**This is a pre- or post-print of an article published in
Kochut, A., Dersch, P.**

**Bacterial invasion factors: Tools for crossing biological
barriers and drug delivery?
(2013) European Journal of Pharmaceutics and
Biopharmaceutics, 84 (2), pp. 242-250.**

Graphical abstract

Bacterial invasion factors: tools for crossing biological barriers and drug delivery?

Annika Kochut and Petra Dersch*

Department of Molecular Infection Biology, Helmholtz Center for Infection Research, 38124 Braunschweig, Germany

*Corresponding author:

Petra Dersch
Dept. of Molecular Infection Biology,
Helmholtz Center for Infection Research
38124 Braunschweig
Germany
Phone: +49-531-6181-5700
FAX: +49-531-6181-5709
e-mail: petra.dersch@helmholtz-hzi.de

Running title: Bacterial invasion factors: tools for drug targeting

Keywords: oral drug delivery, nanoparticles, gastrointestinal system, intestinal barrier, bacterial invasion factors

Abstract

The oral route is the preferential route of drug delivery in humans. However, effective delivery through the gastrointestinal tract is often hampered by the low permeability of the intestinal epithelium. One possibility to overcome this problem is the encapsulation of drugs inside nanoparticulate systems containing targeting moieties with cell invasive properties.

The bioinvasive features of the delivery system could be provided by the attachment of bacterial invasion factors, which promote efficient uptake into host cells and mediate rapid transcytosis of the pathogen through the intestinal epithelium. This review gives an overview of bacterial invasion systems. The molecular structure and function of suitable bacterial invasins, their relative values as targeting agents and possible pitfalls of their use are described. The potential of bioinvasive drug delivery systems is mainly presented on the basis of the well-characterized *Yersinia* invasin protein, which enters M cells to gain access to subepithelial layers of the gastrointestinal tract, but alternative approaches and future prospects for oral drug delivery are also discussed.

Introduction

Over the past decades, multiple biotechnology-based drugs such as peptide and protein therapeutics (e.g. vaccines, hormones, antibodies) became available and many more are expected in the future. Currently, the most effective and most common delivery of these biopharmaceuticals is the parenteral route of administration (injection and intravenous infusion). Yet, in view of its convenience and patient acceptance, oral delivery is the main mode of choice [1, 2]: (i) oral systems are easy to administer (very important in situations where delivery to mass populations is required) and avoids pain and discomfort, (ii) a large variety of dosage forms can be applied and (iii) vaccines, which are delivered orally, generate mucosal immunity, e.g. against enteric pathogens [1]. However, despite the success in oral delivery of small therapeutic molecules such as antibiotics and traditional chemically synthesized compounds, little success has been achieved with macromolecule biopharmaceuticals such as therapeutic proteins. Their delivery through the gastrointestinal (GI) tract is impeded by several biological barriers which lead to a poor bioavailability. The high molecular weight (>700 Da) and hydrophilicity of macromolecular drugs affects permeation and transcellular absorption by passive diffusion. Another major problem is the low permeability of the intestinal epithelial layer. Uptake of drugs by the paracellular pathway, i.e. through intracellular spaces between adjacent cells of the epithelium, is largely inhibited by tight cell-cell junctions, which allow the physical separation of the host from the external environment. The thick mucus layer covering the intestinal epithelium and the different cell composition of the intestinal layer, including enterocytes, goblet cells, Paneth cells, endocrine cells and M cells also hamper efficient targeting and uptake of drugs. Another important problem is the degradation or inactivation of the delivered drugs in the harsh

environment (e.g. strong pH variations, large variety of proteases and other lytic enzymes) of the GI tract, particularly in the stomach and duodenum, or en-route to the site of action. Moreover, inefficient absorption of all drug classes is a problem in patients suffering from GI diseases [3]. As a consequence, high doses of biopharmaceuticals are often required to assure efficient absorption from the epithelium.

There are several technologies that aim to circumvent the above mentioned problems. These include protection and release modification systems where the drug is protected from the harsh environment and released at a particular time following administration. Delivery across the intestinal epithelium could be improved by the development of inert drug carrier systems with targeting molecules with bioadhesive and cell invasive properties. Over the past years, particulate systems like nanoparticles (particulate dispersions with the size of 10-1000 nm) have shown to be very useful for the targeted delivery of various types of drug molecules. Nanoparticles can be made of different materials, such as proteins, polysaccharides or synthetic polymers, and the drug is dissolved, encapsulated or attached to the nanoparticle surface. Furthermore, it was shown that encapsulation of the drug inside nanoparticulate systems can increase the stability of the drug and facilitate controlled and sustained release of the compound [4, 5]. A potential pitfall in the use of nanoparticles is that (i) they can directly transit the GI tract and are rapidly eliminated, or (ii) they provide cellular attachment to sites where cellular internalization and transfer through the intestinal layer may not automatically follow. Thus, for the successful delivery of the drug, the carrier system must promote adherence to sites of the intestinal epithelium that allow efficient absorption and subsequent transcytosis of the nanoparticles into subepithelial tissues. Intestinal „sites“

particularly suitable for particle absorption are M cells. M cells are found in the regions of the follicle-associated epithelium (FAE) that cover the lymphoid structures associated with the mucosal immune system, such as Peyer's patches and isolated lymphoid follicles (ILFs) [6]. They mainly act as antigen sampling cells delivering pathogenic agents and other antigens from the gut lumen to the underlying lymphoid tissues. In contrast to the columnar epithelial cells, they possess a unique glycosylation pattern and their apical membrane is covered by irregular and much broader microvilli, which are thought to facilitate particle adherence and subsequent endo- and phagocytic processes.

Moreover, it could be shown that the apical membranes of M cells express β_1 -integrins – adhesion molecules that are only expressed at the basolateral membranes of adjacent enterocytes [7-9]. Hence, one way to considerably improve adhesion, endo- and transcytosis of drug-containing nanoparticles would be to coat them with ligands capable of interacting with M cell-specific surface components, e.g. glycoproteins or β_1 -integrins. In fact, several studies reported that lectins, such as tomato and bean lectin, which selectively bind to sugar moieties, can be used for site-specific targeting of nanoparticles to the surface of M cells and enterocytes [10, 11]. Tight and long-term adhesion leads to a longer retention period of the particle drug complex at the epithelial cell surface and induces participation in vesicular uptake and transport processes, a prerequisite for oral absorption. Bioinvasion of the drug delivery system could be further improved by the use of viral and bacterial ligands shown to promote host cell entry by the pathogen [12, 13]. In particular invasion factors (invasins) of invasive enteropathogenic bacteria have gained great interest for the development of bioinvasive drug delivery systems. Many of the identified bacterial invasins promote fast and efficient cell entry via β_1 -integrins and

trigger transcytosis of the bacteria through the M cells of the intestinal epithelial layer [14, 15]

The best-characterized bacterial uptake systems are those found in species of the genera *Listeria*, *Salmonella*, *Shigella* or *Yersinia*. The different types and molecular functions of the bacterial uptake pathways are introduced in the following chapters and their potential for the development of site-specific bioinvasive drug delivery systems is discussed.

