Listeria monocytogenes: Clinical and Experimental Update

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Listeria monocytogenes, a small gram-positive bacillus, causes sepsis and meningitis in immunocompromised patients and a devastating maternal/fetal infection in pregnant women. Recent outbreaks demonstrated that L. monocytogenes can cause gastroenteritis in otherwise healthy individuals and more severe invasive disease in immunocompromised patients. Centralized processing in the food industry may be the cause of these large-scale listeriosis outbreaks. The mouse model of listeriosis, which was developed in the 1960s, has been extraordinarily useful for studying T cell–mediated immunity. Contrary to the original concept that macrophages are the principal effector cells in listeriosis, we found that immigrating neutrophils play the predominant role in early liver defenses. At later time points, CD8+ T cells lyse infected hepatocytes by both perforin- and Fas-L/Fas–dependent mechanisms. Of interest, nonclassical major histocompatibility complex (MHC) class Ib–restricted cytolytic activity is expressed early during primary infection, whereas MHC class Ia–restricted activity is predominant through late primary and secondary infections.

Listeria monocytogenes historically has been one of the most important but least recognized microorganisms transmitted by the foodborne route. The bacterium has recently received increasing attention due to large-scale outbreaks associated with contaminated food products. In addition, since its development in the 1960s, experimental listeriosis in mice has served as a prototypic model of infection by intracellular pathogens. The information derived from this model is fundamental to our understanding of the factors that contribute to cell-mediated immunity. This review highlights some of the changes in our understanding of both clinical listeriosis and host defenses to L. monocytogenes.

Clinical Listeriosis

L. monocytogenes is a non-sporulating, gram-positive bacillus that grows on blood agar and produces incomplete β-hemolysis. When cultured at 25°C, it has a characteristic tumbling motion produced by polar flagellae [1]. The microorganism is ubiquitous in soil and water and can be found in the gastrointestinal tracts of animals and up to 5% of healthy human adults.

Two unusual features of this bacterium are its ability to multiply between 1°C and 45°C and to replicate at relatively high salt concentrations. L. monocytogenes can grow at refrigeration temperatures in foods that are preserved in brine, such as soft cheeses. These growth characteristics were illustrated by one of the most devastating outbreaks of listeriosis caused by the ingestion of contaminated Mexican-style soft cheese in Los Angeles in 1985 [2]. L. monocytogenes can be found in a wide variety of foods, including processed meats (e.g., hot dogs), fresh meats and fish from delicatessen counters or supermarkets, raw vegetables, apple cider, and dairy products, including cheeses, butter, and milk.

Clinically, L. monocytogenes causes sepsis and meningitis in immunocompromised individuals (e.g., transplant patients, infants and elderly patients receiving chemotherapy, individuals with diabetes or liver disease, and patients with AIDS). About 25% of the cases of invasive listeriosis occur in pregnant women.

We recently examined the occurrence of listeriosis during pregnancy over a 10-year period in 4 hospitals located in Rhode Island and Massachusetts and reviewed the literature from 1980–2000 (unpublished data; presented at the Infectious Diseases Society of America, 2000). As previously reported, maternal listeriosis resulted in a nonspecific febrile illness that rarely was diagnosed prepartum [2a]. In our series (11 episodes) and in the published reports (180 episodes), 65% of patients had fever, 32% had a “influenza-like” syndrome, 21.5% had back pain, and 10.5% had headache. Of note, 29% had no symptoms at all. Serious illness rarely was noted in any of the pregnant women. Infection, however, often resulted in spontaneous abortion, stillbirth, death of the newborn within hours after birth, or neonatal sepsis. L. monocytogenes has a striking predilection for the placenta and fetus that can result in granulomatosis infantisepica, a devastating illness characterized by microabscesses and granulomas in the liver and spleen. The prognosis is the worst in early pregnancy. Of the surviving infants born to pregnant women with listeriosis, about two-thirds develop neonatal sepsis.

