Published in final edited form as:

Immunobiology. 2016 December ; 221(12): 1407–1417. doi:10.1016/j.imbio.2016.07.004.

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*.

Keywords

Complement; *Listeria monocytogenes*; Foodborne illness; Immune response

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

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Conflict of interest statement

The authors have no financial conflicts of interest.

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 (Taage, 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 evidence that the type I IFNs are harmful during systemic *L. monocytogenes* infections (Auerbuch et al., 2004; Carrero et al., 2004; O'Connell et al., 2004). Mice deficient in the type I IFN receptor (IFNAR) or the type I IFN-inducing transcription factor IRF3 are highly protected against *L. monocytogenes*. Type I IFNs sensitize lymphocytes and myeloid cells to apoptosis (Carrero et al., 2006; Stockinger et al., 2002). Apoptotic lymphocytes are plentiful in the spleens of *L. monocytogenes*-infected wild type mice, whereas few are seen in IFNAR1^{-/-} and IRF3^{-/-} mice (Carrero et al., 2004, 2006; Merrick et al., 1997). While leukocyte depletion is harmful in its own right, apoptotic lymphocytes also trigger IL-10 expression in splenic macrophages and dendritic cells (Carrero et al., 2006). Thus, type I IFNs set in motion a deleterious chain of events that inhibit the innate and adaptive responses to *L. monocytogenes*.

1.3. The complement system

A major element of innate immunity is the complement system. Complement consists of a large collection of secreted proteins that sequentially cleave one another to yield multiple effector molecules. This cascade of protein cleavage is triggered by pathogens *via* one of three pathways (Ricklin et al., 2010) (Fig. 2). While the pathways differ in their initiating factors and the molecules employed, all converge with the formation of a C3 convertase, a complex of complement fragments that activate C3, the cornerstone of the complement cascade. The first pathway identified, the classical pathway, is initiated by the C1 complex, a complex of C1q, C1r, and C1s. The pathway begins when C1q binds to antibody-antigen immune complexes and subsequently undergoes a conformational change. This conformational change triggers the autocatalytic activation of C1r, which in turn cleaves and activates C1s. Finally, C1s cleaves C4 and C2, forming the classical C3 convertase, C4bC2a. In the related lectin pathway, C1q is replaced by mannose-binding lectin (MBL) (Fujita et al., 2004; Wallis et al., 2010). MBL binds mannose moieties on bacterial surfaces and subsequently activates two MBL-associated serine proteases, MASP-1 and MASP-2. The complex of these molecules resembles the C1 complex and can similarly cleave C4 and C2 to generate C3 convertases. The last pathway of complement activation is called the alternative pathway (Harboe and Mollnes, 2008). The alternative pathway is unique in that it does not require a recognition factor like C1q or MBL. Rather, the pathway begins with the spontaneous hydrolysis of C3. In aqueous environments, a low level of C3 hydrolysis occurs constantly. Hydrolyzed C3 interacts with factor B and triggers the latter's cleavage by factor D to form a fluid phase C3 convertase, C3(H₂O)Bb. Under homeostatic conditions, these convertases cleave only a small amount of C3, and the resulting fragments, C3a and C3b, are

rapidly inactivated. However, C3b can attach to bacterial surfaces and join with factor B and properdin to form a stable alternative C3 convertase, C3bBb. The alternative pathway also acts as an amplification loop. C3b produced by any pathway can associate with factor B and properdin to form alternative C3 convertases. In fact, the majority of complement activation *in vivo* is thought to result from this amplification (Harboe and Mollnes, 2008). Finally, C3 convertases from either the classical or alternative pathways can interact with an additional C3b molecule to form C5 convertases (C4bC2aC3b or C3bBbC3b, respectively) that split C5 into C5a and C5b. C5b then concludes the complement cascade through its association with C6, C7, C8 and C9 to form the so-called membrane attack complex (MAC).

Complement activation thus results in the generation of many cleavage fragments. Of these, the major effector molecules are C3b, the MAC (C5b-9), and C3a and C5a. In addition to its role in C3 & C5 convertases, the C3b fragment is also an important opsonizing agent. Complement activation on bacterial surfaces leads to a coating of C3b. C3b is recognized by many complement receptors, including CR1 (CD35), CR3 (CD11b-CD18), and CR4 (CD11c-CD18) (van Lookeren Campagne et al., 2007). C3b-complement receptor binding triggers phagocytosis by cells of macrophage/monocyte lineage and neutrophils. Therefore, C3b plays an important role in the recognition and clearance of bacteria from the bloodstream and tissues. The MAC is also an essential component of host defense against bacteria. Upon inserting into the bacterial membrane, the MAC forms a transmembrane pore that causes bacterial lysis (Frank et al., 1987). Accordingly, complement deficiencies involving the terminal complement components C5-C9 associate with increased susceptibility to infection, especially by the encapsulated bacteria *Neisseria meningitidis* (Skattum et al., 2011). However, Gram positive bacteria like *L. monocytogenes* resist MAC-mediated killing due to their thick protective layer of peptidoglycan (Berends et al., 2013).

The final class of complement effector molecules are C3a and C5a (at times referred to as the complement anaphylatoxins). First identified in 1914 by Friedberger as the product of the reaction between serum and immune complexes that induces anaphylactic shock, it is now appreciated that the anaphylactic activity of activated complement resides in C3a and C5a (Bronfenbrenner, 1915; Wetsel et al., 2000). C3a and C5a act through two G-protein coupled receptors, the C3a receptor (C3aR) and the C5a receptor (C5aR1), respectively. There is also a second C5a receptor, C5aR2, which lacks an intracellular signaling domain and was initially thought to act solely as a “decoy receptor” with anti-inflammatory properties (Gerard et al., 2005; Scola et al., 2009). However, C5aR2 has emerged as having a more diversified and significant role in the pathophysiology of certain diseases, including immune complex lung injury (Chen et al., 2007) and experimental allergic asthma (Zhang et al., 2010). C3a and C5a activities are tightly regulated. This regulation is achieved in the serum by carboxypeptidase N (CPN) and carboxypeptidase R (CPR) (Matthews et al., 2004; Mueller-Ortiz et al., 2009). CPN & CPR are basic carboxypeptidases that cleave C-terminal arginine and lysine residues from peptides. This makes them powerful regulators of C3a and C5a as both have C-terminal arginine residues that are essential for receptor binding. The desarginated form of C3a has little to no biological activity, whereas C5a-desArg retains ~1–10% activity (Reis et al., 2012). However, recent studies suggest that C5a-desArg may retain nearly full activity in some cell types (Liang et al., 2011; Reis et al., 2012).

C3a and C5a have a broad range of biological activities outside of anaphylaxis. This is reflected in the wide tissue and cellular distribution of their receptors. Their greatest expression is seen in cells of the myeloid lineage – monocytes, macrophages, neutrophils, mast cells, basophils, eosinophils and dendritic cells (DCs) (Kirchhoff et al., 2001; Zwirner et al., 1999). In the last two decades, their distribution has been significantly expanded to include many parenchymal cells (Braun et al., 2004; Davoust et al., 1999; Drouin et al., 2001; Haviland et al., 1995; Wetsel, 1995). C3aR and C5aR1 have also been identified on cells of the lymphoid lineage such as T cells, B cells and NK cells (Fischer and Hugli, 1997; Fusakio et al., 2011; Nataf et al., 1999; Werfel et al., 2000). In many of these cells, the anaphylatoxins have chemotactic activity. In particular, C5a has long been recognized as one of the strongest chemotactic agents for neutrophils (Marder et al., 1985). Outside of chemotaxis, the anaphylatoxins also drive immune cell activation. For example, C5a primes the respiratory burst and triggers degranulation in neutrophils (Bender et al., 1983; Goldstein and Weissmann, 1974). A multitude of studies have also established that C5a potentiates the expression of pro-inflammatory cytokines like TNF- α , IL-6 and IL-1 (Okusawa et al., 1988; Schindler et al., 1990; Scholz et al., 1990; Zhang et al., 2007). Thus, C3a and C5a are often characterized as pro-inflammatory molecules due to their ability to recruit leukocytes, trigger immune cell activation, and enhance inflammatory cytokine production.

However, there is also evidence that C3a and C5a can have regulatory effects during the immune response. For example, C5a is a potent suppressor of IL-12 in monocytes and macrophages (Braun et al., 2000a,b; Hawlisch et al., 2005; Wittmann et al., 1999). Supporting these *in vitro* observations, C5aR1 $^{-/-}$ mice infected with the parasite *Leishmania major* have a greater IL-12 mediated Th1 response than infected WT mice and thereby control the parasite more effectively (Hawlisch et al., 2005). Outside of IL-12 regulation, C5a also heightens the expression of the anti-inflammatory cytokine IL-10 in LPS-treated macrophages *in vitro* and during endotoxemia *in vivo* (Bosmann et al., 2012). IL-10 in turn represses the expression of IL-17A, an important mobilizer of neutrophils during bacterial infections (Bosmann et al., 2012). Therefore, C3a and C5a cannot be categorized simply as pro-inflammatory or anti-inflammatory molecules. C3a and C5a are perhaps best described as immune modulating agents (Grailer et al., 2013).

