

Molecular Aspects of *Listeria monocytogenes* Infection

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Abstract

Listeria monocytogenes, a food-borne intracellular animal and human pathogen, interacts with infected host cells both prior to entry and during the intracellular phase of infection. This review is focused on the role of secreted proteins, including listeriolysin O and two distinct phospholipases C, in modulating the signal transduction of infected cells.

Key words: *Listeria monocytogenes*, intracellular signaling, listeriolysin O, phospholipases C

1. Introduction

Listeria monocytogenes (*Lm*) is a facultative intracellular bacterial pathogen widely distributed in the environment that encounters a variety of cell types after being consumed in contaminated food, first in its passage through the digestive tract, then the liver, the circulatory system, and the central nervous system, often with fatal consequences (Schuchat *et al.*, 1997; Vázquez-Boland *et al.*, 2001). In the host organism *Lm* rapidly infects the liver and spleen. In the liver most of the bacteria are killed upon initial encounter with Kupffer cells, resident macrophages in the liver, or with immigrant macrophages. Since some *Lm* can survive this encounter, neutrophils play an important role in resolving these infections (Portnoy *et al.*, 1994; Sheehan *et al.*, 1994). The proportion of surviving bacteria will vary depending on the immunological system state of the host. In studies with isolated macrophages and macrophage-derived cell-lines, the surviving bacteria escape from the primary phagocytic vacuole, grow and divide in the cytoplasm, recruit and polymerize host cell actin, which provides the driving force for movement through the cytoplasm and into nearby cells by means of filopodia-like projections (Tilney and Portnoy, 1989). In these neighboring cells bacteria escape from double-membrane secondary phagosomes and repeat the cycle of growth, recruitment of actin and cell-to-cell spread. *Lm* secretes listeriolysin O (LLO) and two distinct phospholipases C (PLC), one specific for phosphatidylinositol (PI) and glycosyl-PI (GPI) – anchored proteins (PI-PLC) (Leimeister-Wächter *et al.*, 1991; Mengaud *et al.*, 1991; Camilli *et al.*, 1991), and a second broad-range phospholipase (PC-PLC) capable of hydrolyzing a wide variety of mammalian phospholipids, including sphingomyelin (Geoffroy *et al.*, 1991). These three proteins are encoded by genes located in a central virulence gene cluster, and their expression is positively regulated by the transcriptional activator PrfA (Kuhn *et al.*, 1999). A number of studies have provided evidence for the participation of both PLCs in addition to LLO in the induction of host intracellular signaling. In this review we briefly describe the properties of these proteins and their roles in various interactions with different type of infected host cells. Host cell signaling induced by internalins has been reviewed recently (Cossart, 2001; Cossart *et al.*, 2000), and will not be discussed here.

2. Host cell responses to presence of LLO and PLCs

Evidence is accumulating that LLO and PLCs are multifunctional virulence factors with many important roles in the host-parasite interaction other than phagosomal membrane disruption. Exogenous and endogenous exposure to LLO and, in many cases, PLCs which play an accessory role, may induce a number of host cell responses such as cell proliferation and focus formation in transfected fibroblasts (Demuth *et al.*, 1994), activation of the Raf-Mek-mitogen-activated protein (MAP) kinase pathway in epithelial cells

(Tang *et al.*, 1996; Tang *et al.*, 1994; Weiglein *et al.*, 1997), mucus exocytosis induction in intestinal cells (Coconnier *et al.*, 1998), modulation of internalization *via* calcium signaling (Wadsworth and Goldfine, 1999), cytokine expression in macrophages (Nishibori *et al.*, 1996), degranulation and leukotriene formation in neutrophils (Sibeliu *et al.*, 1999), apoptosis in dendritic cells (Guzman *et al.*, 1996), phosphoinositide metabolism, lipid mediator generation (Sibeliu *et al.*, 1996b), NF- κ B activation (Kayal *et al.*, 1999), expression of cell adhesion molecules in infected endothelial cells (Krüll *et al.*, 1997), and expression of Fas ligand on T lymphocytes (Zenewicz *et al.*, 2004). More details of these aspects of activity of LLO and PLCs can be found below.