Although long suspected, food was not strongly implicated in the transmission of L. monocytogenes until a 1981 report described an outbreak in the Maritime provinces of Canada [3] (table 1). The outbreak affected 41 individuals and had a 27%
mortality rate. A case-control study identified coleslaw as the vehicle responsible for the outbreak. *L. monocytogenes* serotype 4b, the outbreak strain, was cultured from coleslaw found in a patient’s refrigerator. The coleslaw had been produced in a regional food-processing plant, but no obvious source for contamination at the plant was found. However, *L. monocytogenes* 4b was cultured from an unopened package of coleslaw obtained from the plant. A review of the sources of cabbages for the plant identified a farmer who also raised sheep. Two sheep at the farm had died of listeriosis, and the farmer had used raw manure from the flock to fertilize the fields where cabbage was grown. The harvested cabbages were then stored in a large cold shed under conditions that would allow *L. monocytogenes* to multiply. The investigators concluded that the outbreak resulted from direct contamination of the cabbages used to make coleslaw by growth in manure derived from infected sheep. This remarkable investigation strongly implicated contaminated food in an outbreak of listeriosis.

Other outbreaks, including those in Massachusetts and Los Angeles relating to pasteurized milk and Mexican-style soft cheese, respectively, confirmed the risk of ingesting contaminated foods [2, 6] (table 1). In each of these outbreaks, listeriosis caused invasive disease with mortality rates that averaged ~30%. These outbreaks illustrate the severe nature of listeriosis, particularly in immunocompromised patients, and highlight the differences between infections caused by *L. monocytogenes* and those caused by other common foodborne pathogens (e.g., *Salmonella* species, *Campylobacter jejuni*, *Vibrio* species, and *Shigella* species). The US Department of Health and Human Services and the Department of Agriculture recently projected there would be 2500 serious cases of listeriosis, with 500 deaths, in the United States each year [7]. By comparison, the Centers for Disease Control and Prevention reviewed 13,056 reported cases of *Salmonella enteritidis* infections in the United States between 1985 and 1991 and found that there were 50 deaths and a mortality rate of 0.38% [8]. Similarly, mortality rates for *Campylobacter* species infections ranged from 0.02% to 0.1%, and rates for *Vibrio* species ranged from 0.005 to 0.01% [9].

It recently has become evident that *L. monocytogenes* can cause a relatively benign gastroenteritis, a common manifestation of point-source outbreaks. One of the first documented outbreaks of gastroenteritis occurred among individuals attending a picnic at a Holstein cow show in Elizabeth, Illinois, in July 1994 (table 1) [8]. *L. monocytogenes* infected 45 healthy adults who drank chocolate milk that was heavily contaminated after pasteurization. Symptoms were those of a self-limited, febrile, diarrheal syndrome; there were no deaths. More recently, an outbreak was reported from Italy in which a cold corn salad served in school cafeterias was implicated as the vehicle contaminated with *L. monocytogenes* (table 1) [9]. At least 93 adults and 1473 children were symptomatic. Diarrhea was reported by 53% of the adults and 40% of the children. Headache, abdominal pain, and nausea were prominent features. Nineteen percent of the patients were admitted to the hospital. There were no cases of sepsis and no deaths.

In the past few years, there has been a striking increase in the number of listeriosis cases related to contaminated processed foods. Some of these contaminated food products have resulted in widespread outbreaks of listeriosis. Because stools are not typically examined for the presence of *L. monocytogenes*, many additional outbreaks may not have been identified. In December 1998, an outbreak of invasive listeriosis was identified in which *L. monocytogenes* was spread by hot dogs and processed meats produced by Bel Mar Foods, a subsidiary of Sara Lee Foods [10]. Over 100 people became ill during the outbreak, and 20 died, and 35 million pounds of meat were recalled. Eight other episodes of contamination occurred in 1999; all resulted in major recalls of processed foods. Subsequently, a multistate outbreak of listeriosis occurred from May to November 2000 and was attributed to delicatessen turkey meat [11]. Twenty-nine cases with 4 deaths and 3 miscarriages/stillbirths were reported in 10 states. Pulsed-field gel electrophoresis and ribotyping determined that the *L. monocytogenes* isolates were identical. Epidemiologic investigation implicated a meat-processing plant in Waco, Texas.

