Microreview

*Bartonella* entry mechanisms into mammalian host cells

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**Summary**

The Gram-negative genus *Bartonella* comprises arthropod-borne pathogens that typically infect mammals in a host-specific manner. *Bartonella bacilliformis* and *Bartonella quintana* are human-specific pathogens, while several zoonotic bartonellae specific for diverse animal hosts infect humans as an incidental host. Clinical manifestations of *Bartonella* infections range from mild symptoms to life-threatening disease. Following transmission by blood-sucking arthropods or traumatic contact with infected animals, bartonellae display sequential tropisms towards endothelial and possibly other nucleated cells and erythrocytes, the latter in a host-specific manner. Attachment to the extracellular matrix (ECM) and to nucleated cells is mediated by surface-exposed bacterial adhesins, in particular trimeric autotransporter adhesins (TAAs). The subsequent engulfment of the pathogen into a vacuolar structure follows a unique series of events whereby the pathogen avoids the endolysosomal compartments. For *Bartonella henselae* and assuming most other species, the infection process is aided at different steps by *Bartonella* effector proteins (Beps). They are injected into host cells through the type IV secretion system (T4SS) VirB/D4 and subvert host cellular functions to favour pathogen uptake. Bacterial binding to erythrocytes is mediated by Trw, another T4SS, in a strictly host-specific manner, followed by pathogen-forced uptake involving the IalB invasin and subsequent replication and persistence within a membrane-bound intra-erythrocytic compartment.

**Introduction**

Currently the genus *Bartonella* comprises 24 different species which subdivide evolutionarily into four lineages. *Bartonella bacilliformis* is the sole representative of the ancestral lineage 1. Members of lineage 2 (e.g. *Bartonella schoenbuchensis* and other ruminant-specific species), lineage 3 (e.g. *Bartonella clarridgeiae* and *Bartonella rochalimae*), and of the most species-rich lineage 4 (e.g. *Bartonella henselae* and *Bartonella quintana*) are considered modern species. As opposed to lineage 1, species of the modern lineages have acquired at least one type IV secretion system (T4SS). T4SSs are ancestrally related to bacterial conjugation machineries used for DNA transfer between bacteria. In the course of *Bartonella* evolution, T4SSs have become host adaptability factors conferring host-specific adhesion or translocation of bacterial effector proteins into host cells (Saenz *et al.*, 2007; Engel *et al.*, 2011).

Clinically most relevant for humans are the human-specific species *B. bacilliformis* and *B. quintana*, and the zoonotic cat-specific species *B. henselae*. *B. bacilliformis* elicits life-threatening biphasic Carrión’s disease with a primary acute stage of haemolytic anaemia called ‘Oroya fever’ and a secondary chronic stage, the ‘ verruga peruana’, characterized by the formation of vasoproliferative lesions. Symptomatic for trench fever caused by *B. quintana* is bacteraemia accompanied by relapsing fever. Infection of immunocompetent patients with *B. henselae* results in ‘cat-scratch disease’, a typically self-limiting swelling of lymph nodes often associated with fever. After infection of immunocompromised patients with *B. henselae* or *B. quintana* severe complications with vasoproliferative lesions known as ‘bacillary angiomatisis’ and eventually damage of the skeleton, brain and inner organs with fatal outcome may arise. The slow-growing bartonellae which partially evade the host immune system and thus typically cause a chronic infec-
Bartonella entry into host cells

Infection of endothelial and other nucleated cells

Bacterial attachment to nucleated cells

The initial steps in the infection of nucleated cells represent adherence to the extracellular matrix (ECM) and to the host cell plasma membrane. Such interactions involve surface proteins mediating contact between bacterial and host cell surfaces, as shown by the release of attached *B. bacilliformis* from fibroblasts after trypsin treatment, and by the reduced infection rate after pretreatment of bacteria with *B. bacilliformis*-specific immune serum (Hill et al., 1992). Primary candidates for a role in this attachment process are pilus-like surface appendages on the bacterial surface that were for *B. henselae* initially mistakenly described as type IV pili (Batterman et al., 1995). These characteristic surface structures of Bartonella were later identified as trimeric autotransporter adhesins (TAAs) (Riess et al., 2004). These non-fimbrial outer membrane adhesion proteins belonging to the type V secretion systems (T5SS) show a modular architecture with a C-terminal membrane anchor that is connected to the neck and head domains via a long stalk composed of multiple repeat units (Linke et al., 2006). To the family of TAAs belong BadA (Bartonella adhesin A) of *B. henselae* and Vomps (variably expressed outer membrane proteins) of *B. quintana*, which share a similar domain composition (Zhang et al., 2004; Muller et al., 2011).

