Polish Journal of Microbiology
2004, Vol. 53, No 2, 75–88

Classes and Functions of *Listeria monocytogenes* Surface Proteins

MAGDALENA POPOWSKA* and ZDZISŁAW MARKIEWICZ

Department of Bacterial Physiology, Institute of Microbiology, Warsaw University
Miecznikowa 1, 02-096 Warsaw, Poland

Received 15 March 2004

Abstract

Listeria monocytogenes is an opportunistic pathogen that causes infections collectively termed listeriosis, which are related to the ingestion of food contaminated with these gram-positive rods. The pathogenicity of *L. monocytogenes* is determined by the following virulence factors: listeriolysin O, protein ActA, two phospholipases C, internalins (InlA and InlB), protein CwhA and a metalloprotease. The bacterium is a model organism in studies on the pathogenesis of intracellular parasites. It is able to penetrate, multiply and propagate in various types of eukaryotic cells and is also able to overcome the three main barriers encountered in the host: the intestinal barrier, the blood-brain barrier and the placenta. Based on *L. monocytogenes* genome sequence analysis 133 surface proteins have been identified. In particular, the large number of proteins covalently bound to murein sets *L. monocytogenes* apart from other gram-positive bacteria. The ability of this pathogen to multiply in various environments as well as the possibility of its interaction with many kinds of eukaryotic cells is, in fact, made possible by the large number of surface proteins.

Key words: *Listeria monocytogenes*, virulence, murein, surface proteins

1. General characteristics of *Listeria monocytogenes*

Listeria monocytogenes is a facultative anaerobe that grows best at 37°C and in neutral or slightly alkaline environment. The bacterium is widespread in nature: waters, soil, rotting parts of plants, animal feces and wastewaters. It has also been isolated from 5% of fecal samples from healthy humans (Farber, 1991). *L. monocytogenes* has also been detected in many food products (Schlech, 2000). The dissemination of the organism is related to the irrigation of rural areas with wastewaters, which results in its presence on vegetation and in products of animal origin. *L. monocytogenes* is pathogenic for people and animals and is the etiological factor of listeriosis, whose nature may be sporadic or epidemic. Infection is usually related to the ingestion of food products contaminated with the bacterium and for this reason listeriosis is considered a food-borne disease (Schlech, 2000).

So far 13 serotypes of *L. monocytogenes* have been identified. On average, 90% of clinical infections are caused by serotypes Ia, Ib and IVb, the latter being the dominating type in Europe (Schlech, 2000). The most frequent forms of the disease are: meningoencephalitis, bacteremia and perinatal listeriosis. *L. monocytogenes* can also cause endocarditis, hepatitis, pleuritis, localized abscesses (*e.g.* in the brain) as well as muscular, skeletal and skin infections. Listeriosis is characterized by low incidence of the disease, but high mortality rate, which can range from 20 to 60% in adults, especially in the case of infections of the central nervous system, or from 54 to 90% for neonates (Hof *et al*., 1997). The group of those at greatest risk embraces pregnant women, neonates, individuals over 60 years of age and people with impaired cell-mediated immunity. The bacterial dose causing the disease in humans is not known, the infectious dose in mammals (monkey) is $\geq 10^9$ cells (Farber, 1991). The incubation period for the disease in humans is from 11 to 70 days (Lorber, 1997).

The pathogenicity of *L. monocytogenes* is largely determined by the following virulence factors (Portnoy *et al*., 1992): the internalins InlA and InlB, listeriolysin O (LLO), protein ActA, two phospholipases C

* Corresponding author: e-mail: magdapop@biol.uw.edu.pl

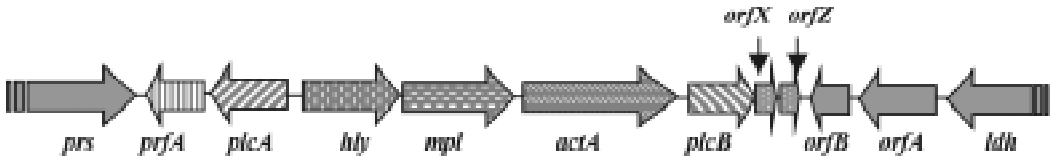

Fig. 1. Physical and transcriptional organization of the central virulence gene cluster of *L. monocytogenes*. For explanations see text.

– a phospholipase specific for phosphatidylinositol (PI-PLC, PlcA) and phospholipase C specific for phosphosfatidylcholine (PlcB, lecithinase), and a metalloprotease. The genes coding for 6 of these factors are located in the bacterial chromosome next to each other in a virulence gene cluster locus (pathogenicity island) and are regulated by the transcription activator PrfA (Fig. 1) (Chakraborty *et al*., 2000; Vazquez-Boland *et al*., 2001). The *inlAB* operon is located elsewhere (Lecuit *et al*., 2001). Moreover, such other determinants as protein CwhA (known earlier as p60 or Iap) (Park *et al*., 2000), catalase, superoxide dismutase, siderophores and protein LmaA are also required for full pathogenic activity of the bacterium.

1.1. Intracellular growth of *Listeria monocytogenes*

L. monocytogenes is a model organism in studies on the pathogenesis of intracellular parasites. It is able to penetrate the cytoplasmic membrane of various eukaryotic cells, such as fibroblasts, epithelial cells, kidney cells and macrophages, grow in them and spread from one cell to another. Detailed studies of these events was possible as a result of the ability of *L. monocytogenes* to grow in macrophage cell lines. The individual stages of the life cycle of *L. monocytogenes* were first described in detail by Tilney and Portnoy (Portnoy *et al*., 2002).

The first phase in the infection of J774 macrophages involves phagocytosis of the bacterial cell, combined with disruption of the cytoplasmic membrane (Cossart *et al*., 2003). This process is mediated by internalins A and B. Almost immediately after internalization of the bacterial cell the phagosome formed combines with the lysozome to digest the enclosed bacterial cell. To avoid the maturation of the phagosome to the phagolysosomal stage, *L. monocytogenes* brings about the disruption of the phagosomal membrane. This is brought about by the low pH inside this compartment, which results in the activation of listeriolysin O (Dramsi and Cossart, 2002) that together with other secreted enzymes (phospholipase B and C) and proteases causes lysis of the phagocytic vacuole and escape of the microorganism to the cytoplasm, where it replicates. In the case of nonphagocytic cells, a zipper-like mechanism is involved in the uptake of *L. monocytogenes*, in that the bacterium sinks into diplike structures on the host cell surface, until is finally engulfed (Cossart, 2002).

While replicating in the cytosol, the cells become covered with actin filaments (Cossart and Lecuit, 1998), which rearrange to form relatively long (up to 40 mm) comet-like tails at one end of the bacterial cell. Some of the cells move towards the plasma membrane where they induce the formation of protrusions or pseudopods (listeriopods) that penetrate neighboring cells and are in turn engulfed by phagocytosis. These events result in the formation of a secondary phagosome surrounded by two membranes, with the inner one originating from the mother cell (Dabiri *et al*., 1990). The activity of listeriolysin O and phospholipase C (Smith *et al*., 1995) specific for phosophatidylocholine results in lysis of the newly formed vacuole and the listeriae escape to the cytoplasm where they can repeat their life cycle (Portnoy *et al*., 2002).

1.2. Therapy of listeriosis and prophylaxis

Recommendations pertaining to choice of drug and duration of therapy in patients with listeriosis are based on results of studies with animal models, susceptibility of *L. monocytogenes* to antibiotics *in vitro* and the results of clinical trials embracing relatively small groups of affected individuals. *L. monocytogenes* is susceptible to most β-lactam antibiotics, except for the cephalosporins (Hof *et al*., 1997). However, new generation cephalosporins are commonly used to treat non-specific symptoms of bacteremia or in the therapy of meningoencephalitis, and for this reason treatment of listeriosis can sometimes be delayed until identification of the causative agent in blood sample or cerebrospinal fluid. The combination of ampicillin (or penicillin) and gentamicin is considered the therapy of choice in the treatment of listeriosis (Jones and MacGowan, 1995; Temple and Nahata, 2000). Ampicillin is considered bacteriostatic towards *L. monocyto-*

genes cells but in concentrations achieved in the cerebrospinal fluid shows delayed bactericidal activity (within 48 hours) (MacGowan *et al*., 1998; Teuber, 1999). An alternative drug for patients intolerable to penicillin is co-trimoxazole (a combination of trimethoprim and sulfamethoxazole). Better outcomes in the case of patients with meningoencephalitis were observed when amoxicillin was used together with co-trimoxazole, compared to the combination of ampicillin and gentamicin (Merle-Melet *et al*., 1996). Erythromycin has also been found effective, especially in the case of pregnant women (MacGowan *et al*., 1998).

