Bartonella and *Brucella*—Weapons and Strategies for Stealth Attack

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Bartonella spp. and *Brucella* spp. are closely related α -proteobacterial pathogens that by distinct stealth-attack strategies cause chronic infections in mammals including humans. Human infections manifest by a broad spectrum of clinical symptoms, ranging from mild to fatal disease. Both pathogens establish intracellular replication niches and subvert diverse pathways of the host's immune system. Several virulence factors allow them to adhere to, invade, proliferate, and persist within various host-cell types. In particular, type IV secretion systems (T4SS) represent essential virulence factors that transfer effector proteins tailored to recruit host components and modulate cellular processes to the benefit of the bacterial intruders. This article puts the remarkable features of these two pathogens into perspective, highlighting the mechanisms they use to hijack signaling and trafficking pathways of the host as the basis for their stealthy infection strategies.

Bartonella spp. and *Brucella* spp. are Gram-negative facultative intracellular bacteria causing bartonellosis and brucellosis, respectively. These infections are typically chronic, ranging from subclinical courses to progressively debilitating and possibly life-threatening diseases. With a few exceptions in the genus *Bartonella* where the host reservoir is human, *Bartonella* spp. and *Brucella* spp. are zoonotic pathogens transmitted from mammals to humans. Wild animals are an important reservoir for the multiple species of both pathogenic genera. However, cats represent the major source for human infection by *Bartonella* (Breitschwerdt and Kor-

dick 2000), whereas human infections by *Brucella* typically originate from livestock (Pappas et al. 2006). Therefore, individuals handling animals or infected material have a higher risk of getting infected either through direct contact or by aerosols. For *Brucella*, persons consuming infested animal products, often milk and its derivatives, are also at risk of getting infected (Pappas et al. 2006).

Via remarkably analogous mechanisms (Batut et al. 2004), *Bartonella* and *Brucella* invade various cell types using (1) cell-surface molecules that mediate host–pathogen interactions, such as adhesion proteins and receptors from

H. Ben-Tekaya et al.

the bacterial and host side, respectively; and (2) type IV secretion systems (T4SS) that translocate bacterial proteins termed effectors (Backert and Meyer 2006; Llosa et al. 2009). Importantly, effectors subvert intracellular trafficking and both innate and acquired immune responses (Roop et al. 2009; Harms and Dehio 2012; von Bargen et al. 2012), thus maneuvering host processes in favor of the pathogen.

BARTONELLA AND BRUCELLA: CLOSELY RELATED PATHOGENS WITH DISTINCT INFECTION SEQUELS

Bartonella spp. and *Brucella* spp. evolved from a common ancestor and belong to the Rhizobiales order of the α -proteobacteria (Gillespie et al. 2011), which includes microbes that co-evolved with animal or plant hosts either as pathogens or as symbionts (Fig. 1). The *Bartonella* genus is composed of 24 species sharing a core genome and subdivided based on phylogenomic analysis in four phylogenetic lineages, lineage 1 being the most ancestral lineage with *Bartonella bacilliformis* as sole species (Saenz et al. 2007; Engel and Dehio 2009; Engel et al. 2011). The *Brucella* genus includes 10 species defined according to their preferential host (Moreno et al. 2002) and divided, based on phylogenomic analysis, in four clades: *Brucella abortus*–*Brucella melitensis*, *Brucella suis*–*Brucella canis*, *Brucella ovis*, and *Brucella ceti* (Wattam et al. 2009), with *Brucella ovis* being a potential common ancestor (Foster et al. 2009).

Each species of the genera *Bartonella* or *Brucella* infects one or few closely related mammals as reservoir host(s) wherein they persist and elicit diverse infection outcomes (Fig. 1). *B. bacilliformis* and *Bartonella quintana* are an exception in having humans as the reservoir host (Maguina et al. 2009). In their mammalian reservoir hosts, *Bartonella* spp. typically cause an intraerythrocytic bacteremia with an often asymptomatic course (Chomel et al. 2009), whereas *Brucella* provoke animal brucellosis, a chronic infection characterized by abortion probably due to the infection of trophoblasts, infertility, or birth of weak infected offspring (Hignett et al. 1966; Samartino and Enright

1993). Animal brucellosis is a significant economic burden in the endemic regions that span from central Asia to South America through the Middle East and the Mediterranean region (Pappas et al. 2006; Roop et al. 2009). Transmission of *Bartonella* is mediated by blood-sucking (hematophagous) arthropod vectors (Billeter et al. 2008; Tsai et al. 2011), whereas for *Brucella*, transmission is direct between mammals via contact with bacteria-rich material (Moreno and Moriyon 2006).

The epidemiology of *Bartonella* and *Brucella* infections as well as the interaction of these bacteria with and persistence within specific host cells suggest some host specificity that determines the selective incidence in certain mammals (Dehio and Sander 1999; Breitschwerdt and Kordick 2000; Tsolis 2002). This host specificity is governed by the intraerythrocytic persistence and the prevalence and ecology of the hematophagous vectors for *Bartonella* (Harms and Dehio 2012) and possibly by effectors translocated via the T4SS and distinctive gene sets localized to islands acquired by phage-mediated integration for *Brucella* (Paulsen et al. 2002; Andersson and Kempf 2004; de Jong and Tsolis 2012). When incidentally transmitted to humans, *Bartonella* and *Brucella* cause chronic infections that are frequently misdiagnosed, misreported, and therefore underestimated (Schwartzman 1996; Velho et al. 2003; Pappas et al. 2006).

