Review

# Innate and adaptive immunologic functions of complement in the host response to *Listeria monocytogenes* infection

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ABSTRACT

*Listeria monocytogenes* is a leading cause of foodborne-illness associated mortality that has attracted considerable attention in recent years due to several significant outbreaks. It has also served as a model organism for the study of intracellular pathogens. For these reasons the host response to *L. monocytogenes* has long been the subject of investigation. A potent innate and adaptive immune response is required for containment and clearance of *L. monocytogenes*. However, some elements of this response, such as type 1 interferons, can be detrimental to the host. Recent studies have revealed novel functions for the complement system, an ancient arm of innate immunity, in this process. Here we review the role of complement in the host response to *L. monocytogenes*.

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## 1. Introduction

Foodborne illness has been a scourge of mankind from antiquity to the present day. Its death toll has left an indelible mark on human history. Although improvements in sanitation have greatly reduced its incidence, food poisoning continues to be a major problem today. As many as 1 in 6 Americans are sickened annually by contaminated food (CDC, 2010; Scallan et al., 2011). One of the most serious foodborne illnesses is listeriosis. The causative agent of listeriosis, the Gram positive bacillus *Listeria monocytogenes*, was first

identified in 1926 (Murray et al., 1926). Despite its early discovery, its route of transmission was not recognized until the early 1980s when an outbreak of listeriosis was linked to a coleslaw manufacturing plant (Schlech et al., 1983). The threat of *L. monocytogenes* to the food supply stems from several factors. First, *L. monocytogenes* is widely dispersed in the environment. Samples of soil, ground water, and fecal material from domestic animals often contain *L. monocytogenes* (Wing and Gregory, 2002). These materials frequently taint manufactured food products. Second, *L. monocytogenes* is endowed with remarkable hardiness. It tolerates both high salinity and acidity, treatments used in food preparation to limit bacterial growth (Cossart, 2011). Finally, in contrast to other pathogenic bacteria, *L. monocytogenes* proliferates at temperatures as low as 4° C (Taege, 1999). For these reasons, strict protocols for food preparation are enforced by regulatory agencies in the United States and abroad. Unfortunately, breakdowns in these protocols are common, resulting in outbreaks of listeriosis. A prime example occurred in 2011 with cantaloupes from Jensen Farms in Colorado (CDC, 2013). The CDC identified 147 cases, resulting in 33 deaths and one miscarriage. As a consequence, *L. monocytogenes* was responsible for the deadliest outbreak of foodborne illness in U.S. history.

Healthcare providers tend to view listeriosis as an uncommon condition (CDC, 2013). Healthy adults are resistant to *L. monocytogenes*, developing only mild gastroenteritis upon exposure. However, in the elderly, immunocompromised, and patients with chronic illness, listeriosis results in severe systemic disease associated with sepsis and/or meningitis (Allerberger and Wagner, 2010). The mortality rate following hospitalization is extremely high (20–30%) in comparison to more common foodborne illnesses such as salmonellosis and shigellosis (Wing and Gregory, 2002). Because of this, listeriosis is the third leading cause of death from food poisoning in the United States and the second leading cause in the European Union (CDC, 2013; Allerberger and Wagner, 2010). A second susceptible group are pregnant women and their unborn children (Wing and Gregory, 2002; Allerberger and Wagner, 2010). *L. monocytogenes* breaches the placental barrier and causes severe infections in the fetus, with outcomes including abortion, stillbirth or neonatal sepsis/meningitis. Therefore, listeriosis causes severe illness across the full span of human life, from the unborn to the elderly.