1.3. Features of the *Listeria* genome

The complete nucleotide sequences of *Listeria monocytogenes* EGD genome (Acc. No AL591824) and a strain of the non-pathogenic *L. innocua* (Acc. No AL592022) were published in 2001 (Glaser *et al*., 2001).

The G+C content of the chromosome is 39% for *L. monocytogenes* EGD. In the case of strain EGD 54 regions with lower, and 6 with higher G+C content were also determined. It is suggested that the regions with different G+C content than the rest of the chromosome were acquired *via* lateral transfer from other bacterial species (Glaser *et al*., 2001). Analysis of these regions shows that they code virulence and factors and surface proteins implicated in the adaptation and life cycle of the pathogen, which is able to live in various environmental conditions and different host cells (Buchrieser *et al*., 2003).

All identified open reading frames (ORFs) take up 90.3% of the chromosome of both *Listeria* species. About 9.5% of the genes are regarded as specific for *L. monocytogenes*, and these genes code proteins related to virulence and adaptation to varied environmental conditions, Genes specific for *L. innocua* approximate 5%. For 35.3% genes of *L. monocytogenes* no potential function has yet been determined. *L. monocytogenes* has more surface proteins (with LPXTG sequence motif) than other gram-positive bacteria (Table I). 41 *L. monocytogenes* proteins with the LPXTG motif have been identified, 19 of which simultaneously have the LRR domain. Also, 7.3% of all *L. monocytogenes* genes, that is 209, are transcription regulators. The best-characterized regulator that activates many known virulence genes in *L. monocytogenes*, is protein PrfA, which is not present in *L. innocua.* In both *Listeria* genomes there is a large number of genes coding surface and secreted proteins, transporters and transcription regulators, which allow the bacterial cell to adapt to changing environmental conditions. The main difference between the two species lies in their surface protein composition (Buchrieser *et al*., 2003).

Table I
Surface proteins of several gram-positive bacterial species

Bacterial species	Genome size (Mb)	Proteins with LPXTG motifs	Proteins with GW modules
Listeria monocytogenes	2.94	41	9
Listeria innocua	1.01	31	9
Bacillus halodurans	4.20	3	0
Bacillus subtilis	4.21	1	0
Lactococcus lactis	2.37	9	0
Staphylococcus aureus	2.88	17	1
Streptococcus pneumoniae	2.04	11	0
Streptococcus pyogenes	1.85	13	0

2. Overview of murein structure

Bacterial cells, with very few exceptions, have a cell wall, whose main component is murein (syn. peptidoglycan). This polymer is composed of glycan chains in which alternating *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid are β-1,4 bound (1→4). The structure of the glycan chains is relatively conserved, though certain modifications are known. The pentapeptide of the precursor, which may become shorted on incorporation into murein, is composed of amino acids in configuration both L and D. The D-lactyl groups of muramic acid in murein of the cell wall are usually amidated with L-alanyl-γ-D-glutamyl-L-diaminoacyl-D-alanine stem tetrapeptides (or tripeptides). The peptide part of murein is far more varied and its composition is species-specific, but such factors as age of the cells and even growth conditions

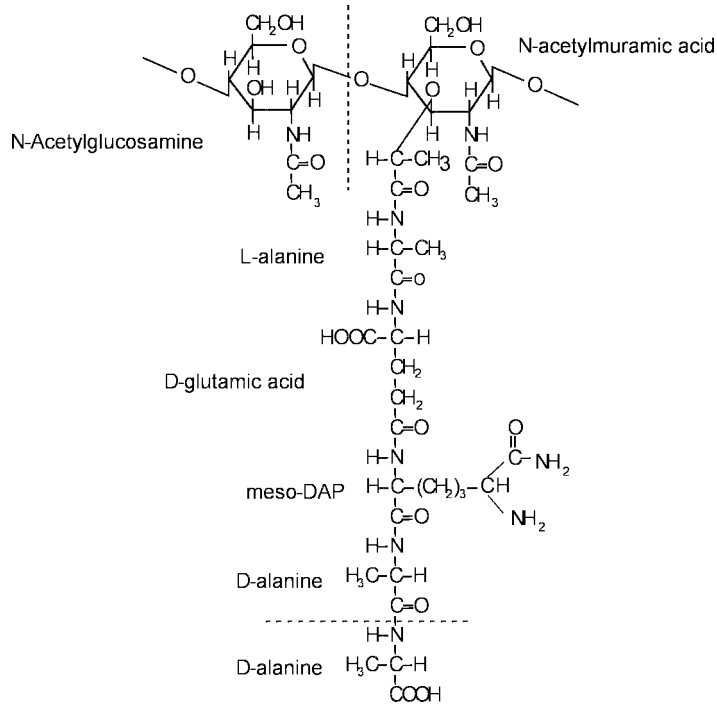

Fig. 2. Structure of the disaccharide-pentapeptide monomer of *L. monocytogenes* murein

may play an important role. In the murein, neighboring peptide chains may be peptide-bound either directly, between the diamino amino acid of one chain and usually D-alanine in position 4 of the other chain (4→3 mureins), or indirectly *via* a shorter or longer interpeptide bridge. Some bacteria, like *Mycobacterium tuberculosis*, have (3→3) mureins with crosslinks between adjacent *meso*-diaminopimelic acid (*m*-DAP) residues, whereas others have mureins in which both (4→3) and (3→3) crosslinks occur. The crosslinkage of the murein of gram-negative is usually 20–30%, whereas in the case of gram-positive bacteria this value is much higher and can approach 100%.

The primary structure of the murein of *L. monocytogenes* murein resembles that of *Escherichia coli* and, like *E. coli* murein, contains (*m*-DAP) acid as the diamino amino acid in the peptide side chain (Fig. 2). Also, like in the case of *E. coli*, both (4→3) and (3→3) crosslinks occur. Other modifications of *L. monocytogenes* murein include amidation of free (*m*-DAP) residues and partial de-*N*-acetylation of glucosoamine (this laboratory, unpublished). Also, typically for gram-positive bacteria, the cell wall of *L. monocytogenes* contains lipoteichoic acids, which in the case of the best studied serotype 1/2 are composed of polyribitol phosphate with N-acetylglucosamine and rhamnose substituents on ribitol and are bound to the muramic acid moiety of murein, lipoteichoic acids and numerous proteins, described in detail below, that are either anchored in the cytoplasmic membrane or cell wall or peptide-bound to the murein (Baba and Schneewind, 1998).

3. Proteins in the gram-positive cell wall

Proteins are rather common in the cell wall of gram-positive bacteria where they can carry out many diverse functions. Their number, as well as functions, differ depending on given species and even strain. They can be involved in the adherence of the bacterial cells, protect them from phagocytosis by leukocytes, take part in motility and some of them show enzymatic activity. There are several known mechanisms of the exposure of these proteins on the cell surface and the nature of their interaction with the cell wall in gram-positive bacteria. Each of these mechanisms depends on the nature of a given protein and the role it plays in the cell (Navarre and Schneewind, 1999).

