INFECTION AND IMMUNITY, Dec. 1995, p. 4595–4599 Vol. 63, No. 12
0019-9567/95/$04.00+0

Listeria monocytogenes Can Grow in Macrophages without the Aid of Proteins Induced by Environmental Stresses

TOMOKO HANAWA,* TOMOKO YAMAMOTO, AND SHIGERU KAMIYA

Department of Microbiology, Kyorin University School of Medicine, Mitaka, Tokyo 181, Japan

Received 3 July 1995/Returned for modification 14 August 1995/Accepted 20 September 1995

Listeria monocytogenes **is a facultative intracellular pathogen which is able to survive and grow within phagocytic cells. Some facultative intracellular bacteria have been shown to respond to the hostile environment within phagocytic cells by producing a set of stress proteins. Since *L. monocytogenes* has a mechanism for intracellular survival that is distinct from those of other bacteria, we studied the phenotypic response of the bacterium to phagocytosis by macrophages. After phagocytosis of *L. monocytogenes* EGD by J774-1 macrophage cells, the microorganism rapidly increased in numbers about 20-fold during an incubation period of 5 h. In this phase of phagocytosis, the selective induction of 32 proteins was observed by two-dimensional gel electrophoresis. The responses to the environmental stresses of heat and hydrogen peroxide were also studied, and it was found that 14 heat shock proteins and 13 oxidative stress proteins were induced. Five of the induced proteins were common to both heat and oxidative stresses. By amino acid sequencing analysis, homologs of DnaK and GroEL were confirmed among the heat shock proteins. A comparison of the autoradiograms of the two-dimensional gels revealed that none of these stress proteins were among the proteins induced by *L. monocytogenes* within the macrophages. This behavior is entirely different from that shown by other facultative intracellular pathogens. Stress proteins known to be induced by environmental stresses were absent in intracellularly grown *L. monocytogenes* in the present study. This absence could be due to the mechanism by which the microorganisms rapidly escape from this stressful environment at a very early phase of phagocytosis.**

Listeria monocytogenes is a gram-positive, food-borne human pathogen which causes listeriosis in immunocompromised individuals and pregnant women (12). In a murine model of infection, *L. monocytogenes* is a facultative intracellular pathogen (14) which is able to grow in professional phagocytic cells, such as macrophages, and nonprofessional phagocytic cells, including epithelial cells and fibroblasts (10, 16, 23, 30). Intracellular growth within professional phagocytic cells has been thought to be important in the expression of the pathogenicity of intracellular parasites. Intracellular parasites utilize a variety of mechanisms to survive within phagocytes, e.g., inhibition of phagosome-lysosome fusion, escape from the phagosome into the cytoplasm, resistance to or inactivation of oxygen intermediates and lysosomal enzymes, and inhibition of the triggering of a complete respiratory burst (2, 6, 15, 17).

Recently, it has been demonstrated in *Salmonella typhimurium* that bacterial stress proteins possibly play an important role in survival and growth within macrophages (4, 18). Furthermore, it has been reported that some facultative intracellular pathogens, e.g., *S. typhimurium*, *Yersinia enterocolitica*, and *Legionella pneumophila*, generally express elevated levels of a set of proteins, including major stress proteins, within macrophages after phagocytosis (4, 41, 42). These are not induced within nonprofessional phagocytic cells, such as epithelial cells (4). The induction of the stress response in bacteria within phagocytic cells is reasonable, because the intracellular environment of a macrophage is very hostile so far as such antimicrobial factors as acidic pH, oxidative products, and lysosomal proteins and peptides are concerned. It is speculated that the enhanced expression of stress proteins may give facultative intracellular bacteria a selective advantage for survival in phagocytes, resulting in their proliferation and dissemination throughout the host (4, 41, 42).

We have been interested in the function of stress proteins induced by phagocytosis in the expression of pathogenicity by facultative intracellular bacteria (40, 41). *L. monocytogenes* is well known to have a mechanism for survival in macrophages that is distinct from those of other bacteria, such as *Y. enterocolitica*, *S. typhimurium*, and *L. pneumophila* (37). To discover whether the induction of stress proteins in intracellular bacteria is generally accompanied by phagocytosis, an approach similar to the one which we had previously developed for *Y. enterocolitica* (41) was used to examine the modulation in protein synthesis, including induction of stress proteins, by *L. monocytogenes* in response to the intracellular environment of macrophages. We initially examined the response to such environmental stresses as heat shock and hydrogen peroxide and found that at least 22 stress proteins were induced by heat shock and hydrogen peroxide. We then analyzed the modulation of protein synthesis by intracellular *L. monocytogenes* and found that 32 proteins were induced and that many proteins were repressed during growth in J774-1 macrophage cells. However, contrary to the observations made with other bacteria, the detectable induction of stress proteins was not observed in intracellular *L. monocytogenes* after phagocytosis. The successful adaptation for survival without the induction of stress proteins will be discussed.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Hemolytic strain EGD (serotype 1/2a) and nonhemolytic strain ATCC 15313 were kindly provided by M. Mitsuyama (Niigata University School of Medicine, Niigata, Japan). Routine culture was carried out in brain heart infusion (Nissui Co., Tokyo, Japan) at 30°C with aeration.