Human bartonellosis ranges from the benign self-limiting manifestation of cat scratch disease (CSD) caused by infection with *Bartonella henselae* and possibly *Bartonella clarridgeiae* (Kordick et al. 1997), to a devastating hemolytic anemia caused by *B. bacilliformis*. *B. henselae* spreads in the feline reservoir by cat fleas (*Ctenocephalides felis*) (Chomel et al. 1996) and is transmitted to humans probably via inoculation of contaminated flea feces during a cat scratch or bite (Chomel et al. 2009). Symptoms of CSD are mainly lymphadenopathy (lymph node swelling) and occasionally fatigue and fever (Lamps and Scott 2004). *B. quintana* causes trench fever, a disease with persistent bacteremia that spreads under poor hygiene conditions by body lice (*Pediculus humanus corporis*) and oc-

Figure 1. Phylogeny, T4SS, and epidemiology of *Bartonella* and *Brucella*. At least one type IV secretion system (T4SS; VirB, Vbh, and Trw) is present in all *Bartonella* species except for *Bartonella bacilliformis*. A related VirB T4SS is also present in *Brucella*.

H. Ben-Tekaya et al.

asionally by cat fleas (Rolain et al. 2003). The symptoms are a 5-d cyclic fever, bone pain, and headache (Byam and Lloyd 1920; Ohl and Spach 2000). A sequel of CSD and trench fever is bacillary angiomatosis: an outgrowth of vasoproliferative tumors affecting mainly the skin but also the liver (bacillary peliosis), spleen, bone marrow, eyes, or other organs and leading to extraerythrocytic bacteremia, neuroretinitis, infective endocarditis, and various neurological symptoms such as encephalitis (Maguina et al. 2009). *B. bacilliformis*, which is endemic in the Andes, where the natives show persistent asymptomatic bacteremia and might thus serve as a reservoir for infection (Ricketts 1948), is transmitted by the sand fly (*Lutzomyia verrucarum*), and is the sole *Bartonella* pathogen eliciting a biphasic life-threatening illness called Carrion's disease. During the acute phase of this disease, which is termed Oroya fever and is rarely developed by the natives of the endemic regions, *B. bacilliformis* can infect a majority of the erythrocytes (Maguina et al. 2001), triggering a hemolytic anemia attributable to the hemophagocytosis of the infected erythrocytes by mononuclear phagocytes in spleen, lymph nodes, and liver (Walker and Winkler 1981; Maguina et al. 2009). This results in fever, hepatosplenomegaly (enlargement of the liver and spleen), lymphadenopathy, anemia (Fisman 2000; Janka 2007), and ultimately fatal suffocation or septicemia killing more than 80% of the untreated invalids (Ricketts 1948; Maguina et al. 2009). The second, chronic phase of Carrion's disease, called verruga peruana, is characterized by vasoproliferative skin lesions that clinically resemble bacillary angiomatosis caused by *B. henselae* and *B. quintana* (Maguina et al. 2009).

Human brucellosis or Malta fever is a neglected, debilitating, relapsing, and often travel-related febrile disease. It is endemic in several regions where animal brucellosis is prevalent and is estimated to be the major bacterial zoonosis worldwide. Malta fever is demanding to treat with antibiotics, and there are no vaccines for humans that prevent infection (Memish and Balkhy 2004; Young 2005; Pappas et al. 2006; Ariza et al. 2007; Pappas 2010). It is transmitted by contact with infected animals, most often

goats, sheep, or camels infected with *B. melitensis*, cattle infected with *B. abortus*, or swine infected with *B. suis*. In rare cases, marine mammals infected with *Brucella pinnipedialis* or *B. ceti* (Sohn et al. 2003; Foster et al. 2007; Whatmore et al. 2008), dogs infected with *B. canis*, desert wood rats infected with *Brucella neotomae*, and sheep infected with *B. ovis* are carriers (Corbel 1997). *Brucella inopinata*, isolated from brucellosis and chronic pneumonia patients (Scholz et al. 2010; Tiller et al. 2010), and *Brucella microti*, isolated from vole and foxes (Scholz et al. 2008), are recently uncovered strains whose transmission to humans is unclear. Symptoms of the acute phase of human brucellosis are flu-like periodic fevers with muscle and joint pain followed by lymphadenopathy, hepatosplenomegaly, and development of granulomas at multiple sites of the body (Franco et al. 2007a). Common complications, some of which are fatal if not promptly diagnosed and treated, are arthritis, liver abscess formation, neurobrucellosis, endocarditis, and even abortion in the endemic regions (Khan et al. 2001; Young 2005; Franco et al. 2007b).

