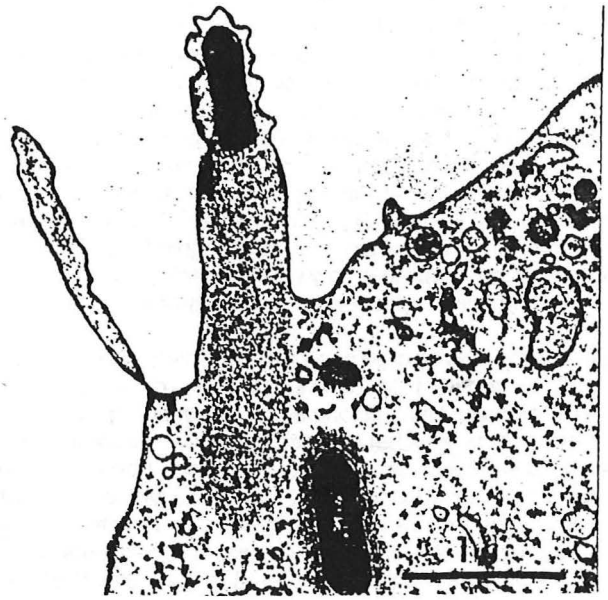
LISTERIA MONOCYTOGENES:

OFT FORGOTTEN, ALWAYS DANGEROUS



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The topic of today's Grand Rounds is the facultative intracellular bacterial pathogen *Listeria monocytogenes* and the clinical syndromes, collectively referred to as listeriosis, that it causes. I have appended "Oft Forgotten, Always Dangerous" to the title because listeriosis is an infection that tends to "fall between the cracks". The disease is common enough that we are all aware of it, yet it is rare enough, with an overall incidence of approximately three cases per one million persons in the U.S. (1,2), that we tend to forget about it in the heat of clinical battle. Recognition of this latter point is particularly important in the age of broad-spectrum β-lactam antimicrobials (e.g., "third generation" cephalosporins) which have generally poor activity against *L. monocytogenes* (3). The importance of listeriosis to contemporary society was demonstrated vividly by the well publicized 1985 epidemic in southern California associated with Mexican-style soft cheese (4,5). In that epidemic, 142 cases of human listeriosis resulted in 48 deaths; 30 of these were in fetuses and neonates. While we will discuss the epidemiology of listeriosis in detail later in this Grand Rounds, this epidemic is mentioned at the outset because it exemplifies many key aspects of this bacterium: (1) its origins as a commensal and/or pathogen of animals, (2) its being a frequent contaminant of many common food products, (3) its use of the gastrointestinal tract as a portal of entry, and (4) its proclivity to cause disease in pregnant women and immunocompromised hosts.

Interestingly, though this pathogen has been recognized for more than sixty years, only within the last decade have many important features of the epidemiology, pathogenesis, and clinical presentation of listeriosis been clarified. The study of the pathogenesis of listerial infections also has become fertile ground for immunologists and molecular microbiologists and I shall try to give you an overview of the exciting recent developments in this area of investigation. Those who desire to learn more than I can cover in today's presentation are referred to the two excellent reviews of *L. monocytogenes* and listeriosis which were published earlier this year (1,5) as well as the excellent chapter in the Principles and Practice of Infectious Diseases (2).

HISTORY OF LISTERIOSIS

While a comprehensive understanding of listeriosis may be relatively recent, listeriosis is far from a newly recognized disease (Table 1). Late in the nineteenth century French and German researchers identified gram-positive rods in tissues from patients probably infected with *L. monocytogenes* (6). In 1919, Hulphers isolated the organism from the necrotic liver of a rabbit and named it *Bacillus hepatis*. In that same year the bacterium was isolated from the cerebrospinal fluid of a patient with meningitis. Interestingly, it was considered a "diphtheroid" at the time of isolation, a potential pitfall which continues to plague unsuspecting clinical microbiologists and clinicians; not until 1942 was this isolate recognized as *L. monocytogenes* (7). In 1926, the bacterium was associated with an epidemic of sepsis among laboratory rabbits and was renamed *Bacterium monocytogenes* because of the profound monocytosis it elicited these infected

animals (6,7). In the 1930's it was identified increasingly as a commensal and pathogen of many species of animals and reports of human infections, including perinatal infections, also appeared at this time. Some of these isolates were from patients with mononucleosis syndromes, and for a period *L. monocytogenes* was believed to be the cause of infectious mononucleosis. Since these early reports, *L. monocytogenes* has not been associated with this syndrome (5,7). In 1940, the organism received its present name. As far as listeriosis is concerned, the modern era began in 1967 with the report by Louria *et al.* (8) describing a new association between listeriosis and malignancy. Soon thereafter other reports began to appear associating listeriosis with immunocompromised states, including those of iatrogenic origin, and the notion became established that *L. monocytogenes* was predominantly an opportunistic pathogen (2,9).