1.4. Complement and *L. monocytogenes*

There has long been interest in the role of complement in listeriosis. As early as 1974 it was reported that heat labile factors in serum enhance the killing of *L. monocytogenes* by human monocytes, providing the first indication of an anti-listerial function for complement (Steigbigel et al., 1974). Subsequent work definitively established that the cell wall of *L. monocytogenes* activates complement (Baker et al., 1977). Incubation of the cell wall fraction with animal serum resulted in reduced hemolytic activity and the generation of a neutrophil chemotactic factor. The molecular weight of the chemotactic factor was approximately 15 kDa, a value consistent with the molecular weight of C5a. It was further demonstrated that this complement activation occurred through the alternative pathway. The production of the chemotactic factor was sensitive to EDTA, an inhibitor of both the classical and alternative pathway, but not EGTA, an inhibitor of the classical pathway alone. This study thus provided both clear evidence that *L. monocytogenes* activates complement

and the first suggestion that the resulting complement C3a and C5a peptides participate in the host response.

2. Complement opsonizes *L. monocytogenes*

When bacteria enter the bloodstream they are rapidly opsonized by serum factors such as immunoglobulins and complement. In rodent models, *L. monocytogenes* is removed from the circulation at an extremely rapid rate by KCs in the liver (Gregory et al., 1996). Presumably, opsonization drives this early clearance through the promotion of phagocytosis. Many groups have attempted to determine the relative importance of immunoglobulin and complement in this process with mixed results. Consistent with the work of Baker et al. (1977), several groups have determined that the opsonization of *L. monocytogenes* occurs largely through complement activation *via* the alternative pathway in mouse serum (Drevets and Campbell, 1991; van Kessel et al., 1981). In man, however, contrasting results were reported. For example, in human serum C3 was found to deposit on the surface of *L. monocytogenes* through alternative pathway activation (Croize et al., 1993). On the other hand, two groups observed that the IgG fraction of human serum drives opsonization and that complement was dispensable as heat inactivation had no effect in their assays (MacGowan et al., 1983; Peterson et al., 1977). Finally, another report indicated a role for both immunoglobulin and complement in *L. monocytogenes* opsonization by human serum (Bortolussi et al., 1986). In their experiments, optimal opsonization required both heat labile activity and the IgM (but not IgG) fraction of serum. In line with this, zymosan-treated serum lacking alternative pathway activity could opsonize *L. monocytogenes*, whereas C4 inactivator-treated serum lacking classical pathway activity could not. It is noteworthy that in these studies low concentrations of serum (3%) were used in an attempt to mimic conditions found at sites of infection rather than those in the circulation. When higher concentrations of serum (10%) were tested in their assays, heat inactivation did not eliminate opsonization (Bortolussi et al., 1986). The reason for the discrepancies between these studies is not entirely clear. As this variation arose in human studies in which serum was prepared from multiple donors and in different countries, the differences may reflect the exposure status of the serum donors. In contrast, *L. monocytogenes* exposure may be uncommon in laboratory animals, resulting in a lack of *L. monocytogenes*-specific immunoglobulin and thus a complete dependence on complement. Regardless, it is clear that complement can opsonize *L. monocytogenes*.

3. CR3 and *L. monocytogenes*

Multiple complement receptors bind to C3b-coated bacteria and trigger their phagocytosis (van Lookeren Campagne et al., 2007). Early work revealed a critical role for CR3 in the host response to listeriosis *in vivo* (Rosen et al., 1989). Mice treated with an anti-CR3 monoclonal antibody developed massive bacterial proliferation in their liver and spleen and complete mortality at an infectious dose that otherwise caused a modest sublethal infection in untreated animals. Careful histological analysis demonstrated a failure of neutrophils and monocytes to organize around infective foci in the liver. The work also ruled out a role for CR3 in early bloodstream sterilization. CR3 blockade had no effect on the kinetics of bacterial clearance from the blood, indicating that KCs are not dependent on CR3 to

phagocytose *L. monocytogenes*. However, it must be recognized that as an integrin receptor, CR3 also has complement-independent functions. Multiple ligands for CR3 outside of the complement system have been identified, including ICAM-1 and extracellular matrix proteins (Ehlers, 2000). Thus, it is unclear what extent of this phenotype (organization of neutrophils and monocytes around infective foci) was complement-dependent.

Subsequent studies from Drevets et al. established that CR3 contributes to listerial phagocytosis and killing by macrophages *in vitro* (Drevets and Campbell, 1991; Drevets et al., 1992; Drevets et al., 1993). Incubation with serum was found to greatly enhance the phagocytosis of *L. monocytogenes* by macrophages (Drevets and Campbell, 1991). When macrophages were treated with an anti-CR3 monoclonal antibody, the effect of serum treatment was lost. Neutralization of C3 similarly reduced phagocytosis, demonstrating that C3b/C3bi-CR3 interactions were responsible for the enhanced phagocytosis. It was later found that listericidal macrophages such as proteose peptone-elicited macrophages utilize CR3 for bacterial phagocytosis to a greater extent than non-listericidal macrophages such as those elicited by thioglycollate (Drevets et al., 1992). Furthermore, listericidal macrophages restricted *L. monocytogenes* to the phagosome, whereas in non-listericidal thioglycollate-elicited macrophages, *L. monocytogenes* lysed phagosomes and spread throughout the cytoplasm. These observations lead to the hypothesis that CR3 promotes listerial killing *via* phagosomal containment. To test this hypothesis, peptone-elicited macrophages were treated with anti-CR3 mAbs, and their ability to kill *L. monocytogenes* was assessed (Drevets et al., 1993). As in the case of phagocytosis, CR3 blockade dose-dependently inhibited bacterial killing. In fact, at high doses of anti-CR3 peptone-elicited macrophages were converted into permissive hosts. However, while blocking CR3 diminished listerial killing, it did not result in bacterial escape from the phagosome. Thus, complement and CR3 promote the phagocytosis and killing of *L. monocytogenes* in murine macrophages through an undetermined mechanism independent of phagosomal containment.

4. CR1g and *L. monocytogenes*

As mentioned previously, *L. monocytogenes* is rapidly cleared from the bloodstream by KCs in the liver following i.v. injection. Since complement opsonizes *L. monocytogenes* through alternative pathway activation on the bacterial surface, its participation in this process seemed likely. However, KCs express little CR3 (Lee et al., 1986), and blocking experiments indicated a lack of CR3 involvement (Rosen et al., 1989). This discrepancy was resolved in 2006 with the discovery of an additional complement C3b receptor, the Complement Receptor of the Immunoglobulin superfamily (CR1g) (Helmy et al., 2006). The expression of CR1g is restricted to tissue macrophages such as KCs and a subset of peritoneal macrophages. To examine the contribution of CR1g to early listerial clearance, KCs were isolated from WT and CR1g^{-/-} mice one hour after i.v. infection. KCs from CR1g^{-/-} mice contained substantially less *L. monocytogenes* than WT KCs, demonstrating a role for CR1g in the clearance of opsonized *L. monocytogenes* from the blood. This reduced KC uptake resulted in a redistribution of *L. monocytogenes* within CR1g^{-/-} mice, with less *L. monocytogenes* depositing in the liver and more in the spleen and lungs as early as 10 min post-injection in comparison with WT mice. CR1g^{-/-} mice were also more susceptible to *L. monocytogenes*-induced mortality. Thus, CR1g is at least partially responsible for the early

clearance of *L. monocytogenes* from the blood. In its absence, *L. monocytogenes* is re-directed from the liver, a site of rapid early bacterial clearance *via* recruited neutrophils, to more permissive sites of infection like the spleen and lung. In addition, CRiG also contributes directly to the listericidal activity of macrophages. Treatment of macrophages with either an agonistic anti-CRiG antibody or C3b dimers enhances their ability to kill *L. monocytogenes* (Kim et al., 2013). This CRiG-mediated induction of bactericidal activity was linked to elevated phagosome-lysosome fusion. CRiG associates with CLIC3, a chloride ion channel, which increases the chloride ion concentration and acidification of phagosomes and is required for CRiG-mediated killing. Therefore, at least two complement C3 receptors, CR3 and CRiG, contribute to listerial killing by macrophages.

