Chapter 4
Adhesins of *Bartonella* spp.

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Abstract Adhesion to host cells represents the first step in the infection process and one of the decisive features in the pathogenicity of *Bartonella spp. B. henselae* and *B. quintana* are considered to be the most important human pathogenic species, responsible for cat scratch disease, bacillary angiomatosis, trench fever and other diseases. The ability to cause vasculoproliferative disorders and intraerythrocytic bacteraemia are unique features of the genus *Bartonella*. Consequently, the interaction with endothelial cells and erythrocytes is a focus in *Bartonella* research. The genus harbours a variety of trimeric autotransporter adhesins (TAAs) such as the *Bartonella* adhesin A (BadA) of *B. henselae* and the variably expressed outer-membrane proteins (Vomps) of *B. quintana,* which display remarkable variations in length and modular construction. These adhesins mediate many of the biologically-important properties of *Bartonella* spp. such as adherence to endothelial cells and extracellular matrix proteins and induction of angiogenic gene programming. There is also significant evidence that the laterally acquired Trw-conjugation systems of *Bartonella* spp. mediate host-specific adherence to erythrocytes. Other potential adhesins are the filamentous haemagglutinins and several outer membrane proteins. The exact molecular functions of these adhesins and their interplay with other pathogenicity factors (e.g., the VirB/D4 type 4 secretion system) need to be analysed in detail to understand how these pathogens adapt to their mammalian hosts.

4.1 Introduction

Reports of infections with *B. bacilliformis* date back to the Inca period (Schultz, 1968) and *B. quintana* has been identified in human tissue traced as far back as 4,000 years. *B. quintana* was also detected in the remains of soldiers from Napoleon's

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Grand Army found in Vilinius, Lithuania (Raoult et al., 2006a) and Kassel, Hesse, Germany (von Grumbkow and Hummel, unpublished). More recently, *B. henselae* and *B. quintana* were identified as the cause of bacillary angiomatosis (Relman et al., 1990, 1991). To date, over 20 different *Bartonella* species have been identified (see Table 4.1) and the list of *Bartonella* species and corresponding hosts is growing steadily. The human pathogens *B. henselae* and *B. quintana* and the rat pathogen *B. tribocorum* have been investigated the most. Their pathogenicity and infection biology have been examined in greater detail and the genomes of these species have been sequenced (Alsmark et al., 2004; Saenz et al., 2007).

4.1.1 Cat Scratch Disease

Cat scratch disease (CSD) occurs after transmission of *B. henselae* (or sometimes *B. clarridgeiae*) by bites or scratches of infected cats. After an incubation time of 2–3 weeks, patients suffer from a unilateral lymphadenitis in the lymph draining region near the inoculation site. Immune competent individuals (mainly children) develop CSD which is primarily of self-limiting nature and such patients are usually not treated with antibiotics (Anderson and Neuman, 1997). Additional signs of CSD are headache, fever or splenomegaly. Rarely, so called "Parinaud's syndrome" (oculo-glandular involvement) may complicate the course of infection.

Cats are known as the standard reservoir host for *B. henselae* (Rolain et al., 2003). Nevertheless, a case of human osteomyelits was reported following a dog scratch (Keret et al., 1998) indicating that dogs may also serve as a reservoir for the pathogen.

There are indications for a potential role of ticks in the transmission of *B. henselae* to humans. The prevalence of *B. henselae* DNA in *Ixodes pacificus* (North America) and *I. persulcatus* (Eastern Europe) ticks, respectively (Chang et al., 2001; Hercik et al., 2007; Rar et al., 2005) and in *I. ricinus* or *Dermacentor* ticks (Central Europe) (Sanogo et al., 2003; Podsiadly et al., 2007; Angelakis et al., 2010) indicates that these arthropods may represent a newly emerged vector class. We found *B. henselae* DNA to be present in up to ~40% of tick populations in Europe (Dietrich et al., 2010). DNA of various *Bartonella* spp. was further detected in biting flies, keds and mites (Billeter et al., 2008). Recently, ticks (*I. ricinus*) were experimentally infected with *B. henselae*. A subsequent inoculation of cats with salivary glands of these ticks caused a *B. henselae* bacteraemia (Cotte et al., 2008), further strengthening the plausibility of ticks as a means of infection.

Further functional analysis of the natural course of CSD is hampered by the lack of an appropriate animal model. The subcutaneous infection of C57BL/6 or BALB/c mice appears to represent the most promising infection model: mice developed a massive lymphadenitis strongly resembling human CSD and the presence of an enhanced B-lymphocyte proliferation can be observed in both human patients and in experimentally infected mice (Kunz et al., 2008).