BadA has an estimated length of 100–300 nm and is variably encoded and expressed in different *B. henselae* strains (Riess et al., 2007). BadA mediates binding to ECM proteins and *B. henselae* lacking BadA expression bind less efficiently to collagen I, III, and IV, and to laminin and fibronectin in vitro (Riess et al., 2004). The BadA stalk region is dispensable for binding to collagen, but is essential for the binding of fibronectin (Kaiser et al., 2012). The recombinant BadA head region adheres more strongly to human umbilical vein endothelial cells (HUVECs) than to HeLa 229 cells, which might present a molecular explanation for the endothelial tropism observed for Bartonella (Kaiser et al., 2008).

Vomps A–D of *B. quintana* have a length of 40 nm and are much shorter than BadA of *B. henselae* (Muller et al., 2011). VompA–C display collagen-binding motifs with confirmed collagen IV binding by VompA and C (Zhang et al., 2004). The lack of fibronectin binding by Vomps may originate from the absence of a putative but still undesignated fibronectin-binding domain found in the stalk of BadA (Schulte et al., 2006). Similar to the variability of *B. henselae* BadA, also different *B. quintana* strains show different Vomp expression patterns. In the course of a *B. quintana* infection in a rhesus macaque animal model, progressive gene rearrangements have been described with the complete loss of vompA and/or vompB during prolonged bacteremia. Comparable expression differences could be identified in isolates from human patients (Zhang et al., 2004). Such genetic variation of vomp and badA loci described above could be a means for evading the adaptive host immune response.

The importance of Vomps in host cell binding is questioned by the finding that *B. quintana* adherence to human macrophages and HeLa 229 cells is not reduced in strains devoid of Vomps (Schulte et al., 2006). However, a vomp deletion mutant of *B. quintana* failed to establish a bloodstream infection in a rhesus macaque animal model, which proves that Vomps are crucial infection factors (Zhang et al., 2004).

Further to TAAs, Bartonella express other T5SS, such as Arp and Cfa (for ‘acidic repeat protein’ and ‘CAMP-like factor autotransporter’ respectively) belonging to the...
A group of monomeric inducible Bartonella autotransporters (Iba) (Litwin and Johnson, 2005; Litwin et al., 2007), and the filamentous haemagglutinins (Fha) (for a review see O’Rourke et al., 2011), which belong to the two-partner T5SS. Whether these immunogenic autotransporters exert similar functions in cell adhesion as TAAs remains to be verified.

The current list of bacterial factors with confirmed binding properties to nucleated host cells is limited and more factors are likely to be discovered among the scarcely defined outer membrane proteins (OMPs) so far described for B. bacilliformis (Minnick, 1994) and B. henselae (Burgess and Anderson, 1998; Rhomberg et al., 2004). All nine identified OMPs of B. henselae were reported to bind to HUVECs in vitro (Burgess and Anderson, 1998), and Omp89 was found to interact with fibronectin (Dabo et al., 2006b).

Further surface-exposed components are haemagglutinins (Hbps). For HbpA (Pap31) of B. henselae (Carroll et al., 2000; Zimmermann et al., 2003) an interaction with the serum protein fibronectin was demonstrated and could be outcompeted by heparin (Dabo et al., 2006a). The authors suggest possible parallels to the homologous Neisseria gonorrhoeae opacity protein OpaA involved in bacterial adhesion and entry into host cells by a concerted action involving heparan sulfate proteoglycans, fibronectin and integrin receptors (van Putten et al., 1998). A tight transcriptional regulation of Hbps in response to altered environmental cues was shown in B. quintana and B. henselae (Battisti et al., 2006; Quebatte et al., 2010) and together with the undetected immunogenicity of these proteins (Vigil et al., 2010) suggests that Hbps might account for adaptation to Bartonella residence in the arthropod vector and in the mammalian host system.

Bacterial entry into nucleated cells

Bartonella uptake is most extensively studied in human endothelial cells in which B. quintana (Merrell et al., 1978) and B. henselae (Dehio et al., 1997; Kempf et al., 2000) were found to be intimately associated with actin-enriched cell membrane protrusions during their entry (Dehio et al., 1997). The general requirement of the actin cytoskeleton during the invasion process was demonstrated in epithelial cells as well as endothelial cells (HUVECs), where cytochalasin D abolished entry of B. bacilliformis (Hill et al., 1992) and B. henselae (Dehio et al., 1997), and where inhibition of the small GTPase Rho, a key signalling factor in actin reorganization, prevented B. bacilliformis entry (Verma et al., 2000).