Invasion strategies of bacterial pathogens

The ability to invade and translocate across the intestinal barrier is a typical feature of enteric pathogens. Invasion into cells of the intestinal layer is important to (a) avoid an attack of the host immune system, e.g. by antimicrobial peptides or immunoglobulins, like IgA which are secreted in the gut lumen, and (b) to initiate transcytosis and colonization of deeper host tissues which is crucial to establish and maintain the infection. Importance of the invasion systems is further supported by the fact that most of the enteroinvasive pathogens, including *Listeria* and *Yersinia*, produce multiple host cell uptake systems, which enable them to induce their own phagocytosis in cells that are normally non-phagocytic (e.g. epithelial cells) [16].

A prerequisite for the invasion of eukaryotic cells is the expression of virulence factors, which promote tight adhesion to host cell surface components [17, 18]. Bacterial invasion proteins either remain anchored in the bacterial membrane or are released via a specialized secretion system. They predominantly interact with specific host cell receptors or proteins of the extracellular matrix (ECM), and trigger local actin cytoskeleton rearrangements within the host cells. This leads to the formation of

membrane protrusions which engulf and enclose the pathogen in a membrane-bound vacuole, a process called “induced endocytosis/phagocytosis” [18-20].

Depending on the invasion factor two distinct uptake strategies are initiated: (A) the **trigger mechanism** and (B) the **zipper mechanism** (Fig. 1).

(A) trigger mechanism

The trigger mechanism is an invasion process based on the bacterial type III secretion system (T3SS). Here, bacteria-host contact is accomplished by components of the T3SS, which deliver effector proteins into the host cell to initiate cytoskeletal rearrangements [21, 22]. This invasion process can be subdivided into four distinct steps: (i) the stand-by state, in which the effectors are stored in the bacterial cytoplasm, (ii) bacteria-host cell contact which triggers the secretory processes, (iii) manipulation of signaling proteins (e.g. small Rho GTPases) by delivered effector proteins which leads to the formation of numerous filo- and lamellipodia, called membrane ruffling (Fig. 1), and (d) closing of the membrane invaginations and engulfment of the bacteria [13].

The triggering mechanism is used by species of the genera *Salmonella* and *Shigella* [21, 22]. They are both transmitted by the fecal-oral route, cross the epithelial barrier of the gut mainly through M cells and cause severe gastrointestinal diseases [23, 24]. By expressing a T3SS the bacteria are able to deliver a subset of different effector proteins (*Shigella*: I_{pas} = invasion plasmid antigens; *Salmonella*: S_{ips} = Salmonella invasion proteins), which initialize the internalization process [25, 26]. After breaching the epithelial barrier, they invade and replicate in macrophages and spread to other host cells thus establishing a systemic infection. In contrast to *Salmonella*, which resides and replicates in endocytic vacuoles, *Shigella* lyses the

phagosome and replicates in the host cell cytoplasm [27, 28]. Although, T3SS-mediated cell uptake is very efficient and allows uptake of close-by “non-invasive” particles, use of this pathway for drug delivery systems is hampered by the fact that cell adhesion and actin rearrangements important for the invasion process are promoted by separate components.

(B) zipper mechanism

The zipper mechanism constitutes a receptor-mediated internalization process [29] (Fig. 1). This entry pathway can be divided into three distinct steps: (i) contact of a membrane-anchored bacterial ligand (invasin) with a specific host cell receptor, (ii) ligand-induced activation of the cell receptor (e.g. by receptor clustering or/and phosphorylation) which triggers certain signaling cascades leading to actin cytoskeleton rearrangements and the formation of a phagocytic cup, and (iii) engulfment and enclosure of the pathogen into a membrane-bound vacuole (bacterial phagosome). This process is very efficient, specific for the ligand-coated object and is solely initiated by the interaction of the bacterial ligand with the host cell receptor.

Listeria monocytogenes and enteropathogenic *Yersinia* species are representatives which use the zipper mechanism to promote host cell uptake [13, 29, 30]. *L. monocytogenes* causes listeriosis with a 30% mortality rate in humans characterized by gastroenteritis, fetoplacental infections as well as infections of the central nervous system [31]. *Listeria* invades into many different cell types *in vitro*, both phagocytic and non-phagocytic cells, whereby the zipper-type invasion process is mediated by different surface proteins, called internalins [30]. Enteropathogenic yersiniae such as *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are food-borne pathogens which interact specifically with M cells in the small intestine to breach the mucosal

barrier of the gut. In the underlying Peyer's Patches, the yersiniae replicate mainly extracellularly and spread to systemic organs, like liver and spleen and cause a wide range of gastrointestinal diseases called yersiniosis [32, 33]. Similar to *Listeria*, *Yersinia* encodes several invasive molecules (InvA, Ifp, YadA), which interact with different cell receptors and are differentially expressed during different stages of the infection [34-36].

As the 'zippering'-inducing bacterial invasion factors provide one of the most direct, specific and efficient manners of host cell invasion, they are of particular interest for use in the oral delivery of bioactive molecules and gene-based vaccines [37-39]. Apart from the *Listeria* and *Yersinia* uptake factors, other 'zippering'-inducing bacterial invasion factors have been identified in non-enteric pathogens [19, 20]. They were found to promote colonization of various different host tissues and/or organs which make them also attractive of therapeutic products. A selection of the most potential bacterial invasins for drug delivery systems is given in Table 1, and described in further detail in the following chapter.

Bacterial invasins – modular factors made for host cell entry

Since the host cell adhesion and entry process represents the first critical step for the pathogen to initiate and establish an infection, it is not surprising that most bacterial invasins are very efficient and highly specific for certain cell types, i.e. for those expressing the appropriate host cell receptor. Bacterial invasion factors could be identified in Gram-positive and Gram-negative bacteria [13, 19, 20]. They are very diverse and belong to different families of surface proteins (Fig. 1B), which often contain domains that are either homologous to each other or homologous to eukaryotic adhesion proteins. Invasins of Gram-negative bacteria are autotransporters

consisting of a membrane anchor/channel that exposes their adhesive and invasive structures onto the bacterial cell surface. Adhesion and invasion can be promoted by extracellular loops of the membrane anchor/channel or by an exported extracellular domain ('passenger domain') [40, 41]. In Gram-positive bacteria, invasion factors are usually anchored with the C-terminus on the bacterial surface via an LPXTG motif. Several members of different classes of bacterial invasins interact with β_1 -integrin receptors which play essential roles in cell-cell and cell-matrix interactions. Since β_1 -integrins are present on M cells in the intestinal epithelium and on several other cell types, they are valuable tools for targeted drug delivery via different administration routes. The best-characterized examples of bacterial invasins include: (A) internalins (InlA, InlB) from *Listeria*, (B) the opacity-associated proteins (Opa/Opc) from *Neisseria*, (C) fibronectin-binding proteins (FnBPs, SfbI) from *Staphylococcus* and *Streptococcus* and (D) Invasin (InvA) and YadA from *Yersinia* spp. (Fig. 2, Table 1) [19].

(A) internalins from *Listeria*

The listerial internalin proteins are invasion factors required for the adhesion and invasion in many different cell types. Internalins are surface proteins with a modular architecture composed of an N-terminal domain containing leucine-rich repeats (LRRs) which are responsible for the interaction with the respective host cell receptor thus leading to bacterial internalization [42]. From the internalin family, only internalin A (InlA) and internalin B (InlB) have been characterized in detail [30]. InlB mediates uptake of the bacteria into endothelial cells and hepatocytes via interaction with the cellular receptors c-Met (or hepatocyte growth factor, HGF) and/or gC1q-R (complement receptor) [43-45]. It could be shown that InlB alone is sufficient to promote internalization into mammalian cells by coating either non-invasive *E. coli* or inert

latex particles with recombinant InlB [46]. InlA promotes efficient invasion of *Listeria* into intestinal epithelial cells and their passage through the intestinal barrier. The transmembrane adhesion molecule E-cadherin, which is usually required for adherens junctions of the enterocytes, serves as the specific receptor for InlA [47-49].