Table 4.1 *Bartonella* spp.: reservoirs, vectors, human diseases (modified from Dehio (2005) and Kaiser et al. (2011))

Bartonella spp.	Reservoir	Vector	Human diseases
Human-specific spp.:			
B. bacilliformis	Human	Sandfly	Carrión's disease: Oroya fever, verruga peruana
B. quintana	Human, [dog? (Kelly et al., 2006)]	Body louse [cat flea, ticks (Rolain et al., 2003)]	Trench fever, endocarditis, bacillary angiomatosis
Zoonotic spp.:			
B. alsatica	Rabbit	Unknown	Endocarditis, lymphadenitis (Raoult et al., 2006b)
B. clarridgeiae	Cat	Cat flea	Cat scratch disease
B. elizabethae	Rat	Unknown	Endocarditis, neuroretinitis
B. grahamii	Mouse, vole	Rodent flea (Bown et al., 2004)	Neuroretinitis
B. henselae	Cat, dog (Morales et al., 2007; Keret et al., 1998)	Cat flea (ticks?)	Cat scratch disease, bacillary angiomatosis, endocarditis, neuroretinitis, bacteraemia
B. koehlerae	Cat	Unknown	Endocarditis
B. rochalimae	Foxes, raccoons, coyotes (Henn et al., 2009)	Fleas (Henn et al., 2009)	Bacteraemia, fever (Eremeeva et al., 2007)
B. tamiae	Rats (?)	Mites (?)	Bacteraemia, fever (Kosoy et al., 2008)
B. vinsonii subsp. *arupensis*	Mouse	Ticks (?)	Bacteraemia, fever, endocarditis (?)
B. vinsonii subsp. *berkhoffii*	Dog	Ticks (?)	Endocarditis
B. washoensis	Ground squirrel	Unknown	Myocarditis, endocarditis (?)
Animal-specific spp.:			
B. birtlesii	Mouse	Unknown	Unknown
B. bovis (= B. weissii)	Cattle, cat	Unknown	Unknown
B. capreoli	Roe deer	Unknown	Unknown
B. chomelii	Cattle	Unknown	Unknown
B. doshiae	Vole	Unknown	Unknown

Table 4.1 (continued)

Bartonella spp.	Reservoir	Vector	Human diseases
B. peromysci	Deer, mouse	Unknown	Unknown
B. phoceensis	Rat	Unknown	Unknown
B. rattimassiliensis	Rat	Unknown	Unknown
B. schoenbuchensis	Roe deer	Unknown	Unknown
B. talpae	Vole	Unknown	Unknown
B. taylorii	Mouse, vole	Rodent flea (Bown et al., 2004)	Unknown
B. tribocorum	Rat	Unknown	Unknown
B. vinsonii subsp. *vinsonii*	Vole	Unknown	Unknown

4.1.2 Carrión's Disease

Carrión's disease is characterized by a biphasic course of infection consisting of a primary bacteremic phase ("Oroya fever") and a subsequent chronic vasculoproliferative condition ("Verruga peruana"). The infection is caused by *B. bacilliformis* and is clinically characterized by an intraerythrocytic bacteraemia often followed by fatal haemolytic anaemia (mortalilty: ~40–90%). The second chronic phase of infection (Verruga peruana) leads to vascular tumours that arise from the chronic colonization of endothelial cells with *B. bacilliformis* (Schultz, 1968). Experimentally, it has been demonstrated that *B. bacilliformis* triggers the proliferation of endothelial cells in vitro. This result has been reproduced in vivo by implanting *B. bacilliformis*-extract containing sponge discs in rats and observing the resulting neoangiogenic processes (Garcia et al., 1990). Humans seem to be the only reservoir host for this pathogen and transmission between humans occurs through sandflies (*Phlebotomus verrucarum*). The disease is endemic in the Andes (Peru, South America).

4.1.3 Trench Fever

B. quintana is the causative agent of Trench fever. Reports describing this disease date back to the time of the Napoleonic military campaign in Russia (Raoult et al., 2006a) and the disease also emerged during the First and Second World Wars (Anderson and Neuman, 1997). Blood sucking body lice (*Pediculus humanus*) transmitted the pathogen between soldiers living under the poor hygienic conditions common in trench warfare. At the end of the twentieth century, Trench fever re-emerged among homeless patients in Seattle (USA) and Marseille (France) often suffering from pediculosis (louse infestation) (Spach et al., 1995; Brouqui et al., 1999). Humans were long thought to be the exclusive host for *B. quintana*. However, it has been recently described that dogs represent a further mammalian reservoir (Kelly et al., 2006).

Following initial infection, Trench fever is characterized by a sudden onset of fever followed by subsequent periodical relapses ("five-day-fever") which are paralleled by an intraerythrocytic bacteraemia (Foucault et al., 2004). *B. quintana* was detected in erythroblasts residing in the bone marrow and these cells may serve as a possible niche for a persistent infection from where the pathogen can re-enter the bloodstream (Rolain et al., 2003a). There is also evidence that human bone marrow represents a possible niche for *B. henselae* (Mändle et al., 2005).

