FRI BRIEFINGS

Virulence Characteristics of *Listeria monocytogenes*

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INTRODUCTION

Listeria monocytogenes has again become a major public health concern. After a series of high profile outbreaks in the 1980s some steps were taken to reduce contamination of food, and it appeared that this pathogen was under control. But a spate of recent outbreaks and recalls have demonstrated that this bacterium still poses a significant health risk. This is partly due to *L. monocytogenes* widespread occurrence in nature and to its ability to grow at refrigeration temperatures. The virulent nature of listeriosis, with a fatality rate of 20% or more, has particularly attracted the attention of the public and food safety professionals. Approximately 76,000,000 cases of foodborne illness are estimated to occur in the U.S.A. each year, and of these about 2,500 (<1%) are caused by *L. monocytogenes*. Nevertheless, listeriae are responsible for approximately 27.6% of the total deaths attributed to foodborne illness. In comparison, foodborne salmonellosis has a fatality rate of only 0.04%. Even though salmonellae account for more than 500 times as many cases of foodborne illness as *L. monocytogenes*, they cause only about 54 more deaths per year (*69*).

A significant proportion of the population, estimated at 3–10%, harbor listeriae in their gastrointestinal tract without showing any signs of illness (*91*). *Listeria monocytogenes* occasionally causes outbreaks with symptoms usually associated with foodborne illness such as nausea and non-bloody diarrhea. Several foods, including corn, chocolate milk, shrimp, and rice salad, have been reported as vehicles (*93*). In a 1994 outbreak in Illinois, 45 people became sick after consuming chocolate milk containing a high concentration of *L. monocytogenes* serotype 1/2b ($8.8 \times 10^8 - 1.2 \times 10^9$ cfu per ml). None of the victims had an underlying chronic disease or immune deficiency, and the one pregnant woman affected delivered a healthy baby 5 days later (*20*).

More commonly, however, *L. monocytogenes* causes no evident gastrointestinal lesions or symptoms but rather makes its presence known by severe, life-threatening symptoms involving the central nervous system or the unborn fetus of a pregnant woman. Pregnant women with listeriosis may experience relatively mild flu-like symptoms themselves. However, *L. monocytogenes* can spread within their bodies and readily cross the placenta to infect the

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unborn child. Abscesses develop in the liver, lungs and other fetal organs, and frequently the result is spontaneous abortion or stillbirth. If the baby survives birth, it may be seriously ill with meningitis. Meningitis is also common in adult victims of listeriosis.

Most, but not all, serious cases of listeriosis occur in people who are pregnant, elderly, or have some underlying disease that depresses their immune function. Statistics from a number of studies demonstrate that healthy people are less likely to get listeriosis and more likely to recover if they do contract this disease. Of 820 patients with listerial infection of the central nervous system in the U.S.A. and England, a majority had a recognized underlying health problem. But a significant number (36%) were apparently quite healthy before becoming infected with *L. monocytogenes* (*72*). Of 74 cases reported in Finland, 49 patients had an underlying serious disease (cancer, diabetes, or kidney transplant), 14 were pregnant, and 11 were non-pregnant and previously healthy. Although all patients were seriously ill with septicemia or meningitis, all of the previously healthy, non-neonatal patients survived; in contrast, only 68% of the patients with compromised immune systems survived (*97*). A high fatality rate was also observed for previously ill persons among a group of 225 cases of listeriosis in France; no deaths occurred among those who were <65 years old and previously healthy (*36*).

Several reviews and a recent book provide much more information on *L. monocytogenes* and its characteristics, its presence in foods, and the development of listeriosis in susceptible individuals (*29,65,91,93,102*). A draft assessment of the risk to public health from foodborne *L. monocytogenes* in some ready-to-eat foods has recently been published by FDA/CFSAN (*8*).

Why is *L. monocytogenes* such a dangerous pathogen? That is the question addressed in this report. The answer requires reviewing data on the bacterium itself – how it causes infection and escapes destruction by the immune system. Different strains of *L. monocytogenes* may vary in their ability to cause disease. In addition, there is information on the immunological defense system of the body and how, in some people, it may malfunction or be inadequate to stem the spread of listeriae through the blood stream and into the central nervous system.

