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Summary: *Listeria monocytogenes* is a foodborne pathogen well known for its adaptability to diverse environment and host niches and its high fatality rate among infected immunocompromised populations. Infection in the immunocompetent host occurs but risk factors for the disease primarily points to abnormalities in cell-mediated and innate immunity as major predispositions to listeriosis. After ingestion of contaminated food, this pathogen is able to cross the intestinal, blood-brain and placental barrier and leads to gastroenteritis, meningitis and maternofetal infections which may result in abortion and spontaneous stillbirth.

Despite the extensive use of this bacterium in the study of cell-mediated immunity and intracellular growth, our understanding of the host, pathogen and environmental factors that impact the pathogenesis of listeriosis is still incomplete.

This review will summarize current knowledge, including our own efforts, about pathogen, host and environmental factors that influence, and contribute to the pathogenesis of *Listeria monocytogenes* infection.

Key words: *Listeria monocytogenes,* listeriosis, mouse model, *Acanthamoeba castellanii.*

INTRODUCTION

Listeria is a genus of rod-shaped, motile, facultative intracellular, Gram-positive bacteria that are ubiquitously distributed in the environment. Of the six species included in the genus, only *L. monocytogenes* (LM) and *L. ivanovii* are pathogenic and cause disease, while *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi* are generally considered to be saprophytic. Weak β-haemolysis on blood agar is one of the features to differentiate pathogenic LM from harmless strains like *L. innocua* (Fig. 1).

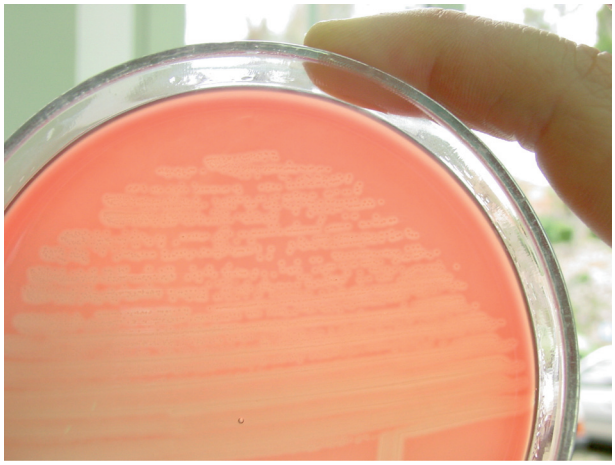

Figure 1. LM is beta-hemolytic on sheep blood agar plates but often produce only narrow zones of hemolysis that frequently do not extend much beyond the edge of the colonies. (Photography from the collection of Department of Microbiology, Medical Faculty University of Rijeka)

LM has become recognised as an important cause of human foodborne infection during recent decades. Bacteria can act as a saprophyte or a pathogen, depending on its environment. LM tolerates a wide range of harsh environmental conditions including high salt, acidic pH, and low temperatures which actually serve as an effective enrichment for the organism. LM can multiply at refrigeration temperatures, thereby challenging an important defense against food-borne pathogens, refrigeration.

There are 13 recognised serotypes of LM that can cause disease, but most human isolates belong to only three serotypes: 1/2a, 1/2b and 4b. After ingestion, LM

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is capable of crossing the intestinal, placental and blood-brain barriers in humans and causing infections ranging from asymptomatic carriage to severe mother-to-child infections as well as infections of the central nervous system, with a mortality rate of about 30%. LM is an invasive bacterial pathogen capable of multiplying inside many host cells, including macrophages, enterocytes and hepatocytes. It induces its own internalisation by host cells, followed by replication within the cytoplasm and direct transfer to another cell, hiding itself from the humoral immune host response.

MOUSE MODEL OF *LISTERIA MONOCYTOGENES* PATHOGENESIS AND HOST IMMUNE RESPONSE TO INFECTION

Area of microbial pathogenesis has become increasingly important due to emerging and re-emerging infectious agents, the increasing incidence of food-borne illnesses, opportunistic and nosocomial infections, or the purposeful release of highly infectious agents into the environment. An important weapon in the battle of infectious diseases is a basic understanding of the mechanisms of pathogenesis of infectious agents and the interaction of the pathogen with the host. LM is a model organism for cellular microbiology and host-pathogen interaction studies (1).