The *B. tribocorum* rat infection model has proved a reliable in vivo model, mimicking the course of *B. quintana* infections in humans (Schülein et al., 2001). After an intravenous infection, the pathogen is rapidly cleared from the bloodstream before a periodic relapsing bacteraemia occurs. Using a signature-tagged mutagenesis approach, the underlying pathogenicity mechanisms were elucidated in greater detail revealing a broad variety of essential pathogenicity factors [e.g., genes of VirB/D4 T4SS and others (Saenz et al., 2007)] and some of these genes are also

crucial for the intracellular persistence in endothelial cells and macrophages (Kyme et al., 2005). BadA-deficient strains of *B. tribocorum* (Saenz et al., 2007) or strains of *B. quintana* lacking the variably expressed outer-membrane proteins (Vomps) (Mackichan et al., 2008) were non-bacteraemic in the rat and macaque infection models, respectively.

4.1.4 Bacillary Angiomatosis and Peliosis Hepatis

Of all human pathogenic bacteria, only pathogens of the genus *Bartonella* are capable of inducing vasculoproliferative disorders. The earliest known example of a vasculoproliferative condition caused by *Bartonella* spp. is the chronic phase of Carrión's disease [Verruga peruana (Schultz, 1968)]. This disorder is geographically limited to endemic areas in the Andes (Peru) and is mainly of historical and local significance. Today, *B. henselae* and *B. quintana* are of more global significance as they can induce vasculoproliferative disorders in immunosuppressed patients (e.g., AIDS patients). These vasculoproliferations are designated "bacillary angiomatosis" when appearing as cutaneous manifestations or peliosis hepatis. Both bacillary angiomatosis and peliosis hepatis are histologically characterized as lobular proliferations of blood-filled capillaries and result from chronic local infections with *B. henselae* or *B. quintana* (Relman et al., 1990; 1991). Antibiotic treatment with cell-permeable compounds (e.g., macrolides) leads to eradication of the pathogen and subsequently to a complete regression of the angiomatosis.

To date, pathomechanisms operating in bacillary angiomatosis can only be analysed using cell culture infection models [including endothelial cell proliferation assays and spheroids (Kempf et al., 2001; Kirby, 2004; Garcia et al., 1990; Scheidegger et al., 2009)] as no animal model yet exists. Whereas the exposure of endothelial cells to *B. bacilliformis* leads directly to cell proliferation (Garcia et al., 1990), the induction of neoangiogenic events caused by *B. henselae* seems to be a multistep process. It can now be assumed that three separate mechanisms work synergistically: (i) triggering of endothelial cell proliferation, (ii) inhibition of endothelial cell apoptosis, and (iii) angiogenic reprogramming of infected host cells. Investigation of pathological angiogenesis in the field of cancer research provided valuable insight for examining *Bartonella*-induced angiogenesis: for example, hypoxia-inducible factor 1 (HIF-1) and vascular endothelial growth factor (VEGF) have been known for at least a decade to direct angiogenic cascades (Maxwell et al., 2001; Pugh and Ratcliffe, 2003). Such angiogenic events are also influenced by *Bartonella* spp.: here, a *B. henselae* infection drives the activation of HIF-1 and the subsequent secretion of angiogenic growth hormones (e.g. VEGF) in infected host cells (Kempf et al., 2001, 2005; Riess et al., 2004).

Interestingly, only *Bartonella* adhesin A (BadA) expressing *B. henselae* initiate a proangiogenic host response but the details of the underlying molecular mechanisms remain unclear (Kempf et al., 2001, 2005; Riess et al., 2004). Theoretically, both HIF-1 activation (dependent on BadA expression of *B. henselae*) and inhibition of endothelial cell apoptosis [dependent of the function of the VirB/D4 type 4

secretion system (T4SS) in *B. henselae*] may stimulate bacteria-induced angiogenesis in *Bartonella* infections cooperatively.

The purpose of such angiogenic stimulation may be explained from an evolutionary standpoint: as *B. henselae* grows faster in the presence of host cells than on inert cultivation media (e.g. Columbia blood agar), it appears that *Bartonella* spp. stimulates the growth of endothelial cells to ensure its own ecologically privileged environment (Kempf et al., 2000, 2002).

4.2 *Analysis of* Bartonella *spp. Pathogenicity Factors*

To date, the body of knowledge about pathogenicity factors of the genus *Bartonella* is small. Progress has been slow due to the technical difficulties in performing genetic studies on these slow growing microorganisms. Moreover, suitable animal models for in vivo pathogenicity studies exist to only a limited extent for bacteraemic diseases [e.g. the *B. tribocorum* rat infection model, the *B. birtlesii* mouse infection model (Schülein et al., 2001; Vayssier-Taussat et al., 2010)] and are completely absent for vasculoproliferative disorders. Two pathogenicity factors have been the primary focus of ongoing research: the VirB/D4 T4SS which translocates *Bartonella* effector proteins (Beps) into mammalian host cells and the family of trimeric autotransporter adhesins (TAAs) of which BadA of *B. henselae* (Riess et al., 2004) (see Fig. 4.1) and the Vomps of *B. quintana* (Zhang et al., 2004) are prominent members. More recently, the Trw T4SS system has also been implicated to play an important role in the infection process of host erythrocytes (Seubert et al., 2003; Vayssier-Taussat et al., 2010).