VIRULENCE OF DIFFERENT STRAINS

Despite the abundance of *L. monocytogenes* in the environment, relatively few people become seriously ill. This is certainly the result of the efficient immune response mounted by most healthy people. But, is it also true that many of the listeriae in the environment are avirulent or only weakly virulent and that only a few serotypes are responsible for most of the serious infections that occur? An analysis of the serovars of *L. monocytogenes* isolated from 1363 patients demonstrated that serovar 4b was the most common, being present in 64% of cases, whereas serovars 1/2a, 1/2b, and 1/2c were detected in 15%, 10%, and 4% of cases, respectively. Serovar 4b was more common in pregnancy-associated cases, and serovar 1/2b occurred more often in non-pregnant individuals with severe underlying disease (*68*). However, when tested in pregnant mice, infectivity of serovars 1/2a and 1/2b did not differ significantly from 4b (*58*).

In another set of experiments, several strains of *L. monocytogenes* belonging to serogroup 4 were compared for their virulence to mice and also for their production of some virulence factors. Strains 4b, 4ab, and 1/2a were most virulent to mice and to Caco-2 cells in culture. The lower virulence observed in strains 4a, 4c, 4d, and 4e correlated with lower levels of production of the virulence factor, ActA protein, and also with actin tail formation. Some strains also produced relatively low levels of listeriolysin O and internalin (*101*).

Analysis of variations in listerial strains by use of RAPD (random amplification of polymorphic DNA) revealed that only a few genotypes of *L. monocytogenes* were common among isolates from cases of human listeriosis in Japan (*45*). Other analytical techniques which examined DNA sequences of some listerial virulence genes indicated that there are at least three evolutionary lineages of

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L. monocytogenes (*84,107*). All the tested isolates from human outbreaks and some isolates from sporadic cases belonged to lineage I, and no human isolates were part of lineage III (*107*). Using this type of DNA sequence data, 117 *L. monocytogenes* isolates from smoked fish and smoke-processing facilities were analyzed and only 37% belonged to lineage I. Of the strains tested in cytotoxicity assays, 17% exhibited an avirulent or weakly virulent activity (*75*).

Listeria monocytogenes strains involved in invasive and noninvasive listeriosis outbreaks have also been characterized by PCR fingerprinting techniques. Differences were detected among the strains, suggesting that there may be differences in their DNA sequences and possibly in the expression of different virulence factors (*31*).

These results suggest that not all *L. monocytogenes* found in the environment (or in food) present the same threat to human health. However, there is at present no easy way to distinguish which strains might pose a health risk. A final caveat should also be noted: *L. monocytogenes* exposed to mildly acidic conditions develops acid tolerance and also become better able to invade and proliferate in cultured cells than non-acid-tolerant bacteria (*15*). Other environmental factors may also increase or decrease the virulence of *L. monocytogenes* serovars.

INVASION AND SPREAD OF *L. MONOCYTOGENES*

Overview

The reason that *L. monocytogenes* causes severe illness can be traced to its ability to induce its own phagocytosis (uptake) by host cells, followed by replication within those cells and direct transfer to another cell. Since *L. monocytogenes* remains within host cells, it spreads through the body while protected from many host defenses, including antibodies and complement. Listeriae ingested with food are taken up by enterocytes or M cells near Peyer's patches in the small intestinal lining and then multiply in underlying phagocytic cells. Bacteria are carried from the intestine in macrophage cells in blood or lymph to the liver and spleen where most of them are killed by neutrophils acting with Küpffer cells. If the host's T cell–mediated immune response is inadequate, listeriae multiply in hepatocytes and macrophages and are carried in the blood to various organs, particularly the brain and/or uterus where they penetrate the blood–brain barrier and the placental barrier. An intricate series of virulence factors are produced by *L. monocytogenes* to facilitate each step of this invasion process. The genes coding for many of these factors are clustered together on the chromosome and regulated by the *prfA* gene (*16,56,87*).