Table I. Early Chronology of Listeriosis

1919	*L. monocytogenes* first isolated (called *Bacillus hepatis*)
1926	Associated with monocytosis in rabbits (renamed *Bacterium monocytosis)*
1935	First case of *L. monocytogenes* meningitis in U.S.
1936	First perinatal case of listeriosis
1940	Renamed *Listeria monocytogenes*
1967	Associated with immunocompromised states

CLINICAL MICROBIOLOGY

L. monocytogenes is a small (0.5 μm x 1-3 μm) gram-positive, nonspore-forming facultatively anaerobic rod. Ultrastructurally and biochemically, the cell wall of *L. monocytogenes* is similar to that of other gram-positive bacteria in that it consists of a thick homogeneous layer (peptidoglycan, techoic acids, and lipotechoic acids) surrounding the cytoplasmic membrane (1,2,7). The bacterium possesses peritrichous flagella which give rise to the characteristic tumbling motility which is seen when it is grown between 20° and 25° C. The bacterium can be isolated easily from clinical specimens obtained from normally sterile body fluids (e.g. blood, CSF) using a variety of nonselective media, although large volumes sometimes must be cultured to compensate for the relatively low concentration of organisms (2). Selective media must be used when isolating it from specimens containing other bacteria (e.g. stool), selective media (1). The bacterium grows at a wide range of temperatures (-0.4° to 45°C); its ability to grow at relatively low temperatures is the basis for the "cold enrichment" technique for isolating it from specimens containing other bacterial species (1). While "cold enrichment" has

been supplanted as a means of isolating *L. monocytogenes* from complex mixtures of bacteria, it continues to be a major contributory factor in the development of high levels of contamination in refrigerated foodstuffs implicated in listerial epidemics (1,5). Colonies of *L. monocytogenes* demonstrate a characteristic blue-green sheen when viewed obliquely by transmitted light. The presence of a narrow zone of β-hemolysis is extremely useful in differentiating colonies of *L. monocytogenes* from morphologically similar "diphtheroids" (*Corynebacterium* species) (1,2). Colonies of *L. monocytogenes* also may be confused with *Erysipelothrix rhusiopathiae*, β-hemolytic streptococci, and enterococci. In clinical specimens examined by gram-stain, it can be mistaken for more common gram-positive cocci, such as the pneumococcus, and in over-decolorized specimens for gram-negative coccobacilli such as *Haemophilus influenzae* (2).

As already indicated, the taxonomy of *L. monocytogenes* (and other members of the *Listeria* genus) has undergone considerable transition over the years. *L. monocytogenes* was the only recognized species until 1961 and included both pathogenic and nonpathogenic species; thereafter, nonpathogenic strains were grouped into a new species, *L. innocua*. Serovar 5 strains, which show a distinctively strong β-hemolysis, were grouped into a separate species, *L. ivanovii*, in 1984; this species is pathogenic for animals but does not appear to cause disease in humans. All other *Listeria* species are nonpathogenic. Serotyping of *L. monocytogenes* is based upon somatic (O) and flagellar (H) antigens and is important for epidemiology; of the 11 serotypes, three (Ia, Ib, and IVb) cause 90% of clinical infections (1,5).

A particularly important, as well as controversial, aspect of *L. monocytogenes* concerns its "thermal resistance", namely, the organism's ability to resist killing by heat. Accurate delineation of this property is of paramount importance in preventing the transmission of *L. monocytogenes* since, as will be discussed shortly, foods are the major vehicles of transmission of this pathogen. One basic question addressed by these studies is whether, given the fact that *L. monocytogenes* is a frequent contaminant of raw milk, commercial pasteurization is sufficient to eliminate it from milk. In fact, this became a major concern following a listeria epidemic in Massachusetts traced to properly pasteurized milk (1,5,10). To complicate this issue further, such processes not only must be capable of eliminating intracellular as well as extracellular organisms; the former are present (often at concentrations in excess of 10^3 CFU/ml) in milk from cows with mastitis. The bottom line (at least as near as I can tell) from a number of studies in which *L. monocytogenes* either was added directly to milk or in which milk was obtained from cows inoculated with *L. monocytogenes* (to obtain milk containing intracellular organisms) is that it can survive for several minutes in milk heated to 60° C but is rapidly killed at temperatures at or above 70° C (1,11). Thus high temperature short time (HTST) pasteurization (71.7° C, 15 seconds) is the preferred process for eliminating *L. monocytogenes* (1). Because milk contaminated by *L. monocytogenes* smells and tastes normally, sensitive methods for the rapid detection of *L. monocytogenes* in food clearly are necessary. Heating of solid foods (e.g. meats, poultry) for several minutes at temperatures at or above 70° C also appears sufficient to kill *L. monocytogenes* (1).

PATHOGENESIS OF LISTERIOSIS

Although cutaneous and conjunctival routes have been documented (e.g., as a result of occupational exposure in veterinarians and abattoir workers) (1,12,13), the GI tract is now considered to be the portal of entry in nearly all cases of acquired listeriosis in adults (1,5,14). In addition to the epidemiological data for this (described below), mice inoculated orally with large numbers (10^8 CFU) of listeria regularly develop invasive disease (1,14). Estimates of infectious inocula derived from foodborne outbreaks suggest that at least 10^3 (and sometimes as many as 10^6) bacteria must be ingested to produce disease in humans (1). The presumably frequent ingestion by humans of foods containing relatively low numbers of organisms (see below) suggests that small inocula are generally ineffective at causing disease in healthy persons. It has also been suggested that our frequent exposure to these small inocula provides a form of natural protective immunity (7). Although GI tract colonization occurs frequently (transiently, in between 1% and 5% of the population), invasive listeriosis is rare (2,5). Factors such as the virulence of the organism and the status of the host's cellular immune system are believed to be critical determinants of whether invasive disease will occur (1,7).