3.1. Surface proteins of *Listeria monocytogenes*

Analysis of the genome sequence of *L. monocytogenes* has allowed to determine the number and types of surface proteins of this bacterium (Cabanes *et al*., 2002), which are more abundant than in any other bacteria with known genome sequence. Among the *L. monocytogenes* surface proteins it is possible to distinguish

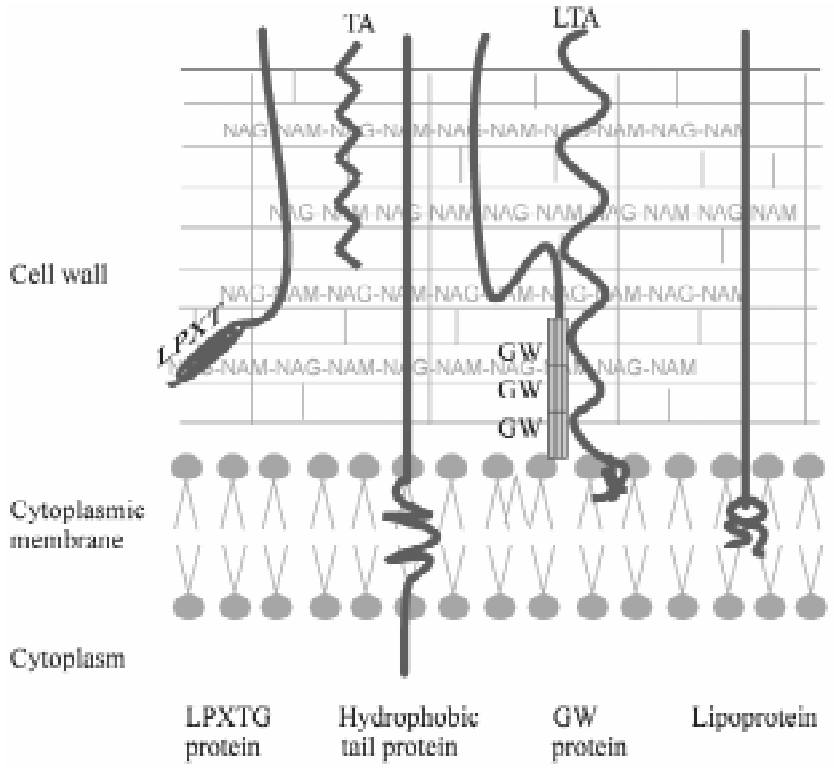

Fig. 3. The major types of surface proteins found in *L. monocytogenes*: Proteins with LPXTG carboxy-terminal sorting signal; proteins anchored by a hydrophobic tail motif; proteins containing GW modules; lipoproteins. LTA – lipoteichoic acid, TA – teichoic acid. For explanations see text.

three major groups of proteins, that are characterized by specific structural features (Fig. 3). These are: 1. Proteins covalently linked to murein through their C-terminal domain (proteins with LPXTG sequence motif), 2. Proteins non-covalently bound by their C-terminal domain (GW proteins, hydrophobic tail proteins, CwhA-like proteins) and 3. Proteins linked to cell wall structures *via* their amino-terminal region (lipoproteins) (Dhar *et al*., 2000).

3.2. Proteins covalently linked to cell wall murein

3.2.1. Proteins containing the LPXTG motif

It seems, at least so far, that the only mechanism allowing covalent binding of surface proteins to the cell murein of gram-positive bacteria requires a specific C-terminal signal (Fischetti *et al*., 1990), *i.e.* the LPXTG motif, which is strongly conserved and followed by a hydrophobic stretch of roughly 20 amino acids and a tail of positively charged amino acids. This sorting sequence keeps the protein in the cytoplasmic membrane until it is cleaved off between the TG residues of the LPXTG motif, followed by the formation of a peptide bond between threonine and diamino amino acid of murein. This reaction is catalyzed by a protein that has been termed a sortase (Navarre and Schneewind, 1999).

The LPXTG motif has been found in over 100 bacterial surface proteins (Table I). The number of proteins of this type in *L. monocytogenes* (Buchrieser *et al*., 2003) is much higher than in many other gram-positive species, *e.g.* 17 in *Staphylococcus aureus* (Kuroda *et al*., 2001), 13 in *Streptococcus pyogenes*, 11 in *S. pneumoniae*, 9 in *Lactococcus lactis* and 3 in *Bacillus subtilis* (Kunst *et al*., 1997).

3.2.2. Proteins with LRR domain

The first identified protein of this group, which at the same time contains the LPXTG motif was internalin InlA (Jonquieres *et al*., 1998). The second protein (no LPXTG motif) was InlB (Jonquieres *et al*., 1999). *inlA* and *inlB* are the most thoroughly studied members of the so-called internalin multigene family. The genes are located in operon *inlAB,* which is transcribed from 4 promoters, only one of which is under the control of the regulator PrfA (Dramsi *et al*., 1997). Genetic inactivation of either *inlA* or *inlB* leads to a significant decrease in the virulence of *L. monocytogenes*.

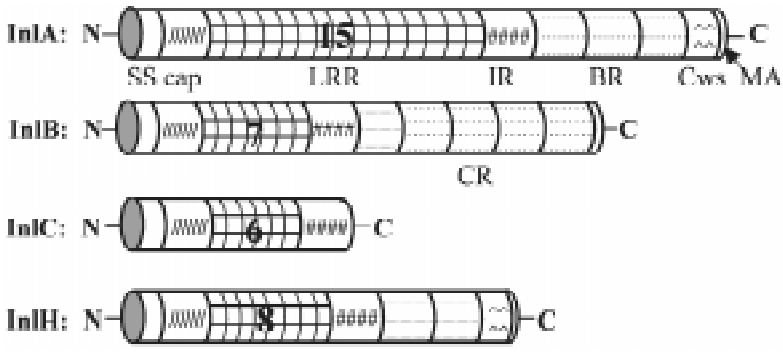

Fig. 4. Schematic domain organization of full-length InlA, InlB, InlC and InlH from *L. monocytogenes*. Homologous regions are the same sign coded. SS – signal sequence; cap – the cap domain; LRR – LRR domain; IR – inter-repeat-region; BR – B-repeats; Cws – cell wall spanning region; MA – membrane anchor; CR – C-repeats. Numerals indicate number of tandem repeats in the LRR domain. For explanations see text

Four other internalin-like proteins were identified more recently: InlE, InlF, InlG and InlH (Engelbrecht *et al*., 1998; Raffelsbauer *et al*., 1998). However, otherwise than internalin InlA, these four proteins are not involved in invasion but are important for colonization of host tissues *in vivo* (Portnoy *et al*., 2002). More recently, a new *L. monocytogenes* internalin-like protein, Lmo2026, has been identified, with a suggested role of the protein in invasion and multiplication in the brain (Autret *et al*., 2001). All these proteins belong to a large family which contains an amino-terminal LRR (leucine-rich repeat) domain, which is followed by a conserved IR (inter-repeat) region – a conserved region of repeats with similarity to immunoglobulin – several other repeats and the motif LPXTG (Fig. 4). It is not yet know whether the Ig-like domain plays any role in specific contacts with eukaryotic cell surface proteins or only has a role in stabilizing the LRR region. A comparison of the amino acid sequences of the known internalins indicates that the IR region is strongly conserved in these proteins (Schubert *et al*., 2002). The LRR and IR domains also occur in other bacterial proteins, which are mostly virulence factors. The fusion of these two domains in InlB and other internalins is an example of optimal adaptation of bacterial pathogens to their eukaryotic hosts in the course of evolution.

Based on different lengths and consensus sequences in proteins from various kinds of cells at least 7 subfamilies of proteins with LRR repeats can be distinguished (Kajava and Kobe, 2002). Most of them contain a conserved N-terminal region with an LRR domain, preceded by a signal sequence - SS as well as an inter-repeat (IR) sequence that is like an immunoglobulin fold (Schubert *et al*., 2002). In some cases a second repeat region of up to three repeats of about 70 amino acids called B repeats may be present. The LRR domain of the listerial proteins consist of tandem repeats of 20–22 amino acids, being typical for the shorter bacterial sequences, with a large number of leucine or isoleucine residues. The C-terminal part of the internalin proteins contains a domain that anchors the protein on the cell surface or is absent, as in the case of secreted proteins. It has tandem ca 80 amino acid repeats that are highly basic and start with the dipeptide GW (Lecuit *et al*., 1997).

The LRR domain is involved in many processes, such as adhesion, and signaling, for instance, but in general it can be said that it provides a versatile structural framework for the formation of protein-protein interactions. The consensus sequence of the 20 amino acids, being the repeat in the LRR domain has been determined: LXXLXLXXNXIXXIXXLXXL (Kajava and Kobe, 2002; Schubert *et al*., 2002).