Virulence factors

Internalins

Listeria monocytogenes is taken into host cells by a process of phagocytosis. Some cells, such as macrophages, are "professional" phagocytic cells which normally engulf bacteria and dying cells, while other epithelial and endothelial cells are "nonprofessional" phagocytes. These latter cells do not normally phagocytize other cells, but they can be induced to do so. Internalin A was first identified as a listerial surface protein that is required for the penetration of *L. monocytogenes* into non-phagocytic cells, such as epithelial cells (*33*). A related protein, internalin B, plays a role in invasion of hepatocytes in the liver (*6,25,33,37,80*). There is still some controversy in the literature as to whether one or the other of these proteins is more important in the invasion of different cell types and whether either is necessary for entry into macrophages. Internalin A on the surface of listerial cells binds to a surface protein, E-cadherin, on the surface of host epithelial cells. This interaction apparently stimulates the phagocytosis of *L. monocytogenes* cells (*56*).

Surface protein p104

In addition to the internalins, another surface protein on *L. monocytogenes*, p104, has recently been identified and shown to play a role in adhesion to intestinal cells (*79*).

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Listeriolysin O

As listeriae are engulfed, they are enclosed within a vacuole that is surrounded by a membrane. Professional phagocytic cells begin almost immediately to kill the listeriae within the vacuoles, and survival of *L. monocytogenes* depends on escaping from the vacuole. Listeriolysin O (LLO), a bacterial pore-forming toxin, is essential for lysing the vacuolar membrane and allowing *L. monocytogenes* to escape into the cytoplasm of the cell. LLO is necessary for establishing infection in mice, and its activity is enhanced by the acidic pH in the vacuole (*4,11,17,67,88*). Mutant *L. monocytogenes* that do not produce listeriolysin can survive within vacuoles of non-professional phagocytes for a while, but they do not multiply and go on to infect other cells because they cannot escape from the vacuoles.

If LLO lyses the membrane surrounding the vacuole, why does it not also attack the cell membrane, causing destruction of the cell and release of listeriae from the cell? This would be dangerous for *L. monocytogenes* because the bacteria would no longer be protected within the host cell from attack by antibodies. Analysis of the LLO molecule revealed that it contains a series of 27 amino acids at one end and that this sequence is very similar to PEST sequences often found on proteins in humans and other animals. In these organisms, the PEST sequence is a starting place for protein–protein interactions and, as such, often indicates proteins slated for degradation. It appears that once LLO has done its job of perforating the vacuolar membrane, it is then recognized by enzymes in the cytoplasm of the cells and is destroyed before it can damage the cell membrane (*23*).

In addition to its pore-forming function, LLO participates in other reactions related to pathogenesis of *L. monocytogenes*. Infection of murine spleen and bone marrow dendritic cells by *L. monocytogenes* results in cell death by apoptosis. Mutant bacteria that do not produce LLO do not induce apoptosis whereas purified LLO can induce this programmed cell death (*40*). LLO also can act as an inflammatory stimulus by inducing endothelial cell activation (*26,50*) and neutrophil activation (*96*).

ActA protein

Once *L. monocytogenes* has escaped from the vacuole into the cytoplasm, it replicates. In order for these bacteria to move directly to another cell, a surface protein, ActA, induces polymerization of globular actin molecules to form polarized actin filaments. Bacterial cells move along these filaments to the cell membrane and cause portions of the membrane to bulge outwards, forming structures called listeriopods (*54,71*). These protuberances are engulfed by adjacent cells, thereby allowing dissemination of *L. monocytogenes* without exposure to antibodies or other immunoactive molecules. Other cellular factors are believed to be important in this actin-based motility, but they have not been well studied. ActA may also facilitate uptake of *L. monocytogenes* cells that do not produce internalins into certain types of cells (*54,56*).

Phospholipases

Two distinct phospholipases C synthesized by *L. monocytogenes* are phosphatidylinositol-specific phospholipase C (PI-PLC) and a broad-range or phosphatidylcholine-specific phospholipase C (PC-PLC). Both apparently play a role in the invasion and spread of *L. monocytogenes* because bacteria with mutations in the genes coding for these enzymes are less virulent to mice than wild-type bacteria. Experiments with strains mutant for one or both phospholipase encoding genes demonstrate that PI-PLC aids in escape from the primary vacuole while PC-PLC is active during cell-to-cell spread of bacteria (*98*). PC-PLC damages vacuolar membranes in some cell types, such as epithelial cells, and can therefore be a substitute for LLO (*63*). PC-PLC also functions in the cell-to-cell spread of listeriae in the brain during cerebral listeriosis (*94*).