3. Molecular mechanisms of intracellular signaling induced by LLO and PLCs

3.1. Calcium signaling

Ca^{2+} is a very important second messenger in a great variety of eukaryotic cell signaling processes (exocytosis, contraction, metabolism, gene transcription, fertilization and proliferation) and is an important regulator of actin microfilaments (Berridge *et al.*, 2000). In case of infection of macrophage-like J774 cells with wild-type *Lm* three elevations of intracellular calcium (Ca^{2+}) was observed. The first begins within 1 min after addition of a washed suspension of bacteria in PBS to a monolayer of J774 cells. After a brief decline to near basal levels, Ca^{2+} increases again at 5–7 min post-infection (p.i.), declines and then increases for a prolonged period starting at 15 min p.i. LLO mutant produces none of these calcium fluxes. A PI-PLC mutant produced a relatively weak signal beginning at 15 min that lasted for about 15 min, and a PC-PLC mutant produced the first signal in Ca^{2+} , but not the others. The first and second elevations of Ca^{2+} occur when very few wild-type bacteria have been internalized, and these calcium signals are seen in cells containing no bound or internalized bacteria (Wadsworth and Goldfine, 1999). Thus, it appears that calcium signaling can be induced by secreted factors. Pretreatment of host cells with the receptor-operated calcium channel blocker SK&F96365 or by removal of extracellular calcium by chelation with EGTA prevented all three Ca^{2+} elevations. Pretreatment with thapsigargin, an inhibitor of calcium release from intracellular stores, prevented only the second and third elevations. Together, these results indicated that the first calcium elevation resulted from influx of extracellular calcium through a channel and that the latter two elevations reflected release of calcium from intracellular stores, which required the initial calcium influx. Correlation between calcium signaling pattern and adhesion, internalization of bacteria was observed. The entry of wild-type *Lm* into J774 cells is slower than the entry of the LLO and PI-PLC mutants. Like heat-killed bacteria, these mutants are taken up rapidly so that from 50 to 70% of bacteria associated with the host cells are internalized within 1 min after infection compared to 10–15% of the wild type. Preventing the elevation of Ca^{2+} with either SK&F96365 or thapsigargin increased the rate of entry, but not to that observed with the LLO and PI-PLC mutants. Treatment with either inhibitor also resulted in greatly decreased efficiency of escape of wild-type *Lm* from the primary phagocytic vacuole (Wadsworth and Goldfine, 1999). Calcium signaling was also observed during infection of epithelial cell line Hep-2 with *Lm* (Dramsı and Cossart, 2003). In this case wild type *Lm*, but not nonpathogenic species *L. innocua* or *Lm* mutant strain defective in listeriolysin O production, was able to induce Ca^{2+} fluxes. In opposition to macrophage-like cells, increase of Ca^{2+} observed rapidly after infection of epithelial cells, facilitates *Lm* entry into these cells. Pretreatment of host cells with calcium channel antagonists or chelation of extracellular calcium markedly reduced *Lm* entry. In contrast, chelation of cytosolic Ca^{2+} or blockade of Ca^{2+} release from intracellular stores did not affect invasion. These results suggest that *Lm*-induced mobilization of extracellular Ca^{2+} by LLO and activation of downstream Ca^{2+} – dependent signaling are required for efficient cell invasion (Dramsı and Cossart, 2003). Long-lasting oscillations of intracellular Ca^{2+} level caused by LLO applied extracellularly were also demonstrated in case of human embryonic kidney cells (Repp *et al.*, 2002). This was presumably resulted from a pulsed influx of extracellular Ca^{2+} through pores that are formed by LLO in the plasma membrane. Unfortunately, it was not tested how changes in Ca^{2+} affect infection of kidney cells.