Labeling of heat shock proteins. For heat shock experiments, 0.2 ml of an overnight culture was used to inoculate 10 ml of brain heart infusion broth. Bacteria were grown at 30°C to an optical density at 600 nm of 0.5. After centrifugation at 7,000 × *g* for 20 min at room temperature, the cell pellet was

* Corresponding author. Mailing address: Department of Microbiology, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181, Japan. Phone: 81-422-47-5511 (ext. 3464). Fax: 81-422-44-7325.

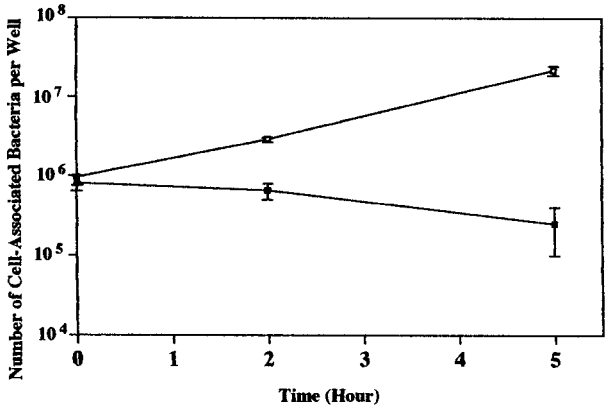

FIG. 1. Growth of *L. monocytogenes* strains in mouse macrophage-like cell line J774-1. A total of 5×10^5 J774-1 cells were challenged with hemolytic strain EGD (□) and nonhemolytic strain ATCC 15313 (■). The numbers of viable bacteria at each time were determined as described in Materials and Methods. Results are expressed as the averages of three determinations. Error bars show standard deviations.

washed and then suspended in 1 ml of methionine-free RPMI 1640 (GIBCO BRL, Grand Island, N.Y.) prewarmed to 30°C. The culture was incubated with shaking for 10 min at 45°C prior to the addition of 10 μCi of [^{35}S]methionine (specific activity, 1,000 μCi/mmol) per ml. Labeling was carried out at the same temperature for 20 min. The cells were harvested by centrifugation at 15,000 × *g* for 5 min at 4°C and suspended in 100 μl of sample buffer for a nonequilibrium pH gradient system (26). The bacterial cells were treated for 25 s at 20 kHz with an ultrasonic disintegrator. After centrifugation, the supernatant was used for gel electrophoresis.

Hydrogen peroxide treatment. Overnight (18-h) cultures were used to inoculate 10 ml of fresh brain heart infusion medium, and the cells were grown at 30°C to an optical density at 600 nm of 0.4. After centrifugation (7,000 × *g* for 20 min at room temperature), the cell pellet was washed and suspended in 1 ml of methionine-free RPMI 1640. The bacterial cells were incubated for 15 min in the presence of 50 mM hydrogen peroxide and then labeled with [^{35}S]methionine (10 μCi) for 30 min. Samples were centrifuged (15,000 × *g* for 5 min at 4°C), and the cell pellet was suspended in the sample buffer for a nonequilibrium pH gradient system (26). The bacterial cells were treated for 25 s at 20 kHz with an ultrasonic disintegrator. After centrifugation, the supernatant was subjected to gel electrophoresis.

Macrophage assay for virulence. J774-1 cells from a continuous macrophage-like cell line derived from a reticulum cell sarcoma (32) were provided by the Japanese Cancer Research Resources Bank and grown in RPMI 1640 supplemented with 10% fetal calf serum. A total of 5×10^5 J774-1 cells in each well of a 24-well plate were challenged with *L. monocytogenes* EGD or ATCC 15313 at a multiplicity of infection of 5. The plates were centrifuged at 250 × *g* for 10 min at room temperature to enhance and synchronize infection. The cells were incubated for 20 min at 37°C to permit phagocytosis, and the free bacteria were removed by three washes with phosphate-buffered saline (PBS). RPMI 1640 supplemented with 2% fetal calf serum and 5 μg of gentamicin per ml was added, and the cells were then incubated at 37°C. The macrophages were sampled at various times by aspirating the medium, rinsing each well with 0.5 ml of PBS, lysing the macrophages with 0.5 ml of distilled water, and then rinsing each well with 0.5 ml of PBS. Triplicate samples were plated individually on brain heart infusion agar plates after appropriate dilutions.