With consolidation of the food industry and centralized food processing, the risk of contamination with *L. monocytogenes* has increased despite major efforts by the Food and Drug Administration and the food industry. Until adequate methods for sterilizing processed foods are implemented, the risk will continue.

**Experimental Model of Listeriosis**

In 1962, George Mackaness first described an experimental model of listeriosis in mice [12]. Mackaness was attempting to develop a model of immunity relevant to tuberculosis, a noto-
riously difficult microorganism to study experimentally. Since its inception, murine listeriosis has proven to be one of the most useful experimental models in history for defining mechanisms that underlie immunity and host defenses to infectious diseases. Fundamental concepts, such as macrophage activation, the role of CD4+ and CD8+ T cells, major histocompatibility (MHC) restriction, adoptive transfer of T cell–mediated immunity, and the function of cytokines, were derived from or expanded in this model. Furthermore, the virulence factors expressed by L. monocytogenes, including those that allow attachment and entry into a cell, escape from phagolysosomes, movement within the cytoplasm, and infection of adjacent cells, were first described in this model.

According to the Mackaness model, bacteria inoculated intravenously are cleared rapidly from the bloodstream by the liver [12]. Sixty to ninety percent of the bacteria are killed within the first 6–12 h of infection. Multiplication subsequently occurs during the next 48–72 h before ultimate sterilization of the liver begins. Most bacteria taken up in the liver were believed to be ingested and killed by resident macrophages (i.e., Kupffer’s cells). After the initial 12 h, it was assumed that the surviving microorganisms multiplied intracellularly within macrophages that exhibited a low antimicrobial potential. It was postulated that such cells were activated eventually by cytokines (originally termed macrophage activation factor and macrophage inhibition factor) produced by sensitized T cells. Humoral immunity was believed to play no role.

This model has proven to be inaccurate in several ways. First, a variety of cell types in addition to activated T cells produce cytokines that are essential for early host defenses. Using SCID mice that lack normal T cell function, Bancroft and co-workers [13–16] demonstrated the importance of cytokines produced in the absence of T cells (i.e., interferon [IFN]-γ, interleukin [IL]-1, tumor necrosis factor [TNF]-α, and IL-12) to innate host defenses to L. monocytogenes. More recently, Edelson and colleagues [17, 18] demonstrated that monoclonal antibody specific for listeriolysin O (LLO), a secreted pore-forming protein produced by L. monocytogenes, provided protection against lethal listerial infections. Humoral immunity, therefore, may play a significant role in host defenses. Last, one of the basic assumptions of the Mackaness model was that Kupffer’s cells phagocytosed and killed the bulk of microorganisms first taken up by the liver. Using techniques to isolate and analyze Kupffer’s cells and to eliminate either Kupffer’s cells or neutrophils, we showed that Kupffer’s cells do not phagocytose most of the inoculum in the liver [19, 20]. Rather, according to our model (figure 1), L. monocytogenes cleared by the liver adhere to the Kupffer’s cell surface [21].

The factors that mediate attachment and/or entrance of L. monocytogenes into phagocytes and epithelial cells are a matter of active investigation. It is known that entry of L. monocytogenes into a variety of cells is facilitated by interaction of the bacteria with cell surface E cadherin, heparan sulfate proteoglycan receptors, and type I macrophage scavenger receptors [22–24]. Recently, Lecuit et al. [25] elucidated the factors that facilitate entry of L. monocytogenes into epithelial cells. They showed that entrance into human epithelial cells is mediated by the interaction of internalin expressed by the organism and E cadherin; however, a specific amino acid substitution in murine E cadherin rendered E cadherin irrelevant in the uptake of L. monocytogenes by mouse cells. This substitution may account for the diminished capacity of murine Kupffer’s cells to internalize L. monocytogenes. Indeed, we reported that internalin did not affect listerial infection of hepatic cells in vivo [26]. A second receptor recently shown to be a factor in listerial infections is the type I/II macrophage scavenger receptor expressed by macrophages, including Kupffer’s cells [24, 27–30]. It was reported that this receptor is important in both uptake of L. monocytogenes and prevention of the organism from lysing the phagolysosome and entering the cytoplasm.