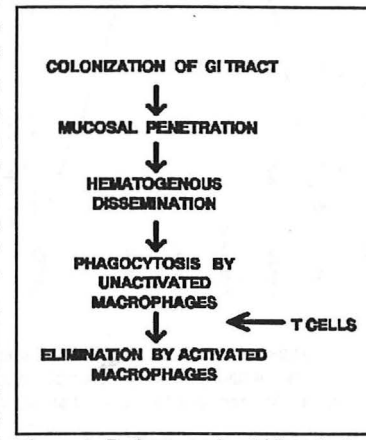

Figure 1. Pathogenesis of Listeriosis

Figure 1 depicts, in simplified form, the stages of the pathogenesis of human listeriosis. Penetration through the intestine is the primary event in the infectious process, an event Rácz (15) termed the "epithelial phase" of listerial infection. In his landmark electron microscopy studies of guinea pigs orally challenged with *L. monocytogenes*, he demonstrated (1) that the bacteria are taken up within phagosomal vacuoles of the intestinal epithelial cells, (2) that they appear to escape into the cytoplasm by dissolving the phagosomal membranes and (3) they appear to spread to adjacent cells without entering the intercellular space. Using cultured intestinal epithelial cells, Sansonetti *et al.* (14,16) described a very similar sequence of events and showed that uptake was mediated by "directed" phagocytosis (i.e., it could be prevented by cytochalasin D). As will be shown below, similar events transpire within infected, but unactivated, professional phagocytes (17-19).

Following the intestinal phase, the bacteria disseminate hematogenously and invade parenchymal cells of various target organs, particularly the placenta, the central nervous system, the liver and spleen. In the mouse model, more than 90% of the total body burden of organisms localize in the liver and spleen, the two major

reticuloendothelial organisms (14,20). In his pioneering experiments, Mackaness (20,21) proved that control of the bacterium in these two organs is entirely dependent upon the cellular immune response. Using a now classic mouse model, he showed that, initially, bacterial replication proceeds largely unchecked in the livers and spleens of mice inoculated intravenously with *L. monocytogenes*. However, after the third day of infection, the bacteria were effectively phagocytized by macrophages and he correlated this with the onset of delayed hypersensitivity (measured by footpad swelling) to *L. monocytogenes* (Fig. 2). He also correlated these findings with in vitro studies demonstrating prolific replication of *L. monocytogenes* within peritoneal macrophages of normal mice but limited replication in macrophages from convalescent, immune animals. Subsequently, he demonstrated that it was possible to transfer the "listeria-resistant state" to normal mice by infusing splenic lymphocytes from convalescent animals 4 or more days after infection (21). Soon thereafter, Lane and Unanue (22) showed that pretreatment of the transferred lymphoid cells with antiserum directed against the theta antigen of T cells abrogated the transfer of protective immunity, thereby confirming that T cells were, in some way, responsible for inducing the listericidal activity in the macrophages.

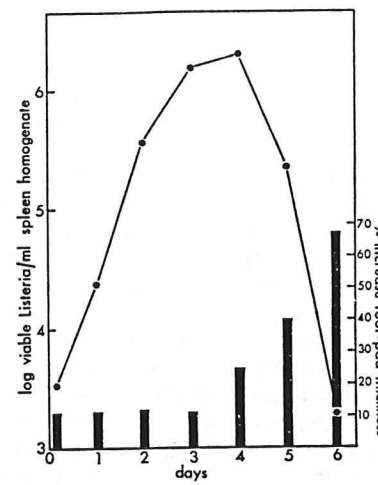

Figure 2. Mean spleen counts of *L. monocytogenes* after sublethal infection vs. percentage increase of foot-pad thickness.

To a large extent, much of the ensuing research into listerial pathogenesis has been an attempt to elucidate at the cellular and molecular levels the events outlined by Rácz and Mackaness. Among the most important discoveries are that the hemolysin of *L. monocytogenes*, lysteriolysin O (LLO), is the major virulence factor of the organism and that its secretion is essential for promoting the intracellular growth and T cell recognition of the organism (14). This 58-kilodalton sulfhydryl-activated enzyme is one of a family of bacterial pore-forming cytolysins of which streptolysin O (a product of *Streptococcus pyogenes*) is the prototype. The importance of LLO as a major virulence factor has been demonstrated both in vivo and in vitro. Gaillard et al. (23) and Kathariou et al. (24) used transposon mutagenesis to produce isogenic hemolysin-negative (Hly-) mutants of a virulent strain of *L. monocytogenes*. Both groups found that the loss of expression of this single gene produced organisms that were avirulent in mice (Fig. 3), while spontaneous (Hly+) revertants regained virulence in the mouse model. Hly- mutants also failed to grow in cultured cells of either epithelial (23) or macrophage origin (25). When examined by EM, it was found that the Hly- mutants remained within the phagosomal vacuoles