Labeling of intracellular bacteria. A total of 2×10^6 J774-1 cells in each well of a six-well plate were challenged with bacteria at a multiplicity of infection of 50. Phagocytosis was allowed by the procedure described above. After three washes with PBS, methionine-free RPMI 1640 supplemented with 50 μg of cycloheximide per ml and 5 μg of gentamicin per ml was added. Intracellular bacteria were labeled with [^{35}S]methionine (10 μCi) at 30°C. Macrophages were lysed with distilled water supplemented with 100 mM EDTA and protease inhibitors, 1 μM leupeptin, 1 μM pepstatin, and 500 μM phenylmethylsulfonyl fluoride. The bacterial cells were collected by centrifugation and solubilized.

Electrophoresis. A portion of each lysate was run in the nonequilibrium pH gradient system described by O'Farrell et al. (26) for the first dimension and then on a sodium dodecyl sulfate (SDS)–12.5% polyacrylamide gel as described by Laemmli (21) for the second dimension. After electrophoresis, the gels were dried and exposed to film (RX100; Fuji Photo Film Co., Tokyo, Japan) at room temperature for 2 to 7 days. Bacterial proteins were also analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) as described before (40).

Microsequencing of proteins. To analyze the N-terminal protein sequence, proteins were separated by two-dimensional electrophoresis and transferred onto a polyvinylidene difluoride membrane (Millipore) by electroblotting from the gels. The membrane pieces from 5 to 10 gels were sequenced on a protein sequencer (473A; Applied Biosystems, Foster City, Calif.).

RESULTS

Intracellular growth of *L. monocytogenes* within macrophages. We initially tested the efficiency of using a continuous macrophage cell line to analyze the interaction of *L. monocytogenes* with macrophages by the method previously demonstrated (30), with some modifications. Figure 1 is a graph of the growth of *L. monocytogenes* strains in the macrophage-like cell line J774-1. After phagocytosis of *L. monocytogenes* EGD, the number of bacteria increased about 20-fold during the initial 5 h. However, a nonhemolytic strain, ATCC 15313, was readily killed and could not grow during the 24 h after phagocytosis (data not shown). These growth curves are typical of the intracellular fate of *L. monocytogenes* within phagocytic cells, in that a hemolytic strain can survive and multiply but a nonhemolytic strain fails to grow (30). We therefore concluded that the combination of the J774-1 cell line and these bacterial strains was an appropriate bacterium-macrophage interaction system for this study.

Response of *L. monocytogenes* to environmental stress conditions. Since the stress proteins of *L. monocytogenes* are not fully understood, we performed several experiments to determine the stress proteins induced by heat shock and oxidative stress.

(i) Heat shock. *L. monocytogenes* EGD and ATCC 15313 were grown to mid-log phase at 30°C, shifted to 45°C for 10 min, and then labeled with [^{35}S]methionine for 20 min. A comparison of the protein profiles of shifted and unshifted bacteria by SDS-PAGE indicates that 12 proteins were induced after the temperature shift (Fig. 2). These proteins were constitutively produced at low concentrations in bacterial cells grown at steady state. These may be essential for growth but may be needed at higher concentrations during growth under stressful conditions. Furthermore, we analyzed the proteins in the heat-shocked cells and normally grown cells by two-dimensional gel electrophoresis (Fig. 3A and B). Autoradiographs of two-dimensional gels showed that the synthesis of at least 14 bacterial proteins in strain EGD was markedly elevated in

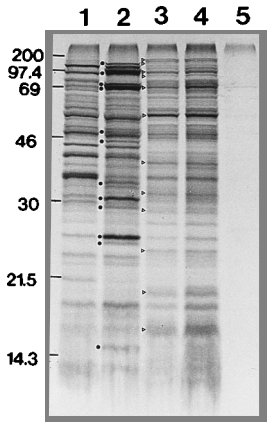

FIG. 2. Autoradiograph of SDS-PAGE gel with ^{35}S-labeled *L. monocytogenes* proteins. *L. monocytogenes* EGD cells were labeled with [^{35}S]methionine for 20 min at 30°C (lane 1) or were transferred to 45°C, incubated for 10 min, and then labeled with [^{35}S]methionine for 20 min at 45°C (lane 2). After phagocytosis, strain EGD was labeled for 2 to 3 h (lane 3) or 3 to 4 h (lane 4). Strain ATCC 15313 was labeled for 3 to 4 h (lane 5). △, proteins induced during growth within macrophages (lanes 3 and 4); ●, proteins induced under heat shock conditions (lane 2). The molecular masses in kilodaltons are indicated at the left.

