during and after a 72-h period of starvation in uninfected mice. The number of CFUm per femur, expressed as a percentage of values in fed mice, tended to increase during starvation and decrease for 4 days of refeeding (Fig. 2). The changes did not vary more than 25% from the base line at any period. Results represent combined data from seven experiments.

Effect of starvation on splenic CFUm. The number of CFUm in spleens fell to a nadir of 12% of the values in fed mice by the end of the starvation period (Fig. 3). The recovery of counts occurred rapidly during the refeeding period. Results from six experiments were combined.

M-CSF levels in serum samples during starvation and

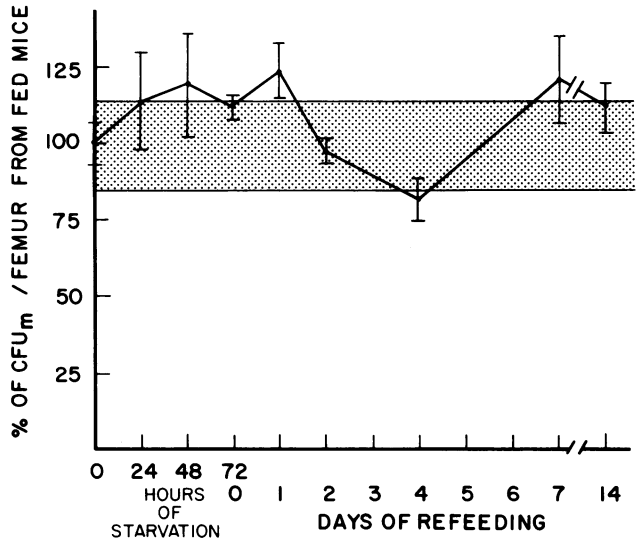

FIG. 2. CFUm per femur during starvation and refeeding. Mice were starved for 72 h and then refed. The number of CFUm per femur, expressed as a percentage of values from fed mice (mean ± 1 standard error of the mean), was determined at various times during the experiments. The 95% confidence limits (▨) were 85 and 115%. Specific values for individual animals ranged from 3,060 to 13,500 colonies per organ.

refeeding. M-CSF is believed to be an important regulatory factor in monocyte production. The M-CSF levels in serum samples, therefore, were measured by a radioimmunoassay technique at various times during starvation and refeeding (Fig. 4). The concentrations in the samples were somewhat decreased at the end of starvation and during the first 2 days of refeeding. Afterward, there was a gradual increase in values.

M-CSF concentrations in serum samples from starved and fed mice during listerial infection. It was demonstrated previously that serum M-CSF levels rise during the first 24 h of listerial infection and remain elevated during the course of infection (13). To determine whether starved mice had an enhanced M-CSF response during infection, we measured the concentration of M-CSF in serum samples at various times during listerial infection. M-CSF levels tended to rise more in fed mice than in starved mice and tended to remain higher throughout the infection period (Fig. 5). Differences between fed and starved mice were significant by Student's *t* test only on day 2 ($P < 0.05$). Levels in both groups of mice remained elevated during infection compared with the base lines, and both returned toward normal by day 15. The results of two experiments were combined.

CFUm in bone marrow and spleens of infected mice. Experiments were performed to determine whether the number of CFUm in starved mice was increased during listerial infection. The number of CFUm in the bone marrow of starved mice was better preserved than that in fed mice during the initial stages of infection (Table 1 shows data from a representative experiment). Fed mice had a greater number of CFUm, however, in the later stages of infection. In spleens, the number of CFUm in starved mice was greater than that in fed mice at day 4 of infection. In other experiments, the greatest difference was observed at day 2. Like bone marrow, the spleens of fed mice showed greater numbers of CFUm than did the same organs of starved mice later in the infection.