Although other animals, such as guinea pigs, seem to be better suited to study the immune response to LM, mice have been proven the most useful model for immunological studies due to availability of knock-out mice deficient in specific genes (2). Hence, most of our knowledge of how the immune system functions has been learned from experimental infections of mice using LM and the subsequent analysis of the innate and adaptive immune responses (3).

Mouse studies typically employ intravenous (i.v.) or intraperitoneal injection of LM, which results in systemic infection. Previously, we found that after i.v. injection bacteria quickly disseminate to the liver and spleen and successful clearance from infected organs depends on the appropriate onset of host immune responses (4). During the first three days of infection, different innate immune cells e.g. monocytes, neutrophils, natural killer (NK), and dendritic cells, mediates bactericidal mechanisms that minimize bacterial proliferation. CD8+ T cells are subsequently recruited and responsible for clearance of *Listeria* from the host, within 10-11 days of infection. At different time points we determined the number of colony forming units (cfu) of bacteria in the liver of infected animals and paralled with the plasma levels of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) measured by enzyme immunoassay. INF-γ production occured in the early phase but was more pronounced after day 4, following the appearance of specific immunity. Mice produced measurable amounts of plasma TNF-α immediately after being given viable LM, peaking on day 2 when the greatest number of bacteria was present in the examined organs. The quantity of IL-6 increased and decreased in concordance with clearance of LM and the clinical status of the animals (5).

In primary *Listeria* infection protection is achieved through production of proinflammatory cytokines (IFN-γ, TNF) crucially involved in the modulation of the antimicrobial activity of macrophages. However, in secondary infection protection is mediated primarily by CD8+ cytotoxic T lymphocytes. Determination and comparison of infection kinetics between different mouse strains is also an important method for identifying host genetic factors that contribute to immune responses against LM. Comparison of host responses to different *Listeria* strains is also an effective way to identify bacterial virulence factors that may serve as potential targets for antibiotic therapy or vaccine design (6).

LISTERIA MONOCYTOGENES AS A FOOD-BORNE PATHOGEN

Since the 1980s when several food-associated outbreaks were identified in Europe and North America, LM has been recognized as emerging foodborne pathogen. The infectious dose is still uncertain but there are indications that the minimum number of bacteria that must be ingested to represent a significant risk of disease is low, possibly less than 10^3 cells/g, depending on host and strain. Animal studies using rhesus monkeys and guinea pigs have both estimated LD_{50} of approximately 10^7 LM cfu similar to the FAO/WHO estimation of human LD_{50} of 1.9×10^6 cfu based on outbreak data (7, 8, 9).

In an attempt to mimic natural listeriosis we studied the pathogenesis of listeriosis in BALB/c mice following intragastric inoculation of bacteria (10). Animals received high inoculum of 10^8 cfu of LM after overnight fasting, or were repeatedly challenged with intra-gastric administration of low bacterial dose for 3 consecutive days. We have found that equally pronounced systemic infection can be achieved by single high dose or repeated application of low bacterial dose. Repeated administration of low dose, exhibited cummulative effect, and increased the severity of liver infection, resulting in a pronounced necrotizing hepatitis and extreme metabolic liver disfunction according to high levels of serum aminotransferases. In addition, LM was isolated from the brain tissue of all infected mice, even though histological examinations revealed

no or only minor lesions (11). These data suggest two hypothesis for human infection. A single high dose or prolonged consumption of low doses of LM may both result in clinically evident illness.

Listeristatic control measures, such as antimicrobial substances, can prevent LM from growing in food. Over the past decade many naturally occurring phenolic phytochemicals from herbs and spices have been shown to possess antimicrobial activity against food--borne pathogens. Thus, the antimicrobial potential of pure phenolics: hydroxytyrosol, tyrosol, 4-hydroxyphenylacetic acid, caffeic acid, gallic acid and 3, 4-dihydroxyphenylacetic acid as well as total polyphenols extracted from Croatian Ascolana monocultivar extra virgin olive oil was assessed in our laboratory using broth dilution and time-kill curve methods. Also, antilisterial activity of main pure phenolic compounds, such as hydroxytyrosol in olive oil, epicatechin in cocoa and carnosic acid in rosemary was each compared withantilisterial activity of their extracts. Rosemary extract, as well as carnosic acid, showed strong, while olive oil and particularly cocoa phenolic extract and epicatechin showed lower antilisterial activity (12). Our results suggest that rosemary, olive oil, and cocoa polyphenols posses potent antilisterial properties, and therefore could be used to protect human or animal health and also as alternative food additives for the prevention of food contamination with LM.