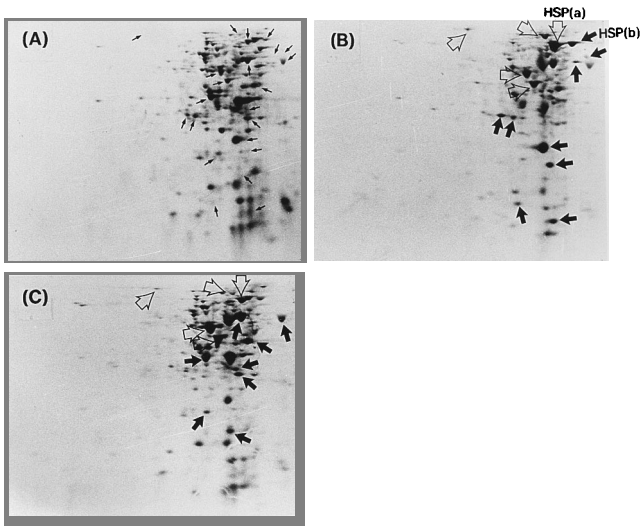

FIG. 3. Autoradiographs of two-dimensional electrophoresis gels of ^{35}S-labeled proteins from *L. monocytogenes*. Bacterial proteins of strain EGD were labeled with [^{35}S]methionine in methionine-free RPMI 1640 at 30°C (A) or 45°C (B) or in the presence of 50 mM hydrogen peroxide (C) as described in Materials and Methods. Arrows show the stress proteins induced by heat shock (B) or hydrogen peroxide (C). Small arrows (A) show the positions of proteins whose synthesis was elevated by exposure to heat or hydrogen peroxide. Open arrows (B and C) indicate the proteins induced by both heat and hydrogen peroxide. The N-terminal amino acid sequences of two heat shock proteins, HSP(a) and HSP(b), are shown in Fig. 4.

heat-shocked bacteria compared with that in the bacteria grown at 30°C (Fig. 3B). Although the protein profile of strain ATCC 15313 grown at 30°C is considerably different from that of strain EGD, all of the heat shock proteins of strain EGD were also induced in strain ATCC 15313 (data not shown). We selected two prominently induced proteins among the 14 heat shock proteins of the EGD strain and characterized them. These proteins, designated HSP(a) and HSP(b), have molecular masses of 67 kDa and 72 kDa, respectively (Fig. 2 and 3). We analyzed their sequences as described in Materials and Methods. The N-terminal amino acid sequences showed that HSP(a) and HSP(b) correspond to GroEL and DnaK homologs, respectively (Fig. 4).

(ii) Oxidative stress. We also analyzed, by two-dimensional gel electrophoresis, the bacterial proteins induced by exposure to hydrogen peroxide (Fig. 3A and C). The levels of at least 13 proteins were markedly elevated in bacteria under oxidative stress, compared with the levels of those proteins in bacteria

L. monocytogenes	HSP(a)	AKNIKFSENAGYAML
		: :::: : :::
B. subtilis	HSP60	AKEIKFSEEARRAML
		: :::: : :::
S. aureus	HSP60	VKQLKFSEDARQAML
L. monocytogenes	HSP(b)	SKIAGINLGTVDEAV
		:: ::
B. subtilis	HSP70	SKVIGID
		:: ::
S. aureus	HSP70	SKIIGIDLGTTNSXV

FIG. 4. Alignment of the N-terminal amino acid sequences of HSP(a) and HSP(b) (shown in Fig. 3B) and those of their homologs. Conserved amino acids are indicated by colons. The amino acid sequences of the homologs of *B. subtilis* and *S. aureus* were reported by Völker et al. (38) and Ohta et al. (27), respectively.

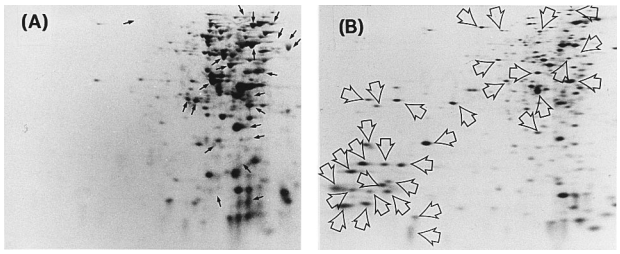

FIG. 5. Autoradiographs of two-dimensional electrophoresis gels with ^{35}S-labeled proteins from *L. monocytogenes* EGD grown in methionine-free RPMI 1640 at 30°C (A) and in J774-1 macrophage cells at 2 to 3 h after phagocytosis (B). Small arrows show the positions of stress proteins induced by exposure to heat and/or hydrogen peroxide (A). Open arrows show the proteins induced during incubation in macrophages (B).

grown at 30°C (Fig. 3B). Five of the 13 oxidative stress proteins were also detected as heat shock proteins (Fig. 3B and C).