(B) Opa/Opc proteins from *Neisseria*

The neisserial Opa/Opc proteins are variable outer membrane proteins responsible for the tight binding of the pathogens *Neisseria gonorrhoeae* and *Neisseria meningitidis* to human cells. Opa/Opcs are integral proteins and possess eight membrane-spanning domains arranged as anti-parallel β -strands, which form a membrane-embedded β -barrel with four extracellular loops [50, 51]. Opa/Opc proteins can bind to several receptors on human cells: they can interact with Heparansulphate proteoglycans (HSPGs) expressed on different cell types and serve as receptors for growth factors or ECM-proteins [52]. Opa/Opc proteins are also able to interact with certain ECM-proteins, e.g. vitronectin (Vn) and fibronectin (Fn) bound to $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins. This so-called “bridging mechanism” leads to integrin receptor clustering and activation, and triggers actin rearrangements required for uptake [53]. Moreover, several carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) serve as receptors for neisserial Opa proteins [54]. Upon CEACAM binding, Opa-expressing bacteria can be internalized by several cell types, including professional phagocytes, epithelial and endothelial cells [55]. Taken together, *Neisseria* species use a versatile set of invasin-receptor interactions to enable the internalization of the pathogen into different human cell types.

(C) FnBPs from *Staphylococcus/Streptococcus*

The fibronectin-binding proteins (FnBPs) are protein adhesins belonging to the class of microbial surface components recognizing adhesive matrix molecules (MSCRAMM). The host cell invasion by *Staphylococcus* and *Streptococcus* species can be accomplished by several mechanisms but binding to the ECM molecule Fn constitutes the most efficient strategy. Similar to the neisserial Opa/Opc proteins, ECM-bridging induces uptake via β_1 -integrin receptors [56, 57]. The most prominent proteins FnBPA and FnBPB can be found in almost all clinical isolates of *Staphylococcus aureus* [58]. *Streptococcus pyogenes* expresses at least five different proteins with Fn-binding activity, including the well-characterized protein SfbI (*S. pyogenes* fibronectin-binding protein I). All these adhesins were shown to promote adhesion to Fn and internalization into endothelial and epithelial cells. By using inert latex particles coated with recombinant SfbI, it could be demonstrated that the bacterial adhesin *per se* is able to provoke the internalization process into epithelial HEP-2 cells [59].

(D) InvA and YadA proteins from *Yersinia* spp.

The enteropathogenic species *Y. enterocolitica* and *Y. pseudotuberculosis* produce two of the best-characterized and most efficient invasion factors, invasin (InvA) and YadA (Fig. 2, 3). Both invasion factors belong to very different groups of auto-transporter adhesins/invasins, and their cell binding specificity and invasion efficiency can strongly differ among the different species due to small variations in their cell binding domains [60].

YadA is a trimeric protein that forms lollipop-shaped projections which cover the bacterial surface [61]. The C-terminal membrane anchor domain is responsible for

oligomerization and translocation of the N-terminal „passenger” domain (Fig. **1B, 2**). The passenger domain is composed of a stalk and a head region which mediate binding to Fn, different collagens (Co) and laminins [36, 61, 62]. Variations within the head region can change the efficiency of cell entry through ECM-bound β_1 -integrins. It has been reported that an additional 21 aa region in the YadA head domain of *Y. pseudotuberculosis* mediates a much stronger adhesion to Fn bound on $\alpha_5\beta_1$ -integrin receptors, but a weaker interaction to co-bound $\alpha_2\beta_1$ -integrins compared to YadA of *Y. enterocolitica*, which results in a much higher cell uptake rate [63].

The outer membrane protein invasin (InvA) interacts directly with different members of the β_1 -integrin family [64]. Most strikingly, this interaction occurs with a substantially higher binding affinity compared with their natural ligands (e.g. Fn), and promotes host cell uptake which is much more efficient than uptake mediated through ECM-bridging (e.g. Fn-bound β_1 -integrins) [65-67]. InvA from *Y. pseudotuberculosis* consists of an N-terminal β -barrel structure that anchors the protein in the outer membrane (Fig. **3**). The extracellular C-terminal domain forms an elongated, rod-like structure composed of four immunoglobulin-like domains (D1-D4) and the cell-binding domain D5 (Fig. **3**) [68]. The last 192 C-terminal amino acids are sufficient for receptor binding and bacterial uptake by epithelial cells [69], and an aspartate residue at position 911 within D5 was shown to be crucial for the internalization process [70]. Since domain D5 is structurally very similar to the integrin binding domain in Fn, it is assumed that this residue mimics the conserved aspartate from the RGD motif of the natural ligand Fn for integrin binding [71-73]. Furthermore, it could be shown that domain D2 of InvA triggers multimerization of the protein. This triggers clustering of the integrin receptors and enhances the efficiency of the uptake process [74].

Use of bacterial invasion factors for drug delivery

Although bacterial invasion factors represent efficient machineries for uptake into eukaryotic cells, one major question remains: Are they also valuable tools for targeted drug delivery? The following features of the bacterial invasion factors strongly favor their use: (i) Different binding specificities of the bacterial ligands would theoretically allow directed targeting of the pharmaceutical compounds to specific cell types or cell subsets. (ii) The general high affinity of invasins to their cell receptors would promote efficient nanoparticle binding and internalization, even when the integrin receptors are occupied by their natural ligands. For example, the affinity of invasin from *Y. pseudotuberculosis* to β_1 -integrin receptors is about 1000-fold higher than the affinity of the natural ligand Fn [64, 66, 67, 75]. (iii) The majority of bacterial invasins interact with surface molecules (e.g. integrins, IgCAMs or cadherins) involved in cell-cell interactions and adhesion to ECM proteins. These receptors are expressed on many host cells and their engagement by bacterial ligands is often accompanied by a loss of cell-cell barrier functions which would enhance the drug delivery process [72, 76].

A major pitfall is that all potential ligands of Gram-negative bacteria are anchored in the bacterial outer membrane. Several bacterial invasins, such as the neisserial Opa/Opc proteins require the formation of a β -barrel structure for proper exposure of their cell-binding domains. However, most invasion proteins are still functional when the membrane domain is deleted. In fact, for InvA/YadA (*Yersinia*), InlA/InlB (*Listeria*), FnBPA (*Staphylococcus*) and SfbI (*Streptococcus*) it has been demonstrated that coating of non-anchored or truncated versions of the invasin proteins on

inert latex particles is sufficient to cause internalization into mammalian cells [46, 59, 74, 77].