5. C1q, C1q receptors, and *L. monocytogenes*

In addition to the well documented roles of C3b/Cbi in promoting the phagocytosis and clearance of *L. monocytogenes*, two studies have demonstrated *in vitro* that C1q can also opsonize *L. monocytogenes* and mediate the enhanced uptake and phagocytosis by macrophages (Alvarez-Dominguez et al., 1993, 2000). Opsonization of *L. monocytogenes* by C1q was inhibited by diaminopimelic acid, L-rhamnose, and N-acetyl-muramyl-L-alanyl-D-isoglutamine dipeptide, indicating that C1q binds preferentially to polysaccharides contained in the cell wall skeleton of *L. monocytogenes*. Type I collagen did not inhibit C1q binding to *L. monocytogenes*, suggesting that C1q attaches to the bacterial cell wall *via* its globular head domains. How *L. monocytogenes* bound C1q engages macrophages for phagocytosis was not examined in these investigations; however, it would not be surprising if the type I membrane glycoprotein C1q receptor (C1qRp/CD93) that binds the collagen stalk domain of C1q and enhances phagocytosis by numerous myeloid cell types was involved (Nepomuceno and Tenner, 1998).

gC1q-R is a ubiquitously expressed, highly anionic protein that was initially identified as a receptor for the globular heads of C1q (Ghebrehwet et al., 1994). It subsequently has been found to bind numerous other ligands, including vitronectin, and high molecular weight kininogen, and is at times referred to as the hyaluronic acid binding protein-1 (HABP-1) or p32 (reviewed in Ghebrehwet and Peerschke, 1998). It is predominately localized to the mitochondrial matrix, but it is found also in other cellular regions, including the endoplasmic reticulum and nucleus, as well as the cell surface where it is thought to be associated with lipid rafts (Dembitzer et al., 2012). As discussed earlier, *L. monocytogenes* invades cells *via* binding of bacterial surface proteins InlA and InlB to host cellular receptor E-cadherin or the receptor tyrosine kinase Met, respectively (Fig. 1). In 2000, gC1q-R was discovered to bind InlB, and antibodies directed against gC1q-R impaired entry of *L. monocytogenes* into mammalian cells *in vitro*, indicating that in addition to Met, gC1q-R may be another important molecule that facilitates entry of *L. monocytogenes* into host cells (Braun et al., 2000a,b). However, gC1q-R is not attached to cellular membranes *via* a cytoplasmic tail, suggesting that the important signaling pathways activated on interaction with InlB are transduced through another cofactor. It also has been reported that interaction between gC1q-R and InlB GW domains antagonize rather than enhance InlB signaling (Marino et al., 2002; Banerjee et al., 2004; Pizarro-Cerda et al., 2012). These findings plus the fact that C1q competes with InlB for binding to gC1q-R (Braun et al., 2000a,b), indicate

that the overall impact and exact role of gC1q-R in *L. monocytogenes* infection needs further clarification.

6. Complement and the adaptive response to *L. monocytogenes*

For many years, immunologists regarded innate and adaptive immunity as non-overlapping spheres. The involvement of innate immunity in the early containment of infections was recognized, but the adaptive response, a separate entity, was responsible for complete bacterial clearance. However, it is now widely appreciated that the two systems are fundamentally linked (Fearon and Locksley, 1996). The innate response initiates and shapes the direction of adaptive immunity. As a key component of innate immunity, complement is thought to participate in the adaptive T cell response to pathogens (Heeger and Kemper, 2012). Early studies of complement in viral infections demonstrated a requisite role for C3 and C5aR1 in T cell activation in response to both influenza virus and lymphocytic choriomeningitis virus (Kim et al., 2004; Kopf et al., 2002; Suresh et al., 2003). Complement regulates adaptive immunity through its actions on both antigen presenting cells (APCs) and T cells. APCs activate T cells *via* the engagement of antigen-loaded MHC molecules with antigen-specific T cell receptors, co-stimulatory molecules, and T cell polarizing cytokines. Upon exposure to antigen-specific T cells or TLR agonists, APCs mature and develop increased T cell stimulatory capacity. Consistent with a role for complement in this process, C3, C3aR, and C5aR1-deficient APCs express lower levels of class II MHC and co-stimulatory molecules CD80 and CD86 than WT APCs and have reduced T cell stimulatory capability (Li et al., 2008; Peng et al., 2006; Peng et al., 2007; Peng et al., 2009; Zhou et al., 2006). A requirement for T cell expression of C3, C5, C3aR and C5aR1 has also been reported (Lalli et al., 2008; Strainic et al., 2008). In these studies, resting T cells were found to express low levels of complement and complement receptors. Upon T cell activation, a substantial induction of C3, C3aR and C5aR1 expression occurred (Strainic et al., 2008). Like APCs, T cells deficient in C3aR and C5aR1 produced lower levels of the co-stimulatory molecules CD28 and CD40L following T cell activation (Strainic et al., 2008). Furthermore, C3aR and C5aR1 activated Akt in response to CD28 ligation, an integral component of Th1 differentiation (Strainic et al., 2008). As a consequence, the proliferation of C3aR^{-/-} C5aR1^{-/-} T cells following CD3/CD28 ligation was severely impaired. C5aR1-mediated activation of Akt also has a pro-survival role as it limits the induction of apoptosis in response to T cell activation (Lalli et al., 2008). This was linked to the up-regulation of Bcl-2 and down-regulation of the Fas receptor. Thus, complement may act directly on APCs and T cells at multiple levels to promote T cell immunity

To date, three studies have demonstrated a requirement for C3 in T cell activation during listeriosis. In the first study, C3^{-/-} mice were shown to develop fewer antigen-specific CD4⁺ and CD8⁺ T cells than WT mice following *L. monocytogenes* challenge (Nakayama et al., 2009). To investigate the mechanism by which C3 contributes to T cell activation, the phenotype of C3^{-/-} DCs was characterized. Again in contrast to prior work, Nakayama et al. (2009) found the maturation state of *L. monocytogenes*-infected C3^{-/-} DCs was comparable to WT DCs. In line with this, C3 deficiency did not impede the ability of DCs to activate CD8⁺ T cells *in vitro*. Nakayama et al. subsequently examined whether C3 directly

regulates T cell proliferation in listeriosis. Adoptive transfer experiments in which C3^{-/-} CD8⁺ T cells were introduced into *L. monocytogenes*-infected WT mice revealed comparable levels of T cell proliferation in donor (C3^{-/-}) and recipient (C3^{+/+}) cells. As transfer into a C3 sufficient environment allows C3^{-/-} T cells to activate normally it would appear that they do not have an intrinsic defect in proliferation. However, when purified CD8⁺ T cells were stimulated with plate-bound anti-CD3, less proliferation was observed in C3^{-/-} CD8⁺ T cells than in C3^{+/+} CD8⁺ T cells. Consistent with the findings of Nakayama et al., found that CD8⁺ T cell expansion was only one-third in C3^{-/-} mice infected with recombinant *L. monocytogenes* expressing OVA and deficient in Act A compared to infected WT mice (Tan et al., 2014). Interestingly, the infected C3^{-/-} mice in this study exhibited less CD8⁺ T cell contraction than infected WT mice. Reduced contraction in the C3^{-/-} mice was accompanied by an increase in CD8⁺ long-lived memory precursor cells and a decrease in IL-12 and IFN- γ , indicating that C3 may be involved in the regulation of CD8⁺ T cell memory, possibly through the regulation of key cytokines that are important in the differential regulation of CD8⁺ T cell subsets. Although both of these studies made a strong case for the importance of C3 in generating a robust T cell response to *L. monocytogenes*, neither of them delineated the C3 activation products (C3-derived or downstream of C3) that were responsible for their findings.

Work by Verschoor et al. also confirmed the requirement of C3 for optimal CD8⁺ T cell responses during listeriosis (Verschoor et al., 2011). However, their studies unearthed a substantially different mechanism. Whereas Nakayama et al. (2009) indicated in their studies that C3 acts directly on T cells to promote their proliferation during listeriosis, Verschoor et al. (2011) found that C3 drives T cell responses by targeting *L. monocytogenes* to APCs in the spleen. In order for a productive *L. monocytogenes* infection to develop within the spleen, CD8 α +DCs must be present in the red pulp (Edelson et al., 2011; Neuenhahn et al., 2006). Shortly after i.v. injection, *L. monocytogenes* is detected almost exclusively within these cells. The importance of complement in pathogen clearance from the circulation in other models of infection led Verschoor et al. (2011) to examine how C3 contributes to this phenomenon during *L. monocytogenes* infection. Consistent with a role for C3 in this process, C3 was required for optimal colonization of the spleen by *L. monocytogenes*. C3^{-/-} CD8 α +DCs were found to contain far less *L. monocytogenes* than C3^{+/+} CD8 α + DCs. Importantly, the targeting of *L. monocytogenes* to CD8 α + DCs by C3 was found to be dependent on platelet- *L. monocytogenes* aggregation. Platelets bind circulating *L. monocytogenes* through a newly identified C3b receptor, the glycoprotein GPIb. As with C3 deficiency, depletion of platelets or GPIb deficiency impaired the delivery of *L. monocytogenes* to CD8 α + DCs. Among the various DC subsets, CD8 α + DCs are uniquely capable of cross-presenting exogenous antigens through class I MHC to CD8⁺ T cells. In keeping with this, the impaired uptake of *L. monocytogenes* by CD8 α + DCs in the C3^{-/-} mice resulted in reduced CD8⁺ T cell expansion. Thus, C3 drives the development of an adaptive anti-listerial response *in vivo* by diverting a portion of *L. monocytogenes* away from the macrophages of the reticuloendothelial system to splenic CD8 α + DCs. This model therefore diverges from that of Nakayama et al. (2009) and others in which a direct role for complement in T cell activation has been proposed. Regardless, it is clear that C3 contributes

to the development of T cell immunity during listeriosis, possibly through both direct and indirect effects on T-cells.