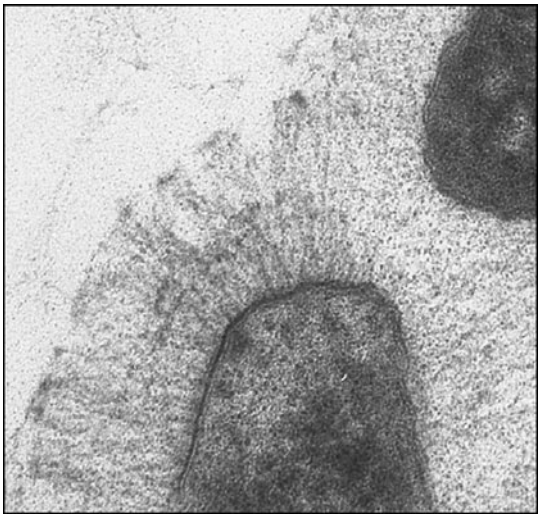

Fig. 4.1 BadA expression of *B. henselae*. Note the remarkable BadA expression forming a dense layer on the bacterial surface (length ~240 nm). Transmission electron microscopy by Heinz Schwarz (Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany)

4.2.1 The VirB/D4 T4SS

T4SSs act like "molecular syringes" in Gram-negative bacteria and "inject" bacterial proteins or DNA either into host cells or other bacteria. Effector proteins of T4SS are secreted through a secretion channel spanning the inner and outer membrane. Originally, T4SS evolved from bacterial conjugation systems and are often essential bacterial virulence factors. Through delivery of particular bacterial effectors, host cell functions are modulated for the benefit of the pathogen (Christie et al., 2005).

The VirB T4SS of *Agrobacterium tumefaciens* is the best characterized T4SS (Chilton et al., 1977) and shows high homologies to that of *Bartonella* spp. (Padmalayam et al., 2000; Schülein and Dehio, 2002). The major function of the *B. henselae* VirB/D4 T4SS seems to be the modulation of endothelial cell functions (Schmid et al., 2004). It is responsible for (i) "invasome formation" (uptake of bacterial agglomerates formed by rearrangements of the host cell actin cytoskeleton), (ii) proinflammatory gene programming [activation of nuclear factor (NF)-κB, interleukin-8 secretion, ICAM-1 and E-selectin expression] and (iii) inhibition of apoptosis in endothelial cells. Moreover, (iv) the VirB/D4 T4SS seems to modulate the mitogenic effects of *B. henselae* on endothelial cells (Schmid et al., 2004; Schülein et al., 2005). So far, seven T4SS-dependent *Bartonella* effector proteins (Beps) have been identified (Schülein et al., 2005) of which the biological functions have been shown in greater detail for two: BepA and BepG. BepA mediates the inhibition of apoptosis in endothelial cells (Schmid et al., 2006) and is also responsible for capillary sprout formation in a more complex infection model (endothelial spheroids) whereas BepG counteracts such sprouting (Scheidegger et al., 2009). The parallel capability of *B. henselae* both to stimulate and to repress angiogenic events through the release of individual effector proteins suggests that the process of *B. henselae*-triggered neoangiogenesis is the result of a finely-balanced interaction between different bacterial effector molecules. To date, the structure and organization of the *B. henselae* VirB/D4 T4SS can only be modelled, as the protein complex has never been visualized (Pulliainen and Dehio, 2009).

4.2.2 Bartonella *Adhesin A (BadA)*

Adherence to the host is the first and most decisive step in a bacterial infection. To ensure efficient host adhesion, bacteria express surface protein complexes with the ability to bind specific molecular host components. These complexes ("adhesins") can differ greatly in their composition and may be classified as pili, fimbriae, membrane-anchored fibres and proteins attached to the cell wall (Hultgren et al., 1993; Hoiczyk et al., 2000; Niemann et al., 2004; Foster and Hook, 1998; Koretke et al., 2006). The family of trimeric autotransporter adhesins {TAAs, other designations: *n*on-*f*imbrial *a*dhesins [NFAs (Hoiczyk et al., 2000)], *o*ligomeric *c*oiled-coil *a*dhesins [Ocas (Henderson et al., 2004; Barocchi et al., 2005)]} has been defined by structure and sequence similarity (Linke et al., 2006; Szczesny and Lupas, 2008).