Figure 1. Bacteria cleared from the bloodstream by the liver are bound extracellularly by Kupffer’s cells and killed by immigrating neutrophils. I, L. monocytogenes bound to the surface of Kupffer’s cells induce CD54 expression and cytokine production. II. The interaction of complementary adhesion molecules (CD54 and CD11b/CD18) promotes the accumulation of neutrophils at the site of L. monocytogenes deposition in the liver. III. Neutrophils infiltrating the liver kill extracellular bacteria and secrete soluble proinflammatory factors. TNF, tumor necrosis factor; IL, interleukin; MIP, macrophage inflammatory protein.
The proinflammatory response induced by *L. monocytogenes* in the liver may be initiated by the interaction of *L. monocytogenes* with receptors (e.g., Toll-like receptors [TLRs], expressed on the surface of Kupffer’s cells). TLRs, described originally in *Drosophila* species, are important in embryonic development and in the immune response of adult flies [31]. In humans and mice, TLRs play a significant role in both innate immunity and the regulation of specific immune responses. TLR4s in mice, for example, govern the response of cells to lipopolysaccharide [32]. A loss-of-function mutation in the *tlr4* gene accounts for the hyporeactivity of the C3H/HeJ and C57BL/10ScCr mouse strains to lipopolysaccharide and renders these strains highly susceptible to infections by gram-negative organisms [33]. TLR2, on the other hand, mediates the response to peptidoglycan and lipoproteins [34]. Human TLR2 promotes monocyte activation by *L. monocytogenes* [35].

The interactions of bacterial products with TLRs induce signal transduction pathways that lead to the activation of transcription factors (e.g., NF-κB) [36]. NF-κB activation in turn promotes the production of proinflammatory cytokines. Consequently, the interaction of *L. monocytogenes* with TLRs expressed by Kupffer’s cells may induce the production of cytokines involved in innate host defenses (e.g., IL-1, IL-6, and TNF-α). *L. monocytogenes* can also up-regulate the expression of intercellular adhesion molecule 1, an integrin-binding protein expressed on the surface of a variety of cell types [37]. These events promote the accumulation of neutrophils, the principal microbicidal cell population in the liver during the first 24–48 h of infection. In addition to killing the bulk of *L. monocytogenes* during the initial hours of infection, neutrophils can produce cytokines (IL-1, IL-6, TNF-α), chemokines (macrophage inflammatory protein [MIP]-1α, MIP-1β, and MIP-2), and other soluble factors (leukotriene B4) [38–40]. Thus, the interaction of Kupffer’s cells with *L. monocytogenes* and neutrophils is the critical event during the first 6–12 h after injection.

A fraction of the *L. monocytogenes* taken up by the liver escapes the antimicrobial activity of neutrophils by entering hepatocytes and multiplying intracellularly. *L. monocytogenes* has a remarkable set of virulence factors that enables it to enter cells, multiply, and infect other cells [41, 42]. Once inside cells, the bacterium, via the action of a pore-forming molecule, LLO, escapes from phagosomes into the cytoplasm, where replication occurs. Mutants lacking LLO are avirulent and remain within the phagosomes. In the cytoplasm, a protein encoded by the *actA* gene and expressed on the surface of *L. monocytogenes* promotes the polymerization of host cell actin, which forms a “halo” around each organism. As actin polymerization becomes polarized, producing what appears to be a “comet tail” in electron micrographs, the bacterium is propelled through the cytoplasm by a platform of stabilized polymerizing actin. The bacterium moves with surprising speed and eventually pushes against the cell membrane, creating a filopodium containing the microorganism. The filopodium abutting a second cell is then phagocyted. Inside the adjacent cell, the bacterium dissolves the surrounding double membrane, using LLO and phospholipases, and the cycle is repeated. These virulence factors enable *L. monocytogenes* to escape normal host defenses and suggest why the microorganism poses a particular threat to immunocompromised individuals.