BARTONELLA AND BRUCELLA: HOMOLOGOUS VIRULENCE SYSTEMS FOR SNEAKY HOST-CELL INVASION

Bartonella and *Brucella* encode conserved virulence factors that are essential for host-cell invasion as shown by in vitro and in vivo studies (Table 1). These include the VirB T4SS and adhesion molecules.

Machineries Indispensable for Host Interactions

VirB Type IV Secretion Systems: Master Virulence Factors

VirB T4SS are central virulence factors dictating the success of infection. The VirB T4SS of *Bartonella* species (lineages 3 and 4) and *Brucella* species are related but distinct in their subunit composition: VirB2–VirB11 and VirB1–VirB12, respectively. They bridge the bacterial inner membrane, periplasm, and outer mem-

Table 1. Virulence factors of *Bartonella* and *Brucella*

	<i>Bartonella</i>	<i>Brucella</i>
T4SSs		
VirB	Confined to lineages 3 and 4: VirB2–VirB11 and coupling protein VirD4 Essential for infection	VirB1–VirB12 Essential for infection
Vbh	Confined to lineage 2 Putative role in infection	—
Trw	Confined to lineage 4 Mediates erythrocyte invasion Essential for persistence	—
VirB T4SS effectors	Beps: inhibit apoptosis; promote angiogenesis; promote invasome formation in <i>B. henselae</i>	Btp1: modulates microtubules' dynamics and TLR4-mediated signaling CstA: interacts with Sec24A PrpA: elicits T-cell-independent IL-10 secretion RicA: interacts with Rab2-GDP BPE005, BPE123, BPE275, BPE043, BvfA, VceA, VceC, BPE043, BPE152: unclear function
Regulatory systems		
Two-component regulatory systems (TC systems)	BatR/BatS Regulate essential gene transcription (i.e., <i>virB</i>)	BvrR/BvrS Regulate essential gene transcription (i.e., <i>virB</i>)
Quorum-sensing systems	—	VjbR/BlxR Regulate numerous genes (i.e., <i>vceA</i> , <i>vceC</i>)
Adhesion molecules		
Flagella	Confined to lineages 1–3 Mediate motility, erythrocytes' adhesion and invasion Escape recognition by TLR5	Act as T3SS? Essential for virulence
Trimeric autotransporters	BadA, Vomps Essential for infection	BtaE Essential for infection
Hemin-binding proteins	Hbp Essential for growth	OMP25/31
LPS		
Lipid A	Pentaacylated with long fatty acid side chain Inhibits TLR4 proinflammatory response and promotes interaction with DCs	Heptaacylated with long fatty acid side chain Inhibits TLR4 proinflammatory response
Core-polysaccharide	Uncharacterized function(s)	Inhibits complement activation and DC's maturation
O-polysaccharide	Uncharacterized function(s)	Inhibits T-cell activation

brane and extend into extracellular pili. This multiprotein complex functions as a translocation pore through which effector proteins are transferred into host cells, possibly by a piston mechanism (O'Callaghan et al. 1999; Sieira et

al. 2000; Cascales and Christie 2003; Hwang and Gelvin 2004; Christie et al. 2005; Schroder and Dehio 2005; Zygmunt et al. 2006; Saenz et al. 2007; Wallden et al. 2010; Engel et al. 2011; de Jong and Tsolis 2012). As a canonical T4SS, the

H. Ben-Tekaya et al.

Bartonella VirB system comprises an effector-recognition component: the coupling protein VirD4 (Schroder and Dehio 2005), which has no homolog in *Brucella*, and which instead expresses the unique VirB12 (O'Callaghan et al. 1999). The genes of the VirB T4SSs are encoded as operons (Berger and Christie 1994), which either locate together with effector genes (*Bartonella* lineage 4) or not (*Bartonella* lineage 3 and *Brucella*) (Schulein et al. 2005; Engel et al. 2011; de Jong and Tsois 2012).

Because biosynthesis of the multiprotein VirB T4SS and its translocated effectors is costly for the bacterial cells, expression is tightly regulated on the transcriptional level. For instance, in *B. henselae*, the *virB* operon and the genes encoding the coupling protein and the translocated effectors are maximally induced at physiological pH (Quebatte et al. 2010), whereas the *virB* operon expression in virulent *Brucella* strains is maximally induced in acidic milieu reminiscent of the lumen of *Brucella*-containing vacuoles (BCVs) (Boschiroli et al. 2002; Rouot et al. 2003).

The well-studied family of bacterial two-component regulatory systems (TC) is composed of pairs of a histidine kinase and an interacting response regulator. The surface-exposed histidine kinase becomes autophosphorylated upon sensing of an extracellular stimulus. The phosphate group is subsequently transferred to the response regulator, which, in turn, triggers transcriptional changes (Casino et al. 2010). The VirB T4SS of *Bartonella* and *Brucella* are transcriptionally controlled by homologous TCs, that is, BatR/BatS in *Bartonella* is most active at pH7.4 (Quebatte et al. 2010), and BvrR/BvrS in *Brucella* is most active at low pH (Martinez-Nunez et al. 2010; Viadas et al. 2010; Lacerda et al. 2013).