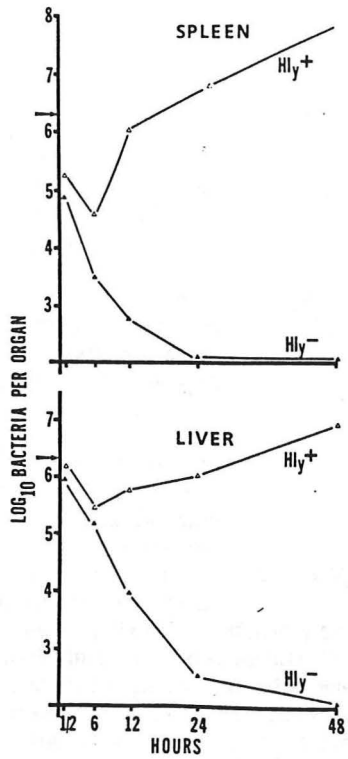

Figure 3. In vivo growth of hemolysin-negative (Hly-) mutant and hemolysin-positive (Hly+) strains of *L. monocytogenes*.

where they were degraded (16,25); thus the hemolysin appears to be critical to dissolution of the phagosomal membrane by *L. monocytogenes* and escape into the cytoplasm where the bacterium replicates.

Perhaps the most impressive demonstration of the importance of LLO to *L. monocytogenes* virulence resulted when the structural gene for the enzyme was cloned into the avirulent gram-positive *Bacillus subtilis* (26). Following uptake by macrophages, the Hly+ recombinant *B. subtilis* was able to escape from macrophage phagosomes and replicate efficiently within the cytoplasm (26). However, while a single gene product was capable of turning a common soil organism (i.e., *B. subtilis*) into an intracellular parasite in vitro, the Hly+ *B. subtilis* was not virulent in mice (26). Thus, LLO is necessary <u>but not sufficient</u> for virulence of *L. monocytogenes*.

Another important finding is the remarkable mechanism by which *L. monocytogenes* spreads from cell to cell. As mentioned above, Racz (15) interpreted his electron micrographs of *L. monocytogenes* in guinea pig intestinal epithelial as showing that the bacteria spread from cell to cell without ever becoming extracellular. He hypothesized that pseudopods containing bacteria might extend from one cell and be phagocytosed by adjacent, uninfected cells. Portnoy and his collaborators (17,19) have confirmed and extended this by describing a unique means of intercellular spread in which the bacterium appropriates the actin cytoskeleton (Fig. 4). Following emergence from the phagosome, the bacterium secretes a filamentous material which acts as a condensation nucleus for actin filaments (17). The actin filaments arrange themselves at one pole of the bacterium into a long tail termed an actin "comet". The "comet" then moves to the cell surface with the tail oriented to the cell center. Once near the cell surface, it forms a pseudopod-like extension which is phagocytosed by a neighboring macrophage. The bacterium is eventually free to replicate within the cytoplasm of the

second cell. Thus, *L. monocytogenes* can remain entirely intracellular while spreading from intercellularly, all the while remaining completely inaccessible to host humoral defense mechanisms.

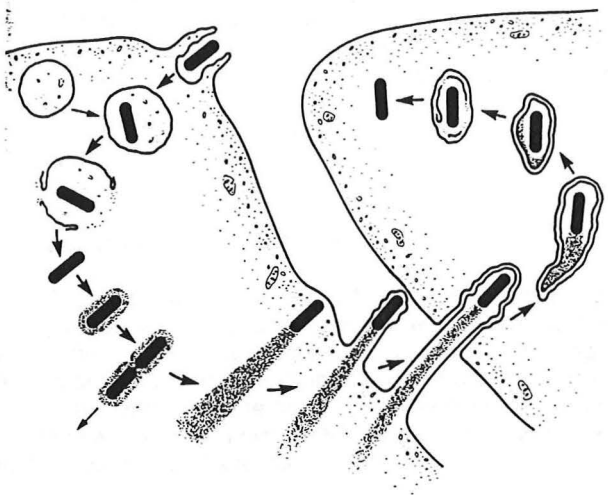

Figure 4. Stages in the intercellular spread of *L. monocytogenes*.

The final research developments I will touch upon concern our vastly improved understanding of the specific components of the cellular immune system which provide protective immunity against infection with *L. monocytogenes*. First it, has been demonstrated that cytokines, specifically, interferon-γ and tumor necrosis factor-α, are essential for inducing the listericidal state; mice treated with antibodies against either cytokine prior to challenge with *L. monocytogenes* are extremely susceptible to infection (27,28). The in vivo data with interferon-γ correlate nicely with in vitro studies showing that activation of macrophages with this cytokine prevents the bacterium from dissolving the phagosomal membrane (18). It has been hypothesized that interferon-γ induces the production of compounds in the vacuole (e.g., superoxides and other oxygen radicals) which alter the redox potential within the phagosomal vacuole, thereby depriving the hemolysin of the reducing environment required for its enzymatic activity (18).

The T cell subtypes which mediate protective immunity also are being increasingly better defined. While both helper and cytotoxic cells contribute to acquired resistance, recent evidence suggests that cytotoxic T cells are more important subset for acquired immunity (29-31). Because neither heat-killed nor viable Hly- bacteria induce protection, the concept has emerged that secretion of LLO by viable Hly+ bacteria is necessary in order to activate the class I pathway and induce protective cytoxic T cells (31,32). Portnoy and co-workers (31) have shown that cytotoxic T cells respond in vitro only to viable Hly+ cells and that cytotoxic T cells can be a source of the interferon-γ. Recently, evidence has emerged that, in addition to its role as a virulence factor, LLO may be an immunodominant antigen for induction of both helper and cytotoxic T cells (33,34). Cytolytic T cell clones capable of killing a macrophage cell line infected with virulent *L.*

monocytogenes lines recognized naturally processed peptides derived from LLO (34). These findings have important implications regarding strategies for the development of a listeria vaccine vectors, such as the vaccinia virus (35) are now available for targeting LLO and other *L. monocytogenes* vaccinogens to the cytoplasmic compartment of host cells. The fact that protective immunity against *L. monocytogenes* appears to be more dependent upon cytotoxic than helper T cells may explain the observation that listeriosis is relatively uncommon in AIDS patients compared to other opportunistic pathogens such as *Pneumocystis carinii* or *Cryptococcus neoformans* (36).