Experimental evidence indicates that T cells play a major role in disrupting the cycle of intracellular listerial replication. While CD4+ T cells may have some importance, experiments using MHC class I– and MHC class II–deficient mice demonstrated that CD8+ T cells are the principal T cell effector critical for host defenses to *L. monocytogenes* [43, 44]. A potential mechanism for CD8+ T cell function is IFN-γ production. Adoptive transfer experiments using CD8+ T cells derived from IFN-γ–deficient mice, however, demonstrated that IFN-γ production by CD8+ T cells is not the sole effector mechanism [45, 46].

Immune CD8+ T cells lyse *L. monocytogenes*–infected host cells in vitro; this is a likely mechanism underlying CD8+ T cell activity in vivo [44, 47, 48]. CD8+ T cells exhibit two major cytolytic mechanisms [49]. One is dependent upon perforin, a protein found in T cell granules. Upon T cell activation, perforin is released as a monomer, inserts into the target cell membrane, and polymerizes to form pores that allow enzymes (serine proteases collectively called granzymes) to enter and initiate apoptosis [50, 51].

A second mechanism is dependent upon the interaction of Fas ligand (FasL, CD95L) with Fas (CD95). Fas is a transmembrane protein that belongs to the TNF/hnerve growth factor receptor superfamily [52]. FasL, expressed by activated T cells, can induce apoptosis in target cells upon interacting with Fas [53]. Kagi et al. [54] showed that clearance of *L. monocytogenes* from the spleen was delayed during primary infection of perforin knockout (PKO) mice. In addition, the secondary immune response was markedly reduced, and CD8+ T cells from PKO donor mice poorly conferred immunity in adoptive-transfer experiments.

Using perforin- and Fas-deficient mice, we determined that resistance to primary infection depended upon both mechanisms; animals deficient in perforin and Fas were highly susceptible to listeriosis [48]. Each mechanism appeared to exert a greater effect at different times during infection. This observation is supported by in vitro studies indicating that perforin-mediated mechanisms are more important early during primary infection, whereas Fas-L/Fas–mediated mechanisms appear more important late during primary infection [48] (unpublished observations). In contrast, the lytic activity expressed by CD8+ T cells obtained following secondary infection and co-cultured with *L. monocytogenes*–infected hepatocytes was wholly dependent upon perforin. Recent studies demonstrating the capacity of *L. monocytogenes*–specific T cell clones derived from PKO mice to conferred immunity in a Fas- and INF-γ–independent but TNF-α–dependent manner support the existence of additional CD8+ T cell–mediated protective mechanisms that remain to be delineated [55].
CD8+ T cells recognize “foreign” peptide sequences expressed on target cells in association with classical, MHC class Ia molecules encoded by three loci in mice: H-2K, H-2D, and H-2L. The highly polymorphic nature of these loci accounts for the frequent failure of MHC class Ia–restricted T cells derived from one mouse strain to recognize target cells from another strain. To date, investigators have identified four L. monocytogenes–related peptides that are presented by H2-Kd molecules and recognized by CD8+ T cells [56–58]. Flow cytometric analysis of CD8+ T cells stained with phycoerythrin-conjugated peptide-H2-Kd tetrameric complexes demonstrated that amino acid residues 91–99 of LLO (LLO91–99) is a dominant epitope, inducing the largest expansion of CD8+ T cells in BALB/c mice infected with L. monocytogenes [59, 60]. The other three peptides, two derived from p60 (a murine hydrolase) and a third derived from a secreted metalloprotease, induced lesser responses. Analyses of the CD8+ T cells obtained from mice following secondary challenge revealed a similar pattern (i.e., the greatest expansion of the T cell subset specific for LLO91–99).