Changes in protein synthesis by *L. monocytogenes* during incubation within J774-1 macrophage-like cells. To examine protein expression under intracellular conditions, the bacterial proteins synthesized within J774-1 cells were labeled with [^{35}S]methionine in the presence of cycloheximide to prevent macrophage protein synthesis and then analyzed by one- or two-dimensional gel electrophoresis (Fig. 2 and 5). SDS-PAGE revealed that at least 12 proteins with sizes ranging from 10 to 200 kDa were induced under intracellular conditions. The elevated level of protein expression was maintained from 30 min to 240 min after phagocytosis. These proteins were not detected in *L. monocytogenes* grown in RPMI 1640 (Fig. 5). When macrophage-sensitive strain ATCC 15313 was phagocytized and radiolabeled, neither bacterial proteins nor host proteins incorporated [^{35}S]methionine. We previously showed that neither bacterial proteins nor macrophage proteins incorporated [^{35}S]methionine when the heat-inactivated bacteria were phagocytized as a control (41). This result suggested that cycloheximide completely inhibited macrophage protein synthesis (Fig. 2). Furthermore, the proteins from the phagocytized bacteria and the proteins from bacteria grown in the medium were subjected to two-dimensional gel electrophoresis. The protein profile of the phagocytized bacteria was apparently different from that of bacteria grown in RPMI 1640. The results revealed that the synthesis of at least 32 bacterial proteins was significantly elevated in phagocytized bacteria compared with that in bacteria grown in culture medium. To examine whether these so-called macrophage-induced proteins are also induced by stress conditions in vitro, we compared the protein profiles by autoradiogram of macrophage-induced proteins and proteins induced by environmental stress in vitro. Visual scanning of the autoradiograms of two-dimensional gels revealed that neither heat shock proteins nor oxidative proteins were included in the macrophage-induced proteins. This behavior of *L. monocytogenes* is entirely different from that of other facultative intracellular pathogens previously reported (4, 41, 42).

DISCUSSION

During the infection process, bacteria are exposed to various stimuli, e.g., a sudden increase in temperature upon entering a mammalian host and oxidative or nonoxidative microbicidal stress associated with exposure to phagocytic cells. For most pathogenic bacteria, the mechanism by which bacteria withstand and survive these stressful environments is a fundamental question for their pathogenesis. Facultative intracellular

bacteria generally induce a large set of proteins, including some stress proteins, in response to intracellular stress when they grow in professional phagocytic cells (4, 41, 42). While it is not clear which stimuli actually induce the change in protein synthesis, it is believed that these induced proteins probably give intracellular bacteria a selective advantage for survival in phagocytes. In this study, we analyzed the modulation of protein expression by *L. monocytogenes* in response to phagocytosis by macrophages and then examined whether stress-induced protein synthesis is observed following phagocytosis.

In many reports, heat shock proteins have been revealed to function as molecular chaperones or proteases in a variety of bacteria (25). Recently, some reports have shown that the mechanism of heat shock response in gram-positive bacteria is different from that in gram-negative bacteria (1). Since the stress response of *L. monocytogenes* has not been fully studied, we initially investigated stress proteins induced by heat shock and oxidative stress. Following heat shock, the induction of 14 stress proteins, including GroEL and DnaK homologs, was detected (Fig. 3B and 4). Some of the heat shock proteins have been shown to be induced by other stresses, like oxidative stress (5, 39). We showed that at least 13 proteins were induced by exposure to 50 mM hydrogen peroxide (Fig. 3B). The electrophoretic analysis with two-dimensional gels revealed that five of these proteins, including the GroEL homolog, correspond to proteins induced by heat shock. Protein analysis indicated that the N-terminal amino acid sequences of the GroEL and DnaK homologs are highly conserved in such gram-positive bacteria as *L. monocytogenes*, *Bacillus subtilis*, and *Staphylococcus aureus* (Fig. 4).