Genome sequence studies have shown that the internalin multigene family of *L. monocytogenes* consists of 24 genes. Besides five internalins that have been known for some time, 14 ORFs have been identified in the genome sequence that code for proteins with a signal sequence, an LRR domain and an LPXTG motif. Thirteen of these proteins have a second repeat domain and 6 the conserved IR domain. The large number of listerial proteins with the LRR domain and LPXTG motif distinguishes *L. monocytogenes* from among other bacteria. In addition, the *L. monocytogenes* genome codes for 5 proteins which contain the LRR domain but do not have the LPXTG motif. One of these is InlB, which contains the GW sequence and attaches to the bacterial cell surface by a different mechanism (Cabanes *et al*., 2002). Four other proteins are secreted proteins with the domain LRR, which include InlC (Dramsi *et al*., 1997; Engelbrecht *et al*., 1998).

Internalin A

Internalin A (InlA) is composed of 800 amino acids and is required for the entry of *L. monocytogenes* into only a few types of cells, including cells of the eukaryotic line Caco-2. The N-terminal part of the

protein contains a typical for all internalins LRR region, followed by the IR region, but its C-terminus contains two and a half repeats of 75 amino acids, followed by a region that contains the LPXTG motif. Amino acids from 36 to 78 form a domain composed of three α-helixes – a cap domain. The LRR domain contains 15 and a half repeats of a 22 amino acid sequence, followed by a Ig-like domain between 415 and 495 aa. In terms of structure, this region is the most elastic part of the internalin domain, and when comparing InlA, InlB and InlH, only small differences in this region can be observed. The surface receptor for InlA is E-cadherin – or more specifically its extracellular part – which is expressed in epithelial cells (Jonquieres *et al*., 1998; Schubert *et al*., 2002). E-cadherin is a transmembrane glycoprotein, which contains 5 extracellular adherin domains, a transmembrane α-helix and intracellular domain. Intracellular E-cadherin is involved in the process of the formation of the actin cytoskeleton. The extracellular N-terminal domain of E-cadherin (EC1) is responsible for specific trans-interactions between identical cadherins of progeny cells, and is also the site recognized by InlA (Schubert *et al*., 2002). InlA forms a complex with this domain and specifically rearranges it. The reaction is Ca-dependent and involves Pro16, which is important for intramolecular rearrangements. The substitution of Pro16 in human EC1 by glutamine results in immunity to bacterial invasion (Lecuit *et al*., 1999).

The interaction between InlA and E-cadherin leads to phagocytosis and not adherence. Why this is so is not entirely clear and so far, *L. monocytogenes* is the only pathogen that has been found to use E-cadherin to enter into cells. Other bacteria as a rule use other adhesion molecules, such as integrins. Using truncated forms of internalin AS in the noninvasive *L. innocua*, it was established that the LRR and IR regions are sufficient to mediate attachment and phagocytosis (Lecuit *et al*., 1997). The carboxy-terminal part of internalin A contains the motif LPXTG, which is responsible for the anchoring of the protein in the cell wall. Small amounts of internalin A can also be found in the culture supernatant, but most of the molecules are anchored via covalent linkages in the cell wall.

Internalin B

Besides InlA discussed above, *L. monocytogenes* also uses InlB to exploit another mammalian signaling pathway to enter the cell. The LRR region at the N-terminus of InlB embraces 213 amino acids, from 36 to 242. Amino acids 1–35 form a signal sequence that is cleaved off, so in the mature protein the LRR domain takes up the whole N-terminus. This region contains a hydrophilic cap composed of two β- and three α-helixes and eight LRRs. InlB, like many other internalin proteins, also contains a B repeat. Although the whole domain, including the B repeat, is necessary for efficient internalization, the N-terminal 213 residue region is sufficient to induce the entry of bacteria into cells and to activate signal transduction pathways (Shen *et al*., 2000). The cap at the N-terminus resembles a calcium-binding domain in calmodulin and related proteins but whether it binds calcium in InlB is still the subject of controversy (Bierne and Cossart, 2002; Marino *et al*., 2002). The amino acid sequence in this region is strongly conserved in all known internalins. InlB, in contrast to InlA, is loosely attached to the cell wall and is partially released to the environment (Jonquieres *et al*., 1999).

InlB has been found to bind to three different kinds of receptors on mammalian cells. The receptor first identified was gC1qR, a glycoprotein with molecular weight 33 kDa that acts as a receptor for C1q, the first component of the complement cascade and occurs mainly in mitochondria and in the nucleus but can also be found on the surface of eukaryotic cells and in body fluids (Braun and Cossart, 2000). Binding is mediated by the Gly-Trp repeats of InlB (Marino *et al*., 2002). The second receptor is known as Met, which is a tyrosine kinase receptor. Met is a heterodimer composed of an extracellular alpha subunit and transmembrane beta subunit. Met is recruited and phosphorylated at the site of listerial entry. Notably, it has been found that Met and E-cadherin (see InlA above) co-localize when co-expressed in cells. Finally, the third type of ligand recognized by InlB are glycosaminoglycans, which "decorate" the proteoglycans that occur on the surfaces of all types of mammalian cells (Jonquieres *et al*., 1999). In this case too, binding is mediated by the Gly-Trp repeats. For activation of the receptors the N-terminal part of InlB, consisting of 213 amino acids out of the 595 amino acids of the whole protein (67 kDa) is required. This region, which contains the LRR motif is necessary and sufficient to activate phosphatidylinositol 3-kinase and bring about cytoskeletal rearrangements (Lecuit *et al*., 1999). To elucidate this effect the X-ray crystal structure of this domain was studied (Schubert *et al*., 2002; Kajava and Kobe, 2002). It was found that Ca is bound within it in a specific manner that may allow the metal can act as a bridge between InlB and the receptor on the surface of a mammalian cell (Bierne and Cossart, 2002).

An analogous system occurs in InlH, where the LRR domain is longer by one 22 amino acid repeat, and an identical Ca-binding region is present. The C-terminal domain is between amino acids 263 and 343. Although

the involvement of InlH in the survival of *L. monocytogenes* in the course of mouse infection has been established, the mechanism and function of InlH in the course of infection have so far not been elucidated.

Calcium binding in the region of α-helix 1 and α-helix 2 does not cause conformational changes and plays no structural role. The binding of magnesium ions by α-helix 1 in the absence of calcium ions has also been shown. The Ca-binding region has been postulated as the site of protein-protein interactions. The metal has been proven to be required in the process of adhesion to host cells, which is a fairly common mechanism in pathogens that induce phagocytosis. The C-terminal region of InlB contains three highly basic tandem repeats of about 80 amino acids, beginning with the dipeptide GW (Bierne and Cossart, 2002). The GW repeats are involved in loose association of the protein with the bacterial surface, mainly though non-covalent interactions with lipoteichoic acid present on the surface of most gram-positive bacteria. Interestingly, they also confer on InlB the unusual property of adhering to bacteria when added to the bacterial growth medium.

Internalin C

InlC is a secreted protein with molecular weight of 30 kDa with five consecutive leucine-rich repeats, which is required for the full virulence of *L. monocytogenes* in the mouse cell infection model. Similar small, secreted internalins were identified in the culture supernatant of *L. ivanovii*. The expression of gene *inlC* occurs *via* two different modes – independent of the transcription activator PrfA and dependent on it. The main promoter from which *inlC* is transcribed is strictly dependent on PrfA and contains a PrfA-binding site at position –40 from the transcription start site. The gene *inlC* is not located adjacent to other virulence genes and is flanked by two so-called housekeeping genes, *rplS* and *infC*. In *L. ivanovii* gene i-*inlC* with 92% similarity to *inlC* has been identified. InlC, like InlA and InlB, contains the typical LRR motif (Engelbrecht *et al*., 1998). No InlC-binding receptor has yet been found on the surface of eukaryotic cells, but it is equally possible that such a receptor may occur inside the host cells, especially since the synthesis of this particular internalin is preferentially induced in the cytosol of mammalian cells.

3.3. Proteins with the RGD motif

The *L. monocytogenes* protein Lmo1666, besides the LPXTG motif and 10 PKD repeats contains a RGD (Arg-Gly-Asp) motif (Cabanes *et al*., 2002). This motif has been found in proteins participating in adhesion to eukaryotic cells and has been shown to be the core recognition sequence for many integrins. They are present in a variety of integrin ligands, including pathogen surface proteins from *Leishmania* and *Bordetella pertussis* (Finlay and Cossart, 1997). These data may indicate the role of protein Lmo1666 in the invasion of the host cells. Besides protein Lmo1666, the RGD motif has also been found in two other *L. monocytogenes* surface proteins: ActA and in a lipoprotein with unknown function, Lmo0460. The role of the RGD sequence in the former is unknown but probably does not mediate the attachment of *L. monocytogenes* to the host cell (Alvarez-Dominguez *et al*., 1997).