Fn-binding bacterial ligands such as SfbI, FnBPA and YadA promote tight adhesion to their target cells, but the uptake efficiency is lower compared to the other invasins, e.g. InvA of *Y. pseudotuberculosis*, which interacts directly with β_1 -integrin receptors. InvA-expressing bacteria are specifically targeted to β_1 -integrins expressing M cells and are rapidly and efficiently transported into subepithelial layers [7]. Thus, it is not surprising that mainly InvA has been used to test its use for bioinvasive drug carrier systems. Although gut proteases target invasin *in vivo* [78], it is possible to use smaller and more resistant protein fragments harboring only the invasive domain of InvA. In 1998, it was shown that latex particles coupled with a 192-residue InvA fragment are internalized into host cells and distributed systemically in rats [77, 79, 80]. InvA was further used for different types of gene carriers. For example, engineered InvA-coated *E. coli* and soluble InvA fragments attached to DNA-binding proteins were used for gene delivery into cultured cells [81-86].

Several factors have been shown to influence InvA-mediated drug delivery systems and can be used and/or must be considered in order to optimize the system. For the production of a nanoparticle-based drug delivery system, large amounts of the protein are required. Recombinant invasin fragments can easily be produced in high amounts by genetic engineering. Nonetheless, the fragments need to be coated onto the carrier particle in a way that does not compromise their bioactivity. This means covalent attachment methods must be optimized to ensure correct orientation of the invasive ligand. Efficiency of particle uptake is further influenced by (i) the invasin fragment and density on the particle, (ii) the stability of its attachment to the carrier, and (iii) the type of nanoparticle (size, mobility, surface charge). Attaching

increasing concentrations of invasin onto a latex nanoparticle was shown to increase cell entry and drug carriage. However, after a certain ligand density was reached, less efficient delivery was observed [87]. Use of a longer 497-residue InvA fragment could be also beneficial for uptake, as it induces invasin self-association and β_1 -integrins clustering which enhances uptake efficiency [74, 88]. On the other hand, a larger InvA fragment might be more prone to degradation by intestinal proteases [78]. Moreover, it could be shown *in vitro* that invasin from *Y. pseudotuberculosis* induces the apical expression of β_1 -integrins on Caco-2 cells and stimulates the uptake of nanoparticles via invasin-dependent transcytosis [80].

Another drawback might be the generation of unwanted immune responses. Invasin-binding to epithelial cells *in vitro* was found to activate gene expression and to induce the production and secretion of multiple proinflammatory cytokines, including IL-8, IL-1 α , IL-1 β , monocyte chemoattractant protein 1 (MCP-1), granulocyte macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor alpha (TNF- α) [89]. Invasin has also successfully been used as a vaccine adjuvant, and ovalbumin-InvA-coated microparticles were found to induce ovalbumin-specific CD8 T-cell responses and CD4 T-cell responses (IFN- γ , IL-4) [90, 91]. These properties must not necessarily be disadvantageous for an InvA-based drug delivery system. Firstly, no or only a very weak immune response to InvA was detected in patients suffering from yersiniosis [92]. Since β_1 -integrins are expressed on certain phagocytes (e.g. dendritic cells), immune tolerance could be induced [93]. Secondly, cyto- and chemokine induction could induce a more potent immune response and enhance the efficacy of delivered vaccines. In fact, a noninvasive *Shigella* mutant ($\Delta ipaB$) equipped with invasin caused polymorphonuclear cell infiltration in the lung. However, it did not induce the production of large amounts of proinflammatory

cytokines and it was safer and more effective than the conventional live vaccine [94]. Thirdly, InvA-mediated IL-8 induction, which is typically accompanied by a transient separation of cell-cell contacts, was shown to facilitate access into deeper tissues and improve dissemination [95]. In this regard, IL-8 produced by InvA and many other bacterial invasion factors (e.g. YadA) could support the invasive function.

Summary and future perspectives

The oral route constitutes the preferred route for drug delivery but many drugs, like peptides and proteins, are still poorly available after oral administration. To overcome this problem, innovative approaches to develop bioadhesive and bioinvasive drug delivery systems, in which inert nanoparticles are coated with cell adhesive or invasive bacterial ligands (invasins) as targeting agents have been initiated. Most pathogenic bacteria have evolved strategies by which they are able to invade host cells, and first efforts have been made to use bacterial invasion factors as ligands for targeted oral drug delivery [96]. In particular the cell entry factor invasin (InvA) of *Y. pseudotuberculosis* has repeatedly been used for the construction of bioinvasive test carrier systems [79, 97, 98]. Use of InvA for drug targeted oral drug delivery seems ideal as: (i) InvA promotes the most efficient uptake pathway so far known, (ii) InvA can be easily produced and functionally coated on nanoparticles and (iii) InvA specifically targets the drug-containing particles to M cells of the gastrointestinal epithelium which possess natural ingestion mechanisms and are in the proximity of lymphatic tissues which might support immune responses against delivered vaccines.

Although use of bacterial invasins has great potential for particle-based oral drug delivery, many issues need to be considered and addressed to improve this

technology. This not only includes the properties of the carrier and the bioactivity of the ligand, it also depends on the effectiveness of a drug delivery system. Bacterial invasins, which promote uptake via β_1 -integrins, would restrict access to M cells and would limit effectiveness. Targeting of other cell types might only be possible under specific conditions, for instance concomitantly to intestinal inflammation (e.g. IBD), when β_1 -integrins become accessible on the apical side of enterocytes due to an opening of the tight-junctions [99]. In this respect, invasin-coated nanoparticles might provide a new possibility to target pharmaceutical compounds for the treatment of IBD.

Since the number of M cells within the intestinal epithelium is usually very low, alternative drug delivery systems targeting different and more abundant intestinal cells are also desirable. Promising alternative candidates for targeting of drug carriers are the listerial internalin proteins. Nanoparticles coated with a fragment of InlA promote uptake into human enterocytes (Caco-2) in a monolayer system [100]. Moreover, expression of InlA by the food-grade bacterium *Lactococcus lactis* (used as an antigen delivery vehicle) rendered the cocci capable of invading epithelial cells *in vitro* as well as *in vivo* using a guinea pig infection model [101]. A recent study further demonstrated that InlA interacts with E-cadherin receptors which are accessible around mucus-expelling goblet cells (GCs), extruding enterocytes at the tip and lateral sides of intestinal villi, and in villus epithelial folds. The InlA-promoted uptake leads to rapid transcytosis across the intestinal epithelium, and release in the lamina propria by exocytosis [102]. Development and analysis of InlA-based drug delivery systems in mouse models was previously hampered by the fact that InlA-mediated invasion was restricted to human cells due to a single amino acid exchange in the murine E-cadherin receptor [103]. However, a mouse model expressing huma-

nized E-cadherin was quite recently developed, which now allows *in vivo* testing [103, 104].

The inquiry of available genomes of bacterial pathogens further reveals that the choice of alternative bacterial invasins for drug delivery is far from being exhausted. Numerous putative adhesion and invasion factors have been identified in enteric and non-enteric bacterial pathogens and their analysis will offer additional possibilities for the development of efficient and safe particulate systems in the future. In many cases, more than 10 putative adhesion/invasion factors are encoded by a particular pathogen. For example, genes of four additional InvA-type proteins have been identified in *Yersinia* genomes, and two of the proteins promote selective binding to differentiated enterocytes *in vitro* and contribute to *Y. pseudotuberculosis* virulence [35]. In addition, several other *Yersinia* proteins with homology to different types of adhesins/invasins (e.g. YadA, pertactins) were identified and are currently under investigation – some of which have already been shown to have important cell adhesive functions [105-107]. Whether any of the bacterial ligands will be used for drug delivery in the future is hardly predictable, but their potential for the development of drug delivery system is unquestionable. A more detailed knowledge about their molecular function and *in vivo* activity will help us to evaluate and prove their merits as drug targeting agents.