L. monocytogenes cells which are phagocytized dissolve the phagosomal or endosomal membrane and escape into the cytoplasm of the macrophage (10). Then, the bacteria migrate by cell-to-cell spreading. The migration requires the induction of polymerization of F-actin and coating with actin filaments (36, 37). Recent studies by transposon mutagenesis have elucidated several genes involved in the intracellular survival of *L. monocytogenes*. Listeriolysin O, coded for by the *hly* gene, is involved in the lysis of the phagosome vacuole (3, 11, 19). ActA, a surface-associated protein coded for by the *actA* gene, mediates actin assembly within the host cell (9, 20). The *plcA* gene encodes a phosphatidylinositol-specific phospholipase C (22, 34). The *mpl* gene encodes metalloprotease (8, 24). The gene *plcB* is a determinant for a second phospholipase C with lecithinase activity (33). Expression of five clustered virulence genes, *plcA*, *hly*, *mpl*, *actA*, and *actB*, is transcriptionally regulated by PrfA (3), and therefore the proteins coded for by these genes are called PrfA-dependent proteins. Since all of these genes are essential for survival in phagocytic cells and the full expression of virulence by *L. monocytogenes* (31), the operon must be efficiently expressed within phagocytic cells. In this study, we observed the induction of 12 proteins on SDS-polyacrylamide gels and of 32 proteins on two-dimensional gels (Fig. 2 and 5). The molecular sizes of the major PrfA-dependent proteins, including ActA, Mpl, digested ActA, LLO, digested Mpl, PlcA, and PlcB, were determined to be 90, 70, 60, 57, 34, 32, and 29 kDa, respectively (35). It is likely that the PrfA-dependent proteins are included in the macrophage-induced proteins detected on the SDS-polyacrylamide gel (Fig. 2). Many macrophage-induced proteins were detected in the basic region of the two-dimensional gel (Fig. 5). A similar result was obtained with *Y. enterocolitica* grown within macrophages (40).

By visual scanning of two-dimensional gels, neither heat shock proteins nor oxidative stress proteins were detected in *L. monocytogenes* grown within macrophage cells. The absence of induction was confirmed up to 4 h after phagocytosis. This absence of any detectable increase in the levels of stress proteins during the early stages of phagocytosis is entirely different from the results that have been demonstrated with other facultative intracellular bacteria, such as *S. typhimurium*, *Y. enterocolitica*, and *L. pneumophila* (4, 41, 42). Recently, the fate of *L. monocytogenes* in murine bone marrow-derived macrophages during the early stages of infection was analyzed by cytochemical and immunolabeling approaches at the electron microscopy level (7). All phagosomes were immediately acidified after phagocytosis; therefore, intracellular growth of *L. monocytogenes* appears to be the result of a competition between the expression of the hydrolytic activity of these cells following phagosome-lysosome fusion and the capacity of bacteria to escape from the acidified phagosomal component before phagosome-lysosome fusion. Once bacteria succeed in escaping into the cytoplasm, they would not be exposed to any stressful component. If stress proteins are induced in phagocytized *L. monocytogenes*, this induction might occur during a very early and short period of phagocytosis before escape into the cytoplasm, because we could not detect induction of any stress protein from 30 min to 1 h after phagocytosis (data not shown). It is possible that this presumptive stress response very early in phagocytosis may slightly contribute to survival of *L. monocytogenes* within the macrophage. This assumption is supported by the observation that there is no significant difference in virulence between a catalase-negative mutant and the parental strain (11).

It seems that *L. monocytogenes* grows more rapidly in macrophage-phagocytes than does *L. pneumophila*, *S. typhimurium*, or *Y. enterocolitica*. These bacteria grow slowly within macrophage cells and increase approximately 10-fold during a 24-h incubation after infection (25, 28, 29, 39), while *L. monocytogenes* increases 20-fold within 4 h after phagocytosis, as can be seen from Fig. 1. This distinct behavior is not dependent on the source of the macrophages. These bacteria do not escape into the cytoplasm and survive and replicate within the phagosome or phagosome-lysosome by various mechanisms, such as inhibition of phagosome-lysosome fusion or a respiratory burst by the host macrophage (17). Since these bacteria are exposed to various microbicidal mechanisms in the phagosome or phagosome-lysosome for a long period, they should respond to the hostile conditions by inducing a set of stress proteins. A critical role of stress proteins for survival of intracellular bacteria in host phagosomes was originally demonstrated with *S. typhimurium* in macrophages, in which the HtrA-deficient mutant showed great sensitivity to phagocytosis by macrophages and a great increase in the 50% lethal dose in mice compared with that of the parental strain (18). However, *L. monocytogenes* probably has adapted to survive and multiply without the induction of stress proteins because of its ability to escape rapidly from the stressful phagosome into the cytoplasm of the macrophage.

ACKNOWLEDGMENTS

We thank J. Kumada for technical assistance. We also thank D. Ang (University of Geneva) for critical reading of the manuscript.