EPIDEMIOLOGY OF LISTERIOSIS

L. monocytogenes is found throughout the environment, having been isolated from soil, water, sewage, and decaying vegetation (1); in one survey, it was recovered from 21% of plant and soil samples (5). The organism can persist at high levels for prolonged periods in a number of different environmental sites, a finding of considerable importance with respect to the use of fecal material containing the organism as fertilizer (5). In fact, an outbreak of listeriosis in Nova Scotia was related directly to coleslaw which was made from cabbage fertilized with such contaminated fecal material (37).

L. monocytogenes is a major pathogen of animals; in other words, listeriosis is a "zoonosis" which often occurs in "epizootic" form. The organism has been isolated from cattle, pigs, sheep, chickens, and many other species in which it causes syndromes similar to those of humans (e.g., abortions and meningoencephalitis or "circling disease") or asymptomatic gastrointestinal carriage (1,5-7,38). It is generally believed that animals are the primary reservoir for the bacterium and that humans become infected incidentally via direct contact or, more commonly, indirect contact with contaminated foodstuffs, water, etc (1,5,7). Although suggested by experimental studies in animals, only within the last decade have investigations of large outbreaks provided convincing evidence for this conjecture.

L. monocytogenes has been found in a wide range of foods, including dairy products, meats, and poultry (1,5,39). Of all foods, cheeses appear to be among the most frequently contaminated and among the most frequently implicated in cases of human listeriosis (1,39). This is probably because *L. monocytogenes* is capable of surviving the process by which many naturally ripened cheeses, particularly soft cheeses, are made. Levels of *L. monocytogenes* as high as 10^7 CFU/g have been found in some naturally contaminated cheeses (1,5). Given that fecal carriage of *L. monocytogenes* by many mammals and birds is so common, it is not surprising that numerous isolations from raw meats and poultry have been reported (1). In some surveys, up to 30% of meat and 60% of poultry samples have yielded *L. monocytogenes* in numbers ranging from 20 to 10^3 per gram (1). The numbers of organisms in refrigerated foods can increase by up to two logs during a ten day period (1).

Table 2. Foodborne outbreaks due to *L. monocytogenes*

Location (yr)	No. of cases (no. of deaths)	No. perinatal/no. nonperinatal	Foods associated	No. of immuno-compromised individuals
Boston (1979)	20 (5)[a]	0/20	Raw celery, tomatoes, lettuce[b]	10
New Zealand (1980)	29 (9)	22/7	Shellfish, raw fish[b]	0
Maritime Provinces (1981)	41 (17)	34/7	Coleslaw	0
Massachusetts (1983)	49 (14)	7/42	Pasteurized milk[b]	42
California (1985)	142 (48)	93/49	Jalisco cheese	48
Canton de Vaud, Switzerland (1983–1987)	122 (31)	63/59	Raw milk, cheese	–[c]
Philadelphia (1986–1987)	36 (16)	4/32	Ice cream,[b] salami[b]	24
Connecticut (1989)	9 (1)	2/7	Shrimp[b]	0
United Kingdom (1987–1989)	>300 (?)	NK[d]	Paté[b]	NK

As mentioned earlier, listeriosis is surprisingly uncommon in light of its high frequency as a foodborne contaminant. In the various countries reporting listerial infection, its incidence averages about 1 case per 100,000 population annually (1). Interestingly, listeriosis is predominantly a disease of industrialized countries. In fact, I was unable even to find it referenced in a major textbook of tropical medicine! The two major epidemiological patterns of listeriosis are epidemic and sporadic disease; the major foodborne outbreaks are shown in Table 2. In most of these outbreaks the association with food was confirmed by (1) case-control study implicating a particular food, (2) recovery of a single strain of *L. monocytogenes* from patients, and (3) recovery of the epidemic strain from the implicated food product. Data from the 1981 Canadian outbreak provided the first conclusive evidence that the disease in humans is primarily foodborne and that the GI tract is the portal of entry (37). A second large outbreak (case fatality rate 29%) occurred in Massachusetts in the summer of 1983 and involved predominantly immunosuppressed, nonpregnant adults (10). This epidemic was traced to a specific brand of pasteurized milk. Because no evidence was found that the pasteurization process at the incriminated plant was breached, concerns arose that the pasteurization process itself might have been ineffective. As discussed above, these concerns have largely been discounted.

