**Title:** 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 1980’s, 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 the fact that *L. monocytogenes* is widespread in nature and to its ability to grow at refrigeration temperatures. However, 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. 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. (Mead et al. 1999)

A significant proportion of the population, estimated at 3-10%, harbor listeriae in their gastrointestinal tract without showing any signs of illness. (Ryser ET and Marth EH 1999) *L. 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. (Schlech 2000) 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 X 10^8 - 1.2 X 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. (Dalton et al. 1997)
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 crosses the placenta to infect the 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. 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*. (Mylonakis et al. 1998) 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. (Skogberg et al. 1992) A high fatality rate was also observed for previously ill persons among a group of 225 cases of listeriosis in France while no deaths occurred among those who were <65 years old and previously healthy. (Goulet and Marchetti 1996)

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. (Farber and Peterkin 1991; Marth EH 1988; 1999; Schlech 2000; Southwick and Purich 1996) 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. (Center for Food Safety and Applied Nutrition (FDA) and the Food Safety and Inspection Service (USDA) 2001)

Why is *L. monocytogenes* such a dangerous pathogen? That is the question which will be addressed in this report. To answer this question will require 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 will be 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 bloodstream 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 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 – present in 64% of cases while 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 while serovar 1/2b occurred more often in non-pregnant individuals with severe underlying disease. (McLauchlin 1990) However, when tested in pregnant mice, infectivity of serovars 1/2a and 1/2b did not differ significantly from 4b. (Lammerding *et al.* 1992)

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. (Sokolovic *et al.* 1996)

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. (Inoue *et al.* 2001) Other analytical techniques which examined DNA sequences of some listerial virulence genes indicated that there are at least three evolutionary lineages of *L. monocytogenes*. (Rasmussen *et al.* 1995;Wiedmann *et al.* 1997) 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. (Wiedmann *et al.* 1997) 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. (Norton DM *et al.* 2001)

*L. 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. (Franciosa G *et al.* 2001)
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 develop acid tolerance and also become better able to invade and proliferate in cultured cells than non-acid tolerant bacteria. (Conte *et al.* 2000) 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 Kpffer 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. (Cossart and Lecuit 1998;Kuhn M and Goebel W 1999;Renzoni et al. 1999)

**VIRULENCE FACTORS**

**Internalins.** *L. 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 which is required for the penetration of *L. monocytogenes* into non-phagocytic cells, such as epithelial cells. (Gaillard *et al.* 1991) A related protein, internalin B, plays a role in invasion of hepatocytes in the liver. (Braun *et al.* 1998;Dramsi *et al.* 1995;Gaillard *et al.* 1991;Greiffenberg *et al.* 1998;Parida *et al.* 1998) 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 \textit{L. monocytogenes} cells. (Kuhn M and Goebel W 1999)

**Surface protein p104.** In addition to the internalins, another surface protein on \textit{L. monocytogenes}, p104, has recently been identified and shown to play a role in adhesion to intestinal cells. (Pandiripally \textit{et al.} 1999)

\textbf{Listeriolyisn O.} As listeriae are engulfed, they are enclosed within a vacuole which is surrounded by a membrane. Professional phagocytic cells begin almost immediately to kill the listeriae within the vacuoles and so survival of \textit{L. monocytogenes} depends on escaping from the vacuole. Listeriolyisn O (LLO), a bacterial pore-forming toxin, is essential for lysing the vacuolar membrane and allowing \textit{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. (Beauregard \textit{et al.} 1997; Conlan and North 1992; Cossart \textit{et al.} 1989; McKay and Lu 1991; Roll and Czuprynski 1990) Mutant \textit{L. monocytogenes} which do not produce listeriolyisn 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 \textit{L. monocytogenes} since 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. (Decatur and Portnoy 2000)

In addition to its pore-forming function, LLO participates in other reactions related to pathogenesis of \textit{L. monocytogenes}. Infection of murine spleen and bone marrow dendritic cells by \textit{L. monocytogenes} results in cell death by apoptosis. Mutant bacteria which do not produce LLO do not induce apoptosis while purified LLO can induce this programmed cell death. (Guzman \textit{et al.} 1996) LLO also can act as an inflammatory stimulus by inducing endothelial cell activation (Drevets 1998; Kayal \textit{et al.} 1999) and neutrophil activation (Sibelius \textit{et al.} 1999).