REFERENCES

1. **Abu Kwaik, Y., B. I. Eisenstein, and N. C. Engleberg.** 1993. Phenotypic modulation by *Legionella pneumophila* upon infection of macrophages. Infect. Immun. **61:**1320–1329.
2. **Armstrong, J. A., and P. D'Arcy Hart.** 1971. Response of cultured macrophages to *Mycobacterium tuberculosis* with observations on fusion of lysosomes with phagosomes. J. Exp. Med. **134:**713–740.
3. **Bohne, J., Z. Skolovic, and W. Goebel.** 1994. Transcriptional regulation of

prfA and PrfA-regulated virulence genes in *Listeria monocytogenes*. Mol. Microbiol. **11:**1141–1150.
4. **Buchmeier, N. A., and F. Heffron.** 1990. Induction of *Salmonella* proteins upon infection of macrophages. Science **248:**730–732.
5. **Christman, M. F., R. W. Morgan, F. S. Jacobson, and B. N. Ames.** 1985. Positive control of a regulation for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell **41:**753–762.
6. **Dabri, G. A., J. M. Sanger, D. A. Portnoy, and F. S. Southwick.** 1990. *Listeria monocytogenes* moves rapidly through the host-cell cytoplasm by inducing directional actin assembly. Proc. Natl. Acad. Sci. USA **87:**6068–6072.
7. **de Chastellier, C., and P. Berche.** 1994. Fate of *Listeria monocytogenes* in murine macrophages: evidence for simultaneous killing and survival of intracellular bacteria. Infect. Immun. **62:**543–553.
8. **Domann, E., M. Leimeister-Wächter, W. Goebel, and T. Chakraborty.** 1991. Molecular cloning, sequencing, and identification of a metalloprotease gene from *Listeria monocytogenes* that is species specific and physically linked to the listeriolysin gene. Infect. Immun. **59:**65–72.
9. **Domann, E., J. Wehland, M. Rhode, S. Pisor, W. Goebel, M. Hartl, M. Leimeister-Wachter, M. Wuenscher, and T. Chakraborty.** 1992. A novel bacterial virulence gene in *Listeria monocytogenes* required for host cell microfilament interaction with homology to the proline-rich region of vinculin. EMBO J. **11:**1981–1990.
10. **Gaillard, J.-L., P. Berche, J. Mounier, S. Richard, and P. Sansonetti.** 1987. In vitro model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell line Caco-2. Infect. Immun. **55:**2822–2829.
11. **Gaillard, J. L., P. Berche, and P. Sansonetti.** 1986. Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria monocytogenes*. Infect. Immun. **52:**50–55.
12. **Gellin, B. G., and C. V. Broome.** 1989. Listeriosis. JAMA **261:**1313–1320.
13. **Georgopoulos, C., K. Liberek, M. Zylicz, and D. Ang.** 1994. Properties of the heat shock proteins of *Escherichia coli* and the autoregulation of the heat shock response, p. 209–249. *In* R. I. Morimoto, A. Tissières, and C. Georgopoulos (ed.), The biology of heat shock proteins and molecular chaperones. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
14. **Hahn, H., and S. H. E. Kaufman.** 1981. The role of cell-mediated immunity in bacterial infections. Rev. Infect. Dis. **3:**1221–1250.
15. **Hart, P. D., J. A. Armstrong, C. A. Brown, and P. Draper.** 1972. Ultrastructural study of the behavior of macrophages toward parasitic mycobacteria. Infect. Immun. **5:**803–807.
16. **Havell, E. A.** 1986. Synthesis and secretion of interferon by murine fibroblasts in response to intracellular *Listeria monocytogenes*. Infect. Immun. **54:**787–792.
17. **Horwitz, M. A.** 1983. The Legionnaires' disease bacterium (*Legionella pneumophila*) inhibits phagosome-lysosome fusion in human monocytes. J. Exp. Med. **158:**2108–2126.
18. **Johnson, K., I. Charles, G. Dougan, D. Pickard, P. O'Gaora, G. Costa, T. Ali, I. Miller, and C. Hormaeche.** 1991. The role of a stress-response protein on *Salmonella typhimurium* virulence. Mol. Microbiol. **5:**401–407.
19. **Kathariou, S., P. Metz, H. Hof, and W. Goebel.** 1987. Tn*916*-induced mutations in the hemolysin determinant affecting virulence of *Listeria monocytogenes*. J. Bacteriol. **169:**1291–1297.
20. **Kocks, C., E. Gouin, M. Tabouret, P. Berche, H. Ohayon, and P. Cossart.** 1992. *Listeria monocytogenes*-induced actin assembly requires the *actA* gene product, a surface protein. Cell **68:**521–531.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