In *Brucella*, the loci encoding the VirB T4SS and other virulence factors like flagella and outer membrane proteins are controlled by additional regulatory systems such as the quorum-sensing LuxR-type regulators VjbR and BlxR, the starvation-sensing stringent response regulator Rsh (RelA/SpoT homolog), and small regulatory RNAs (sRNA) (Roop et al. 2009; von Bargen et al. 2012).

The VirB-translocated *Bartonella* effector proteins (Bep; named BepA–BepG in *B. henselae*) display a composite domain architecture characterized by an amino-terminal effector domain and a carboxy-terminal secretion signal (Schmid et al. 2004; Dehio 2005; Schulein et al. 2005; Engel et al. 2011). The carboxy-terminal secretion signal is bipartite and composed of a Bep intracellular delivery domain (BID) of approximately 140 amino acids and a short, nonconserved, positively charged tail sequence (Schulein et al. 2005). Besides mediating T4SS-dependent translocation into host cells, some BID domains can also directly modulate host-cellular pathways. For instance, the BID domain of BepA promotes apoptosis inhibition and angiogenesis (Schmid et al. 2006; Scheidegger et al. 2009). The amino-terminal effector domain is best exemplified by the FIC (filamentation induced by cyclic AMP) domain, which is present in some effectors of lineage 3 and most effectors of lineage 4 (Engel et al. 2011). This domain mediates AMPylation, the covalent transfer on an adenyl monophosphate (AMP) moiety onto the hydroxyl side chains of host target proteins like the small GTPases of the Rho family (Palanivelu et al. 2011; Engel et al. 2012). Effectors lacking an FIC domain show duplicated BID domains and/or amino-terminal tyrosine motifs that get phosphorylated inside host cells by Src family kinase(s) (Schulein et al. 2005; Selbach et al. 2009).

The few described putative *Brucella* effectors have unclear functions and only one common feature: a nonconserved carboxy-terminal positively charged amino acid sequence considered essential for VirB T4SS-dependent translocation (von Bargen et al. 2012). Examples are (1) VceA and VceC, which belong to the VjbR regulon, consequently having their expression coregulated with the VirB T4SS (de Jong et al. 2008); (2) BPE123, whose translocation additionally depends on a Sec-secretion signal at the amino terminus; and (3) BPE005, BPE275, and BPE043 (Table 1) (Marchesini et al. 2011). Other putative effectors with indefinite positively charged amino acid carboxy-terminal sequences are (1) RicA, which is the sole effector having an assigned host partner, the guanosine di-

phosphate (GDP)-bound form of the small GTPase Rab2 (de Barsey et al. 2011); (2) Btp1 (TcpB in *B. melitensis*), which regulates innate immune responses via its Toll/interleukin-1 receptor (TIR) domain (Cirl et al. 2008; Salcedo et al. 2008) and possibly modulates microtubule dynamics (Radhakrishnan et al. 2011); (3) PrpA, a proline-racemase family member, which regulates immune responses (Spera et al. 2006); (4) CstA (conserved Sec24A-targeted protein A), which controls *Brucella* intracellular trafficking (de Barsey et al. 2012); and (5) BvfA, which is required for *Brucella* intracellular replication (Lavigne et al. 2005).

Instead of the VirB T4SS, some *Bartonella* species encode a VirB homologous (Vbh) T4SS (Fig. 1) (Dehio 2008). Vbh was likely the first T4SS acquired by the *Bartonella* lineage and is present in all species of lineage 2. It is believed to have been functionally replaced within lineages 3 and 4 by the subsequently acquired VirB T4SS (some lineage 4 species still encode non-functional remnants of Vbh) (Fig. 1). Vbh components display protein sequence identity of 40%–80% with their VirB homologs, suggesting that the two systems have only recently diverged from a common ancestor (Saenz et al. 2007). Further to the Vbh system, the *vbh* locus encodes a putative effector protein with the canonical FIC–BID architecture of the VirB-translocated Beps, advocating a potential role in virulence (Engel et al. 2011).

Adhesion Molecules: Key for Host–Pathogen Interaction

Adhesion molecules are often multifunctional membrane-exposed proteins and complexes necessary for *Bartonella* attachment to host cells (O'Rourke et al. 2011). Homologs of some of these adhesins are also present in *Brucella*, although their function is less scrutinized (von Bargen et al. 2012). They interact with host extracellular matrix (ECM) components (e.g., fibronectin, collagen) and receptors (e.g., integrins for *Bartonella* and sialic acid-bound receptors for *Brucella*) (Fig. 2). Such molecules include the Trw T4SS (for *Bartonella*), flagella, autotransporter adhesins, the ATP-binding cas-

sette (ABC) systems, the hemin-binding proteins, and further outer membrane proteins (OMP) such as in *Bartonella* OMP89 and OMP43, a homolog of *Brucella* OMP2b (Dabo et al. 2006).