\textbf{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 bulge outwards to form structures called listeriopods. (Kocks *et al.* 1992; Moors *et al.* 1999) 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 which do not produce internalins into certain types of cells. (Kocks *et al.* 1992; Kuhn M and Goebel W 1999)

**Phospholipases**

Two distinct phospholipases C are synthesized by *L. monocytogenes*: 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. (Smith *et al.* 1995) PC-PLC damages vacuolar membranes in some cell types, such as epithelial cells, and can therefore be a substitute for LLO. (Marquis *et al.* 1995) PC-PLC also functions in the cell-to-cell spread of listeriae in the brain during cerebral listeriosis. (Schluter *et al.* 1998)

**Metalloprotease**

PI-PLC is synthesized in an active form while 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. (Marquis *et al.* 1997)

**Clp proteases and ATPases**

In order to adapt to adverse environmental conditions (high or low pH, temperature, osmotic conditions), many bacteria have chaperone proteins which 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 which aids in disruption of the vacuolar membrane and the intracellular survival of listeriae. (Rouquette *et al.* 1998) 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. (Nair et al. 2000) 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. (Gaillot et al. 2000)

Protein p60

Protein p60 is murein hydrolase enzyme which 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 that it is important for phagocytosis of *L. monocytogenes* by some cell types. (Kuhn M and Goebel W 1999)

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. (Kuhn M and Goebel W 1999) 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. Listeria 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. (Buncic et al. 1996; Conte et al. 1994; Datta and Kothary 1993) 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. (McCarthy 1991) 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. (Santiago et al. 1999)
Acidity. Although it has been reported that exposure to low pH (4.5-4.9) reduces production of listeriolysin O (Datta and Kothary 1993) and diminishes invasion of Caco-2 cells in culture (Galdiero et al. 1997), 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. (Conte et al. 2000) Following incubation at pH3.5, acid tolerant L. monocytogenes were isolated which were more virulent to mice and more resistant to the influx of neutrophils and macrophages mobilized to protect the mice. (Gahan and Hill 1999; O'Driscoll et al. 1996)

Iron. Growth in an iron-rich medium enhanced the invasiveness of L. monocytogenes for Caco-2 cells by increasing the expression of internalin genes (Conte et al. 1996) Availability of iron also affects expression of the ActA protein. (Conte et al. 2000)

INVASION PROCESS IN DIFFERENT TISSUES

Intestine. L. 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. (Okamoto et al. 1994) Large numbers of listeriae (10^8-10^9 cells) are normally required to produce infection in the healthy animal models tested: rats and mice (Czuprynski and Haak-Frendscho 1997) and cynomolgus monkeys (Farber et al. 1991). In experiments with a ligated rat ileal loop system, gross intestinal lesions were only observed when very large doses (≥10^9 listerial cells) were inoculated in a loop. (Pron et al. 1998) 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 X 10^{11} cfu of L. monocytogenes per person in a 1994 outbreak in Illinois. (Dalton et al. 1997) 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. (Okamoto et al. 1994) 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. (Hess et al. 1996; Pandiripally et al. 1999; Yoshikai 1999) 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. (Czuprynski and Haak-Frendscho 1997; Guzman et al. 1995; Marco et al. 1997; Pron et al. 1998)

In addition to the virulence factors produced by *L. monocytogenes* and the host defense mechanisms (to be discussed later), the other obvious factor which 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. (Sprong RC et al. 1999) 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. (Southwick and Purich 1996) 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. (Huang et al. 2000; Tuomanen 1996) 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. (Greiffenberg et al. 1998) Other research using HBMEC cells from a different source indicated that internalin was not required for infection by *L. monocytogenes*. (Wilson and Drevets 1998) It appears that in the presence of serum, internalin is not required but in its absence, internalin is necessary for invasion. (Drevets 1999) Invasion of HBMEC stimulated 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 internalin B-dependent reaction. (Dons et al. 1999) 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. (Jin et al. 2001)
**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 of *L. monocytogenes*. (Altieri *et al.* 1999) 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. (Menudier *et al.* 1994) 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. (Smith 1999) 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 and this probably explains why the majority of adults exposed to this bacterium do not become sick. (Okamoto *et al.* 1994) 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. (Kuhn and Goebel 1998;Kuhn *et al.* 1999) Cell-mediated immunity is probably the main host defense against listeriosis with CD4\(^+\) T helper lymphocytes activating macrophages. (Czuprynski CJ 1994)