PREGNANCY-ASSOCIATED LISTERIOSIS

LM infection is relatively rare in healthy adults — although asymptomatic fecal carriage occurs in approximately 3% of healthy humans they seem to be able to eliminate LM from the intestines very efficiently. Risk factors for the disease primarily points to abnormalities in cell-mediated and innate immunity as major predispositions to listeriosis. The high-risk Y.O.P.I. (13) populations for listeriosis include the Young (fetus or newborn), Old, Pregnant, and Immunocompromised persons such as transplant patients, and HIV infected individuals. However, about one-third of all reported listeriosis cases happen during pregnancy. When a pregnant woman acquires an infection, the health of both the woman and her fetus can be endangered. Pregnant women usually experience a mild, flu-like illness, but the infection increase an infant risk of prematurity, low birth weight and long-term disability because of brain affection.

In our study, using a mouse model of pregnancy--associated listeriosis we have done a careful analysis of the immune response, defining some of the factors that influence pregnant women's susceptibility to LM infection (14). We studied the kinetics of bacterial clearance, histopathological changes in maternal and fetal tissues, as well as, cytokine/chemokine pattern during the course of experimental infection. Evaluating the growth of listeria in liver of gestating mice, we have showed that listeria could still be isolated many days after their disappearance from the same organs of virgin mice. Primary infection persisted in gestating animals almost twice longer than in the virgin controls. The liver infection was resolved in 9–10 days in control versus 17 days in pregnant mice (15).

Pregnant BALB/c mice challanged i.v. showed failure of maternal anti-listerial immune response at the systemic (significantly reduced serum IFN-γ levels) and local level (reduced expression of proinflammatory cytokines and chemokines in the liver tissue) leading to devastating necrotizing hemorrhagic hepatitis. The aggravated course of the infection could be attributed to a suppressed transcription and production of anti-listerial, proinflammatory cytokines and chemokines, namely IFN-γ, TNF-α, interleukin-12p40, inducible nitric oxide synthase, murine monokine induced by interferon-gamma, and interferon-gamma-inducible protein-10 (16).

So, during the course of Listeria infection, the maternal immune system of pregnant woman is faced with a rather ironic choice: to response in Th1 manner and eliminate an intracellular infection, or to maintain Th2 response to protect her pregnancy. But in attempting to solve the infection she may loos her fetuses, and in attempting to protect the fetus by maintaining Th2 bias, she may herself succumb to the infection.

PERINATAL LISTERIOSIS

Intra-amniotic infection can result from LM that crosses over from the maternal circulation to the amniotic sac or by ascending bacteria that inhabit the genital tract. If a mother becomes infected with LM, the fetus is affected in more than 90% of cases. When bacteria reach the fetus through the placenta, the placenta will often show evidence of infection, including a leukocytic infiltrates, microabscesses, and infarction (Fig. 2). Sequelae of intrauterine infection include spontaneous abortion (2nd/3rd trimester), stillbirth, preterm labor, and neonatal sepsis.

It is well known that the placenta plays an integral role in the pathogenesis of congenital infections. In our mouse model of pregnancy-associated listeriosis, placental colonization involved all or only some of the placentas in the same uterus. The severity of placental infection predicted whether the fetus was aborted or resorbed. LM were frequently detected in the tissues of the resorbing fetuses while only occasionally from the

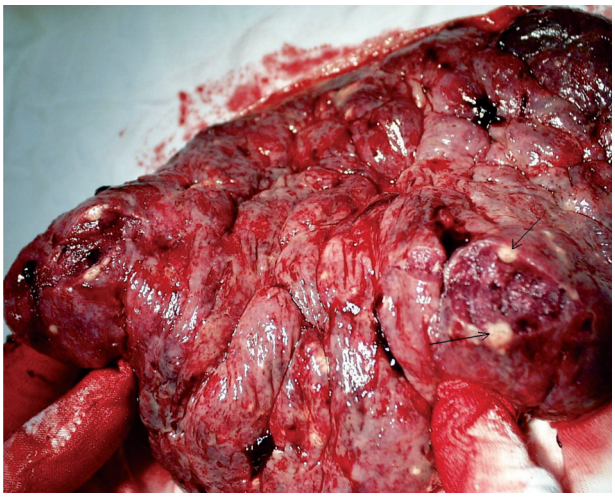

Figure 2. The maternal surface of 38-week placenta from 31 year old primigravida with LM infection. Transverse cut reveals white-yellowish nodules (black arrows) in intervillous space. (Photography from the collection of Department of Pathology, Medical Faculty University of Rijeka)