Trw T4SS. Trw is a T4SS that functionally diversified to an adhesion complex crucial for persistence of all *Bartonella* lineage 4 species (Schroder and Dehio 2005). The *trw* locus comprises an operon encoding a typical T4SS but as a particularity displays multiple tandem-repeated gene copies encoding variant forms of the extracellular pilus component. The *Bartonella* Trw T4SS is closely related to the canonical Trw conjugation system of the conjugative plasmid R388 and was thus probably gained by horizontal gene transfer (Seubert et al. 2003). The Trw T4SS mediates erythrocyte adhesion of lineage 4 species in a host-specific manner. The subsequent invasion process requires the invasion-associated locus, containing IalA and IalB, and/or the IalAB-independent erythrocyte invasion locus, Iiv (Seubert et al. 2003; Dehio 2004; Schroder and Dehio 2005; Saenz et al. 2007; Vayssier-Taussat et al. 2010; Deng et al. 2012).

Flagella. Flagella are bacterial nanomachines that mediate motility. The filament of flagella that is composed of a single protein, flagellin, is assembled by the basal body by a pathway homologous to the type 3 secretion system (T3SS) (Hueck 1998; Kirov 2003). In motile *Bartonella* species (lineages 1–3, except *Bartonella bovis*), flagella are considered to mediate erythrocyte internalization as a mechanical force or as adhesion molecules, as shown exemplarily for *B. bacilliformis* (Walker and Winkler 1981; Merinaugh and Ihler 1992). *Bartonella* species of lineage 4 that are nonmotile owing to the loss of flagella do mediate erythrocyte interaction via the Trw T4SS, which suggests that Trw has functionally replaced flagella for establishing interaction with erythrocytes (Dehio 2008). *Brucella* are nonmotile bacteria, yet flagella genes are essential for their virulence (Lestrade et al. 2003). Debatably, the basal body may function as a T3SS rather than establishing a motile nanomachine (Fretin et al. 2005).

Autotransporter Adhesins. This family mediates adhesion to nucleated cells and interac-

H. Ben-Tekaya et al.

Figure 2. Intracellular life of *Bartonella* and *Brucella*. *Bartonella* and *Brucella* invade cells in vacuolar structures (BCV) or, in the case of *B. henselae*, as an aggregate in invasomes. Once internalized, these structures interact with specific host components and compartments subverting their function. AIM, Autophagy initiation markers; COPII, coatomer protein II; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; ERES, ER exit sites; MVB, multivesicular bodies; T4SS, type IV secretion system.

tions with ECM, belongs to the type 5 secretion system (T5SS), and comprises two-partner systems (TPSs), classical monomeric autotransporter adhesins (CAAs), and trimeric autotransporter adhesins (TAAs). TPS filamentous haemagglutinins in *Bartonella* are putative adhesins encoded by multiple genes, indicating functional redundancy (O'Rourke et al. 2011). CAAs and TAAs are characterized by an amino-terminal signal peptide, a passenger domain, and a carboxy-terminal translocation domain (van Ulsen 2011). CAAs important for infection are in *Brucella* are OmaA (outer membrane autotransporter A) (Bandara et al. 2005) and BmaC (Posadas et al. 2012), and in *Bartonella* are Arp (acidic repeat protein) (Litwin et al. 2007) and Cfa (cAMP-like factor autotransporter) (Litwin and Johnson 2005), two members of the Iba (inducible *Bartonella* autotransporter) group whose expression is induced during infection. TAAs are essential for virulence and have a head–stalk–anchor structure forming modular length–adaptable trimerized cell surface-exposed filaments (Linke et al. 2006). Examples are BtaE in *Brucella* (Ruiz-Ranwez et al. 2013) and in *Bartonella*, *B. henselae* adhesin A (BadA) and the homologous *B. quintana* variably expressed outer membrane proteins (Vomps) (Zhang et al. 2004; Muller et al. 2011). *Bartonella* TAAs additionally induce angiogenesis and serum resistance (O'Rourke et al. 2011). Interestingly, BadA expression on the bacterial surface masks the activity of the VirB T4SS (Lu et al. 2013), but these two major virulence factors are differentially regulated on the transcriptional level via the TC system BatS/BatR, indicating that the two act at different stages of the infection process.

ATP-Binding Cassette Transporter Systems.

ABC transporters are a family of transport systems that hydrolyze adenosine triphosphate (ATP) (Davidson and Chen 2004; Davidson et al. 2008). Examples are the heme HutABC/HmuV (Parrow et al. 2009) and iron FatBCD and Sit/YfeABCd (Battisti et al. 2006) uptake systems as well as the *ugp*-encoded glycerol 3-phosphate importers (Saenz et al. 2007) in *Bartonella*. Such an importer is in *Brucella* surface protein 41 (SP41), which probably interacts with

sialylated host receptors (Castaneda-Roldan et al. 2006).

Hemin-Binding Proteins. Hemin-binding proteins (Hbps in *Bartonella* and OMP25/31 in *Brucella*) constitute a family of porin-like outer membrane proteins that bind hemin (Battisti et al. 2006; Dabo et al. 2006; Delpino et al. 2006; Caro-Hernandez et al. 2007). Hemin is essential for *Bartonella* growth (Sander et al. 2000), rendering these bacteria the most hemin-dependent microbes.