3.2. Induction of host phospholipases

The ability of wild-type *Lm* to induce calcium fluxes in J774 macrophage-like cells and the finding that the second brief elevation and the third prolonged elevation were inhibited by factors, which deplete calcium stores, indicated that these calcium fluxes resulted from the release of Ca^{2+} from intracellular stores

(Wadsworth and Goldfine, 1999). An important mediator of Ca^{2+} release from the endoplasmic reticulum is inositol-1, 4, 5- P_3 (IP_3), a product of hydrolytic activity of eukaryotic calcium-activated PLC on PI-4, 5- P_2 (PIP_2). Distinct elevation of IP_2 and IP_3 in murine bone marrow-derived macrophages infected with wild-type *Lm* was shown, but not with an LLO⁻ mutant. Infection of J774 cells prelabeled with [³H]-inositol with wild-type *Lm* resulted in elevation of [³H]-IP within 10 min, followed by more rapid release at 20–30 min. Much less [³H]-IP formation was observed upon infection with mutants deficient in either LLO or PI-PLC, but loss of PC-PLC did not affect IP formation. It appears that PI-PLC with the aid of LLO rapidly hydrolyzes host PI, resulting in release of diacylglycerol (DAG) and IP (Goldfine *et al.*, 2000). Since the *Lm* PI-PLC does not hydrolyze PIP_2 , release of IP_3 , unlike release of IP, is an indicator of host PLC activation (Goldfine and Knob, 1992; Griffith and Ryan, 1999). IP_3 elevation occurred within 10 min of infection of J774 cells with wild-type *Lm*, but not with an LLO mutant. IP_3 elevation was somewhat reduced with a PI-PLC mutant. Earlier studies with human umbilical vein endothelial cells (HUVEC), which had not internalized *Lm* during the course of these experiments, showed that a combination of LLO and *Lm* PI-PLC secreted by recombinant *Listeria innocua* strains, resulted in the formation of products of phosphoinositide hydrolysis resulting from activation of host PLC isoforms (Sibeliu *et al.*, 1996a). DAG was also observed to accumulate rapidly. It appears that host PLC isoforms were activated since a similar response was elicited by LLO alone (Sibeliu *et al.*, 1996b). It was proposed a model for the delivery system for PI-PLC in which LLO enable PI-PLC translocation into cytosol of infected cells and, which is analogous to the type III secretion system of gram-negative bacteria (Sibeliu *et al.*, 1996a; Madden *et al.*, 2001). In this model pores formed by LLO permit access of PI-PLC to PI in the inner leaflet of the host cell plasma membrane, resulting in the formation of IP, and a separate induction of host phospholipases. From studies with J774 cells, it appears likely that LLO induces a potent phosphoinositide response, in the absence of sustained calcium elevation, since the double PI-PLC, PC-PLC deletion mutant of *Lm*, which produced no intracellular Ca^{2+} elevation (Wadsworth and Goldfine, 1999), evoked the release of IP_2 and IP_3 , but an LLO⁻ strain did not (Goldfine *et al.*, 2000). In both HUVEC and human neutrophils *Lm* and *L. innocua* strains expressing LLO induced the release of platelet activating factor and products of arachidonate metabolism, prostaglandin I_2 or leukotrienes, which indicates activation of host phospholipase A_2 (Sibeliu *et al.*, 1996a; Sibeliu *et al.*, 1996b; Sibeliu *et al.*, 1999). In HUVEC, a combination of LLO and PI-PLC were required for maximal responses, but in neutrophils, the response was mainly attributed to LLO.