The internalization process of bartonellae into endothelial cells is best studied in B. henselae for which two closely intertwined entry routes were described. After initial penetration of HUVECs, B. henselae is found in Bartonella-containing vacuoles (BCVs). Each of these membrane-delimited endocytic structures contains a single up to a few bacteria. In agreement with earlier observations (Zbinden et al., 1995), BCVs are perinuclearly enriched (Dehio et al., 1997). This process is subsequently arrested by the injection of Bartonella effector proteins (Beps; B. henselae express seven Beps: BepA–G) through the VirB/D4 T4SS into the host cytoplasm. Either BepG alone (Rhomberg et al., 2009) or a combination of BepC and BepF is sufficient for blocking BCV-mediated endocytosis (Truttmann et al., 2011b). Such an inhibition of the endocytic route might account for an accumulation of extracellular bacteria at the host cell plasma membrane and leads to their rearward transport across the leading lamella resulting in the formation of a cell surface-associated bacterial aggregate, its engulfment and its final internalization by the host cell in a so-called ‘invasome’. Invasomes are characteristically tightly surrounded by actin fibres which are anchored to focal adhesion sites. Accordingly, actin depolymerization results in the abrogation of the invasome formation process (Dehio et al., 1997). Actin cytoskeleton remodeling proteins such as the Rho family GTPases Rac1 and Cdc42, their effectors Scar/WAVE and WASP, respectively, and the actin nucleation and branching complex Arp2/3 as downstream effector are essential for invasome formation (Rhomberg et al., 2009; Truttmann et al., 2011b), comparable to the requirement of Rac1, Cdc42 and PAK-1 kinase for entry of B. bacilliformis into endothelial cells (Verma and Ihler, 2002). Exclusively involved in the BepC-BepF-dependent invasome-formation pathway is the actin-severing protein cofillin-1 (Truttmann et al., 2011b).

The invasome-mediated uptake of B. henselae appears to be controlled by bi-directional host cell signalling cascades. B. henselae bind to inactive and active forms of the integrin β1 receptors on the host cell surface, while only the extended active conformation of this integrin does mediate invasome formation. Talin-1, which binds to integrins at the cytoplasmic side, is essential for inside-out activation of integrin β1, while its property to link integrins to actin is dispensable for invasome formation. Furthermore, also the tyrosine kinases Src and FAK are needed for successful invasome formation (Truttmann et al., 2011a).

Intriguingly, B. henselae internalized into BCVs display unusual trafficking within the endocytic network as those compartments are devoid of late endocytic and lysosomal markers. Also B. henselae do neither localize to the endoplasmic reticulum (ER) and the Golgi, nor to caveolin-1-positive structures (Kyme et al., 2005). The final subcellular destination of the pathogen in the host cell remains to be characterized. B. henselae neutralized by heat or paraformaldehyde, or opsonized bacteria scarcely...
enter the host and if so, they are transported along the conventional endocytic pathway and get exposed to the lysosomal-degradative environment. A subset of bacterial proteins involved in avoiding the lysosomal-degradative pathway has been identified by isolation of *B. henselae* mutants that, other than wild type, traffic to lysosomes. Mutants were affected in genes for virulence-associated protein VapA5, haemin-binding protein HbpD, D-serine/ D-alanine/glycine transport protein CycA, and an unknown protein (Kyme *et al.*, 2005).

Analogously to endothelial cells, also in macrophages *B. henselae*-containing compartments display a delay in the acquisition of lysosomal markers when compared with other endocytosed material (Kyme *et al.*, 2005). The mechanism by which *Bartonella* avoids or delays the fusion of BCVs with phagolytic vesicles to promote its own survival has yet to be unravelled.

**Infection of erythrocytes**

As opposed to nucleated cells, there is no evidence for active membrane transport in erythrocytes. Nevertheless bartonellae can invade erythrocytes of different age (Schulein *et al.*, 2001). Bacterial uptake must therefore be actively triggered by the pathogen. Consistently, energy depletion in bacteria impeded invasion of *B. bacilliformis* into erythrocytes (Walker and Winkler, 1981). One of likely several energy-dependent steps in *B. bacilliformis* uptake into erythrocytes is the initial attachment to the erythrocyte membrane. A block of the proton motive force by N-ethylmaleimide or the inactivation of respiration by KCN indeed reduces bacterial binding to the erythrocyte surface (Benson *et al.*, 1986; Scherer *et al.*, 1993) and other energy-consuming steps may play a role in invasion.

Adherence of bartonellae to the erythrocytic surface

Ahead of bacterial entry, the small extracellular factor deformin (or ‘DF’ for deformation factor) of *B. bacilliformis* or *B. henselae* induces indentations of the erythrocytic membrane in which bacteria might localize prior to their internalization (Mernaugh and Ihler, 1992; Xu *et al.*, 1995; Iwaki-Egawa and Ihler, 1997).