Strategies for Host Invasion

Lessons Learned from In Vitro Studies

Our understating of cellular invasion processes during *Bartonella* and *Brucella* infections is greatly based on in vitro infection models. These comprise primary cells and immortalized cell lines: professional phagocytes (e.g., macrophages, monocytes, dendritic cells), nonprofessional phagocytic cells (e.g., epithelial cells, fibroblasts, endothelial cells), erythrocytes for *Bartonella*, and trophoblasts for *Brucella* (Anderson et al. 1986; Benson et al. 1986; Kordick and Breitschwerdt 1995; Roop et al. 2009; Harms and Dehio 2012; von Bargen et al. 2012; Salcedo et al. 2013). Entry into cells leads to the establishment of BCVs (*Bartonella*/*Brucella*-containing vacuoles), structures formed when *Bartonella* and *Brucella* are taken up as single bacteria or as small bacterial clumps (Moreno and Gorvel 2004; Eicher and Dehio 2012). Some *Bartonella* species additionally enter host cells in aggregates of hundreds of bacteria, leading to the establishment of an invasion structure known as the invasome (Fig. 2) (Dehio et al. 1997).

Uptake into BCVs or Invasomes: Alternative Routes of Bacterial Entry Exemplified by *B. henselae*

At early stages of *B. henselae* interaction with host cells (also at subsequent stages in the case of VirB-T4SS deficiency), individual bacteria trigger a zipper-like entry mechanism involving Rho, Rac, Cdc42, tyrosine phosphorylation, and/or $\alpha 5 \beta 1$ -integrin (Fig. 2) (Hill et al. 1992; Brouqui and Raoult 1996; Williams-Bouyer and

H. Ben-Tekaya et al.

Hill 1999; Truttmann et al. 2011a). Once internalized, BCVs induce focal adhesion-anchored stress fibers and possibly replicate in the juxta-nuclear region, where they presumably interact with the Golgi apparatus (Kempf et al. 2000; Verma et al. 2000, 2001; Verma and Ihler 2002; Kyme et al. 2005).

Once the VirB T4SS-translocated effectors reach a critical intracellular concentration, the formation of BCVs by individual bacterial uptake is blocked. Bacteria then accumulate on the cell surface, which is followed by bacterial aggregation by host-cell-driven surface transport, and subsequent internalization into a structure termed the invasome. This unique bacterial uptake structure is reminiscent of bacterial aggregates in bacillary angiomatosis lesions (Dehio et al. 1997; Dehio 2003). Invasome formation is triggered by VirB-translocated effectors via redundant pathways, which depend on either BepG or the combination of BepC and BepF. In each case, this results in the formation of a compact F-actin ring composed of condensed stress fibers, and membrane protrusions on the host-cell surface (Fig. 2) (Rhomberg et al. 2009; Truttmann et al. 2011b). This process is dependent on integrin- β 1 (ITGB1) binding and activation via Talin-1 inside-out signaling and on integrin- β 1-mediated outside-in signaling by focal adhesion kinase (FAK), Src, vinculin, and paxilin (Truttmann et al. 2011b). Invasome rearrangement of the F-actin cytoskeleton involves Arp2/3-dependent cortical F-actin polymerization by Rac1/Scar1/WAVE, Cdc42/WASP pathways, and additionally by cofilin-1 in the case of BepC and BepF (Rhomberg et al. 2009; Truttmann et al. 2011b,c).

Uptake of *Brucella* in BCVs

Contrary to *Bartonella*, the VirB T4SS of *Brucella* is not expressed during bacterial uptake and thus does not contribute to this process. Entry is mediated by surface-exposed bacterial and host membrane complexes at cholesterol- and glycosylphosphoinositide (GPI)-rich host-cell membrane domains (lipid rafts) (Pizarro-Cerda et al. 1998; Watarai et al. 2002, 2003; Kim et al. 2004; Nakato et al. 2012) in a phospho-

inositide (PI)-3 kinase, actin, ezrin, Rho, Rac, Cdc42, and/or Toll-like receptor (TLR)4-dependent manner (Fig. 2) (Guzman-Verri et al. 2001; Chaves-Olarte et al. 2002; Watanabe et al. 2009). Once inside cells, BCVs' lipid raft organization is disrupted by the bacterial membrane periplasm-enriched *Brucella* virulence factor β -1,2 cyclic glucan, which promotes acidification and development of autophagosome properties (Pizarro-Cerda et al. 1998; Ugalde 1999; Arenas et al. 2000; Arellano-Reynoso et al. 2005; Starr et al. 2008). Acidification requires the recruitment of early endosome (Rab5, EEA1), late endosome (Rab7), and lysosome (Lamp1) markers, and is necessary for the activation of virulence factors such as *virB* genes (Porte et al. 1999; Boschioli et al. 2002) essential for interactions with early secretory pathway components (Sar1/Sec24A/COPII, Rab2, and its partners) and endoplasmic reticulum-derived ribosome-rich structures (Comerci et al. 2001; Celli et al. 2003, 2005; Fugier et al. 2009) where *Brucella* replicate and persist before egressing in an autophagy initiation molecules and PI3 kinase-dependent manner (Roop et al. 2009; Starr et al. 2012).