3.3. Mobilization of isoforms of host protein kinase C

Since DAG and calcium are known activators of PKC, it was postulated that hydrolysis of PI by bacterial PI-PLC leads to the production of DAG, and thus activation of PKC isoforms in infected host cells (Wadsworth and Goldfine, 1999). The four major isoforms of PKC found in the J774 murine macrophage-like cells are α , βI , βII and δ (Smith *et al.*, 1997). Of these, α , βI , βII are “classical” isoforms activated by intracellular calcium and/or DAG, while PKC δ is a “novel” isoform which is activated by DAG, but is Ca^{2+} – independent (Ron and Kazanietz, 1999). PKC δ has been implicated in phosphorylation and opening of Ca^{2+} channels in eukaryotic cells (Levin *et al.*, 1997). It was observed that PKC δ moves to a peripheral location within 30 s of addition of a washed suspension of wild-type *Lm* or the PC-PLC mutant to J774 cells, but not after addition of either LLO or PI-PLC deletion mutants. Studies performed using calcium channel blocker show that this translocation is calcium-independent. On the other hand, it was inhibited by 3 h pretreatment with phorbol myristate acetate (PMA), which downregulates PKCs. Pretreatment with PMA or rottlerin, an inhibitor of PKC δ , abolished the first calcium signal, consistent with the role of PKC δ in activating calcium channels (Wadsworth and Goldfine, 2002). Further studies revealed rapid translocation of PKC βI and βII to early endosomes after infection of J774 cells with wild-type *Lm* or the PC-PLC mutant. The translocation of PKC βII was seen from 30 s to 3 min p.i., while that of PKC βI occurred between 1 and 4 min p.i. No PKC βI or βII translocation was observed after infection with a LLO mutant. PKC βI , but not PKC βII , translocation to early endosomes occurred after infection with a PI-PLC mutant. PKC translocation like the first calcium elevation occurred in cells that had not internalized *Lm* (Wadsworth and Goldfine, 2002). Translocation of PKC βII to early endosomes is calcium- and presumably DAG-dependent since it was blocked by treatment with the calcium channel blocker and required bacterial PI-PLC. Translocation of PKC βI , in contrast, did not appear to require elevated intracellular Ca^{2+} , since it was seen upon infection with a double phospholipase mutant (*plcA*, *plcB*), which produced no calcium signal in J774 cells. DAG was presumably produced upon infection with the double phospholipase mutant by activation of host phosphoinositide-specific PLC (Wadsworth and Goldfine, 1999; Wadsworth and Goldfine, 2002). These

results lead to a proposed signaling pathway in macrophage-like cells in which LLO and PI-PLC cooperate in cleavage of host PI, resulting in translocation of PKC α to the cell membrane. PKC δ mobilization leads to Ca^{2+} influx, which in combination with DAG, results in translocation of PKC β II to early endosomes. *Lm* mutants that do not secrete phospholipases, but do secrete LLO, can activate PKC β I through activation of host phospholipases, which also produce DAG. As noted above, inhibition of calcium signaling resulted in increased early association of wild-type *Lm* with J774 cells and increased uptake of associated bacteria. PI-PLC or LLO mutants associate with J774 cells and enter more rapidly than the wild type, and drugs that block calcium signaling had minimal effects on uptake of these mutants (Wadsworth and Goldfine, 1999). Hispidin, which inhibits PKC β I selectively, also increased the association of wild-type *Lm* with J774 cells. The percent of wild-type bacteria internalized was also increased. It appears that PKC signaling modulates uptake of *Lm* into these macrophage-like cells. As was the case for inhibitors of calcium signaling, hispidin also greatly decreased the efficiency of escape of both wild-type *Lm* and PI-PLC mutant from the primary vacuole (Wadsworth and Goldfine, 2002).

3.4. Induction of host phospholipase D (PLD) activity

Activation of eukaryotic PLD is often obtained with agonists that activate PLC isoforms, and in some systems activation of PLD occurs downstream of PLC (Singer *et al.*, 1997). Infection of J774 cells with wild-type *Lm* and with mutants lacking either or both of the *Lm* PLCs resulted in activation of host PLD. Of the strains tested, only that lacking LLO failed to activate PLD. This increase in PLD activity occurred 15 min p.i. and, therefore, after translocation of PKC β I, β II, and δ . It is either coincident with or following activation of host polyphosphoinositide-specific PLC (Goldfine *et al.*, 2000). The activation of PLD in J774 cells coincides with the entry of wild-type *Lm* into host cells and the beginning of vacuolar maturation (Wadsworth and Goldfine, 1999). Inhibition of PLD by 2,3-diphosphoglycerate was accompanied by decreased efficiency of escape from the primary vacuole (Goldfine *et al.*, 2000). Since the loss of PI-PLC by mutation also results in diminished efficiency of escape, but does not affect PLD activation, PI-PLC probably contributes to the escape process through another pathway. There is a tight coupling between phagocytosis of *Mycobacterium tuberculosis* as well as opsonized zymosan particles and PLD activation in human macrophages (Kusner *et al.*, 1996). PLD activation and the product of its activity, phosphatidic acid, are thought to play important roles in intracellular vesicle transport, but the mechanisms have not been established (Exton, 1997).