TAAs are present in α-, β-, and γ-proteobacteria, are clearly linked with pathogenicity and are expressed in human, animal- and plant-pathogens [e.g. *Xanthomonas* adhesin A (XadA) from *Xanthomonas oryzae* (Ray et al., 2002)]. The best examined TAA is the *Yersinia* adhesin A (YadA) of *Yersinia enterocolitica* [first identified in 1982 as "P1" protein (Bolin et al., 1982)] and it is the prototype for other TAAs such as the ubiquitous surface proteins A1 and A2 (UspA1, UspA2) of *Moraxella catarrhalis* (Hoiczyk et al., 2000) and *Neisseria* adhesin A (NadA) of *Neisseria meningitidis* (Comanducci et al., 2002).

On the bacterial surface, all TAAs assemble in a similar way best described as a trimeric "lollipop-like" structure. TAAs are constructed modularly, combining conserved elements designated as "membrane anchor", "stalk", "neck" and "head" (Linke et al., 2006) (see Fig. 4.2). TAAs are defined by their C-terminal membrane anchor domain which is responsible for the autotransport activity and is highly homologous throughout the TAA family. The C-terminal membrane anchor domains

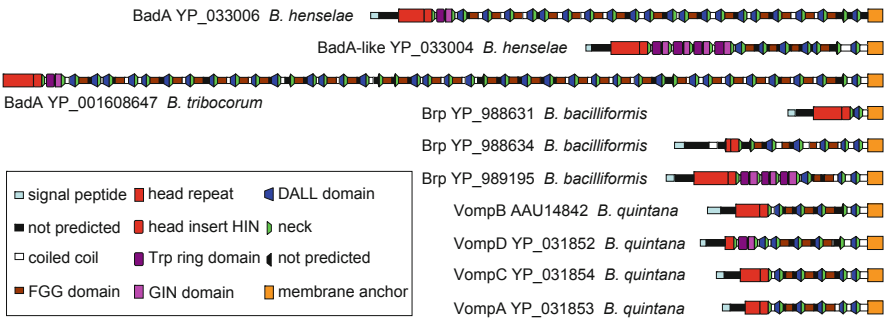

Fig. 4.2 Domain organisation of BadA homologues within the genus *Bartonella*. Homologues to BadA were found using BLAST (Altschul et al., 1997) against the sequenced genomes of *B. henselae* Houston-1, *B. quintana* Toulouse, *B. bacilliformis* KC583, and *B. tribocorum* CIP 10547. The domain composition was determined using the tool daTAA (Szczesny and Lupas, 2008). All domains are drawn to scale (sequence length). The annotation in the figure is from BadA of *B. henselae* Marseille, which harbours an intact copy of the BadA gene. In *B. henselae* Houston-1, a frame shift deletion in the membrane anchor supposedly prevents BadA expression. In addition, two incomplete TAA genes are found in the *B. henselae* Houston-1 genome (not depicted), and one shorter but intact BadA-like TAA with a long, repetitive head domain. The BadA gene of *B. tribocorum* is the longest so far observed TAA in *Bartonella*. This gene is incomplete as it lacks a signal peptide necessary for inner membrane secretion; this is possibly a sequence error as BadA has been shown to be functional in that strain (Saenz et al., 2007). Of the four Vomp genes [VompA-D; reported in (Zhang et al., 2004)], only three are found in the genome of *B. quintana* Toulouse (NC_010161.1). The annotation of VompB was made on the basis of the sequence from *B. quintana* JK31 (Zhang et al., 2004), the other Vomps were annotated from the *B. quintana* Tolouse genome (Alsmark et al., 2004). Although much shorter, VompD is related to BadA, while the other three Vomps lack the Trp-ring and the GIN domain (Szczesny et al., 2008). In *B. bacilliformis*, three Brp genes are found that differ in length and head domain structure; one of them is highly similar in its head structure to the shorter BadA-like TAA of *B. henselae*, while the others are more similar to VompA-C lacking Trp-ring and the GIN domain [reprinted in a modified format from: Kaiser et al. (2011) *Bartonella* spp.: throwing light on uncommon human infections. Int J Med Microbiol 301: 7–15]

of *Haemophilus* influenzae adhesin A (HiA) and YadA of *Y. enterocolitica* (Meng et al., 2006; Grosskinsky et al., 2007) are known to be secreted into the periplasm. Following this, they form a trimeric 12-stranded β-barrel pore in the outer membrane through which the "effector" domains are transported. Other domains (head and stalk) are present in most TAAs but may be rearranged in a variable order similar to "Lego bricks" (see Fig. 4.3).

TAAs of the genus *Bartonella* share some remarkable features which differentiate them from the TAAs of other bacteria: (i) they vary enormously in length, (ii) often more than one TAA gene variant or gene fragment is present in the genome and (iii) they are highly conserved within the genus.