It was once presumed that CD8+ T cell recognition of L. monocytogenes–infected host cells was restricted exclusively by MHC class Ia molecules. During the past few years, several laboratories, including our own, have reported that nonclassical, MHC class Ib–restricted T cell responses to L. monocytogenes also occur [61–64]. While more numerous than the classical MHC class I genes, the MHC class Ib genes are much less polymorphic and, thus, more likely to encode molecules shared by histoincompatible mouse strains. To date, the MHC class Ib (H2–M3)–restricted CD8+ T cell responses to three N-formylated peptides (f-MIVIL, f-MIGWII, and f-MIVTLF) have been identified in L. monocytogenes–infected mice [65]. Flow cytometric analyses of cells stained with phycoerythrin-conjugated peptide/MHC class I tetrameric complexes indicate that expansion of these H2–M3–restricted CD8+ T cell populations occur earlier during primary listerial infections than do the MHC class Ia–restricted populations [66].

In contrast to MHC class Ia–restricted CD8+ T cells, however, only minimal expansion of the MHC class Ib–restricted populations occurs during secondary infection. We similarly found that CD8+ T cells derived from mice early during primary listerial infection (i.e., on day 6) exhibited MHC class Ib– and Ia–restricted cytolytic activity in vitro [44, 47, 64] (unpublished observation). The lytic activity expressed by CD8+ T cells obtained late during primary infection (on day 11) or following secondary challenge,

![Figure 2](image-url)

**Figure 2.** CD8+ T cell–mediated lysis of L. monocytogenes–infected hepatocytes. *Early primary infection,* major histocompatibility (MHC) class Ia– and Ib–restricted CD8+ T cells recognize L. monocytogenes–infected hepatocytes and induce apoptosis dependent upon perforin and granzymes. *Late primary infection,* MHC class Ia–restricted CD8+ T cell–mediated, FasL (CD95L)-dependent lysis of L. monocytogenes–infected hepatocytes. *Secondary infection,* MHC class Ia–restricted CD8+ T cells induce apoptosis in infected hepatocytes by a perforin-dependent mechanism. Active and inactive FasL molecules expressed on the surface of CD8+ T cells are depicted as trimers and monomers, respectively.
however, was restricted exclusively by MHC class I molecules. In accordance with these findings, Bouwer et al. [67] reported more recently that the number of MHC class Ib–restricted CD8+ T cells quantified by ELISPOT analysis of IFN-γ production increased significantly during primary infection but not during secondary challenge with *L. monocytogenes*. Thus, a small MHC class Ib–restricted T cell population capable of cytokine production but not cytolytic activity appears to persist in mice following recovery from a primary listerial infection.

It is relevant to note that the CD8+ T cell populations that accumulate at different tissue sites differ significantly in terms of their T cell receptor repertoire. Recently, for example, Huleatt et al. [68] reported that the frequency of the MHC class Ia–restricted T cell response to *L. monocytogenes* in the lamina propria was 4- to 5-fold greater than in the spleen, while the frequency of the MHC class Ib (H2–M3)–restricted response was diminished. A relatively high frequency of LLO-specific, MHC class Ia–restricted T cells remained in the lamina propria on day 30 following oral infection, suggesting that the lamina propria serves as an important repository for *L. monocytogenes*–specific memory CD8+ T cells, providing long-term protection against reinfection by this foodborne pathogen.

Figure 2 illustrates our concept of CD8+ T cell function in terms of both lytic mechanisms and MHC restriction expressed at various times during infection of the liver. Early during primary infections, MHC class Ia–restricted CD8+ T cells and MHC class Ib–restricted CD8+ T cells induce apoptosis in infected hepatocytes by a perforin-mediated mechanism. Late during primary infections, the cytolytic activity is limited to MHC class Ia–restricted CD8+ T cells; MHC class Ib–restricted T cells, however, may continue to elaborate cytokines. MHC class Ia–restricted CD8+ T cells induce perforin-dependent cytolytic activity during secondary infections.

*L. monocytogenes* is a remarkable bacterium. It continues to cause significant morbidity and mortality in immunocompromised patients and in recent years has become an increasing threat as a foodborne pathogen. Equally important, for the past 40 years, experimental listeriosis has provided startling insights into the mechanisms that underlie cell-mediated host defenses.

References