The largest epidemic of listeriosis in North America occurred in 1985 in Los Angeles. A case-control study implicated a particular brand of Mexican soft cheese which became contaminated with *L. monocytogenes* serotype IVb during processing. While the pasteurizer in the plant was found to be working properly, FDA investigators found that the amount of raw milk delivered to the pasteurizer occasionally exceeded its capacity. Other potential opportunities for contamination of pasteurized milk with raw milk also were noted. The prolonged incubation period determined from this epidemic (mean of 31 days), much longer than other foodborne diseases, provides one indication of the difficulties involved in identifying relevant food exposures in sporadic cases of listeriosis. The 1986-7 Philadelphia outbreak was interesting because it involved several different types of foods and multiple *L. monocytogenes* serotypes (40). Since many of the infected individuals reported previous gastrointestinal tract symptoms, the investigators

hypothesized that a co-infecting GI tract pathogen may have been responsible for converting asymptomatic gastrointestinal tract colonization to invasive disease. In support of this, Lorber (41) recently reported a case of a farmer who developed a listerial brain abscess following shigellosis.

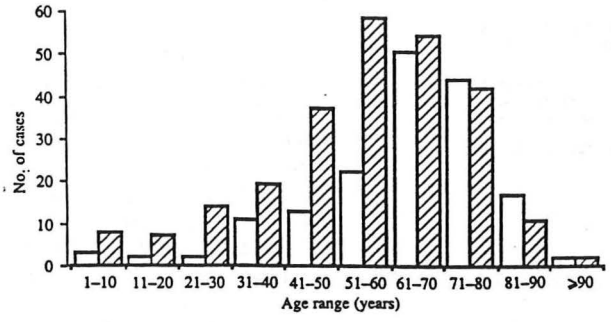

Figure 5. Age distribution of nonpregnancy-associated cases of listeriosis

Several nosocomial outbreaks also have been reported. Most of these involved index cases of neonatal listeriosis in which the pathogen was transmitted by inadequate hand washing or other breaches in nursing technique to other infants in the same nursery; the secondarily infected neonates developed the form of disease called late-onset neonatal listeriosis (5). One nosocomial outbreak in Costa Rica was traced to contaminated mineral oil used to bathe newborn infants; the oil presumably was contaminated from an infant with early onset listeriosis born to a mother with amnionitis (42).

As a result of the Los Angeles outbreak, the Centers for Disease Control initiated active surveillance for listeriosis in 1986. In one population-based survey conducted in LA County and five other states, it was determined that 0.7 sporadic cases occur per 100,000 population (approximately 1700 cases per year) resulting in 450 adult and 100 fetal/perinatal deaths per year (5,7). Not surprisingly, the incidence of sporadic listeriosis increases with age; in one study, 84% of the nonperinatal cases were over 50 years old and 41% were over 70 years (Fig. 5)(43). Investigations of many sporadic cases have identified contaminated food products, often "ready-to-eat" foods, supporting the belief that food-borne transmission is the predominant cause of sporadic as well epidemic cases (1,39).

CLINICAL SYNDROMES

As noted previously, exposure to *L. monocytogenes* most commonly results in a transient, asymptomatic carrier state characterized by fecal excretion of the organism (1,2,14). The clinical syndromes arising from infection with *L. monocytogenes* are shown in Figure 6. Both in neonates and adults, the large majority of these infections manifest

as bacteremia and/or involvement of the central nervous system (usually meningitis). In a large British series involving 722 patients, 34% and 66% of listeria cases were pregnancy- and nonpregnancy-associated, respectively (43). Approximately 75% of the non-pregnancy associated listeria cases had one or more predisposing conditions (43). Nonimmunocompromised individuals were substantially more likely to present with meningitis without bacteremia (43).

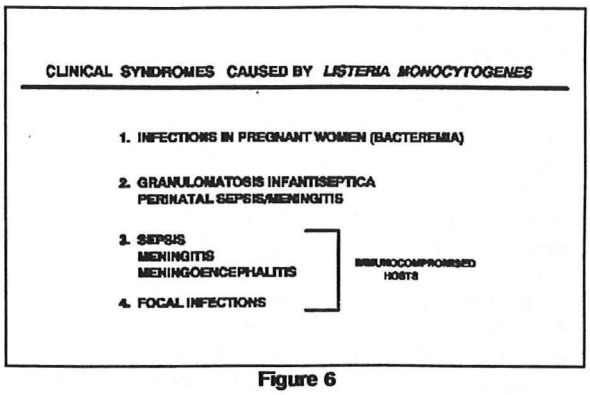

Figure 6

Listeriosis during pregnancy and in the fetus/neonate. Listeriosis during pregnancy is encountered most frequently during the third trimester, although cases as early as the second month of gestation have been documented (44,45). It is probable, though unproven, that listeriosis also is a significant cause of early fetal wastage (45). Listeriosis in the setting of pregnancy is distinctive in that the maternal bacteremia is usually asymptomatic or results only in a mild flu-like illness while bacteremic infection of the fetoplacental unit results in stillbirth, late abortion, or prematurity with variable symptoms of listeriosis (sepsis, pneumonia, meningitis) in the live neonate. Exceptions to this do occur since cases of maternal listerial meningitis during pregnancy have been reported (46) as have cases in which maternal bacteremia (even untreated) was not followed by infection of the fetus (44,47).