The TAA of *B. henselae*, BadA, is the best understood adhesin of the genus. It was first described as a "type IV-like pilus" (Batterman et al., 1995) but further investigation revealed that it indeed represents a member of the TAA family (Riess et al., 2004). BadA is an extraordinarily large TAA (~3,000 amino acid residues resulting in a size of ~328 kDa per monomer) with a measured length

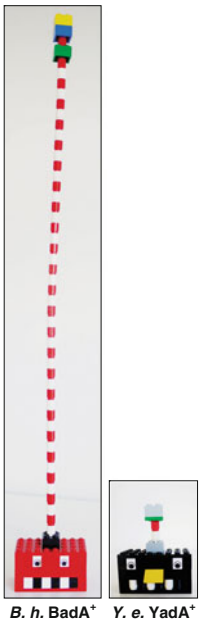

Fig. 4.3 Domain composition of BadA ("Lego brick-concept"): Model of the modular domain organisation of the TAAs *Yersinia* adhesin A (YadA) of *Y. enterocolitica* and *Bartonella* adhesin A (BadA) of *B. henselae*. In principle, certain "bricks" [membrane anchor (YadA: *grey*, BadA: *black*), neck-stalk repeats (*white-red*) and the head domain (YadA: *green*/*gray*, BadA: *green*/*red*/*blue*/*yellow*)] are present in all TAAs. Such "bricks" act as modules sharing similar functions (e.g., membrane anchor: anchoring the adhesin in the OM; neck(s): trimerization motif(s); head: adhesion to host cells). There is evidence that such "bricks" can be removed from TAAs (Kaiser et al., 2008) or even exchanged between e.g., α- and γ-proteobacteria (Schmidgen, Linke and Kempf, unpublished data)

of ~240 nm (Fig. 4.1). It was demonstrated that BadA is essential for the adhesion of *B. henselae* to host cells and extracellular matrix proteins, particularly to fibronectin, laminin and collagens. Moreover, expression of BadA is clearly linked to the capacity of *B. henselae* to trigger a proangiogenic host cell response via activation of the transcription factor HIF-1 and the secretion of angiogenic cytokines [e.g. VEGF, interleukin-8 (Kempf et al., 2001, 2005; Riess et al., 2004)]. While testing sera of patients suffering from *B. henselae* infections, it was discovered that such sera regularly (~70–80%) contained BadA-specific antibodies (Wagner et al., 2008; Riess et al., 2004). Therefore, BadA also seems to be an immunodominant protein and this finding might be used to improve current laboratory protocols for serological diagnostic approaches.

Most of the enormous length of BadA (~240 nm) is formed by multiple repeats of the neck-stalk elements, which are rich in coiled-coil structures. This repetition results in a long and fibrous BadA structure of much greater length than the prototypical YadA from *Y. enterocolitica* (~23 nm) (Hoiczyk et al., 2000). The purpose of the BadA stalk and the significance of its extreme length has not been elucidated. PCR analysis of nine *B. henselae* strains revealed surprising variations in stalk length within the species (Riess et al., 2007). Furthermore, the BadA stalk appears to be important in binding extracellular matrix proteins such as fibronectin, as experiments with a BadA mutant strain lacking almost the entire stalk impaired the fibronectin binding function (Kaiser et al., 2008). However, it is not yet clear whether the stalk of BadA contains a defined binding site for fibronectin or whether it simply represents a "spacer", separating the head at a defined distance from the bacterial cell surface.

While the length of the BadA stalk varies between different *B. henselae* strains, the head domain seems to be of conserved length and high sequence identity (Riess et al., 2007). Correspondingly, the Bad A head domain has been identified as the active element involved in most of BadA functional properties including autoagglutination, binding to extracellular matrix and host cells and triggering of proangiogenic host cell responses. Structurally, the head is composed of three subdomains: (i) an N-terminal domain, (ii) a Trp-ring and (iii) a GIN domain (Szczesny et al., 2008). The N-terminal domain of Bad A is homologous to the prototypical YadA head repeats whereas the Trp-ring and GIN domain (although arranged in opposite order) show high structural but no sequence similarity to the Hia of *H. influenzae*. Although the biological properties of the BadA head have been well analysed (Kaiser et al., 2008) it has not yet been possible to attribute specific functions to individual subdomains.

Genes encoding BadA-homolgous TAAs can be found in all other *Bartonella* species investigated so far (see Fig. 4.2) suggesting a conserved role of these adhesins in *Bartonella* pathogenicity (Zhang et al., 2004; Saenz et al., 2007; Gilmore et al., 2005). In the case of *B. quintana*, four different adhesins (Vomp A-D) are present. Although it is not clear whether all of these Vomps are expressed under natural conditions (Zhang et al., 2007), Vomp D exhibits the closest predicted structural similarity to BadA. Autoagglutination, collagen binding and VEGF secretion of host cells are attributed to Vomp expression (Zhang et al., 2004;

Schulte et al., 2006). Furthermore, Vomp-defective *B. quintana* and BadA-defective *B. tribocorum* mutants do not cause chronic bacteraemia in their respective animal models (Saenz et al., 2007; Mackichan et al., 2008).