In their capacity to adhere to and invade erythrocytes, bartonellae are confined to their reservoir host. Host specificity of this process is conferred by the T4SS Trw (Seubert *et al.*, 2003; Vayssier-Taussat *et al.*, 2010) which is expressed exclusively by bartonellae of lineage 4 which comprises most of the modern *Bartonella* species (Saenz *et al.*, 2007; Engel *et al.*, 2011). The components of the supramolecular Trw complex that mediate the observed host specificity might be the surface-exposed T4SS plus components TrwJ and TrwL (Vayssier-Taussat *et al.*, 2010).

*B. bacilliformis* and the species of *Bartonella* lineages 2 and 3 which do not bear a Trw, are found to be flagellated instead (Harms and Dehio, 2012). It is thus conceivable to assume that flagella play a prominent role in erythrocyte adherence of these *Bartonella* lineages, but got functionally replaced by the Trw system in *Bartonella* lineage 4. Indeed, a block of flagella by antibodies diminishes the association with erythrocytes (Scherer *et al.*, 1993). Non-motile *B. bacilliformis* do not attach to erythrocytes at all (Walker and Winkler, 1981), yet flagella isolated from *B. bacilliformis* do not bind to erythrocytes. The role of flagella in erythrocytic adherence thus remains controversial.

**Invasion of erythrocytes**

Entry of *Bartonella* into erythrocytes (Cuadra and Takano, 1969; Benson *et al.*, 1986) is an ill-defined process. *In vitro* the actual penetration step of the blood cell membrane by *B. bacilliformis* is observed only several hours after the occurrence of indentations (Mernaugh and Ihler, 1992) and might present a bacterium-induced ‘forced endocytosis’ (Hill *et al.*, 1992). Deformin activity in combination with bacterial motility might favour host membrane fusion events at the neck of the deformin-induced invaginations, which subsequently results in pathogen-loaded vacuolar compartments in the erythrocytic cytoplasm (Benson *et al.*, 1986). However, non-flagellated *Bartonella* species as *B. henselae* and rat-specific *Bartonella tribocorum* also invade erythrocytes (Kordick and Breitschwerdt, 1995; Schulein *et al.*, 2001).

Also essential for erythrocyte penetration is the combined effect of the invasion associated locus proteins A and B (IaA/IaB) (Coleman and Minnick, 2001), which are conserved among all species of the genus (Mitchell and Minnick, 1997; Vayssier-Taussat *et al.*, 2010). Expression of IaA/IaB from *B. bacilliformis* or *B. henselae* in *Escherichia coli* confers a minimal invasiveness for erythrocytes without conferring increased attachment (Mitchell and Minnick, 1995; Minnick *et al.*, 1996). Despite the controversy about the IaB localization, its structure suggests that it is an OMP which might thus interact with the erythrocytic surface (Harms and Dehio, 2012). The importance of IaA in the infection process is less clear. IaA is a (di)nucleoside polyphosphate hydrolase presumably aiding bacterial survival by reducing levels of stress-induced dinucleotides during invasion (Cartwright *et al.*, 1999; Conyers and Bessman, 1999).

For a later intra-erythrocytic stage, the interaction of *B. bacilliformis* with the human erythrocyte membrane proteins actin, spectrin and glycoporphin has been demonstrated, but the role of this interaction in the infection process has not been further characterized (Iwaki-Egawa and Ihler, 1997; Buckles and McGinnis Hill, 2000).
Conclusions and outlook

Figure 1 summarizes our current understanding of cell adhesion and entry processes of bartonellae in endothelial and erythrocytic cells. For the invasion of endothelial and other nucleated cells, the present knowledge is largely limited to findings in *B. henselae*. The phenotypic description of the entry process is slowly being substantiated by mechanistic effects on host and pathogen side.

Prominent is the importance of the VirB/D4-translocated Beps on the pathogen side resulting in actin rearrangements in the host cell. While a few bacterial factors essential for erythrocytic adhesion and invasion have been identified, the cell biological processes underlying erythrocyte infection are still elusive on a molecular level. The structural components on the erythrocytic surface determining host-specific infection via interaction with the Trw system remain to be determined. Further it is open how
Bartonella force entry into erythrocytes in the absence of dedicated endocytic pathways, and how erythrocytic membrane integrity is maintained during this invasion process.

Bartonella are typical stealth pathogens causing chronic infection, which goes along with mitogenic stimulation and immune evasion mechanisms, subjects which are beyond the scope of this review, but were recently summarized elsewhere (Harms and Dehio, 2012; Pullliainen and Dehio, 2012). A future challenge is to integrate the different facets of host interactions mediated by Bartonella into a global model of the infection process. This might require systems-level approaches with suitable in vitro and in vivo infection models in order to define host and pathogen prerequisites for a successful infection.

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