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 (Altimira *et al.* 2000) and fatty acids (de Pablo *et al.* 2000) 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 combating *L. monocytogenes* infections. During early stages of listeriosis, neutrophils and macrophages migrate to the spleen and liver destroying most of the listerial cells which 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 which invade these organs. (Czuprynski and Haak-Frendscho 1997; Guleria and Pollard 2000; Lopez *et al.* 2000; Lopez *et al.* 1999; Unanue 1997) Although neutrophils and macrophages can attach to and lyse cells containing listeriae, some macrophages do not kill listeriae. Those macrophages which allow survival of listeriae contain too much or too little intracellular iron and have interleukin-10 on their surface and this is known to suppress macrophage function. (Fleming and Campbell 1997) 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.

**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*. (Czuprynski CJ 1994) Serum IL-6 levels are also increased exponentially in mice with increasing numbers of *L. monocytogenes*. (Kim *et al.* 2001)

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. (Arnold and Konig 1998; Kayal *et al.* 1999; Nishibori *et al.* 1996; Rose F *et al.* 2001)

Gamma-interferon, produced by natural killer cells after stimulation by IL-12, plays a protective role against neuroinvasion in mice. (Jin *et al.* 2001; Liu and Kurlander 1995) Gamma-interferon levels also increase in other tissues in mice with increasing numbers of *L. monocytogenes* up to a concentration of $3 \times 10^6$ CFU per spleen. When listerial concentrations exceeded this number, interferon levels decreased. (Kim *et al.* 2001)

**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. (Okamoto et al. 1994) Infected cells with *L. monocytogenes* in the cytoplasm process listerial proteins such as listeriolyisin O into peptides and present them with major histocompatibility molecules Ia and Ib on the cell surface. CD8+ T cells recognize Listeria-infected cells and lyse them. (Bouwer et al. 1997; Czuprynski CJ 1994; Pamer et al. 1997; Wing et al. 2000) This is a major route for destruction of infected hepatocytes in mice. (Jiang et al. 1997) Other experiments with mice demonstrated that CD8+ cells besides helping to clear primary listerial infection also generate a memory T cell population which can provide significant protection against secondary infection. (Seaman et al. 2000)

Mouse macrophages infected with listeriae, which were dead or did not produce listeriolyisin 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. (Unanue 1997)

Similarly functioning T cells have been identified in humans. (Guo et al. 1995; Jouen-Beades et al. 1997)

**EFFECTS OF PREGNANCY**

Pregnancy presents a complex immunological problem for the mother's body. The fetus contains traits from the father which 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 which 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. (Smith 1999)

Infants also respond inadequately to listerial infection. Examination of the in vitro immune response to *L. monocytogenes* by one year-old infants who had 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*. (Issekutz et al. 1984)

Some experiments with mice indicated that the immune response was impaired in the fetoplacental unit but not in other tissues, such as liver and spleen. (Redline and Lu 1987) 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. (Redline and Lu 1988) 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. (Guleria and Pollard 2000) 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. (Klink and Rudnicka 1995) 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 which would be able to respond to new infections. The aging process decreases the efficiency of B-cell and T-cell mediated immunity. (Chandra 1992; Chandra 1997; Klontz *et al.* 1997; Smith 1998)

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. (Hensler *et al.* 1997) 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. (Chandra 1992; Pike and Chandra 1995) 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. (Altimira *et al.* 2000) Finally, results from several studies indicate that long-term, regular exercise helps maintain immune function, including T-cell function, in the elderly. (Paw *et al.* 2000; Venjatraman and Fernandes 1997)

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 and this may permit the survival and penetration of *L. monocytogenes*. (Klontz *et al.* 1997)

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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