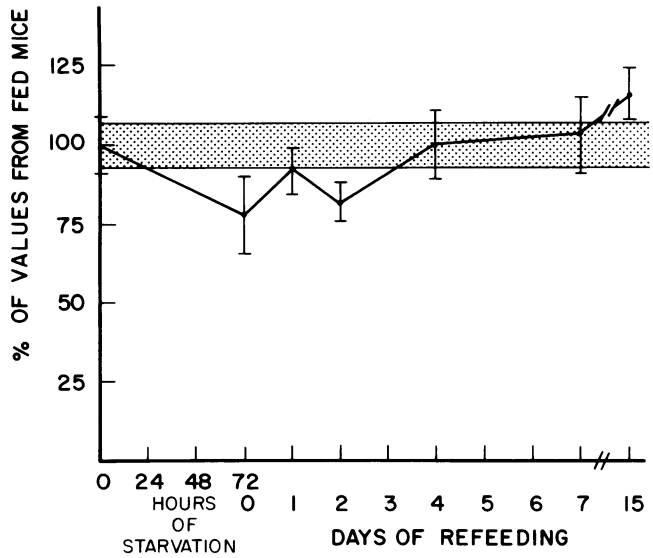

FIG. 4. M-CSF levels in serum samples during starvation and refeeding. M-CSF levels were measured by a radioimmunoassay and are expressed as the mean ± 1 standard deviation for five mice. The 95% confidence limits (▨) were 93 and 107%. Specific values ranged from 525 to 1,100 U/ml.

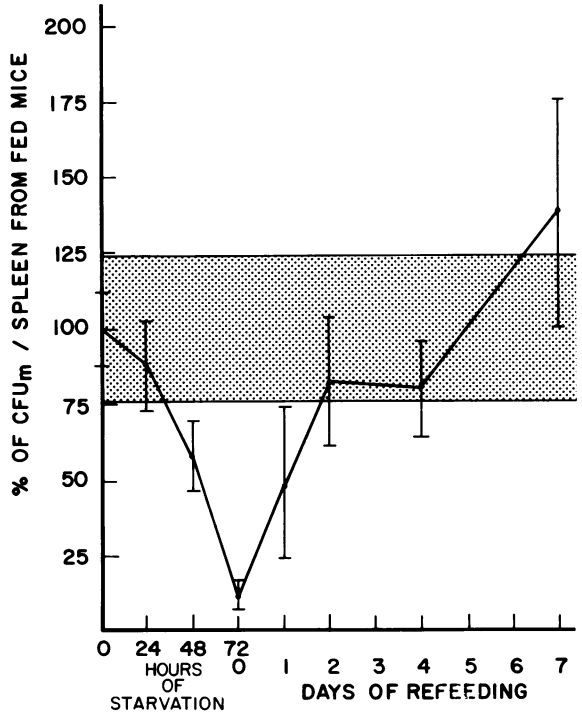

FIG. 3. CFUm per spleen during starvation and refeeding. CFUm are expressed as a percentage of values from fed mice (mean ± 1 standard error of the mean). The 95% confidence limits (▨) were 76 and 124%. Specific values ranged from 303 to 3,100 colonies per organ.

DISCUSSION

The data presented in this article characterize the changes in two important parameters of monocyte production during starvation. Both CFUm in bone marrow and the spleen and M-CSF, a secretory glycoprotein, are believed to be major determinants of macrophage production. Since our previous data suggested that protection in starved mice was mediated by a replicating cell population, presumably monocytes (10), we believed that regulatory factors in monocyte production might be enhanced by starvation.

The number of CFUm in bone marrow showed a slight increase during starvation, but the changes were not striking. By contrast, the CFUm in spleens actually decreased during the period of food deprivation. This reduction paralleled an overall decrease in cellularity in the spleen and thymus. In a previous study, the number of leukocytes fell in spleens to 50% and in thymuses to 30% of normal values after 48 h of starvation (15). In that study, the recovery of overall cellularity occurred by day 7, as did the recovery of the number of CFUm in the present experiments. In starved, uninfected mice, therefore, we did not observe changes in CFUm that might be expected to enhance monocyte production.

During the early stages of infection, however, the number of CFUm in the bone marrow and spleens of starved mice was significantly higher than in those of fed mice. These findings suggest that the starved mice had an initial increased capacity for monocyte production. The number of CFUm in the bone marrow of starved, infected mice was not actually increased and may have simply represented an epiphenomenon related to the depression of the numbers of CFUm