3.4. Proteins with PKD repeats

Eleven *L. monocytogenes* proteins with the motif LPXTG contain PKD repeats at their C-terminal end (Cabanes *et al*., 2002). The domain PKD was first identified in the human protein polycystin-1 (PKD1) encoded by gene *PKD1*, whose function is unknown (Huan and van Adelsberg, 1999). The protein contains a signal peptide, the LRR domain, lipoprotein A module (LDL-A), a calcium-dependent domain and 16 PKD repeats consisting of about 80 amino acids each. PKD domains have also been found in extracellular regions of proteins from higher organisms, bacteria and archeons. It has been shown that domain PKD contains an Ig-like fold and this type of domain has been shown to form ligand binding sites in cell surface proteins (like in the case of IR sequences of internals – see above), and it is therefore suggested that PKD domains carry out a similar function. Two internalin proteins of *L. monocytogenes* Lmo0331 and Lmo0333 contain both LRR and PKD domains, which may indicate their role in adhesion or signaling.

3.5. Sortases

Surface proteins with the LPXTG motif are anchored in the cell wall by specific cysteine proteases that have been termed sortases (Cossart and Jonquieres, 2000). The first studied sortase of *Staphylococcus aureus* is a 206 amino acid protein, with a potential N-terminal signal peptide that could also act as a membrane

anchor (Ilangovan *et al*., 2001). It also has a cysteine at position 184, within a catalytic TLXTC signature, which has been shown to be critical for the functioning of the enzyme. Sortase recognizes the sequence LPXTG of proteins translocated through the cell wall, catalyzes its proteolysis and subsequent covalent attachment of the protein to murein. The purified enzyme catalyzes the formation of a hydroxylamine-sensitive acyl enzyme intermediate, which in the presence of murein precursors allows a transpeptidation reaction to proceed (Ton-That *et al*., 1999). Sortase thus has both protease and transpeptidase activities. The enzyme cleaves the sequence LPXTG between threonine and glycine and the carboxyl group of threonine is then amide-linked to the amino group of cross-bridges within murein precursors. In *S. aureus* the pentaglycine interpeptide bridge is covalently bound to the lysine of the side peptide of the cell wall murein. This general scheme applies to all proteins covalently linked to murein but since the structure of bacterial murein is species-specific, the LPXT part of the protein may bind differently in the case of different bacteria. For example, *Streptococcus pyogenes* murein contains a dialanine bridge to which the protein binds, whereas in *L. monocytogenes* the binding has been found to be between the threonine of LPXT and *m*-(DAP) acid (Cossart and Jonquieres, 2000). The sortase genes of various bacteria are frequently located next to the gene that codes for their substrate.

Analysis of the genome sequence of *L. monocytogenes* has identified two sortase genes, one being similar to *srtA* of *Staphylococcus aureus*, and consequently also termed *srtA* and the other *srtB*, at a distance of 1300 kb from *srtA* in the *Listeria* genome (Garandeau *et al*., 2002). The sequence around cysteine 184 (based on *S. aureus* numbering) in the *L. monocytogenes* SrtA was strikingly conserved compared to the *S. aureus* sortase with the TLXTC motif being evident. The enzyme is 222 amino acids long and in the *L. monocytogenes* genome its structural gene is flanked by genes coding for proteins similar to *Bacillus subtilis* ORFs, Yhfl of unknown function and YxiJ, a 3-methyladenine DNA glycosylase. The construction of a *L. monocytogenes* mutant lacking functional SrtA sortase showed that the enzyme is indispensable for the anchoring of the invasion protein internalin A to murein. The mutation also prevented the proper sorting of several other murein-anchored proteins with the LPXTG motif. The *srtA* mutant is defective in entering epithelial cells and its virulence in the mouse model is strongly attenuated. The second *L. monocytogenes* sortase gene, *srtB*, is adjacent to two genes coding LPXTG proteins (Bierne *et al*., 2004). The enzyme is 246 amino acids long and is 23% identical to SrtA, which is lower than could be expected. It too, contains the TLXTC sequence and putative signal sequence region but in addition contains two stretches of 13 and 31 amino acids that are not present in SrtA. The second *S. aureus* sortase, SrtB, is needed to anchor proteins with the NPQTN motif. Gene *srtB* is part of an iron-regulated region, which carries genes for surface proteins with the LPXTG motif and the NPQTN domain and an iron transporter gene (Jonsson *et al*., 2003). In *L. monocytogenes* gene *srtB* is also part of an area that codes for LPXTG proteins and an iron transporter but does not contain any gene for proteins with the NPQTN motif. Moreover, it is not known whether this region is subject to similar regulation. These and other observations suggest the existence of two, if not more, distinct families of sortases in gram-positive bacteria (Paterson and Mitchell, 2004).

3.6. Proteins non-covalently linked to the cell wall

3.6.1. Proteins interacting with LTA

A prime example of this type of protein is *L. monocytogenes* internalin B, which has been described in detail above. It interacts with lipoteichoic acids (LTAs) *via* the GW modules (dipeptide Gly-Trp) at its C-terminal end (Bierne and Cossart, 2002). The conserved GW modules also interact with glycosaminoglycans on mammalian cells. InlB is partly anchored in the wall, using lipoteichoic acid as a ligand (Jonquieres *et al*., 1999). This attachment is relatively weak and consequently internal B can be released from the cells on incubation in InlB Tris/HCl with high concentration, even though the enzyme cannot be released from the cell wall after its digestion with muramidase.

Eight GW modules are also present in the well-characterized cell surface amidase of *L. monocytogenes*. The larger number of modules presumably allow for tighter binding to the cell surface (Navarre and Schneewind, 1999). Genome sequence analysis has identified a further seven other proteins in *L. monocytogenes* containing the GW motif. Interestingly, six of them, similarly to Ami (Lmo2558) (Jacquet *et al*., 2002) contain the amidase domain (Lmo1215, Lmo1216, Lmo2203, Lmo1521, Lmo2591, lmo1076). Internalin InlB is the only protein of this group that has both GW modules and an LRR domain. In *L. innocua* there are 9 GW proteins and GW modules similar to the listerial ones have been found in the autolysin Atl from *Staphylococcus aureus* (Oshida *et al*., 1995) and in three other surface murein hydrolases: AtlC from *Staphylococcus caprae*, AtlE from *S. epidermidis* and Aas from *S. saprophyticus* (Heilmann *et al*., 2003).

These four autolysins share the same organization, two amidase domains linked GW modules (Cabanes *et al*., 2002). These observations may suggest that the mechanism of autolysin anchoring on the cell surface using specific GW modules is common to staphylococci and listeriae (Baba and Schneewind, 1998).

3.6.2. Cwha and Cwha-like proteins

L. monocytogenes protein CwhA (previously Iap or p60) participates in the invasion of host cells but also has murein hydrolase activity and has been shown to be participate in cell division (Park *et al*., 2000). The authors of an early paper maintained that a mutation in the structural gene for CwhaA (*iap*) was lethal for *L. monocytogenes* (Wuenscher *et al*., 1993). However, subsequent reports (Wisniewski and Bielecki, 1999; Pilgrim *et al*., 2003) showed that the deletion of *iap* was not lethal but led, amongst others, to impaired cell division and actin-based motility. Protein CwhA contains two LysM domains, a SH3 domain (bacterial Src homology 3) and the C-terminal domain NLPC/P60. Domain LysM is present in many enzymes degrading cell wall murein. The main function of this domain is binding to murein. The bacterial SH3 domain (SH3b) is homologous to eukaryotic SH3 domains and is also found in CwhA-like proteins in other *Listeria* species (Whisstock and Lesk, 1999) as well as in other bacteria, such as *Bacillus subtilis*, *Escherichia coli*, *Chlamydia trachomatis*, *Haemophilus influenzae*, *Helicobacter pylori*, *Staphylococcus aureus* and *Streptococcus pyogenes*. The function of this domain has not yet been determined but the murein hydrolase lysostaphin from *Staphylococcus simulans* contains a C-terminal SH3 domain which is responsible for the attachment of the protein to the cell wall (Baba and Schneewind, 1998). This observation suggests such a role for other bacterial proteins containing the SH3b domain.