Acknowledgements

We thank Dr. Manfred Rohde for scanning microscopy and Dr. Fabio Pisano for critically reading the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (SFB621) and the Fonds der Chemischen Industrie.

References

- [1] M.N.V.R. Kumar, Handbook of particulate drug delivery., American Scientific Publishers, 2008.
- [2] G. Russell-Jones, Intestinal receptor targeting for peptide delivery: an expert's personal perspective on reasons for failure and new opportunities., Ther. Deliv., 2 (2011) 1575-1593.
- [3] Y. Goto, H. Kiyono, Epithelial barrier: an interface for the cross-communication between gut flora and immune system, Immunological Reviews, 245 (2012) 147-163.
- [4] A. Vila, A. Sanchez, M. Tobio, P. Calvo, M.J. Alonso, Design of biodegradable particles for protein delivery, J. Control. Rel., 78 (2002) 15-24.
- [5] L. Mu, S.S. Feng, A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS, J. Control. Rel., 86 (2003) 33-48.
- [6] A. Siebers, B.B. Finlay, M cells and the pathogenesis of mucosal and systemic infections, Trends Microbiol, 4 (1996) 22-29.
- [7] M.A. Clark, B.H. Hirst, M.A. Jepson, M-cell surface beta1 integrin expression and invasin-mediated targeting of *Yersinia pseudotuberculosis* to mouse Peyer's patch M cells, Infect Immun, 66 (1998) 1237-1243.
- [8] A. Marra, R.R. Isberg, Invasin-dependent and invasin-independent pathways for translocation of *Yersinia pseudotuberculosis* across the Peyer's patch intestinal epithelium, Infect Immun, 65 (1997) 3412-3421.
- [9] R. Schulte, S. Kerneis, S. Klinke, H. Bartels, S. Preger, J.P. Kraehenbuhl, E. Pringault, I.B. Autenrieth, Translocation of *Yersinia enterocolitica* across reconstituted intestinal epithelial monolayers is triggered by Yersinia invasin

- binding to beta1 integrins apically expressed on M-like cells, *Cellular Microbiology*, 2 (2000) 173-185.
- [10] N. Hussain, P.U. Jani, A.T. Florence, Enhanced oral uptake of tomato lectin-conjugated nanoparticles in the rat, *Pharm Res*, 14 (1997) 613-618.
- [11] E.J. Haltner E., Lehr CM, Lectins and bacterial invasion factors for controlling endo- and transcytosis of bioadhesive drug carrier systems, *Eur J Pharma and Biopharma* 44 (1997) 3-13.
- [12] Y. Fujimura, R.L. Owen, M cells as portals of infection: clinical and pathophysiological aspects, *Infect Agents Dis*, 5 (1996) 144-156.
- [13] P. Cossart, P.J. Sansonetti, Bacterial invasion: the paradigms of enteroinvasive pathogens, *Science*, 304 (2004) 242-248.
- [14] M.R. Neutra, A. Frey, J.P. Kraehenbuhl, Epithelial M cells: gateways for mucosal infection and immunization, *Cell*, 86 (1996) 345-348.
- [15] M.R. Neutra, Current concepts in mucosal immunity. Role of M cells in transepithelial transport of antigens and pathogens to the mucosal immune system, *Am J Physiol*, 274 (1998) G785-791.
- [16] R.J. Lamont, Bacterial invasion of host cells., in: *Advances in molecular and cellular microbiology*, Cambridge University Press, 2004.
- [17] D.R. Ofek I, Bacterial adhesion to cells and tissues., in, Chapman & Hall, 1994.
- [18] G.E. Soto, S.J. Hultgren, Bacterial adhesins: common themes and variations in architecture and assembly, *J Bacteriol*, 181 (1999) 1059-1071.
- [19] P. Dersch, Molecular and cellular mechanisms of bacterial entry into host cells, in: *Host response mechanisms in infectious diseases*, Basel, Karger, 2003, pp. 183-209.

- [20] J. Pizarro-Cerda, P. Cossart, Bacterial adhesion and entry into host cells, *Cell*, 124 (2006) 715-727.
- [21] J.E. Galan, Salmonella interactions with host cells: type III secretion at work, *Annu Rev Cell Dev Biol*, 17 (2001) 53-86.
- [22] P.J. Sansonetti, Rupture, invasion and inflammatory destruction of the intestinal barrier by Shigella, making sense of prokaryote-eukaryote cross-talks, *FEMS Microbiol Rev*, 25 (2001) 3-14.
- [23] M.A. Clark, M.A. Jepson, N.L. Simmons, B.H. Hirst, Preferential interaction of *Salmonella typhimurium* with mouse Peyer's patch M cells, *Res Microbiol*, 145 (1994) 543-552.
- [24] B.D. Jones, N. Ghori, S. Falkow, *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches, *J Exp Med*, 180 (1994) 15-23.
- [25] M. Ogawa, Y. Handa, H. Ashida, M. Suzuki, C. Sasakawa, The versatility of Shigella effectors, *Nat Rev Microbiol*, 6 (2008) 11-16.
- [26] S. Mattoo, Y.M. Lee, J.E. Dixon, Interactions of bacterial effector proteins with host proteins, *Curr Opin Immunol*, 19 (2007) 392-401.
- [27] P.J. Sansonetti, J.P. Di Santo, Debugging how bacteria manipulate the immune response, *Immunity*, 26 (2007) 149-161.
- [28] D. Zhou, L.M. Chen, L. Hernandez, S.B. Shears, J.E. Galan, A *Salmonella* inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host cell actin cytoskeleton rearrangements and bacterial internalization, *Mol Microbiol*, 39 (2001) 248-259.
- [29] A. Marra, R.R. Isberg, Common entry mechanisms. Bacterial pathogenesis, *Curr Biol*, 6 (1996) 1084-1086.

- [30] P. Cossart, M. Lecuit, Interactions of *Listeria monocytogenes* with mammalian cells during entry and actin-based movement: bacterial factors, cellular ligands and signaling, EMBO J, 17 (1998) 3797-3806.
- [31] J.A. Vazquez-Boland, M. Kuhn, P. Berche, T. Chakraborty, G. Dominguez-Bernal, W. Goebel, B. Gonzalez-Zorn, J. Wehland, J. Kreft, *Listeria* pathogenesis and molecular virulence determinants, Clin Microbiol Rev, 14 (2001) 584-640.
- [32] P. Dube, Interaction of *Yersinia* with the gut: mechanisms of pathogenesis and immune evasion, Curr Top Microbiol Immunol, 337 (2009) 61-91.
- [33] P.D. Barnes, M.A. Bergman, J. Mecsas, R.R. Isberg, *Yersinia pseudotuberculosis* disseminates directly from a replicating bacterial pool in the intestine, J Exp Med, 203 (2006) 1591-1601.
- [34] R.R. Isberg, D.L. Voorhis, S. Falkow, Identification of invasin: a protein that allows enteric bacteria to penetrate cultured mammalian cells, Cell, 50 (1987) 769-778.
- [35] F. Pisano, A. Kochut, F. Uliczka, R. Geyer, T. Stolz, T. Thiermann, M. Rohde, P. Dersch, *In vivo*-induced InvA-like autotransporters Ifp and InvC of *Yersinia pseudotuberculosis* promote interactions with intestinal epithelial cells and contribute to virulence, Infect Immun, 80 (2012) 1050-1064.
- [36] Y. El Tahir, M. Skurnik, YadA, the multifaceted *Yersinia* adhesin, International journal of medical microbiology : IJMM, 291 (2001) 209-218.
- [37] S.E. Autenrieth, I.B. Autenrieth, *Yersinia enterocolitica*: subversion of adaptive immunity and implications for vaccine development, Int J Med Microbiology 298 (2008) 69-77.