3.5. Activation of NF- κ B

NF- κ B is an important transcription factor that mediates the response to infection through regulation of genes involved in the immune response (Siebenlist *et al.*, 1994). In its inactive form NF- κ B is found in the cytoplasm bound to inhibitor proteins. Stimulation of cells by various extracellular agents such as viruses, oxidants, inflammatory cytokines and lipopolysaccharide leads to phosphorylation cascade and subsequent degradation of inhibitor proteins. Thus NF- κ B is released from the complex and translocates into the nucleus, where it regulates the expression of many targets including proinflammatory cytokines. *Lm* infection has been shown to activate NF- κ B in a number of different cell types. In P388D₁ murine macrophages a biphasic activation of NF- κ B was observed. Transient NF- κ B activation was induced by adhesion of *Lm* to P388D₁ cells, which also occurred upon addition of nonvirulent *L. innocua* or by addition of listerial lipoteichoic acid to these cells (Hauf *et al.*, 1997; Hauf *et al.*, 1994). The second phase of NF- κ B activation was persistent and appeared to require a signal induced during escape of *Lm* from the phagosome or during intracellular replication, as NF- κ B activation was significantly lower when cells were infected with phospholipase (*plcA* or *plcB*) or ActA mutants (Marquis *et al.*, 1997; Hauf *et al.*, 1997; Marquis and Hager, 2000). In the Caco-2 epithelial cell line, *Lm* infection induced a monophasic activation of NF- κ B connected to lipoteichoic acid activation occurred prior to entry of the bacteria. This activation occurred prior to entry of the bacteria since addition of agents blocking bacterial invasion, failed to inhibit NF- κ B activation (Hauf *et al.*, 1999). Infection of HUVEC results in translocation of NF- κ B to the nucleus and upregulation of adhesion molecules such as E-selectin, VCAM-1 and ICAM-1 and LLO is required for full NF- κ B activation (Drevets, 1997; Schwarzer *et al.*, 1998; Kayal *et al.*, 1999). There is unclear that bacterial phospholipase expression is required for NF- κ B activation since such evidence correlated with the ability to produce ceramide was reported in one studies (Schwarzer *et al.*, 1998), but not in the other study that examined this requirement (Kayal *et al.*, 1999).

4. Conclusions

It appears that *Lm* manipulates intracellular signaling in different type of host cells at all steps of infection including the initial phase, in which bacteria interact either through secreted proteins or by contact; after internalization, when bacteria reside in an endocytic vacuole; upon release into the cytosol; and during cell-to-cell spread, when *Lm* resides briefly in the double-membrane secondary vacuole. LLO and PLCs play a crucial role in modulating the signal transduction of infected cells providing the optimal level of invasion and intracellular growth, and thus these exotoxins are multifunctional virulence factors with many important roles in the host-parasite interaction other than phagosomal membrane disruption.