The NLPC/P60 domain (100–110 amino acids), was first determined in Cwha and in the *E. coli* lipoprotein precursor NlpC. The function of this domain is not clear but it has since been found in several other lipoproteins and bacterial surface proteins. A search for the NLPC/P60 domain in the *L. monocytogenes* genome revealed a similar sequence in three other proteins, one of which, termed P45, has been characterized as a protein with murein hydrolyzing activity. The enzymes is both present on the cell surface of *L. monocytogenes* and secreted to the growth medium of the bacterium. For this reason the gene encoding it has been named *spl* (secreted protein with lytic property) (Schubert *et al*., 2000). Proteins cross-reacting with a monoclonal antibody that reacts with P45 were present in strains of all seven *Listeria* species investigated, except *L. grayi*. Protein Lmo0394, besides the NLPC/P60 domain also contains a SH3 domain. The third listerial protein containing the NLPC/P60 domain is Lmo1104. CwhA-like proteins have also been found in *L. innocua*, as well as in other gram-positive bacteria, such as *B. subtilis*, *L. lactis*, *S. aureus*, *M. leprae* and *M. tuberculosis* where they presumably function as murein hydrolases (Cabanes *et al*., 2002).

3.6.3. Proteins with hydrophobic tail

Eleven proteins of *L. monocytogenes* have a carboxyl terminus consisting of a hydrophobic domain, followed by positively charged amino acids and this „tail" serves to attach the proteins to the bacterial cell surface (Domann *et al*., 1992). The best studied of these proteins is ActA, which plays a crucial role in actin-based motility of listeriae since it is involved in the polymerization of actin fibers in the host cell. Expression of the ActA polypeptide is controlled by the PrfA regulator protein and its structural gene is located between the metalloprotease (*mpl*) and phosphatidylcholine-specific phospholipase C (*plcB*) genes. (Pistor *et al*., 1995). The C-terminal region of the protein contains a hydrophobic stretch of 22 amino acids followed by a tail with positive charge. Protein *L. monocytogenes* SvpA (Lmo2185) – described in 2001, is also attached to the cell surface by means of a hydrophobic tail, similarly to ActA and is required for the escape of *L. monocytogenes* from the phagosome formed inside a macrophage which is indispensable for the intracellular survival of the bacterial and implicates the protein in virulence (Borezee *et al*., 2001). The *L. monocytogenes* genome encodes 9 other proteins (Lmo0058, Lmo0082, Lmo0528, Lmo0552, Lmo0586, Lmo0701, Lmo0821, Lmo2061 and Lmo2186) with C-terminal hydrophobic region. Protein ActA and Lmo0082 are the only ones of this group that are not present in *L. innocua* (Buchrieser *et al*., 2003).

3.7. Lipoproteins

Bacterial lipoproteins are characterized by a specific signal peptide, which as a rule is shorter than the "classical" signal sequences, contains more hydrophobic amino acids in its central region and is followed by a cysteine. These proteins are synthesized in the form of a prolipoprotein, which is then cleaved by a lipoprotein-specific peptidase (proliprotein peptidase or peptidase II), to produce the mature form of the

protein. Lipoproteins can be anchored by their fatty acids in the cytoplasmic membrane, though free periplasmic forms are known, *e.g.* in *Escherichia coli*. Moreover, sometimes, like in the case of the *E. coli* lipoproteins, known as Braun's lipoprotein, the peptide part of the lipoprotein can be covalently attached to the diaminopimelic acid of the murein.

Bacterial lipoproteins are involved in initiation of the immune response in mammalian cells (Aliprantis *et al*., 1999). They are capable of activating many types of cells, including monocytes, macrophages, neutrofils and B cells, thus playing a significant role in pathogenesis. In *L. monocytogenes* 68 genes (that is 2.5% of all *L. monocytogenes* genes) coding lipoproteins have been identified. The number of these genes in *L. monocytogenes* is the highest among all pathogenic gram-positive bacteria, *e.g. M. tuberculosis* codes for 65 lipoproteins (1.7% of all genes). In the case of the *Mycoplasma pneumoniae* and *M. genitalium* genomes 6.8% and 4.5% of all open reading frames, respectively, code for lipoproteins (Himmelreich *et al*., 1996), though of course the size of the genomes of these two species chromosomes is very much smaller than that of *L. monocytogenes*. Only a few of *L. monocytogenes* lipoproteins have been studied and their putative roles identified. Lipoprotein TcsA has been found to activate T cells (Sanderson *et al*., 1995), whereas lipoprotein OppA (Borezee *et al*., 2000), similarly to its counterparts in many other bacterial species, *e.g. Escherichia coli*, participates in the transport of oligopeptides and is required for growth of the cells at low temperature. Other *L. monocytogenes* lipoproteins that can participate in host cell-pathogen interactions, like Lmo1847 and Lmo1800, have been identified but their specific roles remain unclear. The latter is likely a tyrosine phosphatase.

3.8. Diverse proteins similar to surface proteins of other gram-positive species

L. monocytogenes protein, Lmo1799 that contains the LPXTG motif also contains 226 Ala-Asp (AD) tandem repeats. Proteins with Ser-Asp (SD) repeats have been found in bacteria belonging to the genus *Staphylococcus*. In *S. aureus*, CifA that binds to fibrinogen contains a SD repeat region which is predicted to span the cell wall and expose the ligand on the outer surface of the bacterium. The AD repeats in Lmo1799 could play a similar role, though the role of the putative protein in *L. monocytogenes remains* to be elucidated.

Another *L. monocytogenes* protein containing the LPXTG motif, Lmo0842, is present also in *L. innocua* and shows similarity to other proteins, R28 from *Streptococcus pyogenes*, Rib from *S. agalactiae* and Esp from *Enterococcus faecalis* (Shankar *et al*., 1999). All four proteins contain the motif LPXTG and 6 to 12 repeats of about 80 amino acids that show high sequence identity (up to 42% identity). R28 is an adhesin that may play a role in virulence. Esp is a surface protein with as yet unknown function, which participates in the formation of biofilms by *E. faecalis*. The putative protein Lmo0842 could thus play a role in virulence and/or the formation of biofilms.

4. Conclusions

The *L. monocytogenes* genome is unusual, compared to most other bacteria, in that 4.7% of all its genes code for a total of 133 surface proteins. The genes encoding these proteins are predominantly located in the first 25% of the *L. monocytogenes* genome, which region would seem to have higher plasticity and greater capacity for accommodating lateral gene transfer. However, the answer to the question why this particular region stands out is, at this stage, purely speculative.

The large number of surface proteins and varied systems of their anchoring in the cell wall is very likely related to the ability of *L. monocytogenes* to survive in a broad range of environmental conditions that are frequently detrimental, and to interact with diverse types of eukaryotic host cells. Surface proteins play an important role in interactions between a microorganism and its environment as well as in the consecutive stages of the infection process. Among the main virulence factors of *L. monocytogenes* are the surface proteins internalin B, which is required for the induction of phagocytosis and penetration of the bacteria into macrophage cells, and protein ActA, which stimulates the accumulation and polymerization of actin into fibers in the host cell and subsequently enables the movement of *L. monocytogenes* in the cytoplasm and the migration of the bacteria from one eukaryotic cell to another. Most of the known virulence genes of *L. monocytogenes* are regulated by protein PrfA – a transcription regulator that binds to the palindromic sequence TTAACAnnTGTTAA (PrfA-box), located in the promoter region (Bockmann *et al*., 2000). Genome sequence analysis revealed the presence of 19 genes coding surface proteins preceded by the

PrfA-box sequence: 10 of them code proteins with the LPXTG motif, 2 proteins with the GW motif, 1 a hydrophobic protein and the remaining 6 lipoproteins (Buchrieser *et al*., 2003; Glaser *et al*., 2001). 25% genes coding proteins with the motif LPXTG are preceded by PrfA-box sequence, whereas this sequence has been found in the case of only 10% of all *L. monocytogenes* genes. These data suggest that proteins with the LPXTG motif are involved in virulence and being surface proteins, responsible for contact with an eukaryotic cell, initiate a cascade of events ultimately resulting in infection. Consequently, they would be an ideal target for modern-day chemotherapeutics.