7. Role of the C3a and C5a receptors in listeriosis

As discussed above, a considerable body of research has stressed the importance of C3 and complement-mediated opsonization in host defense against *L. monocytogenes*. On the other hand, the role of C3a and C5a has scarcely been examined. To fill this void, our laboratory recently investigated the role of C3aR and C5aR1 in a mouse model of systemic *L. monocytogenes* infection. While C5aR1's function has been extensively probed in various infectious disease models, few studies have assessed the importance of C3aR during infection (Mueller-Ortiz et al., 2006). Recently, the work of Mueller-Ortiz et al. has demonstrated a novel protective role for C3aR during listeriosis (Mueller-Ortiz et al., 2014). C3aR^{-/-} mice were found to have impaired resistance to *L. monocytogenes*, with increased bacterial burden and reduced survival in comparison with WT mice. Although C3aR contributes to optimal cytokine production in many models, a broad analysis of serum cytokine and chemokine levels revealed a non-essential role for C3aR in the production of protective cytokines or chemokines such as TNF- α and IFN- γ during listeriosis. As discussed earlier, splenocyte apoptosis is a major feature of *L. monocytogenes* infection that negatively impacts bacterial clearance through the induction of IL-10 expression. Since C3aR and C5aR1 have been reported to promote splenocyte and T-cell survival under homeostatic conditions (Liszewski et al., 2013) and during T cell activation (Lalli et al., 2008; Strainic et al., 2008), it was reasoned that C3aR might protect against *L. monocytogenes*-induced splenocyte death. In accordance with this hypothesis, splenocyte apoptosis was significantly increased and total splenocyte numbers were significantly reduced in *L. monocytogenes*-infected C3aR^{-/-} mice (Mueller-Ortiz et al., 2014). This splenocyte depletion was not restricted to T cells as nearly all splenocyte subsets were affected. In line with the work of Strainic et al., this increased susceptibility to apoptosis in C3aR^{-/-} splenocytes was associated with lower levels of the anti-apoptotic molecule Bcl-2 and higher levels of the pro-apoptotic receptor Fas (Mueller-Ortiz et al., 2014). To test whether the elevated apoptosis seen in C3aR^{-/-} mice was responsible for the phenotype, C3aR^{-/-} mice were treated with the apoptosis inhibitor Z-VAD before infection. Indeed, Z-VAD reduced bacterial burden in the liver, splenocyte apoptosis and serum cytokines including IL-10 to levels comparable to that of WT mice (Mueller-Ortiz et al., 2014). Thus, C3aR protects the host against listeriosis by inhibiting splenocyte death. A similar phenotype was recently described in C3aR^{-/-} mice infected with the intracellular bacterium *Chlamydia psittaci* (Dutow et al., 2014). C3aR^{-/-} mice were more susceptible to pulmonary *C. psittaci* infections and had significantly fewer T cells and B cells in their draining lymph nodes than WT mice. Taken together, this data may demonstrate a broad pro-survival role for C3aR in leukocytes during intracellular bacterial infections.

While these studies indicated a clear protective role for C3aR in the host response to *L. monocytogenes*, C5aR1's role remained uncertain. As C5a represses IL-12 expression (Braun et al., 2000a,b) and inhibits the Th1 response to the intracellular parasite *Leishmania major* (Hawlish et al., 2005), a detrimental role for C5aR1 in listeriosis could be envisioned. However, there was also reason to suspect a protective role for C5aR1. Several

decades ago it was reported that A/J mice, a mouse strain naturally deficient in C5, are more susceptible to *L. monocytogenes* (Czuprynski et al., 1985; Gervais et al., 1984). Of the two C5 fragments, the loss of C5b is an unlikely candidate for this susceptibility due to the resistance of Gram positive bacteria to MAC-mediated lysis (Berends et al., 2013). Thus, deficiency of C5a was most likely responsible for the increased susceptibility of A/J mice. Our work in C5aR1 deficient mice now supports this hypothesis (Calame et al., 2014). C5aR1^{-/-} mice were highly susceptible to *L. monocytogenes* infection, with higher bacterial burdens and reduced survival in comparison to WT mice. C5aR1 deficiency resulted in profound depletion of all splenocyte compartments as a result of increased splenocyte apoptosis. As with C3aR, C5aR1 was not required for the production of critical cytokines or chemokines. Instead, several pieces of evidence argue that C5aR1 conveys protection by inhibiting type 1 IFN expression. Serum levels of both IFN- α and IFN- β are significantly elevated in C5aR1^{-/-} mice. Similarly, TRAIL, a downstream target of type 1 IFN and a major driver of *L. monocytogenes*-induced splenocyte apoptosis, was significantly upregulated in splenic NK cells in C5aR1^{-/-} mice. Finally, blockade of the type 1 IFN receptor rescued C5aR1^{-/-} mice from *L. monocytogenes*-induced mortality (Calame et al., 2014). Subsequent work from our lab has clarified the mechanism by which C3a and C5a regulate type 1 IFN expression. These data demonstrate that C3a and C5a, *via* direct signaling through their specific receptors, suppress IFN- β expression by modulation of a distinct innate cytosolic surveillance pathway involving DDX41, STING, and other downstream molecular targets of *L. monocytogenes*-generated c-di-AMP (Mueller-Ortiz et al., abstract presented at XXVI International Complement Workshop, Kanazawa, Japan). Thus, suppression of type 1 IFN can be added to the list of regulatory functions of the C3a-C3aR and C5a-C5aR1 axes and a means by which C3aR and C5aR1 signaling protects against *L. monocytogenes*-induced apoptosis.

8. Conclusions

L. monocytogenes remains a major cause of morbidity and mortality from foodborne illness. The work reviewed here demonstrates multiple protective roles for the complement system in host defense against listeriosis from opsonization and intracellular killing to the promotion of leukocyte survival. *L. monocytogenes* has served as a platform for the discovery of several novel functions of complement, including the recognition of CRIg, C3-glycoprotein GPIIb/platelet-mediated bacterial clearance from the bloodstream, and C3a/C3aR and C5a/C5aR1 inhibition of type 1 IFN expression (summarized in Table 1). Undoubtedly, further discoveries in complement will be fueled by research involving *L. monocytogenes*. As there is presently substantial clinical interest in the use of complement inhibitors for the treatment of disease, it will be important to consider their impact on the immune response to bacteria such as *L. monocytogenes*.

Acknowledgments

This work was supported by National Institutes of Health Public Service Grant RO1 AI025011 (to R.A.W.) and the Hans J. Muller-Eberhard and Irma Gigli Distinguished Chair in Immunology.

Abbreviations

InlA	internalin A
InlB	internalin B
LLO	listeriolysin O
NK	natural killer
IFN	interferon
IFNAR	type 1 interferon receptor
MBL	mannose-binding lectin
MAC	membrane attack complex
CPN	carboxypeptidase N
CPR	carboxypeptidase R
DC	dendritic cell
WT	wild type
KC	Kupffer cell
CR1	complement receptor 1
CR3	complement receptor 3
CR4	complement receptor 4
CRlg	complement receptor of the immunoglobulin superfamily
APC	antigen presenting cell

References

- Allerberger F, Wagner M. Listeriosis: a resurgent foodborne infection. *Clin Microbiol Infect.* 2010; 16:16–23. [PubMed: 20002687]
- Alvarez-Dominguez C, Carrasco-Marin E, Leyva-Cobian F. Role of complement component C1q in phagocytosis of *Listeria monocytogenes* by murine macrophage-like cell lines. *Infect Immun.* 1993; 61:3664–3672. [PubMed: 8359889]
- Alvarez-Dominguez C, Carrasco-Marin, Lopez-Mato PE, Leyva-Cobian F. The contribution of both oxygen and nitrogen intermediates to the intracellular killing mechanisms of C1q-opsonized *Listeria monocytogenes* by the macrophage-like IC-21 cell line. *Immunology.* 2000; 101:83–89. [PubMed: 11012757]
- Auerbuch V, Brockstedt DG, Meyer-Morse N, O’Riordan M, Portnoy DA. Mice lacking the type I interferon receptor are resistant to *Listeria monocytogenes*. *J Exp Med.* 2004; 200:527–533. [PubMed: 15302899]
- Baker LA, Campbell PA, Hollister JR. Chemotaxis and complement fixation by *Listeria monocytogenes* cell wall fractions. *J Immunol.* 1977; 119:1723–1726. [PubMed: 410883]