Literature

- Berridge M.J., P. Lipp and M.D. Bootman. 2000. The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell Biol.* **1**: 11–21.
- Camilli A., H. Goldfine and D.A. Portnoy. 1991. *Listeria monocytogenes* mutants lacking phosphatidylinositol-specific phospholipase C are avirulent. *J. Exp. M.* **173**: 751–754.
- Cocconnier M.-H., E. Dliissi, M. Robard, C.L. Laboisie, J.-L. Gaillard and A.L. Servin. 1998. *Listeria monocytogenes* stimulates mucus exocytosis in cultured human polarized mucosecreting intestinal cells through action of listeriolysin O. *Infect. Immun.* **66**: 3673–3681.
- Cossart P. 2001. Met, the HGF-SF receptor: another receptor for *Listeria monocytogenes*. *Trends Microbiol.* **9**: 105–107.
- Cossart P., D.A. Portnoy, V.A. Fischetti, R.P. Novick, J.J. Ferretti and J.A. Rood. 2000. *Gram-positive pathogens*, p. 507–515. ASM Press, Washington, D.C.
- Demuth A., T. Chakraborty, G. Krohne and W. Goebel. 1994. Mammalian cells transfected with the listeriolysin gene exhibit enhanced proliferation and focus formation. *Infect. Immun.* **62**: 5102–5111.
- Dramsı S. and P. Cossart. 2003. Listeriolysin O-mediated calcium influx potentiates entry of *Listeria monocytogenes* into the human Hep-2 epithelial cell line. *Infect Immun.* **71**: 3614–3618.
- Drevets D.A. 1997. *Listeria monocytogenes* infection of cultured endothelial cells stimulates neutrophil adhesion and adhesion molecule expression. *J. Immunol.* **158**: 5305–5313.
- Exton J.H. 1997. New developments in phospholipase D. *J. Biol. Chem.* **272**: 15579–15582.
- Geoffroy C., J. Raveneau, J.L. Beretti, A. Lecroisey, J.A. Vazquez-Boland, J.E. Alouf and P. Berche. 1991. Purification and characterization of an extracellular 29-kilodalton phospholipase C. from *Listeria monocytogenes*. *Infect. Immun.* **59**: 2382–2388.
- Goldfine H. and C. Knob. 1992. Purification and characterization of *Listeria monocytogenes* phosphatidylinositol-specific phospholipase C. *Infect. Immun.* **60**: 4059–4067.
- Goldfine H., S.J. Wadsworth and N.C. Johnston. 2000. Activation of host phospholipases C and D in macrophages after infection with *Listeria monocytogenes*. *Infect. Immun.* **68**: 5735–5741.
- Griffith O.H. and M. Ryan. 1999. Bacterial phosphatidylinositol-specific phospholipase C: structure, function, and interaction with lipids. *Biochim. Biophys. Acta.* **1441**: 237–254.
- Guzman C.A., E. Domann, M. Rhode, D. Bruder, A. Darji, S. Weiss, J. Wehland, T. Chakraborty and K.N. Timmis. 1996. Apoptosis of mouse dendritic cells is triggered by listeriolysin, the major virulence determinant of *Listeria monocytogenes*. *Mol. Microbiol.* **20**: 119–126.
- Hauf N., W. Goebel, F. Fiedler and M. Kuhn. 1999. *Listeria monocytogenes* infection of Caco-2 human epithelial cells induces activation of transcription factor NF-kappaB/Rel-like DNA binding activities. *FEMS Microbiol. Lett.* **178**: 117–122.
- Hauf N., W. Goebel, F. Fiedler, Z. Sokolovic and M. Kuhn. 1997. *Listeria monocytogenes* infection of P388D1 macrophages results in a biphasic NF-kB (RelA/p50) activation induced by lipoteichoic acid and bacterial phospholipases and mediated by Ikb a and Ikb b degradation. *Proc. Natl. Acad. Sci. USA.* **94**: 9394–9399.
- Hauf N., W. Goebel, E. Serfling and M. Kuhn. 1994. *Listeria monocytogenes* infection enhances transcription factor NF-kappaB in P388D1 macrophage-like cells. *Infect. Immun.* **62**: 2740–2747.
- Kayal S., A. Lilienbaum, C. Poyart, S. Memet, A. Israel and P. Berche. 1999. Listeriolysin O-dependent activation of endothelial cells during infection with *Listeria monocytogenes*: activation of NF-kB and upregulation of adhesion molecules and chemokines. *Mol. Microbiol.* **31**: 1709–1722.
- Krüll M., R. Nost, S. Hippenstiel, E. Domann, T. Chakraborty and N. Suttorp. 1997. *Listeria monocytogenes* potently induces up-regulation of endothelial adhesion molecules and neutrophil adhesion to cultured human endothelial cells. *J. Immunol.* **159**: 1970–1976.
- Kuhn M., W. Goebel, E.T. Ryser and E.H. Marth. 1999. *Listeria*, listeriosis and food safety, p. 97–130. Marcel Dekker, New York.
- Kusner D.J., C.F. Hall and L.S. Schlesinger. 1996. Activation of phospholipase D is tightly coupled to the phagocytosis of *Mycobacterium tuberculosis* or opsonized zymosan by human macrophages. *J. Exp. M.* **184**: 585–595.
- Leimeister-Wächter M., E. Domann and T. Chakraborty. 1991. Detection of a gene encoding a phosphatidylinositol specific phospholipase C that is co-ordinately expressed with listeriolysin in *Listeria monocytogenes*. *Mol. Microbiol.* **5**: 361–366.
- Levin R., A. Braiman and Z. Priel. 1997. Protein kinase C induced calcium influx and sustained enhancement of ciliary beating by extracellular ATP. *Cell Calcium* **21**: 103–113.