Uptake in Erythrocytes: A Route to Persistence

A hallmark of *Bartonella* infection is invasion of erythrocytes using the Trw T4SS or flagella as adhesion factors (Harms and Dehio 2012). These processes depend on host-cell-surface glycosyl moieties, actin, spectrin, band 3 protein, glycophorin A, and/or glycophorin B (Walker and Winkler 1981; Iwaki-Egawa and Ihler 1997; Buckles and McGinnis Hill 2000; Chomel et al. 2009; Vayssier-Taussat et al. 2010; Deng et al. 2012). A potential amphiphilic small molecule, deformin, deforms the erythrocytes' membrane initiating "forced endocytosis" of *Bartonella* in vacuoles, where they shortly replicate but persist (Benson et al. 1986; Iwaki-Egawa and Ihler 1997; Derrick and Ihler 2001).

Lessons Learned from In Vivo Studies

Bartonella and *Brucella* infection spreads from the site of inoculation to distinct immune-priv-

ileged persistence niches that are essential for transmission to other hosts. During dissemination to the persistence niches, these pathogens may colonize other host niches. The identities of the primary niches remain elusive, although dendritic cells, and in the case of *Bartonella* also endothelial cells, have been proposed to represent such primary niches (Harms and Dehio 2012; von Bargen et al. 2012). The persistence niches are erythrocytes for *Bartonella* (Schulein and Dehio 2002) and the reticuloendothelial system for *Brucella* (Ficht 2003). Dissecting the sequence of events from the primary to the persistence niches is fundamental to understand the progression from an acute infection to a chronic disease. For this, clinical data and mostly laboratory in vivo models, in which different infection stages can be monitored, are essential. A widely used *Brucella* in vivo model is mouse, which largely recapitulates natural animal brucellosis (Silva et al. 2011). Several *Bartonella* species also have a small animal model reflecting infection either (1) in the reservoir host (Fig. 1), for example, rat for *Bartonella tribocorum* (Schulein and Dehio 2002) and mouse for *Bartonella birtlesii* and *Bartonella grahamii* (Koesling et al. 2001; Marignac et al. 2010); or (2) in a nonreservoir host as an incidental infection, for example, the lymphadenopathy mouse model for *B. henselae* (Kunz et al. 2008), mirroring human infection by zoonotic species (in this case, CSD). In vivo models also proved to be a significant tool in understanding immune responses during the course of infection.

BARTONELLA AND BRUCELLA: ANALOGOUS VIRULENCE FACTORS FOR DIVERSE APPROACHES TO MODULATE IMMUNE RESPONSES

Bartonella and *Brucella* possess conserved virulence factors essential for chronic infection. These include LPS and for some species flagella, which promote survival for longer periods in macrophages, interfere with dendritic cell (DC) maturation, and modulate immunity (Kohler et al. 2003; Roop et al. 2004; Dehio et al. 2012).

LPS Modifications: Proficiency to Escape Immune Responses

Lipopolysaccharides (LPSs) are conserved sheaths of Gram-negative bacteria composed of an endotoxin component lipid moiety (lipid A) that provides membrane anchoring, a core, and an extracellular polysaccharide chain (O-polysaccharide). They are pathogen-associated molecular pattern (PAMP), eliciting an immune response. However, *Bartonella* and *Brucella* LPSs have unique structural modifications that fail to display endotoxin activity (Table 1) (Moreno et al. 1981; Rasool et al. 1992; Matera et al. 2008). Similar chemical modifications aiming at detoxifying LPSs and thus dampening the immune response are used by other Gram-negative bacteria such as *Helicobacter pylori* (Miller et al. 2005).

Bartonella and *Brucella* lipid A structure is characterized by a short glycosyl backbone (diaminoglucose), a pentaacylation for *Bartonella* and a heptaacylation for *Brucella*, as well as a very long fatty acid side chain (Iriarte et al. 2004; Zahringer et al. 2004; Lapaque et al. 2006; Matera et al. 2008). This lipid A is poorly recognized by TLR4, thereby impeding a broad inflammatory response (Miller et al. 2005). *B. quintana* LPS is further a potent antagonist of TLR4 signaling (Popa et al. 2007).

Depending on the presence or absence of the O-polysaccharide, *Brucella* strains are, respectively, smooth human-infective or rough (Haag et al. 2010). *Brucella* O-polysaccharide inhibits T-cell activation, probably via sequestration of MHCII in large macromolecules in infected macrophages (Moreno et al. 1981; Forestier et al. 1999, 2000; Barquero-Calvo et al. 2007).