- [38] K. Schulze, C. Olive, T. Ebensen, C.A. Guzman, Intranasal vaccination with SfbI or M protein-derived peptides conjugated to diphtheria toxoid confers protective immunity against a lethal challenge with *Streptococcus pyogenes*, *Vaccine*, 24 (2006) 6088-6095.
- [39] W.L. Yu, H. Dan, M. Lin, InlA and InlC2 of *Listeria monocytogenes* serotype 4b are two internalin proteins eliciting humoral immune responses common to listerial infection of various host species, *Current Microbiology*, 56 (2008) 505-509.
- [40] H.H. Niemann, W.D. Schubert, D.W. Heinz, Adhesins and invasins of pathogenic bacteria: a structural view, *Microbes and infection / Institut Pasteur*, 6 (2004) 101-112.
- [41] K.A. Kline, S. Falker, S. Dahlberg, S. Normark, B. Henriques-Normark, Bacterial adhesins in host-microbe interactions, *Cell host & microbe*, 5 (2009) 580-592.
- [42] M. Hamon, H. Bierne, P. Cossart, *Listeria monocytogenes*: a multifaceted model, *Nat Rev Microbiol*, 4 (2006) 423-434.
- [43] S. Dramsi, I. Biswas, E. Maguin, L. Braun, P. Mastroeni, P. Cossart, Entry of *Listeria monocytogenes* into hepatocytes requires expression of *inlB*, a surface protein of the internalin multigene family, *Mol Microbiol*, 16 (1995) 251-261.
- [44] S.K. Parida, E. Domann, M. Rohde, S. Muller, A. Darji, T. Hain, J. Wehland, T. Chakraborty, Internalin B is essential for adhesion and mediates the invasion of *Listeria monocytogenes* into human endothelial cells, *Mol Microbiol*, 28 (1998) 81-93.
- [45] A. Lingnau, E. Domann, M. Hudel, M. Bock, T. Nichterlein, J. Wehland, T. Chakraborty, Expression of the *Listeria monocytogenes* EGD inlA and inlB

- genes, whose products mediate bacterial entry into tissue culture cell lines, by PrfA-dependent and -independent mechanisms, *Infect Immun*, 63 (1995) 3896-3903.
- [46] L. Braun, H. Ohayon, P. Cossart, The InlB protein of *Listeria monocytogenes* is sufficient to promote entry into mammalian cells, *Mol Microbiol*, 27 (1998) 1077-1087.
- [47] J.L. Gaillard, P. Berche, C. Frehel, E. Gouin, P. Cossart, Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci, *Cell*, 65 (1991) 1127-1141.
- [48] M. Lecuit, H. Ohayon, L. Braun, J. Mengaud, P. Cossart, Internalin of *Listeria monocytogenes* with an intact leucine-rich repeat region is sufficient to promote internalization, *Infect Immun*, 65 (1997) 5309-5319.
- [49] J. Mengaud, H. Ohayon, P. Gounon, R.M. Mege, P. Cossart, E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells, *Cell*, 84 (1996) 923-932.
- [50] K.S. Bhat, C.P. Gibbs, O. Barrera, S.G. Morrison, F. Jahnig, A. Stern, E.M. Kupsch, T.F. Meyer, J. Swanson, The opacity proteins of *Neisseria gonorrhoeae* strain MS11 are encoded by a family of 11 complete genes, *Mol Microbiol*, 5 (1991) 1889-1901.
- [51] B. Malorny, G. Morelli, B. Kusecek, J. Kolberg, M. Achtman, Sequence diversity, predicted two-dimensional protein structure, and epitope mapping of neisserial Opa proteins, *J Bacteriol*, 180 (1998) 1323-1330.

- [52] T. Chen, R.J. Belland, J. Wilson, J. Swanson, Adherence of pilus- Opa+ gonococci to epithelial cells in vitro involves heparan sulfate, J Exp Med, 182 (1995) 511-517.
- [53] J.P. van Putten, T.D. Duensing, R.L. Cole, Entry of OpaA+ gonococci into HEp-2 cells requires concerted action of glycosaminoglycans, fibronectin and integrin receptors, Mol Microbiol, 29 (1998) 369-379.
- [54] T. Chen, E.C. Gotschlich, CGM1a antigen of neutrophils, a receptor of gonococcal opacity proteins, Proc Natl Acad Sci USA 93 (1996) 14851-14856.
- [55] C.R. Hauck, T.F. Meyer, F. Lang, E. Gulbins, CD66-mediated phagocytosis of Opa52 *Neisseria gonorrhoeae* requires a Src-like tyrosine kinase- and Rac1-dependent signalling pathway, EMBO J, 17 (1998) 443-454.
- [56] K. Dziwanowska, J.M. Patti, C.F. Deobald, K.W. Bayles, W.R. Trumble, G.A. Bohach, Fibronectin binding protein and host cell tyrosine kinase are required for internalization of *Staphylococcus aureus* by epithelial cells, Infect Immun, 67 (1999) 4673-4678.
- [57] V. Ozeri, I. Rosenshine, D.F. Mosher, R. Fassler, E. Hanski, Roles of integrins and fibronectin in the entry of *Streptococcus pyogenes* into cells via protein F1, Mol Microbiol, 30 (1998) 625-637.
- [58] S.J. Peacock, N.P. Day, M.G. Thomas, A.R. Berendt, T.J. Foster, Clinical isolates of *Staphylococcus aureus* exhibit diversity in fnb genes and adhesion to human fibronectin, J Infect, 41 (2000) 23-31.
- [59] G. Molinari, S.R. Talay, P. Valentin-Weigand, M. Rohde, G.S. Chhatwal, The fibronectin-binding protein of *Streptococcus pyogenes*, SfbI, is involved in the internalization of group A streptococci by epithelial cells, Infect Immun, 65 (1997) 1357-1363.

- [60] J.C. Leo, M. Skurnik, Adhesins of human pathogens from the genus *Yersinia*, *Advances in experimental medicine and biology*, 715 (2011) 1-15.
- [61] E. Hoiczyk, A. Roggenkamp, M. Reichenbecher, A. Lupas, J. Heesemann, Structure and sequence analysis of *Yersinia* YadA and *Moraxella* UspAs reveal a novel class of adhesins, *EMBO J*, 19 (2000) 5989-5999.
- [62] J. Eitel, P. Dersch, The YadA protein of *Yersinia pseudotuberculosis* mediates high-efficiency uptake into human cells under environmental conditions in which invasin is repressed, *Infect Immun*, 70 (2002) 4880-4891.
- [63] T. Heise, P. Dersch, Identification of a domain in *Yersinia* virulence factor YadA that is crucial for extracellular matrix-specific cell adhesion and uptake, *Proc Natl Acad Sci USA*, 103 (2006) 3375-3380.
- [64] R.R. Isberg, J.M. Leong, Multiple beta 1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells, *Cell*, 60 (1990) 861-871.
- [65] G. Tran Van Nhieu, R.R. Isberg, Bacterial internalization mediated by beta 1 chain integrins is determined by ligand affinity and receptor density, *EMBO J*, 12 (1993) 1887-1895.
- [66] G.T. Van Nhieu, E.S. Krukoni, A.A. Reszka, A.F. Horwitz, R.R. Isberg, Mutations in the cytoplasmic domain of the integrin beta1 chain indicate a role for endocytosis factors in bacterial internalization, *J Biol Chem*, 271 (1996) 7665-7672.
- [67] G.T. Van Nhieu, R.R. Isberg, The *Yersinia pseudotuberculosis* invasin protein and human fibronectin bind to mutually exclusive sites on the alpha 5 beta 1 integrin receptor, *J Biol Chem*, 266 (1991) 24367-24375.