Metalloprotease

PI-PLC is synthesized in an active form whereas PC-PLC is produced as an inactive precursor. A bacterial zinc-dependant metalloprotease and a host cell cysteine protease are required to cleave off part of the precursor and activate the phospholipase (*64*).

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Clp proteases and ATPases

In order to adapt to adverse environmental conditions (high or low pH, temperature, osmotic conditions), many bacteria have chaperone proteins that assist in the proper refolding of proteins or assembly of protein subunits and proteases which process proteins that cannot be altered conformationally. Some of the Clp (caseinolytic proteins) group of proteins, which act both as chaperones and as proteolytic enzymes, have been identified as having a role in pathogenesis of *L. monocytogenes*. ClpC ATPase is a general stress protein that aids in disruption of the vacuolar membrane and the intracellular survival of listeriae (*90*). ClpC also modulates expression of the ActA protein and the internalins, apparently acting at the transcriptional level. Another ATPase, ClpE, also plays a role in listerial pathogenesis (*73*). ClpP serine protease is required for growth under stress conditions and has been shown to affect the activity of listeriolysin O and escape from vacuoles (*34*).

Protein p60

Protein p60 is murein hydrolase enzyme that catalyzes a reaction during the final stage of cell division of *L. monocytogenes*. It is normally present on the cell surface as well as being secreted into the surrounding medium. Analyses of mutations in the gene coding for this protein indicate it is important for phagocytosis of *L. monocytogenes* by some cell types (*56*).

All of these virulence factors participate in specific ways in the infection process, and in addition each may also affect host cell signal transduction in ways that enhance the spread of infection (*56*). Studies with cultures of different cell types have also demonstrated that some of these factors may be more important for infecting some cell types than others. There are still many avenues of research to pursue before we completely understand all the reactions and factors involved in virulence.

Environmental parameters affecting virulence

Growth temperature, pH, and availability of iron have been shown to affect the expression of some virulence factors in *L. monocytogenes*, but some of these in vitro results may not be relevant to human infectivity.

Temperature

Listeriae can survive and grow at low temperatures (4–25°C), but under these conditions listeriolysin O production is reduced or abolished. However, it takes only 2 hours at 37°C for LLO levels to return to normal (*7*,*12*,*21*). Therefore, it is likely that listeriae in refrigerated foods would recover their infectivity during passage through the intestinal tract of warm-blooded animals. Heat stress (56°C for 20 min) decreased pathogenicity of *L. monocytogenes* for mice along with decreasing listeriolysin activity (*66*). Although growth at a higher temperature (42°C) increased expression of *Listeria* adhesion proteins, there was no corresponding increase in virulence because relatively few adhesion molecules are required for attachment and infection (*92*).

Acidity

Although it has been reported that exposure to low pH (4.5–4.9) reduces production of listeriolysin O (*21*) and diminishes invasion of Caco-2 cells in culture (*35*), other reports indicate that acid-adapted listeriae are more capable of infecting Caco-2 and macrophage-like cells and proliferating in them than non-acid-stressed listeriae (*15*). Following incubation at pH 3.5, acid-tolerant *L. monocytogenes* were isolated that were more virulent to mice and more resistant to the influx of neutrophils and macrophages mobilized to protect the mice (*32*,*76*).

Iron

Growth in an iron-rich medium enhanced the invasiveness of *L. monocytogenes* for Caco-2 cells by increasing the expression of internalin genes (*14*). Availability of iron also affects expression of the ActA protein (*13*).

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Invasion process in different tissues

Intestine

Listeria monocytogenes ingested with food must first traverse the intestinal wall before it can cause severe systemic illness characteristic of listeriosis. Experiments with rodents demonstrated that both the normal intestinal flora and the activity of macrophages and neutrophils in the intestine inhibit infection with *L. monocytogenes* (*77*). Large numbers of listeriae (10^8–10^9 cells) are normally required to produce infection in the healthy animal models tested: rats and mice (*18*) and cynomolgus monkeys (*28*). In experiments with a ligated rat ileal loop system, gross intestinal lesions were only observed when very large doses ($\geq 10^9$ listerial cells) were inoculated in a loop (*83*). This is similar to outbreaks of gastroenteritis among healthy people caused by highly contaminated foods, such as the chocolate milk which delivered a median dose of 2.9×10^{11} cfu of *L. monocytogenes* per person in a 1994 outbreak in Illinois (*20*). However, much lower numbers of *L. monocytogenes* were required to cause illness when rats were treated either with an antibiotic to kill the resident intestinal bacteria or with a drug which inhibits T cell activity (*77*). It is apparently also true that a relatively low dose of listeriae can cause listeriosis in immunocompromised people.