Brucella core polysaccharide inhibits DC maturation, TLR4–MD2 recognition, and complement activation, thus complement-mediated bacterial lysis (Conde-Alvarez et al. 2012). DC maturation is also inhibited by the *Brucella* T4SS effector Btp1, which, owing to its TIR domain, mimics host TLR signaling, specifically dampening TLR2 and TLR4 signaling and subsequent NF- κ B activation. The Btp1 TIR domain mediates interaction with the death domain of Myd88 (myeloid differentiation factor

H. Ben-Tekaya et al.

88) and/or TIRAP/MAL (TIR-associated protein), leading to TIRAP/MAL ubiquitination, then degradation (Cirl et al. 2008; Salcedo et al. 2008; Radhakrishnan et al. 2009; Sengupta et al. 2010; Chaudhary et al. 2012). Conversely, the glucose polymer β 1,2-cyclic glucan activates DC in a TLR4 Myd88-dependent manner, thus counteracting Btp1-mediated inhibition of DC maturation (Martirosyan et al. 2012). Whether *Bartonella* has a similar DC maturation fine-tuning as *Brucella* is unclear, but *Bartonella* is hitherto known to stimulate DC maturation in a TLR2-dependent manner (Vermi et al. 2006; Pulliainen and Dehio 2012).

Flagellin Mutations: Adeptness to Escape Immune Responses

The conserved amino-terminal D1 domain of flagellin that mediates flagella assembly is also detected by TLR5, thereby triggering pro-inflammatory responses (Ramos et al. 2004). However, the flagellated *Bartonella* species and also a few other pathogens like *H. pylori* fail to elicit such immune responses thanks to amino acid changes in the D1 domain that dampen recognition by TLR5 and subsequent NF- κ B activation. These flagellin mutations can interfere with flagella function and thus are always associated with compensatory mutations that preserve motility (Andersen-Nissen et al. 2005).

Additional Strategies to Subvert Immune Responses

Besides manipulating DC maturation and consequently T-cell-mediated immune responses, *Bartonella* and *Brucella* can also control T-cell-independent immune responses. For instance, the *Brucella* effector PrpA elicits T-cell-independent B-lymphocyte polyclonal activation and anti-inflammatory IL-10 cytokine secretion (Spera et al. 2006), which is also induced by an unknown *Bartonella* component (Matera et al. 2003, 2008). It is thus likely that both *Bartonella* and *Brucella* induce a transient and partial immune suppression to establish chronic infection (Couper et al. 2008; Harms and Dehio 2012).

Immune suppression is apparently also required for vasoproliferative tumor-like lesion formation during bacillary angiomatosis, a clinical manifestation caused by *B. henselae* or *B. quintana* infection in immunocompromised patients (Maguina et al. 2009). These lesions are characterized by small vessels lined with endothelial cells and are an amalgam of bacteria, polymorphonuclear neutrophils, macrophages, and endothelial cells, the latter two being effective producers of angiogenic factors (Cotell and Noskin 1994; Kostianovsky and Greco 1994; Manders 1996; Qian and Pollard 2010). In these lesions, *Bartonella* induces (1) autocrine angiogenesis in an angiopoietin-2, IL-8, ICAM-1, and/or Bcl-2-related protein A1-dependent manner (Cerimele et al. 2003; McCord et al. 2006); and/or (2) a VirB T4SS-dependent or -independent paracrine angiogenic loop, thus stimulating the secretion of the potent angiogenic stimulus, vascular endothelial growth factor (VEGF) (Kempf et al. 2001; Resto-Ruiz et al. 2002; Schulte et al. 2006). The VirB T4SS-dependent angiogenesis in *B. henselae* is the net effect of pro- and anti-angiogenic activities of BepA/BepD and BepG, respectively (Scheidegger et al. 2009). In a G α s-dependent manner, BepA binds adenylyl cyclase, consequently elevating cAMP levels and probably inhibiting NF- κ B-dependent caspase activation and DNA fragmentation, two hallmarks of apoptosis (Kirby and Nekorchuk 2002; Schmid et al. 2006; Pulliainen et al. 2012). Similar to *Bartonella*, apoptosis suppression is a characteristic feature of *Brucella* infection. It is T4SS- and LPS-dependent and the result of (1) transcriptional regulation of pro- and anti-apoptotic genes such as bcl-2 family A1 and caspase-3, and (2) inhibition of mitochondrial cytochrome *c* release and reactive oxygen species, thus dampening caspase activation (Gross et al. 2000; Eskra et al. 2003; Tolomeo et al. 2003; He et al. 2006).

CONCLUDING REMARKS

Pathogens of the closely related α -proteobacterial genera *Bartonella* and *Brucella* encode, on the one hand, homologous virulence devices and factors (i.e., VirB T4SS, LPS) and, on the other

hand, specific effectors and strategies to invade, proliferate, and persist into host cells and organisms and thus engender distinctive chronic, occasionally fatal infections. Further investigation of virulence factors, deciphering their functions, and elucidating the host interaction partners and mechanisms involved in vitro and in vivo as well as in the global context of infection will be crucial to unveil the causes and processes of the persisting zoonosis. Such a goal requires concerted approaches that integrate basic sciences, veterinary sciences, and clinical medicine. This endeavor would improve diagnostics and therapeutics in animals and humans, allow the generation of animal and human vaccines, and eventually minimize outbreaks and eradicate the disease in the sporadic and endemic regions, respectively.

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H. Ben-Tekaya et al.

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