Two clinical forms of neonatal listeriosis have been described-early and late onset; a third "overlap" form, intermediate, is also often described. Severely infected infants manifest a form of the disease called *granulomatosis infantisepticum* which is characterized by granulomatous involvement of numerous organs (2). Late-onset neonatal listeriosis usually occurs in full-term infants of uncomplicated pregnancies; the manifestations of the infection become apparent days to weeks after birth (2,44,45). Cases of late-onset listeriosis are not the result of maternal bacteremia. Instead, they are believed to result either from acquisition of the organism from the mother's genital tract at the time of birth or from some exogenous, even nosocomial source. The distribution of 210 cases of neonatal listeriosis from Great Britain is shown in figure 7 (44). Intrauterine death (IUD) occurred in 42 of these, while 70% of the remaining 168 were early onset. Two distinctive differences between the early and late onset cases in this series were the much greater incidence of meningitis and the much lower incidence of pneumonia in the late onset group (44). The overall mortality rate of the 168 infants alive at birth was 35%!

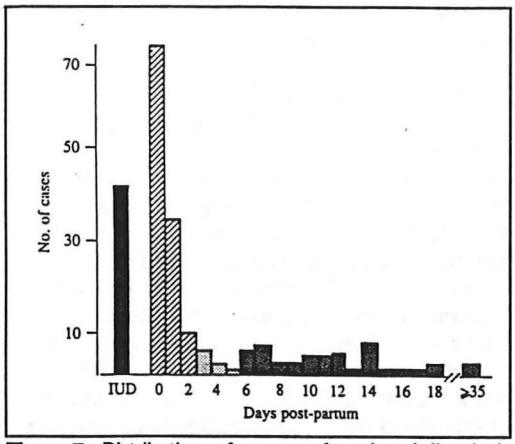

Figure 7. Distribution of cases of perinatal listeriosis relative to days postpartum. IUD, (intra-uterine death).

Nonperinatal Infections. In their classic reviews of *L. monocytogenes* and listeriosis, neither Gray (6) nor Hoeprich noted (38) an association between listeriosis and the immunocompromised state. However, in 1967 Louria et al. (8) presented a series of 18 patients with listerial infection in which all had underlying neoplastic disease, usually involving lymphatic tissues, and a majority were taking corticosteroids. Of the 18 patients, 6 had isolated bacteremia, 8 had both bacteremia with meningitis, 2 had isolated meningitis, 1 had bacteremia and pleural effusion, and the final patient had bilateral knee arthritis with bacteremia and meningitis. Over the next decade, listeriosis was noted increasingly as an infectious complication in immunocompromised patients. This trend resulted largely from more aggressive treatment of various malignancies and the more widespread use of immunosuppressive medications in transplant patients and patients with autoimmune disorders. Other chronic illnesses such as diabetes, chronic renal failure, and alcoholism/chronic liver disease also predispose to listeriosis.

Renal transplant patients currently represent the largest single group of immunocompromised patients at risk for listeriosis (2). In one series, all of the renal transplant patients were taking immunosuppressive medications, particularly corticosteroids (and almost always in doses greater than or equal to 30 mg of prednisone daily) (48). Patients were at greatest risk during the first two months after transplantation. Three-quarters of the cases occurred during the first year following transplantation although cases as late as five years post-transplantation were seen (48). Interestingly, it was noted early in the AIDS epidemic that *L. monocytogenes* is uncommon among AIDS patients (36). However, in the past several years, small series of listeriosis in AIDS patients have appeared (49-51). Thus, while the incidence of listeriosis in AIDS patients is surprisingly low compared to other opportunistic pathogens, its incidence is manyfold greater than that in the general population.

Primary bacteremia is a common form of listeriosis in nonpregnant, immunocompromised adults, occurring in approximately one-third of patients (9,43,48). These patients can have a spectrum of illness ranging from fever and "flu-like" syndrome without localizing signs to frank sepsis with hypotension (2,7,9). Chest X-ray abnormalities, including cavitary pneumonia, have been described in a small proportion of patients with primary bacteremia (43,48). In one small series from the University of

Pittsburgh, bacteremic patients presented with severely abnormal hepatic transaminases, thereby mimicking acute viral hepatitis (52).

Meningitis and meningoencephalitis (meningitis with focal findings) comprise 50% or more of listerial infections in most series (2,9,43,48), and in a series from Sloan-Kettering Memorial Hospital *L. monocytogenes* was the most common cause of meningitis in patients with underlying neoplastic disease (53). While capable of causing an acute meningitis comparable in severity to those caused by the pneumococcus and meningococcus, listerial meningitis is also known for its subacute presentations. The CSF laboratory parameters in patients with listeria meningitis may differ substantially from those in patients with other forms of bacterial meningitis. Approximately one-third of patients with listerial meningitis have a lymphocytic predominance in their CSF (48). CSF protein levels are usually, though not universally, elevated but often only to levels associated with forms of "aseptic" meningitis. CSF glucose levels are often only mildly depressed or even within normal range, and in one large study, a severely depressed CSF glucose was a poor prognostic indicator (54). Patients also have been described with normal CSF values at presentation (43,55). Less common listerial infections of the central nervous system include cerebritis and frank brain abscess (43,48,56). Though less common than other causes of brain abscess in immunocompromised persons, *L. monocytogenes* clearly must be considered in the differential diagnosis of such patients.

L. monocytogenes is an uncommon cause of endocarditis, representing a very small proportion of all reported cases of listeriosis (7,9). Approximately half of such patients have underlying cardiac lesions, including prosthetic valves, while many, though not all, of the remainder have a variety of debilitating illnesses (9,57). Listerial endocarditis is clinically indistinguishable from more common causes and usually presents with subacute disease (9,57). Other focal infections (e.g., osteomyelitis, arthritis, liver abscess, and endophthalmitis) occur mainly in immunocompromised persons and are the result of metastatic implantation during bacteremia (2,7,43,48).