4.2.3 Does an Interplay Between the VirB/D4 T4SS and BadA Exist?

The VirB/D4 T4SS and BadA of *Bartonella* spp. probably represent two of the most important pathogenicity factors of this genus. The functions these structures perform are crucial for infection and, in the case of *B. henselae*, both proteins may provide important elements in the multi-step process leading to neoangiogenesis. It can be hypothesized that BadA and the VirB/D4 T4SS may interact during the infection process resulting in a synergistic relationship in which BadA not only provides initial adhesion but also maintains the optimal distance between bacterium and host for effective VirB/D4 T4SS protein translocation.

Examples of such relationships already exist, for example between the type III secretion system (T3SS) and YadA of *Y. enterocolitica*. As with T4SS, the T3SS translocation systems are used by many Gram-negative bacteria for transmission of bacterial effector proteins from the bacterial cytosol into the host cell [e.g., *Yersinia* outer proteins (Yops)]. Modified from an ancestral flagellum structure, the T3SS is made up of a transmembrane basal body and a needle ("injectosome") emerging from the bacterial surface through which the effector proteins are transported. During assembly, the length of the T3SS is controlled by the "*Yersinia* size controller" (YscP), which acts as a molecular ruler alongside the growing needle machinery. Studies comparing YadA and the T3SS in *Y. enterocolitica* indicated that the YadA and T3Ss have to be the same length for effective Yop translocation in host cells (Mota et al., 2005).

One might argue that a similar relationship between the VirB/D4 T4SS and BadA of *B. henselae* is unlikely as the enormous length of BadA would imply an equally long VirB/D4 T4SS. Even though it may be difficult to imagine effective protein translocation over such a distance, T4SSs spanning such a gap are known. For example, the T-pilus of *A. tumefaciens* is even longer at ~1,400 nm (Aly and Baron, 2007). Although much is known about the functions of the VirB/D4 in *B. henselae*, the length of this structure is still unknown. Until more information exists, the plausibility of such a correlation for VirB/D4 T4SS and BadA in *B. henselae* cannot be conclusively determined.

4.2.4 Filamantous Haemagglutinin of Bartonella *spp.*

Genomic sequencing of *B. henselae* revealed the presence of multiple sequences coding for filamentous haemagglutinins (*fhaB1-8,* differing in length) and their corresponding partner secretion proteins (*fhaC/hecB*) indicating the possible existence of further adhesins in this species (Alsmark et al., 2004).

To date it is still unclear whether any of the *fha* genes of *B. henselae* play a role in pathogenicity or whether these genes are even expressed. An STM-study of *B. tribocorum* (also containing ten Fha homologues) did not reveal an obvious role for Fha in pathogenicity (Saenz et al., 2007); however, it needs to be mentioned that with such an STM-approach it is almost impossible to identify a phenotype. Theoretically it would be necessary to knock out all eight *fha* genes before an effect on pathogenicity might be observed. In fact, any kind of mutagenesis approach to this problem would be frustrated by the need to disable multiple genes simultaneously. Nevertheless, despite the technical problems they cause in analysis, the existence of so many versions of this gene may actually be an indicator of its importance: most likely, such "backup" measures against loss would be only afforded to genes of evolutionary significance.

In *B. henselae* the *fha* sequences are found on two genomic islands flanked by tRNAs and integrase genes suggesting that their presence is a result of phage interactions (Alsmark et al., 2004). Fha homologues are found in the genomes of both plant- and animal- pathogenic bacteria in the plant pathogen *Erwinia chrysanthemi* and the animal pathogen *Bordetella bronchiseptica,* Fha homologues are known to play an important role in the infection process (Rojas et al., 2002; Nicholson et al., 2009). The location of *fhaC/hecB* and *fhaB* within a genomic island of the *B. henselae* genome is consistent with the phylogenetic analysis of these homologues, which indicates horizontal transfer across species (Rojas et al., 2002).

Despite the lack of knowledge about the biological function of *B. henselae* Fha, parallels to the Fha of *Bordetella pertussis* (the pathogen causing whooping cough in humans) can be drawn. The Fha of *B. pertussis* represents the best-characterized member of the Fha protein family due to its importance in virulence and its use as a component in most acellular *B. pertussis* vaccines (Pines et al., 1999). The gene combination *fhaB* and *fhaC/hecB* represents a typical two partner secretion system in which *fhaB* codes for the Fha protein and *fhaC/hecB* for an excretion protein specifically dedicated to transport of Fha across the outer membrane. Fha of *B. pertussis* is activated through the two-component regulatory system BvgAS and is initially produced as a 367 kDa precursor protein which is extensively modified over the course of its transport to form a highly immunogenic 220-kDa hairpin-shaped protein at maturity. Transport across the cytoplasmic membrane is accomplished through a sec-signal peptide-dependent pathway followed by secretion across the OM through the Fha specific FhaC protein which forms a transmembrane β-barrel (Clantin et al., 2007). The protein reaches final maturity at the cell surface, where it is further modified and may be released in part due to interaction with the serine protease SphB1 (Mazar and Cotter, 2006; Coutte et al., 2001, 2003b).