22. **Leimeister-Wachter, M., E. Domann, and T. Chakraborty.** 1991. Detection of gene encoding a phosphatidylinositol specific phospholipase C that is co-ordinately expressed with listeriolysin in *Listeria monocytogenes*. Mol. Microbiol. **5:**361–366.
23. **Mackaness, G. B.** 1962. Cellular resistance to infection. J. Exp. Med. **116:**381–406.
24. **Mengaud, J., C. Geoffroy, and P. Cossart.** 1991. Identification of a new operon involved in *Listeria monocytogenes* virulence: its first gene encodes a protein homologous to bacterial metalloproteases. Infect. Immun. **59:**1043–1049.
25. **Miyamoto, H., S. Yoshida, H. Taniguti, M. H. Qin, H. Fujino, and Y. Mizuguti.** 1993. Protein profiles of *Legionella pneumophila* Philadelphia-1 grown in macrophages and characterization of a gene encoding a novel 24kDa *Legionella* protein. Microb. Pathog. **15:**469–484.
26. **O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell.** 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell **12:**1133–1142.
27. **Ohta, T., K. Saito, M. Kuroda, K. Honda, H. Hirata, and H. Hayashi.** 1994. Molecular cloning of two new heat shock genes related to the *hsp70* genes in *Staphylococcus aureus*. J. Bacteriol. **176:**4779–4783.
28. **Papp-Szabò, E., M. Firtel, and P. D. Josephy.** 1994. Comparison of the sensitivities of *Salmonella typhimurium oxyR* and *katG* mutants to killing by human neutrophils. Infect. Immun. **62:**2662–2668.
29. **Pearlman, E., A. H. Jiwa, N. C. Engleberg, and B. I. Eisenstein.** 1988. Growth of *Legionella pneumophila* in a human macrophage-like (U937) cell line. Microb. Pathog. **5:**87–95.
30. **Portnoy, D. A., P. S. Jacks, and D. J. Hinrichs.** 1988. Role of hemolysin for intracellular growth of *Listeria monocytogenes*. J. Exp. Med. **167:**1459–1471.
31. **Portnoy, D. A., T. Chakraborty, W. Goebel, and P. Cossart.** 1992. Molecular determinants of *Listeria monocytogenes* pathogenesis. Infect. Immun. **60:**1263–1267.
32. **Ralph, P., J. Prichard, and M. Cohn.** 1975. Reticulum cell sarcoma: an effector cell in antibody-dependent cell-mediated immunity. J. Immunol. **114:**898–905.
33. **Raveneau, J., C. Geoffroy, J.-L. Beretti, J.-L. Gaillard, J. E. Alouf, and P. Berche.** 1992. Reduced virulence of a *Listeria monocytogenes* phospholipase-deficient mutant obtained by transposon insertion into the zinc metalloprotease gene. Infect. Immun. **60:**916–921.
34. **Schwan, W. R., A. Demuth, M. Kuhn, and W. Goebel.** 1994. Phosphatidylinositol-specific phospholipase C from *Listeria monocytogenes* contributes to intracellular survival and growth of *Listeria innocua*. Infect. Immun. **62:**4795–4803.
35. **Sokolovic, Z., J. Riedel, M. Wuenscher, and W. Goebel.** 1993. Surface-associated, PrfA-regulated proteins of *Listeria monocytogenes* synthesized under stress conditions. Mol. Microbiol. **8:**219–227.
36. **Tilney, L. G., P. S. Connelly, and D. A. Portnoy.** 1990. Actin filament nucleation by the bacterial pathogen, *Listeria monocytogenes*. J. Cell Biol. **111:**2979–2988.
37. **Tilney, L. G., and D. A. Portnoy.** 1989. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. J. Cell Biol. **109:**1597–1608.
38. **Völker, U., S. Engelmann, B. Maul, S. Riethdorf, A. Völker, R. Schmid, H. Mach, and M. Hecker.** 1994. Analysis of the induction of general stress proteins of *Bacillus subtilis*. Microbiology **140:**741–752.
39. **Walkup, L. K. B., and T. Kogoma.** 1989. *Escherichia coli* proteins inducible by oxidative stress mediated by the superoxide radical. J. Bacteriol. **171:**1476–1484.
40. **Yamamoto, T., T. Hanawa, S. Y. Murayama, and S. Ogata.** 1994. Isolation of thermosensitive mutants of *Yersinia enterocolitica* by transposon insertion. Plasmid **32:**238–243.
41. **Yamamoto, T., T. Hanawa, and S. Ogata.** 1994. Induction of *Yersinia enterocolitica* stress proteins by phagocytosis with macrophage. Microbiol. Immunol. **38:**295–300.
42. **Zuber, U., and W. Schumann.** 1994. CIRCE, a novel heat shock element involved in regulation of heat shock operon *dnaK* of *Bacillus subtilis*. J. Bacteriol. **176:**1359–1363.

Editor: A. O'Brien