- Madden J.C., N. Ruiz and M. Caparon. 2001. Cytolysin-mediated translocation (CMT): A functional equivalent of type III secretion in gram-positive bacteria. *Cell* **104**: 143–152.
- Marquis H., H. Goldfine and D.A. Portnoy. 1997. Proteolytic pathways of activation and degradation of a bacterial phospholipase C during intracellular infection by *Listeria monocytogenes*. *J. Cell. Biol.* **137**: 1381–1392.
- Marquis H. and E.J. Hager. 2000. pH-regulated activation and release of a bacteria-associated phospholipase C during intracellular infection by *Listeria monocytogenes*. *Mol. Microbiol.* **35**: 289–298.
- Mengaud J., C. Braun-Breton and P. Cossart. 1991. Identification of phosphatidylinositol-specific phospholipase C activity in *Listeria monocytogenes*: a novel type of virulence factor. *Mol. Microbiol.* **5**: 367–372.
- Nishibori T., H. Xiong, I. Kawamura, M. Arakaea and M. Mitsuyama. 1996. Induction of cytokine gene expression by listeriolysin O and roles of macrophages and NK cells. *Infect. Immun.* **64**: 3188–3195.
- Portnoy D.A., V.L. Miller, J.B. Kaper and R.R. Isberg. 1994. Molecular genetics of bacterial pathogenesis, p. 279–293. American Society for Microbiology, Washington, D.C.
- Repp H., Z. Pamukci, A. Koschinski, E. Domann, A. Darji, J. Birringer, D. Brockmeier, T. Chakraborty and F. Dreyer. 2002. Listeriolysin of *Listeria monocytogenes* forms Ca²⁺-permeable pores leading to intracellular Ca²⁺ oscillations. *Cell. Microbiol.* **4**: 483–491.
- Ron D. and M.G. Kazanietz. 1999. New insights into the regulation of protein kinase C and novel phorbol ester receptors. *FASEB J.* **13**: 1658–1676.
- Schuchat A., K. Robinson, J.D. Wenger, L.H. Harrison, M. Farley, A.L. Reingold, L. Lefkowitz and B.A. Perkins. 1997. Bacterial meningitis in the United States in 1995. *New England J.M.* **337**: 970–976.
- Schwarzer N., R. Nöst, J. Seybold, S.K. Parida, O. Fuhrmann, M. Krüll, R. Schmidt, R. Newton, S. Hippenstiel and E. Domann. 1998. Two distinct phospholipases C of *Listeria monocytogenes* induce ceramide generation, nuclear factor- κ B activation, and E-selectin expression in human endothelial cells. *J. Immunol.* **161**: 3010–3018.
- Sheehan B., C. Kocks, S. Dramsi, E. Gouin, A.D. Klarsfeld and P. Cossart. 1994. Molecular and genetic determinants of the *Listeria monocytogenes* infectious process. *Curr. Topics Microbiol. Immunol.* **192**: 187–216.
- Sibeliu U., T. Chakraborty, B. Krögel, J. Wolf, F. Rose, R. Schmidt, J. Wehland, W. Seeger and F. Grimminger. 1996a. The listerial exotoxins listeriolysin and phosphatidylinositol-specific phospholipase C synergize to elicit endothelial cell phosphoinositide metabolism. *J. Immunol.* **157**: 4055–4060.