Similarly, knowledge of the mechanism of the attachment of surface proteins to murein, mediated by sortase, as well as the increasing knowledge of the function of surface proteins in pathogenesis, is currently the base for murein studies aimed at creating a "magic bullet" acting on proteins involved in the first stage of infection – the adhesion of the bacterial cell to the eukaryotic host cell. One of such targets would thus be the internalins as proteins inducing and initiating this process. Another, of arguably greater importance, would be the family of sortase enzymes, which play a crucial role in the sorting of proteins to cell wall structures of gram-positive bacteria and therefore are of prime importance for pathogenesis. Moreover, these proteins are ubiquitous in all gram-positive pathogens studied thus far.

Literature

Alvarez-Dominguez C., J.A. Vazquez-Boland, E. Carrasco-Marin, P. Lopez-Mato and F. Leyva-Cobian. 1997. Host cell heparan sulfate proteoglycans mediate attachment and entry of *Listeria monocytogenes*, and the listerial surface protein ActA is involved in heparan sulfate receptor recognition. *Infect. Immun.* **65**: 78–88.

Aliprantis A.O., R.B. Yang, M.R. Mark, S. Suggett, B. Devaux, J.D. Radolf, G.R. Klimpel, P. Godowski and A. Zychlinsky. 1999. Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science* **285**: 736–739.

Autret N., I. Dubail, P. Trieu-Cuot, P. Berche and A. Charbit. 2001. Identification of new genes involves in the virulence of *Listeria monocytogenes* by signature-tagged transposon mutagenesis. *Infect. Immun.* **69**: 2954–2065.

Baba T. and O. Schneewind. 1998. Targeting of muralytic enzymes to the cell divsion site of Gram-positive bacteria: repeat domains direct autolysin to the equatorial surface ring of *Staphylococcus aureus*. *EMBO J.* **17**: 4639–4646.

Bierne H. and P. Cossart. 2002. InlB, a surface protein of *Listeria monocytogenes* that behaves as an invasion and growth factor. *J. Cell Science* **115**: 3357–3367.

Bierne H., C. Garandeau, M.G. Pucciarelli, C. Sabet, S. Newton, F. Garcia-Del Portillo, P. Cossart and A. Charbit. 2004. Sortase B, a New Class of Sortase in *Listeria monocytogenes*. *J. Bacteriol.* **186**: 1972–1982.

Bockmann R., C. Dickneite, W. Goebel and J. Bohne. 2000. PrfA mediates specific binding of RNA polymerase of Listeria monocytogenes to PrfA-dependent virulence gene promoters resulting in a transcriptionally active complex. *Mol. Microbiol.* **36**: 487–497.

Borezee E., E. Pellegrini, J.L. Beretti and P. Berche. 2001. SvpA, a novel surface virulence-associated protein required for intracellular survival of *Listeria monocytogenes*. *Microbiology* **147**: 2913–2923.

Braun L. and P. Cossart. 2000. Interactions between *Listeria monocytogenes* and host mammalian cells. *Micro. Infect.* **2**: 803–811.

Buchrieser C. and Ch. Rusniok. 2003. The *Listeria* Consortium, Kunst F., Cossart P., Glaser P.: Comparison of the genome sequences of *Listeria monocytogenes* and *Listeria innocua*: clues for evolution and pathogenicity. *FEMS Immun. Med. Microbiol.* **35**: 2007–213.

Cabanes D., P. Dehoux, O. Dussurget, L. Frangeul and P. Cossart. 2002. Surface proteins and the pathogenic potential of *Listeria monocytogenes*. *Trends in Microbiol.* **5**: 238–245.

Chakraborty T., T. Hain and E. Domann. 2000. Genome organization and the evolution of the virulence gene locus in Listeria species. *Int. J. Med. Microbiol.* **290**: 167–174.

Cossart P. and R. Jonquieres. 2000. Sortase, a universal target for therapeutic agents against Gram-positive bacteria? Commentary. *PNAS* **10**: 5013–5015.

Cossart P. and M. Lecuit. 1998. Interactions of *Listeria monocytogenes* with mammalian cells during entry and actin-based movement: bacterial factors, cellular ligands and signaling. *EMBO J.* **17**: 3797–3806.

Cossart P., J. Pizarro-Cerda and M. Lecuit. 2003. Invasion of mammalian cells by *Listeria monocytogenes*: Functional mimicry to subvert cellular functions. *Trends in Cell Biol.* **1**: 23–31.

Cossart P. 2002. Molecular and cellular basis of the infection by *Listeria monocytogenes*, an overview. *Int. J. Med. Microbiol.* **291**: 401–409.

Dabiri G.A., J.M. Sanger, D.A. Portnoy and F.S. Southwick. 1990. *Listeria monocytogenes* moves rapidly through the host-cell cytoplasm by inducing directional actin assembly. *Proc. Natl. Acad Sci. USA*. **87**: 6068–6072.

Dhar G., K.F. Faull and O. Schneewind. 2000. Anchor structure of cell wall surface proteins in *Listeria monocytogenes*. *Biochemistry* **39**: 3725–3733.

Dramsi S., P. Dehoux, M. Lebrun, L. Goossens and P. Cossart. 1997. Identification of four new members of the internalin multigene family of *Listeria monocytogenes* EGD. *Infect. Immun.* **65**: 1625–1625.

Dramsi S. and P. Cossart. 2002. Listeriolysin O: a genuine cytolysin optimized for an intracellular parasite. *J. Cell Biol.* **156**: 943–6.

Engelbrecht F., C. Dickneite, R. Lampidis, M. Götz, U. DasGupta and W. Goebel. 1998. Sequence comparison of the chromosomal regions encopassing the internalin C genes (*inlC*) of *Listeria monocytogenes* and *L. ivanovii*. *Mol. Gen. Genet*. **257**: 186–197.

Farber J.M. 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev*. **55**: 476–511.

Finlay B.B. and P. Cossart. 1997. Exploitation of mammalian host cell functions by bacterial pathogens. *Science* **276**: 718–725.

Fischetti V.A., V. Pancholi and O. Schneewind. 1990.Conservation of a hexapeptide sequence in the anchor region of surface proteins from Gram-positive cocci. *Mol. Microbiol*. **4**: 1603–1605.

Garandeau C., H. Reglier-Poupet, I. Dubail, J.L. Beretti, P. Berche and A. Charbit. 2002. The sortase SrtA of Listeria monocytogenes is involved in processing of internalin and in virulence. *Infect. Immun*. 70: 82–90.

Glaser P. and The *Listeria* Consortium. 2001. Comparative Genomics of *Listeria* species. *Science* **294**: 849–852.

Heilmann Ch., G. Thumm, G.S. Chhatwal, J. Hartleib, A. Uekötter and G. Peters. 2003. Identification and characterization of a novel autolysin (Aae) with adhesive properties from *Staphylococcus epidermidis*. *Microbiol*. **149**: 2769–2778.

Himmelreich R., H. Hilbert, H. Plagens, E. Pirkl, B.C. Li and R. Herrmann. 1996. Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. *Nucleic Acids Res*. **24**: 4420–4449.

Hof H., T. Nichterlein and M. Kretschmar. 1997. Management of listeriosis. *Clin. Microbiol. Rev*. **10**: 345–357.

Huan Y. and J. van Adelsberg. 1999. Polycystin-1, the PKD1 gene product, is in a complex containing E-cadherin and the catenins. *J. Clin. Invest*. **104**: 1459–1468.

Ilangovan U., H. Ton-That, J. Iwahara, O. Schneewind and R.T. Clubb. 2001. Structure of sortase, the transpeptidase that anchors proteins to the cell wall of *Staphylococcus aureus*. *Proc. Natl. Acad Sci. USA*. **98**: 6056–6061.

Jacquet C., E. Gouin, D. Jeannel, P. Cossart and J. Rocourt. 2002. Expression of ActA, Ami, InlB, and listeriolysin O in *Listeria monocytogenes* of human and foof origin. *Appl. Environ. Microbiol*. **68**: 6616–22.

Jones E.M. and A.P. MacGowan. 1995. Antimicrobial chemotherapy of human infection due to *Listeria monocytogenes*. *Eur. J. Clin. Microbiol. Infect. Dis*. **14**: 165–175.

Jonsson I.M., S.K. Mazmanian, O. Schneewind, T. Bremell and A. Tarkowski. 2003. The role of *Staphylococcus aureus* sortase A and sortase B in murine arthritis. *Microbes Infect*. **5**: 775–780.