THERAPY OF LISTERIOSIS

No large controlled trials have ever been conducted to determine the optimal treatment regimens for different forms of listeriosis. For this reason, certain aspects of treatment have remained controversial (2). Nevertheless, therapeutic principles have emerged from in vitro and animal studies in conjunction with historical experience over the past several decades.

In the 1950's it was discovered that penicillin and ampicillin are efficacious for the treatment of listeriosis, including meningitis. In 1972, Moellering et al. (58) and used modern microbiological methods to study the minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) for different antibiotics against *L.*

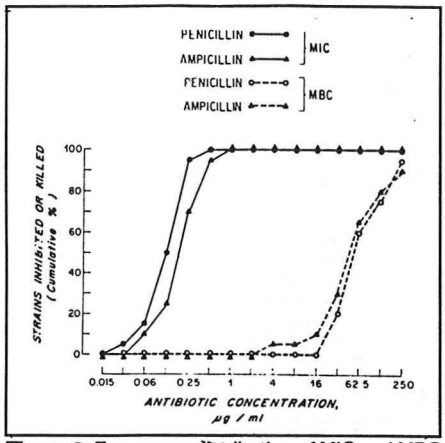

Figure 8. Frequency distribution of MIC and MBC values for ampicillin and penicillin against 20 *L. monocytogenes* strains

monocytogenes. They demonstrated that the MICs of twenty different clinical strains for both penicillin and ampicillin were well within the concentrations achievable in serum and CSF (Fig. 8). However, the MBCs for the same antibiotics were considerably above the MICs, a condition now referred to as "tolerance" (Fig. 8). Gentamicin, on the other hand, showed a much narrower spread between the MICs and MBCs for these clinical strains (58). Of particular importance, combinations of penicillin or penicillin plus gentamicin showed enhanced killing against nearly all of the strains; the concentrations of gentamicin required for synergy can be achieved in the CSF using intravenously administered antibiotic. These basic findings have been confirmed by other investigators (3,59-61), and the superiority of the β-lactam-gentamicin combination over penicillin or ampicillin alone has been demonstrated in the mouse model of listeria sepsis and the rabbit model of experimental meningitis (59,62). In some in vitro studies, the MICs and MBCs for ampicillin were lower than those of penicillin (3,7). Ampicillin also performed better than penicillin in the rabbit model of experimental *L. monocytogenes* meningitis (62), a finding which was supported by two retrospective analyses of listeria meningitis (54,55). On the basis of these cumulative data, ampicillin is generally preferred to penicillin and many authorities recommend the ampicillin-gentamicin combination (2,7). This combination is also rational if one considers the need for bactericidal antimicrobial therapy in the setting of meningitis, especially in immunocompromised individuals. Although some authorities recommend the routine use of intrathecal gentamicin for listeria meningitis (2), in my opinion, intrathecal aminoglycoside should be reserved for refractory cases of meningitis (63).

It is important to note, that, unlike penicillin and ampicillin, cephalosporins, particularly the second and third-generation cephalosporins, have poor activity in vitro against *L. monocytogenes* (Table 3) (3), and treatment failures with these agents (especially in meningitis) have been reported (7). Trimethoprim with or without sulfamethoxazole has excellent in vitro bactericidal activity against *L. monocytogenes* and this combination has done well in clinical practice, including patients with meningitis (64,65). Vancomycin also has excellent activity against *L. monocytogenes* and is another alternative for penicillin-allergic patients (66). Rifampin, an antibiotic which achieves good concentrations within phagocytes and frequently synergizes with other β-lactams, is theoretically attractive for combined therapy of listeriosis (67). However, in vitro and animal model studies have failed to support the effectiveness of β-lactam-rifampin combination therapy (59).

Table 3. Activity of some β-lactam antibiotics against *L. monocytogenes*

Antibiotics	Range of MICs (mg/l)	Break point values (mg/l)
Penicillin G	0.06–2	0.25–16
Oxacillin	0.5–4	2
Ampicillin	< 0.03–1	4–16
Amoxicillin	0.06–0.5	4–16
Carbenicillin	2–8	128
Piperacillin	0.12–8	16–128
Imipenem	< 0.03–4	4–8
Cephalothin	0.25–16	8–32
Cefoxitin	16–64	8–32
Cefotaxime	1–> 128	4–32
Cefoperazone	8–64	4–32
Ceftriaxone	4–> 128	4–32
Latamoxef	8–128	4–32

The recommended durations of therapy for different forms of listeriosis are shown in Table 4. Noting the tendency for listerial meningitis to relapse, some authorities recommend that meningitis patients receive a minimum of 3 weeks of therapy. Interestingly, AIDS patients with listeriosis have not required maintenance therapy, in contrast to the situation with another intracellular bacterial infection, salmonellosis, which requires much longer and even chronic therapy to prevent relapses (49-51).

Table 4. Duration of Treatment of Listeriosis

Clinical Syndrome	Length of Treatment (wks.)
Neonatal Listeriosis	2
Listeriosis in Pregnancy	2
Primary Bacteremia	2-3
Meningitis	2-3
Endocarditis	4-6
Other Focal	4-6

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