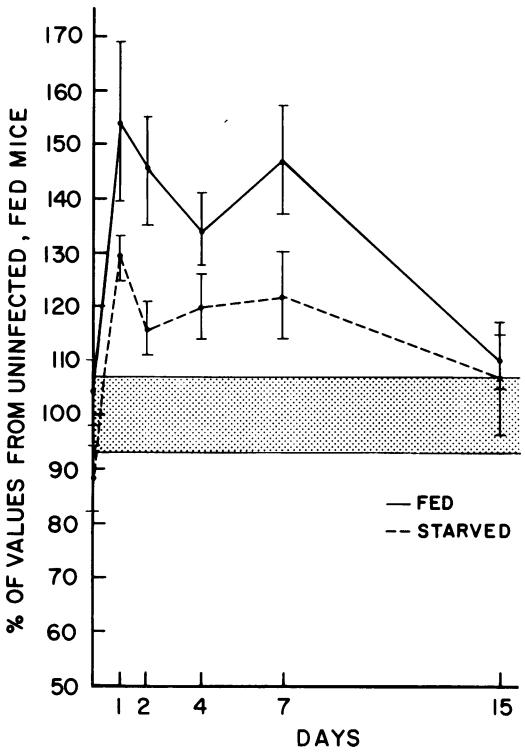

FIG. 5. M-CSF concentration in serum samples of fed and starved mice during *Listeria* infection. Mice were fed or starved for 72 h and then inoculated with *L. monocytogenes*. M-CSF levels are expressed as the mean ± 1 standard error of the mean. The 95% confidence limits (▒) were 93 and 107%. Specific values for individual mice ranged from 525 to 2,080 U/ml.

by high bacterial loads. The increased number of CFUm in the spleens of starved, infected mice, however, was a striking rise over the base line and represents an enhanced response to infection. The importance of this finding is emphasized by the fact that the spleen, a major site of bacterial multiplication during listeriosis, was found in this study to have lower numbers of *L. monocytogenes* in starved mice.

Other data support the concept that precursor cells are important in host defenses in the starvation model. We have performed experiments previously indicating that low-dose, whole-body irradiation inhibits the early protection induced by starvation (10). This indicates that a proliferating cell population is important in host defenses during the initial phases of infection. We have also used Sr^{89} to specifically irradiate bone marrow, the presumed source of CFUm in the spleen. The protection in Sr^{89}-treated, starved mice was eliminated as well (unpublished results). These data plus the present experiments support the concept that macrophage precursors such as CFUm are important in host defenses. Nonetheless, until specific techniques, such as monoclonal antibodies, to eliminate CFUm and other precursors are available, it will not be possible to determine precisely the role of these precursors.

TABLE 1. Bone marrow and spleen CFUm in fed and starved mice infected with *L. monocytogenes*

Day of infection	Mean CFUm ± 1 SD (% of control)[a] in:			
	Bone marrow		Spleen	
	Fed	72-h starved	Fed	72-h starved
2	46 ± 6	78 ± 2[b]	182 ± 40	125 ± 25[c]
4	42 ± 5	112 ± 10[b]	376 ± 87	1,246 ± 150[b]
7	82 ± 8	61 ± 7[d]	645 ± 78	610 ± 120
10	—[e]	—	586 ± 115	174 ± 19[b]
14	91 ± 6	71 ± 8[d]	—	—

[a] Numbers of CFUm were determined in mice inoculated on day 0 with 10^4 *L. monocytogenes*. Results are expressed as percentages of the corresponding values for fed, uninfected mice and are from five samples.
[b] $P < 0.001$ by Student's t test compared with results for fed mice.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] —, Not done.

M-CSF promotes the proliferation and differentiation of monocytic progenitor cells. M-CSF also enhances tissue macrophage metabolism and function, including protein and DNA synthesis (1), release of interleukin-1 (4) and oxygen reduction products (8), and antitumor activity (14). Base-line levels of M-CSF in serum samples are approximately 800 to 1,200 U/ml. During listerial infection, levels rise rapidly to approximately 1,600 U/ml during the first 24 h and remain elevated during infection (13). In *Listeria*-immune mice, M-CSF levels peaked significantly earlier than in nonimmune mice after bacterial challenge (9). M-CSF is therefore an acute-phase reactant that may regulate, in part, monocyte-macrophage production and function during acute listeriosis. Starvation alone, however, did not appear to increase M-CSF levels in serum samples. Concentrations were slightly decreased during food deprivation and returned to normal values over 3 to 4 days. During acute listerial infection, M-CSF levels increased in starved mice but were not as elevated as in fed, infected mice. This suggests that there was not an increased reserve of M-CSF in starved mice that could be mobilized during the stress of infection.

Experiments by Young and Cheers are relevant to our findings (16). These workers measured CSF activity in serum and the number of progenitor cells in mice that were genetically resistant or sensitive to *Listeria* sp. CSF levels during infection appeared to correlate with the bacterial load. In addition, resistant mice had a greater number of progenitor cells in bone marrow than did sensitive mice during infection, although this dropped somewhat early in the infection. The number of progenitor cells in the spleens of resistant mice was greater than that in sensitive mice at the base line and during infection. The presence of increased numbers of progenitor cells in spleens, therefore, may be a marker for increased resistance and may possibly represent an important host defense mechanism.

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