Several adhesion molecules, including p60 and p104, and internalins aid in the attachment of *L. monocytogenes* to intestinal cells (*43,79,109*). Peyer's patches in the intestinal lining of rodents are an important point of entry for *L. monocytogenes*. These areas are composed of lymphoid cells overlaid with specialized epithelium containing M cells that actively take up a number of pathogenic organisms including viruses, bacteria, and protozoa. Listeriae multiply rapidly in Peyer's patches and are then carried in dendritic cells or macrophages through the lymphatic system to mesenteric lymph nodes and then via the blood stream to the liver, spleen, placenta, and central nervous system. After an oral dose of *L. monocytogenes* to mice, listeriae were detected in mesenteric lymph nodes after 6 hours and in the liver and spleen after 24 hours (*18,41,62,83*).

In addition to the virulence factors produced by *L. monocytogenes* and the host defense mechanisms (to be discussed later), the other obvious factor that could affect growth and invasiveness of listeriae is the partially digested food present in the intestine. Not much research has been done in this area, but one set of experiments demonstrated that a diet high in milk fat inhibited intestinal colonization of listeriae in rats (*103*). Digestion of fats produces fatty acids and monoglycerides which are known to be potent inhibitors of Gram-positive bacteria. A high milk fat diet did not inhibit growth of *Salmonella enteritidis*, a Gram-negative bacterium. Other food components or characteristics may also alter the pathogenic potential of *L. monocytogenes*.

Nerve cells and brain

Central nervous system infections are one of the most serious manifestations of listeriosis. *L. monocytogenes* may cause meningitis, frequently accompanied by seizures, or rhomboencephalitis, an infection of the brain stem (*102*). In order to infect the central nervous system, listeriae must penetrate the blood–brain barrier, which maintains biochemical homeostasis within the brain and spinal cord. This barrier consists of epithelial cells bound together with tight junctions which prevent the passage of even small molecules like antibiotics (*44,104*). However, *L. monocytogenes* can invade these human brain microvascular endothelial cells (HBMEC) in culture. Once within these cells, bacteria replicate and go on to invade adjacent cells. Listeriae can also invade the microvascular endothelial cells directly from infected macrophages which may have been carried to the brain in the blood stream from the intestine (*37*). Other research using HBMEC cells from a different source indicated that internalin was not required for infection by *L. monocytogenes* (*108*). It appears that in the presence of serum, internalin is not required, but in its absence internalin is necessary for invasion (*27*). Invasion of HBMEC stimulates leukocyte recruitment and inflammatory responses, which leads to tissue damage.

Infection of nerve cells in the brain apparently occurs infrequently but sensory nerve cells in culture were readily invaded by *L. monocytogenes* in an

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internalin B–dependent reaction (*24*). Bacteria were able to move along the axons of these nerve cells in either direction, indicating that dissemination of these bacteria from peripheral sites to the central nervous system along sensory nerves may be a possibility. Data from experiments in which mice were infected at the snout with *L. monocytogenes* revealed that infection traveled along peripheral nerves to the central nervous system (*48*).

Uterus and fetus

Macrophages containing *L. monocytogenes* circulate throughout the body. At the placenta, listeriae from macrophages can infect endothelial cells and then the fetus, thereby precipitating premature labor or death of the fetus. Infants can also be infected during passage through the birth canal and develop sepsis or meningitis several days later. Although the amniotic fluid surrounding the fetus kills or stops the growth of some bacteria, it appears to have no adverse effect on *L. monocytogenes* (*1*). The severity of listeriosis in pregnant mice depends on the stage of pregnancy when infection occurs. Those infected early in pregnancy (prior to day 14) were likely to abort or die of encephalitis whereas those infected later were less likely to become ill (*70*). It appears that the opposite is true of humans: Listeriosis is more common during the third trimester of pregnancy than during the previous 6 months (*100*). However, bacteriological analysis is not usually performed on spontaneously aborted fetuses so the actual incidence of listeriosis in early miscarriages is unknown.