tissues of the aborted fetuses. At days 2 and 3 p.i., the placenta was characterized by large, hemorrhagic necrosis in association with numerous bacteria which covered the entire organ, while the inflammatory reaction was confined to single granulocytes, but T cells and other cellular elements necessary for effective antilisterial defence were absent. It was obvious that immune response at the materno-fetal interface was insufficient to control bacterial proliferation in placental tissue. During the course of infection, TNF-α and occasionally, IFN-γ were transcribed in placental tissue. Increased levels of these anti-listerial cytokines were not sufficient to control bacterial growth, but may eventually contribute to spontaneous fetal loss and poor pregnancy outcome.

In order to determine the importance of TNF-α and its receptor 1 (TNFR1) signalling in antilisterial immunity, neonatal, two days old, TNFR1 knock-out (KO) mice, and age-matched C57BL/6 wild type (WT) controls were infected with attenuated actA deficient LM. The course of infection, pathological changes, cellular response and expression of different mediators in neonatal liver and brain were studied (17). 100% of the TNR1 KO mice succumbed while all WT mice survived the infection. Induction of protective immune response in WT pups resulted in the prompt control of infection with an attenuated actA mutant LM, accompanied by enhanced hepatic expression of mRNA for IFN-γ, TNF-α, and IL-10. Conversely, the lack of TNFR1 signalling in TNFR1KO neonatal mice resulted in substantial changes in the profile of inflammatory mediators and ultimately fatal outcome of the infected pups, which experienced increasingly severe hepatitis, meningitis, and brain abscesses. In addition to the exaggerated production of inflammatory mediators, large necrotic lesions consisting of granulocytes and macrophages were scattered throughout the liver of these mice. TNFR1KO neonates were unable to clear neutrophils and switch from the innate immune response to a specific reaction mediated by T-cells. The loosely associated lymphocyte and macrophage infiltrates in the liver of TNFR1KO mice failed to form granulomas and to prevent progressive infection. The course of listeriosis and mortality rate of TNFR1 KO mice shows the critical importance of TNFR1 for the efficient antilisterial host protection during the neonatal period (18).

ENVIRONMENTAL LIFE OF *LISTERIA MONOCYTOGENES*

LM is widely distributed in natural and man-made environments such as soil and vegetation, freshly harvested grass, polluted water, sewage, fecal material, animal feed (silage and straw), etc. It can also be found in food especially dairy, processing environments on walls, floors, drains, ceilings, equipments, etc. Despite ubiquitous distribution of LM in the nature and ability to survive in different harsh conditions, the way of its environmental transmission and ecology is still uncelar.

One important aspect of environmental survival of LM may be involved in the interplay with free-living amoebae, a common representative of natural ecosystems. Amoebae may act as a predators, as well as an environmental reservoirs, and vehicles for the transmission of different facultative and obligate intracellular pathogens, like *Vibrio cholerae* (19), *Chlamydia pneumoniae* (20), *Legionella pneumophila* (21), *Francisella tularensis* (22), etc. However, the ability for in-

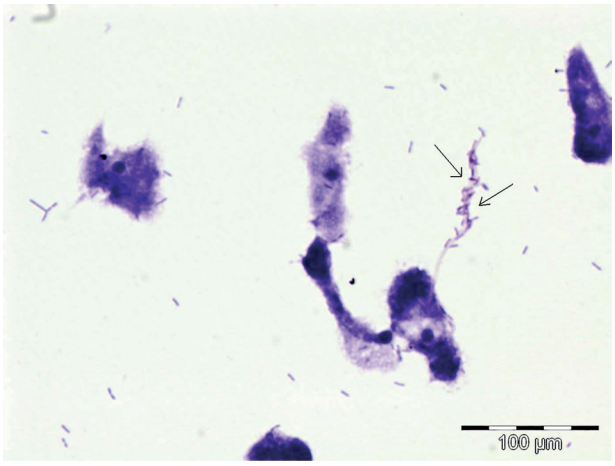

Figure 3. Giemsa-stained smear showing Acanthamoeba castellanii trophozoite exhibited specific ail shaped aggregation of LM cells (black arrows) after co-cultivation assay (x 1000)

tracellular multiplication and survival of LM in free-living protozoa is contraversial (23, 24, 25).