- Banerjee M, Copp J, Vuga D, Marino M, Chapman T, Van Der Geer P, Ghosh P. GW domains of the *Listeria monocytogenes* invasion protein InlB are required for potentiation of Met activation. *Mol Microbiol.* 2004; 152:257–271.
- Bender JG, McPhail LC, Van Epps DE. Exposure of human neutrophils to chemotactic factors potentiates activation of the respiratory burst enzyme. *J Immunol.* 1983; 130:2316–2323. [PubMed: 6300243]
- Berends ETM, Dekkers JF, Nijland R, Kuipers A, Soppe JA, van Strijp JAG, Rooijackers SHM. Distinct localization of the complement C5b-9 complex on Gram-positive bacteria. *Cell Microbiol.* 2013; 15:1955–1968. [PubMed: 23869880]
- Bortolussi R, Issekutz A, Faulkner G. Opsonization of *Listeria monocytogenes* type 4b by human adult and newborn sera. *Infect Immun.* 1986; 52:493–498. [PubMed: 3084384]
- Bosmann M, Sarma JV, Atefi G, Zetoune FS, Ward PA. Evidence for anti-inflammatory effects of C5a on the innate IL-17A/IL-23 axis. *FASEB J.* 2012; 26:1640–1651. [PubMed: 22202675]
- Braun L, Cossart P. Interactions between *Listeria monocytogenes* and host mammalian cells. *Microbes Infect.* 2000; 2:803–811. [PubMed: 10955961]
- Braun L, Ghebrehwet B, Cossart P. gC1q-R/p32, a C1q-binding protein, is a receptor for the InlB invasion protein of *Listeria monocytogenes*. *EMBO J.* 2000a; 19:1458–1466. [PubMed: 10747014]
- Braun MC, Lahey E, Kelsall BL. Selective suppression of IL-12 production by chemoattractants. *J Immunol.* 2000b; 164:3009–3017. [PubMed: 10706689]
- Braun MC, Reins RY, Li TB, Hollmann TJ, Dutta R, Wetsel RA, Teng BB, Ke B. Renal expression of the C3a receptor and functional responses of primary human proximal tubular epithelial cells. *J Immunol.* 2004; 173:4190–4196. [PubMed: 15356170]
- Bronfenbrenner J. The nature of anaphylatoxin. studies on immunity II. *J Exp Med.* 1915; 21:480–492. [PubMed: 19867884]
- Calame DG, Mueller-Ortiz SL, Morales JE, Wetsel RA. The C5a anaphylatoxin receptor (C5aR1) protects against *Listeria monocytogenes* infection by inhibiting type 1 IFN expression. *J Immunol.* 2014; 193:5099–5107. [PubMed: 25297874]
- Carr KD, Sieve AN, Indramohan M, Break TJ, Lee S, Berg RE. Specific depletion reveals a novel role for neutrophil-mediated protection in the liver during *Listeria monocytogenes* infection. *Eur J Immunol.* 2011; 41:2666–2676. [PubMed: 21660934]
- Carrero JA, Calderon B, Unanue ER. Type I interferon sensitizes lymphocytes to apoptosis and reduces resistance to *Listeria* infection. *J Exp Med.* 2004; 200:535–540. [PubMed: 15302900]
- Carrero JA, Calderon B, Unanue ER. Lymphocytes are detrimental during the early innate immune response against *Listeria monocytogenes*. *J Exp Med.* 2006; 203:933–940. [PubMed: 16549598]
- Centers for Disease Control and Prevention. CDC reports 1 in 6 get sick from foodborne illnesses each year [Press release]. 2010. Retrieved from <http://www.cdc.gov/media/pressrel/2010/r101215.html>
- CDC. Vital signs: *Listeria* illnesses, deaths, and outbreaks—United States, 2009–2011. *MMWR.* 2013; 62:448–452. [PubMed: 23739339]
- Chen NJ, Mirtsos C, Suh D, Lu YC, Lin WJ, McKerlie C, Lee T, Baribault H, Tian H, Yeh WC. C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. *Nature.* 2007; 446:203–207. [PubMed: 17322907]
- Conlan JW, North RJ. Neutrophils are essential for early anti-*Listeria* defense in the liver, but not in the spleen or peritoneal cavity, as revealed by a granulocyte-depleting monoclonal antibody. *J Exp Med.* 1994; 179:259–268. [PubMed: 8270870]
- Cossart P. Met, the HGF-SF receptor: another receptor for *Listeria monocytogenes*. *Trends Microbiol.* 2001; 9:105–107. [PubMed: 11239771]
- Cossart P. Illuminating the landscape of host-pathogen interactions with the bacterium *Listeria monocytogenes*. *Proc Natl Acad Sci U S A.* 2011; 108:19484–19491. [PubMed: 22114192]
- Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol.* 2008; 180:5771–5777. [PubMed: 18424693]
- Croize J, Arvieux J, Berche P, Colomb MG. Activation of the human complement alternative pathway by *Listeria monocytogenes*: evidence for direct binding and proteolysis of the C3 component on bacteria. *Infect Immun.* 1993; 61:5134–5139. [PubMed: 8225590]

- Czuprynski CJ, Canono BP, Henson PM, Campbell PA. Genetically determined resistance to listeriosis is associated with increased accumulation of inflammatory neutrophils and macrophages which have enhanced listericidal activity. *Immunology*. 1985; 55:511–518. [PubMed: 4018836]
- Dai WJ, Kohler G, Brombacher F. Both innate and acquired immunity to *Listeria monocytogenes* infection are increased in IL-10-deficient mice. *J Immunol*. 1997; 158:2259–2267. [PubMed: 9036973]
- Dalrymple SA, Lucian LA, Slatery R, McNeil T, Aud DM, Fuchino S, Lee F, Murray R. Interleukin-6-deficient mice are highly susceptible to *Listeria monocytogenes* infection: correlation with inefficient neutrophilia. *Infect Immun*. 1995; 63:2262–2268. [PubMed: 7768607]
- Davoust N, Jones J, Stahel PF, Ames RS, Barnum SR. Receptor for the C3a anaphylatoxin is expressed by neurons and glial cells. *Glia*. 1999; 26:201–211. [PubMed: 10340761]
- Dembitzer FR, Kinoshita Y, Burstein D, Phelps RG, Beasley MB, Garcia R, Harpaz N, Jaffer S, Thung SN, Unger PD, Ghebrehwet B, Peerschke EI. gC1qR: expression in normal and pathologic human tissues: differential expression in tissues of epithelial and mesenchymal origin. *J Histochem Cytochem*. 2012; 60:467–474. [PubMed: 22638269]
- Drevets DA, Campbell PA. Roles of complement and complement receptor type 3 in phagocytosis of *Listeria monocytogenes* by inflammatory mouse peritoneal macrophages. *Infect Immun*. 1991; 59:2645–2652. [PubMed: 1906842]
- Drevets DA, Canono BP, Campbell PA. Listericidal and nonlistericidal mouse macrophages differ in complement receptor type 3-mediated phagocytosis of *L. monocytogenes* and in preventing escape of the bacteria into the cytoplasm. *J Leukoc Biol*. 1992; 52:70–79. [PubMed: 1640177]
- Drevets DA, Leenen PJ, Campbell PA. Complement receptor type 3 (CD11b/CD18) involvement is essential for killing of *Listeria monocytogenes* by mouse macrophages. *J Immunol*. 1993; 151:5431–5439. [PubMed: 8228236]
- Drouin SM, Kildsgaard J, Haviland J, Zabner J, Jia HP, McCray PB, Tack BF, Wetsel RA. Expression of the complement anaphylatoxin C3a and C5a receptors on bronchial epithelial and smooth muscle cells in models of sepsis and asthma. *J Immunol*. 2001; 166:2025–2032. [PubMed: 11160252]
- Dutow P, Fehlhaber B, Bode J, Laudeley R, Rheinheimer C, Glage S, Wetsel RA, Pabst O, Klos A. The complement C3a receptor is critical in defense against *Chlamydia psittaci* in mouse lung infection and required for antibody and optimal T cell response. *J Infect Dis*. 2014; 209:1269–1278. [PubMed: 24273177]
- Ebe Y, Hasegawa G, Takatsuka H, Umezu H, Mitsuyama M, Arakawa M, Mukaida N, Naito M. The role of Kupffer cells and regulation of neutrophil migration into the liver by macrophage inflammatory protein-2 in primary listeriosis in mice. *Pathol Int*. 1999; 49:519–532. [PubMed: 10469395]
- Edelson BT, Bradstreet TR, Hildner K, Carrero JA, Frederick KE, Wumesh KC, Belizaire R, Aoshi T, Schreiber RD, Miller MJ, Murphy TL, Unanue ER, Murphy KM. CD8 α + dendritic cells are an obligate cellular entry point for productive infection by *Listeria monocytogenes*. *Immunity*. 2011; 35:236–248. [PubMed: 21867927]
- Ehlers MR. CR3: a general purpose adhesion-recognition receptor essential for innate immunity. *Microbes Infect*. 2000; 2:289–294. [PubMed: 10758405]
- Fearon DT, Locksley RM. The instructive role of innate immunity in the acquired immune response. *Science*. 1996; 272:50–53. [PubMed: 8600536]
- Fischer WH, Hugli TE. Regulation of B cell functions by C3a and C3a(desArg): suppression of TNF- α , IL-6, and the polyclonal immune response. *J Immunol*. 1997; 159:4279–4286. [PubMed: 9379023]
- Frank MM, Joiner K, Hammer C. The function of antibody and complement in the lysis of bacteria. *Rev Infect Dis*. 1987; 9(Suppl 5):S537–S545. [PubMed: 3317749]
- Fujita T, Matsushita M, Endo Y. The lectin-complement pathway—its role in innate immunity and evolution. *Immunol Rev*. 2004; 198:185–202. [PubMed: 15199963]
- Fusakio ME, Mohammed JP, Laumonnier Y, Hoebe K, Kohl J, Mattner J. C5a regulates NKT and NK cell functions in sepsis. *J Immunol*. 2011; 187:5805–5812. [PubMed: 22058413]