HOST DEFENSES

Animals defend themselves from bacterial attack using three strategies: (1) general, non-specific resistance factors, (2) cell-mediated immunity, and (3) antibody-mediated immunity. Antibody-mediated immunity does not appear to play a significant role in protection from listeriosis. Non-specific host defenses for foodborne pathogens include stomach acidity, which is lethal to many bacteria in food, and the normal gut microflora which are usually so numerous that they occupy all available niches in the intestine and consume essential nutrients, thereby preventing incoming bacteria from getting established. *L. monocytogenes* is known to be a poor competitor; this probably explains why the majority of adults exposed to this bacterium do not become sick (*77*). Infection of epithelial and macrophage cells by *L. monocytogenes* strongly modulates the complex host cell signalling systems affecting the production and activity of a number of non-specific proteins and cells which counteract or support listerial infections (*55,56*). Cell-mediated immunity is probably the main host defense against listeriosis with $CD4^+$ T helper lymphocytes activating macrophages (*19*).

Activity of immune system cells and proteins against *L. monocytogenes* will be described below. The effectiveness of immune reactions in different individuals is modified by genetic and nutritional factors. For example, dietary selenium (*2*) and fatty acids (*22*) have been shown to affect the ability of mice to react to listerial infections. Pregnancy and aging also affect the effectiveness of the immune response as described below.

Neutrophils and macrophages

Evidence indicates that a rapid inflammatory response is a key ingredient in combatting *L. monocytogenes* infections. During early stages of listeriosis, neutrophils and macrophages migrate to the spleen and liver, destroying most of the listerial cells that have arrived there from the small intestine. Some listeriae escape by invading hepatocyte cells. Neutrophils are also recruited to the utero-placental unit and the central nervous system to kill listeriae invading these organs (*18,38,60,61,105*). Although neutrophils and macrophages can attach to and lyse cells containing listeriae, some macrophages do not kill listeriae. Macrophages that allow survival of listeriae contain too much or too little intracellular iron and have interleukin-10 on their surface. This is known to suppress macrophage function (*30*). An early inflammatory response is important for limiting growth and spread of *L. monocytogenes* in the body, but it is not sufficient to destroy all listeriae.

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Cytokines

Cytokines are small molecules, including interleukins (IL), interferons (IFN), and tumor necrosis factor (TNF), which coordinate cells and activities of the immune system. A number of these cytokines promote anti-*Listeria* reactions. These include IL-1, IL-2, IL-12, TNF-α, and IFN-γ. Other cytokines, IL-4 and IL-10, diminish resistance to *L. monocytogenes* (*19*). Serum IL-6 levels are also increased exponentially in mice with increasing numbers of *L. monocytogenes* (*51*).

Interaction of endothelial cells with *L. monocytogenes* increases synthesis of IL-8 and granulocyte-macrophage colony-stimulating factor (GM-SCF) which attract T-lymphocytes, monocytes, and neutrophils. IL-6, which induces T cell differentiation and proliferation, is also produced. Listeriolysin O appears to be the stimulus for increased production of these proinflammatory cytokines because mutant cells which do not synthesize this virulence factor do not provoke as much cytokine activity (*3,50,74,89*).

Gamma-interferon, produced by natural killer cells after stimulation by IL-12, plays a protective role against neuroinvasion in mice (*48,59*). Gamma-interferon levels also increase in other tissues in mice with increasing numbers of *L. monocytogenes* up to a concentration of 3×10^6 CFU per spleen. When listerial concentrations exceeded this number, interferon levels decreased (*51*).

T cells

T cell–mediated specific cellular immunity, if functioning properly, can detect and eliminate the *L. monocytogenes* cells which evaded the neutrophils and macrophages. Treatment of mice with cyclosporin A, an inhibitor of T cell functions, one day before infection with listeriae caused high mortality rates compared to infection of control mice (*77*). Infected cells with *L. monocytogenes* in the cytoplasm process listerial proteins such as listeriolysin O into peptides and present them with major histocompatibility molecules Ia and Ib on the cell suface. $CD8^+$ T cells recognize *Listeria*-infected cells and lyse them (*5,19,78*). This is a major route for destruction of infected hepatocytes in mice (*47*). Other experiments with mice demonstrated that $CD8^+$ cells help clear primary listerial infection and also generate a memory T cell population which can provide significant protection against secondary infection (*95*).