- [68] Z.A. Hamburger, M.S. Brown, R.R. Isberg, P.J. Bjorkman, Crystal structure of invasin: a bacterial integrin-binding protein, *Science*, 286 (1999) 291-295.
- [69] S. Rankin, R.R. Isberg, J.M. Leong, The integrin-binding domain of invasin is sufficient to allow bacterial entry into mammalian cells, *Infect Immun*, 60 (1992) 3909-3912.
- [70] J.M. Leong, P.E. Morrissey, A. Marra, R.R. Isberg, An aspartate residue of the *Yersinia pseudotuberculosis* invasin protein that is critical for integrin binding, *EMBO J*, 14 (1995) 422-431.
- [71] E. Ruoslahti, RGD and other recognition sequences for integrins, *Annu Rev Cell Dev Biol*, 12 (1996) 697-715.
- [72] R.O. Hynes, Integrins: bidirectional, allosteric signaling machines, *Cell*, 110 (2002) 673-687.
- [73] C. Brakebusch, R. Fassler, beta 1 integrin function *in vivo*: adhesion, migration and more, *Cancer Metastasis Rev*, 24 (2005) 403-411.
- [74] P. Dersch, R.R. Isberg, A region of the *Yersinia pseudotuberculosis* invasin protein enhances integrin-mediated uptake into mammalian cells and promotes self-association, *EMBO J*, 18 (1999) 1199-1213.
- [75] G.T. Van Nhieu, R.R. Isberg, Bacterial internalization mediated by beta 1 chain integrins is determined by ligand affinity and receptor density, *EMBO J*, 12 (1993) 1887-1895.
- [76] C.R. Hauck, F. Agerer, P. Muenzner, T. Schmitter, Cellular adhesion molecules as targets for bacterial infection, *Eur J Cell Biol*, 85 (2006) 235-242.
- [77] A. Wiedemann, S. Linder, G. Grassl, M. Albert, I. Autenrieth, M. Aepfelbacher, *Yersinia enterocolitica* invasin triggers phagocytosis via beta1 integrins, CDC42Hs and WASp in macrophages, *Cell Microbiology*, 3 (2001) 693-702.

- [78] J. Trcek, M.F. Oellerich, K. Niedung, F. Ebel, S. Freund, K. Trulzsch, Gut proteases target Yersinia invasin in vivo, BMC Res Notes, 4 (2011) 129.
- [79] N. Hussain, A.T. Florence, Utilizing bacterial mechanisms of epithelial cell entry: invasin-induced oral uptake of latex nanoparticles, Pharm Res, 15 (1998) 153-156.
- [80] E.G. Ragnarsson, I. Schoultz, E. Gullberg, A.H. Carlsson, F. Tafazoli, M. Lerm, K.E. Magnusson, J.D. Soderholm, P. Artursson, *Yersinia pseudotuberculosis* induces transcytosis of nanoparticles across human intestinal villus epithelium via invasin-dependent macropinocytosis, Lab Invest, 88 (2008) 1215-1226.
- [81] M.E. Hemler, VLA proteins in the integrin family: structures, functions, and their role on leukocytes, Annu Rev Immunol, 8 (1990) 365-400.
- [82] E. Fortunati, E. Ehlert, N.D. van Loo, C. Wyman, J.A. Eble, F. Grosveld, B.J. Scholte, A multi-domain protein for beta1 integrin-targeted DNA delivery, Gene Ther, 7 (2000) 1505-1515.
- [83] R.J. Critchley, S. Jezzard, K.J. Radford, S. Goussard, N.R. Lemoine, C. Grillot-Courvalin, G. Vassaux, Potential therapeutic applications of recombinant, invasive *E. coli*, Gene Ther, 11 (2004) 1224-1233.
- [84] K. Narayanan, P.E. Warburton, DNA modification and functional delivery into human cells using *Escherichia coli* DH10B, Nucl Acids Res, 31 (2003) e51.
- [85] C. Grillot-Courvalin, S. Goussard, F. Huetz, D.M. Ojcius, P. Courvalin, Functional gene transfer from intracellular bacteria to mammalian cells, Nat Biotechnol, 16 (1998) 862-866.
- [86] I. Fajac, S. Grosse, J.M. Collombet, G. Thevenot, S. Goussard, C. Danel, C. Grillot-Courvalin, Recombinant *Escherichia coli* as a gene delivery vector into airway epithelial cells, J Control Rel, 97 (2004) 371-381.

- [87] A.T. Florence, N. Hussain, Transcytosis of nanoparticle and dendrimer delivery systems: evolving vistas, *Adv Drug Deliv Rev*, 50 Suppl 1 (2001) S69-89.
- [88] P. Dersch, R.R. Isberg, An immunoglobulin superfamily-like domain unique to the *Yersinia pseudotuberculosis* invasin protein is required for stimulation of bacterial uptake via integrin receptors, *Infect Immun*, 68 (2000) 2930-2938.
- [89] D. Kampik, R. Schulte, I.B. Autenrieth, *Yersinia enterocolitica* invasin protein triggers differential production of interleukin-1, interleukin-8, monocyte chemoattractant protein 1, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor alpha in epithelial cells: implications for understanding the early cytokine network in *Yersinia* infections, *Infect Immun*, 68 (2000) 2484-2492.
- [90] C.L. Finstad, C.Y. Wang, J. Kowalski, M. Zhang, M.L. Li, X.M. Li, W.G. Xia, M.C. Bosland, K.K. Murthy, A.M. Walfield, W.C. Koff, T.J. Zamb, Synthetic luteinizing hormone releasing hormone (LHRH) vaccine for effective androgen deprivation and its application to prostate cancer immunotherapy, *Vaccine*, 22 (2004) 1300-1313.
- [91] O.T. Buhler, C.A. Wiedig, Y. Schmid, G.A. Grassl, E. Bohn, I.B. Autenrieth, The *Yersinia enterocolitica* invasin protein promotes major histocompatibility complex class I- and class II-restricted T-cell responses, *Infect Immun*, 74 (2006) 4322-4329.
- [92] N. Fortineau, J.L. Beretti, P. Berche, M. Simonet, Lack of antibody response to invasin in humans with yersiniosis, *Clin Diagn Lab Immunol*, 1 (1994) 235-237.
- [93] T.K. van den Berg, M. van der Ende, E.A. Dopp, G. Kraal, C.D. Dijkstra, Localization of beta 1 integrins and their extracellular ligands in human lymphoid tissues, *Am J Pathol*, 143 (1993) 1098-1110.