Jonquieres R., H. Bierne, F. Fiedler, P. Gounon and P. Cossart. 1999. Interaction between the protein InlB of *Listeria monocytogenes* and lipoteichoic acid: a novel mechanism of protein association at the surface of gram-positive bacteria. *Mol. Microbiol*. **34**: 902–914.

Jonquieres R., H. Bierne, J. Mengaud and P. Cossart. 1998. The *inlA* gene of *Listeria monocytogenes* LO28 harbors a nonsense mutation resulting in release of internalin. *Infect. Immun*. **7**: 3420–3422.

Kajava A. and B. Kobe. 2002. Assessment of the ability to model proteins with leucine-rich repeats in light of the latest structural information. *Protein Science* **11**: 1082–1090.

Kunst F., N. Ogasawara and I. Moszer *et al*. 1997.The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature* **390**: 249–256.

Kuroda M., T. Ohta and I. Uchiyama *et al*. 2001. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* **357**: 1225–1240.

Lecuit M., S. Vandormael-Pournin, J. Lefort, M. Huerre, P. Gounon, C. Dupuy, C. Babinet and P. Cossart. 2001. A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. *Science* **292**: 1722–1725.

Lecuit M., S. Dramsi, C. Gottardi, M. Fedor-Chaiken, B. Gumbiner and P. Cossart. 1999. A single amino acid in E-cadherin responsible for host specificity towards the human pathogen *Listeria monocytogenes*. *EMBO J*. **18**: 3956–3963.

Lecuit M., H. Ohayon, L. Braun, J. Mengaud and P. Cossart. 1997. Internalin of *Listeria monocytogenes* with an intact leucine-rich repeat region is sufficient to promote internalization. *Infect. Immun*. **65**: 5309–5319.

Lorber B. 1997. Listeriosis. *Clin. Infect. Dis*. **24**: 1–11.

MacGowan A.P., M. Wootton, K. Bowker, H.A. Holt and D. Reeves. 1998. Ampicillin-aminoglycoside interaction studies using *Listeria monocytogenes*. *J. Antimicrob. Chemother*. **41**: 417–418.

Marino M., M. Banerjee, R. Jonquieres, P. Cossart and P. Ghosh. 2002. GW domains of the *Listeria monocytogenes* invasion Protein InlB are SH3-like and mediate binding to host ligands. *EMBO J*. **21**: 5623–34.

Merle-Melet M., L. Dossou-Glete, P. Maurer, P. Meyer, A. Lozniewski, O. Kuntzburger, M. Weber and A. Gerard. 1996. Is amoxicillin-cotrimoxasole the most appropriate antibiotic regiment for *Listeria meningoencephalitis*. Review of 22 cases and the literature. *J. Infect*. **33**: 79–85.

Navarre W.W. and O. Schneewind. 1999. Surface proteins of Gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiol. Mol. Biol. Rev*. **63**: 174–229.

Oshida T., M. Sugai, H. Komatsuzawa, Y-M. Hong, H. Suginaka and A. Tomasz. 1995. A *Staphylococcus aureus* autolysin that has an N-acetylmuramoyl-L-alanine amidase domain and an endo-β-N-acetylglicosaminidase domain: cloning, sequence analysis and charaterization. *Proc. Natl. Acad Sci. USA*. **92**: 285–289.

Park J.H., Y.S. Lee, Y.K. Lim, S.H. Kwon, C.U. Lee and B.S. Yoon. 2000. Specific binding of recombinant *Listeria monocytogenes* p60 protein to Caco-2 cells. *FEMS Microbiol. Lett*. **186**: 35–40.

Paterson G.K. and T.J. Mitchell. 2004. The biology of Gram-positive sortase enzymes.*Trends Microbiol.* **12**: 89–95.

Pilgrim V., A. Kolb-Maurer, L. Gentschev, W. Goebel and M. Kuhn. 2003. Deletion of the gene encoding p60 in *Listeria monocytogenes* leads to abnormal cell division and los of actin-based motility. *Infect. Immun*. **71**: 3473–3484.

Pistor S., T. Chakraborty, U. Walter, J. Wehland. 1995. The bacterial actin nucleator protein ActA *of Listeria monocytogenes* contains multiple binding sites for host microfilament proteins. *Curr. Biol*. **5**: 517–525.

Portnoy D.A., V. Auerbuch and I.J. Glomski. 2002. The cell biology of *Listeria monocytogenes* infection: the intersection of bacterial pathogenesis and cell-mediated immunity. *J. Cell Biol*. **3**: 409–414.

Portnoy D.A., T. Chakraborty, W. Goebel and P. Cossart. 1992. Molecular determinants of *Listeria monocytogenes* pathogenesis. *Infect. Immun*. **60**: 1263–1267.

Raffelsbauer D., A. Bubert, F. Engelbrecht, J. Scheinpflug, A. Simm and J. Hess. 1998. The gene cluster inlC2DE of *Listeria monocytogenes* contains additional new internalin genes and is important for virulence in mice. *Mol. Gen. Genet*. **260**: 144–158.

Sanderson S., D.J. Campbell and N. Shastri. 1995. Identification of a CD4+ T cell-stimulating antigen of pathogenic bacteria by expression cloning. *J. Exp. Med*. **182**: 1751–1757.

Schlech W.F. 2000. Foodborne listeriosis. *Clin. Infect. Dis*. **31**: 770–5.

Schubert K., A.M. Bichlmaier, E. Mager, K. Wolff, G. Ruhland and F. Fiedler. 2000. P45, an extracellular 45 kDa protein of *Listeria monocytogenes* with similarity to protein p60 and exhibiting peptidoglycan lytic activity. *Arch. Microbiol*. **173**: 21–28.

Schubert W-D., C. Urbanke, T. Ziehm, V. Beier, M.P. Machner, E. Domann, J. Wehland, T. Chakraborty and D.W. Heinz. 2002. Structure of internalin, a major invasion protein of *Listeria monocytogenes*, in complex with its human receptor E-cadherin. *Cell* **111**: 825–836.

Shankar V., A.S. Baghdayan, M.M. Huycke, G. Lindahl and M.S. Gilmore. 1999. Infection-derived *Enterococcus faecalis* strains are enriched in *esp*, a gene encoding a novel surface protein. *Infect. Immun*. **67**: 193–200.

Shen Y., M. Naujokas, M. Park and K. Ireton. 2000. InlB-dependent internalisation of *Listeria* is medited by the Met receptor tyrosine kinase. *Cell* **27**: 501–510.

Smith G.A., H. Marquis, S. Jones, N.C. Johnston, D.A. Portnoy and H. Goldfine. 1995. The two distinct phospholipases C of *Listeria monocytogenes* have overlapping roles in escape from a vacuole and cell-to-cell spread. *Infect. Immun*. **63**: 4231–7.

Temple M.E. and M.C. Nahata. 2000. Treatment of listeriosis. *Ann. Phamacother*. **34**: 656–61.

Teuber M. 1999. Spread of antibiotic resistance with food-borne pathogens. *Cell Mol. Life Sci*. **56**: 75–63.

Ton-That H., G. Liu, S.K. Mazmanian, K.F. Faull and O. Schneewind. 1999. Purification and characterization of sortase, the transpeptidase that cleaves surface proteins of *Staphylococcus aureus* at the LPXTG motif. *Proc. Natl. Acad Sci. U S A*. **96**: 12424–12429.

Vazquez-Boland J.A., M. Kuhn, P. Berche, T. Chakraborty, G. Dominguez-Bernal, W. Goebel, B. Gonzales-Zorn, J. Wehland and J. Kreft. 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev*. **14**: 584–640.

Whisstock J.C. and A.M. Lesk. 1999. SH3 domains in prokaryotes. *Trends Biochem. Sci*. **24**: 132–133.

Wiśniewski J.M. and J.E. Bielecki. 1999. Intracellular growth of *Listeria monocytogenes* insertional mutant deprived of protein p60. *Acta Microbiol. Pol*. **48**: 317–329.

Wuenscher M.D., S. Kohler, A. Bubert, U. Gerike and W. Goebel. 1993. The *iap* gene of *Listeria monocytogenes* is essential for cell viability, and its gene product, p60, has bacteriolytic activity. *J. Bacteriol*. **175**: 3491–501.