Mouse macrophages infected with listeriae, which were dead or did not produce listeriolysin O, kept the bacterial cells sequestered in the vacuole and therefore did not express antigens which reacted with $CD8^+$ T cells. However, these cells readily activated $CD4^+$ T cells which then stimulated production of IFN and IL-2 (*105*).

Similarly functioning T cells have been identified in humans (*39,49*).

Effects of pregnancy

Pregnancy presents a complex immunological problem for the mother's body. The fetus contains traits from the father that are antigenically foreign to the mother, and therefore her immune system should reject the fetus. This does not occur, however, because the mother's cell-mediated immunity is downregulated by increasing progesterone levels during pregnancy. Although this immunomodulation allows the fetus to survive, it also increases susceptibility to intracellular pathogens that are normally attacked by the cellular immune system. In addition to *L. monocytogenes*, other intracellular pathogens such as *Coxiella burnetii*, *Toxoplasma gondii*, and hepatitis E virus may cause severe illness in pregnant women and/or their fetuses (*100*).

Infants also respond inadequately to listerial infection. Examination of the in vitro immune response to *L. monocytogenes* by one-year-old infants who previously had a severe listerial infection at birth revealed that they produced neither antibodies nor a cell-mediated response to *L. monocytogenes*. In other words, their immune systems had no memory of encountering this pathogen previously. In contrast, the mothers of these infants did respond immunologically to a challenge with *L. monocytogenes* (*46*).

Some experiments with mice indicated that the immune response was impaired in the fetoplacental

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unit but not in other tissues, such as liver and spleen (*85*). Some monocytes and macrophages were observed in the placental region but they were not present at the foci of listerial infection and macrophages were not appropriately activated (*86*). Immunochemical staining confirmed that macrophages were not present in the placenta proper nor were the macrophage inflammatory protein or the monocyte chemoattractant protein detectable in the placenta (*38*). Other studies with mice indicated that antigen-presenting cells and T lymphocytes did not form cell clusters which are required for antigen recognition and cell proliferation (*52*). All of these deficiencies in immune function permit the growth of *L. monocytogenes* in the placenta and fetus.

Effects of age

A number of factors may affect the immune response and susceptibility to listerial infection in the elderly. Results from numerous studies comparing the immune response of people ≥ 65 years of age with that of young adults indicate that T cell functions (proliferative responses, interleukin-2 production, antibody production) tend to decline with age. In addition, there is a decline in the number of naive T cells that would be able to respond to new infections. The aging process decreases the efficiency of B cell– and T cell–mediated immunity (*9,10,53,99*).

Major surgery, poor nutrition, and lack of exercise can also diminish cell-mediated immune responses. Surgery causes a transient decrease in T cell proliferation and cytokine production (*42*). Surveys have indicated that diets of as many as one-third of the elderly are deficient in some vitamins and/or trace elements. In a clinical trial, persons receiving supplements of vitamins and minerals had a significantly higher immune status and levels of IL-2 and $CD4^+$ T cells as compared to unsupplemented controls (*9,82*). Selenium may be one of the important nutrients, as selenium-deficient mice developed neurological symptoms more frequently when exposed to *L. monocytogenes* than did selenium-replete mice (*2*). Finally, results from several studies indicate that long-term, regular exercise helps maintain immune function, including T cell function, in the elderly (*81,106*).

Gastrointestinal function may also be altered in the elderly. Gastric acidity levels are decreased by atrophic gastritis, infection with *Helicobacter pylori*, and the use of antacids. This reduction in acidity lowers an important barrier to the establishment of intestinal foodborne infections. Gastrointestinal motility may also decline, thereby reducing clearance of pathogens from the intestinal tract. Finally, use of antibiotics to treat illness destroys much of the competing microflora in the gastrointestinal tract, permitting the survival and penetration of *L. monocytogenes* (*53*).

Although all elderly people probably have less efficient immune responses than healthy young adults, the extent of an individual's immune deficiency, and therefore susceptibility to infections such as listeriosis, depends on gastrointestinal function as well as nutritional and lifestyle practices and overall health.

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