In our study, the interaction between the protozoan *Acanthamoeba castellanii* (ATCC 30234) and LM was investigated by viable counts and gentamicin assay, under different temperature regiment. Survival of bacteria and amoeba was checked at regular intervals, coupled with microscopy (26). Co-culture experiments under different laboratory temperatures suggest that *A. castellanii* acts as a predator on internalized listeria and that different temperatures have minor effect on its intracellular survival. At 22°C listeria was internalized, but after 4h of co-cultivation we were not able to recover any viable intracellular bacteria. An interesting observation during coincubation assays at room temperature, was the occurrence of a specific attachment of LM to an *Acanthamoeba* cell. LM accumulated on the outer surface of amoebae, resulting in formation of tail-shaped large aggregates at the uroid pole (Fig. 3). Similar phenomenon was described for *Hartmanella* spp. in the presence of monotrichous bacteria (27) as well as for some water-borne bacteria (28). The catching of bacteria seems to be part of a general phagocytosis mechanism.

In co-culture incubated at 35°C, listeria was ingested but was not able to establish an intracellular lifestyle within the eucaryotic host (26). LM had no killing effect on the amoebae but did cause rapid protozoan encysment, probably thanks to listeriolysin production. Although free-living amoeba and listeria may be found in the same environment it seems that LM is not maintained nor transferred in the environment by association with *A. castellanii*. However, the presence of amoeba enhanced the growth of LM according to higher numbers of extracellular bacteria when cultured with amoebae compared with growth in their absence. Higher numbers were likely sustained on metabolic waste products released during co-culture. Thus, it can be speculated that such cointeraction may be of importance for LM survival in its natural environment.

CONCLUSION

The ubiquity and saprophytic nature of LM and asymptomatic fecal carriage reduces the likelihood of complete elimination of bacterium from its natural environment. However, Food Safety standards and policies were developed to break the chain between host, environment and pathogen and help minimize the risk of foodborne illnesses. Efforts to reduce contamination are followed by declines in incidence of human illness. A concerted research effort has been directed toward an improved understanding of the LM bacterium and its pathogenic mehanisms. It is well recognized that *in vivo* approaches are required for full understanding of bacterial pathogenesis. In this review, we describe the utility of mouse model of infection for studying pathogenesis and immune response to the facultative intracellular pathogen LM. Using different combinations of mouse and bacterial strains, as well as different routes of infection, murine model of listeriosis may be used to explore a complete picture of LM infection and immunity. In addition, early diagnosis and better understanding pathogenesis of listeriosis and the relationship between host and bacteria, can contribute to the prevention, improve treatment and ameliorate the often severe consequences of this deadly disease.

A LIST OF ABBREVIATIONS

cfu = colony forming units
FAO = Food and Agriculture Organization
IL= interleukin
i.v. = intra venous
LD = lethal dose
LM = *Listeria monocytogenes*
NK = natural killer
TNF-α = tumor necrosis factor-alpha
TNFRKO = tumor necrosis factor receptor knockout
WHO = World Health Organization
WT = wild type

Sažetak

LISTERIOZA: PATOGEN, DOMAĆIN I OKRUŽENJE

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Listeria monocytogenes je patogen prisutan u prehrambenim proizvodima, dobro poznat po svojoj prilagodljivosti na različita okruženja i domaćine i po visokoj stopi smrtnosti među zaraženim populacijama imunokomprovitovanih. Infekcija se može javiti i kod imunokompetentnih domaćina, ali u faktore rizika pre svega spadaju abnormalnosti ćelijski posredovanog ili urođenog imuniteta, što je glavna predispozicija za oboljevanje od listerioze. Nakon konzumiranja kontaminirane hrane, ovaj patogen je sposoban da prođe crevnu, krv-

no-moždanu i placentalnu barijeru i prouzrokuje gastroenteritis, meningitis i intrauterine infekcije koje mogu izazvati abortus i spontanu mrtvorođenost.

Uprkos studijama koje ispituju ćelijski posredovan imunitet i intraceularni rast ove bakterije, razumevanje faktora kao što su domaćin, patogen i okruženje, koji mogu uticati na patogenezu listerioze, je još uvek nepotpuno.

Ovaj pregledni članak sumira aktuelna saznanja, uključujući i naša sopstvena nastojanja u proučavanju patogena, domaćina i okruženja kao faktora koji utiču i doprinose patogenezi infekcije koju izaziva *Listeria monocytogenes*.

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