The Fha of *B. pertussis* contains three confirmed binding domains: (i) two Arg-Gly-Asp triplets (RGD), which bind to monocytes and macrophages and to human bronchial epithelial cells via Very Late Antigen 5 (VLA-5) (Ishibashi and Nishikawa, 2002; 2003), (ii) a carbohydrate recognition domain (CRD) (Prasad et al., 1993), which binds to ciliated respiratory epithelial cells and macrophages and (iii) a heparin-binding domain, which mediates adherence to non-ciliated epithelial cells (Hannah et al., 1994). Due to the lack of suitable animal models, the biological

functions of *B. pertussis* Fha are mainly deduced from the Fha of *B. bronchiseptica*: here, Fha is crucial for tracheal colonization of rats suggesting its main role is as an adhesin (Cotter et al., 1998). Additionally, Fha is involved in autoagglutination and modulation of the inflammatory host response (Coutte et al., 2003a; McGuirk and Mills, 2000; Inatsuka et al., 2005). The length of *B. pertussis* Fha (3,590 aa; NP_880571.1) was calculated to be ~50 nm (Makhov et al., 1994). This suggests that a putative Fha of *B. henselae* (e.g., FhaB3, 2,653 aa, YP_033500.1) might be of similar length. It is not clear how *B. henselae* Fha mediates host cell interactions in the presence of BadA (~240 nm in length). However, several strains of *B. henselae* do not express BadA (Riess et al., 2007). Therefore, Fha might play an important role in the infection process when BadA is not expressed by *B. henselae*.

4.2.5 The Trw Type 4 Secretion System of Bartonella *spp.*

The function of the Trw T4SS system has been examined in several *Bartonella* species. Trw systems are assumed to have evolved from plasmid conjugation systems acquired through horizontal gene transfer from other bacterial species. Over time, co-evolution with mammalian hosts altered the original biological function from conjugation to host cell interaction leading to subsequent selection to match specific host cell structures (Nystedt et al., 2008). The first indications of the Trw system function were gained from the *B. tribocorum* rat infection model in which *trwE* mutants were characterized by a non-bactaeremic phenotype (Seubert et al., 2003). Recently, using a signature-tagged mutagenesis approach, a *B. birtlesii* mouse infection model revealed mutants impaired in establishing an intraerythrocytic bacteraemia. The corresponding genes encoded components of the Trw T4SS, demonstrating that this virulence factor is directly involved in adherence to erythrocytes. Ectopic expression of Trw of *B. tribocorum* (rat-specific) in *B. henselae* (cat-specific) or *B. quintana* (human-specific) expanded the host range for erythrocyte infection to rat, clearly demonstrating that Trw mediates host-specific erythrocyte infection (Vayssier-Taussat et al., 2010). The analysis of the exact erythrocyte binding partners of the Trw T4SS is currently ongoing (M. Vayssier-Taussat, personal communication). Cumulatively, the Trw T4SS appears to be the determining factor mediating host specific erythrocyte infections.

4.2.6 Less Characterized Adhesins of Bartonella *spp.*

Finally, several *B. henselae* outer membrane proteins (the 28, 32, 43, 52 and 58 kDa OMPs) may also act as adhesins in the course of infection, as they have been shown to bind to endothelial cells in vitro (Burgess and Anderson, 1998). ICAM-1 might be a binding partner of the 43 kDa protein which exhibits a strong affinity to endothelial cells (Burgess et al., 2000). Furthermore, exposure of endothelial cells to purified *B. henselae* outer membrane proteins resulted in NF-κB activation and increased expression of adhesion molecules (E-selectin, ICAM-1) (Fuhrmann et al., 2001).

However, detailed data for the role of these potential adhesions in the host cell infection process are still missing.

4.3 Conclusions and Outlook

Their ability of *Bartonella* spp. to cause vascularpoliferative disorders and intraerythocytic bacteraemia is unique among bacterial pathogens. The VirB/D4 T4SS and TAAs (e.g., BadA, Vomps, BrpA) represent important virulence factors which may work synergistically in host cell infection processes. Other potential adhesins are represented by the Trw-system, the filamentous haemagglutinins and several outer membrane proteins. The exact molecular functions of *Bartonella*-adhesins and their interplay with other pathogenicity factors (e.g., the VirB/D4 T4SS, the Trw T4SS or Fha) need to be analysed in greater detail.

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