- Gerard NP, Lu B, Liu P, Craig S, Fujiwara Y, Okinaga S, Gerard C. An anti-inflammatory function for the complement anaphylatoxin C5a-binding protein, C5L2. *J Biol Chem*. 2005; 280:39677–39680. [PubMed: 16204243]
- Gervais F, Stevenson M, Skamene E. Genetic control of resistance to *Listeria monocytogenes*: regulation of leukocyte inflammatory responses by the Hc locus. *J Immunol*. 1984; 132:2078–2083. [PubMed: 6699408]
- Ghebrehiwet B, Peerschke EIB. Structure and function of gC1q-R a multiligand binding membrane protein. *Immunobiology*. 1998; 199:225–238. [PubMed: 9777408]
- Ghebrehiwet B, Lim BL, Peerschke EIB, Willis AC, Reid KBM. Isolation, cDNA cloning, and overexpression of a 33-kDa cell surface glycoprotein that binds to the globular heads of C1q. *J Exp Med*. 1994; 179:1809–1821. [PubMed: 8195709]
- Goldstein IM, Weissmann G. Generation of C5-derived lysosomal enzyme-releasing activity (C5a) by lysates of leukocyte lysosomes. *J Immunol*. 1974; 113:1583–1588. [PubMed: 4473046]
- Grailer JJ, Bosmann M, Ward PA. Regulatory effects of C5a on IL-17A, IL-17F, and IL-23. *Front Immunol*. 2013; 3:387. [PubMed: 23316190]
- Gregory SH, Sagnimeni AJ, Wing EJ. Bacteria in the bloodstream are trapped in the liver and killed by immigrating neutrophils. *J Immunol*. 1996; 157:2514–2520. [PubMed: 8805652]
- Hamon MA, Ribet D, Stavru F, Cossart P. Listeriolysin O: the swiss army knife of *Listeria*. *Trends Microbiol*. 2012; 20:360–368. [PubMed: 22652164]
- Harboe M, Mollnes TE. The alternative complement pathway revisited. *J Cell Mol Med*. 2008; 12:1074–1084. [PubMed: 18419792]
- Havell EA, Moldawer LL, Helfgott D, Kilian PL, Sehgal PB. Type I IL-1 receptor blockade exacerbates murine listeriosis. *J Immunol*. 1992; 148:1486–1492. [PubMed: 1531668]
- Haviland DL, McCoy RL, Whitehead WT, Akama H, Molmenti EP, Brown A, Haviland JC, Parks WC, Perlmutter DH, Wetsel RA. Cellular expression of the C5a anaphylatoxin receptor (C5aR): demonstration of C5aR on nonmyeloid cells of the liver and lung. *J Immunol*. 1995; 154:1861–1869. [PubMed: 7836770]
- Hawlich H, Belkaid Y, Baelder R, Hildeman D, Gerard C, Kohl J. C5a negatively regulates toll-like receptor 4-induced immune responses. *Immunity*. 2005; 22:415–426. [PubMed: 15845447]
- Heeger PS, Kemper C. Novel roles of complement in T effector cell regulation. *Immunobiology*. 2012; 217:216–224. [PubMed: 21742404]
- Helmy KY, Katschke KJ, Gorgani NN, Kljavin NM, Elliott JM, Diehl L, Scales SJ, Ghilardi N, van Lookeren Campagne M. CR1g: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell*. 2006; 124:915–927. [PubMed: 16530040]
- Huang S, Hendriks W, Althage A, Hemmi S, Bluethmann H, Kamijo R, Vilcek J, Zinkernagel RM, Aguet M. Immune response in mice that lack the interferon-gamma receptor. *Science*. 1993; 259:1742–1745. [PubMed: 8456301]
- Humann J, Lenz LL. Activation of naïve NK cells in response to *Listeria monocytogenes* requires IL-18 and contact with infected dendritic cells. *J Immunol*. 2010; 184:5172–5178. [PubMed: 20351186]
- Kelly JP, Bancroft GJ. Administration of interleukin-10 abolishes innate resistance to *Listeria monocytogenes*. *Eur J Immunol*. 1996; 26:356–364. [PubMed: 8617304]
- Kim AH, Dimitriou ID, Holland MC, Mastellos D, Mueller YM, Altman JD, Lambris JD, Katsikis PD. Complement C5a receptor is essential for the optimal generation of antiviral CD8+ T cell responses. *J Immunol*. 2004; 173:2524–2529. [PubMed: 15294968]
- Kim KH, Choi BK, Song KM, Cha KW, Kim YH, Lee H, Han IS, Kwon BS. CR1g signals induce anti-intracellular bacterial phagosome activity in a chloride intracellular channel 3-dependent manner. *Eur J Immunol*. 2013; 43:667–678. [PubMed: 23280470]
- Kirchhoff K, Weinmann O, Zwirner J, Begemann G, Gotze O, Kapp A, Werfel T. Detection of anaphylatoxin receptors on CD83+ dendritic cells derived from human skin. *Immunology*. 2001; 103:210–217. [PubMed: 11412308]
- Kocks C, Gouin E, Tabouret M, Berche P, Ohayon H, Cossart P. L. *monocytogenes*-induced actin assembly requires the actA gene product a surface protein. *Cell*. 1992; 68:521–531. [PubMed: 1739966]

- Kopf M, Abel B, Gallimore A, Carroll M, Bachmann MF. Complement component C3 promotes T cell priming and lung migration to control acute influenza virus infection. *Nat Med.* 2002; 8:373–378. [PubMed: 11927943]
- Kurihara T, Warr G, Loy J, Bravo R. Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J Exp Med.* 1997; 186:1757–1762. [PubMed: 9362535]
- Labow M, Shuster D, Zetterstrom M, Nunes P, Terry R, Cullinan EB, Bartfai T, Solorzano C, Moldawer LL, Chizzonite R, McIntyre KW. Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. *J Immunol.* 1997; 159:2452–2461. [PubMed: 9278338]
- Lalli PN, Strainic MG, Yang M, Lin F, Medof ME, Heeger PS. Locally produced C5a binds to T cell-expressed C5aR to enhance effector T-cell expansion by limiting antigen-induced apoptosis. *Blood.* 2008; 112:1759–1766. [PubMed: 18567839]
- Lecuit M, Dramsi S, Gottardi C, Fedor-Chaiken M, Gumbiner B, Cossart P. A single amino acid in E-cadherin responsible for host specificity towards the human pathogen *Listeria monocytogenes*. *EMBO J.* 1999; 18:3956–3963. [PubMed: 10406800]
- Lecuit M, Vandormael-Pournin S, Lefort J, Huerre M, Gounon P, Dupuy C, Babinet C, Cossart P. A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. *Science.* 2001; 292:1722–1725. [PubMed: 11387478]
- Lee SH, Crocker P, Gordon S. Macrophage plasma membrane and secretory properties in murine malaria. Effects of *Plasmodium yoelii* blood-stage infection on macrophages in liver spleen, and blood. *J Exp Med.* 1986; 163:54–74. [PubMed: 3001215]
- Li K, Anderson KJ, Peng Q, Noble A, Lu B, Kelly AP, Wang N, Sacks SH, Zhou W. Cyclic AMP plays a critical role in C3a-receptor-mediated regulation of dendritic cells in antigen uptake and T-cell stimulation. *Blood.* 2008; 112:5084–5094. [PubMed: 18812470]
- Liang S, Krauss JL, Domon H, McIntosh ML, Hosur KB, Qu H, Li F, Tzekou A, Lambris JD, Hajishengallis G. The C5a receptor impairs IL-12-dependent clearance of *Porphyromonas gingivalis* and is required for induction of periodontal bone loss. *J Immunol.* 2011; 186:869–877. [PubMed: 21149611]
- Liszewski MK, Kolev M, Le Friec G, Leung M, Bertram PG, Fara AF, Subias M, Pickering MC, Drouet C, Meri S, Arstila TP, Pekkarinen PT, Ma M, Cope A, Reinheckel T, Rodriguez de Cordoba S, Afzali B, Atkinson JP, Kemper C. Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. *Immunity.* 2013; 39:1143–1157. [PubMed: 24315997]
- MacGowan AP, Peterson PK, Keane W, Quie PG. Human peritoneal macrophage phagocytic, killing, and chemiluminescent responses to opsonized *Listeria monocytogenes*. *Infect Immun.* 1983; 40:440–443. [PubMed: 6403471]
- Mackaness GB. Cellular resistance to infection. *J Exp Med.* 1962; 116:381–406. [PubMed: 14467923]
- Marder SR, Chenoweth DE, Goldstein IM, Perez HD. Chemotactic responses of human peripheral blood monocytes to the complement-derived peptides C5a and C5a des Arg. *J Immunol.* 1985; 134:3325–3331. [PubMed: 3884709]
- Marino M, Banerjee M, Jonquieres R, Cossart P, Ghosh P. GW domains of the *Listeria monocytogenes* invasion protein InlB are SH3-like and mediate binding to host ligands. *EMBO J.* 2002; 21:5623–5634. [PubMed: 12411480]
- Matthews KW, Mueller-Ortiz SL, Wetsel RA. Carboxypeptidase N: a pleiotropic regulator of inflammation. *Mol Immunol.* 2004; 40:785–793. [PubMed: 14687935]
- Merrick JC, Edelson BT, Bhardwaj V, Swanson PE, Unanue ER. Lymphocyte apoptosis during early phase of *Listeria* infection in mice. *Am J Pathol.* 1997; 151:785–792. [PubMed: 9284827]
- Mueller-Ortiz SL, Hollmann TJ, Haviland DL, Wetsel RA. Ablation of the complement C3a anaphylatoxin receptor causes enhanced killing of *Pseudomonas aeruginosa* in a mouse model of pneumonia. *Am J Physiol Lung Cell Mol Physiol.* 2006; 291:L157–L165. [PubMed: 16461429]
- Mueller-Ortiz SL, Wang D, Morales JE, Li L, Chang J, Wetsel RA. Targeted disruption of the gene encoding the murine small subunit of carboxypeptidase N (CPN1) causes susceptibility to C5a anaphylatoxin-mediated shock. *J Immunol.* 2009; 182:6533–6539. [PubMed: 19414808]