- Sibeliu U., F. Rose, T. Chakraborty, A. Darji, J. Wehland, S. Weiss, W. Seeger and F. Grimminger. 1996b. Listeriolysin is a potent inducer of the phosphatidylinositol response and lipid mediator generation in human endothelial cells. *Infect. Immun.* **64**: 674–676.
- Sibeliu U., E.C. Schulz, F. Rose, K. Hattar, T. Jacobs, S. Weiss, T. Chakraborty, W. Seeger and F. Grimminger. 1999. Role of *Listeria monocytogenes* exotoxins listeriolysin and phosphatidylinositol-specific phospholipase C in activation of human neutrophils. *Infect. Immun.* **67**: 1125–1130.
- Siebenlist U., G. Franzoso and K. Brown. 1994. Structure, regulation and function of NF- κ B. *Annu. Rev. Cell Biol.* **10**: 405–455.
- Singer W.D., H.A. Brown and P.C. Sternweis. 1997. Regulation of eukaryotic phosphatidylinositol-specific phospholipase C and phospholipase D. *Annu. Rev. Biochem.* **66**: 475–509.
- Smith E.R., P.L. Jones, J.M. Boss and A.H. Merrill. 1997. Changing J774A.1 cells to new medium perturbs multiple signaling pathways, including the modulation of protein kinase C by endogenous sphingoid bases. *J. Biol. Chem.* **272**: 5640–5646.
- Tang P., I. Rosenshine, P. Cossart and B.B. Finlay. 1996. Listeriolysin O activates mitogen-activated protein kinase in eukaryotic cells. *Infect. Immun.* **64**: 2359–2361.
- Tang P., I. Rosenshine, P. Tang and B.B. Finlay. 1994. *Listeria monocytogenes*, an invasive bacterium, stimulates MAP kinase upon attachment to epithelial cells. *Mol. Biol. Cell* **5**: 455–464.
- Tilney L.G. and D.A. Portnoy. 1989. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. *J. Cell Biol.* **109**: 1597–1608.
- Vázquez-Boland J.A., M. Kuhn, P. Berche, T. Chakraborty, G. Dominguez-Bernal, W. Goebel, B. González-Zorn, J. Wehland and J. Kreft. 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiol. Rev.* **14**: 584–640.
- Wadsworth S.J. and H. Goldfine. 1999. *Listeria monocytogenes* phospholipase C-dependent calcium signaling modulates bacterial entry into J774 macrophage-like cells. *Infect. Immun.* **67**: 1770–1778.
- Wadsworth S.J. and H. Goldfine. 2002. Mobilization of protein kinase C in macrophages induced by *Listeria monocytogenes* affects its internalization and escape from the phagosome. *Infect. Immun.* **70**: 4650–4660.
- Weiglein L., W. Goebel, J. Troppmair, U.R. Rapp, A. Demuth and M. Kuhn. 1997. *Listeria monocytogenes* infection of HeLa cells results in listeriolysin O-mediated transient activation of the Raf-MEK-MAP kinase pathway. *FEMS Microbiol. Lett.* **148**: 189–195.
- Zenewicz L.A., J.A. Skinner, H. Goldfine and H. Shen. 2004. *Listeria monocytogenes* virulence proteins induce surface expression of Fas ligand on T lymphocytes. *Mol. Microbiol.* **51**: 1483–1492.