## 1.1. Life cycle of L. monocytogenes

Aside from its clinical significance, *L. monocytogenes* has been of great importance to the scientific community as a model organism for the study of intracellular pathogens. Accordingly, its life cycle and virulence factors are extensively described (Portnoy et al., 2002; Vazquez-Boland et al., 2001) (Fig. 1). *L. monocytogenes* readily enters non-professional phagocytes through a family of cell surface proteins called internalins. For example, the best characterized internalin, internalin A (InlA), binds E-cadherin and triggers cytoskeletal remodeling and bacterial internalization (Braun and Cossart, 2000). As E-cadherin is a junctional protein expressed by epithelial cells, InlA allows *L. monocytogenes* to penetrate the intestinal epithelial barrier. Curiously, murine E-cadherin does not act as a receptor for InlA (Lecuit et al., 1999). This explains the poor infectivity of *L. monocytogenes* by gastric lavage in mice. In line with this, transgenic mice expressing human E-cadherin are more susceptible to intragastric infection than WT mice, and mutant *L. monocytogenes* expressing a modified InlA that binds murine E-cadherin are 1000-fold more capable of infecting mice through the intragastric route (Lecuit et al., 2001; Wollert et al., 2007). Similarly, internalin B triggers internalization through its recognition of the host receptor tyrosine kinase Met (Cossart, 2001). Once inside the cell, *L. monocytogenes* secretes several virulence factors to lyse the phagosome. Of primary importance is the pore-forming molecule listeriolysin O (LLO) (Hamon et al., 2012). LLO-deficient *L. monocytogenes* strains are avirulent as they cannot leave the phagosome. *L. monocytogenes* also secretes phospholipases that, together with LLO, release bacteria into the nutrient-rich cytosol (Vazquez-Boland et al., 2001; Portnoy et al., 2002). Within the cytosol *L. monocytogenes* hijacks host actin filaments to move about the cell. This is achieved through the virulence factor ActA (Kocks et al., 1992). By polymerizing actin, ActA propels bacteria through the cell and ultimately allows their intercellular spread through protrusions of the host cell membrane into neighboring cells. Taken together, these factors make *L. monocytogenes* an extremely efficient pathogen by allowing it to live within the cell and evade immune recognition.

## 1.2. Host response to L. monocytogenes

The host response to *L. monocytogenes* has also been the subject of extensive study. Much of the work to date has focused on the adaptive immune response. A T cell response involving both CD4+ and CD8+ T cells is required for sterilizing immunity during both primary and secondary infection (Pamer, 2004; Unanue, 1997). In contrast, humoral immunity does not make a significant contribution, likely as a consequence of the bacterium's capacity for intercellular spread (Mackaness, 1962; North, 1973). CD4+ T cells confer protection through the secretion of IFN-γ, which increases the bactericidal capabilities of macrophages (Pamer, 2004; Zenewicz and Shen, 2007). Similarly, CD8+ T cells have bactericidal activity through a combination of cytokine production and cytolytic activity (Zenewicz and Shen, 2007).

While adaptive immunity is required for total clearance of *L. monocytogenes*, a potent innate immune response must precede it to provide bacterial containment and activate lymphocytes. In fact, the earliest response occurs within minutes of its injection into the bloodstream. Tissue macrophages rapidly sterilize the blood by phagocytosing the circulating bacteria (North, 1974). Kupffer cells (KCs), the tissue macrophages of the liver, play a major role in this process, and indeed the vast majority of *L. monocytogenes* is sequestered in this organ (Gregory et al., 1996). Neutrophils quickly infiltrate the liver and contribute to bacterial clearance (Carr et al., 2011; Conlan and North, 1994; Gregory et al., 1996; Rogers and Unanue, 1993). In contrast, they are dispensable for bacterial control in the spleen (Carr et al., 2011; Conlan and North, 1994). Cells of monocyte/macrophage lineage are paramount as their depletion or defective mobilization results in profound failure to clear the *L. monocytogenes* from either organ (Ebe et al., 1999; Kurihara et al., 1997; Serbina et al., 2003). Furthermore, many acute inflammatory cytokines contribute to the early host response to *L. monocytogenes*. Numerous studies have revealed essential roles for TNF-α, IL-6, IL-12, IFN-γ, and the IL-1 family (Dalrymple et al., 1995; Havell et al., 1992; Huang et al., 1993; Labow et al., 1997; Pfeffer et al., 1993; Rothe et al., 1993; Tripp et al., 1994). In addition to their ability to mobilize and activate neutrophils, monocytes and macrophages, these cytokines also drive the expression of IFN-γ by NK cells, providing an early innate source of that critical macrophage activating cytokine (Humann and Lenz, 2010; Tripp et al., 1993).

Although the overall direction of the innate immune response is protective during listeriosis, certain elements are detrimental. The anti-inflammatory cytokine IL-10 acts broadly to curtail inflammation and thereby limit immunopathology (Couper et al., 2008; Ouyang et al., 2011). Therefore, IL-10 acts as a double edge sword during infection. On one hand it can limit immune-mediated injury, but on the other hand it can dampen the immune response to pathogens. Examples of infectious models that fall on each side of the blade are plentiful (Couper et al., 2008). In listeriosis models, however, IL-10 is largely detrimental (Dai et al., 1997; Kelly and Bancroft, 1996; Wagner et al., 1994). Similarly, there is ample