- Mueller-Ortiz SL, Morales JE, Wetsel RA. The receptor for the complement C3a anaphylatoxin (C3aR) provides host protection against *Listeria monocytogenes*-induced apoptosis. *J Immunol.* 2014; 193:1278–1289. [PubMed: 24981453]
- Murray EGD, Webb RA, Swann MBR. A disease of rabbits characterized by a large mononuclear leukocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. sp.). *J Pathol.* 1926; 29:407–439.
- Nakayama Y, Kim SI, Kim EH, Lambris JD, Sandor M, Suresh M. C3 promotes expansion of CD8+ and CD4+ T cells in a *Listeria monocytogenes* infection. *J Immunol.* 2009; 183:2921–2931. [PubMed: 19648268]
- Nataf S, Davoust N, Ames RS, Barnum SR. Human T cells express the C5a Receptor and are chemoattracted to C5a. *J Immunol.* 1999; 162:4018–4023. [PubMed: 10201923]
- Nepomuceno RR, Tenner AJ. C1qRp, the C1q receptor that enhances phagocytosis, is detected specifically in human cells of myeloid lineage, endothelial cells, and platelets. *J Immunol.* 1998; 160:1929–1935. [PubMed: 9469455]
- Neuenhahn M, Kerkisiek KM, Nauwerth M, Suhre MH, Schiemann M, Gebhardt FE, Stemberger C, Panthel K, Schroder S, Chakraborty T, Jung S, Hochrein H, Russmann H, Bocker T, Busch DH. CD8alpha+ dendritic cells are required for efficient entry of *Listeria monocytogenes* into the spleen. *Immunity.* 2006; 25:619–630. [PubMed: 17027298]
- North RJ. Cellular mediators of anti-*Listeria* immunity as an enlarged population of short-lived, replicating T cells. Kinetics of their production. *J Exp Med.* 1973; 138:342–355. [PubMed: 4198199]
- North RJ. T cell dependence of macrophage activation and mobilization during infection with *Mycobacterium tuberculosis*. *Infect Immun.* 1974; 10:66–71. [PubMed: 4210333]
- O'Connell RM, Saha SK, Vaidya SA, Bruhn KW, Miranda GA, Zarnegar B, Perry AK, Nguyen BO, Lane TF, Taniguchi T, Miller JF, Cheng G. Type I interferon production enhances susceptibility to *Listeria monocytogenes* infection. *J Exp Med.* 2004; 200:437–445. [PubMed: 15302901]
- Okusawa S, Yancey KB, van der Meer JW, Endres S, Lonnemann G, Heftner K, Frank MM, Burke JF, Dinarello CA, Gelfand JA. C5a stimulates secretion of tumor necrosis factor from human mononuclear cells in vitro: comparison with secretion of interleukin 1 beta and interleukin 1 alpha. *J Exp Med.* 1988; 168:443–448. [PubMed: 3260938]
- Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu Rev Immunol.* 2011; 29:71–109. [PubMed: 21166540]
- Pamer EG. Immune responses to *Listeria monocytogenes*. *Nat Rev Immunol.* 2004; 4:812–823. [PubMed: 15459672]
- Peng Q, Li K, Patel H, Sacks SH, Zhou W. Dendritic cell synthesis of C3 is required for full T cell activation and development of a Th1 phenotype. *J Immunol.* 2006; 176:3330–3341. [PubMed: 16517700]
- Peng Q, Li K, Anderson K, Farrar CA, Lu B, Smith RAG, Sacks SH, Zhou W. Local production and activation of complement up-regulates the allostimulatory function of dendritic cells through C3a-C3aR interaction. *Blood.* 2007; 111:2452–2461. [PubMed: 18056835]
- Peng Q, Li K, Wang N, Li Q, Asgari E, Lu B, Woodruff TM, Sacks SH, Zhou W. Dendritic cell function in allostimulation is modulated by C5aR signaling. *J Immunol.* 2009; 183:6058–6068. [PubMed: 19864610]
- Peterson PK, Verhoef J, Schmeling D, Quie PG. Kinetics of phagocytosis and bacterial killing by human polymorphonuclear leukocytes and monocytes. *J Infect Dis.* 1977; 136:502–509. [PubMed: 409787]
- Pfeffer K, Matsuyama T, Kundig TM, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Kronke M, Mak TW. Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock: yet succumb to *L. monocytogenes* infection. *Cell.* 1993; 73:457–467. [PubMed: 8387893]
- Pizarro-Cerda J, Kuhbacher A, Cossart P. Entry of *Listeria monocytogenes* in mammalian epithelial cells: an updated view. *Cold Spring Harb Perspect Med.* 2012; 2:1–17.

- Portnoy DA, Auerbuch V, Glomski JJ. The cell biology of *Listeria monocytogenes* infection: the intersection of bacterial pathogenesis and cell-mediated immunity. *J Cell Biol.* 2002; 158:409–414. [PubMed: 12163465]
- Reis ES, Chen H, Sfyroera G, Monk PN, Kohl J, Ricklin D, Lambris JD. C5a receptor-dependent cell activation by physiological concentrations of desarginated C5a: insights from a novel label-free cellular assay. *J Immunol.* 2012; 189:4797–4805. [PubMed: 23041570]
- Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol.* 2010; 11:785–797. [PubMed: 20720586]
- Rogers HW, Unanue ER. Neutrophils are involved in acute, nonspecific resistance to *Listeria monocytogenes* in mice. *Infect Immun.* 1993; 61:5090–5096. [PubMed: 8225586]
- Rosen H, Gordon S, North RJ. Exacerbation of murine listeriosis by a monoclonal antibody specific for the type 3 complement receptor of myelomonocytic cells. Absence of monocytes at infective foci allows *Listeria* to multiply in nonphagocytic cells. *J Exp Med.* 1989; 170:27–37. [PubMed: 2501445]
- Rothe J, Werner L, Lotscher H, Lang Y, Koebel P, Kontgen F, Althage A, Zinkernagel R, Steinmetz M, Bluethmann H. Mice lacking the tumor necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. *Nature.* 1993; 364:798–802. [PubMed: 8395024]
- Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis.* 2011; 17:7–15. [PubMed: 21192848]
- Schindler R, Gelfand JA, Dinarello CA. Recombinant C5a stimulates transcription rather than translation of interleukin-1 (IL-1) and tumor necrosis factor: translational signal provided by lipopolysaccharide or IL-1 itself. *Blood.* 1990; 76:1631–1638. [PubMed: 2207333]
- Schlech WF, Lavigne PM, Bortolussi RA, Allen AC, Haldane EV, Wort AJ, Hightower AW, Johnson SE, King SH, Nicholls ES, Broome CV. Epidemic listeriosis—evidence for transmission by food. *N Engl J Med.* 1983; 308:203–206. [PubMed: 6401354]
- Scholz W, McClurg MR, Cardenas GJ, Smith M, Noonan DJ, Hugli TE, Morgan EL. C5a-mediated release of interleukin 6 by human monocytes. *Clin Immunol Immunopathol.* 1990; 57:297–307. [PubMed: 2208809]
- Scola A, Johswich K, Morgan BP, Klos A, Monk PN. The human complement fragment receptor, C5L2, is a recycling decoy receptor. *Mol Immunol.* 2009; 46:1149–1162. [PubMed: 19100624]
- Serbina NV, Salazar-Mather TP, Biron CA, Kuziel WA, Pamer EG. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity.* 2003; 19:59–70. [PubMed: 12871639]
- Skattum L, van Deuren M, van der Poll T, Truedsson L. Complement deficiency states and associated infections. *Mol Immunol.* 2011; 48:1643–1655. [PubMed: 21624663]
- Steigbigel RT, Lambert LH, Remington JS. Phagocytic and bacterial properties of normal human monocytes. *J Clin Invest.* 1974; 53:131–142. [PubMed: 4202669]
- Stockinger S, Materna T, Stoiber D, Bayr L, Steinborn R, Kolbe T, Unger H, Chakraborty T, Levy DE, Muller M, Decker T. Production of type I IFN sensitizes macrophages to cell death induced by *Listeria monocytogenes*. *J Immunol.* 2002; 169:6522–6529. [PubMed: 12444163]
- Strainic MG, Liu J, Huang D, An F, Lalli PN, Muqim N, Shapiro VS, Dubyak GR, Heeger PS, Medof ME. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naïve CD4⁺ T cells. *Immunity.* 2008; 28:425–435. [PubMed: 18328742]
- Suresh M, Molina H, Salvato MS, Mastellos D, Lambris JD, Sandor M. Complement component 3 is required for optimal expansion of CD8⁺ T cells during a systemic viral infection. *J Immunol.* 2003; 170:788–794. [PubMed: 12517942]
- Taege AJ. Listeriosis: recognizing it, treating it, preventing it. *Cleveland Clin J Med.* 1999; 66:375–380.
- Tan Y, Li Y, Fu X, Yang F, Zheng P, Zhang J, Guo B, Wu Y. Systemic C3 modulates CD8⁺ T cell contraction after *Listeria monocytogenes* infection. *J Immunol.* 2014; 193:3426–3435. [PubMed: 25187659]
- Tripp CS, Wolf SF, Unanue ER. Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice

- with listeriosis, and interleukin 10 is a physiologic antagonist. *Proc Natl Acad Sci U S A*. 1993; 90:3725–3729. [PubMed: 8097322]
- Tripp CS, Gately MK, Hakimi J, Ling P, Unanue ER. Neutralization of IL-12 decreases resistance to *Listeria* in SCID and C.B-17 mice. Reversal by IFN- γ . *J Immunol*. 1994; 152:1883–1887. [PubMed: 7907107]
- Unanue ER. Studies in listeriosis show the strong symbiosis between the innate cellular system and the T-cell response. *Immunol Rev*. 1997; 158:11–25. [PubMed: 9314070]
- van Kessel KP, Antonissen AC, van Dijk H, Rademaker PM, Willers JM. Interactions of killed *Listeria monocytogenes* with the mouse complement system. *Infect Immun*. 1981; 34:16–19. [PubMed: 6795123]
- van Lookeren Campagne M, Wiesmann C, Brown EJ. Macrophage complement receptors and pathogen clearance. *Cell Microbiol*. 2007; 9:2095–2102. [PubMed: 17590164]
- Vazquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Dominguez-Bernal G, Goebel W, Gonzalez-Zorn B, Wehland J, Kreft J. *Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev*. 2001; 14:584–640. [PubMed: 11432815]
- Verschoor A, Neuenhahn M, Navarini AA, Graef P, Plaumann A, Seidlmeier A, Nieswandt B, Massberg S, Zinkernagel RM, Hengartner H, Busch DH. A platelet-mediated system for shuttling blood-borne bacteria to CD8 α ⁺ dendritic cells depends on glycoprotein GPIb and complement C3. *Nat Immunol*. 2011; 12:1194–1201.
- Wagner RD, Maroushek NM, Brown JF, Czuprynski CJ. Treatment with anti-interleukin-10 monoclonal antibody enhances early resistance to but impairs complete clearance of *Listeria monocytogenes* infection in mice. *Infect Immun*. 1994; 62:2345–2353. [PubMed: 8188357]
- Wallis R, Mitchell DA, Schmid R, Schwaible WJ, Keeble AH. Paths reunited: initiation of the classical and lectin pathways of complement activation. *Immunobiology*. 2010; 215:1–11. [PubMed: 19783065]
- Werfel T, Kirchhoff K, Wittmann M, Begemann G, Kapp A, Heidenreich F, Gotze O, Zwirner J. Activated human T lymphocytes express a functional C3a receptor. *J Immunol*. 2000; 165:6599–6605. [PubMed: 11086104]
- Wetsel RA, Kildsgaard J, Haviland, DL. Complement anaphylatoxins (C3a, C4a, C5a) and their receptors (C3aR, C5aR/CD88) as therapeutic targets in inflammation. In: Lambris, JD., Holers, VM., editors. *Contemporary Immunology: Therapeutic Interventions in the Complement System*. Humana Press; Totowa, NJ: 2000. p. 113–154.
- Wetsel RA. Structure, function and cellular expression of complement anaphylatoxin receptors. *Curr Opin Immunol*. 1995; 7:48–53. [PubMed: 7772282]
- Wing EJ, Gregory SH. *Listeria monocytogenes*: clinical and experimental update. *J Infect Dis*. 2002; 185(Suppl 1):S18–24. [PubMed: 11865436]
- Wittmann M, Zwirner J, Larsson V, Kirchhoff K, Begemann G, Kopp A, Gotze O, Werfel T. C5a suppresses the production of IL-12 by IFN- γ -primed and lipopolysaccharide-challenged human monocytes. *J Immunol*. 1999; 162:6763–6769. [PubMed: 10352296]
- Wollert T, Pasche B, Rochon M, Deppenmeier S, van den Heuvel J, Gruber AD, Heinz DW, Lengeling A, Schubert WD. Extending the host range of *Listeria monocytogenes* by rational protein design. *Cell*. 2007; 129:891–902. [PubMed: 17540170]
- Zenewicz LA, Shen H. Innate and adaptive immune responses to *Listeria monocytogenes*: a short overview. *Microbes Infect*. 2007; 9:1208–1215. [PubMed: 17719259]
- Zhang X, Kimura Y, Fang C, Zhou L, Sfyroera G, Lambris JD, Wetsel RA, Miwa T, Song W. Regulation of toll-like receptor-mediated inflammatory response by complement in vivo. *Blood*. 2007; 110:228–236. [PubMed: 17363730]
- Zhang X, Schmudde I, Laumonnier Y, Pandey MK, Clark JR, Konig P, Gerard NP, Gerard C, Wills-Karp M, Kohl J. A critical role for C5L2 in the pathogenesis of experimental allergic asthma. *J Immunol*. 2010; 185:6741–6752. [PubMed: 20974988]
- Zhou W, Patel H, Li K, Peng Q, Villiers MB, Sacks SH. Macrophages from C3-deficient mice have impaired potency to stimulate alloreactive T cells. *Blood*. 2006; 107:2461–2469. [PubMed: 16304047]

Zwirner J, Gotze O, Begemann G, Kapp A, Kirchhoff K, Werfel T. Evaluation of C3a receptor expression on human leucocytes by the use of novel monoclonal antibodies. *Immunology*. 1999; 97:166–172. [PubMed: 10447728]

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Fig. 1.

Life cycle and virulence factors of *Listeria monocytogenes*. A) *L. monocytogenes* initially enters the host cell through phagocytosis. To access the intracellular compartment of non-phagocytic cells such as those of the intestinal epithelium, *L. monocytogenes* binds E-cadherin through internalin A (InlA) and/or Met through internalin B (InlB). This binding triggers the uptake of *L. monocytogenes* into a phagosome. B) Once inside the phagosome, *L. monocytogenes* secretes the poreforming toxin listeriolysin O (LLO) and phospholipase C (PLC). LLO and PLC lyse the phagosomal membrane, releasing *L. monocytogenes* into the cytosol where it replicates freely. C) *L. monocytogenes* exploits the host cell's actin cytoskeleton through the virulence factor ActA. ActA polymerizes actin monomers to propel *L. monocytogenes* through the cytoplasm. This propulsion ultimately allows for its intercellular spread *via* membrane protrusions.

Fig. 2.

Overview of the Complement System. Bacteria like *L. monocytogenes* can activate the complement system in one of three ways. In the classical pathway, antibodies (Ab) bind to the bacterial surface and undergo a conformational change allowing C1q to bind their Fc region. The activated C1 complex then cleaves C4 and C2 to generate the classical C3 convertase, C4bC2a. In the lectin pathway, mannose binding lectin (MBL) binds mannose residues on the surface of *L. monocytogenes*. MBL can then form a complex with MASP-1 and MASP-2 that cleaves C4 and C2 similar to the C1 complex. Finally, in the alternative pathway, the spontaneous hydrolysis of C3 leads to the formation of a fluid phase C3 convertase C3(H₂O)Bb that can cleave C3. C3b then deposits on the surface of *L. monocytogenes* and binds factor B (fB) to form the alternative pathway C3 convertase, C3bBb. This convertase allows for the complement amplification loop. Ultimately, all

pathways converge on C3 and result in the generation of three types of complement effector molecules: C3b, C3a and C5a, and the membrane attack complex (C5b-9).

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Table 1

Biological functions mediated by complement components and their receptors during the host response to *L. monocytogenes* infection.

Complement Component or Receptor	Biological Functions	References
C1q	Opsonization and phagocytosis by macrophages	Alvarez-Dominguez et al. (1993)
gC1qR	Binds <i>Lm</i> surface Invasion Protein InlB	Braun et al. (2000a,b)
C3	C3 dependent CD8+ T-cell proliferation and CD8+ T-cell contraction	Nakayama et al. (2009) and Tan et al. (2014)
C3b/Cbi	Opsonization resulting in biological functions on binding CR3, CRIg, or GPIb (see below)	Croize et al. (1993)
CR3	Phagocytosis by macrophages and Kupffer cells but does not result in phagosomal containment	Drevets et al. (1993)
CRIg	High expression of Kupffer cells responsible for early clearance of opsonized <i>Lm</i> from blood	Helmy et al. (2006)
GPIb	Expression on platelets facilitates delivery of opsonized <i>Lm</i> to splenic CD8α+ DCs	Verschoor et al. (2011)
C3a/C3aR	Induction of prosurvival signals impairing <i>Lm</i> induced apoptosis of leukocytes	Mueller-Ortiz et al. (2014)
C5a/C5aR1	Suppression of Type 1 Interferon expression thereby inhibiting <i>Lm</i> induced apoptosis of splenocytes	Calame et al. (2014)