- [94] T. Suzuki, Y. Yoshikawa, H. Ashida, H. Iwai, T. Toyotome, H. Matsui, C. Sasakawa, High vaccine efficacy against shigellosis of recombinant noninvasive *Shigella* mutant that expresses *Yersinia* invasin, *J Immunol*, 177 (2006) 4709-4717.
- [95] B.A. McCormick, A. Nusrat, C.A. Parkos, L. D'Andrea, P.M. Hofman, D. Carnes, T.W. Liang, J.L. Madara, Unmasking of intestinal epithelial lateral membrane beta1 integrin consequent to transepithelial neutrophil migration in vitro facilitates inv-mediated invasion by *Yersinia pseudotuberculosis*, *Infect Immun*, 65 (1997) 1414-1421.
- [96] E. Haltner, E. J.H., C.M. Lehr, Lectins and bacterial invasion factors for controlling endo- and transcytosis of bioadhesive drug carrier systems, *Eur. J. Pharm. Biopharm.*, 44 (1997) 3-13.
- [97] R.N. Palumbo, C. Wang, Bacterial invasin: structure, function, and implication for targeted oral gene delivery, *Curr Drug Deliv*, 3 (2006) 47-53.
- [98] G.F. Dawson, G.W. Halbert, The *in vitro* cell association of invasin coated polylactide-co-glycolide nanoparticles, *Pharm Res*, 17 (2000) 1420-1425.
- [99] K.L. Edelblum, J.R. Turner, The tight junction in inflammatory disease: communication breakdown, *Curr Opin Pharmacol*, 9 (2009) 715-720.
- [100] Rowland R.E.S., Taylor, P.W., Florence A.T., Attachment, uptake and transport of nanoparticles coated with an internalin A fragment in Caco-2 cell monolayers, *J Drug Del Sci TEch*, 15 (2005) 313-317.
- [101] V.D. Guimaraes, J.E. Gabriel, F. Lefevre, D. Cabanes, A. Gruss, P. Cossart, V. Azevedo, P. Langella, Internalin-expressing *Lactococcus lactis* is able to invade small intestine of guinea pigs and deliver DNA into mammalian epithelial cells, *Microbes and infection / Institut Pasteur*, 7 (2005) 836-844.

- [102] G. Nikitas, C. Deschamps, O. Disson, T. Niault, P. Cossart, M. Lecuit, Transcytosis of *Listeria monocytogenes* across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin, *J Exp Med*, 208 (2011) 2263-2277.
- [103] M. Lecuit, S. Dramsi, C. Gottardi, M. Fedor-Chaiken, B. Gumbiner, P. Cossart, A single amino acid in E-cadherin responsible for host specificity towards the human pathogen *Listeria monocytogenes*, *EMBO J*, 18 (1999) 3956-3963.
- [104] M. Lecuit, Understanding how *Listeria monocytogenes* targets and crosses host barriers, *Clin Microbiol Infect*, 11 (2005) 430-436.
- [105] J.D. Lenz, M.B. Lawrenz, D.G. Cotter, M.C. Lane, R.J. Gonzalez, M. Palacios, V.L. Miller, Expression during host infection and localization of *Yersinia pestis* autotransporter proteins, *J Bacteriol*, 193 (2011) 5936-5949.
- [106] P.C. Strong, S.J. Hinchliffe, H. Patrick, S. Atkinson, O.L. Champion, B.W. Wren, Identification and characterisation of a novel adhesin Ifp in *Yersinia pseudotuberculosis*, *BMC Microbiol*, 11 (2011) 85.
- [107] S. Forman, C.R. Wulff, T. Myers-Morales, C. Cowan, R.D. Perry, S.C. Straley, *yadBC* of *Yersinia pestis*, a new virulence determinant for bubonic plague, *Infect Immun*, 76 (2008) 578-587.

Figure Legends

Figure 1: Invasion strategies of bacteria: Trigger- and Zipper-mechanism

(A) Trigger mechanism. (1) Schematic drawing of the trigger uptake mechanism, illustrating the formation of large membrane projections – membrane ruffling; (2) T3SS provides the molecular basis for the trigger mechanism; (3) EM picture showing a *S. typhimurium* bacterium entering a fibroblast cell. **(B)** Zipper mechanism. (1) Schematic drawing of the uptake process, showing membrane engulfment of the bacteria; (2) interaction between invasion proteins and eukaryotic cell receptors provides the molecular basis for the zipper mechanism; (3) electron microscopy picture of *S. pyogenes* entering an endothelial cell. T3SS: type III secretion system; IM: inner membrane; OM: outer membrane; PM: plasma membrane (EM pictures were kindly provided by Dr. Manfred Rohde, HZI Braunschweig).

Figure 2: Bacterial invasion proteins

Schematic drawings of the modular structures of selected bacterial invasins. **(A)** Opa protein from *Neisseria*, **(B)** SfbI from *S. pyogenes*, **(C)** InlA from *L. monocytogenes*, and **(D)** YadA from *Yersinia*. SP: signal peptide; AR: aromatic region; S1 / S2: spacer 1/2; PR: proline repeats; FnBR: fibronectin-binding repeats; WM: wall-membrane region; LRR: leucine-rich repeats; IR: interrepeat-region; BR: B-repeats; CWS: cell-wall spanning region; MA: membrane anchor.

Figure 3: Invasin from *Y. pseudotuberculosis*

(A) The domain structure of invasin is illustrated; the black bar underneath the scheme represents the structure of invasin shown in **B**; **(B)** structure of the external cell-binding domain; **(C)** Invasin-coated latex particles bound to human epithelial cells

(SP: signal peptide; Big: bacterial immunoglobulin-like; CTLD: C-type lectin-like domain).

Table 1: Bacterial invasion factors

Pathogen	Adhesin / invasin	Receptor	Associated disease
<i>Listeria monocytogenes</i>	InlA, InlB	E-cadherin, c-Met, gC1q-R	listeriosis
<i>Neisseria gonorrhoeae</i> <i>Neisseria meningitidis</i>	Opa proteins	HSPG, Vn, Fn, CEACAMs	gonorrhoea, meningitis
<i>Staphylococcus aureus</i>	FnBPA, FnBPBB	Fn, β_1 -integrins	wound infections, endocarditis, TSS
<i>Streptococcus</i> spp.	Sfbl	Fn, β_1 -integrins, collagen	meningitis, pneumonia, scarlet fever, pharyngitis
<i>Yersinia enterocolitica</i> <i>Yersinia pseudotuberculosis</i>	InvA, Ifp, YadA	β_1 -integrins, Fn, collagen, Laminin	gastroenteritis, mesenteric lymphadenitis

Inl: Internalin; Opa: Opacity-associated proteins; HSPG: Heparansulphate proteoglycan; Vn: Vitronectin; Fn: Fibronectin; CEACAM: carcinoembryonic antigen-related cell adhesion molecule; FnBP: Fibronectin-binding protein; TSS: toxic shock syndrome; Sfbl: *S. pyogenes* fibronectin-binding protein I; InvA: InvasinA; YadA: *Yersinia* adhesin A

Fig. 1

[Click here to download high resolution image](#)

Fig. 1: Kochut & Dersch 2012

Fig. 2

[Click here to download high resolution image](#)

Fig. 2: Kochut & Dersch 2012

Fig. 3
[Click here to download high resolution image](#)

Fig. 3: Kochut & Dersch 2012